

Diurnal patterns of carbon dioxide, water vapour, and energy fluxes in pineapple [*Ananas comosus* (L.) Merr. cv. Red Spanish] field using eddy covariance

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Abstract

We analyzed the eddy covariance measurements of momentum, mass, and energy taken daily throughout five consecutive seasonal courses (*i.e.* 840 d after planting) of a pineapple [*Ananas comosus* (L.) Merr. cv. Red Spanish] field growing in the Orinoco lowlands. This field provides an opportunity for micrometeorological studies because of the flat and windy site; the seasonal weather including ENSO effects and the Crassulacean Acid Metabolism (CAM) physiology of the crop were additional attributes. Soil CO₂ flux was quantified and added to the net ecosystem exchange in order to obtain the canopy flux (F_C). The canopy CO₂ flux partially followed the four phases of CAM *sensu* Osmond (1978). The daily pattern of gaseous exchange in pineapple showed a continuum spectrum in which a major proportion of CO₂ uptake occurring during the daytime was common and in which the CAM expression was related to day and nocturnal CO₂ uptake. However, the benefits of CO₂ uptake at low water cost were constrained by the limited nocturnal CO₂ uptake. Seasonal and ontogenetic changes affected the energy exchange as well as the partitioning of available energy into sensible (Q_H) and latent (Q_{LE}) heat. When the hourly net radiation (Q_{Rn}) reached its maximum value, latent heat flux (Q_{LE}) to available energy throughout the vegetative and reproductive stages was 0.65, 0.05, 0.30, 0.11, and 0.33 for the 1997 wet season, 1997/98 dry season, 1998 wet season, 1998/99 dry season, and 1999 wet season, respectively. Throughout the growth period, we found the pivotal role of surface conductance (g_s) in both Q_{LE} and F_C. Furthermore, the canopy responded to environmental changes. During the wet seasons the g_s was strongly influenced by humidity mole fraction deficit and was usually lower than aerodynamic conductance, whereas during the dry seasons, soil water deficit limited evapotranspiration and production rates. For the fully canopy cover, the hourly trend of marginal water cost of pineapple carbon gain in the dry seasons indicated that g_s became sufficiently efficient to reduce the amount of water transported per unit of carbon gain. In the wet season, the coupling of CO₂ uptake and stomatal conductance was more effective in maintaining a higher proportionality between Q_{LE} and g_s.

Additional key words: air humidity; CAM; energy flux density; Orinoco lowlands; southern oscillation phenomenon; surface conductance; temperature; wind velocity.

Introduction

In drought-prone domains, there is interest in crops that assimilate CO₂ via the Crassulacean Acid Metabolism (CAM) pathway in order to integrate marginal lands into production. This is possible because CAM crops can maintain a positive carbon balance, even at low water potential (Osmond 1978). Therefore, the comparative advantage of CAM systems and their potential for food production are always undergoing intensive examination.

Pineapple is the most widely distributed CAM crop (Sideris *et al.* 1948, Ekern 1965, Zhang and Bartholomew 1993). This crop is planted over wide areas of savannas where interacting stressful factors are a common environmental feature. Under these conditions, the eco-physiological advantage of CAM species can be related to metabolic plasticity in the CAM response to daily or seasonal changes (Borland and Taybi 2004, Lüttge 2004).

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Adaptive features in the pineapple cultivars have been associated with their functional advantages in limiting environments (Bartholomew and Kadzimin 1977). Specifically, the inverted pattern of stomatal movement in pineapple is coupled with a low transpiration ratio (50–166) (Neales *et al.* 1968, Aubert 1970, Bartholomew and Kadzimin 1976). This pattern is markedly regulated by thermoperiod, photoperiod, and plant water status (Yoder 1969, Yoder and Bartholomew 1969). Critical adaptations for the survival of pineapple during stressful periods result in a deadlock for carbon exchange as the CO₂ uptake is considerably reduced during the noon hours (Osmond 1978). Under experimental conditions, this reduction in CO₂ uptake can reach 58–88 % of the total CO₂ absorption (Neales *et al.* 1968, Neales 1973). In savanna conditions, adaptation of pineapple cultivars to strongly interacting stress factors is critical for survival and agricultural output. However, available results are scarce for understanding and predicting pineapple performance under field conditions. Water use in field by CAM crops is scarcely known, especially under variable environment, particularly water supply (Ekern 1965). CAM plants are efficient users of water compared to C₃ and even C₄ plants (Lüttge 2004) but need assessing in field because of feed-back mechanism, which may decrease advantage of CAM mechanism. The overall framework of this study was to analyze the effect of diurnal conditions on the ecological functioning (*i.e.* CO₂ exchange and evapotranspiration) of a pineapple cultivar Red Spanish growing during consecutive seasonal courses in the Orinoco lowlands. We monitored diurnal energy balance, CO₂ exchange, and the evapotranspiration process of rain-fed pineapple cultivated in savanna.

Knowledge of the physiology of gaseous exchange in CAM plants has mostly been gained on the basis of leaves or whole plants using methods relying on measurements with assimilation chambers and carbon isotope ratios under field or experimentally controlled conditions (Joshi *et al.* 1965, Neales *et al.* 1968, 1980, Bartholomew and Kadzimin 1977, Nose *et al.* 1977, 1981, 1986, Sale and Neales 1980, Cote *et al.* 1993, Zhu *et al.* 1999, Keller and Lüttge 2005). However, feedbacks between physiological processes and the environment around the leaves do not usually operate and gaseous exchange may be effectively reduced by changes in stomatal conductance (Jarvis and McNaughton 1986). Few analyses of atmospheric conditions and their influence on pineapple evapotranspiration have been performed at the crop surface by lysimeters (Ekern 1965). Measurements of CO₂ exchange for a CAM community placed in sealed rooms have been carried out at the Biosphere 2 Center in Arizona (Nobel and Bobich 2002). Recent micrometeorological approaches can provide suitable research means. This is because pineapple growth can affect surface forcing conditions, and consequently also CO₂ exchange and the partitioning of available energy into the densities

of sensible (Q_H) and latent heat fluxes (Q_{LE}) (Baldocchi 1994a,b, San José *et al.* 2003). Here, gaseous exchange and energy flux density are measured over a pineapple canopy throughout all the life-cycle stages. In this case, micrometeorological approaches and models for the analysis of gaseous exchange are of particular interest because of their ability to predict surface control of energy transfer in terms of canopy and environmental factors (Jarvis and McNaughton 1986). However, they have not been used to analyze the coupling of the CAM plants and the atmosphere. In this sense, pineapple is an ideal CAM crop to examine atmospheric control upon surface conductance (*g_s*) with concern for the effect of diurnal conditions. As a consequence, although an increasing number of studies of gaseous exchange in CAM crops are being undertaken, the results are often inconclusive.

This study was carried out within the Orinoco savanna (0.4×10¹² m²) of northern South America. Across these savannas, an appreciable climatic gradient occurs mainly related to variation in mean annual precipitation and the length of the dry season, which increases from 2 to 5 months (San José *et al.* 1985, San José and Montes 1991). These remarkable seasonal conditions are reinforced by their oligotrophic soils with low water-holding capacity (San José *et al.* 1982, 1985). Under these limiting conditions, the rain-fed and fertilized pineapple yields 35 t per ha (Michelena 1992), which is considerably lower than the maximum yield (103 t per ha) reported for pineapple (Romero *et al.* 1973). Therefore, there is a need to increase understanding of diurnal CO₂ and water vapour transfer processes associated with increased productivity of pineapple, taking into account daily differences across growth duration. A notable feature of the Orinoco savanna is its high potential for expanding agricultural activities. This provided an opportunity to study rain-fed pineapple adaptation over consecutive seasonal courses. Understanding the role of the transfer processes is necessary for predicting how land management could affect crop functioning.

