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Biotropica, Vol. 11, No. 1 (Mar., 1979), 70-77.

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Abaxial Anthocyanin Layer in Leaves of Tropical Rain Forest Plants: Enhancer of Light Capture in Deep Shade

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ABSTRACT

The permanent pigmentation of the leaves of tropical rain forest herbs with anthocyanin has traditionally been viewed as a mechanism for enhancing transpiration by increased heat absorption. We report measurements to $\pm 0.1^\circ\text{C}$ on four Indo-malaysian forest species polymorphic with respect to color. There were no detectable differences in temperature between cyanic and green leaves. In deeply shaded habitats, any temperature difference would arise from black-body infrared radiation which all leaves absorb and to which anthocyanins are transparent. Reflectance spectra of the lower leaf surfaces of these species revealed increased reflectance around 650-750 nm for cyanic leaves compared with green leaves of the same species. In all species anthocyanin was located in a single layer of cells immediately below the photosynthetic tissue. These observations provide empirical evidence that the cyanic layer can improve photosynthetic energy capture by back-scattering additional light through the photosynthetic tissue.

LEAVES OF TROPICAL FOREST PLANTS show a wide range of color combinations and patterning effects. Divergence from a "normal" uniform green may be summarized in three categories: (1) young leaves of woody plants often have, transiently, intense cyanic coloration throughout their tissue; (2) many species have permanently patterned leaves in which cyanic, chlorotic, or non-pigmented regions occur as spots, bands, margins, or patches; (3) species (nearly always herbaceous) restricted mainly to deeply shaded habitats often show permanent anthocyanin coloration of the lower (normally abaxial) surface.

The first of these categories has recently been the subject of unpublished investigation by Lowry and Lee. Here our attention turns to the third category, seen in herbaceous shade plants in tropical forests. Richards (1952) described this phenomenon as one of the general features of shady forest habitats throughout the tropics. Although similar distributions of anthocyanins also occur in certain aquatic plants, we shall confine our discussion here to rain forest plants.

Many earlier workers attempted to attribute functional significance to the red undersurfaces of leaves. Haberlandt (1914) discussed the phenomenon at length, and Kerner and Oliver (1895) described some of their own research along with that of others.

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Smith (1909) reported temperature increases in red leaves. These authors discussed possible protective effects of anthocyanins against intense sunlight, but ruled against the importance of any such protective effects in a habitat with little, if any, direct sunlight. They thought that the best explanation was that the red leaf undersurfaces enhanced the absorption of heat in order to increase rates of transpiration. By this time, it was generally known that such leaves had nearly all their stomata located on the undersurfaces. Haberlandt (1914) reviewed the early experimental evidence for this hypothesis, including the demonstration of temperature increase in red leaves after they had been placed next to a candle, but doubted if such effects were relevant in the rain forest. Since that time, additional research has given no better understanding of the phenomenon.

Recently, however, ecology and crop physiology has benefited from application of biophysical principles which increase our understanding both of leaf function and of the leaf as an organ of energy transfer (Gates, 1965, 1976).

In this paper, current biophysical thinking, new data, and our own results are integrated to bear on the problem of cyanic layers of leaf undersurfaces. As a result we largely eliminate traditional hypotheses and arrive at another, for which some empirical evidence is provided.

We can abandon the suggestion of a radiation-

protection role. Although leaf anthocyanin may be associated with a high-ultraviolet light environment (Caldwell 1971), light intensity reaching the forest floor is less than 1 percent of that in the canopy, and there is virtually no damaging UV light present (Yoda 1974, Bjorkman 1972a).

That the red color of the leaf undersurface might lead to increased temperature near stomata, nearly all of which are found on the under surface, and thus to increased rate of transpiration, remains a formal possibility which though improbable in view of new radiation data can at least be tested by direct measurement under field conditions.

It is now known unequivocally that light is the limiting factor for growth of tropical forest-floor plants (Bjorkman 1972a). Not only is the intensity low, but the light is poor in photosynthetically active wavelengths, those available being mainly in the red region (Federer and Tanner 1966). It is thus to be expected that plants in this habitat will show adaptations to enhance utilization of this light. In fact, we have shown that the metallic-blue iridescence of the forest-floor plant *Selaginella willdenowii*, and the similar iridescence of several other species of the same habitat, is the result of an optical interference effect which increases absorption of red light at the expense of greater reflection of blue light (Lee and Lowry 1975).

