THE ECOLOGY OF AN ELFIN FOREST IN PUERTO RICO, 9. CHEMICAL STUDIES OF COLORED LEAVES

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ONE OF THE SPECTACULAR FEATURES of tropical vegetation is the frequent occurrence of brightly colored leaves. The color may be found in young foliage, produced either regularly or seasonally, or in the mature foliage of certain taxa as common in cultivation as *Codiaeum variegatum* var. *pictum* or *Acalypha wilkesiana*. Color development in the fall or with the change from dry to rainy season is less frequent. The red colors are assumed to be due to the dominance of anthocyanin pigments and may appear in young leaves before the chlorophyll develops, in mature leaves in segments of the blade or partially masked by chlorophyll, and in senescent leaves after the chlorophyll has been destroyed. Red color may also be a pathological symptom frequently associated with phosphorous deficiency. Macmillan (1952) stated "anthocyanin may appear temporarily in the young leaves, and, if abundant before the chlorophyll is largely developed, a bright red immature foliage results. This is very evident in many tropical trees, e.g. *Mesua ferrea*, a species of *Calophyllum*, *Eugenia*, *Cinnamomum* etc. The coloration is at times so vivid that from a distance such trees appear to be in flower." Anthocyanins are usually red in acid solution and may become purplish to blue as the pH is increased. A previous paper (Howard, 1969) has shown that the pH of the plant sap of the component species of the elfin forest on Pico del Oeste ranges from 2.5 to 6.5. Anthocyanins are often associated as well with the occurrence of sugars in the plant cells and, in temperate areas, with the occurrence of frost or low temperatures. Crocker (1938) stated "anthocyanins appear in many plant cells mainly in the early spring and in autumn at times of low temperatures; under these conditions soluble sugars are also abundant in plant organs. Arthur finds that low temperature favors the development of anthocyanin in the apple without a change in sugar content. He also points out that the small amount of pigment found in cells calls for relatively little sugar as a building material and concludes that temperature probably acts directly rather than through sugar accumulation." More recent work has shown an association of color due to anthocyanin with a shortened photoperiod and suggests that it may be regulated by a phytochrome system. Finally it is evident to anyone familiar with a fall season in New England that potential for the development of color is also inherent in certain plants.

The elfin forest on Pico del Oeste displays a localized brilliance in the vivid colors of young leaves and in the flush growth of many of the woody components. The development of color with age was noted only in *Miconia pachyphylla*, *Mecranium amygdalinum*, and *Calycogonium squamulosum*. Perhaps the greatest year-round color, however, is found in many plants of the bromeliad genus *Vriesea*.

We were not able to attempt tests of the ratio of red to far-red light as a factor in the leaf color we observed in the various plants of the cloud-dominated environment. We were able, however, to establish a small laboratory through the courtesy of Mr. Joseph Martinson, in which we attempted simple tests to examine the chemical bases previously proposed for the color we observed. Tests were run to determine the sugar content of young and old leaves, and of the red- and green-leaved forms of Vrieseas, and that of the water soluble phosphorus.

The epiphyte *Vriesea sintenisii* occurs throughout the forest: on the branches of isolated trees, on upper branches of trees forming the canopy, with many young plants on the horizontal branches or, occasionally, on the ground in cut-over areas. The plants that are exposed to the sky exhibit a brilliant red color. Within the forest, on shaded branches, and frequently on the ground other plants of the same species lack the red color and are pale green in appearance. Although the color difference is intense, the red plants may have the leaf bases green within the rosette but very few plants could be truly called intermediate, that is, partly green and partly red. The principal variation is in the intensity of red. The color difference is also apparent at a very young age. Seedling plants in exposed areas, with developing leaves, have red color at the tips while the protected or shaded seedlings are all green. The intensity of color does not appear to vary throughout the year or to suggest a photoperiod variation. However, the maximum variation in daylength during the year in Puerto Rico is only 2 hours and 18 minutes.

Gleason and Cook (1927) do not mention *Vriesea sintenisii* in their description of elfin forest types, and previous workers on the family or on the flora of Puerto Rico have not described the color variations or suggested any taxonomic value for them. Our initial encounter with these two color forms of *Vriesea sintenisii* suggested that two ecotypes were present.