The present work was undertaken to: (1) analyze the effect of environmental savanna conditions on diurnal CO₂ flux and the partitioning of available energy into latent heat and sensible exchanges over a pineapple canopy using the eddy covariance method; and (2) assess the processes controlling CO₂ exchange and evapotranspiration under the different surface conditions generated by canopy structure and growth duration throughout the life-cycle stages. Those results could be useful in formulating a more comprehensive ecological study of CAM crops on the surface and environmental processes that control energy transfer during daytime and night-time periods. Although the magnitudes of the fluxes reported are relevant to similar sites, the processes described may be representative of lowlands subjected to daily variations and planted with pineapple.

Materials and methods

Study-site was selected at the experimental station (9°38'N, 63°37'W) of Santa Barbara (National Institute for Agricultural Research) (Edo. Monagas), Venezuela. The landscape is a conserved mesa (Hedberg and Pyre 1944) with alluvial highlands. Site altitude was 195 m. The soil is an infertile sandy loam belonging to the Ultisols and specifically to the Arenic Kanhaplustuls according to U.S. Taxonomy (Cabrera de Bisval *et al.* 1993). The climate is characterized by a mean annual precipitation of 1 018 mm and a growing season spanning May to November with 685 mm. The annual mean temperature is 25.9 °C and annual class A pan evaporation is 1 548 mm.

Pineapple community: A 36 ha plot (600×600 m) was selected such that the source area/footprint of the flux measurements remained within the area. At the centre of the plot, the performance of the eddy covariance equipment was tested by measuring and relating the ratio of the standard deviation of the vertical wind speed (σ_w) to the friction velocity (u_*). This ratio was related to the atmospheric stability as predicted by Monin-Obukhov similarity theory. For unstable conditions, this ratio is measured at a rate proportional to $[(z - d)/L]^{1/3}$ as indicated by Kaimal and Finnigan (1994), where z represents the height above zero-plane displacement (*i.e.* d) and L the Monin-Obukhov stability length. Determinations of these parameters will be explained in following sections. The up-wind fetch was 424.2 m.

The 36-ha plot was plowed and planted (6 June 1997) with basal and axillary buds of pineapple cv. Red Spanish. The planting distance was 0.5 m between individuals and 0.6 m between rows (33 000 plants per ha). Fertilizer was split into five applications. Fertilizer applications were typical of those applied in the zone for commercial pineapple. At 30 d after planting, the soil was fertilized with 300 kg per ha of 12-12-17/2 NPK fertilizer. Thereafter, the soil was fertilized with commercial urea in split applications of 46.7 kg(N) per ha at 119, 370, 524, and 554 d after planting (DAP). Thus, during the crop life-cycle, the total N application was 222.8 kg(N) per ha. The split applications were carried out during leaf growth and just before flower inductions as outlined in Lacoëuilhe (1978). At the 30–40 leaf stage, flowering was forced with *Ethephon* when the crop was 22 months old. The fruits were harvested on 24 Sept. 1999 (*i.e.* 840 DAP).

Micrometeorological measurements: The eddy covariance technique (Aubinet *et al.* 2000) was used to simultaneously measure the mass and energy flux densities over the pineapple field from crop establishment (120 DAP) to harvesting time at 840 DAP. The carbon budget of the stand was calculated as outlined in Ruimy *et al.* (1995). The analysis was carried out throughout five consecutive seasonal courses. During the 1997 wet

season (0–220 DAP), the crop was established (0–120 DAP), and thereafter it grew vegetatively throughout the 1997/98 dry (221–340 DAP), 1998 wet (341–600 DAP), 1998/99 dry (601–664 DAP), and the beginning of the 1999 wet (665–690 DAP) seasons. Reproduction onset was forced and measurements were taken until harvesting (840 DAP) at the end of the 1999 wet season.

The flux density of short-wave radiation was measured over the plot with two pyranometers (*LI-200X*, *LI-COR*, Lincoln, NE, USA and *CM3*, *Kipp and Zonen*, Delft, Holland) facing up. The albedo was measured with a pyranometer (*CM3*, *Kipp and Zonen*) facing down. The net radiation (Q_{Rn}) (*i.e.* net all-wave radiation balance) over the crop was determined with two net radiometers (Funk-type, model *SRI.4*, *Solar Radiation Instruments* and *Q-7.1* *Campbell Scientific*, Logan, UT, USA). At the soil surface, the net radiation was measured with a tube radiometer (*TRL/M3*, *Delta-T Devices*, Cambridge, England) and a net pyrrometer (Funk-type, model *S.R.I.4*, *Solar Radiation Instruments*, CSIRO, Australia). The signals from the instruments were recorded every second and averaged every minute on a data-logger (*21X*, *Campbell Scientific*). Hourly data represent an average of 60 consecutive readings. All times were local apparent times.

The eddy covariance system was assembled according to Moncrieff *et al.* (1997). It encompassed a 3-dimensional sonic anemometer (*Solent A1002R*, *Gill Instruments*, Lymington, UK) for measuring the components of wind and a fast-responding closed-path gas analyzer (IRGA) *LI-6262* (*LI-COR*, Lincoln, NE, USA) to measure carbon dioxide and latent heat flux densities. The inlet tube was mounted 1.6 m above the canopy. Air was conducted continuously through 6-mm diameter tubing (*Dekabon 1300*, *Deame & Co.*, Glasgow, Scotland) at a flow rate of 6 000 cm³ per min into the gas analyzer. It was pulled with a pump (*CAPEX V2X*, *Charles Austen Pumps*, Surrey, UK) and the flow rate was maintained with a mass flow controller (*Tylan FC2900B*, *Tylan General*, Swindon Wilts, UK). The calibration of the analyzers was checked every 2 d using a water vapour generator (*WG 602*, *Analytical Development Company*, Hertfordshire, UK) and accurate CO₂ concentration from a primary gas calibration cylinder prepared by *LI-COR*. The accuracy of this gas was periodically checked by gas prepared using precision gas-mixing pumps (*Wösthoff*, Bochum, Germany). The system was controlled and fluxes calculated in real time by the software *Edisol* (Moncrieff *et al.* 1997). This software also allows the storage of raw data on a laptop computer for post-processing. *Edisol*'s algorithms permit corrections from non-ideal frequency response of the system on the basis of combined transfer functions as proposed by Moore's scheme (Moore 1986). The maximum corrected signal was less than 8 % of the fluxes. In addition to these corrections, the CO₂ and water

vapour delay times between measuring changes in air velocity (w') (*i.e.* sonic anemometer) and recording the simultaneous change in scalar concentration (c') at the IRGA were calculated, and maximum correlation coefficient (*i.e.* $w' c'$) was set in order to calculate the fluxes (Massheder and Moncrieff 1997). These results were inter-compared with those measured in a second sonic system involving a second thermocouple attached to a Krypton hygrometer (KH20) and a sonic anemometer (model CA27 from Campbell Scientific) as proposed by Villalobos (1997) for studying the effect of sensor separation and corrections on eddy covariance fluxes. During two weeks, the results indicated that the fluxes were similar throughout unstable and stable conditions.

At the plot, soil heat flux (Q_G) was measured using a network of ten heat flux plates (S.R.I.9. *Solar Radiation Instruments*, CSIRO, Victoria, Australia), which were placed 0.08 m below soil surface. The net storage of energy (Q_{SE}) in the soil column was determined from the temperature profile (TP) taken above each soil heat flux plot.

At the plot, instruments were installed near the centre, and measurement height was maximized by using the source area/footprint analysis as outlined in Schuepp *et al.* (1990). Additionally, air-intake tubes were fixed to a metal mast at 12 levels above the crop and within foliage. These hoses were connected to a type 161 gas handling system (*Analytical Development Company*, Hertfordshire, UK) and CO_2 in the air was measured with an infrared gas analyzer (LI-6262, *LI-COR*) to calculate changes in CO_2 storage fluxes. In addition to measuring the vertical changes in the gaseous exchange, two masts were placed in the field at distances of 10 and 25 m from the central mast, and the eddy covariance systems were used to analyze changes in gaseous exchange with distance downwind (*i.e.* advection) as outlined in San José (1992). Before the field measurements, the equipment was inter-compared and figures connected for variations.

