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Photosynthetic photon flux density, carbon dioxide concentration, and vapor pressure deficit effects on photosynthesis in cacao seedlings

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Abstract

Independent short-term effects of photosynthetic photon flux density (PPFD) of 50–400 µmol m^{-2} s^{-1}, external CO$_2$ concentration (C_a) of 85–850 cm^3 m^{-3}, and vapor pressure deficit (VPD) of 0.9–2.2 kPa on net photosynthetic rate (P_N), stomatal conductance (g_s), leaf internal CO$_2$ concentration (C_i), and transpiration rates (E) were investigated in three cacao genotypes. In all these genotypes, increasing PPFD from 50 to 400 µmol m^{-2} s^{-1} increased P_N by about 50 %, but further increases in PPFD up to 1 500 µmol m^{-2} s^{-1} had no effect on P_N. Increasing C_a significantly increased P_N and C_i while g_s and E decreased more strongly than in most trees that have been studied. In all genotypes, increasing VPD reduced P_N, but the slight decrease in g_s and the slight increase in C_i with increasing VPD were non-significant. Increasing VPD significantly increased E and this may have caused the reduction in P_N. The unusually small response of g_s to VPD could limit the ability of cacao to grow where VPD is high. There were no significant differences in gas exchange characteristics (g_s, C_i, E) among the three cacao genotypes under any measurement conditions.

Additional key words: leaf internal CO$_2$ concentration; net photosynthesis; stomatal conductance; *Theobroma cacao*; transpiration rate.

Introduction

Cacao (*Theobroma cacao* L.), the source of chocolate, is a high value crop grown by nearly 6 million resource poor farmers of tropical regions. It is native to rain forests of the Amazon basin and other tropical areas of South and Central America and is grown as an understory plant. Botanically cacao is considered to be a shade rather than a sun plant. Cacao grows well in moderate shade and young plants especially need shade to reduce water and nutrient stress (Raja Harun and Kamariah 1983, Wood and Lass 2001). In full sun, yields of cacao are higher in early years of growth, but the trees tend to be shorter-lived than the shade grown plants (Ahenkorah *et al.* 1974). Serrano and Biehl (1999) have shown that cacao is unable to adapt to high irradiances. In unshaded plantations cacao trees showed signs of photoinhibitory stress and with lack of water supply and fertilizer such stress can reduce productivity. Invariably, new plantations are established by planting cacao, along with banana and a range of tropical native trees, to provide shade as cacao grows. However, universal agreement does not exist regarding the exact amount of shade required to maximize cacao production.

In the tropical forest understory plants usually receive a photosynthetic photon flux density (PPFD) of between 5 and 25 µmol m^{-2} s^{-1} or 1 or 2 % of that received at canopy levels (Liang *et al.* 2001). Miyaji *et al.* (1997) reported that in Bahia Brazil, irradiance at noon time above the cacao canopy ranged between 30 and 100 % of full sunlight, and 4–10 % at ground level. Increasing irradiance from 2 to 25 % of full daylight increased the CO$_2$ assimilation rate in cacao from 1.8 to 5.8 µmol m^{-2} s^{-1} and irradiances near 100 % of maximum sunlight decreased CO$_2$ assimilation rates (Lemee 1955). Okali and Owusu (1975) reported that in Ghana saturation of cacao leaves occurred at 20 % of full sunlight and photosynthetic rate declined at irradiances greater than 30 % of full sunlight. Earlier reports have shown that the PPFD for maximum photosynthesis in cacao occurs at about 350–400 µmol m^{-2} s^{-1}, which is about 20 % of the intensity of full sunlight (Hutcheon 1976, Raja Harun and Hardwick 1988). Baligar *et al.* (2005) reported that increasing PPFD from 65 to 190 µmol m^{-2} s^{-1} increased

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shoot and root growth, relative growth rate, and net photosynthetic rate (P_N) of cacao. However, 1 050 µmol m^{-2} s^{-1} of PPFD was detrimental to cacao growth.