Another problem concerning the light available for photosynthesis in the rain-forest floor habitat is the inconstant diurnal distribution of light. Most of the light available and used for photosynthesis in the deep-shade habitat is provided by relatively brief durations of light in small areas known as sunflecks, as noted by Bjorkman (1972a) and Boardman (1977). Is it more advantageous for shade-plants to follow a strategy that optimizes the use of sunflecks, or the lower intensities of the shade which are continuous? Those species which we have studied grow in habitats so deeply shady that sunflecks are relatively of less importance than for slightly more open habitats and for plants of the next higher strata. It should be remembered, however, that sunflecks are not entirely random phenomena, but to a degree are repetitive. They are affected by cloud movement, by the configuration of foliage and branches occupying the space above the forest floor, by the effect of wind, growth, decay, predation, tree-fall resulting in canopy gaps, and by other phenomena. In equatorial latitudes, the solar radiation will track over essentially the same path each day of the year. Thus there will be a discrete zone of enhanced net light intensity in some zones relative to others which do not receive these

repetitive "fleck-tracks." In habitats where light intensity is very close to the lower limit for plant growth there will be effects caused by this variation in available light, but as it is essentially a non-predictable habitat, there can be no uniform selection pressure. We take up this topic further in the last part of this paper.

It is necessary to ask whether the cyanic zone could have any effects in increasing photosynthetic energy capture. The location of the pigment, the known optical properties of anthocyanins, and the absence of any suggestion that absorbed energy can be transferred from the molecule, all indicate that the only possible influence of the cyanic layer on photosynthesis would be to enhance reflectance. Light which would otherwise pass out through the lower surface of the leaf could instead be reflected back through the photosynthetic tissue.² Reflectance measurements with the precise determination of pigment location reported here show that this is possible.

We note finally that polyphenols (including anthocyanins) are frequently of general and specific antimicrobial activity (McLure 1976). Attribution of other functions would not eliminate the possibility of these pigments having a defensive role, but the distribution of both pigment and plant makes this seem improbable.

MATERIALS AND METHODS

Four species, all Angiosperms, were studied (table 1). The monocotyledons are represented by one family, while the other three are dicotyledons. In three species (*Begonia pavonina*, *Forrestia mollis*, and *Allomorpha malaccensis*) there were individual plants with acyanic (green) leaves as well as individuals with red leaves. This color polymorphism provided a convenient form of experimental "control." It also has interesting evolutionary adaptational implications, as discussed below.

The plants were studied within 12 hours of col-

²A situation exists in the distribution of marine algae, especially in the clear waters of tropical bays, in which as light penetrates the water, intensity decreases and energy-wavelength (spectral distribution) changes. Red algae (Rhodophyceae) contain the absorptive pigments phycoerythrin and phycocyanin which mediate by reflecting red, absorbing green-yellow-orange (500-620 nm) and blue light and then releasing it for absorption by the chlorophylls, thus enhancing the photosynthetic performance, through more efficient light-gathering. This physiological capability in fact helps to determine the broad patterns of algal species distributions. The phycoerythrin and phycocyanin absorb and transfer more of the long wave-length light normally not used. If this algal ecosystem be compared with forest, it may be seen that a similarity exists both in the physical-optical and the biological-ecological aspects (Halledal 1968, Bjorkman 1972b).

lection from their natural habitats. Anatomical comparisons were made with fresh, hand-cut transverse sections. We were able to observe the location of anthocyanins in cell layers in these sections.

We measured leaf reflectance with an Acta 5 spectrophotometer with total fluorescence attachment. A blank with a 45° angle cut across its face replaced the quartz cuvette, and light reflectance at a fixed 60° angle of incidence could be measured from samples placed against this face. Slices of fresh leaves (1 cm²) were placed on the blank and compared to the reflection of a magnesium oxide blank. The scans (fig. 2) are representative of numerous such tests, and the values are relative to the optically opaque blanks.

Anthocyanins were extracted from dried specimens with a 1 percent concentrated hydrochloric acid in dioxan for spectral measurements. Chemical characterization, where attempted, was by visual methods (Harborne 1967). Visible spectra were measured with a Spectronic 70 spectrophotometer. Leaf surface temperatures were measured with a Grant telethermoprobe thermometer, sensitive to 0.1° C.