At the plot, hourly soil CO_2 efflux was measured over the pineapple life-cycle stages by randomly positioning a respiratory chamber (LI-6000-09, *LI-COR*) connected to an infrared gas analyzer (LI-6200, *LI-COR*). Every hour, four replicate samples were processed in the area. For each measurement, soil temperature was taken by placing a thermocouple 0.1 m below the soil surface.

Volumetric water content was measured daily at 0.6 m depth in intervals of 0.15 m by using time domain reflectometry (TDR) (6050X1 Trase System, *Soil Moisture Equipment*, CA, USA). At each soil depth, three probes were buried horizontally and readings were taken by using a multiplexer (6022 Trase System). During the crop life-cycle, the site was equipped with an automatic weather station (CM6 Campbell Scientific, Logan, UT, USA) to measure the values of ancillary meteorological features (*i.e.* solar radiation, precipitation, air temperature, relative humidity, pressure, wind speed, and direction).

Data gap-filling due to system stops was performed in 7 % of the total number of days. In order to take account of the data gaps, a parametrization was used as outlined in Aubinet *et al.* (2000, 2002) when meteorological data was available. In the case of unavailable data, the missing flux was replaced by interpolation.

Conductance pathway to the transfer processes: The conductance approach based on the Penman equation (Monteith *et al.* 1965) was applied for comparative analysis of the processes controlling the transfer of energy. The aerodynamic conductance for water vapour (g_a) was calculated from wind data taking into account the additional boundary layer conductance to water vapour and CO_2 fluxes relative to the momentum flux (Thom 1975). It can be expressed as:

$$g_a^{-1} = \frac{u}{u_*^2} + \frac{1}{ku_*} \left(\ln \frac{Z_0}{Z_H} + \Psi_M - \Psi_H \right) \quad (1)$$

where u is the horizontal wind speed as measured at the reference height above the surface, u_* is the friction velocity as measured by the eddy covariance system, k is von Karman's constant, and Z_0/Z_H is the ratio of the surface roughness parameter for momentum (Z_0) and heat (Z_H) transfers (Verma 1989), which takes into account the sink and source distribution of momentum, heat, and mass as well as the difference in transfer mechanism. The Z_0/Z_H rate was taken as 10, a typical value for homogeneous canopy (Garraat and Hicks 1973), and Ψ_M and Ψ_H are the adiabatic correction factors for momentum and heat, respectively (Paulson 1970). These were calculated by the integrated momentum and heat profile functions.

Surface conductance (g_s) was calculated from inversion of the Penman-Monteith equation (Kelliher *et al.* 1993), which encompasses the latent (Q_{LE}) and sensible heat (Q_H) flux densities as well as the specific humidity deficit of air (D_a). Thus, g_s is calculated for the entire surface:

$$g_s^{-1} = \left(\frac{\epsilon + 1}{g_a} \right) \left(\frac{\epsilon A}{(\epsilon + 1) Q_{LE}} - 1 \right) + \frac{\rho_a D_a}{Q_E} \quad (2)$$

where ϵ is the rate of change of the latent heat content of saturated air with changes in sensible heat content [*i.e.* $(\lambda/C_p) dq_{sat}/dT_a$, λ is the latent heat of vaporization, C_p is the specific heat of the air, q_{sat} , the saturation specific humidity at a reference height above the surface, T_a is air temperature]; A is the available energy for the canopy (A_c) and soil (A_s) (*i.e.* $A = A_c + A_s$), ρ_a is the density of air, and Q_E is the surface evaporation rate. This is an equation with latent heat flux density for the total LE density (*i.e.* contribution from canopy and soil) and the available energy for the entire surface (A).

Microclimatic features: Canopy surface temperature (T_c) (*i.e.* air temperature at the surface canopy) was determined as outlined in Adams *et al.* (1991). The

equation to calculate T_c becomes:

$$T_c = T_a + \frac{Q_H}{\rho_a C_p g_a} \quad (3)$$

where Q_H is the sensible heat flux. In four plants selected at random, canopy temperature was measured by attaching four copper-constantan thermocouples to the adaxial leaf insertion at the stem.

Canopy-to-air humidity fraction mole difference (D_c) was calculated as the difference between the saturation humidity fraction mole at the canopy surface and the humidity fraction mole of the air. The former was determined from the evaporation rate, the air humidity fraction mole, and g_a .

The environmental effect on g_s was analyzed by non-linear least square optimization as outlined in Jarvis (1976). This analysis is used to empirically determine response of g_s to radiation, canopy temperature, air humidity mole fraction deficit, and soil volumetric water content. Also, the model developed by Cowan and Farquhar (1977) was applied for a comparative analysis of the processes controlling the transfer of energy. We examined in detail the influences of changes in environmental conditions on the g_s behaviour by calculating the marginal unit water cost of canopy carbon gain (Λ) as outlined in Lloyd *et al.* (1991) for field application.

Results and discussion

During the measuring period (*i.e.* 840 d after planting from 6 June 1997 to harvesting), the recorded meteorological data (Fig. 1) indicated that the accumulated precipitation was 2 789 mm. The meteorological patterns during the years 1997 and 1998 were drier and hotter than those prevailing in the Orinoco lowlands over the 33 year-climadiagram. After crop planting, there was a bimodal pattern of precipitation with four marked rainless periods (*i.e.* 100–105, 128–159, 185–191, and 217–219 DAP) and accumulated rainfall of 792 mm. The observed severe rainfall variability and displacement of the centre of precipitation were associated with a warm, sunny, and hot front with low rain, which is characteristic of the El Niño/Southern Oscillation phenomenon (ENSO). By contrast, the conditions during the year 1999 were similar to the normal climatic pattern.

The turbulent energy fluxes (*i.e.* sensible heat, Q_H , plus latent heat, Q_{LE}) and the available energy (*i.e.* net radiation, Q_{Rn} , less the net storage heat flux, Q_{ASH}) over the flat, homogeneous, and short pineapple stand were determined simultaneously, and the results compared by regression. The effect of the random error in the independent variables was addressed on the regression statistics (Meek and Prueger 1998). The statistical test showed a significant slope of 0.99; $r^2 = 0.81$; Fs ANOVA = 146; and root mean square = 18 W m⁻². The methods indicate a closure at the site and from these findings;

The value of Λ remains constant when the sensitivities of the rates of transpiration and carbon canopy assimilation (F_c) to variation in surface conductance [*i.e.* ($\Lambda = \delta Q_{LE}/\delta g_s)/(\delta F_c/\delta g_s)$] is a constant over a specific time (Cowan and Farquhar 1977). Therefore, g_s responds directly to change in the environmental conditions (Cowan 1982). The term is calculated as:

$$\Lambda = \delta Q_{LE}/\delta F_c = \frac{g_s^2 D_a (C_a - \Gamma)}{1.6 F_c^2} \quad (4)$$

where C_a is the atmospheric CO₂ concentration, and Γ (*i.e.* the CO₂ compensation concentration) was measured as a function of canopy temperature (T_c) using a field-based photosynthesis system. The canopy flux (F_c) was calculated by adding net ecosystem exchange to soil flux. The method was applicable to the pineapple canopy on the basis of the following theoretically satisfactory requirements. Here, the internal CO₂ concentration (C_i) and F_c were partially depending on the CAM mode and relatively independently on D_a . The aerodynamic conductance was sufficiently large for it to be ignored and the relationship in a closed canopy between $\delta Q_{LE}/\delta g_s$ and D_a was adequate. It was assumed that the carboxylation efficiency and chloroplastic mole fraction of CO₂ was linear, and transpiration had low impact upon canopy temperature.

