

## PHYSIOLOGICAL ADAPTATIONS FOR MAINTAINING PHOTOSYNTHESIS UNDER WATER STRESS IN CACAO

by

ROBERT J. JOLY

*Department of Horticulture, Purdue University, West Lafayette, Indiana 47907, U.S.A.*

### SUMMARY\*

The objective of this study was to measure the effect of water stress in cacao seedlings with respect to net assimilation rate, stomatal conductance, and transpiration during periods of gradually decreasing soil and leaf water potentials. Net photosynthesis (Pn) of non-stressed seedlings was characterized in terms of the level of photosynthetically active radiation (PAR), leaf age, and leaf water potential. Net assimilation rates were measured by use of a closed-system portable infrared gas analyzer. Pn of 9-month-old non-stressed seedlings is low ( $1.5$  to  $4.5$  mol  $\text{CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ ). Seedlings exhibit increasing Pn up to approximately  $350$  to  $400$   $\mu\text{E m}^{-2}$   $\text{s}^{-1}$ . Increasing the irradiance up to  $750$   $\mu\text{E m}^{-2}$   $\text{s}^{-1}$  had no further effect on photosynthetic rate. Leaves from the most recently hardened flush (Flush 1) have the highest level of photosynthetic activity relative to older leaf flushes. They exhibit a rate of  $3.65$  mol  $\text{CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ , approximately double that of the next older flush of leaves<sup>2</sup>. Flushes 3 and 4 exhibit very low activity (less than  $1.1$  mol  $\text{CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ ) and are not different from each other. The very low Pn in the older leaf flushes may be at least partly explained by an apparent increase in stomatal resistance in these leaves. An experiment was designed to test the effect of leaf water potential ( $\psi$ ) on photosynthetic activity. The regression of net assimilation on leaf water potential shows a moderately strong relation ( $r = -.69$ ) between Pn and  $\psi$ . There is a dramatic impairment of Pn and a very large increase in stomatal resistance as leaf water potential falls between approximately  $-8$  and  $-13$  bars. The relationship between stomatal conductance and net photosynthesis is described.

### ADAPTATIONS PHYSIOLOGIQUES POUR MAINTENIR LA PHOTOSYNTHESE SOUS STRESS HYDRIQUE DU CACAOYER

#### RESUME

L'objectif de cette étude était de mesurer l'effet du stress hydrique sur les plantules de cacaoyers, du point de vue du taux net d'assimilation, de la transpiration et de la résistance des stomates pendant les périodes où les potentiels d'eau du sol et des feuilles diminuaient graduellement. La photosynthèse nette (Pn) de plantules qui ne subissaient pas de stress était caractérisée quant aux niveaux de radiation actifs photosynthétiquement (PAR), de l'âge des feuilles, de l'humidité, de la température et du comportement diurne. Les taux nets d'assimilation étaient mesurés au moyen d'un analyseur portatif à circuit fermé, aux infra-rouges à gaz. La Pn de plantules de 9 mois qui n'avaient pas subi de stress était faible ( $0,07$  à  $0,22$  mg  $\text{CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ ). Les plantules manifestaient une augmentation de Pn jusqu'à environ  $400$   $\mu\text{E m}^{-2}$   $\text{s}^{-1}$ , et la plupart des plants observés parvenaient à la saturation lumineuse à cette intensité. Certains individus continuaient à accroître l'assimilation nette jusqu'à plus de  $800$   $\mu\text{E m}^{-2}$   $\text{s}^{-1}$ . Les feuilles de la poussée foliaire la plus récemment affirmée (poussée 1) avaient le niveau le plus élevé d'activité photosynthétique, en comparaison avec les poussées foliaires plus anciennes. Le taux manifesté était de  $0,16$  mg  $\text{CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ , environ le double de celui des poussées foliaires immédiatement plus anciennes. Les poussées 3 et 4 manifestaient une très faible activité (moins de  $0,05$  mg  $\text{CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ ) et ne différaient pas l'une de l'autre. La très faible Pn des plus vieilles poussées foliaires peut s'expliquer au moins en partie par une augmentation apparente de la résistance des stomates dans ces feuilles. Les tendances diurnes étaient caractérisées, ce qui permettra d'éviter de se méprendre sur des changements provoqués par le stress hydrique dans les expériences de l'avenir, croyant qu'il s'agit de changements joints aux rythmes diurnes. Une analyse de Pn en fonction du temps est présentée. L'appareil de photosynthèse répond rapidement à la première lumière de la journée; les plantules manifestent des taux très élevés de Pn seulement 15 minutes après l'extinction des lumières. Le Pn moyen après une heure d'éclairage à  $400$   $\mu\text{E m}^{-2}$   $\text{s}^{-1}$  était de  $0,160$  mg  $\text{CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ . Dans les plantules qui n'avaient pas subi de stress, la Pn demeurait stable à ce taux pendant toute la journée. Une expérience a été conçue pour mettre à l'épreuve l'effet du potentiel hydrique des feuilles ( $\psi$ ) sur l'activité photosynthétique. La régression de l'assimilation nette sur le potentiel hydrique des feuilles manifeste un rapport modérément fort ( $r = -0,94$ ) entre Pn et  $\psi$ . Il y a une réduction très forte du Pn et une augmentation très nette de la résistance des stomates lorsque le potentiel hydrique des feuilles tombe entre environ  $-6$  et  $-13$  bars. L'importance de la variabilité génétique dans la réponse photosynthétique au déficit hydrique est décrite pour quatre sources de cacaoyers.

