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ULTRASTRUCTURAL BASIS AND DEVELOPMENTAL CONTROL OF BLUE IRIDESCENCE IN SELAGINELLA LEAVES[1]

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ABSTRACT

The iridescent blue color of several *Selaginella* species is caused by a physical effect, thin-film interference. Predictions for a model film have been confirmed by electron microscopy of *S. willdenowii* and *S. uncinata*. For the latter species iridescence contributes to leaf absorption at wavelengths above 450 nm and develops in environments enriched with far-red (730 nm) light. This evidence supports the involvement of phytochrome in the developmental control of iridescence.

IRIDESCENT COLOR, caused by optical effects and not pigmentation, is a well-studied phenomenon among animals. Its existence in plants, however, is little appreciated. Some benthic marine algae produce blue to violet iridescence, and the physical basis has been investigated in several taxa (Gerwick and Lang, 1977; Pederson, Roomans and Hofsten, 1980). Blue iridescence appears on the upper leaf surfaces of a few land plants from the shady environments of humid tropical forests (Richards, 1952). The physical basis of this coloration is thin-film interference (Lee, 1977).

Blue iridescence is most common in the genus *Selaginella.* The two taxa analysed here are native to the extreme shade of humid tropical forests: *S. willdenowii* (Desv.) Bak. in Southeast Asia and *S. uncinata* Spr. in South China. In both species blue iridescence develops on leaves in shade beneath foliage. The green leaves that develop in response to more direct sunlight do not become blue when subjected to this shade, but blue leaves gradually turn to green with age or exposure to more direct light (pers. observ.). The filtering action of the forest foliage produces an environment deficient in energy for photosynthesis, with only 0.1–0.3% of the light above the canopy (Björkman and Ludlow, 1972; Bazzaz and Pickett, 1980). Previous studies with *S. willdenowii* suggested that interference at the leaf surface, while constructively increasing blue-light reflectance, destructively decreased reflectance at the upper end of the action spectrum of photosynthesis (Lee and Lowry, 1975). This decreased reflectance contributes to the absorption of wavelengths relatively more abundant in extreme forest shade. Furthermore, the foliage shifts the ratio of red (R) to far-red (FR) radiation, which may affect phytochrome equilibria and thus influence plant development (Tasker and Smith, 1977). Numerous studies have demonstrated the effects of FR-enriched light on plant morphogenesis, most probably mediated by phytochrome (Morgan and Smith, 1981).

Here we present a model for the thickness and location of an interference filter and describe the ultrastructural basis of iridescence in *Selaginella willdenowii* and *S. uncinata.* We also give evidence for the control of development and selective advantage of iridescence in *S. uncinata.*

MATERIALS AND METHODS—For ultrastructural studies, plants were obtained from the Jardin Botanique, in Montpellier. Small branches were fixed in 4% glutaraldehyde in 0.1 *M* cacodylate at pH 7.2 for 1 hr. Small leaf pieces were fixed for another hour. After rinsing in buffer, material was post-fixed in 1% OsO_4 in cacodylate overnight at 3 C, washed in distilled H_2O and dehydrated in acetone. Material was embedded in "ultralow viscosity" resin (Ladd) for *S. willdenowii* and Epon for *S. uncinata.* Fixation for scanning electron microscopy (SEM) was as the preceding, with dehydration in ethanol, CO_2 critical-point drying, and gold-palladium coating. Prior to taking photographs the microscope was calibrated with a grating. All measurements were

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[2] Dr. Hébant, internationally recognized for his work in structural botany, died in May 1982, as this article was being prepared.