The brilliance of the red form of *Vriesea sintenisii* suggested that the plants might make attractive ornamentals. However, when bright red plants were taken to a lower elevation within the Luquillo Mountains and placed in an area with less cloud cover, the plants died even when supplied with water daily. Red color forms which were returned to Boston and kept in the greenhouse under high humidity, without any adjustment of the natural photoperiod of the Boston area, retained their red color but failed to flourish, remaining in a vegetative state long after plants of comparable size on Pico del Oeste had flowered, shed seeds, and reproduced vegetatively. Red-colored plants taken from the exposed tree tops and placed on the forest floor in the shade gradually lost their red color and by the time of flowering were almost completely green.

The green-colored plants were also subjected to transplant experiments. These, too, failed to survive when transferred to a lower location, while those transported to the greenhouse in Boston grew well in the new location and flowered on schedule when compared with plants on Pico del Oeste. Green-colored plants within the elfin forest were also transferred from their protected positions to exposed positions by strapping branches holding these plants to upper branches of the forest canopy. These green plants died in the exposed positions. Unfortunately we were unable to attempt a gradual transfer of these plants from one position to another. Clearly the green plants were physiologically adapted to shaded location and could not survive an abrupt although seemingly slight change in exposure to greater light. The red plants became adapted to the shaded location with the apparent loss or masking of the red pigment.

A second bromeliad found in the elfin forest was *Guzmania berteroniana*. Plants of *Guzmania* occurred primarily on the trunks of *Prestoea montana* or on the ground in protected areas on the lee slopes of the trail. All plants of *Guzmania* were green and no red forms were seen. Transplant experiments produced results nearly comparable to those for green forms of *Vriesea*. Plants taken to lower elevations died; those taken to Boston have persisted, but the rate of maturation was slower; plants which were transferred to exposed locations in the canopy died even more quickly than did the green forms of *Vriesea*.

When initial sugar tests suggested a higher sugar level in red-leaved plants of *Vriesea*, we continued a comparative study through two years, making analyses of plants in various stages of development.

In each test an average of eight plants was collected in the Pico del Oeste forest and taken to the laboratory. The leaves were all separated and washed thoroughly in running water and hand dried with towels. Roots and rhizomes were discarded. After the fresh weight of the leaves was obtained for each plant they were oven dried at 70°–75° for two days. When the dry weight was obtained the leaves were finely cut, and after a thorough mixing of the fragments of the individual plants, 1 gm. of dry leaves was placed in an Erlenmeyer flask; 100 cc. of distilled water was added; and the mixture was simmered for 30 minutes. When cooled to air temperature, distilled water was added to regain the original volume of 100 cc. The solution was allowed to mix for about two hours, then the sugar content was estimated quantitatively, following the colorimetrical method of Folin and Wu (1920). In a second Folin-Wu tube 0.1 cc. of 1:10 diluted HCl was added and the tube submerged for 5 minutes in a boiling water bath to hydrolize the higher sugars to a hexose before a second colorimetric sugar determination was made. The results are expressed in the following tables as percentages of sugar per gram of dry weight of the plant, and are the average of the eight plants of each sample category. There was no significant variation between the eight samples.

Soluble phosphorus determinations were made from 2 grams of the dried material which was diluted with 100 cc. of distilled water and simmered at 90°C. for 30 minutes; this was cooled to air temperature and distilled water was added to regain the original weight. The soluble phosphorus was determined according to the procedure of Benedict and Theis (1924): 10 cc. of the extract and 10 cc. of a standard were mixed with 1 cc. of a 5% hypochlorate solution to decolorize the brownish mixture; after 24 hours the solution was filtered and 5 cc. of the standard and the unknown were measured in test tubes; the results are expressed as mg.% of dried plant material.

1966 Red-colored Vriesea

Column no.	1	2	3	4	5	Mean
Water	83%	83%	83%	81%	80%	82%
Hexose	1.92%	1.88%	1.88%	1.44%	1.51%	1.73%
Total sugar	2.27%	1.96%	2.05%	1.72%	1.96%	1.99%

1966 Green-colored Vriesea

Water	86%	85%	86%	85%	82%	84.8%
Hexose	1.27%	1.32%	1.29%	1.09%	1.36%	1.26%
Total sugar	1.56%	1.82%	2.36%	1.15%	2.06%	1.79%

Columns: 1. Young plants in vegetative rosettes. 2. Inflorescence present, basal flowers open. 3. Inflorescence mature, basal flower in young fruit stage. 4. Fruit forming, seeds turning black. 5. Fruiting stage, seeds mature.