### ADAPTAÇÕES FISIOLÓGICAS PARA MANUTENÇÃO DA FOTOSÍNTESE DO CACAU SOB CONDIÇÕES DE TENSÃO HÍDRICA

#### RESUMO

O propósito deste estudo foi avaliar o efeito da tensão hídrica em mudas de cacauero no tocante à taxa líquida de assimilação, transpiração e resistência do estoma durante períodos de potenciais hídricos decrescentes no solo e nas folhas. A fotossíntese líquida (Pn) de mudas não submetidas a tensão foi caracterizada em termos de nível de irradiação fotossintética ativa (PAR), idade foliar, umidade, temperatura e comportamento diurno. Os índices de assimilação líquida foram medidos através de um analisador de gás infravermelho portátil de sistema fechado. A Pn de mudas não submetidas a tensão, com 9 meses de idade, foi baixa ( $0,07$  a  $0,22$  mg  $\text{CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ ). As mudas apresentam Pn ascendente até aproximadamente  $400$   $\mu\text{E m}^{-2}$   $\text{s}^{-1}$  e a maioria das plantas observadas atingem saturação de luz nesse grau de intensidade. Algumas plantas individuais continuam a aumentar sua assimilação líquida até algo acima de  $800$   $\mu\text{E m}^{-2}$   $\text{s}^{-1}$ . As folhas das brotações mais recentemente

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solidificadas (Brotação 1) apresentam o mais alto nível de atividade fotossintética relativamente a brotações mais antigas de folhas. Mostram um coeficiente de  $0,16 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , cerca do dobro da brotação de folhas imediatamente mais velha. As brotações 3 e 4 apresentam níveis muito baixos de atividade (menos de  $0,05 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) e não diferem entre si. Os níveis muito baixos de Pn em brotações de folhas mais antigas podem ser explicados, ao menos em parte, pelo aparente aumento de resistência dos estomas dessas folhas. As tendências diurnas foram determinadas de forma que as alterações causadas pela tensão hídrica em experiências futuras não venham a ser confundidas com alterações associadas ao ritmo diurno. Apresenta-se uma análise da Pn em função do tempo. O aparelho de fotossíntese reage rapidamente à primeira luz do dia: as mudas exibem taxas muito altas de Pn apenas 15 minutos após as luzes serem acesas. A Pn média após uma hora de iluminação a  $400 \mu\text{E m}^{-2} \text{ s}^{-1}$  foi de  $0,160 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . Em mudas não submetidas a tensão, a Pn manteve-se estável nesse nível durante todo o dia. Projetou-se uma experiência para testar o efeito do potencial hídrico foliar ( $\psi$ ) sobre a atividade de fotossíntese. A regressão da assimilação líquida sobre o potencial hídrico foliar mostra uma correlação relativamente forte ( $r = -0,94$ ) entre Pn e  $\psi$ . Ocorre uma queda substancial de Pn e um aumento muito grande da resistência estomática à medida que o potencial hídrico foliar baixa para cerca de  $-6$  e  $-13$  bars. Descreve-se aqui o grau de variabilidade genética na reposta fotossintética à água para quatro fontes de cacau.