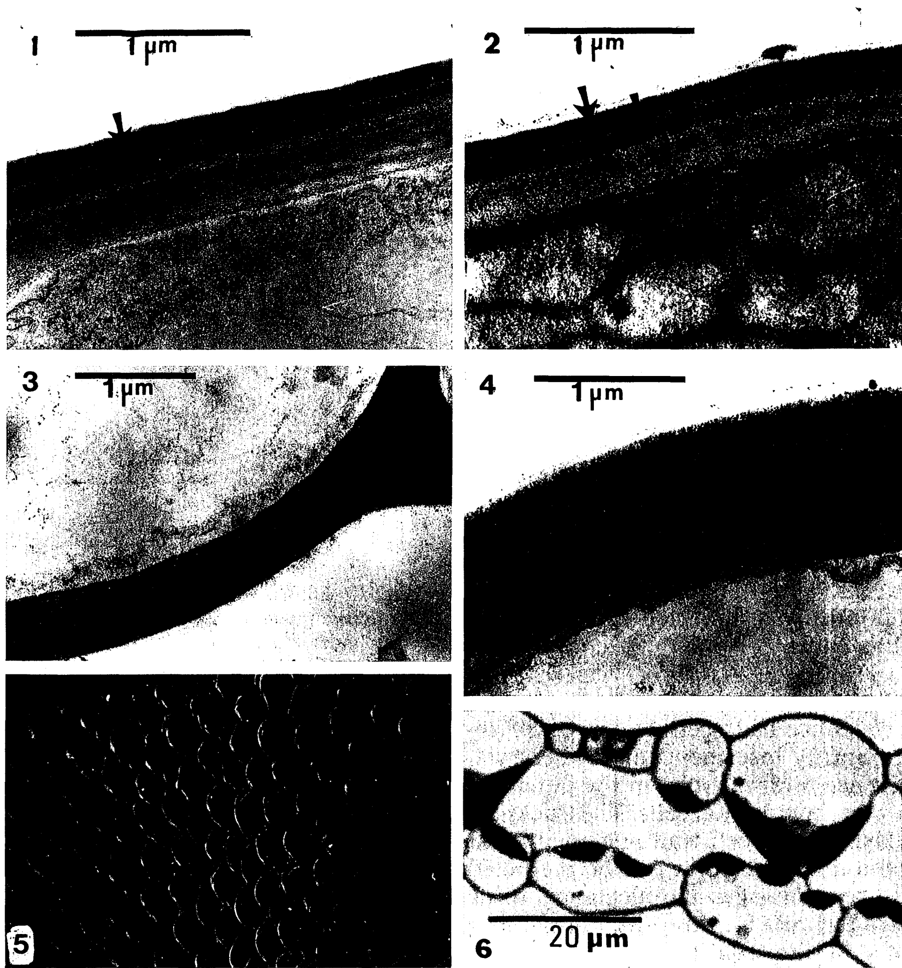

Fig. 1–6. Micrographs of *Selaginella* leaves. **1.** Transverse section of the outer cell wall from the upper epidermis of a blue leaf of *S. willdenowii* by transmission electron microscope (TEM), the two lamellae are indicated by arrows. ×23,150. **2.** Photograph by TEM of *S. uncinata;* transverse section of the outer cell wall from the upper epidermis of a blue leaf; the two lamellae are indicated by arrows. ×22,600. **3.** Transverse section of the outer cell wall from the lower epidermis of a blue leaf of *S. uncinata* by TEM; note the lack of lamellae. ×16,000. **4.** Transverse section of the outer wall of the upper epidermal cell of a green leaf of *S. willdenowii* by TEM, note the absence of lamellae. ×20,000. **5.** The convexly curved upper epidermal cells of *S. willdenowii* by SEM; scale is the same as Fig. 6. ×324. **6.** Transverse leaf section of *S. uncinata* by light microscopy, note the two large chloroplasts at the lower end of each epidermal cell. ×1,050.

made from negatives using a jeweler's magnifying glass accurate to 0.1 mm. Because of the possibility of differences in lamella thickness being caused by sections not perpendicular to the wall surface, we compared the total wall thickness to that of the lamellae; there was no correlation.

Developmental studies and reflectance measurements were conducted in Miami, and commercially obtained plants of *S. uncinata* were grown in closed terraria, in potting soil, at near 100% relative humidity, 12-hr photoperiod, and temperatures of 22–25 C. A high R:FR ratio of 3.0 (Smith, 1982) was achieved with cool white fluorescent bulbs, yellow polythene to reduce blue light, and neutral screen to reduce light levels to 12μE m^{-2} sec^{-1}. A moderate R:FR ratio (1.50) was constructed with the above minus the yellow filter and supplemented with tungsten bulbs at 11 μE m^{-2} sec^{-1}. A low R:FR ratio (0.35) similar to shade under foliage was created by filtering light from 75-W tungsten bulbs through 4.5 cm of H_2O and pieces of 750 CBS filters, at 12 μE m^{-2} sec^{-1}. Ratios were calculated from the emission spectra of lamps, filter spectral characteristics, and light measurements with a Licor quantum radiometer. Diffuse reflectance of

TABLE 1. *Summary of measurements of thicknesses of upper and lower lamellae, cuticle thickness, and total thickness of the outer cell walls of the upper epidermis of blue leaves of two species of* Selaginella

Measurement	*S. uncinata*	*S. willdenowii*
Cell wall thickness	561 ± 121 nm	572 ± 79 nm
Upper lamella + cuticle	120 ± 18 nm	110 ± 12 nm
Upper lamella only	94 ± 18 nm	74 ± 13 nm
Lower lamella	87 ± 13 nm	98 ± 23 nm
N =	22	60

composite leaf samples (10 × 15 mm) was measured in matched integrating spheres coated with barium sulfate (Kodak #6080) with a double beam spectrophotometer at a slit width of 5 nm. The barium sulfate coating was used as the reference. Curves of blue and green leaves were generated from the means of five separate measurements, and the difference in reflectance at 600 nm was subjected to analysis by the student's *t* test.