1967 Red-colored Vriesea

Column no.	1	2	3	Mean
Water	80%	85%	80%	82%
Hexose	1.5%	1.9%	1.8%	1.7%
Total sugar	1.8%	2.1%	2.0%	2.0%
Soluble phosphorus	23mg%	30mg%	21mg%	24mg%

1967 Green-colored Vriesea

Water	83%	86%	83%	84%
Hexose	1.2%	1.5%	1.4%	1.4%
Total sugar	1.4%	1.7%	1.6%	1.6%
Soluble phosphorus	26mg%	24mg%	25mg%	25mg%

Columns: 1. Young plants. 2. Inflorescence mature. 3. Fruit mature.

Guzmania berteroniana

Column no.	1	2	3	4	Mean
Water	86%	85%	87%	88%	86%
Hexose	1.0%	1.1%	2.0%	1.2%	1.3%
Total sugar	1.3%	1.3%	2.2%	1.2%	1.5%
Soluble phosphorus	24mg%	25mg%	25mg%	n.e.	25mg%

Columns: 1. Young vegetative rosettes. 2. Mature vegetative plants. 3. Inflorescence mature. 4. Fruits mature.

The water content of the leaf tissue of the components of the elfin forest has been given in a previous paper (Howard, 1969). Tissues tested contained from 93 percent water to 44 percent water. *Vriesea* ranked in the lower portion of the upper third of all the plants tested in water content. All the plants tested which revealed a higher water content were understory plants. The red color-form of *Vriesea*, being primarily a canopy epiphyte had a slightly lower water content than did the green-colored *Vriesea* which occurred in protected locations. The water content of the plant tended to diminish as the plant matured and was lowest when the plant was in fruiting condition. The total sugar in the green-colored form increased to the flowering period, decreased during the development of the fruit, but increased as the fruit matured.

Hexose and total sugar content proved to average higher in red-colored *Vriesea* and the total sugar content was approximately 40 percent higher in the red than in the green forms during the growing season.

In *Guzmania* the water content averaged higher than in *Vriesea*, increasing slightly in the life cycle to the fruiting stage. Hexose and total sugars increased in *Guzmania* during the development of the inflorescence and was 100 percent higher in the flowering cycle, exceeding the red form of *Vriesea*.

The amount of soluble phosphorus averaged about the same in the red and green Vrieseas. However, it seemed to increase in the red *Vriesea* during the flowering period and decrease in the green forms during the same period.

The amount of soluble phosphorus is evidently not a limiting factor in the coloration found in the red form of *Vriesea sintenisii*.

In *Guzmania*, soluble phosphorus levels remain fairly constant through the life cycle and were approximately the same as those of *Vriesea*. Since both bromeliads would receive inorganic materials as wind-blown debris collected in the leaf bases directly, it appears the supply or utilization of phosphorus is equally available to the plants no matter what their position in the forest structure.

The distinctive coloration of young developing leaves in tropical forests is mentioned in many previous studies. An earlier study (Howard, 1969) indicated red, orange, pink, yellow, and bronze tones in the young leaves of specific plants within the elfin forest of Pico del Oeste. Many taxa which did not produce colored leaves when young did show a lighter green or a yellow-green color to the young foliage. Tests for hexose sugars and for total sugars were made on selected leaves from individual taxa. One to four brightly colored leaves were taken, as available, from a flush of growth and tests on these were compared with material from older leaves of the same branch. The following results were obtained:

TAXON	Color of young leaf	Percent water		Percent hexose sugar		Percent all sugars	
		yg lf	old lf	yg lf	old lf	yg lf	old lf
Calycogonium squamulosum	pale red	75	74	4.3	2.6	—	—
Calyptranthes krugii	yellow-green	61	48	5.7	4.45	7.85	4.40

Cleyera albopunctata	yellow	70	57	8.35	8.75	8.35	8.90
Eugenia borinquensis	deep red	70	55	5.62	5.80	5.70	8.05
Gonocalyx portoricensis	pink	89	75	2.8	2.3	—	—
Hillia parasitica	light green	83	81	2.86	4.45	3.57	5.00
Hornemannia racemosa	orange-pink	67	55	4.96	8.50	6.08	8.88
Ilex sintenisii	yellow-green	70	63	7.05	7.30	9.55	8.00
Mecranium amygdalinum	yellow-green	69	70	coagulates copper reagent			
Marcgravia sintenisii							
juvenile leaves	red	82	78	7.6	5.7	—	—
		86	81	7.20	8.84	7.90	8.86
adult leaves	red	84	69	3.4	3.8	—	—
		89	69	7.10	10.00	7.35	10.10
Miconia pachyphylla	red-purple	62	56	coagulates copper reagent			
Miconia foveolata	red-pink	71	68	7.31	5.73	7.82	7.55
Miconia pycnoneura	red-purple	60	53	coagulates copper-reagent			
Ocotea spathulata	bronze-red	62	68	7.7	6.7	—	—
Tabebuia rigida	red-purple	83	70	6.4	3.7	—	—
Torralbasia cuneifolia	yellow-green	71	63	8.80	9.55	8.84	10.89
Wallenia yunquensis	wine-red	75	63	9.80	7.85	10.00	8.90

