The Selective Advantages of Anthocyanins in Developing Leaves of Mango and Cacao

David W. Lee; Susan Brammeier; Alan P. Smith

Biotropica, Vol. 19, No. 1 (Mar., 1987), 40-49.

Stable URL:
http://links.jstor.org/sici?sici=0006-3606%28198703%2919%3A1%3C40%3ATSAOAI%3E2.0.CO%3B2-4

Biotropica is currently published by The Association for Tropical Biology and Conservation.

The Selective Advantages of Anthocyanins in Developing Leaves of Mango and Cacao[1]

David W. Lee and Susan Brammeier

Department of Biological Sciences, Florida International University, Miami, Florida 33199, U.S.A.

and

Alan P. Smith

Department of Biology, University of Miami, Coral Gables, Florida 33133, U.S.A.; Smithsonian Tropical Research Institute, Balboa, Panama

ABSTRACT

Although the developing leaves of mango and cacao contain appreciable concentrations of anthocyanins not present in mature leaves, these compounds are a small proportion of the total phenolic concentration. For this and other reasons anthocyanins do not seem to be important in the developing leaves as: (1) a screen against ultraviolet radiation; (2) a mechanism for elevating leaf temperature; (3) a means of defense or aposematic coloration against herbivory; and (4) a part of any postulated physiological mechanism, as photosynthesis. Anthocyanic coloration may be a by-product of the metabolism of other flavonoid compounds in these rapidly growing organs.

PERHAPS THE MOST CONSPICUOUS CHARACTERISTIC of tropical trees is the frequently brilliant coloration associated with the development of foliage. It is an exceedingly common phenomenon in trees at equatorial latitudes (Richards 1952) and not conspicuous among temperate trees, although many do produce young leaves with some anthocyanins present (Price & Sturgess 1938). The correlation between the incidence of this pigmentation and latitude on different continents, independent of taxonomic affinity, suggests a common selective advantage for its appearance. Yet, a fully satisfactory explanation has never been given. Here we report on detailed analyses of leaf development in mango (*Mangifera indica* L.) and cacao (*Theobroma cacao* L.), and discuss the results in relationship to various hypotheses concerning the selective advantage of anthocyanic coloration in young leaves of tropical trees.

The earliest explanation for anthocyanic pigmentation in tropical trees was given by Smith (1909), who measured leaf temperatures with a primitive thermocouple device, and showed that the leaves were significantly warmer than adult leaves. He speculated that warmer leaves might develop more rapidly. Conflicting reports have subsequently appeared on temperature differences (Nagornaya & Kotsur 1970, McClure 1975).

Anthocyanin production in leaves is associated with various physiological processes in plants (Harborne 1965, McClure 1975), the basis for other hypotheses on the functions of anthocyanins in flushing leaves. Anthocyanins may be produced in response to various environmental stresses, including nutrient deficiency, water stress, physical damage, and fungal attack (Levin 1971). In senescing autumn leaves of temperate plants anthocyanins may be associated with the movement of sugars from leaves to other parts of the plant. A doubtful association between anthocyanins and sugars of the developing leaves of montane plants in Puerto Rico led Wagner *et al.* (1969) to suggest that anthocyanins assist in sugar transport. Anthocyanins are, by definition, certain flavonoid compounds (anthocyanidins) complexed to sugars. How anthocyanins could assist in sugar transport, or any of the above processes, has never been explained. Anthocyanins may be synthesized in response to fungal attack (Harborne 1965), but these pigments are not nearly as active against fungal pathogens as other flavonoid compounds, and there is no relationship to disease for the flushing leaves of tropical trees. Allegations of the activity of anthocyanins in photosynthesis have not been supported by good evidence (McClure 1975). However, Rauf and Sharma (1980) measured increased Hill activity in developing leaves of mango, and speculated that anthocyanins may play a role in this activity.

Many flavonoids are bitter or toxic. An additional hypothesis concerning the function of anthocyanins in flushing leaves is that anthocyanins might protect against herbivory. However, anthocyanins are safe enough to be used as a food coloring, and are not toxic to higher animals. Along with other phenolic compounds, anthocyanins could only be considered toxic in their tendency to complex with, and render, proteins indigestible (Harborne 1979). Anthocyanins could function aposematically, warning potential herbivores of the presence of toxic compounds (see Janzen 1979).

Anthocyanins in many plants are synthesized in re-

[1] Received 31 March 1984, revision accepted September 30, 1986.

sponse to intense radiation, and the principal response is to UV light (Lindoo & Caldwell 1978, Yatsuhashi *et al.* 1982). The long-observed correlation between intense radiation and anthocyanic coloration has been the basis for the hypothesis that anthocyanins might help prevent UV-B (280–315 nm) induced damage (Caldwell 1981). In addition to their absorbance in visible light (at around 525 nm) anthocyanins also absorb strongly in the UV and could absorb potentially damaging radiation. Although this strong association certainly exists, it is difficult to critically test such an hypothesis. Bünning (1947) was the first to suggest that anthocyanins might protect the susceptible young leaf tissue to the more intense UV light climate at equatorial latitudes. Because of a generally thinner layer of stratospheric ozone above equatorial latitudes the UV light climate is demonstrably harsher (Caldwell *et al.* 1980). Young leaves should be more susceptible to UV damage because they are optically more transparent. They lack a fully developed epidermal layer and cuticle that can intercept UV radiation before it damages internal tissues (Lautenschlager-Fleury 1955; Gausman *et al.* 1975; Robberecht & Caldwell 1978, 1980). Furthermore, young leaves lack intercellular spaces that contribute to the backscattering of light out of the leaf (Allen *et al.* 1973). Lee and Lowry (1980) tested this hypothesis with Malaysian species by measuring leaf specular reflectance and pigment concentrations. Reflectance increased and anthocyanin levels decreased as leaves matured, consistent with the hypothesis of protection against UV. However, their measurements of leaf optics included only reflectance at a fixed angle of 45°, and they were not able to distinguish between the importance of anthocyanins and other UV-absorbing phenolics present in young and old leaves. The hypothesis is a viable one, but remains to be adequately tested.