RESULTS AND DISCUSSION—*The interference filter model*—Since iridescent leaves of *Selaginella* temporarily lose their color when wet, the interference filter must be in the outer cell wall of the upper epidermal cells. The thickness of a thin layer in the cell wall responsible for the production of blue color can be predicted from measurements of peak reflectance, using the standard formula for thin-film interference (Jenkins and White, 1957):

$$\text{thickness} = \frac{\lambda}{4\mu \cos \partial}$$

where ∂ = refracted angle of light in the filter, μ = the refractive index of the film, and λ = the peak wavelength for constructive interference. We assumed a refractive index of μ = 1.45 for the outer portion of the cell wall based on the measurements of Woolley (1975). The following calculations include previous measurements of reflectance of *S. willdenowii* (Lee and Lowry, 1975) and measurements reported here for *S. uncinata* (Fig. 7).

S. willdenowii

$$\frac{405}{4 \times 1.45 \times 0.873} = 80 \text{ nm}$$

S. uncinata

$$\frac{410}{4 \times 1.45 \times 1.0} = 71 \text{ nm}$$

The phase shift of the filter depends on whether μ in the region beneath is higher (giving a thickness of 142 or 160 nm) or lower (71 or 80 nm). Assuming the region of the cell wall farthest from air to be more fully hydrated (and with lower μ) we predict a film thickness in the upper epidermal cell wall of 71–80 nm, and we predict the absence of such layers in the walls of green leaves or the outer wall of the lower epidermis of blue leaves.

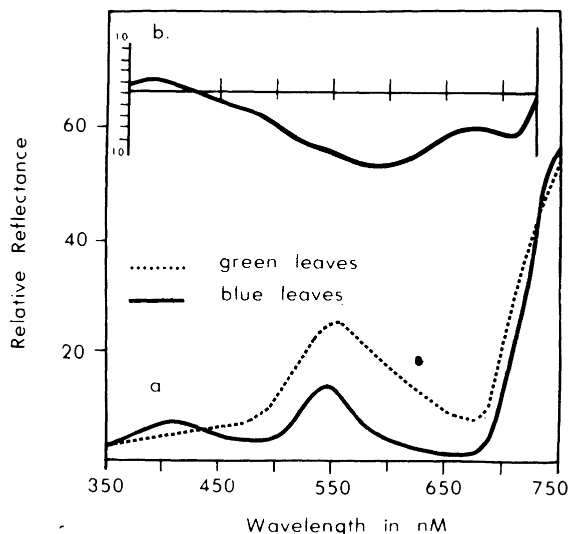

Fig. 7. Diffuse reflectance of leaves of *S. uncinata*. **a.** Blue foliage grown under R:FR = 0.35 and green foliage grown under R:FR = 1.50; the differences between the curves at 600 nm was significant at a level of 0.05. **b.** Differences between the two leaves.

In transmission electron micrographs we observed two lamellae of the predicted thickness, at the predicted position. These layers were found only in blue leaves of both species (Table 1, Fig. 1, 2). The lamellae were absent from green leaves and from the outer cell wall of the lower epidermis of blue leaves (Fig. 3, 4). The small deviations from predicted values could be caused by differences for μ in the lamellae, by shrinkage or expansion during fixation (Browning and Gunning, 1977), or by error in estimating magnification. As has been shown for animals, such parallel layers should intensify color production (Fox, 1976). The loss of iridescence upon wetting may be explained by the penetration of water to the electron-transparent layer between the lamellae (Fox and Wells, 1971). Residual iridescence, which is seen under a dissecting microscope, would be expected from lack of water penetration through the inner lamella.

Plants subjected to long-term exposure (4 weeks and more) of different R:FR ratios grew into strikingly different forms. These morphological differences are presently under investigation, and only the differences in blue iri-

descence are reported here. Only plants grown in a R:FR ratio of 0.35 produced intensely blue leaves (Fig. 7). These treatments were reversible; branches excised from one treatment and rooted in a different growth chamber produced the form characteristic of the new chamber on its new growth. These results demonstrate the effect of FR radiation on the development of blue iridescence, and thus implicate phytochrome in its developmental control.

Both *Selaginella* species possess structural and physiological adaptations to extreme shade (see Jagels, 1970). The upper epidermal cells of their leaves are lens-shaped (Fig. 5) with large chloroplasts adjacent to the interior walls (Fig. 6). *Selginella willdenowii* has one large cone-shaped chloroplast in each epidermal cell, while *S. uncinata* has two (Haberlandt, 1912; Ogara, 1972). Haberlandt argued that the presence of chloroplasts in the epidermis (which is a rare condition) would increase the efficiency of light absorption, as would light refraction by the curved cell surfaces (see also Tanno and Webster, 1982). Measurements for *S. uncinata* of decreased leaf reflectance above 450 nm and especially at the upper end of the action spectrum of photosynthesis (Fig. 7), are consistent with the earlier measurements for *S. willdenowii* (Lee and Lowry, 1975).

Leaf iridescence is but one of many striking characteristics of plants in the tropical humid forest understory. These results suggest that light quality, as well as quantity, may be important in regulating the developmental response of plants to extreme shade in these environments. In the case of *S. uncinata* the response should enhance the capacity of the plant to absorb energy for photosynthesis.

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