Plasticity in photosynthesis of bat laurel (*Prunus polystachya*) in tropical rainforest of Singapore

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Abstract
Rainforests are one of the most important habitats on Earth and contain an extraordinary amount of global biodiversity. However, due to human disturbance we have lost a great portion of primary forests. Thankfully, due to the rising awareness of the importance of rainforests, secondary rainforests are regenerating. This has provided a new environmental condition for different species, as it is a much higher light environment than primary forests. Plants must adjust their traits in order to adapt to the new light conditions. This study investigates the plasticity in photosynthetic characteristics of *Prunus polystachya*. We predicted that understorey leaves will have a higher chlorophyll content, higher overall carbon fixation, and higher photosynthetic induction. Because understorey leaves receive little light, they rely heavily on sun flecks for photosynthesis. Therefore, they should have photosynthetic traits that will maximise all the sunlight they receive. We found that understorey leaves do indeed have a higher chlorophyll density and carbon fixation amount, but the hypothesis of understorey leaves having a higher photosynthetic induction was not supported. These findings could assist in determining how different species excel under different conditions, leading to the prediction of floristic composition of primary and secondary forests. In the field of forest conservation, it will assist in predicting the species turnover rate between primary and secondary rainforests by having knowledge of which species will need protection and which we should focus on in the regeneration of secondary forests.

Key Words: Light gradient, primary & secondary forests, phenotypic plasticity

Introduction
Rainforests in South-East Asia have been heavily exploited and cleared for human activities and economic purposes over the past century (Achard *et al.* 2002). Although only an eighth of the plant species in the rainforest have actual economic value to humans, such as timber, medicine and edibles, the land has also been cleared for agriculture (Primack and Lovejoy 1995). Connor and McCoy (1979) predicted that if the exploitation...
is maintained, we would only be able to preserve about 50 per cent of the original species in the rainforests.

Two major phenomena have resulted from human disturbances within the South-East Asian forests: forest fragmentation and the regeneration of secondary forests. The original forest areas have been reduced into many smaller fragments, increasing light levels around the forest edges (Skole and Tucker 1993). Secondary forests are forests that have regrown over an area that has experienced disturbance, resulting from both natural processes and human activity (Corlett 1994), and are usually a much higher light environment when compared to the primary forests.

The changing of light conditions of the rainforests ultimately influences a plant’s photosynthetic characteristics. In different light environments plants will have different photosynthetic characteristics in order to maximise growth and use of the available sunlight. Generally, in a primary forest there are three main light environments: canopy, understory and gaps. The canopy is the top layer of the forest and always receives full light (Ghazoul and Sheil 2010). In the understory of a primary forest there is very little light (Montgomery and Chazdon 2001), and plants must rely on sun flecks and the minimal light that penetrates the canopy (Way and Pearcy 2012). And finally, gap environments provide a much higher light intensity for much longer throughout the day – the micro-climates in gap environments are much closer to the conditions that are found in the canopy (Ghazoul and Sheil 2010). In both forest fragments and secondary forests, the increase in higher light environments is causing the loss of shade tolerant and primary forest species (Cramer et al. 2007; Michalski et al. 2007).

Fortunately, plants can adjust their characteristics in response to the ever-changing environment. Phenotypic plasticity is defined as the differential response of a genotype to different environments (Rozendaal et al. 2006). Even sun and shade leaves from an individual could present a difference in leaf traits, and we already know that we could use this information to predict a number of factors such as growth or performance of the plant by looking at their leaf traits (Popma and Bongers 1988; Poorter and Bongers 2006). For instance, leaves in the understory receive a number of bursts of sunlight for a short duration throughout the course of the day. To avoid any wastage of light, plants could increase their rate of photosynthetic induction so that the leaves reach maximum photosynthesis faster. With more photosynthesis occurring, more carbon is consumed from the atmosphere for growth and maintenance of the leaves. Therefore, sun leaves are exposed to more light and so will assimilate more carbon than shade leaves; however, they will also require more carbon for maintenance of the photosynthetic machinery.

