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Regulation of photosynthetic pigments in tropical understory and gaps

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Regulation of Photosynthetic Pigments In Tropical Understory and Gaps

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ABSTRACT

A plant can manipulate its absolute and relative amounts of photosynthetic pigments in different light environments (Hopkins, 1995, Goncalves and Vieira, 2001). Leaf samples from ten gap and ten understory plants were collected from *Calyptrogyne brachystachys* (Arecaceae)*, Heliconia monteverdensis* (Heliconiaceae)*,* and *Piper ariteum* (Piperaceae), and their chlorophylls a, b, and carotenoid concentrations were found. The total concentration of chlorophylls a, b, total chlorophyll and carotenoids were significantly higher ($p = <0.05$) in the understory leaf samples of *C. brachystachys* and *P. auriteum*, but higher in the gap samples of *H. monteverdensis*. Ratios of chlorophyll *a* to chlorophyll *b* were significantly greater for gap samples of all three species. A higher concentration of chlorophyll and carotenoids in the understory plants of *C. brachystachys* and *P. auriteum* suggests they are adjusting the absolute and relative amounts of pigments to make use of sparse light in the understory. *H. monteverdensis* utilizes its photosynthetic pigments slightly differently, acting much like a canopy plant.

RESUMEN

Una planta puede manipular absolutamente la cantidad relativa de pigmentos fotosintéticos en ambientes con diferente luz (Hopkins, 1995, Goncalves y Vieira, 2001). Muestras de hojas de 10 plantas en el sotobosque y en claros de bosque fueron colectadas de *Calyptrogyne bracystachys* (Arecaceae)*, Heliconia monteverdensis* (Heliconiaceae)*,* y *Piper ariteum* (Piperaceae), y se midieron las concentraciones de clorofila a y b, y carotenoides. La concentración total de clorofila a, clorofila b y carotenoides fue significativamente mayor en las muestras del sotobosque para *C. brachystachys* y *P. auriteum*, pero mayor en los claros de bosque para *H. monteverdensis*. Proporciones de clorofila a: clorofila b fueron mayores en las muestras del claro de bosque para las tres especies. Una mayor concentración de clorofila y carotenoides en las plantas del sotobosque *C. brachystachys* y *P. auritum* sugieren que estas ajustan la cantidad relativa y absoluta de pigmentos para usar la luz escasa en el sotobosque. *H. monteverdensis* utiliza los pigmentos fotosintéticos un poco diferentes a las otras dos especies, comportándose más como una planta de dosel.

INTRODUCTION

Tropical understory plants live in an environment that has low light, high relative humidity, low wind, and moderate temperature (Rundel and Gibson, 1996). Solar radiation levels in the understory are between 5 and 10 μmol m^{-2} s^{-1} and less than 0.5% of sunlight reaches the forest understory (Pearcy 1983). Sunflecks, brief periods of a direct beam of solar radiation, provide between 10-78% of the total photon flux density on the forest floor, having profound changes on the plant's photosynthetic process (Chazdon 1986b). Contrastingly tropical understory plants that grow in light gaps receive between 400-1500 μmol m^{-2} s^{-1} of solar radiation (Kursar and Coley, 1999). These two environments with different light allotments may affect how a plant photosynthesizes.

Photosynthesis consists of two photosystems (PS), which accept the energy from a photon and use this energy to create adenosine triphosphate. Furthermore light harvesting complexes (LHC), in the photosystems, act like antennae, using chlorophyll to capture the maximum amount of photons and shuttle them to the reaction center. Photosynthetic pigments absorb in the visible light range. The primary and the most abundant pigment for absorbing photons is chlorophyll *a*. There are also two accessory pigments that aid in light absorption, chlorophyll *b* and carotenoids (Raven, Evert, and Eichhorn, 1999). Production of all of these pigments is very costly to the plant, but if an understory plant is to be a viable competitor it must be efficient with the light it receives. Carotenoids are present in all photosynthetic organisms and protect the plant from photo-oxidation by absorbing and dissociating excess energy from chlorophyll. For those plants that end up in a gap they change their photosynthetic pigments, investing mostly in chlorophyll *a* and carotenoids. Chlorophyll *a* will absorb most the light these plants need and the carotenoids may become more important for protection from this new environment, having less of a role as a secondary pigment. Each of these photosynthetic pigments maximum absorption is at different wavelengths, which is how their total concentration can be calculated.