Both cacao and mango are ideal plants for testing these hypotheses because their physiology is relatively well-studied (Alvim 1977, Singh 1977) and genetically uniform varieties are available. Here we report on analyses of leaves at different stages of development: (1) diffuse reflectance and transmittance, permitting estimations of absorptance; (2) concentrations of phenolic compounds; (3) concentrations of anthocyanins; (4) total chlorophyll; (5) leaf temperatures; and (6) stomatal resistances. These measurements, coupled with strategic anatomical observations and a careful survey of the literature, have allowed us to critically evaluate the hypotheses described above.

MATERIALS AND METHODS

We analyzed the foliage of young cacao trees, variety "Amelonado," and mature mango trees, variety "Tommy Atkins," both growing in Miami (latitude 25°40′). We chose only foliage exposed to full light, but the cacao plants were growing under a screen, providing 35 percent of photosynthetic photon flux density (PPFD) of full sunlight. All measurements of different stages of leaf expansion were conducted from March through May of 1983, during periods of active leaf flushing for both species.

ANATOMICAL AND MORPHOLOGICAL ANALYSIS.—We began the analysis with leaves approximately 5 cm in length, the smallest size usable for the optical analysis. Forty leaves of each species were tagged and measured. These were measured during their development for length, area and (eventually) dry weight. Fresh hand sections of leaves at different stages of development were prepared in order to measure leaf thickness, cuticle and epidermal thickness, cell layers and location of anthocyanins. Reported measurements are the means of 10 sections. Stomatal densities were calculated from fresh paradermal sections, counting densities with an ocular grid. These observations were recorded by photography with Ektachrome 64 film.

LEAF OPTICAL ANALYSIS.—Freshly collected leaves were examined as follows. Five leaves of each species at various developmental stages (4–5 cm, 8–8.5 cm, 10–11 cm, 12–16 cm, 18–20 cm, 23–26 cm, 31–36 cm, 35–37 cm — mature, old) were analyzed for diffuse reflectance, diffuse transmittance and thus absorptance, using matched integrating spheres of 5 cm diameter in a double beam spectrophotometer, at 5 nm slit width. Spheres were coated with barium sulfate (Kodak #6048), and the same material was used for the reference sample. Sample size was 10 × 15 mm, suspended within the sphere for reflectance. For developmental studies leaves were measured at 300 and 550 nm, and leaves of approximately 15 cm length were compared to mature leaves by scanning spectrally from 300 to 750 nm. Curves represent the means of five scans. Epidermal peels of mature mango leaves were separated after an overnight incubation in pectinase (Sigma) at 37°C in 1 M acetate buffer at pH 4.0. All methods (including enzymes, mechanical separation, and digestion in calcium oxalate) were unsuccessful in isolating the epidermis of cacao. Isolated mango epidermis was spectrally examined using the methods for intact leaves.

LEAF TEMPERATURES AND DIFFUSION RESISTANCE.—We analyzed flushing and mature leaves of both species on an overcast and a sunny day in April between 1000 and 1230 hr. Leaf temperatures, diffusion resistance and PPFD (incident to the upper leaf surface) were subsequently measured with a Li-1100 diffusion porometer (Li-con, Lincoln, NB 68504, U.S.A.). Leaf angle was measured along the midvein with a level. On each day 10 flushing and 10 mature leaves (from three trees) were measured *in situ*.

CHEMICAL ANALYSIS.—Weighed portions of freshly collected leaves were extracted by grinding in a mortar and pestle with 0.5 N HCl in methanol. Solvent was added until no more pigment was extractable and the total vol-

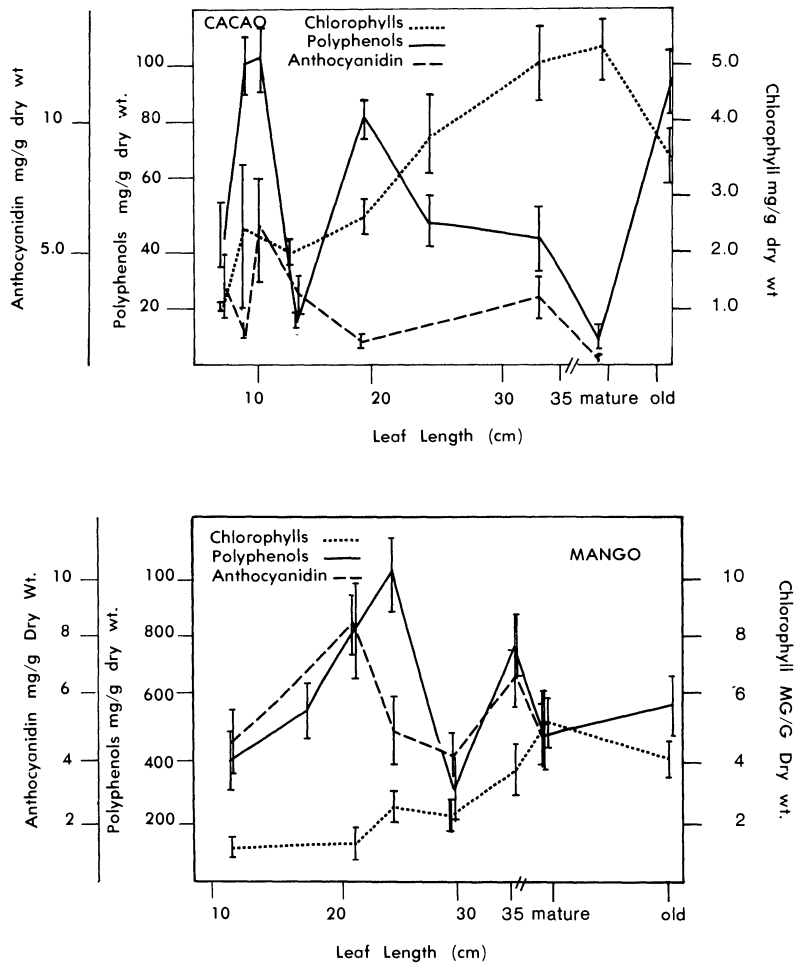

FIGURE 1. Content of total chlorophylls, phenolic compounds and anthocyanidin in developing leaves of mango and cacao. Mature and old leaves are approximately the same length as the largest developing size class.