Evidence of genetic variation in morphological and physiological characteristics has been reported among cacao genotypes (Yapp and Hadley 1994, Daymond *et al.* 2002a,b) and these plant traits could have great implications on the ability of plants to intercept and utilize solar radiation. Interactions between cacao genotypes and environmental variables on growth and P_N have received only limited attention. Only a few studies have examined the differences in P_N among cacao genotypes and hybrids. Criollo, Forastero, and Trinitario are the three main types or groups of cacao genotypes, and Hutcheon (1973) found that all three types grew poorly in full sunlight. However, only a few selected genotypes among these types grew well in full sunlight. In this study Scavina 6 (Criollo) seedlings tolerated full sunlight, while Amelonado (Forestero) seedlings did not. Galyuon *et al.* (1996a,b) have reported the influence of full sunlight (1 800 µmol m^{-2} s^{-1}) and shade (900 µmol m^{-2} s^{-1}) on the growth and photosynthesis and its components in Amelonado and crosses involving Trinidad-selected hybrids (TSH) cacao. In this study leaf size, internode length, total leaf area, and dry matter per plant were significantly reduced while leaf thickness, specific leaf area ratio, P_N, and relative growth rate were reduced under full sunlight. Information is lacking on cacao genotypic response to irradiance at elevated external CO_2 concentration (C_a) and leaf to air VPD. The C_a has risen from a pre-industrial value ≈280 cm^3 m^{-3} in 1900 to the present day level of around 375 cm^3 m^{-3} and is expected to reach ≈700 cm^3 m^{-3} by the end of the 21st century (Bolin 1998, Prentice *et al.* 2001). Such an increase in C_a could have a significant impact on cacao P_N. Loss of shade trees and growing without shade trees could increase cacao exposure to high irradiances and, consequently, decrease photosynthesis of cacao.

The objective of our study was to determine the independent short-term effects of PPFD, C_a, and leaf-to-air VPD on P_N, transpiration rate (E), leaf internal CO_2 concentration (C_i), and stomatal conductance (g_s) in three cacao genotypes.

Materials and methods

Cacao clones CCN 51, LCT EEN 37/A, and VB 1117 were used. CCN 51 is a clone from Ecuador obtained from crosses of ICS-95×IMC-67 (International Cocoa Germplasm Database, 2006). ICS-95 is a Trinitario clone with parental contributions from lower Amazon Forastero and Criollo (Pound 1945, Lanaud *et al.* 2001), whereas IMC-67 is an upper Amazon Forastero collected from Peru (Pound 1945). LCT EEN 37/A is a Forastero clone collected from Ecuador (International Cocoa Germplasm Database, 2006). VB-1117 is an Amelonado clone selected from Almirante, Bahia, Brazil for tolerance to Witches Broom disease (*Monililiophthroa pernicosa*).

Pods of the genotypes were received from Almirante Cacao Research Center, Itejupe Bahia, Brazil. Seeds were removed from pods and planted one per pot in 20-cm diameter plastic pots containing 1.7 kg mixture of sand/perlite/promix medium (2 : 2 : 1 volume). The growth medium was prepared by mixing sand, perlite, and promix in a cement mixer along with required macro and micronutrients to provide supplemental nutrients. Each kg of growth medium received [mg kg^{-1}] 600 N, 600 P, 240 K, 1 012 Ca, 309 Mg, 500 S, 119 Fe, 0.7 B, 17.5 Mn, 7 Cu, 7 Zn, and 0.35 Mo. Nutrients were applied as *Osmocote* (*18-6-12*; *Scotts*, Marysville, OH, USA), triple super phosphate, urea, calcium sulfate, dolomitic lime, and Scott's micromix. Pots were watered as needed and had holes in the growth container to provide adequate bottom drainage. Plants were grown under greenhouse conditions for about 500 d under 400±50 µmol m^{-2} s^{-1} of irradiance, with C_a of 370±30 cm^3 m^{-3}, and night and day temperatures of 20±5 and 32±5 °C, respectively.

Two days before photosynthetic determinations, the plants were moved to a controlled-environmental chamber with daytime growth conditions of 400 µmol m^{-2} s^{-1} irradiance for 12 h, 370±30 cm^3 m^{-3} of CO_2, and 25 °C constant temperature. Daymond and Hadley (2004) found that 25 °C was the optimum temperature for cacao photosynthesis. In the preliminary assessment we confirmed this temperature as optimal for photosynthesis of the cacao genotypes used in this study.