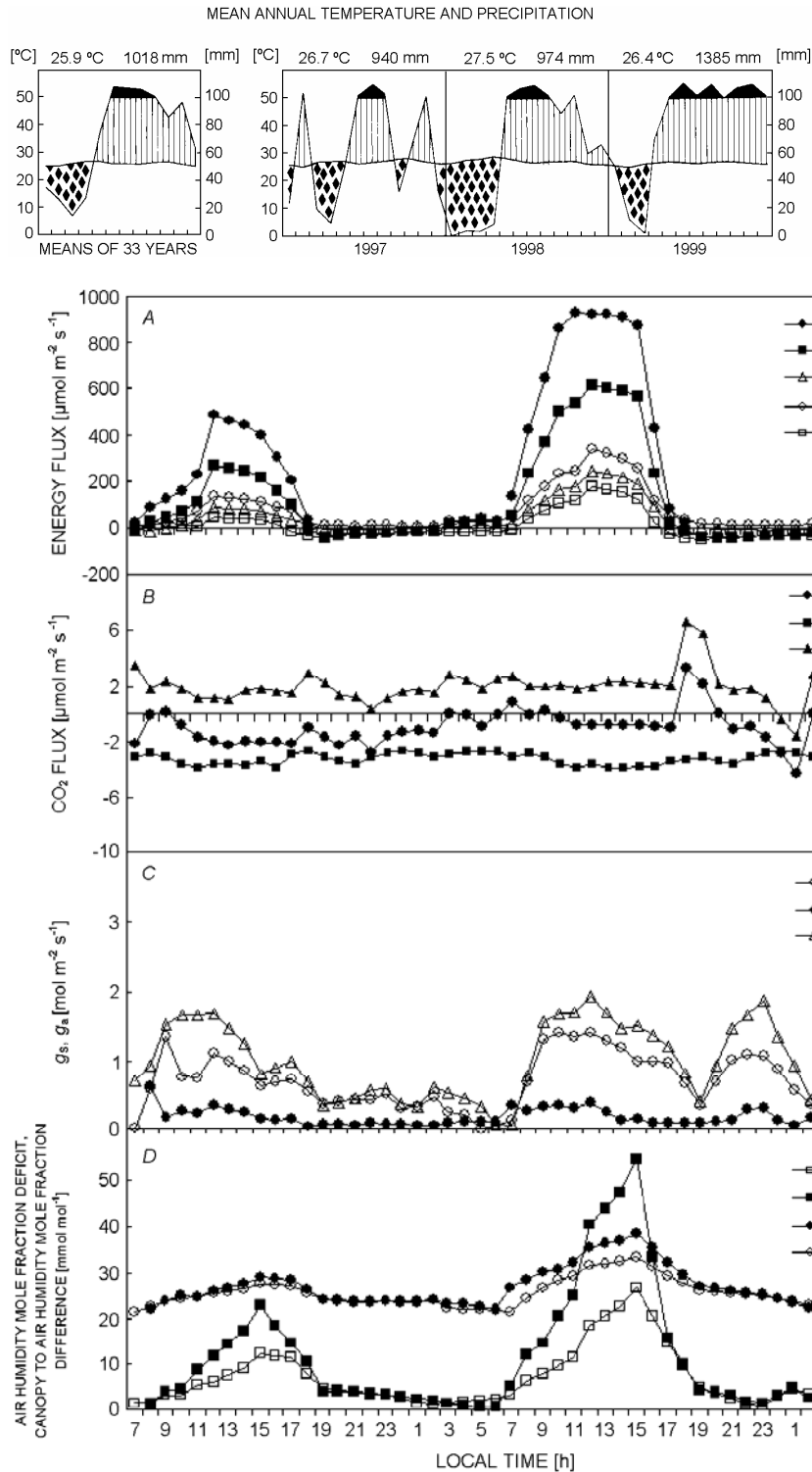
hence the method was adequate for proceeding with the analysis. Even though, the energy closure is relevant to the evaluation of Q_{LE} and Q_H , the atmospheric mechanisms, and the theoretical assumptions are similar for the CO₂ scalar (Paw U *et al.* 2000, Wilson *et al.* 2002). Furthermore, there is a link between CO₂ flux and energy balance closure (Wilson *et al.* 2002). The reasons for the energy balance closure in the pineapple field can be related to (a) the short and homogeneous crop growing in a flat area, (b) the match of flux footprint and sensor measurements, (c) the considerations of storage terms, (d) the absence of advection as indicated by measurements of horizontal fluxes, and (e) the corrections for frequency losses and sensor lag times.

In the Orinoco lowlands, the diurnal patterns of the hourly CO₂ and water vapour fluxes throughout the pineapple life-cycle were closely dependent upon the canopy processes and environmental conditions (Figs. 2–6). The daily patterns of the canopy CO₂ flux in pineapple partially followed the four-phases-model described for CAM species by Osmond (1978), and specifically for pineapple growing under experimental conditions (Nose *et al.* 1977, 1986, Neales *et al.* 1980, Cote *et al.* 1993).

At the early vegetative growth stage (*i.e.* 1997 wet season) (Fig. 2), the pineapple field showed an initial slight CO₂ burst (*i.e.* phase II of CAM *sensu* Osmond 1978) from 06:00 to 07:00 h. Associated with a partial g_s

effect, this phase is considered as a transient between dark CO_2 fixation and the photoperiod, when the storage

behaviour of the CAM vacuole and the phosphoenolpyruvate carboxylase (PEPC) activity are involved



(Kluge *et al.* 1982). Here, the CO₂ burst occurred during early growth when the environmental conditions were characterized by a null gradient in temperature and humidity between the canopy and the air. In experimental conditions, the CO₂ burst in CAM species is enhanced by factors such as the long day conditions, the high irradiance, the low daytime temperature, and water-supply (Kluge 1968). After earlier morning hours, the CO₂ burst in the crop was followed by a slight decrease in CO₂ intake, which would correspond to phase III of Osmond's model. The slight CO₂ uptake depressions related to CAM metabolism occurred independently of the water relations of the crop. Only during the extremely stressful conditions of the dry season, the water losses in pineapple

were markedly reduced (Figs. 3 and 5). During these days, the magnitude of g_s allows a moderated gaseous exchange with the atmosphere, and a feedback response leads to conserve water such as reported for other CAM species growing in extreme habitats (Lange *et al.* 1975). During the phase III, the reduction in the uptake of the CO₂ from the atmosphere was associated to a decrease of both the g_s and the CO₂ gradient between the atmosphere and the calculated C_i , specifically during the warmer hours. Similar trend of gaseous exchange has been reported for pineapple growing under experimental conditions (Cote *et al.* 1989). In CAM species with strictly inverted rhythm, photosynthesis depends entirely upon carbon supply from the onset of malic acid, the

