SIZE-SPECIFIC AND PARTIALLY INDUCIBLE DELAYED GREENING IN *ANTHURIUM HOFFMANNII* (ARACEAE)

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Abstract. Herbivory rates are much higher in tropical forest plant communities than in comparable temperate systems, even with the higher anti-herbivory defenses employed by tropical species. For example, some plant species exhibit delayed greening (i.e., delaying chloroplast production in the youngest leaves until they have built up quantitative defenses). In a tropical oak forest, we explored how the patterns of leaf greening in *Anthurium hoffmanni* (Araceae) varied with stem size and along an herbivory gradient. For each focal plant, we measured the relative size of the first leaf, the stem diameter (the square of which we used as a surrogate for carbon storage), the hue of the newest expanding leaf, and the amount of herbivory on the plant. We predicted that smaller plants would not invest in delayed greening because they would not have sufficient carbon stores to subsidize non-photosynthetic leaves. We found that the smallest plants had no delayed greening. We also hypothesized, and found, that on plants with more herbivore damage, young leaves remained red longer, suggesting that the timing of greening may be phenotypically plastic, and a partly inducible anti-herbivore defense. To our knowledge, this is the first demonstration of apparently adaptive intraspecific variability in delayed greening.

Keywords: herbivory, plant defense, Araceae, *induction, plant development*

INTRODUCTION

Rates of herbivory in tropical forests are higher than in comparable temperate systems, even though tropical species are better defended (Coley and Barone 1996). While most damage in temperate species occurs on mature leaves, tropical species accrue the most damage on young leaves (Coley and Barone 1996; Numata et al. 2003). In addition, tropical plants in sun-lit canopies suffer less damage from herbivory than those in shaded subcanopies (Coley and Barone 1996). The leaf lifespan of tropical shade-tolerant plant species averages 2-4 years, with 70% of lifetime damage occurring during the first two weeks of expansion (Coley and Kursar 1996). Herbivores prefer young leaves because of their lower toughness and high water and nitrogen content, which give them a higher nutritional value than mature leaves (Coley and Kursar 1996). Early damage to leaves from herbivory alters plant growth and reproduction and consequently, the abundance and distribution of plants (Clark & Clark 1985). Therefore, in tropical regions, studies aiming to better understand herbivory defense

strategies need to focus on the defense mechanisms of the youngest leaves (Coley and Kursar 1996).

The young leaves of many shade-tolerant tropical plant species have high concentrations of red anthocyanins, and little or no chlorophyll. In delayed greening, anthocyanin content decreases more gradually than in plants with normal greening, as the young leaves mature and slowly begin to produce chlorophyll (Cai et al. 2005). Previous studies have suggested that delayed greening is an adaptation to reduce the amount and costs of herbivory (Coley 1983, Coley and Barone 1996, Coley and Kursar 1996, Cai et al. 2005). In the developing leaves of plants with delayed greening, chloroplast development occurs only after the leaf has reached a certain size, and is better protected from herbivores by quantitative defenses that take some time to develop (Coley and Barone 1996). Quantitative defenses include compounds such as phenols, tannins and lignins that are present in high concentrations and increase the toughness of leaves, or reduce digestibility (Coley et al. 1985). Young leaves with delayed greening can have *c.* 10-20% less light harvesting proteins, photosynthetic enzymes, chlorophyll, and lipid-rich membranes compared to normally greening leaves. Thus, the resources lost from a young leaf for a given amount of herbivory are reduced compared to older leaves (Coley and Barone 1996). It may be especially advantageous for tropical shade-tolerant species to have delayed greening under conditions of low light and high herbivory (Coley and Barone 1996). Although delayed greening reduces photosynthesis during leaf expansion, the benefits of herbivory reduction can outweigh the costs of reduced photosynthesis (Coley and Kursar 1996).

