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Red coloration of tropical young leaves: a possible antifungal defence?

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ABSTRACT. Many woody species in humid tropical forests synchronously flush entire canopies of young red leaves. Numerous unsuccessful attempts have been made to explain the adaptive value of this visually striking phenomenon. In the humid tropics, fungal attack is a potentially important source of mortality for expanding young leaves. We propose that the anthocyanins responsible for the red coloration of young leaves may play a protective role against invasions by leaf-attacking fungal pathogens.

Fungus-growing leaf cutting ants (*Atta columbica* Guerin) were used in choice tests because they are known to select against leaves or chemicals containing fungicidal properties. In feeding trials with leaf discs from 20 common species, ant preference decreased significantly with increasing anthocyanin content. In feeding trials with pure anthocyanin (3,3′,4′,5,7-pentahydroxyflavylium chloride) presented on oat flakes, ants again showed a significant dosage dependent preference. This suggests that even low concentrations of anthocyanins may be harmful to the fungal colonies of ants. Additional work on the effects of anthocyanin on leaf-attacking fungi is encouraged.

KEY WORDS: anthocyanin, antifungal defence, *Atta columbica*, herbivory, leaf-cutting ants, Panama, tropics, young leaves.

INTRODUCTION

In the humid tropics, many tree species synchronously flush whole canopies of brightly coloured young red leaves (Burgess 1969, Richards 1952). This developmental pattern is so widespread and visually striking that for over 100 years, scientists have investigated the adaptive significance of the red coloration. The phenomenon is extremely common, with 20% to 40% of the woody species in a single site exhibiting some red pigmentation (Opler *et al.* 1980). Red coloration has apparently arisen many times independently, as it is present in a variety of unrelated families.

The red coloration is due to anthocyanin pigments which, depending on pH, methylation, and hydroxyl groups, cause leaves to be various shades of red, blue, or purple (Harborne 1967). Anthocyanins frequently are present in mesophyll and epidermal cells during leaf expansion (McClure 1975) and disappear as the leaf becomes full size and begins to toughen (Harborne 1979a). Surveys have shown that 80% to 93% of the anthocyanins in young leaves are cyanidin-3-glucoside (Harborne 1979a, Price & Sturgess 1938, Swain 1965). In the leaf, anthocyanins occur as glycosides and are stable and soluble in sap. Upon acid hydrolysis or leaf damage, the aglycocidic anthocyanidins are produced.

Hypotheses for adaptive value of anthocyanins

Various hypotheses have been presented suggesting an adaptive function of anthocyanins in developing leaves. Red coloration was first thought to raise leaf temperature, thereby hastening development through increased transpiration and metabolism (Smith 1909). This hypothesis has not been supported by more recent studies (Lee *et al.* 1987).

Another hypothesis first developed for arctic or alpine plants, suggests that anthocyanins function as a screen against harmful UV-B radiation (Caldwell 1981, Caldwell *et al.* 1980, Lee & Lowry 1980). However, measurements on tropical species by Lee and co-workers (Lee *et al.* 1987) suggest that anthocyanins are only a small percentage of the UV screening compounds present in leaves, and probably are not as effective as other phenolic compounds. Furthermore, the majority of species with young leaf anthocyanins do not receive high levels of radiation since the young leaves hang vertically and grow in the shaded understorey with light levels below 1% of full sun (Chazdon & Fetcher 1984).

It has been suggested that anthocyanins function as a defence against herbivores, either through direct toxicity or through warning or cryptic colouration (M. Rothschild pers. comm., Stone 1979). None of these proposals has been supported (Lee *et al.* 1987). Young red leaves show extremely high rates of herbivore damage relative to mature leaves (Coley 1981, 1983), and no difference relative to non-red young leaves (Aide unpublished). Furthermore, no evidence exists for toxic or deterrent properties of anthocyanins against insects or mammals (Harborne 1979b, Lee *et al.* 1987, McClure 1975).

In this paper, we suggest that anthocyanins may protect young leaves against fungal pathogens during the vulnerable period of expansion before the leaves are protected by fully developed cuticles and lignified cell walls. A direct test of the protective role of anthocyanins would be to demonstrate antifungal activity against major pathogens of young tropical leaves. However, the extensive preliminary work of isolating, identifying and culturing fungal pathogens has not been done for wild plants of the humid tropics. We therefore performed choice tests with leaf-cutting ants (*Atta columbica* Guerin) as a preliminary indicator of possible anti-fungal properties of anthocyanins.