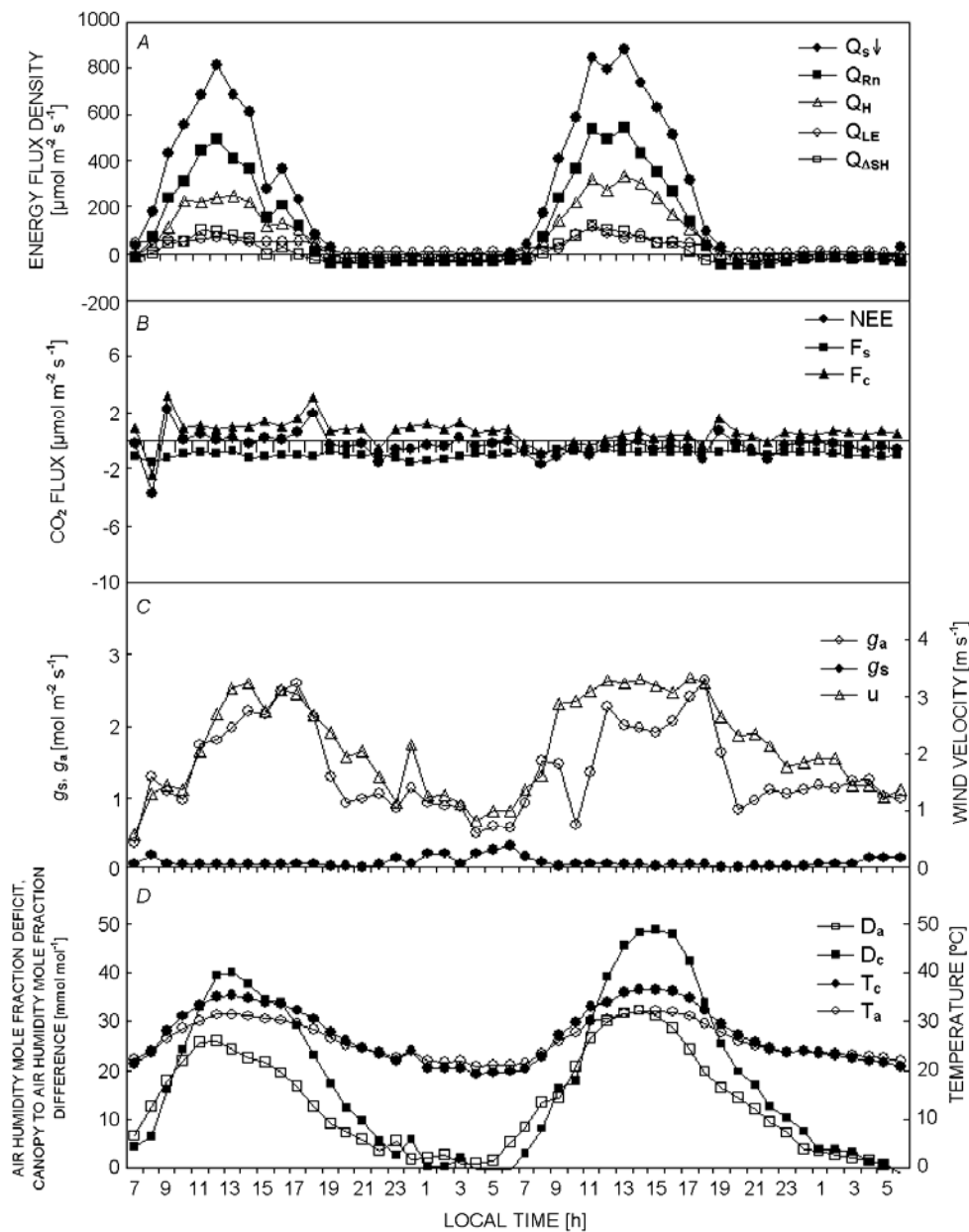


Fig. 3. The same parameters as in Fig. 2, but measured in typical days of the vegetative stage (251–252 DAP).

efflux from the vacuole, and the recycling (Winter and Tenhunen 1982, Friemert *et al.* 1988). In the early vegetative growth stage of pineapple (Fig. 2), a second peak of canopy CO_2 flux (*i.e.* phase IV) was measured at the end of the daytime. During this stage of exogenous CO_2 assimilation, the nocturnally stored malic acid is consumed and transferred from the CO_2 uptake via ribulose-1,5-bisphosphate carboxylase (RuBPC) to CO_2 storage via PEPC (Kluge 1968). The magnitude of phase IV depended upon irradiation. During the stressful conditions of the dry seasons, this phase experienced marked reduction such as has been reported for other pineapple cultivars (Nose *et al.* 1981, Keller and Lüttge 2005). During the earlier growth stage, nocturnal CO_2

uptake (*i.e.* phase I of the Osmond's model) and sporadic short respiratory activity were found. The ratio of night-time CO_2 uptake to daytime canopy CO_2 flux was 2.08. As the vegetative growth proceeded (Fig. 3), the crop experienced the effect of the stressful conditions of the 1997/98 dry season (*i.e.* the ENSO phenomenon), and the length of the growing season was markedly increased from 2.0 to 2.3 years. During these extreme conditions, the daily trend of the surface conductance over the pineapple field showed an inverted rhythm, which was similar to the pattern exhibited by the model of CAM *sensu* Osmond. However, photosynthesis did not depend entirely upon carbon supplied from the decarboxylation of malic acid as a slight CO_2 uptake from the atmosphere

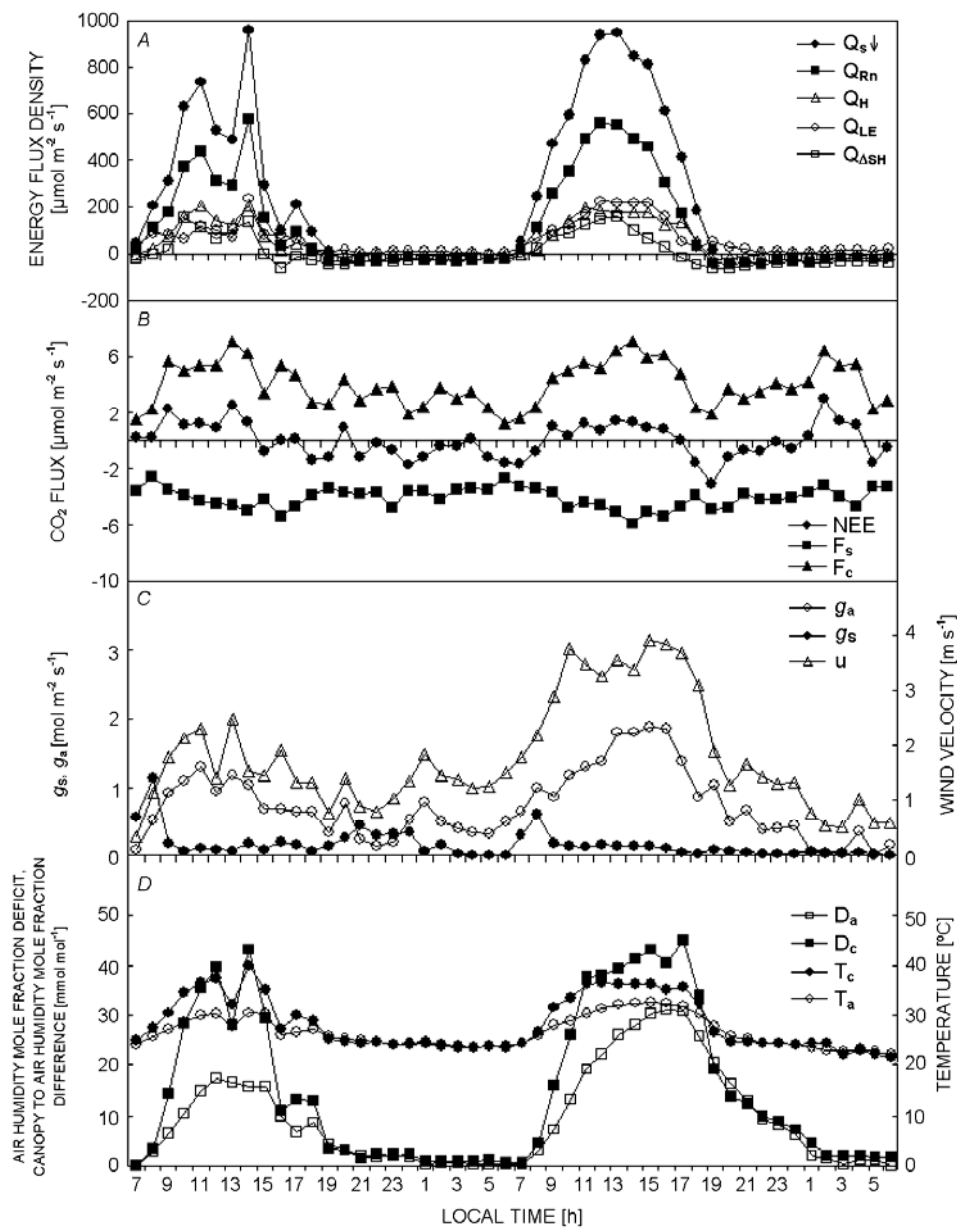


Fig. 4. The same parameters as in Fig. 2, but measured in typical days of the rapid vegetative growth stage (432–433 DAP).

was measured (Fig. 3). At the beginning of daytime, CO_2 efflux was observed when g_s increased. Similar efflux has been measured in a single pineapple leaf and a micro-crop (Neales *et al.* 1980). This efflux occurred when the rate of CO_2 re-fixation by photosynthesis was insufficient to retain the CO_2 released from malate decarboxylation, mitochondrial respiration, and photorespiration (Kluge 1968, Szarek and Ting 1974). Here, the efflux was enhanced by solar radiation and temperature. The radiation effect was shown in individual pineapple plants by Nose *et al.* (1977).