## ADAPTACIONES FISIOLÓGICAS PARA MANTENER LA FOTOSÍNTESIS EN CONDICIONES DE STRESS DE AGUA EN EL CACAO

### RESUMEN

El objeto de este estudio fue el de medir el efecto del "stress" del agua sobre las plántulas de cacao en lo que toca a la tasa neta de asimilación, transpiración y resistencia estomatal durante períodos de disminución gradual de potenciales de agua en el suelo y en la hoja. Las fotosíntesis netas (FN) de plántulas sin stress se caracterizaron en términos de radiación activa fotosintética (RAF), edad de la hoja, humedad, temperatura y comportamiento diurno. Las tasas netas de asimilación se midieron usando un analizador de gases portátil en sistema cerrado por infrarrojo. La FN de plántulas sin stress de 9 meses de edad es baja ( $0,07$  a  $0,22 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Las plántulas exhibieron una FN de hasta aproximadamente  $400 \mu\text{E m}^{-2} \text{ s}^{-1}$ , y la mayoría de las plantas observadas llegan a la saturación de luz a esa intensidad. Algunos individuos continúan aumentando la asimilación neta hasta más de  $800 \mu\text{E m}^{-2} \text{ s}^{-1}$ . Las hojas de los renuevos más recientemente endurecidos (Renuevo 1) tienen el nivel más elevado de actividad fotosintética en relación a los renuevos de hojas más viejos. Exhiben una tasa de  $0,16 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , aproximadamente el doble que los renuevos de hojas que le siguen en edad. Los renuevos 3 y 4 exhiben una actividad muy baja (menos de  $0,05 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) y no son diferentes unos de otros. La FN muy baja en los renuevos de hojas más viejos puede explicarse por lo menos en parte por un aumento aparente de resistencia estomatal en esas hojas. Se caracterizaron las tendencias diurnas de modo que los cambios causados por stress de agua en experimentos futuros no sean confundidos con cambios asociados con ritmos diurnos. Se presenta un análisis de la FN como una función de tiempo. El aparato fotosintético responde rápidamente a la primera luz del día; las plántulas exhiben tasas muy elevadas de FN solamente 15 minutos después de encender las luces. La FN media después de una hora de iluminación a  $400 \mu\text{E m}^{-2} \text{ s}^{-1}$  fue de  $0,160 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . En las plántulas no sometidas a stress la FN se mantuvo estable a esa tasa a lo largo del día. Se concibió un experimento para probar el efecto del potencial de agua en la hoja ( $\psi$ ) sobre la actividad fotosintética. La regresión de la asimilación neta sobre el potencial de agua en la hoja muestra una relación moderadamente fuerte (igual a  $0,94$ ) entre FN y  $\psi$ . Hay un empeoramiento espectacular de FN y un aumento muy grande de resistencia estomatal cuando el potencial de agua en la hoja cae entre aproximadamente  $-6$  y  $-13$  bars. Se describe el alcance de variabilidad genética en la reacción fotosintética al déficit de agua para cuatro fuentes de cacao.

### INTRODUCTION

THE rate of photosynthesis per unit leaf area as well as patterns of carbon allocation among plant organs are major physiological determinants of yield for any fruit crop. Very little is known, however, about the photosynthetic characteristics of tropical trees in general, and few data are available for cacao (*Theobroma cacao* L.). Further, the response of photosynthesis to developing leaf water deficits are even less well understood.

Water stress has profound effects on the photosynthetic performance of many crop species. As tissue relative water content begins to decline, net photosynthesis is often observed to be relatively independent of leaf water potential. But as drought stress intensifies, net photosynthetic  $\text{CO}_2$  uptake rate (Pn) decreases markedly, often reaching zero at high levels of stress. The threshold water potential below which Pn decreases rapidly is of particular interest because it defines a range of soil and plant water contents that will very likely result in large reductions in yield.