With the exception of one plant of *Marcgravia sintenisii* and those of *Eugenia* and *Hornemania*, all taxa tested which had red or orange, brightly colored leaves had a higher hexose sugar content in juvenile foliage than in mature foliage of the same plant. In the taxa which produced yellow or pale green juvenile foliage, the hexose sugar content was lower in juvenile leaves than in mature leaves, with the exception of *Calyptranthes* *krugii*. For total sugars in yellow-green juvenile leaves, the same exception occurs, with the addition of *Ilex sintenisii*.

It is of interest to record that the plant extracts obtained during the month of April from four taxa of the Melastomataceae all contain some chemical substances which form a gelatinous coagulate in the Folin and Wu copper reagent. One sample of *Calycogonium squamulosum*, of the same family, formed the coagulate in March but a second test in April from the same plant did not. A similar reaction was experienced for *Miconia foveolata*.

The contrast between the higher hexose and total sugar content in the brightly colored juvenile leaves and that of the mature green foliage in the woody plants is similar to that condition found in *Vriesea sintenisii* when the anthocyanin-dominant plants were compared with the green color-form.

VARIATIONS IN pH OF CELL SAP

The pH of liquid expressed from leaf tissues was mentioned in relation to vulnerability to insect damage in an earlier paper in this series (Howard, 1969). Where possible liquid was obtained from fully expanded leaves and leaves normally green but which had not completely hardened. The leaves were pressed between clean microscope slides, a drop or two of expressed fluid collected, and the pH checked with a Beckman pH meter. The amount of liquid and the ease with which it could be obtained varied considerably between the plants of the elfin forest of Pico del Oeste, and also varied with the time of year for individual plants. The liquids varied in their color and consistency, and in the rate of color changes upon exposure to air. The results of this survey are presented in the following table:

Variations in the liquid extract of crushed leaves and in the pH of plant sap

	Color or consistency of sap	Feb.	July	Nov.
CYATHEACEAE				
Cyathea pubescens	clear	—	4.6	—
PALMAE				
Prestoea montana	yellow-green	5.1	6.2	5.4
ARACAE				
Anthurium dominicense	clear	5.0	5.5	5.8
BROMELIACEAE				
Guzmania berteroniana	rusty	—	4.0	—
Vriesea sintenisii	clear	—	5.1	—
DIOSCOREACEAE				
Rajania cordata	—	—	—	4.9

ZINGIBERACEAE				
Renealmia antillarum	—	5.2	5.3	4.8
PIPERACEAE				
Peperomia emarginella	—	5.0	5.0	5.5
Peperomia hernandiifolia	—	5.0	4.6	5.1
CHLORANTHACEAE				
Hedyosmum arborescens	orange-brown	4.5	5.3	5.6
MORACEAE				
Cecropia peltata	—	5.1	5.1	5.1
URTICACEAE				
Pilea krugii	lilac	5.3	5.1	6.0
Pilea yunquensis	—	5.5	5.9	6.4
LAURACEAE				
Ocotea spathulata	gelatinous	5.1	4.9	4.9
MELIACEAE				
Trichilia pallida	dirty green	5.6	5.2	5.4
AQUIFOLIACEAE				
Ilex sintenisii	—	5.3	5.2	5.1
CELASTRACEAE				
Torralbasia cuneifolia	—	5.0	4.8	4.7
OCHNACEAE				
Sauvagesia erecta	yellow-green	4.9	4.9	5.6
MARCGRAVIACEAE				
Marcgravia sintenisii	—	4.6	5.4	4.8
THEACEAE				
Cleyera albopunctata	—	3.8	4.1	4.2
GUTTIFERAE				
Clusia grisebachiana	yellow-orange	3.9	3.9	4.3
BEGONIACEAE				
Begonia decandra	cherry-red	2.5	2.5	2.9
MYRTACEAE				
Calyptranthes krugii	—	—	4.0	5.1
Eugenia borinquensis	dirty brown	4.8	4.8	4.8
MELASTOMATACEAE				
Calycogonium squamulosum	lavender	3.4	3.3	3.7
Mecranium amygdalinum	rose-purple	3.2	3.8	3.7
Miconia foveolata	red-purple	3.9	3.9	4.0
Miconia pachyphylla	rose	3.7	4.0	4.3
Miconia pycnoneura	rose	3.3	3.4	3.8
ERICACEAE				
Gonocalyx portoricensis	—	3.3	3.3	3.5
Hornemannia racemosa	—	3.9	4.2	5.3