During the daytime of the reproductive stage (Fig. 6), the canopy CO_2 flux reached its maximum value ($7.2\text{--}8.7\ \mu\text{mol m}^{-2}\text{ s}^{-1}$). This result could be related to an

increase in the assimilation sink (*i.e.* demand function) as reproductive organ development. This stimulating effect on canopy activity by the onset of reproductive organs was reported for *Manihot esculenta* growing under similar lowland conditions (San José and Mayobre 1982). The maximum canopy CO_2 flux in pineapple was limited to a low rate as compared to that reported for C_4 pasture using eddy covariance techniques ($25\text{--}40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$; Grace *et al.* 1998). The low CO_2 uptake in pineapple was associated to remarkably low g_s . This is a common trait of the CAM species that has been explained on the basis of the high resistance to CO_2 diffusion from the atmosphere to the site of carboxylation and the capacity of RuBPC (Combres 1983, Py *et al.* 1984, Cote *et al.*

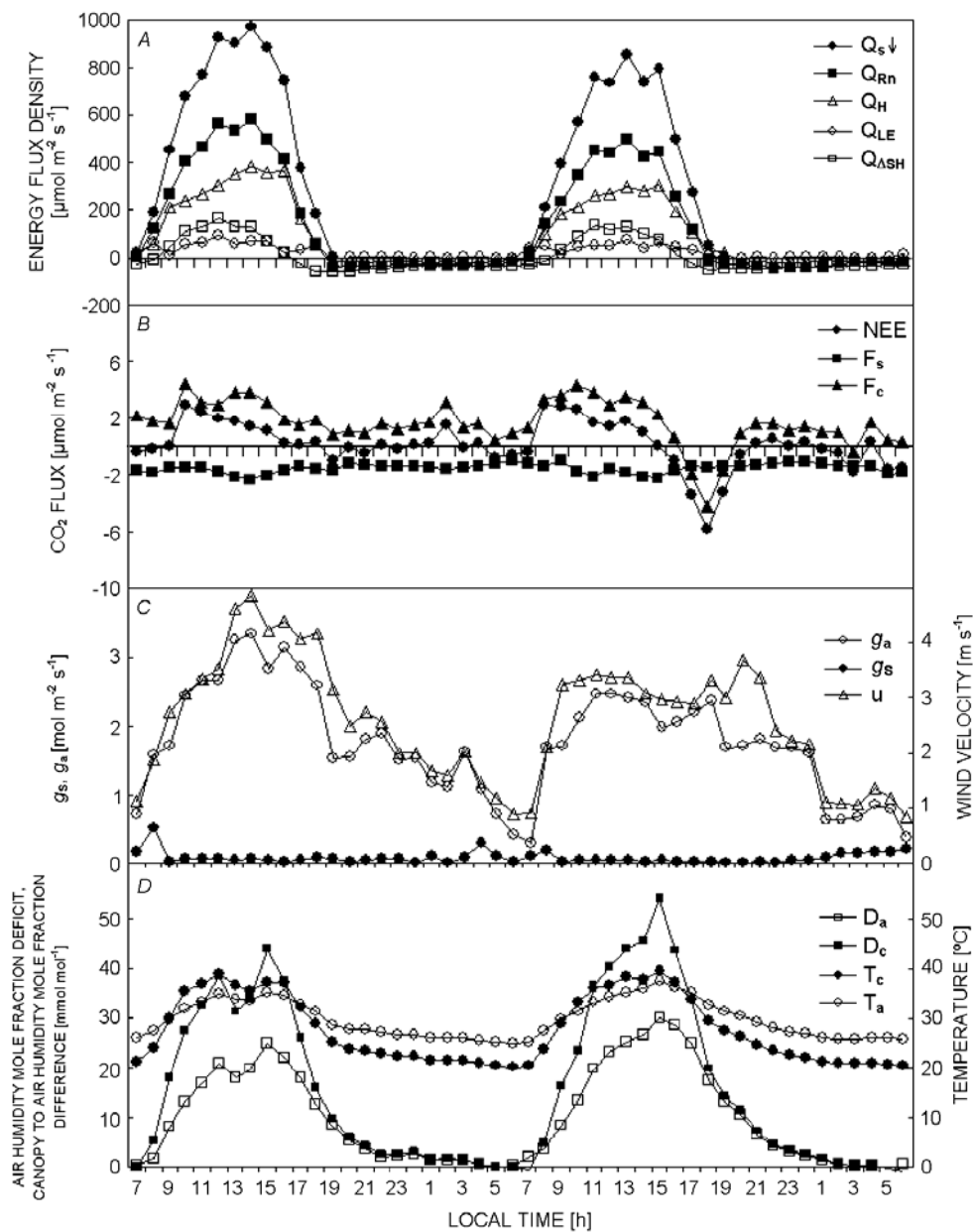


Fig. 5. The same parameters as in Fig. 2, but measured in typical days of the late vegetative growth stage (649–650 DAP).

1993). However, carbon assimilation in pineapple depends not only on the daytime atmospheric CO_2 uptake but also on the nocturnal gross CO_2 uptake (*i.e.* CO_2 from external atmosphere and respiratory recycling) such as shown by Cote *et al.* (1989) using isotopic techniques. In this balance, the respiratory recycling ranges from 30 to 40 % of the total CO_2 fixed into malate *via* PEPC. This recycling depends upon the metabolic control (*i.e.* the regulation of PEP synthesis from glucan, and feedback control of PEPC; Kluge and Ting 1978). Using aforementioned results, the calculated carbon assimilation by the pineapple cv. Red Spanish growing in the Orinoco lowlands would be $22\text{--}23 \mu\text{mol m}^{-2} \text{s}^{-1}$, which is a similar

value as the low extreme determined for the aforementioned C_4 pasture.