ume was recorded after filtration through glass wool. The same procedure was repeated for total chlorophyll, but with 80 percent aqueous acetone as the extracting solvent (MacKinney 1941). Total phenols were determined by use of the Folin-Denis reagent (Ribereau-Gayon 1972), absorbance at 750 nm initially calculated per ml of tissue extract and 1 g dry wt of leaf tissue.

Anthocyanin concentrations were determined by the H_2O_2 method of Swain and Hillis (1959); 3 ml of extract and 1 ml of 3 percent H_2O_2 were incubated for 30 min, concentrations initially calculated as for phenols. To assess the contributions of anthocyanins to total phenolic concentration, petunidin-3-glucoside (for cacao) and cyanidin-3-arabinoside (for mango) were purified by preparative thin layer chromatography (TLC) in darkness on 0.6 mm thick microcrystalline cellulose (Sigma), tertiary butanol : acetic acid : H_2O (3:1:5) and 1 percent aqueous HCl used as solvents. Anthocyanin spots from five 15 cm long immature leaves, eluted in the extraction solvent, were analyzed for their (1) absorbance spectra, (2) peroxide reduction, and (3) total phenolic concentration. Published extinction coefficients (Swain 1976, Fuleki & Francis 1968a, Francis 1982) allowed an estimation of molar concentrations of anthocyanins in solution. We used $\epsilon = 4.40 \times 10^4$ at 525 nm for both species (J. Harborne, pers. comm.). The means of each analysis provided a basis for comparing the absolute concentrations of anthocyanins (given as free anthocyanidin and expressed in mg/g dry wt) and phenolics. Individual compounds were identified (Mabry *et al.* 1970) assisted by previous descriptions of the two taxa (Griffiths 1958, El Sissi & Saleh 1965, Jacquemin 1970).

RESULTS

The flushing activities of the two species are quite different. Branches of cacao normally flush sporadically throughout

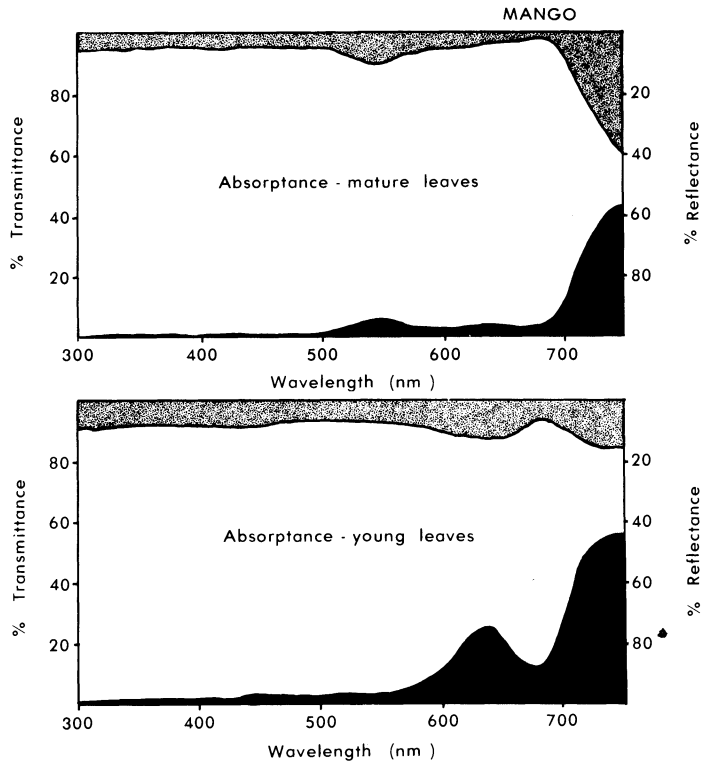

FIGURE 2. Reflectance, transmittance and absorptance of mature and young mango leaves. Solid areas represent transmittance, stippled areas reflectance, and white absorptance. Lines represent the means of measurements of 5 leaves.

the year (Goodall 1950, Greenwood & Posnette 1950, Lemée 1956, Sale 1968, Greathouse *et al.* 1971, Alvim 1977). In Miami leaf development also occurs throughout the year, but infrequently in January, at the coldest time. Mango flushes more sporadically, depending on climatic conditions, flowering and fruiting (Singh 1977). In Miami flushing activity is greatest in March–May (after flowering and before fruit maturation), and during the summer months after fruit has been harvested. The developing leaves are also displayed differently. In mango the leaves are limp during development, suspended vertically. With maturation the leaves become stiff, but are still displayed at angles closer to the vertical (24 ± 5°). In cacao the petioles of developing leaves are bent to display the turgid leaves vertically. When the leaves mature they are displayed more erectly (56 ± 7° from the vertical). Although the leaf development of both species has been described in stages characterized by different colors (Jacquemin 1970) the leaves of both varieties we studied grew rapidly and continually. From an initial leaf length of 5 cm, maximum leaf expansion was completed in one week. The leaves lost their anthocyanic coloration and matured in the following week. For both species the results of the colorimetric analyses (phenols, anthocyanins and total chlorophyll) are seen in Figure 1. The levels of both anthocyanins and total phenols fluctuate during leaf expansion, but in both species the levels of anthocyanins drop by maturity, contrasted by the continued high levels of total phenols. In mature leaves of mango and cacao the H_2O_2 assay detected absorbance differences almost certainly due to interference by other compounds, because analysis by TLC failed to detect any anthocyanins in mature leaf tissue.