RESULTS AND DISCUSSION

All four of the species studied possess a clearly defined layer of anthocyanin-containing cells on the undersurface of the leaf (fig. 1). The layer was one cell thick and was located immediately beneath the chlorenchyma (which was at the bottom of the palisade layer parenchyma). The lower epidermis contained no anthocyanin except in *Forrestia mollis* which, however, has the pigmented epidermis immediately beneath the chloroplast-containing bundle sheath cells. (See fig. 1.)

The currently accepted optical model of the leaf, the Willstätter-Stall theory (see Allen *et al.* 1973), stresses the importance of intracellular spaces in determining the amount of leaf reflectance. Since most of the light entering the plant is reflected by these spaces, we would expect that the light-absorbing pigment would be located in the epidermis of the leaf undersurface, at least if energy absorption were the main consideration. Conversely, if back-scattering of red light to the chloroplasts were most important, then the anthocyanin layer ought to be immediately adjacent to the palisade parenchyma layer which contains the chlorophyllous cells.

Measurements of leaf temperatures of all four species revealed no statistically significant differences in temperature either between the upper and lower surfaces or between red and green undersurfaces of the three polymorphic species (table 2).

All of the pigment extracts absorbed very strongly in the ultra-violet, variously in the visible, and very slightly in the infra-red wave-lengths (fig. 2). The reflectance spectra of these species were similar to those of many other species (Gates *et al.* 1965, Woolley 1971, Gausman and Allen 1973); the upper surface of the leaves absorbed more light than plants occurring in sunny locations. The undersurface of each leaf generally reflected more light than did the upper surface. The red leaf undersurfaces reflected substantially more light between 600 nm and 750 nm than did the green leaf undersurfaces.

Clearly these results do not support the hypothesis of leaf temperature modification. There was no significant increase in temperature under field conditions. Neither the anatomical observations nor the

TABLE 1. *Plants examined in this investigation.*

Species ^a	Voucher specimen	Family	Additional information ^b
1. <i>Begonia pavonina</i> Ridley	KLU.23340	Begoniaceae	Low herb to 25 cm high in damp very shady forest floor habitats in Malaya. Individuals are polymorphic with respect to red leaf color (which is due to cyanidin glycosides).
2. <i>Allomorpha malaccensis</i> Ridley	KLU.23337	Melastomaceae	Shrub eventually reaching 2-3 m high in rain forest understory, Malaya. Only the juvenile plants have leaves with red (purplish) undersurfaces. Individuals are polymorphic with respect to this color. The pigment is a mixture of acylated malvidin and cyanidin glycosides.
3. <i>Forrestia mollis</i> Hasskarl	KLU.23338	Commelinaceae	Herb to 1 m high in damp shady rain forest habitats, S.E. Asia. Individuals are polymorphic with respect to leaf pigment which is cyanidin-3-rhamnoside.
4. <i>Piper porphyrophyllum</i> N.E. Br.	KLU.23339	Piperaceae	Common climber in dense shady rain forests of S.E. Asia. All individuals have undersurfaces red; but only as juvenile phase.

^aSpecies determinations by B. C. S.

^bChemical determinations by J. B. L.