The fully expanded sixth leaf from the top of each plant was selected to measure leaf gas exchange. It was measured using a *CIRAS-1* portable gas exchange system (*PP Systems*, Haverhill, MA, USA), set to use an external air supply, and incorporating a water-jacketed chamber with a mixing fan enclosing a whole leaf, with control of irradiance, temperature, C_a, and water vapor pressure (Bunce 1993). The PPFD levels were controlled by adjusting a rheostat connected to four 150 W low-temperature lamps. The PPFD was determined by a *LI-COR LI-190* quantum sensor inside the leaf chamber. The C_a was controlled by mixing pure CO_2 with air that had been scrubbed to be free of CO_2. The VPD was controlled by mixing air bubbled through a water tank with dry air. Leaf area was determined after the gas exchange measurement by tracing the sampled leaf and running the tracing through a *LI-COR 2000* leaf area meter. Four plants of each genotype were sampled in each experiment unless otherwise noted.

Experiment I – Effect of PPFD: Plants were taken from the growth chamber and a leaf was placed in the measurement chamber. The PPFD was initially adjusted to 400 µmol m^{-2} s^{-1}. During the determinations, leaf temperature and C_a were kept at 25 °C and 370 cm^3 m^{-3}, respectively. After the leaf gas exchange became constant, the PPFD was adjusted in gradual steps to 50, 100, 200, 300, and 400 µmol m^{-2} s^{-1}. During the P_N measurements VPD was maintained in the range of 1.0 to 1.2 kPa. The leaf was allowed to equilibrate at the chosen PPFD for 10 min and P_N and photosynthetic components g_s, E, and C_i were determined.

Experiment II – Effect of C_a on P_N was determined by subjecting the whole leaf to a VPD of 0.7–1.1 kPa, a PPFD of 400 µmol m^{-2} s^{-1}, C_a of 370 cm^3 m^{-3}, with a leaf temperature of 25 °C. When leaf gas exchange became constant, the C_a was adjusted to 850, 680, 240, 160, and 85 cm^3 m^{-3}. At each C_a the leaf was allowed to equilibrate for 10 min and the rate of P_N and its components were determined.

Experiment III – Effect of VPD on P_N was determined on the three cacao clones by placing a leaf in the gas exchange system at a VPD of 1.1 kPa, a PPFD of 400 µmol m^{-2} s^{-1}, C_a of 370 cm^3 m^{-3}, with a leaf temperature of 25 °C. When leaf gas exchange became constant, the VPD was adjusted to 0.9, 1.3, 1.7, and 2.2 kPa.

Statistics: Responses of gas exchange parameters to environment were tested using either linear or quadratic regressions. Analysis of variance was used to test for differences among the three clones at a given measurement condition.

Results and discussion

PPFD effects: Increasing PPFD from 50 to 400 µmol m^{-2} s^{-1} significantly increased P_N, as indicated by the significant quadratic regression (Fig. 1*A*), although the changes were not significant between the PPFDs of 200 and 400 µmol m^{-2} s^{-1}. The P_N at 50 µmol m^{-2} s^{-1} of PPFD was about 2/3 of the maximum of about 3 µmol m^{-2} s^{-1}, indicating that very little radiant energy is required to support photosynthesis. Similarly, Raja Harun and Hardwick (1988) found the saturating irradiance for maximum photosynthesis of cacao was around 400 µmol m^{-2} s^{-1} and 95 % of the maximum photosynthesis occurred at 200 µmol m^{-2} s^{-1}. In another study Hutcheon (1976) reported that saturating PPFD for most cacao leaves was around 350 µmol m^{-2} s^{-1}, although Okali and Owusu (1975) found somewhat higher irradiance was required to saturate sun than shade leaves. Brief periods of high irradiance such as sunflecks are injurious to cacao leaves (Raja Harun and Kamariah 1983, Raja Harun and Hardwick 1988). Baligar *et al.* (2005) found that 1 050 µmol m^{-2} s^{-1} of PPFD was detrimental to shoot and root growth of cacao.