Leaf optical propreties also changed with development in both species. Young leaves had lower absorptances at most wavelengths (Fig. 2 and 3); these changes in optical properties with development are documented at 300 and 550 nm in Figures 4 and 5. In both species higher absorptances occurred at 300 nm because of the presence of phenolic compounds. In cacao, absorptance increased with leaf age. In mango, increases in diffuse reflectance were offset by decreases in diffuse transmittance, resulting in little change in absorptance. For mango the isolated cuticle and epidermis showed considerable absorptance, especially at lower wavelengths (Fig. 6).

The developing leaves of both mango and cacao offered considerably more diffusion resistance than mature leaves (Table 1), and the temperatures of immature and mature leaves were not significantly different, with the exception of mango leaves measured on a cloudy day (a difference of 0.5°C).

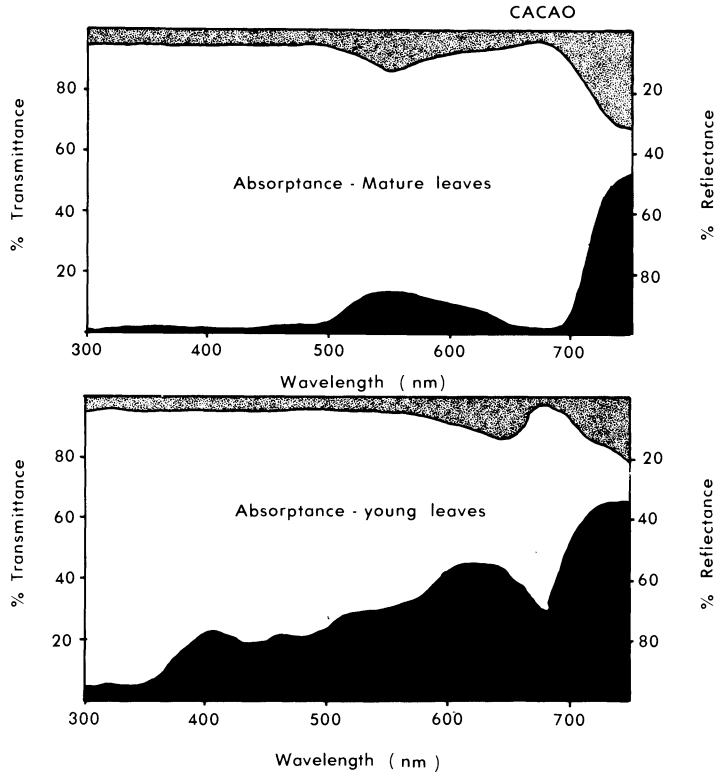

FIGURE 3. Reflectance, transmittance and absorptance of mature and young cacao leaves. Legends are as in Figure 2.

DISCUSSION

LEAF TEMPERATURE.—These data are not consistent with the hypothesis that flushing leaves should absorb more radiation and be warmer than mature leaves. There is little difference in temperature between flushing and mature leaves on cloudy and bright days (Table 1). Differences in absorptance of young versus mature leaves are not consistent with the hypothesis as well. Mature leaves of both species have higher absorptances than flushing leaves. An important role for anthocyanins in light absorptance would be indicated by a large absorptance peak at 525 nm, but only a slight peak is present in both species (Fig. 2 and 3). Anthocyanins absorb well at lower wavelengths of the visible and UV spectra (Fig. 6), but are highly transparent at longer wavelengths of visible radiation as well as the infra-red (Ribereau-Gayon & Josien 1960). Also, all leaves (regardless of pigmentation) are highly reflective in the infra-red (Wong & Blevin 1967).

Pigmentation is only one factor influencing total leaf energy absorptance. The lack of intercellular spaces observed in developing leaves should mean greater diffuse transmittances and reflectances (Allen *et al.* 1973), and lack of a well-developed cuticle and epidermis may reduce or increase diffuse reflectance. The small differences in temperature (only significantly greater in young leaves of mango on the cloudy day, see Table 1) are more likely to be due to differences in evaporative cooling caused by the higher diffusion resistances in the developing leaves. Developing leaves of both species had higher resistances to water loss due to transpiration and/or evaporation. Both species develop stomata exclusively on the leaf undersurfaces. The mature leaves of mango had stomatal densities greater than $1 \times 10^4/cm^2$, and the young leaves of cacao approximately 15 cm in length had densities of $2–5 \times 10^3/cm^2$, clustered along the veins (see also Abo-Hamed *et al.* 1983). Increased diffusion resistances of the flushing leaves must in part be due to lower stomatal densities. However, since diffusion resistance for both species was significantly higher on the sunny rather than overcast day, the stomata of the young leaves of both species can apparently regulate water loss.