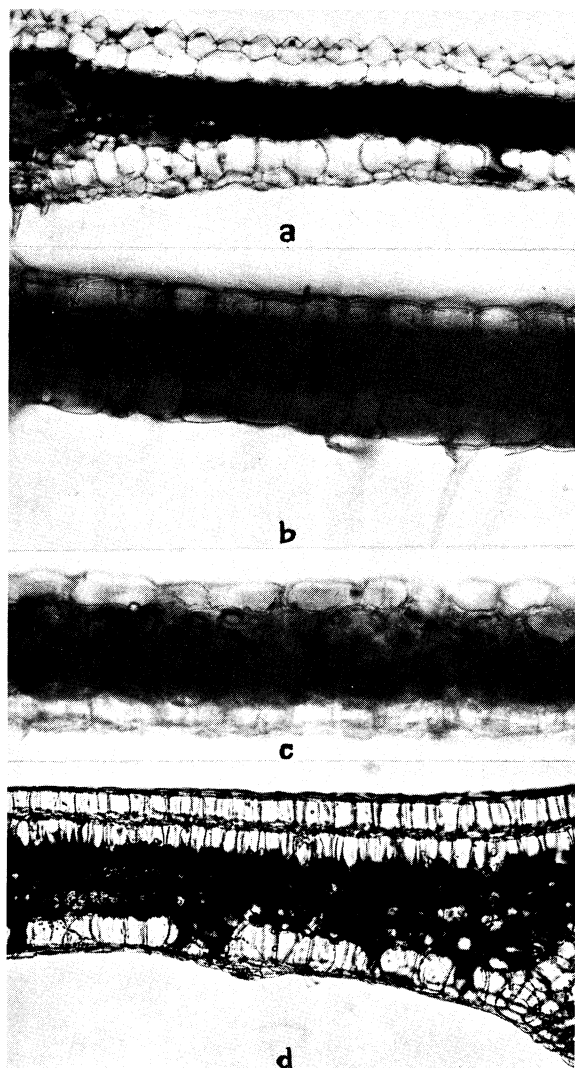


FIGURE 1. Photographs of fresh transverse sections of leaves of: A. *Piper porphyrophyllum* (X 100); B. *Forrestia mollis* (X 100); C. *Allomorpha malaccensis* (X 400); D. *Begonia pavonina* (X 100). Note that the anthocyanin-containing cells are adjacent to the photosynthetic tissue in each case.

spectroscopic properties of anthocyanins and reflectance properties of leaves in the infra-red are compatible with the hypothesis. The undersurfaces of the leaves are parallel to the ground, which radiates black-body infra-red radiation between 4 nm and 50 nm with an optimum around 10 nm (Gates 1965). Thus, if the red undersurfaces are to absorb this energy, they must have specific characteristics which would allow them to do so. Wong and Blevin (1967) have

shown that many leaves absorb virtually all infra-red radiation between 2 nm and 14 nm, even normal green leaves. Secondly, Ribereau-Gayon and Josien (1960) measured the infra-red spectra of the six common anthocyanins and found them to be largely transparent to infra-red light between 4 nm and 14 nm, with specific bands of absorption between 6 nm and 7 nm; thus it is not likely that the red layer would absorb significant amounts of light coming from above; since the anthocyanins are largely transparent to light above 550 nm.

TABLE 2. Temperature measurements of leaves (G and R signify green and red leaves, respectively, U and L denote upper and lower leaf surfaces). Means and standard deviations of 10 measurements are given.

	G		R	
	U	L	U	L
<i>Begonia</i>	25.05 ±0.10	25.10 ±0.12	24.92 ±0.15	25.01 ±0.16
<i>Allomorpha</i>	24.96 ±0.30	25.44 ±0.35	24.87 ±0.20	25.73 ±0.60
<i>Forrestia</i>	24.55 ±0.08	24.76 ±0.40	24.61 ±0.20	25.17 ±0.54
<i>Piper</i>	24.51 ±0.15	24.53 ±0.16	Not applicable	

The data we obtained are consistent with our alternative hypothesis, namely that red light is back-scattered into the chloroplasts by the cyanic layer. In all four species studied, this layer is immediately beneath the chloroplasts. Reflectance spectra show that the red-undersurfaced leaves do reflect appreciably more light at wavelengths of 650-750 nm, even in comparison to the all-green leaves of individuals of the same (polymorphic) species. All of these leaves, as well as any others ever measured (Gates *et al.* 1965, Woolley 1971, Gausman and Allen 1973), reflect more light in the wavelengths above 750 nm; Bjorkman (1972a) argued that plants in the dim light climate of the rain forest floor are likely to possess adaptations which increase their light utilization efficiency. Since a disproportionate amount of the light available to them is above 650 nm, it is not so surprising to find a reflective layer in the plants.

It may seem paradoxical that pigments transparent at a specific wavelength could be a component of a "mirror" that could effectively back-scatter those same wavelengths. Nakayama and Powers (1972) discussed several factors which make the *in vivo* char-