During the reproductive stage (Fig. 6), the nocturnal canopy flux ranged from 5.9 to $7.1 \mu\text{mol m}^{-2} \text{s}^{-1}$. Taking into account the leaf area index (LAI), the maximum values spanned from 1.3 to $1.6 \mu\text{mol}(\text{CO}_2) \text{m}^{-2}(\text{leaf area}) \text{s}^{-1}$. Under experimental conditions, Sale and Neales (1980), Neales *et al.* (1980), and Zhu *et al.* (1999) reported nocturnal values for pineapple spanning from 1.0 to $7.0 \mu\text{mol m}^{-2}(\text{leaf area}) \text{s}^{-1}$. The night flux error was an assessment of the relative effect of the storage and advection processes that take place at night. The need to take these two terms into account has been stressed by

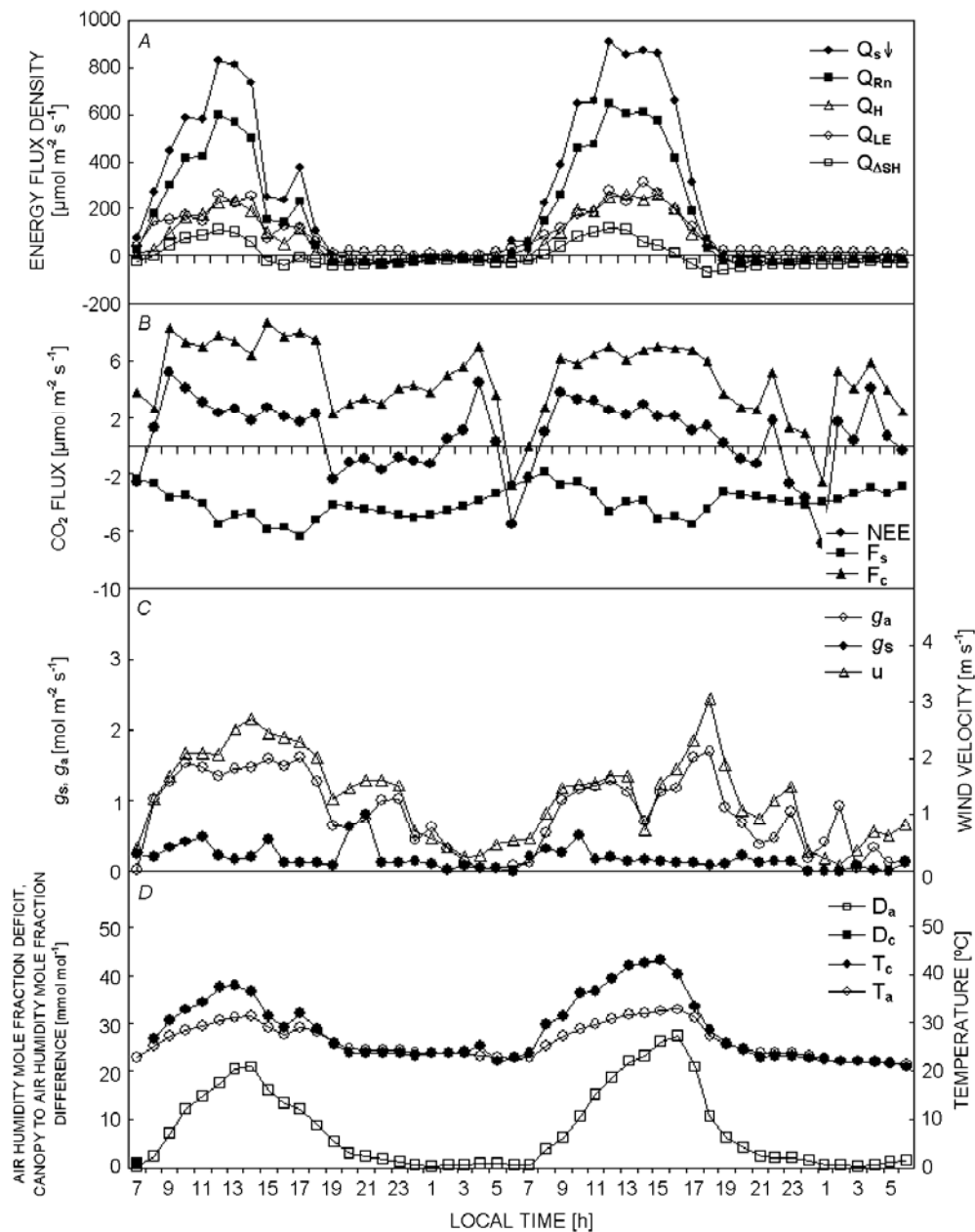


Fig. 6. The same parameters as in Fig. 2, but measured in typical days of the reproductive growth stage (778–779 DAP).

Goulden *et al.* (1996), Grace *et al.* (1996), Aubinet *et al.* (2002), and Kruijt *et al.* (2003). Here, the storage was neglectful during the five consecutive seasons. Thus, the seasonal averaged hourly storage in the daytime and night-time ranged from 0.032 to 0.123 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Therefore, this term did not lead to an appreciable correction. The effect of field heterogeneity and advection shows that the flux differences from the three eddy covariance systems were less than three percent. Therefore, nocturnal fluxes with respect downwind can be considered as isotropic. The effect of night-time mixing in the diurnal pattern of net ecosystem exchange was assessed. Nights were evaluated on the basis of the average value of the friction velocity (u_*) below 0.1 m s^{-1} . Hence during the five consecutive seasonal courses, the percentage of nights with average u_* below 0.1 m s^{-1} was 5.8, 0.0, 17.3, 0.0, and 22.0, respectively. Therefore, the calm nights were absent throughout the dry seasons, whereas during the wet season nights there was a reduced proportion. In the calm nights, less than 40 % of u_* values were below 0.1 m s^{-1} . The corrector applied by replacing data made during the calm nights by parametrization showed that the correction varied between 0 and 4 % of the uncorrected flux. In these conditions, correction of the night flux error was less than 5 % in extreme nights with low turbulence. These results were corroborated by the agreements found between the eddy covariance system and soil chamber measurements. Here, the flux of nocturnal CO_2 uptake was partially controlled by the behaviour of g_s such as has been reported for CAM species (Kluge and Ting 1978). Also the storage capacity of the vacuole and malic acid content in the mesophyte cell vacuoles of the chloroplast are constraining the process (Kluge and Fischer 1967, Ting and Gibbs 1982).

Throughout crop development, the daily pattern of gaseous exchange showed an elastic response since it presented a continuous spectrum. This ranges between two extremes in which major proportion of daytime net CO_2 uptake was common and in which the expression of CAM was related to both diurnal and nocturnal CO_2 uptake. For the typical days of the wet seasons (Figs. 4 and 6) the proportion of canopy CO_2 flux in the night-time in relation to this flux in the day (*i.e.* CAM activity) ranged from 0.34 to 0.48. Therefore, the daily carbon incorporation during the wet seasonal courses was related mainly to daytime CO_2 fixation. Daily trend of net CO_2 exchange and water vapour transfer measured over the pineapple field during the wet seasons did not follow the CAM inverted rhythm reported for this crop growing under controlled conditions with adequate water supply (Neales *et al.* 1968, 1980, Nose *et al.* 1977, Sale and Neales 1980, Cote *et al.* 1993, Zhu *et al.* 1997a,b, 1999). However, the nocturnal CO_2 influx of the community still occurred such as reported for *Agave deserti* (Hartsock and Nobel 1976), *A. americana* (Neales *et al.* 1968), and *Dudleya farinosa* (Bartholomew 1973). In relation to g_s , the typical inverted trend was not observed. However,

when canopy temperature gradient between daytime and night-time was above 16 °C, the nocturnal magnitude of g_s was markedly above that for daytime g_s (Figs. 4 and 6). As the nocturnal canopy to air humidity mole fraction difference increased, the night-time g_s decreased until it reached a minimum value. Thereafter, it increased slightly until pre-dawn hours. The results evidenced that pineapple growth was maximal during the wet seasons when daytime CO_2 uptake via RuBPC predominates over the daily course. This major partitioning taken up in the daytime via RuBPC is a major adaptive advantage for growth of pineapple and other CAM plants because of longer CO_2 uptake (Neales 1973, Borland and Taybi 2004). For the typical days of dry seasons (Figs. 3 and 5), CAM activity in pineapple spanned from 0.31 to 0.90. In stressed pineapple cultivars, Bartholomew (1982) and Keller and Lüttge (2005) have described CO_2 uptake almost exclusively during the dark period (*i.e.* phase I of CAM mode). Here the benefits of CO_2 uptake at low water cost in the Red Spanish pineapple were curtailed by the limited night-time CO_2 fixation. In spite of this limited nocturnal gaseous exchange, the crop was able to maintain the phytomass during the extreme conditions of the dry season. Under these conditions, CAM idling (*sensu strictu*) (Szarek *et al.* 1973, Ting 1985, Lüttge 1987, Griffiths 1988) in the cv. Red Spanish occurred during very few days (Fig. 3). This maintenance mechanism was related partially to internal recycled or night-time re-fixed respiratory CO_2 as the g_s remained markedly low during the daily trend. In spite of the fact that carbon recycling under water stress occurred during few days (Griffiths 1988), the respiratory CO_2 utilization was crucial in maintaining a functional photosynthetic apparatus during the dry seasons.

