An Investigation of Hematochrome Accumulation in the Alga *Phycopeltis hawaiiensis* n. sp.[1]

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The production and accumulation of hematochrome, an orange-red pigment commonly found in the cells of Trentepohliaceae, are thought to depend upon certain external environmental conditions, as well as upon the physiological activity of the cells. According to Senn (1911) and Geitler (1923), the accumulation of hematochrome depends upon the quality and intensity of the light reaching the cells, food supply, moisture, and rate of growth. Senn points out that, in nutritive mineral solutions encouraging active growth, the amount of hematochrome pigment is greatly diminished. In this investigation an attempt was made to determine whether or not there is a correlation between rate of growth and hematochrome accumulation in a heretofore undescribed species of *Phycopeltis* found on the campus of the University of Hawaii.

Phycopeltis hawaiiensis n. sp.

Hematochrome orange-red; thallus circular with two concentric areas differing in hue; margin entire; filaments branching; no sporangia or gametangia produced.

Thallus aurantiaciacus discoideus, centro et regio concentrico exteriore in colore distincte gradato, margine integro, filamentis ramosis saepe oppositis sed trichotomo-simulantibus, est. Sporangia et gametangia desunt.

Holotype, a preparation of material collected June 12, 1953, on the campus of the University of Hawaii, is deposited in the Bernice P. Bishop Museum in Honolulu. Isotypes will be distributed through the Museum.

Method of culturing Phycopeltis

Phycopeltis used in this investigation was found growing on the leaves of a member of the Araliaceae on the campus of the University of Hawaii. In damp, shaded environments the alga reaches its ultimate state of development, forming numerous orange-colored, cushion-like discs on leaves. Upon microscopic examination of a disc, the presence of fungal hyphae living in close association with the alga is revealed. In culture the fungal hyphae can be seen intermingled with the algal filaments. This condition has led many investigators to consider *Phycopeltis* to be a lichen.

Discs were carefully removed from leaves and placed on different sterile media as shown in Table 1. All media contained a base of 1 per cent Knop's nutrient solution (Bold) in 2 per cent agar. The algae were then cultured under natural daylight conditions. The cultures were observed each day under a wide-angle microscope, to discover whether new filaments had developed from the discs.

It is observed from Table 1 that the most rapid growth took place when *Phycopeltis* was cultured on a medium containing Knop's nutrient solution in 2 per cent agar. The presence of sucrose or juice from the leaves upon which the epiphyte grew in the medium reduced the growth rate during the period immediately following inoculation. However, at the end of 80 days all media had produced considerable growth except those containing Knop's solution in agar, juice from leaves upon which the epiphyte was growing, and sucrose. Since Knop's nutrient solution in

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TABLE 1

A List of Media Used in the Cultivation of *Phycopeltis*, Showing the Number of Days Necessary for New Filaments to Appear

MEDIUM	DAYS
Basic	8
Basic and sucrose	16
Basic and juice from leaf upon which the epiphyte grew	12
Basic, juice from leaf upon which the epiphyte grew, and sucrose	*
Basic, juice from leaf upon which the epiphyte grew, and fragmented discs	16
Basic, sucrose, and fragmented discs	22

* Discs did not produce new filaments even after 80 days of incubation.

agar produced the most rapid growth, this medium was used to cultivate the algal material needed for further experimentation.

EXPERIMENTAL PROCEDURE

The addition of growth-affecting substances to the basic media brought about differences in rate of growth and in the predominant color of the algae, although each medium was adjusted to a *p*H of 5.6 and placed under similar environmental conditions. It was observed that, when a mass of *Phycopeltis* filaments is grown on nutrient media, new filaments are sent out horizontally from the center of the mass, forming a nearly circular disc. This being the case, the growth rate of an algal mass could be determined by measuring the change in area covered by the disc, a method previously used by the writer (1952). The diameters of the masses were measured at the time of inoculation and again on the thirty-second day after inoculation. The difference, in millimeters, was then recorded. Four cultures in each class were used in the experiment, and the average increase was computed. Table 2 shows the color and the amount of growth after 32 days of incubation under continuous light produced by fluorescent daylight bulbs and natural daylight. Temperature under which the cultures grew varied from 25°C. in natural daylight to 33°C. in light produced by fluorescent daylight bulbs. Experimental results showed, however, that this temperature variation had no effect upon color change.