MYRSINACEAE				
Ardisia luquillensis	dirty lavender	4.4	4.4	4.4
Grammadenia sintenisii[1]	—	4.1	4.5	4.1
Wallenia yunquensis	—	4.2	4.1	3.5
SAPOTACEAE				
Micropholis garciniaefolia	straw color	4.2	4.1	4.8
SYMPLOCACEAE				
Symplocos micrantha	blue-purple	4.0	4.2	4.8
OLEACEAE				
Haenianthus salicifolius var. *obovatus*	—	5.1	5.2	5.1
CONVOLVULACEAE				
Ipomoea repanda	—	4.5	6.2	5.9
BIGNONIACEAE				
Tabebuia rigida	—	5.0	5.5	5.3
GESNERIACEAE				
Alloplectus ambiguus	—	5.1	4.6	5.5
Gesneria sintenisii	rust-brown	5.3	5.7	5.6
ACANTHACEAE				
Justicia martinsoniana	slimy	5.5	4.1	6.5
RUBIACEAE				
Hillia parasitica	lime-green	4.9	4.7	4.9
Psychotria berteriana	—	5.0	5.7	5.7
Psychotria guadalupensis	brick-red	4.9	5.0	4.9
CAMPANULACEAE				
Lobelia portoricensis	lime-green	5.0	4.8	4.7
COMPOSITAE				
Mikania pachyphylla	—	5.1	5.9	5.8

The leaves from which the liquid was expressed to obtain the records in the preceding table were a normal green color. Where the color is not indicated the liquid was pale green. Accessory pigments, however, were present in several plants and were usually seen in the petiole; yet there was no consistent correlation with the color of the plant liquid obtained. The petioles and lower leaf surface of *Peperomia hernandiifolia, Wallenia yunquensis*, and *Tabebuia rigida* were red-purple in appearance. *Miconia foveolata* had red pubescence and *Miconia pachyphylla* appeared to have an underlying tone of red-purple to the leaf blade. The veins of *Begonia decandra* appeared bright red in fresh condition, and the cherry red color of the expressed liquid suggests this pigmentation was released in crushing. *Psychotria guadalupensis* exhibited a red pig-

[1] In a recent paper Lundell has established a new genus based on this taxon and has made the combination *Cybianthopsis sintenisii* (Urb.) Lundell, Wrightia 4: 68. 1968.

ment in the petioles and inflorescence axis but not in the leaf blade. The brick red color of the liquid extracted was darker than the pigmentation observed in the whole plant.

It was not possible to extract liquid by the hand pressure method used, from any member of the Gramineae, Cyperaceae, or Orchidaceae.

The pH of the extracted liquid ranged from 2.5 in *Begonia decandra* to 6.5 in *Justicia martinsoniana*. In three taxa tested in February, July, and November, the pH was identical. For 12 taxa two identical readings were obtained in the three unit test. The pH was highest in November for 20 taxa, with high readings in July in 10 taxa, and in February for 6. In 16 taxa the lowest pH reading was obtained in February, in 9 taxa during July, and for 4 taxa in November. In all cases except the herbaceous species the material examined was taken from a single marked plant for the tests. In a general observation the greatest number of plants would be in flower in July, in fruit in November, and in dormant condition during February.

The average pH of the expressed fluid of 11 taxa, in which the juvenile leaves are predominantly red, is 4.6 in a range of 3.4 to 5.2. The comparable pH of 6 taxa, in which the young leaves are yellow or yellow-green, is 4.7 with a range of 3.5 to 5.2. The average pH of the remaining 23 taxa of dicotyledonous plants tested, in which the young leaves are not noticeably different in color from the mature foliage, is 4.9 in a range of 2.7 to 6.0. The color of the young leaves and the associated pH appears to be characteristic of the individual species, with no direct correlation evident between the color of the leaf or of the expressed pigment, the pH, or the sugar content.

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