PHYSIOLOGICAL ROLE.—Rauf and Sharma (1980) presented evidence for the role of anthocyanins in the Hill reaction in developing mango leaves. Assuming that the Hill reaction is greater in the immature red leaves, the

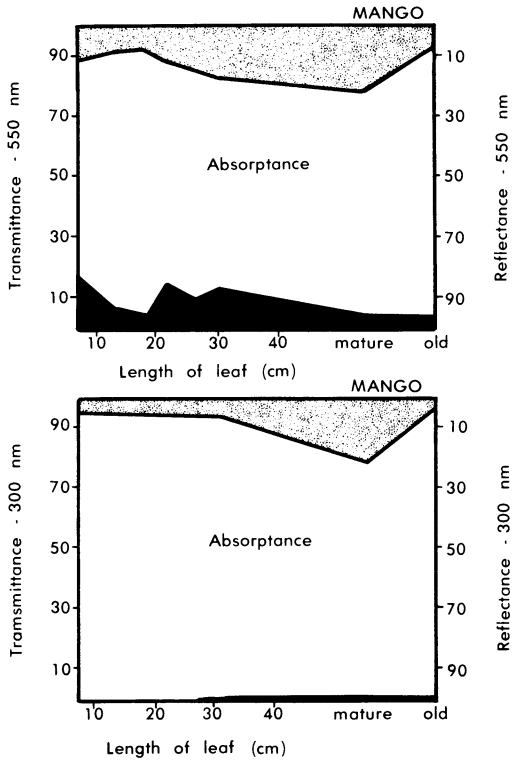

FIGURE 4. Reflectance, transmittance and absorptance of developing mango leaves at 550 nm and 330 nm. Legends are as in Figure 2. Leaves are of the same size classes as Figure 1 and each point is the mean of 5 measurements.

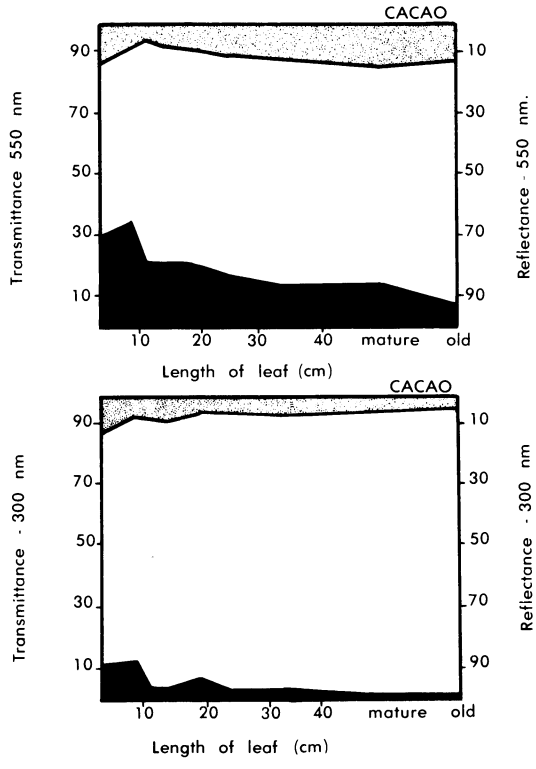

FIGURE 5. Reflectance, transmittance and absorptance of developing cacao leaves at 560 nm and 330 nm. Legends are as in Figure 4.

differences could easily be due to age and not pigmentation. Our anatomical observations confirmed those of Jacquemin (1970) in locating the anthocyanins in discrete regions of the mango leaf (mainly in the second cell layer above the lower epidermis) not clearly associated with photosynthetic tissue. If the anthocyanins assist in sugar transport (as Wagner *et al.* 1969, have suggested) localization of anthocyanins would be expected in areas adjacent to the veins of the flushing leaves. This is certainly the case for cacao, where the areas between the closed reticulations of veins are devoid of anthocyanin, but it is certainly not the case of mango, where the anthocyanins are not particularly associated with the veins. The data are not relevant to the hypothesis of protection against fungal pathogens, but the anthocyanins in the flushing

TABLE 1. *Temperatures and diffusion resistances of flushing and mature leaves of mango and cacao on overcast and sunny days. Values are the means and standard deviations of 10 measurements. Means are considered significantly different at probabilities equal to or greater than 0.05 (analysis by ANOVA).*

	Cacao			Mango		
	Flushing	Mature	*P*	Flushing	Mature	*P*
Cloudy						
Diffusion resistance in S cm^{-1}	13.3 ± 4.0	5.0 ± 1.4	.001	16.4 ± 1.2	7.1 ± 5.3	.001
Leaf T°C	27.7 ± 0.5	27.6 ± 0.4	NS	25.8 ± 0.5	25.3 ± 0.3	.05
Sunny						
Diffusion resistance in S cm^{-1}	51.3 ± 31.5	7.0 ± 1.4	.001	119.3 ± 38.1	5.3 ± 1.2	.001
Leaf T°C	33.4 ± 0.3	33.3 ± 0.2	NS	31.1 ± 3.5	32.3 ± 1.9	NS

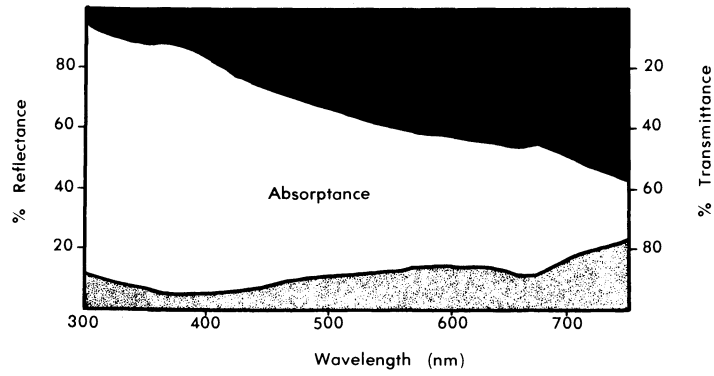

FIGURE 6. Reflectance, transmittance and absorptance of mango mature leaf upper epidermis and cuticle. Solid areas represent transmittance, stippled areas reflectance, and blank areas absorptance, the means of 5 measurements.

leaves of either species are certainly not in an anatomical position to defend against the initial stages of fungal attack.