We studied the relationship between herbivore damage and delayed greening in the shade-tolerant understory plant, *Anthurium hoffmannii* (Araceae) in a tropical oak forest. Previous studies have observed delayed greening, and explored its value as an anti-herbivory defense, and its effects on herbivory among species (Coley 1983, Coley & Barone 1996, Coley & Kursar 1996, Numata et al. 2004, Cai et al. 2005, Coley et al. 2005). However none, to our knowledge, has explored variation in delayed greening within a species or the effects of herbivory on delayed greening within the lifetime of a plant.

We reasoned that two factors, plant size and herbivory intensity, might alter the amount of delayed greening in plants within a species. First, smaller plants have limited carbon stores and may simply be unable to support a new leaf that does not fix carbon: delayed greening in small plants could result

in high mortality. Therefore, we hypothesized that the smallest plants would not delay greening in their young leaves. Second, the size of the expanding leaf at which the plant begins to invest in chloroplast production may vary depending on the level of herbivory experienced by an individual plant. Specifically, we hypothesized that plants experiencing less herbivory would invest in chloroplasts at an earlier stage in the expanding leaf development than plants with higher levels of herbivory. Plants with more herbivory are more likely to lose valuable resources in their expanding leaves, so the benefits of delaying investment in chloroplasts in the developing leaf should outweigh the costs due to forfeited photosynthetic gain.

METHODS

We sampled 82 *A. hoffmanni* plants along the loop trail of the Cuerici Biological Reserve, Costa Rica from 28 to 29 January 2007. We randomly sampled plants of all sizes along a 50 m stretch of trail. We measured stem diameter on each plant directly under the node of the newest leaf. The square of the stem diameter is proportional to stem cross-sectional area; we used this as a surrogate for the carbon storage capacity of each plant.

Sampled plants had 3.36 ± 1.20 leaves per plant (mean ± 1 SE). For each plant, we measured the dimensions of each of its leaves as the length of the central vein and the leaf width at the base of the central vein. The average leaf width and length per plant were highly correlated ($r = 0.9682$, $P < 0.0001$, $n = 80$ leaves). We calculated a leaf size index as the product of the width and length. We used the proportional size of the first leaf, relative to older leaves, to describe the relative size of the newest leaf on each plant:

$$\text{Relative Size of the First Leaf} = \frac{\sum_{i=1}^{N-1}\left(s_x / s_i\right)}{N},$$

where N = total number of leaves on the plant, s_x = size of the newest leaf on the plant, and s_i = size of the ith older leaf. Using this formula, we were able to compare the relative sizes of new leaves among individual plants, in spite of the differences in average leaf size per plant.

We processed leaf images using Adobe Photoshop CS (v. 8.0). From each photo, we took a 50 pixel x 50 pixel sample close to the main vein, which we considered to be

representative of the entire leaf. Then, we applied an Average Blur filter to each sample to create a single, composite mean color from the 2500-pixel sample (Figure 1). From this sample, we extracted numeric values for red and green content as well as hue, using values provided by the software. Hue represents the frequency of light on a color scale, where red has the lowest hue and violet the highest. Red and green values represent the saturation of each color in the image (i.e. the "density" of each color), independent of other color saturations. While individual color values are independent of one another, hue values represent the contribution of all color values (i.e. green, red, and blue all contribute to hue). Hue values represent overall changes in leaf color while green values are probably more specific in describing chlorophyll concentration. We used hue to quantify delayed greening on the newest leaf. We considered a plant with low hue (i.e., red color), on leaves with larger sizes relative to the size of older leaves, to exhibit a high degree of delayed greening.

Figure 1. The gradient of composite sample colors from digital photographs of the youngest leaf of *Anthurium hoffmannii* (Araceae) in Cuericí Biological Reserve, Costa Rica. The lowest hue values are on the left (red) and the highest hues are on the right (green). Composite sample colors were calculated by using an Average Blur filter in PhotoShop 8.0; the colors in the gradient represent the average hue of the entire leaf (n = 120 leaves). The differences in saturation and brightness (i.e. darker greens and reds) do not influence hue.