This study compares the concentration of chlorophyll *a*, chlorophyll *b*, and carotenoids between the understory plants *Calyptrogyne bracystachys* (Aracaceae), *Heliconia monteverdensis* (Heliconiaceae), and *Piper ariteum* (Piperaceae). These species were chosen because they are found in the shaded understory and can persist and even excel in light gaps. *H. monteverdensis* naturally grows in open areas, but once it is established it can persist for many years in the regenerating forest (Janzen, 1983). The calculated chlorophyll and carotenoid concentrations give insight as to how these specific species adjust their pigments in different light environments. Plants in the light gap receive a higher intensity of light and therefore are expected to have lower concentrations of chlorophyll *a* and *b*. alternatively, carotenoids concentration is harder to predict because they are not needed so much as a secondary pigment in this environment but are necessary to protect the plant from photo-oxidation. In addition with less light available in the shaded understory; the plants of this habitat should have higher chlorophyll *b* concentrations than gap plants. This is because understory plants are using their increased chlorophyll *b* to jump start PSII and the whole photosynthetic process when in a sunfleck. Carotenoids in this environment could play a different role, less protection is needed from photo-oxidation, but more carotenoids can help photosynthesis by absorbing light in the 470 nm ranges. Thus adapting with different light conditions in the tropical understory, plants should change their relative abundance of photosynthetic pigments.

MATERIALS AND METHODS

Data collection occurred in the Tropical Cloud Forest of Monteverde, Costa Rica behind the Monteverde Biological Station at approximately 1535 meters in elevation. The methods used for this research follow those used by Wallentine (2006). Ten leaves from each of the study species were collected from the understory and from light gap plants, for a total of 60 samples. Each leaf sample was then cut into a five by five centimeter square using a cardboard stencil and massed. The pigments were extracted by adding 7ml of 85% acetone solution at a pH of 6.5 to shredded pieces of the leaf sample in a test tube. This solution precipitated for 15 minutes and was shaken every five minutes for 30 seconds to make sure the solution mixed properly. Next the samples were centrifuged at 4000 rpm, and the total volume of the decanted solution containing the pigments was recorded. Next, 2 ml of this decanted solution was added to 8 ml of 85% acetone to dilute the sample. A small portion of this mixture was poured into a cuvet and the absorbance was measured at 663, 646, and 470 nm. The concentrations based mass of the leaf of chlorophyll *a*, chlorophyll *b*, and carotenoids was calculated using the following equations from Lichtenthaler and Welber (1983):

$$\text{Chlorophyll } a \text{ (mg/g)} = \frac{[12.21\ (\text{Abs}_{663}) - 2.81\ (\text{Abs}_{646})] \text{ x [Purified Volume (ml)]}}{[200] \text{ x [Mass of Leaf Used (g)]}}$$

$$\text{Chlorophyll } b \text{ (mg/g)} = \frac{[20.13\ (\text{Abs}_{646}) - 5.03\ (\text{Abs}_{663})] \text{ x [Purified Volume (ml)]}}{[200] \text{ x [Mass of Leaf Used (g)]}}$$

$$\text{Carotenoids (mg/g)} = \frac{\{1000\ (\text{Abs}_{470}) - 3.27[\text{chl } a] - 104\ [\text{chl } b]\} \text{ x \{Purified Volume (ml)\}}}{\{45400\} \text{ x \{Mass of Leaf Used (g)\}}}$$

Then the ratios of chlorophyll *a* to chlorophyll *b* are determined by dividing one from the other. Finally a t-test was performed between the pigment concentrations of the light and dark plants, checking for significance.