The P_N values observed for cacao genotypes (1–4 µmol m^{-2} s^{-1}) in this study were similar to those reported for other cacao genotypes and hybrids of various ages grown in different ecosystems and growing conditions (Raja Harun and Hardwick 1988, Balasimha 1993, Galyuon *et al.* 1996b). Bastid and Jimmy (2003) in their review reported that P_N for cacao seedlings of 3 months to more than 12 years of age ranged 0.46–11.40 µmol(CO_2) m^{-2} s^{-1}. Cacao is native to the understory of tropical forests (Wood and Lass 2001) and the P_N values observed in the current experiment with cacao were similar to those found for other understory plants such as *Aechmea magdalenae* and *Pharus latifolius* (Ziska *et al.* 1991) and *Coffea arabica* (Fanjul *et al.* 1985). The maximum P_N recorded in leaves of Arabica coffee, an understory plant similar to cacao, is in the range of 7–11 µmol(CO_2) m^{-2} s^{-1} with average values around 4 (Kumar and Tieszen 1980, Rena *et al.* 1994).

The g_s values observed in the current study were similar in range to the values reported in the literature for other cacao genotypes (Bastid and Jimmy 2003). The g_s was not significantly affected by irradiance over the observed range of 50–400 µmol m^{-2} s^{-1} (Fig. 1*B*). Maintaining high g_s at very low irradiance may be of advantage to understory plants, by allowing photosynthesis to respond rapidly to sunflecks (Kirschbaum and Pearcy 1988).

Increasing PPFD from 50 to 300 µmol m^{-2} s^{-1} did not significantly affect C_i (Fig. 1C). The C_i values of about 180 cm^3 m^{-3} are lower than is typical of C_3 species, which are usually 220 cm^3 m^{-3} or higher (Jones 1983) and would indicate higher than normal water use efficiency in cacao compared to many other tropical plants. Increasing PPFD from 50 to 400 µmol m^{-2} s^{-1} tended to increase E; however, the relationship between E and PPFD was not significant (Fig. 1*D*).

C_a effects: Increasing the C_a from 85 to 680 cm^3 m^{-3} increased the P_N from less than 1 µmol m^{-2} s^{-1} to 4 µmol m^{-2} s^{-1} (Fig. 2*A*). However, changes in P_N were minimal between the C_a of 680 and 850 cm^3 m^{-3}. Because cacao is a C_3 plant, this response of P_N to increasing C_a was expected (Cure and Acock 1986, Kimball *et al.* 2002). The P_N of cacao in our study increased about 33 % between C_a values of 370 and 680 cm^3 m^{-3}, which is typical of C_3 species (Cure and Acock 1986, Kimball *et al.* 2002). Balasimha *et al.* (1991) reported a nearly linear relationship between P_N and C_i in cacao. Anticipated future increases in the C_a should improve cacao photosynthesis unless down-regulation occurs after prolonged exposure to elevated C_a.

Increasing C_a strongly decreased the g_s (Fig. 2*B*) in all cacao genotypes. In the Florida scrub species (*Quercus myrtifolia*), Lodge *et al.* (2001) reported that g_s was approximately 40 % lower in the high (approx. 700 cm^3 m^{-3})

compared to the low (approx. 350 cm^3 m^{-3}) C_a. In leaves of C_3 annual plants the g_s was reduced by about 34 % on average by doubling of the C_a (Cure and Acock 1986). For trees, reviews have found that g_s decreases about 20 % on average over C_a range of 370 to 700 cm^3 m^{-3} (Curtis and Wang 1998, Medlyn *et al.* 2001). In our study the decrease of g_s in cacao is much larger than this average, *i.e.* about 65 %. Such a large decrease in g_s could result in substantial reductions in E (Fig. 2*B,D*) and improve plant water status and drought resistance.

Increasing C_a from 85 to 850 cm^3 m^{-3} increased C_i. However, it is evident from Fig. 2*C* that the ratio of C_i to C_a decreased strongly with increasing C_a. This response contrasts with the usual response of C_3 species, which is for the ratio to be constant with increasing C_a (Morison and Gifford 1983), and results from the unusually large decrease in g_s with C_a already mentioned. Increasing C_a from 85 to 850 cm^3 m^{-3} decreased E from about 0.66 to 0.16 mmol m^{-2} s^{-1}, and the relation between these variables was highly significant (Fig. 2*D*). Most C_3 plants exposed to higher C_a decrease E, reflecting reduced g_s (Cure and Acock 1986). Stomatal conductance to water loss is often reduced as C_a increases, and such effects may alter plant responses to drought and alter WUE (Wong 1979, Eamus 1991). In our study instantaneous leaf WUE increased about 3-fold between 370 and 680 cm^3 m^{-3} of C_a. Therefore increasing C_a will probably improve WUE in cacao.