We quantified herbivory as the mean percent tissue lost on extant leaves for each plant. We visually estimated tissue loss by dividing each leaf into quarters and estimating the percent of leaf area lost to herbivory in each section. We averaged the percent tissue damage over the four quarters for each leaf. Finally, we visually noted the color of the first (newest) leaf (red, transition, or green) and photographed it with a Nikon Coolpix L3 digital camera (5.1 Megapixels).

Statistical Analyses.

To determine whether plants with smaller carbon stores would be less likely to invest in delayed greening, we used two separate regression analyses to test the relationship between stem size and hue and green saturation in the youngest leaves of different plants. To determine how the color in the newest leaf varied with the relative size of the first leaf, we used three separate regression analyses to test the relationships between the

relative size of the first leaf, and hue, red, and green saturation.

To determine the effects of herbivory on delayed greening, we used a multiple regression to test the effects the relative size of the first leaf, mean percent leaf damage, and their interaction on leaf hue. We used an arcsin square root transformation to achieve normality in our mean percent leaf damage data.

RESULTS

In smaller-stemmed plants (presumably with lower carbon stores) there was a greater tendency for new leaves to be green than in larger plants. As stem size increased, hue and green saturation of the youngest expanding leaves decreased (Figure 2a, b). In addition, plants with larger stem diameters had proportionally smaller youngest expanding leaves; the relative size of the first leaf decreased with the square of the stem size ($F_{1,78} = 21.9647$, $r^2 = 0.2197$, $P < 0.0001$). As the relative size of the first leaf increased, leaves had higher hues and were greener (Figure 2c, d). Overall, the relative size of the first leaf accounted for only a small amount of the variation in red saturation ($F_{1,78} = 8.5049$, $r^2 = 0.0983$, $P < 0.005$). In plants with a stem size below 0.5 cm, we did not observe red coloration in any of the youngest expanding leaves.

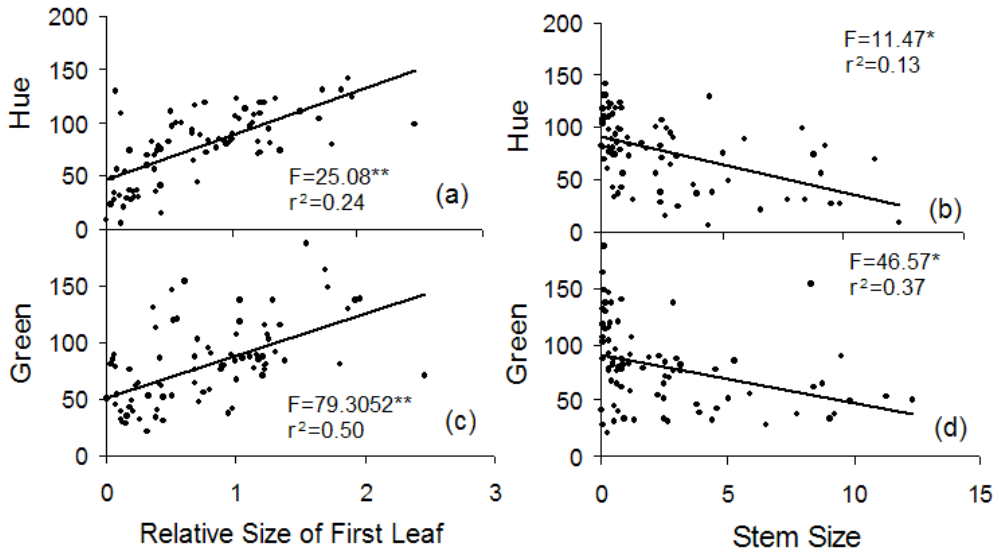

Figure 2. Linear regressions demonstrating negative relationships between stem size (surrogate for carbon stores) and hue and green saturation (a and b) and positive relationships between the relative size of the first leaf and hue and green saturation (c and d) in *Anthurium hoffmannii* at Cuericí Biological Reserve, Costa Rica. A low hue value indicates red coloration and a high hue value indicates green coloration. * indicates $P < 0.005$ and ** indicates $P < 0.0005$. Each point represents one plant (n = 82 plants).