Several species of leaf-cutting ants cut leaf pieces on which they cultivate fungus in underground gardens (Cherrett 1968). The fungus is the sole food source for larvae and composes much of the diet for the workers and queen (Kermarrec *et al.* 1986, Quinlan & Cherrett 1979). Because of this dependence on the fungus, the ants have apparently been selected to avoid cutting leaves with anti-fungal compounds (Howard 1987, Howard & Wiemer 1986, Hubbell & Wiemer 1983, Hubbell *et al.* 1983, 1984).

METHODS

Study site

The work was carried out at the Smithsonian Tropical Research Station on Barro Colorado Island in Panama (9° N, 80° W). The island is classified as tropical moist forest in the Holdridge Life Zone System (Holdridge *et al.* 1971, see also Croat 1978, Leigh *et al.* 1982). All feeding trials with *A. columbica* were done on natural foraging trails radiating from a large nest located near the intersection of Fairchild and Wheeler trails.

Anthocyanin extractions

To quantify the range of anthocyanin concentrations occurring naturally in young leaves, we collected 20 common species (five plants per species) which were flushing young leaves in December 1986. These included species whose leaves appeared almost white, to those whose leaves were a dark red.

Freshly collected young leaves (30–50% of full size) were ground in a mortar and pestle with 80% methanol containing 1% HCl. Extracts were centrifuged to eliminate turbidity, and the volume recorded. Absorbances were measured at 535 nm and compared against absorbances for pure cyanidin (3,3′,4′,5,7-pentahydroxyflavylium chloride; Atomergic Chemicals Corp.). Cyanidin was chosen as a standard since surveys of young leaves have found that 80–90% of the anthocyanins are cyanidin (Harborne 1979a, Price & Sturgess 1938, Swain 1965). All 20 study species showed a peak at 535 nm which is characteristic of cyanidin. Anthocyanin concentrations were calculated per fresh weight, dry weight, and area.

Feeding trials with Atta columbica

Leaf discs. To determine if anthocyanin content affected ant preference, feeding trials were done using punches from fresh young leaves. Leaf discs ($0.32\ cm^2$) were placed in single species piles on the foraging trail. The number of discs removed during 5 minutes was counted. Two trials were conducted on two different days in December 1986.

Oat flakes. We also tested ant responses to the pure leaf anthocyanin, cyanidin (3,3′,4′,5,7-pentahydroxyflavylium chloride), delivered on oat flakes (Quaker). Solutions with different concentrations of cyanidin chloride were prepared in 95% ethanol, and 0.005 ml of solution delivered to each oat flake. Flakes of uniform size (0.0075 g) were chosen so anthocyanin concentrations could be calculated. Ants were presented with a choice between three oat flakes at each station, a control flake (ethanol only) and two flakes containing different anthocyanin concentrations. Removal rates of flakes were monitored for 5 minute intervals. When a flake was removed, it was replaced with a new one. Feeding trials were conducted on two different days. In one trial, we used anthocyanin concentrations of 8.0 and 16.0 $mg\ g^{-1}$ fresh weight (FW). These are equivalent to the intermediate and highest concentrations seen in young leaves. In the other trial, anthocyanin concentrations were 1.7 and 3.5 mg g^{-1} FW. These concentrations give only a faintly pink colour in young leaves.

RESULTS

There was a 10-fold range in concentration among the species surveyed (Table 1). The young leaves of *Cnestidium rufescens* (Connaraceae) are dark red and the concentration of anthocyanins was 7.4% dry weight. The concentrations of anthocyanins in the white young leaves of *Rourea glabra,* also in the Connaraceae, was only 2.0%.

The feeding trials using fresh leaf discs showed a significant negative relationship between the number of discs removed and the concentration of anthocyanin (Figure 1) ($r = -0.48$, $P = 0.03$). Anthocyanin is clearly not the only leaf characteristic influencing ant choice. Many other leaf components may also be involved, such as toughness, water content, nitrogen, and other secondary chemicals (Hubbell *et al.* 1983, 1984). For example, *Talisia* has low anthocyanins and a low removal rate, therefore it must be rejected for other reasons (Figure 1). However, it is interesting that no species with high anthocyanin content was taken. This suggests that although anthocyanin may not be the only compound affecting leaf quality, it is sufficient to deter *A. columbica.*

Since other leaf characteristics besides anthocyanin concentration vary among species and could potentially influence ant choice, we tested ant preference to pure leaf anthocyanin delivered on oat flakes. In both feeding trials, ants preferred the control flakes with solvent only, and distinguished between

Table 1. Anthocyanin concentrations in young leaves of 20 common species on Barro Colorado Island, Panama. Nomenclature follows Croat, 1978.