Temperature, rainfall, solar radiation, relative humidity, and wind have each been shown to modify physiological behavior of cacao and to influence yield. Variation in yield from year to year, however, appears to be affected to a greater degree by rainfall distribution than by any other climatic factor (Alvim, 1981). It is of considerable interest, therefore, to characterize the transpiration and photosynthetic performance of cacao in relation to tissue water status. After such baseline data are analyzed, future efforts could be directed toward the

development of predictive models to estimate yield performance of clones in the field from observed Pn or other gas exchange parameters.

The elucidation of mechanisms by which the water relations of cacao modify photosynthetic behavior must be of central importance in any program designed to predict yield. Water stress reduces crop yield below potential values through both direct and indirect effects. Alvim (1977) has suggested that the high incidence of cherrille wilt and pod loss associated with water stress may be due to reduction in net photosynthesis or to inhibition of translocation of photosynthates to growing pods. Hutcheon (1977a) notes that flowering and fruit set are maximal after the beginning of the wet season when the photosynthetic rate is high. The author concluded that yield is strongly related to photosynthetic production. Few data are available, however, on the relation of plant water status and photosynthesis. Hutcheon (1977b) characterized the effect of leaf water status and stomatal conductance on the rates of photosynthesis and leaf area expansion. He reported that stomatal conductance decreased rapidly as  $\psi$  dropped below  $-15$  bars. Further, water status was strongly related to the rate of leaf area expansion. To our best knowledge, there are no other published reports describing the impact of soil or plant water deficit on net photosynthetic carbon fixation in cacao.

Accordingly, the principal objective of this study was to measure the effect of water stress in cacao seedlings with respect to net assimilation rate, stomatal conductance, and transpiration during periods of gradually

decreasing soil and leaf water potentials. Before this objective could be undertaken, however, it was necessary to describe Pn of non-stressed seedlings in terms of photosynthetically active radiation (PAR) and to test the effect of leaf age on photosynthetic activity. These preliminary data would allow us to choose standard conditions of PAR, temperature, and relative humidity under which to test the effects of water deficit.

## MATERIALS AND METHODS

### Light Saturation

Previous studies have shown that photosynthetic light saturation in cacao occurs at very low irradiances. In order to verify this, we measured net photosynthetic CO<sub>2</sub> uptake at PAR levels ranging from zero to 750  $\mu\text{E m}^{-2} \text{s}^{-1}$  at intervals of approximately 50  $\mu\text{E m}^{-2} \text{s}^{-1}$ .

Seedlings were 9-months-old at the time of measurement and were from a single source (UF 613 PA). Plants were grown from seed in 15 cm plastic pots in a 2:2:1 v/v mixture of perlite, peat moss, and topsoil and grown on benches in the Horticulture greenhouse, Purdue University, West Lafayette, Indiana. Seedlings were grown under approximately 27 C daytime maximum and 23 C night-time minimum temperatures. CO<sub>2</sub> concentration was ambient. Plants were fertilized bi-weekly with a liquid feed program of 200 ppm N, 100 ppm P, 100 ppm K, and monthly with a standard micronutrient mixture. After three months, seedlings were transplanted to 7.6 liter pots and grown at 0.15 m<sup>2</sup> spacing per plant until measurements were made.

Thirty healthy, vigorously growing plants were sampled in order to determine light saturation characteristics. Leaves selected for measurement were from the most recently hardened flush; these were dark green in color and free from any visible defect.

The light source for growth chamber measurements consisted of four 400 W high pressure sodium lamps. Light intensity was varied by varying the distance between light source and plant and by using cheesecloth screens. Intensity was monitored by a LICOR quantum sensor. Leaves selected for measurement were positioned to receive a given level of photosynthetic photon flux density, and the plants were equilibrated at that irradiance for two hours before measurements were made. At least four replications were made for each measurement.

Net CO<sub>2</sub> assimilation was determined by using a LICOR Model LI-6000 portable (closed) photosynthesis system with a tripod-mounted 0.25 liter leaf chamber. Approximately 25 to 30 cm<sup>2</sup> of leaf area was sampled in a typical Pn determination. Each Pn determination was taken as the mean of 10 measurements that were logged at 8 s intervals. Temperature inside the growth chamber where measurements were made was 30 C. Relative humidity was maintained at 55 to 60% by use of a humidifier and by evaporation from trays of pea gravel filled with water.