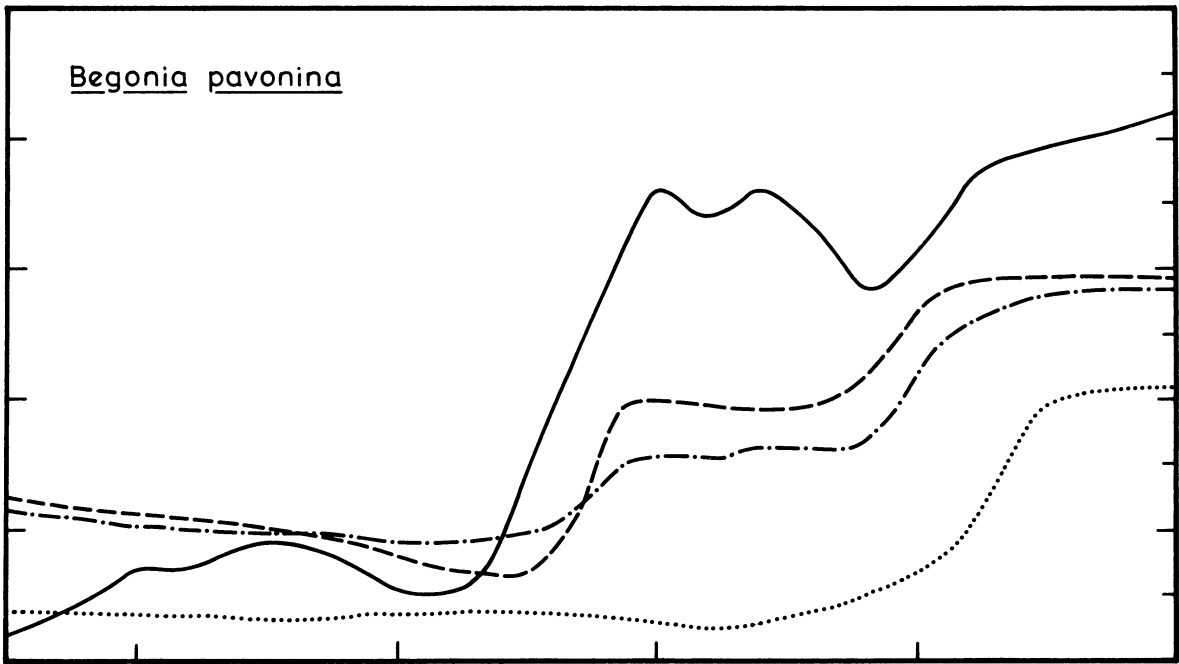
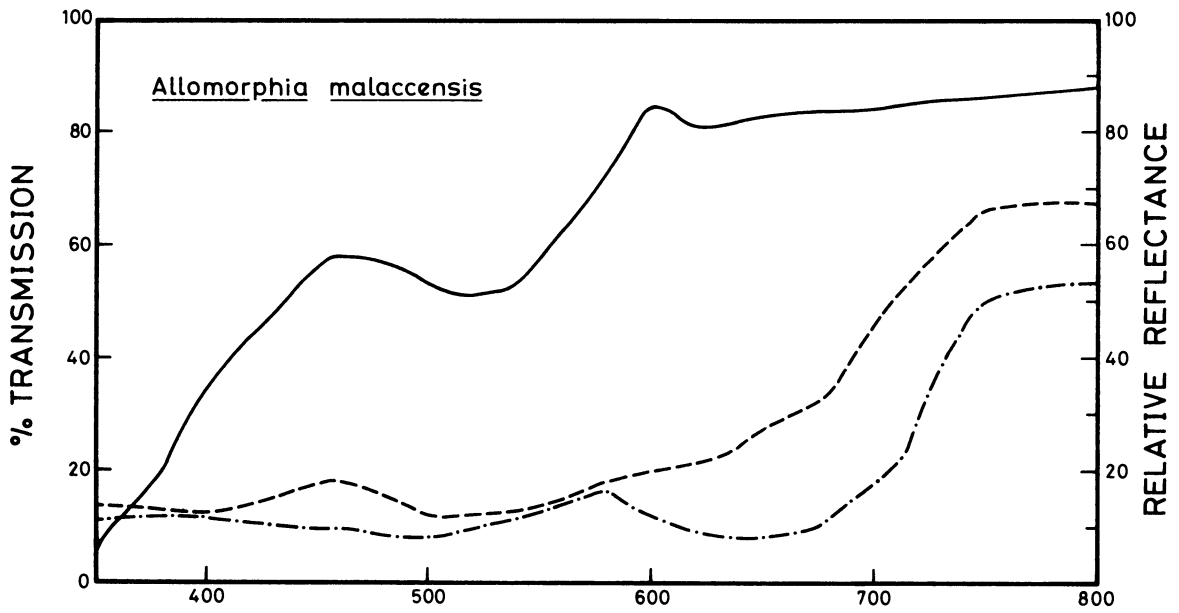