RESULTS

Light quality did in fact change the concentration of the total photosynthetic pigments. The *H. monterverdensis* gap leaves were collected from very large mature plants while the leaves collected for this species from the shade habitat were relatively smaller. The total chlorophyll concentrations ranged between 0.0076 mg/g and 0.0586 mg/g. Shaded leaves of *C. brachystachys* and *P. ariteum* had a higher amount of chlorophyll, while *H. monterverdensis* had a higher amount of chlorophyll in the leaves from the light gap (Figure 1, *C. brachystachys* t = 2.364, dof = 18, p = 0.0295, *H. monteverdensis* t = -11.266, dof = 18, p = <0.0001, *P. ariteum* t = 2.594, dof = 18, p = 0.0183). Comparing the chlorophyll *a* concentrations between the species shows similar trends to the first figure. Chlorophyll *a* is the main pigment and is in very high concentrations in the gap plants of *H. monteverdensis* while the others have a higher concentration in the understory plants. (Figure 2, *C. brachystachys* t = 1.841, dof = 18, p = 0.0822, *H. monteverdensis* t = -11.889, dof = 18, p = <0.0001, *P. ariteum* t = 2.395, dof = 18, p = 0.0277). Concentrations of chlorophyll *b* are much smaller than chlorophyll *a*, with the highest chlorophyll *b* concentration equaling the lowest concentration of chlorophyll *a.* This also shows similar trends to the total chlorophyll concentration graph. Interestingly the *C. brachystachys* understory and *H. monteverdensis* gap have almost equal concentrations, with both being the highest concentration for all samples. (Figure 3, *C. brachystachys* t = 3.780, dof = 18, p = 0.0015, *H. monteverdensis* t = -3.754, dof = 18, p = 0.0015, *P. ariteum* t = 3.228, dof = 18, p = 0.0047). Using data from the two previous graphs a ratio was made between the two chlorophyll concentrations. Differences in the chlorophyll *a* and *b* ratios ranged between 2.1541 mg/g and 4.1066 mg/g. The ratio of chlorophyll *a* to chlorophyll *b* was higher in the gap environment of all three species. The gap plants have much more chlorophyll *a* than *b* making the ratio of the two pigments higher for these samples (Figure 4, *C. brachystachys* t = -8.184, dof = 18, p = <0.0001, *H. monteverdensis* t = -3.493, dof = 18, p = 0.0026, *P. ariteum* t = -3.777, dof = 18, p = 0.0014). The next graph shows that shaded understory leaves in *C. brachystachys* and *P. ariteum* had a greater concentration of carotenoids, while the gap plants of *H. monteverdensis* had a greater concentration. Carotenoid concentrations were at a lowest of 0.0039 mg/g and highest of 0.0347. (Figure 5, *C. brachystachys* t = 4.117, dof = 18, p = 0.0006, *H. monteverdensis* t = -11.362, dof = 18, p = <0.0001, *P. ariteum* t = 2.754, dof = 18, p = 0.0131).

DISCUSSION

A previous study has shown differences in the quality and quantity of light cause some species to compensate with the abundance of chlorophyll and carotenoids in their leaves (Tinoco-Ojanguren and Pearcy, 1995). Wallentine (2006) found that plants in the understory and canopy use different techniques to capture light. Since the canopy leaves have a more stable supply of light it is unnecessary to have such high chlorophyll concentrations. This study found that total chlorophyll concentrations were higher in shaded leaves for *C. brachystachys* and *P. auriteum,* but gap leaves of *H. monteverdensis* had more chlorophyll. Day (1996) showed that mature leaves have higher chlorophyll concentrations, which could explain why *H. monteverdensis* have such a high concentration of chlorophyll. In order to completely capture light from sunflecks, shaded leaves may need to have more chlorophyll.

Looking at the ratios between the concentration of chlorophyll *a* and chlorophyll *b* indicates the range of light that the plant absorbs. Not surprisingly individual chlorophyll *a* and *b* concentrations reflect those of the total chlorophyll concentrations. Chlorophyll *b* is associated with photosystem II (PSII), and the starting point of the whole photosynthetic process (Hopkins, 1995). This study found that the understory plants in the gap had a higher ratio of chlorophyll *a* to chlorophyll *b*. using the graphs of the individual chlorophyll pigments shows that the change in the ratio is due to chlorophyll *b* increasing and not a decrease of chlorophyll *b*. This finding suggests that the shaded understory plants use more chlorophyll b to improve the efficiency of PSII.

One important trend to note is that the total carotenoids concentration mirrors that of total chlorophyll concentration. This could mean that as chlorophyll levels increase in the gap plants carotenoids are used to protect against photo-oxidation, but when chlorophyll concentration is higher in the understory plants carotenoids are used more as secondary pigments. Perhaps the level of chlorophyll *a* is the main factor in determining the role of carotenoids.

