DELAYED GREENING AND THE EFFECT OF LIGHT AVAILABILITY ON CHLOROPHYLL DENSITY IN *DIPLAZIUM URTICIFOLIUM*

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Abstract: Many young leaves are a lighter shade of green than mature leaves, but it is not clear whether this reflects changing chlorophyll densities with age. Based on delayed greening theory, we predicted that younger fronds of the fern *Diplazium urticifolium* would have lower chlorophyll densities than mature fronds. We also hypothesized that younger fronds would have greater chlorophyll densities in high light than in low light, because in high light the greater potential carbon gain would outweigh the risk of loss to herbivory. We found lower chlorophyll density in younger fronds, consistent with delayed greening, but no significant difference in chlorophyll densities between high light and low light environments, possibly due to more herbivory in high light.

Key Words: physiological constraint, growth rate, trade-offs, inducible

INTRODUCTION

Delayed greening, whereby plants withhold chlorophyll from young leaves until they are adequately defended, is a common adaptation to reduce losses to herbivory. Plants depend on chlorophyll to convert light into chemical energy, but chlorophyll is costly, and the youngest leaves are most susceptible to herbivory (Coley and Kursar, 1996).

We investigated how chlorophyll density (g/m^2) in the fern *Diplazium urticifolium* is affected by light availability and age. The youngest frond of *D. urticifolium* has the lightest shade of green, and becomes darker with age. This color change could be explained by increasing density of chlorophyll (Erickson et al. 2001) or changes in frond thickness and physical structure with age.

We hypothesized that younger fronds delay greening because they are less well defended, and losing chlorophyll to herbivory is costly. Thus, we predicted that young fronds would have lower chlorophyll densities than mature fronds.

Delayed greening may be an inducible trait, optimized to an individual plant's environment (Vu et al., 2007). Plants in higher light receive more energy return per unit chlorophyll than plants in low light (Chazdon et al., 1996). We hypothesized that for young fronds in high light, the greater potential energy gain per unit chlorophyll would outweigh the risk of loss to

herbivory, and predicted that chlorophyll densities should be greater in high light than in low light. Finally, we hypothesized that if delayed greening is inducible with respect to light, then between high light and low light chlorophyll densities should differ in young fronds but converge to a similar value as the frond matures.

METHODS

On 23 and 24 Jan 2008 we sampled 24 *D. urticifolium* sporophytes in the forest above the Monteverde Biological Station in Costa Rica. We selected plants by walking a random number of steps (between 1 and 60) along the Sendero Principal Trail, then turning in a random compass direction and picking the first *D. urticifolium* encountered. We collected the youngest frond and a random mature frond of the fern sampled, and recorded whether it was in high light (gap) or low light (under canopy). We photographed the two fronds of each fern on a neutral gray surface in a dark room with an Olympus 770 SW set at ISO 100, using its flash.

To test if fern size had a confounding effect on chlorophyll density, we counted the number of blades (leaflets) on each frond and the length and width of the middle blade. We estimated blade area by multiplying blade length by width, frond area by multiplying blade area by number of blades, and total fern foliage area by multiplying frond area by number of fronds.

We extracted the average RGB values of four randomly selected blades of every frond with the imaging software Gimp 2. Using the formula: chlorophyll density=0.952-1.76*(R-B)/(R+B) (Kawashima and Nakatani, 1998) we estimated the chlorophyll density (g/m^2) of the four blades of each frond. Of the 188 blades, six had negative chlorophyll values; this was not critical as we required only relative densities to test our predictions. There was little variation in chlorophyll density estimates between the four blades of a frond, and we averaged them to obtain a composite value.

RESULTS

Fern size did not significantly affect chlorophyll density in terms of blade area ($F = 1.14$, $df=1, 45$, $P = 0.29$), frond area ($F = 1.13$, $df=1, 45$, $P = 0.29$), or total frond foliage area ($F = 1.63$, $df = 1, 45$, $P = 0.21$).

As predicted, chlorophyll densities were significantly lower in younger than older fronds (two-way ANOVA, $F = 14.6$, $df = 1, 44$, $P < 0.0001$). Contrary to our predictions, there was no significant difference in chlorophyll densities between high light and low light environments ($F = 2.81$, $df = 1, 43$, $P = 0.10$). There was no interaction between light and age

(F = 0.11, df = 1, 43, P = 0.74, Figure 1), further evidence that delayed greening was not inducible in response to light.