Species	Leaf colour	Anthocyanins (mg)		
		g^{-1} FW	g^{-1} DW	dm^{-2}
Cnestidium rufescens Planch. (Connaraceae)	dark red	16.93	73.7	19.56
Ormosia panamensis Benth. ex Seem. (Papilionoideae)	dark purple	12.27	48.6	20.56
Oenocarpus panamanus Bailey (Palmae)	dark red	10.23	26.7	10.70
Clitoria javitensis H.B.K. (Papilionoideae)	dark purple	9.94	41.5	11.75
Inga umbellifera (Vahl) Steud. (Mimosoideae)	medium red	8.96	35.9	13.51
Psidium anglohondurense (Lund.) McVaugh (Myrtaceae)	dark red	7.89	38.2	11.90
Hirea reclinata Jacq. (Malpighiaceae)	medium pink	7.52	34.8	9.13
Protium panamense (Rose) I. M. Johnston (Burseraceae)	light red	6.79	30.4	7.54
Tetragastris panamensis (Engler) O. Kuntze (Burseraceae)	light red	6.75	22.9	6.42
Faramea occidentalis (L.) A. Rich. (Rubiaceae)	light purple	6.21	42.5	7.74
Cupania sylvatica Seem. (Sapindaceae)	medium pink	5.61	32.6	4.92
Guarea sp. nov. 'hairy' (Meliaceae)	medium pink	4.22	23.7	5.98
Licania platypus (Hemsl.) Fritsch (Chrysobalanaceae)	medium pink	4.02	15.1	6.82
Connarus panamensis Griseb. (Connaraceae)	light pink	3.31	13.2	5.28
Trichilia tuberculata (Tr. & Pl.) C. DC. (Meliaceae)	light red	3.04	10.8	5.06
Rourea glabra H.B.K. (Connaraceae)	white	2.65	20.2	2.51
Gustavia superba (H.B.K.) Berg (Lecythidaceae)	white	2.64	20.4	3.98
Rheedia edulis (Seem.) Planch & Tr. (Guttiferae)	light red	2.59	17.8	3.73
Talisia princeps Oliv. (Sapindaceae)	white	2.05	11.3	1.71
Ouratea lucens (H.B.K.) Engler in Mart. (Ochnaceae)	white	1.82	13.3	2.57

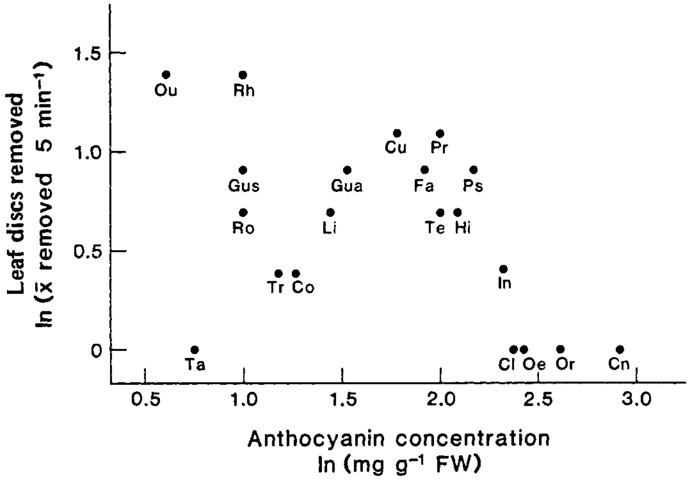

Figure 1. Feeding trial with *Atta columbica* using leaf discs from young leaves of 20 species. The data were log transformed and the 'leaf discs removed' values are the mean of the two trial days. The species are listed in Table 1 and identified here by the first letters of the generic name.

Table 2. Anthocyanin feeding trial with *Atta columbica*. A single classification ANOVA for each trial date shows significant differences between treatments ($F = 19.0$, $P \leqslant 0.001$ for 3 December; $F = 22.9$, $P \leqslant 0.001$ for 7 December). Within each trial date, treatments followed by different letters are significantly different (Student-Newman-Keuls test). Controls are different from each anthocyanin concentration with a significance level of $P < 0.01$. Anthocyanin concentrations are significantly different from each other at $P < 0.05$.