HERBIVORY.—The data are not consistent with the explanation that anthocyanins in flushing leaves provide defense against predation, or that they function as warning coloration. Flushing leaves of cacao are very susceptible to insect attack (Greenwood & Posnette 1950; and pers. obs.). Flushing leaves of mango appear no more resistant to herbivory than mature leaves (pers. obs.). Neither anthocyanin is known to be toxic to animals, although some of the phenolic constitutents may be. The hypothesis could be tested by a detailed comparison of herbivory on anthocyanic developing leaves of tree species, unpigmented mature leaves, and unpigmented developing leaves of other species, as shown by Coley (1983).

UV PROTECTION.—Certain of the data are consistent with this hypothesis. In both species the flushing leaves are colored more brilliantly (and presumably contain higher concentrations of anthocyanins) when the branches have been exposed to direct sunlight (Greenwood & Posnette 1950, pers. obs.). In both species there is a rough correlation between optical properties and anthocyanin content, the latter disappearing when the leaves mature.

The optical properties of leaves of both species change during development, at least partly because of anatomical changes. The developing leaves of both species lack intercellular spaces. Mango leaves approximately 15 cm long are 131 μm in thickness (SD = 8, N = 10), including an epidermis 18 (SD = 2) μm thick with no evidence of a cuticle. The blade is composed of 9 compact files of cells. Mature leaves are 240 (SD = 44) μm thick, including a heavily cuticularized epidermis 24 (SD = 2) μm thick, and a typical spongy mesophyll. In cacao flushing leaves of 15 cm length are 64 (SD = 4) μm in thickness, cells in six compact files, and an epidermis 12 (SD = 2) μm thick. Mature leaves are 96 (SD = 12) μm thick, with a cuticularized epidermis 18 (SD = 3) μm thick, and with typical spongy mesophyll. These observations lead us to conclude that the young leaves of both species would allow more radiation to penetrate the leaf, perhaps making UV protection of paramount importance. In mango the mature epidermis and cuticle absorb a significant amount of radiation (Fig. 6). In both species the young leaves allow more radiation to pass through (greater diffuse transmittance, Figs. 2, 3, 4 and 5).

However, other data are *not* consistent with the hypothesis. The anthocyanins present in immature leaves are not a very significant proportion of the concentration of all phenolic compounds (all of which absorb in the UV and could confer protection against damage by UV-B radiation). The results support this conclusion in three ways. Calculations for total leaf anthocyanidin are approximately 5–10 percent of that for total phenols in both species (Fig. 1). However, these comparisons are based on the known standard of the principal anthocyanin for each species, and individual phenolic compounds will vary in their sensitivity to the colorimetric assay. Secondly, methanol-HCl extracts of young leaves were diluted to allow measurement of absorbance in the UV. Such curves absorbed little in the visible absorbance peak for anthocyanins. Since anthocyanins have similar extinction coefficients at 525 nm and 300 nm and below (Schou 1927, Hayashi 1935, Fuleki & Francis 1968b), at the low pH of the extraction solvent, the preponderance of absorbance in the UV must be due to other phenolic compounds. Thirdly, individual spots of other phenolics eluted from TLC plates absorbed more than the anthocyanins (Fig. 7). These are representative of the many unidentified compounds isolated in developing leaves, 11 spots in mango and 16 in cacao.

The anatomical location of anthocyanins in the flushing leaves is not consistent with the hypothesis as well. In cacao virtually all of the anthocyanin is associated with small veins in the developing leaves, these developing in

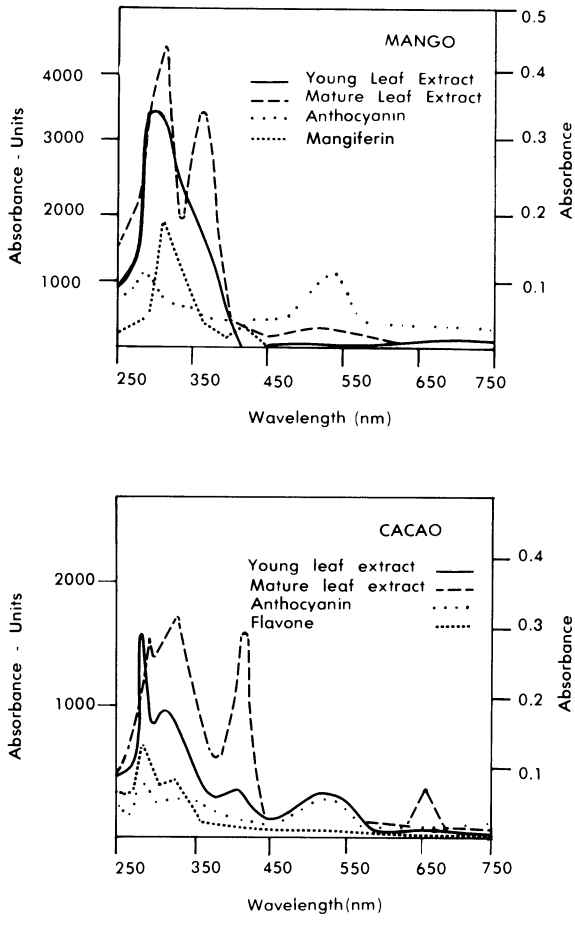

FIGURE 7. Absorbance spectra of leaf extracts of mango and cacao. For whole leaf extraction absorbance units are in g dry wt of tissue in 1 ml of extract. Anthocyanins and flavonoids were eluted from the same TLC plates. Spectra represent the means of three extracts.

the two cell layers adjacent to the lower epidermis. A prediction of the hypothesis would be their location near or in the upper epidermis where they could more efficiently intercept UV-B radiation. Other flavonoids are located near the upper leaf surface (Jacquemin 1970). In mango the anthocyanins are also located near the lower epidermis.

Thus the role of anthocyanins in absorbing UV-B radiation is probably insignificant when compared to other phenolic compounds present in both flushing and adult leaves.