### Leaf Age

In order to assess the within-plant variability in net assimilation, it was essential to characterize leaf age effects on Pn. Six plants were selected on the basis of good leaf retention of the older flush leaves; they were approximately 1.25 m tall and had 35 to 45 leaves. Seedlings were removed from the greenhouse and placed

in the growth chamber prior to dawn on the day of measurement. Leaves were positioned to receive 350  $\mu\text{E m}^{-2} \text{s}^{-1}$  and were equilibrated for two hours at this irradiance. We measured Pn and stomatal conductance (g<sub>s</sub>) within each of four leaf flushes; a single leaf within each leaf flush was sampled three times.

### Plant Water Stress

We measured gas exchange rates in cacao seedlings during three soil drying cycles. In August 1986, 32 seedlings from each of three sources were arranged on greenhouse benches in a split plot design. Seedlings were spaced at 0.15 m<sup>2</sup>/plant. The experiment was analyzed as a split-split plot design with irrigation as whole plots, source of origin as subplots, and time (i.e., sampling date) as the sub-subplot treatment. Irrigation treatments were arranged in a completely randomized design. Three treatment replications were included within this design. A separate ANOVA was performed for each of the three soil-drying treatments. The level of irrigation was either frequent (every one to two days) or no water during the drying periods.

Beginning on 23 September 1986, water was withheld from seedlings in nonirrigated plots; the drying continued through 7 October (Julian days 266–80). Seedlings were sampled every one to two days throughout this period. At the conclusion of this drying period all plants were watered to saturation and then watered every one to two days until a second drying period was initiated on day 294. In total, three drying cycles were applied between 23 September and 26 November, 1986. The Julian dates for each drying cycle are as follows: cycle 1, 266–80; cycle 2, 294–309; cycle 3, 317–30.

On each sample date, measurements of Pn, g<sub>s</sub>, and leaf water potential, were made on 18 seedlings selected at random from the appropriate source and irrigation treatment. Leaf water potential was measured by use of a pressure chamber (Scholander, *et al.*, 1964) on the same leaf used to measure gas exchange characteristics.

## RESULTS AND DISCUSSION

### Light Saturation

Photosynthetic light saturation occurred at 350 to 400  $\mu\text{E m}^{-2} \text{s}^{-1}$  in leaves of the most recently hardened flush (Fig. 1). Increasing the irradiance up to 750  $\mu\text{E m}^{-2} \text{s}^{-1}$

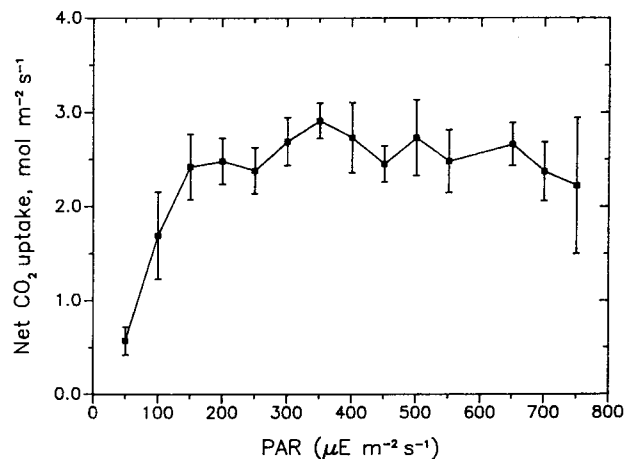


FIG. 1. Effect of level of photosynthetically active radiation (PAR) on net photosynthesis in leaves of 9-month-old container-grown cacao seedlings. Points are means of 4 to 5 determinations  $\pm$  standard errors.

had no further effect on photosynthetic rates. At saturation PAR, net photosynthesis was approximately  $2.75 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . The rates of net photosynthesis observed here are similar in magnitude to values reported by Hutcheon (1977b) who used a  $^{14}\text{CO}_2$  absorption technique and by Harum and Hardwick (1984) who measured Pn by infrared gas analysis.