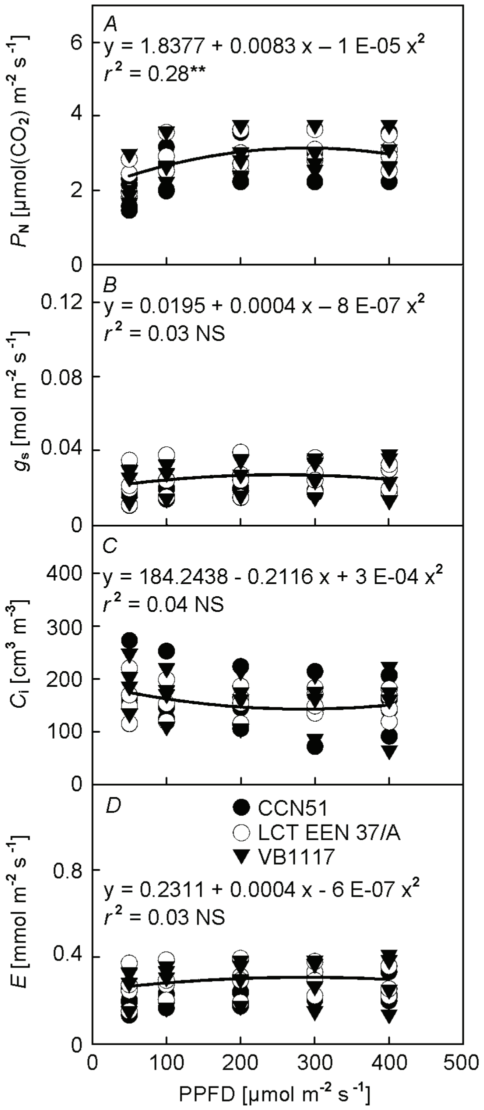

Fig. 1. Effect of photosynthetic photon flux density (PPFD) on (*A*) net photosynthetic rate, P_N; (*B*) stomatal conductance, g_s; (*C*) internal CO_2 concentration, C_i; and (*D*) transpiration rate, E of three cacao genotypes (CCN 51, LCT EEN 37/A, VB1117) at 25 °C and 370 cm^3 m^{-3} of CO_2. Each symbol represents one leaf. Solid line is quadratic best-fit of all data points. *indicates significant at p=0.05; **significant at p=0.01, and NS not significant at p=0.05.

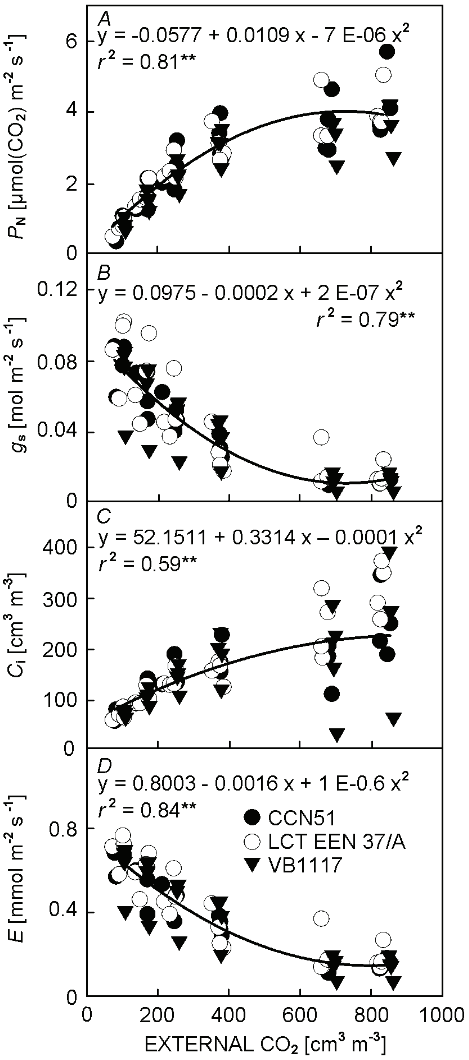

Fig. 2. Effect of external carbon dioxide levels [cm^3 m^{-3}] on photosynthetic characteristics (see legend to Fig. 1) of three cacao genotypes (CCN 51, LCT EEN 37/A, VB1117) at 25 °C and 400 µmol m^{-2} s^{-1} of PPFD. For significance symbols see legend to Fig. 1.