Chlorophyll levels were lower in the light green leaves than in the darker leaves, shown by a negative linear relationship between green brightness (from RGB values) and chlorophyll density, which was calculated independently of green brightness (F = 7.10, df = 1, 45, P = 0.01).

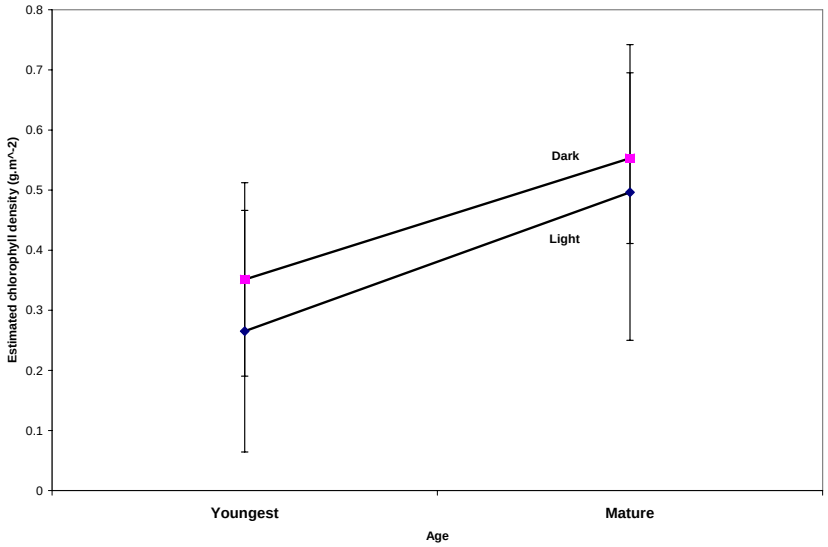

FIGURE 1. Estimates of chlorophyll densities in young and mature fronds of *D. urticifolium* in high light and low light environments at Monteverde, Costa Rica, using RGB color values and a formula from Kawashima and Nakatani (1998).

DISCUSSION

Regardless of fern size and light availability, young fronds had a lighter coloration and lower chlorophyll densities than mature fronds. This is consistent with delayed greening theory, with ferns investing less chlorophyll in their vulnerable young fronds, mitigating losses to herbivory. However, rapid expansion of young fronds could also reduce chlorophyll density (Coley and Kursar, 1996).

While it is generally assumed that leaves have higher chlorophyll density in high light than in low light, both shade tolerant and intolerant plants appear to have converged on a similar chlorophyll density in mature leaves, regardless of light availability (Chazdon et al. 1996). Our results are consistent with chlorophyll densities in mature leaves being relatively fixed, since we did not detect a difference between high light and low light environments.

It has been asserted that delayed greening is an inducible trait in young leaves with respect to herbivory (Vu et al., 2007). Our

result suggests that it is not inducible with respect to light availability in young fern fronds, but data on chlorophyll densities over a range of frond ages would be necessary to confirm this. Also, if there is greater herbivory in high light, that would increase the value of delayed greening in high light, and confound assessment of light as a factor affecting delayed greening.

LITERATURE CITED

Chazdon, R. L., R. W. Pearcy, D. W. Lee and N. Fetcher. 1996. Photosynthetic response of tropical forests plants to contrasting light environments. Pages 16-19 in S. S. Mulkey, R. L. Chazdon and A. P. Smith, editors. Tropical forest plant ecophysiology. Chapman & Hall, New York, NY.

Coley, P. D. and T. A. Kursar. 1996. Anti-herbivore defense of young tropical leaves: physiological constraints and ecological trade offs. Pages 313-327 in S. S. Mulkey, R. L. Chazdon and A. P. Smith, editors. Tropical forest plant ecophysiology.Chapman & Hall, New York, NY.

Erickson, P. B., J. E. Musnicki and D. J. Sugar. 2001. The effects of light environment and herbivory on delayed greening of tropical forest leaves. Dartmouth Studies in Tropical Ecology 2001, pp. 50-51.

Kawashima, S. and M. Nakatani. 1998. An algorithm for estimating chlorophyll content in leaves using a video camera. Annals of Botany 81: 49-54.

Vu, H. L., S. Lei, J. Marlow and J. N. Cech. 2007. Size-specific and partially inducible delayed greening in *Anthurium Hoffmannii* (Araceae). Dartmouth Studies in Tropical Ecology 2007, pp. 121-128.