### Leaf Age

Leaves from the most recently hardened flush (denoted here as Flush 1) have the highest level of photosynthetic activity in these cacao seedlings. They exhibit a rate of  $3.7 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , approximately double that of the next older flush of leaves (Fig. 2). Flushes 3 and 4 exhibit very low activity (less than  $0.75 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and are not different from each other. The very low Pn in the older flushes may be at least partly explained by an apparent decrease in stomatal conductance in these leaves (Fig. 3). Three of the six plants sampled exhibited stomatal conductances of  $0.03 \text{ cm s}^{-1}$  or less in flushes 3 and 4, and this undoubtedly contributed to the low photosynthetic rates observed for those flushes.

### Plant Water Stress

Mean Pn of irrigated plants was relatively constant throughout all three soil drying cycles, varying between

approximately  $2.7$  and  $3.7 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  from 23 September through 26 November, 1986. Pn of nonirrigated seedlings dropped precipitously relative to irrigated controls within 6 to 8 days after water was withheld

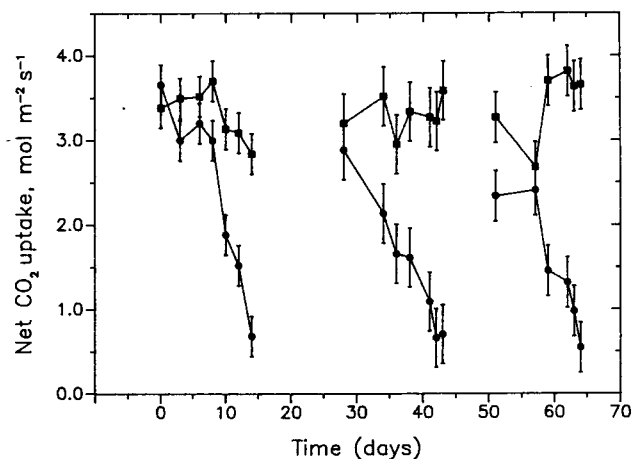


FIG. 4. Net photosynthesis for irrigated (■) and nonirrigated (●) cacao seedlings in each of three soil drying cycles. Soil drying was initiated on Julian days 266, 294 and 317 for cycles 1, 2, and 3, respectively. Breaks between points represent stress recovery periods. Points are means  $\pm$  standard errors ( $n=9$ ).

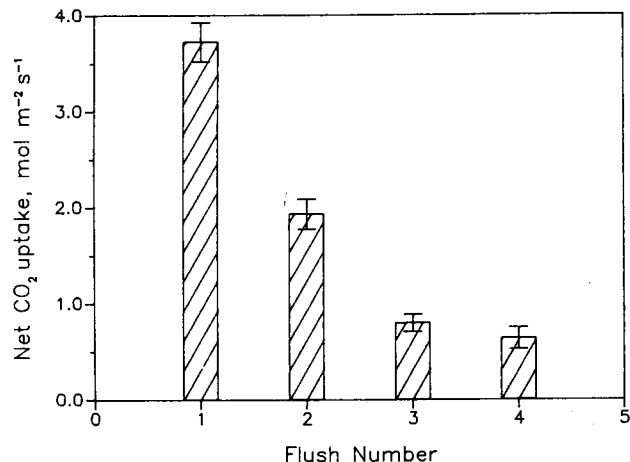


FIG. 2. Effect of leaf age on net photosynthesis in container-grown cacao seedlings. Flush 1 denotes the most recently hardened flush. Bars are means of three replications  $\pm$  standard errors.

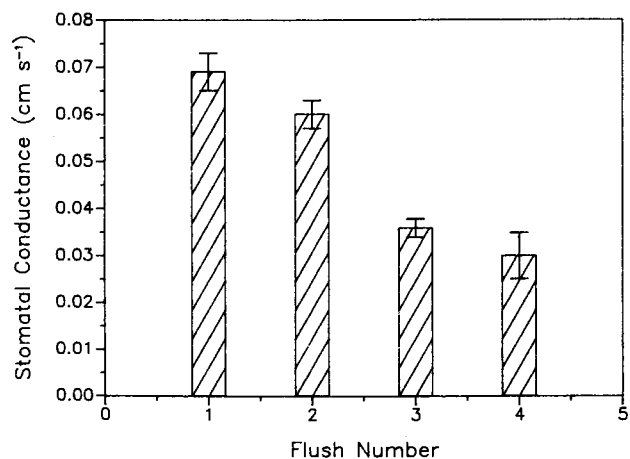


FIG. 3. Effect of leaf age on stomatal conductance in container-grown cacao seedlings. Flush 1 denotes the most recently hardened flush. Bars are means of three replications  $\pm$  standard errors.