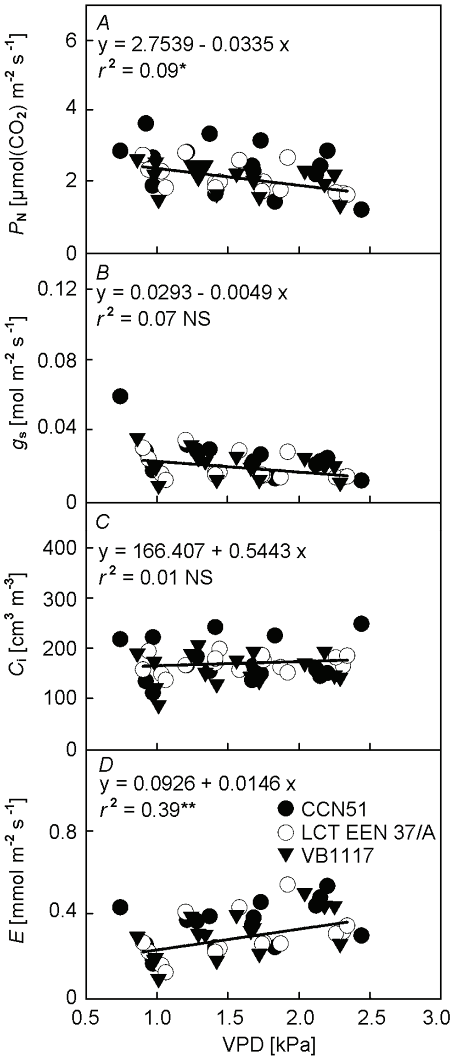

Fig. 3. Effect of vapor pressure deficit (VPD) on photosynthetic characteristics (see legend to Fig. 1) of three cacao genotypes (CCN 51, LCT EEN 37/A, VB1117) at 25 °C and 370 μmol m^{-2} s^{-1} of PPFD. For significance symbols see legend to Fig. 1.

No significant differences occurred among the cacao genotypes for P_N and its components (g_s, C_i, E) over the range of C_a. Intraspecific differences in responses of P_N to CO$_2$ concentrations have not been reported in any species, nor have differences in the response of g_s to CO$_2$. No significant differences occurred among the three genotypes for any of the measured variables under any measurement condition, possibly because of the large plant to plant variation within each genotype.

VPD effects: In all the cacao genotypes studied, P_N decreased slightly, but significantly with increasing VPD (Fig. 3*A*). Balashimha *et al.* (1991) reported that in cacao the P_N was highest during periods of low evaporative demand (low VPD). In coffee P_N also decreased with increasing VPD (Fanjul *et al.* 1985). In our study the reduction in P_N was not directly related to stomatal closure and reduction in C_i, because C_i was unchanged or increased slightly with increasing VPD (Fig. 3*C*). The decrease in P_N could be related to the higher E at high VPD (Fig. 3*D*) (Sharkey 1984).

The g_s in cacao leaves tended to decrease with increasing VPD but the decline was not significant (Fig. 3*B*). This non-significant change in g_s was smaller in cacao than found by Franks and Farquhar (1999) in other rainforest tree species such as *Toona australis* (F. Muell.) Harms, *Argyrodendron peralatum* (Bailey) Edlin, *Idiospermum australiense* (Diels) S.T. Blake, *Dysoxylum gaudichaudianum* (Adr. Juss) Miq, or *Neolitsea dealbata* (R. Br) Merr. These authors found that conductance at 2 kPa VPD averaged 0.55 of that at 1 kPa for five species, compared with the value of about 0.80 observed here for cacao. Cacao was thus unusually ineffective in limiting E at high VPD. This may explain why it does best as an understory species where it is not exposed to the higher VPDs of the overstory environment.

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