Trial date	Trial length (min)	Removal rate oats min^{-1}	Total oats removed	Mean number of oats removed per 5 min				
					Anthocyanin contents mg g^{-1} FW			
				Control	1.7	3.5	8.0	16.0
12/3	347	1.5	347	3.57a	2.30b	1.67c	–	–
12/7	290	3.2	290	7.28a	–	–	4.94b	3.89c

the two anthocyanin concentrations (Table 2). Ants were even sensitive to the lowest concentration tested (1.7 mg g^{-1} FW) which was lower than any measured in leaves (Table 1).

Although ants distinguished between different anthocyanin concentrations presented in a single trial date, this is not true if one compares between days. This may be because overall removal rates of flakes were two times greater on the second trial as compared to the first (Table 2). The second trial followed a day of heavy rain, perhaps causing the ants to be more active, and less selective.

DISCUSSION

These two experiments provide preliminary evidence suggesting that anthocyanins may play an antifungal role for developing leaves, and that this protection may be the reason why young leaves are red. Although the toxicity of anthocyanins has not been well studied, there is some evidence that they can have antibiotic properties (Powers 1964) and are synthesized in response to fungal attack (Cruickshank & Perrin 1964, Harborne 1965, 1979b). Few measurements have been made on rates of fungal attack in nature, but preliminary data suggest that pathogen attack of young leaves is common and in some species can cause orders of magnitude more damage than insects (Arhenius & Langenheim 1983, 1986, Langenheim 1984, Langenheim *et al.* 1978, P. Coley, T. Kursar & L. Gutierrez in prep., R. Dirzo pers. comm.). Protection from fungi would be particularly important in environments like the humid tropics where conditions are exceptionally conducive to fungal growth.

Although leaf-cutter ants can be serious pests of agricultural plants, they are not as significant a source of leaf damage within mature forests (Coley 1981, 1982). It is therefore unlikely that mature forest species have evolved compounds toxic, primarily, to ants. Instead, resistance to leaf-cutting ants may be a by-product of selection for resistance to fungi (Hubbell *et al.* 1984).

Before our hypothesis can be thoroughly tested, extensive data on naturally occurring fungal pathogens must be gathered. Firstly, rates of fungal damage to young tropical leaves must be quantified in order to determine the magnitude relative to other sources of leaf damage such as herbivory. These data would indicate the potential selective strength of fungal pathogens in shaping leaf defensive chemistry. Secondly, fungi must be isolated from young leaves of a range of wild host plants. And thirdly, the toxicity of anthocyanin must be tested directly on these species of leaf-attacking fungi. A powerful first step in testing the latter two points would be to quantify effects of anthocyanins on fungi known to attack leaves of tropical fruit or timber trees.

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Book review

ALVARES, C. & BILLOREY, R. *Damming the Narmada. India's greatest planned environmental disaster.* 1988 Third World Network/APPN, Malaysia. ISBN 967-99908-8-5. 196 pages, Price: US$6.00 (Third World countries), US$9.00 (elsewhere). The book can be obtained from: Consumers' Association of Penang, 87 Cantonment Road, 10250 Penang, Malaysia. Postage per copy: Malaysia, Singapore and Brunei, M$0.50, elsewhere US$1.00 (surface mail), US$3.00 (air mail). Payment should be made in favour of: Consumers' Association of Penang.

This book has been published jointly by the Third World Network and the Asia-Pacific People's Environment Network and is primarily targetted at those interested in Environmental and Third World politics. It is very much a political book aimed at increasing awareness of (and opposition to) the Narmada Valley Project whereby two large dams and a series of smaller ones will be built across the Narmada River in the Indian States of Gujarat and Madhya Pradesh. The authors of the book believe it will lead to the world's largest ever 'planned' environmental and human tragedy. To convince the reader of this the authors have produced an extremely well written and thought provoking book.

The book is split into three sections. The first section is a summary of the authors' objections to the project, detailing the effects there will be on the environment and the people in the area and pointing out the lack of economic and social benefit that will actually result. The second section consists of interviews with the Narmada Project's top officials and the third section is a whole series of Appendices full of facts, assessments and statistics.

I recommend this book to anyone interested in environmental politics; it is one of the best researched book on such issues I have read.

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