Plants employ multiple techniques in order to take advantage of the light environment they belong to. Those growing in an understory light gap have less variable light than those in the shaded understory, and can take advantage of this by creating more chlorophyll *a* to capture the light. Shaded plants have to use different strategies to ensure they will have enough light to grow. *C. brachystachys* and *P. ariteum* alter their pigments to increase their light capturing ability in the understory. One way to do this is to increase the amount of carotenoids and chlorophyll *b* in the leaves to be used as accessory pigments. However, *H. monteverdensis* uses different light capturing techniques, often the opposite of the other two plants. In this way *H. monteverdensis is* acting similarly to a canopy species, by biding its time in the understory and waiting for a gap to reach their full potential. The biochemistry of photosynthesis is still not fully understood and further investigation as to how individual plants function can give insights as to how species, populations, and communities interact.

FIGURES

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FIGURE 1. The total chlorophyll concentration for leaves of three understory species that also persist in treefall gaps. Total chlorophyll represents the total chlorophyll *a* and *b* as measure by spectrophotometry. Blue bars represent the mean total chlorophyll concentration for ten understory individuals, and the purple show means for individuals in treefall gaps in an open canopy. Error bars show the standard deviation. The asterisk shows a significant difference at $p < 0.05$ (see text).

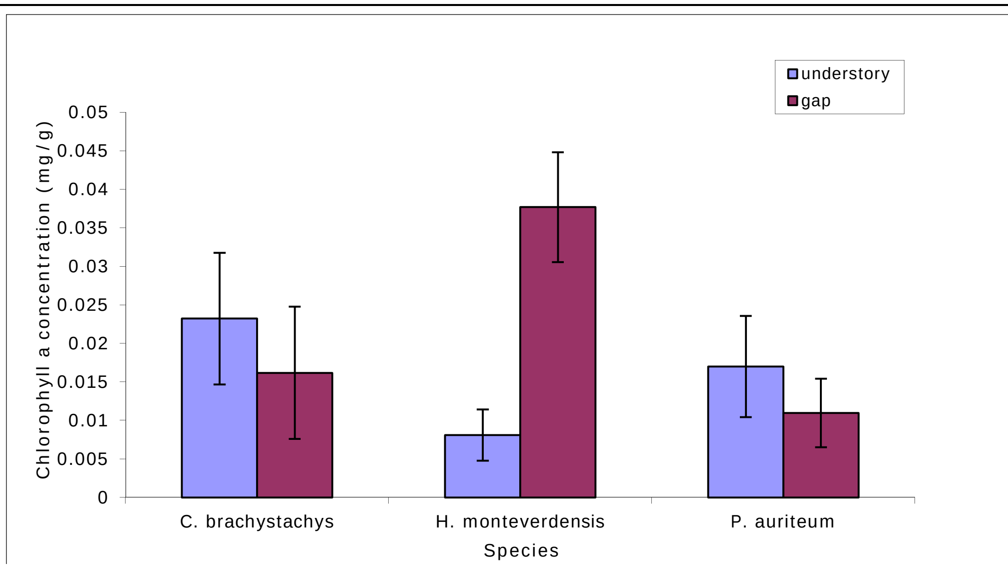

FIGURE 2. The total chlorophyll *a* concentration for leaves of three understory species that also persist in treefall gaps. Chlorophyll *a* concentrations were measure by spectrophotometry. Blue bars represent the mean total chlorophyll concentration for ten understory individuals, and the purple show means for individuals in treefall gaps in an open canopy. Error bars show the standard deviation. The asterisk shows a significant difference at $p < 0.05$ (see text).

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FIGURE 3. The total chlorophyll *b* concentration for leaves of three understory species that also persist in treefall gaps. Chlorophyll *b* concentrations were measured using spectrophotometry. Blue bars represent the mean total chlorophyll concentration for ten understory individuals, and the purple show means for individuals in treefall gaps in an open canopy. Error bars show the standard deviation. The asterisk shows a significant difference at $p < 0.05$ (see text).

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FIGURE 4. The ratio of chlorophyll *a* to *b* concentration for leaves of three understory species that also persist in treefall gaps. Chlorophyll *a* and *b* concentrations were measured using spectrophotometry. Blue bars represent the mean total chlorophyll concentration for ten understory individuals, and the purple show means for individuals in treefall gaps in an open canopy. Error bars show the standard deviation. The asterisk shows a significant difference at $p < 0.05$ (see text).

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FIGURE 5. The total carotenoid concentration for leaves of three understory species that also persist in treefall gaps. Carotenoid concentrations were measured using spectrophotometry. Blue bars represent the mean total chlorophyll concentration for ten understory individuals, and the purple show means for individuals in treefall gaps in an open canopy. Error bars show the standard deviation. The asterisk shows a significant difference at $p < 0.05$ (see text).

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