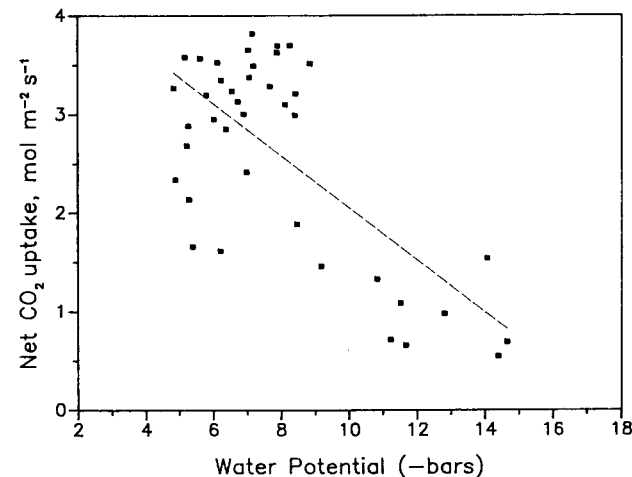


FIG. 5. Effect of leaf water potential on net photosynthesis in container-grown cacao seedlings. Each point is a mean of 9 determinations. Correlation coefficient ( $r$ ) is  $-0.69$ .

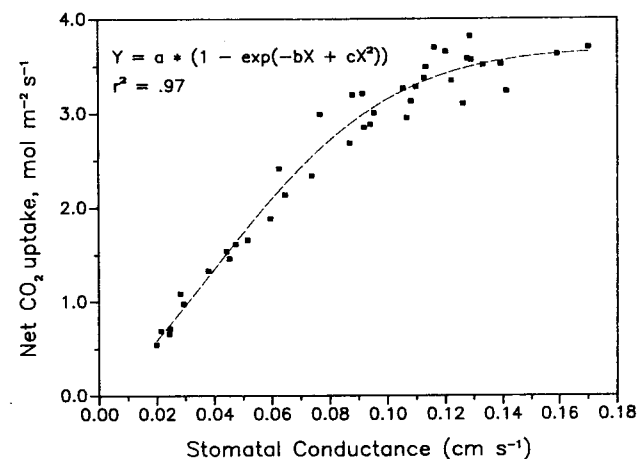


FIG. 6. Relation between stomatal conductance and net photosynthesis in container-grown cacao seedlings. Each point is a mean of 9 determinations.

(Fig. 4). Essentially the same pattern was observed in each of two subsequent soil drying periods. By the end of each of the 13- to 15-day drought treatments, Pn had declined to between 0.5 and 1.0 mol CO<sub>2</sub> m<sup>2</sup> s<sup>-1</sup>. Stressed plants did not appear to recover fully from the effects of the artificial drought treatments. The mean Pn of nonirrigated seedlings was 3.7 mol CO<sub>2</sub> m<sup>2</sup> s<sup>-1</sup> at the beginning of cycle 1, but Pn dropped to 2.9 and then to 2.3 mol CO<sub>2</sub> m<sup>2</sup> s<sup>-1</sup> at the beginning of cycles 2 and 3, respectively.

The relationship between leaf water potential and net CO<sub>2</sub> uptake is shown in Figure 5. Means of both irrigated and nonirrigated treatments are plotted; these represent the combined data of 20 sample dates over the three

drying cycles. Although water potential and Pn are only moderately well correlated ( $r = -0.69$ ), there appears to be a threshold water potential between  $-8.0$  and  $-8.5$  bars, below which Pn drops by 50% or more.

The relationship between stomatal conductance and net photosynthesis is non-linear and can best be described by a second order asymptotic exponential of the form  $P_n = 3.676 * [1 - \exp(-6.06X + 132.43X^2)]$ . Data were combined over all drying cycles and a scatter-plot is given in Figure 6. Net photosynthesis approaches maximal values at stomatal conductances of 0.12 to 0.16 cm s<sup>-1</sup>. This results is in accord with data reported by Hutcheon for a wide range of plant types (both field and container grown).

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