SUMMARY

Data that we have obtained on leaf development in mango and cacao are not consistent with any of the published hypotheses concerning the selective advantage of anthocyanins in the developing foliage of tropical trees, and the pigments by themselves may not have any direct role. It will be important to study other taxa, especially to see if anthocyanins are a small portion of the total phenolic concentration. At least for mango and cacao, one possibility is that the interaction (co-pigmentation) of anthocyanins with other flavonoid pigments may contribute to advantageous *in vivo* spectral qualities (Robinson and Robinson 1931, Chen & Hrazdina 1981, Sweeney *et al.* 1981, Osawa 1982) or to greater *in vivo* stability (Sweeney *et al.* 1981). A third possibility is that anthocyanic coloration may merely be the by-product of the very rapid synthesis of other flavonoid compounds in certain tissues. Mango leaf tissue is the site of synthesis for a great diversity of phenolic compounds; Jacquemin (1970) resolved 26 spots by paper chromatography. In both flushing and mature leaves we resolved 18 spots by TLC, 11 in flushing and 8 in mature leaves. El Sissi and Saleh (1965) observed the increasing contribution of mangiferin to total leaf phenolic composition during development. Griffiths (1958) observed strong qualitative differences between flushing and mature leaves of the phenolic constitutents of cacao leaves. In addition to the absence of anthocyanin from mature leaves there was also a disappearance of epicatechin, along with an increase in p-coumaric acid and neochlorogenic acid. We detected 11 different phenolic compounds from flushing leaves and 8 in mature leaves, only three common to both stages. Given the rapid metabolic turnover of phenolic compounds during leaf development of both species, it seems likely that some functional role is being fulfilled. Unfortunately, the pathway of synthesis of flavonoid compounds in plants is poorly understood (Grisebach 1982), but Guruprasad and Laloraya (1980) have shown that there may be feedback mechanisms that tie the metabolism of anthocyanins to other flavonoids.

Thus a more realistic approach towards the understanding of the function of anthocyanins in flushing leaves would be to study the physiological and biochemical relationships of the different flavonoid compounds to each other. It seems that we are not much closer to solving this problem than we were seventy years ago, but it may be that a central function will be discovered that explains the synthesis of anthocyanins in many plant tissues under stress.

LITERATURE CITED

ABO-HAMED, S., H. A. COLLIN, AND K. HARDWICK. 1983. Biochemical and physiological aspects of leaf development in cacao (*Theobroma cacao* L.). VII. Growth, orientation, surface structure and water loss from developing flush leaves. New Phytol. 95: 9–17.