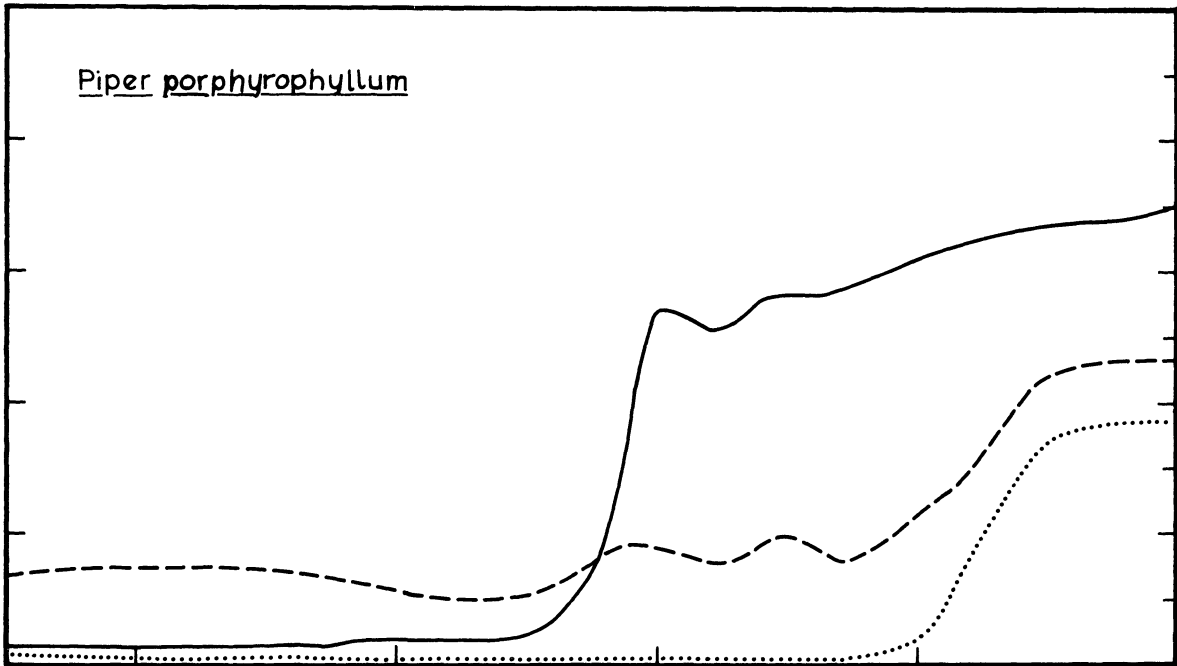
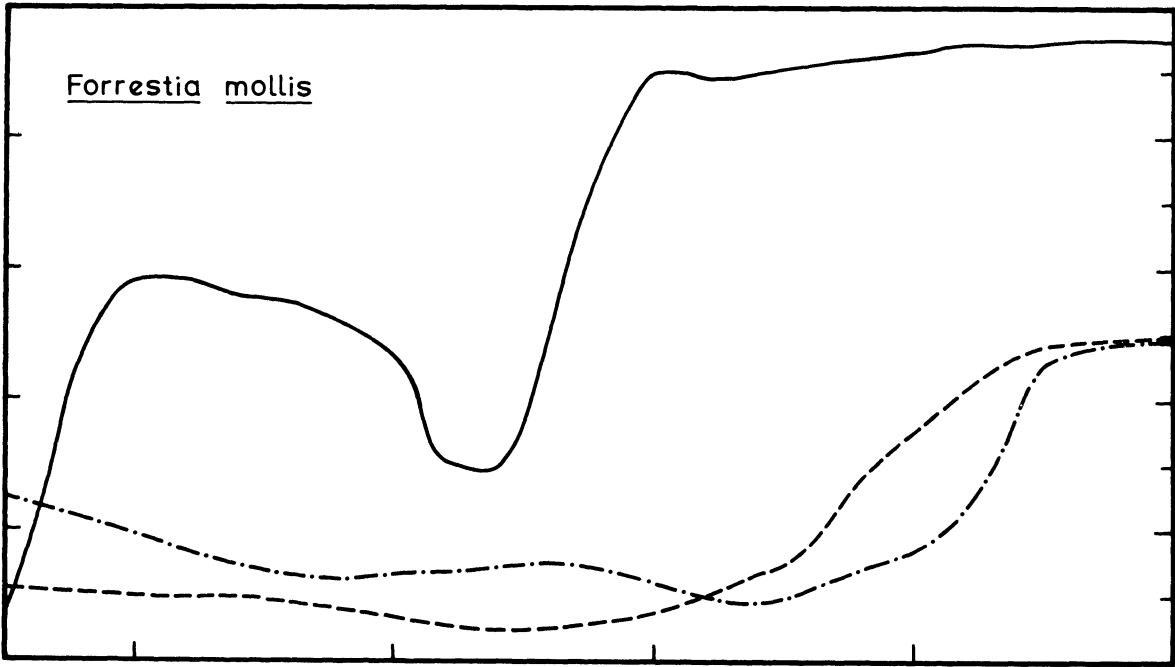