We found that, controlling for relative leaf size, herbivory damage had a marginally significant negative effect on hue (Table 1). Plants with higher herbivory delayed greening in their youngest expanding leaf longer than plants with lower herbivory. For example, a 10% increase in herbivory would result in a 16% decrease in hue.

Table 1. Multiple regression statistics for testing the effects of mean percent herbivory damage (arcsin square-root transformed), relative size of the first leaf (log transformed) and the herbivory*leaf size interaction on hue for *Anthurium hoffmannii* (Araceae) in Cuericí Biological Reserve, Costa Rica (n= 82 plants). A low hue value indicates red coloration and a high hue value indicates green coloration.

Source	DF	SS	F	P
Relative leaf size	1	30168.041	51.6291	<.0001
Leaf damage	1	2096.049	3.5871	0.0620
Relative leaf size*Leaf damage	1	302.911	0.5184	0.4737

DISCUSSION

We found significant variation in patterns of delayed greening across size classes within the herbaceous plant, *Anthurium hoffmannii*. Our results confirmed our first prediction: investment in delayed greening increased with stem size (larger plants had lower hues and green saturation, Figure 2a, b). We suggest that the smallest plants (which had no delayed greening) simply do not have sufficient carbon reserves to subsidize leaves that cannot photosynthesize fully. Thus, delayed greening in small plants may increase their risk of mortality. By exerting strong selection for alternative developmental pathways in the young leaves, this increased risk may inhibit the delays on chlorophyll production that characterize the young leaves of larger plants, in delayed greening species.

While previous studies have quantified the patterns of delayed greening among species, none to our knowledge has documented how delayed greening varies across size classes within a species. Our results suggest that, within a species, the delayed greening anti-herbivory defense may not always be the optimal strategy. Plants may be able to regulate how much delayed greening they exhibit. Further studies could quantify chlorophyll production as individuals of delayed greening species mature, to ensure that the pattern from our static observations also occurs over developmental time.

As the relative size of the youngest leaves increased, we found that greening increased (plants with relatively larger leaves had higher hues and green saturation, Figure 2c, d). We found that both hue and

green saturation increased linearly with the relative size of the first leaf (and correspondingly, red saturation decreased slightly with leaf size). The change in hue as relative leaf size increases is likely due to increasing concentrations of chlorophyll, which masks the red anthocyanins (Cai et al. 2005). While some studies have found that chlorophyll increases linearly with leaf expansion (Kursar and Coley 1992), consistent with our results, other studies have hypothesized that complete chlorophyll production is delayed until the new leaf has amassed sufficient levels of secondary compounds (Coley and Kursar 1996). For example, in *Desmopsis panamensis* (Annonaceae) and *Annona spragueii* (Annonaceae), the newest leaves are white and then abruptly accumulate chlorophyll while species with red leaves gradually accumulate chlorophyll (Kursar and Coley 1992). Future studies could include chemical analyses on the secondary compounds in the youngest expanding leaves of white and red delayed greening species to quantify how photosynthetic and non photosynthetic pigments change with leaf development.

Finally, we found support for our prediction that young leaves would prolong delayed greening when herbivore damage on the plant was high. We showed that there is variability in the phenotypic expression of delayed chlorophyll production that may, at least in part, be influenced by the amount of herbivory an individual plant experiences. Our findings also imply that the importance of herbivory to delayed greening may vary with plant size. The trend for larger plants with high herbivory to have more delayed greening suggests that delayed greening may be an inducible defense. Inducible herbivory defenses may be especially important in the tropics where herbivory on young leaves is high (Coley and Barone 1996). Future studies could test the degree to which delayed greening is inducible, by quantifying the levels of chlorophyll in the new leaves of plants experiencing differing levels of herbivory over time.

While other studies have shown that delayed greening may be beneficial for some species and not for others, our study suggests that optimal delayed greening strategies may also vary within a species and even within an individual. The plastic expression of delayed greening within a species demonstrates the capacity of plants to alter programs of leaf development, in response to variability in damage by natural enemies.

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