ALLEN, W. A., H. W. GAUSMAN, AND A. J. RICHARDSON. 1973. Willstatter-Stoll theory of leaf reflectance evaluated by ray tracing. Appl. Optics 12: 2448–2453.
ALVIM, P. DE T. 1977. Cacao. *In* P. de T. Alvim and T. T. Kozlowski (Eds.). Ecophysiology of tropical crops, pp. 279–313. Academic Press, New York.
BÜNNING, E. 1947. In dem waldern Nord-Sumatras, pp. 100–124. Ferd. Dummlers, Bonn.
CALDWELL, M. M. 1981. Plant responses to ultraviolet radiation. Physiological ecology I. Encyclopedia of plant physiology series II, pp. 170–197. Springer-Verlag, Heidelberg.
———, M. R. ROBBERECHT, AND W. D. BILLINGS. 1980. A steep latitudinal gradient of solar ultraviolet-B radiation in the arctic-alpine life zone. Ecology 61: 600–611.
CHEN, L. J., AND G. HRAZDINA. 1981. Structural aspects of anthocyanin-flavonoid complex formation and its role in plant color. Phytochemistry 20: 297–303.
COLEY, P. D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol. Mon. 53: 209–233.
EL SISSI, H. I., AND N. A. M. SALEH. 1965. Phenolic components of *Mangifera indica* (Part II). Planta Medica 13: 346–352.
FRANCIS, F. J. 1982. Analysis of anthocyanins. *In* P. Markakis (Ed.). Anthocyanins as food colors, pp. 192–207. Academic Press, New York.
FULEKI, T., AND F. J. FRANCIS. 1968a. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. J. Food Sci. 3: 72–77.
———, AND ———. 1968b. Quantitative methods for anthocyanins. 2. Determination of total anthocyanin and degradation index for cranberry juice. J. Food Sci. 33: 78–83.
GAUSMAN, H. W., R. R. RODRIGUEZ, AND D. E. ESCOBAR. 1975. Ultraviolet radiation reflectance, transmittance and absorptance by plant leaf epidermises. Agron. J. 67: 719–724.
GOODALL, D. W. 1950. Growth analysis of cacao seedlings. Ann. Bot. 14: 291–306.
GREATHOUSE, D. E., W. M. LAETSCH, AND B. O. PHINNEY. 1971. The shot growth rhythm of a tropical tree, *Theobroma cacao.* Amer. J. Bot. 58: 281–286.
GREENWOOD, M., AND A. F. POSNETTE. 1950. The growth flushes of cacao. J. Hort. Sci. 25: 164–174.
GRIFFITHS, L. A. 1958. Phenolic acids and flavonoids of *Theobroma cacao* L. Separation and identification by paper chromatography. Biochem. J. 70: 120–125.
GRISEBACH, H. 1982. Biosynthesis of anthocyanins. *In* P. Markakis (Ed.). Anthocyanins as food colors, pp. 69–92. Academic Press, New York.
GURUPRASAD, K. N., AND M. M. LALORAYA. 1980. Effect of pigment precursors on the inhibition of anthocyanin biosynthesis (in radish seedlings) by GA and ABA (giberellic acid and abscisic acid). Plant Science Letters 19: 73–79.
HARBORNE, J. B. 1965. Flavonoids: distribution and contribution to plant colour. *In* T. W. Goodwin (Ed.). Chemistry and biochemistry of plant pigments, pp. 247–278. Academic Press, London.
———. 1979. Flavonoid pigments. *In* G. A. Rosenthal and D. H. Janzen (Eds.). Herbivores: their interactions with secondary plant metabolites. Academic Press, New York.
HAYASHI, K. 1935. Spektrographische Untersuchunger uber die Farbstoffe vom Benzopyryliumtypus. V. Acta Phytochemica 9: 1–24.
JACQUEMIN, H. 1970. Recherches sur les anthocyanes foliares de trois arbres tropicaux (*Mangifera indica* L., *Theobroma cacao* L., *Lophira alata* Banks ex Gaertner F.). O.R.S.T.O.M., Paris, 118 p.
JANZEN, D. 1979. New horizons in the biology of plant defenses. *In* G. A. Rosenthal and D. H. Janzen (Eds.). Herbivores: their interactions with secondary plant metabolites. Academic Press, New York.
LAUTENSCHLAGER-FLEURY, D. 1955. Uber die ultraviolet durchlassigkeit von Blattepiderm. Ber. Schweiz. Bot. Ges. 66: 343–386.
LEE, D. W., AND J. B. LOWRY. 1980. Young leaf anthocyanin and solar ultraviolet. Biotropica 12: 75–76.
LEMÉE, G. 1956. Recherches eco-physiologiques sur le cacaoyer. Revue Gen. de Botanique 63: 41–94.
LEVIN, D. A. 1971. Plant phenolics: an ecological perspective. Amer. Nat. 10: 157–181.
LINDOO, S. J., AND M. M. CALDWELL. 1978. UV-B radiation induced inhibition of leaf expansion and promotion of anthocyanin production. Lack of involvement of the low irradiance phytochrome system. Plant Physiol. 61: 278–282.
MABRY, T. J., K. R. MARKHAM, AND M. B. THOMAS. 1970. The systematic identification of flavonoids. Springer-Verlag, Berlin.
MACKINNEY, G. 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. 140: 315–322.
MCCLURE, J. W. 1975. Physiology and functions of flavonoids. *In* J. B. Harborne, T. J. Mabry, and H. Mabry (Eds.). The flavonoids, pp. 971–1055. Chapman and Hall, London.
NAGORNAYA, R. V., AND N. V. KOTSUR. 1970. Absorption of radiant energy by anthocyanin-containing plants. Nauk. Pr. Ukr. Silskogospod. Akad. 31: 103–112. (Chem. Abstr. 75-85372).
OSAWA, Y. 1982. Copigmentation in anthocyanins. *In* P. Markakis (Ed.). Anthocyanins as food colors, pp. 41–68. Academic Press, New York.
PRICE, J. R., AND V. C. STURGESS. 1938. CCXIV. A survey of anthocyanins. VI. Biochem. J. 32: 1658–1660.
RAUF, A., AND U. SHRAMA. 1980. Hill activity and anthocyanin levels in *Mangifera indica.* Phytochemistry 19: 1270–1271.
RIBEREAU-GAYON, P. 1972. Plant phenolics. Oliver and Boyd, Edinburgh.
———, AND M. L. JOSIEN. 1960. Contribution a l'etude des anthocyanes par spectrophotometre infrarouge. Bull. Soc. Chim. Fr.: 924–937.
RICHARDS, P. W. 1952. The tropical rainforest. Cambridge University Press, London.
ROBBERECHT, R., AND M. M. CALDWELL. 1978. Leaf epidermal transmittance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. Oecologia 32: 277–287.
———, AND ———. 1980. Leaf ultraviolet optical properties along a latitudinal gradient in the arctic-alpine life zone. Ecology 61: 612–619.
ROBINSON, G. M., AND R. ROBINSON. 1931. CLXXXII. A survey of anthocyanins. I. Biochem. J. 25: 1687–1705.

SALE, P. J. M. 1968. Flushing and leaf growth of cacao under controlled temperature conditions. J. Hort. Sci. 43: 475–589.
SCHOU, S. A. 1927. Uber die lichtabsorption einiger Anthocyanidine. Helv. Chim. Acta 10: 907–915.
SINGH, L. B. 1977. Mango. *In* P. de T. Alvim and T. T. Kozlowski (Eds.). Ecophysiology of tropical crops, pp. 479–485. Academic Press, New York.
SMITH, A. M. 1909. On the internal temperatures of leaves in tropical insolation, with special reference to the effect of their colour on temperature; also observations on the periodicity of the appearance of young coloured leaves of trees growing in Peradinaya Gardens. Ann. Roy. Bot. Gard. Peradinaya 4: 229–297.
SWAIN, T. 1976. Nature and properties of flavonoids. *In* T. W. Goodwin (Ed.). Chemistry and biochemistry of plant pigments, 2nd Ed., vol. 2, pp. 425–463. Academic Press, London.
SWAIN, T., AND W. E. HILLIS. 1959. The phenoic constituents of *Prunus domestica.* I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63–68.
SWEENEY, J. G., M. M. WILKINSON, AND G. A. IACOBUCCI. 1981. Effect of flavonoid sulfonates on the photo bleaching of anthocyanins in acid solution. J. Agric. Food Chem. 29: 563–567.
WAGNER, R. J., A. B. WAGNER, AND R. A. HOWARD. 1969. The ecology of an elfin forest in Puerto Rico, 9. Chemical studies of colored leaves. J. Arnold Arboretum 50: 556–565.
WONG, C. L., AND W. R. BLEVIN. 1967. Infra-red reflectances of plant leaves. Aust. J. Biol. Sci. 20: 501–508.
YATSUHASHI, H., T. HASHIMOTO, AND S. SHIMUZU. 1982. Ultraviolet action spectrum for anthocyanin formation in broom sorghum first internodes (*Sorghum bicolor*). Plant Physiol. 70: 735–741.