FIGURE 2. Leaf reflectance and pigment transmission as a function of light wavelength in four plant species (representative scans). Key: straight unbroken line=denotes light transmission through pigment extracts; dotted line=denotes reflectance of leaf upper surface; dash-dot-dash line=denotes reflectance of green leaf lower surface; and dashed line=denotes reflectance of red leaf lower surface. Note the increase in reflectance of red leaf underside for 600 to 750 nm in all species.



acteristics of anthocyanins different from those in extracts. Concentration, pH changes, metal ions, and modifying substances such as pectins can change the nature of the isolated pigments. In synthetic organic pigments (including those similar to anthocyanins in structure), enhanced transmission correlates with high back-scattering characteristics, depending on the physical state of the pigment (Patten 1973). In our four species the anthocyanins are concentrated in very dense layers which help account for the back-scattering effects.

Our present view of the energy relationships of leaves and the energy environment of the rain forest floor is relevant also to another observation. The lack of intercellular spaces between the epidermis and the palisade layer (as observed in our species) minimizes the back-scattering of light and ensures its more efficient absorption. This fact could explain why we find stomata always associated with intercellular spaces only on the undersurfaces. Understanding rain forest understory leaves as optical structures will help us appreciate the evolutionary significance of their anatomical features.

The polymorphism for color, here exploited experimentally, is seen in many other species (Lowry, 1974, Stone 1966). If the adaptive value of such polymorphism is real, as we suggest, one might expect more positive selection for the character. However, tropical rain forests are both complex and dynamic entities; primary rain forest is a mosaic of canopy phases (Whitmore 1975) and natural canopy gaps appear frequently. It is in the more brightly lit area below a canopy gap that net productivity of the forest floor plants will increase, provided they survive the increased temperature and lowered humidity effects; and it is reasonable to expect that they will then have greatly increased seed production, relative to the members of the same population which remain under deep shade. Thus it seems likely that within an area of forest, seed production will be taking place to a disproportionately large extent in small areas with temporarily increased light intensity; but in such areas, the anthocyanin undersurface layers are of less

effect and thus of less adaptive value. Thus we have the interesting situation where a characteristic has a definite strong adaptive value for the majority of the individuals of the population, yet selection for it is weak. For in the bright "gaps" the individuals with all-green leaves are, presumably, equally capable of seed production. The genetic elements which produce red leaf undersurface pigmentation in each new generation are therefore probably in a somewhat fluctuating equilibrium with the elements which produce all-green leaves. According to this hypothesis, color polymorphism is therefore adaptively valuable in itself, as are other instances of polymorphism.

It is important to bear in mind that there are species which have leaves with red undersurfaces in juvenile, but not in adult, plants. It is a fairly common phenomenon in juvenile stages of species in which the adults "emerge" out of the understory into higher strata (Stone 1966). This situation seems to be particularly true for plants which become tall shrubs, trees, or high-climbing lianas. Probably the red color again can be considered as an adaptation, but functional only during the period in which the juvenile 'occupies' a deep-shade habitat.

Finally we note that some species with red leaf undersurfaces are not confined to low-light habitats; for example, *Rhoeo spathacea*. These examples might be considered as cases in our second category (cf. our opening paragraph) of spotted, striped, or patterned leaves. However, in at least one conspicuous example, *Excoecaria bicolor* (Euphorbiaceae), the red pigment is not in fact anthocyanin, which may serve as a warning that mere visual observation does not suffice for identification of the adaptation. Because of the complexity of tropical rain forest, one tends to doubt automatically any too-facile explanation for a particular observation. Yet it is reasonable to assume that a modification found widely and almost uniquely in plants of a particular habitat, not linked to taxonomic affinity, has adaptive significance. We believe that our hypothesis on back-scattering of light by the cyanic layer is both ecologically sound and in accord with observation and experiment.

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