Pineapple strategy switched from water expenditure in the early vegetative growth stage to water-saving mode in the rest of the growth stages. Therefore, the last response was maintained throughout the consecutive seasonal courses, including the wet conditions. Thus, water is conserved for plant growth throughout the seasonal courses. However, the surface control on the daytime Q_{LE} was lower during the wet season as compared to that in the dry season. At the beginning of the wet season, the reversible g_s control prevailed in the stressed cv. Red Spanish since the gaseous exchange capacity was rapidly restored.

The relative effect of g_s on the gaseous exchange (*i.e.* Q_{LE} and F_c) was evaluated by calculating the marginal unit water cost of pineapple carbon gain (*i.e.* $\Lambda = \delta Q_{LE} / \delta F_c$) for the complete canopy cover (Fig. 7) as outlined by Cowan (1982). Results indicate that surface response depended on the season. During the wet season, the pineapple crop features a surface adapted to decreased Λ throughout the daytime. Thus, the sensitivity of Q_{LE} and F_c to g_s followed a similar hourly trend. However, the coupling of CO_2 uptake and g_s was more effective in maintaining a higher proportionality between assimilation

Table 1. Non-linear square optimization parameters (K_1 , K_2 , and K_3) of the hourly surface conductance model for pineapple [*Ananas comosus* (L.) Merr. cv. Red Spanish] field grown in the Orinoco lowlands. Data corresponds to daytime values of the consecutive seasonal courses.

Model	Season	K_1	K_2	K_3
Hourly solar radiation and hourly air humidity mole fraction deficit	1997, wet season	1.726	8.330	0.048
	1998, wet season	2.943	3.786	-2.683
	1999, wet season	2.678	2.378	-0.031
Hourly solar radiation and soil volumetric water content	1997/98, dry season	1.250	1.762	-63.183
	1998/99, dry season	1.053	-1.925	-104.184

and g_s as compared to the proportionality between Q_{LE} and g_s . Therefore, less water was transpired per unit of carbon gained. During the dry season, the relatively lower daytime $\delta Q_{LE}/\delta F_c$ indicates an increase in carbon gain as the soil volumetric water content decreased. During this season, the pineapple surface changed as regards controlling water losses. As daytime proceeded, g_s became sufficiently efficient to reduce the amount of water transported per unit carbon gain. Therefore, the magnitude of $\delta Q_{LE}/\delta F_c$ resulted in much carbon gain as possible in order to increase water-use efficiency under low water availability. This g_s response was related with variations in the hourly solar radiation and soil volumetric water

content after calculating the non-linear optimization parameter of the hourly surface conductance model (Jarvis 1976, Reed *et al.* 1976) (Table 1). The hydraulic signal of Λ could be associated to changes in content of foliar abscisic acid, ABA (Thomas and Eamus 1999). A similar response to soil drought has been reported for *Eucalyptus tetrodonta* growing in northern Australian savannas (Thomas *et al.* 1999). The relatively constant slope of hourly $\delta Q_{LE}/\delta F_c$ during the stress indicates that surface was behaving optimally as postulated by Cowan (1982). An efficient surface to maintain a constant slope of $\delta Q_{LE}/\delta F_c$ can be crucial for pineapple survival during the dry season.

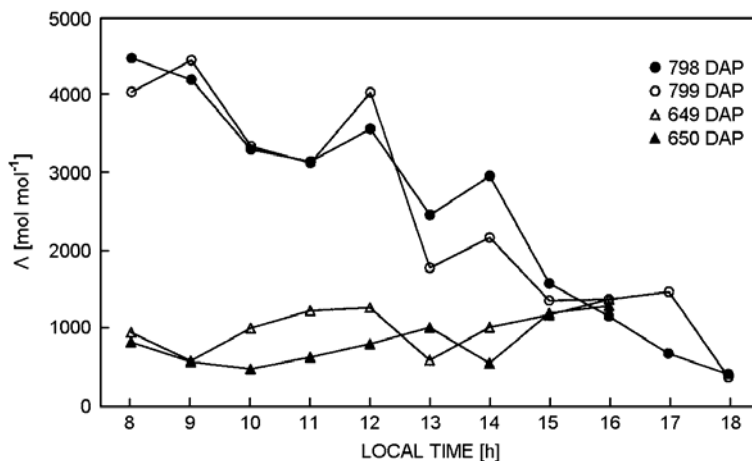


Fig. 7. The marginal unit water cost of pineapple carbon gain ($\Lambda = \delta Q_{LE}/\delta F_c$) [mol mol⁻¹] as a function of the local hour for typical days of the dry (649–650 DAP) and wet (798–799 DAP) seasons of the Orinoco lowlands.

Results of cv. Red Spanish did not show that the CAM mode was affecting markedly the g_s response. This finding is compared unfavourably with the experiment of Neales (1973). At the beginning of daytime, the calculated C_i increased with lowering in rates of transpiration and CO_2 exchange and in g_s . However, the daytime demand function in pineapple increased more than the supply function and the maximum C_i did not reach elevated values (407–470 $\mu\text{mol mol}^{-1}$) such as measured in pineapple under experimental conditions (1 320–5 000 $\mu\text{mol mol}^{-1}$) (Cockburn *et al.* 1979, Spalding *et al.* 1979). This response is modulated by an opposite signal from the D_a and the soil water content deficit through a feedback mechanism. In addition to these physiological adaptations to cope with stressful conditions, structural

canopy characteristics were related to the water-saving mode. These attributes were the leaf display and a high canopy albedo (*i.e.* 0.16–0.17), which reduces the radiation absorption in the hours of high radiation. Also Red Spanish has a well developed water storage tissue in the adaxial leaf surface, forming a palisade tissue. This tissue stores in the vacuoles malic and aspartic acids as well as PEPC (Osmond 1978). In relation to roots, new belowground phytomass is developed in pineapple when soil water becomes available. Similar adaptations were reported in species of cacti (Kausch 1965).

The aforementioned results indicate that rain-fed pineapple was adapted to withstand the extreme seasonal conditions of the Orinoco llanos. The water-saving mode in pineapple was able to reduce transpiration even during

periods with adequate soil water supply. Nevertheless, the extreme conditions markedly constrained pineapple growth and leaf area development. The plasticity and synchronization of the gaseous exchanges with environmental signals were crucial to cope with seasonal variations. In the Orinoco environment, pineapple was subject to changing multiple stresses during its growth cycle. The structural and metabolic features of pineapple allow optimize growth and reproductive output under these changing conditions. Traits such as canopy longevity and g_s behaviour can be viewed as an adjustment to the environmental stress regime. The flexibility of wild pineapple species and cultivars for photon energy use and habitat occupation has been reported by Keller and Lüttge

(2005). Therefore, potential productivity in pineapple will be reached when the supply and demand for carbon (*i.e.* elasticity for carbon uptake), temporal carbon transport, and water conservation are synchronized in interlocking with seasonal environments. Signalling requirements for the co-ordination of carbon fluxes have been proposed by Borland and Taybi (2004).

Our results can improve management of pineapple or predict the response to environmental variations that global change will produce. The effect of the phenomenon referred to as the ENSO was evident on pineapple function. A previous work (Zhu *et al.* 1997a,b) indicates that high concentrations of CO₂ and warm temperatures can modify gaseous exchange in pineapple.

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