When growth substances were added to the basic medium, the algal discs grown thereon for 32 days showed a greater increase in area covered than did those grown for the same length of time on the control medium. Although all algal masses were predominantly green (Fig. 1) at the time of inoculation, a distinct change in color was observed in the algal filaments grown on most of the media containing growth substances. After the 32-day growth period, the hematochrome pigment was predominant in those algae grown upon media containing indole acetic acid and colchicine in both continuous light produced by fluorescent daylight bulbs and in natural daylight (Fig. 2). Algae grown upon the medium containing benzimidazole became orange in color when grown under continuous light produced by fluorescent daylight bulbs but remained green under natural daylight. Benzimidazole had no effect upon the

TABLE 2

Results After Algae Had Grown on Media for 32 Days. Green *Phycopeltis* Masses Were Used as Inocula

MEDIUM	FLUORESCENT (DAYLIGHT)*		NATURAL DAYLIGHT†	
	Color	Diameter increase	Color	Diameter increase
		Mm.		*Mm.*
Basic (controls)	Green	0.05	Green	0.03
Basic plus indole acetic acid 1 gamma/ml.	Orange	0.12	Orange	0.09
Basic plus colchicine 0.1 per cent	Orange	0.15	Orange	0.12
Basic plus benzimidazole 2.5 $\times 10^{-3}$m.	Orange	0.09	Green	0.05

* Continuous for 24 hours each day.
† No light supplied during the night.

TABLE 3

ALGAL MASSES WITH ORANGE OR GREEN PIGMENT PREDOMINANT, INCUBATED ON MEDIA CONTAINING THREE DILUTIONS OF COLCHICINE UNDER CONTINUOUS LIGHT PRODUCED BY FLUORESCENT DAYLIGHT BULBS

MEDIUM	COLOR AT TIME OF INOCULATION	COLOR OF NEW FILAMENTS			
		Days after inoculation			
		4	8	16	24
Basic	Green	G	G	G	G
	Orange	O	O	OG	OG
Basic plus colchicine 0.01 per cent	Green	G	OG	OG	O
	Orange	O	O	O	O
Basic plus colchicine 0.05 per cent	Green	G	OG	O	O
	Orange	O	O	O	O
Basic plus colchicine 0.1 per cent	Green	G	OG	O	O
	Orange	O	O	O	O

hematochrome accumulation in cells grown under natural daylight. The filaments growing on control media did not show a change in color under continuous light produced by fluorescent daylight bulbs or natural daylight.

Since colchicine was shown to have a definite effect upon the growth rate and hematochrome accumulation in *Phycopeltis*, experiments were conducted using dilutions of this chemical. Algal masses showing the predominance of green or orange pigment were grown on media containing three dilutions of colchicine, as shown in Table 3. The cultures were grown under continuous light produced by fluorescent daylight bulbs and examined each day in order to determine any change in color. Observations, however, were recorded at the day intervals shown in Table 3. Four cultures from each class were used in the experiment.

After 24 days of incubation, the orange pigment was predominant in all the algal masses except those grown on the basic medium; however, the change from green to orange required less time when grown upon a medium containing 0.05 per cent or 0.1 per cent colchicine. In the controls (on the basic medium) containing a predominance of green pigment at the time of inoculation, there was no change in color, but those controls with a predominance of orange pigment at the time of inoculation showed green pigment mixed with orange.

SUMMARY

A new species of *Phycopeltis*, *P. hawaiiensis*, has been discovered growing as an epiphyte on leaves of a tree found on the campus of the University of Hawaii, Honolulu, Hawaii.

Phycopeltis discs taken from leaves were cultured on several media. Those grown on a medium containing Knop's nutrient solution in 2 per cent agar produced the most rapid growth. The addition of organic substances such as sucrose or juice from the leaves upon which the epiphyte was growing reduced the growth rate during that period immediately following inoculation. After 80 days, however, all cultures were growing at approximately the same rate, except one. This suggests that the medium containing Knop's nutrient solution in 2 per cent agar more closely duplicated the natural nutritional requirements; therefore, little adjustment was necessary. On the other hand, the media containing additional organic substances did not duplicate the natural nutritional requirements as closely; therefore, an adjustment period was necessary. This brought about a delay in the initial growth. As a rule, pigmented algae growing on agar medium do not need additional organic compounds. There also seems to be a limit to how much a medium can be enriched with organic compounds and, at the same time, produce growth. Media enriched with sucrose and juice from the leaves upon which *Phycopeltis* grew produced no growth even after 80 days of incubation.

In all cases except one there was a greater accumulation of hematochrome pigment and an increase in growth rate when certain

growth-affecting substances (indole acetic acid, colchicine, or benzimidazole) were added to the medium. The culture containing benzimidazole and grown in natural daylight did not show a greater accumulation of hematochrome pigment or an increase in growth rate. No attempt is made to explain the biochemical effect of growth-affecting substances upon *Phycopeltis*. It is shown, however, that a greater accumulation of hematochrome pigment can be brought about by increasing the growth rate. In nutrient media, hematochrome accumulation in *Phycopeltis* is correlated with growth rate. The algal masses that showed the greatest increase in area covered also contained the greater amount of hematochrome pigment.

Green *Phycopeltis* filaments incubated for 24 days upon media containing colchicine became orange in color, whereas orange filaments treated in like manner did not change in color. The green controls remained green, but the orange controls showed the presence of green filaments. It is quite conclusive that colchicine increases the accumulation of hematochrome pigment in *Phycopeltis*.

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