As the environmental conditions differ between primary and secondary forests, the floristic structure of the two types of forests is greatly different (Brearley et al. 2004). A recent study by Ngo et al. (2016) has shown that, although there are no signs of forest degradation within the rainforests of Singapore, there was a very high species turnover rate and fluctuation between the primary and secondary forest species. This could be linked to the different plasticity margins between plant traits and species. As some species of plants would have higher plasticity in their photosynthesis traits, for instance, they would be able to adapt to the high light environment of secondary forests and therefore dominate any primary forest species that are shade adapted and less plastic in the regenerating habitat. By looking at plant trait plasticity we can predict how likely it is
for a particular species to succeed in the changed habitat and ultimately determine the floristic change over patterns between primary and secondary forests.

The overall objective of this study was to compare photosynthesis characteristics between gap and understorey leaves of *Prunus polystachya*. The following predictions were made: 1) Understorey leaves will have a higher chlorophyll density; 2) Understorey leaves will have a higher net carbon fixation overall as they have a lower maintenance cost; 3) Understorey leaves will have a faster photosynthetic induction rate when compared to leaves from the gap environment.

**Methods**

**Study site and species**

The study site chosen for this study was the Bukit Timah Nature Reserve located in Singapore (1°21'14"N, 103°46'37"E). One of the least seasonal places in the world, it has an average temperature of 27°C and mean annual rainfall of 2,597 mm with no month receiving under 140 mm (Corlett 1990). The reserve has an area of 163 hectares and is home to 40 per cent of fauna and flora in Singapore, containing at least 2,100 native vascular plant species (National Parks Board 2018).

The species chosen for this study is the bat laurel, *Prunus polystachya*, an evergreen tree that is endemic to Singapore and occurs in both primary and secondary forests. *P. polystachya* can grow up to 36 m tall and in altitudes from sea level to 600 m (Kalkman 1998).

**Sample collection**

A total of 11 individuals were sampled, of which five were from gaps and six from understorey sites. One branch was collected from each individual, cut from as close to the main stem as possible. Once the sample was cut, the stem was immediately put under water and a further approximately 5 cm cut off to avoid embolism.

Gap environments were defined as individuals in an open area with no canopy directly above, while if there were no visible large gaps allowing sunlight through it was defined as an understorey environment.

Leaf age was controlled to minimise the variance between the chlorophyll density and leaf mass per area. From the branches collected, the second leaf from the tip of the branches was measured. Once the sample branches were in the bucket, chlorophyll densities were measured with a chlorophyll meter, three times for each leaf to get an average, from the area where the Li-Cor measurements were to be taken.

**Carbon fixation**

To measure the rate of carbon fixation, we used a Li-Cor 6400 infrared gas analyser (Li-Cor BioSciences, Lincoln, NE, USA) and put the leaves through cycles of high light and low light. First, we left the leaf to sit in low light until the photosynthetic rate was constant. We then put each leaf through three bursts of high light and low light cycles. We attempted to simulate an environment with sunflecks as leaves would experience in both
gap and understory leaves. Each high and low light interval was 30 seconds long, and the high light intensities were 1,500, 500 and 1,300 \mu mol m^{-2}s^{-1} while the low light intensity was held constant at 10 \mu mol m^{-2}s^{-1}. The sum of each maximum point of carbon assimilation rate was calculated for each individual leaf to give an indication of the total carbon fixation.

**Photosynthetic induction measurement**

The Li-Cor 6400 was used to measure the photosynthesis characteristics of the samples. The sampled leaves were first left on low light setting of 10 \mu mol m^{-2}s^{-1} for at least 5 minutes until the photosynthesis rate was at constant before the light conditions are changed. This is similar to the previous experiment with the difference that high light intensities were constant throughout this part of the experiment, as we were trying to keep the curves looking similar so that the induction rate would be the same.

To measure the photosynthesis induction rate, the samples are put through three cycles of one minute of high light (1,000 \mu mol m^{-2}s^{-1}) and then one-minute low light (10 \mu mol m^{-2}s^{-1}). To calculate the induction rate, we measure the time it took to reach the maximum rate of photosynthesis and determine the halfway point and the time it took to reach that halfway value of the maximum photosynthesis rate of that particular curve.

**Statistical analysis**

Three measurements of chlorophyll content were recorded for each sample using a chlorophyll meter, and an average taken. The average was then calculated to get one value for each gap and understory environment and an unpaired t-test was conducted to determine if there is a significant difference between the two averages. Standard deviation was also calculated for both gap and understory to see the spread of data within each category.

For both the photosynthesis induction and carbon fixation experiment, an average for gap and understory environments were calculated and once again using an unpaired t-test to determine if there is a significant difference between the collected data between gap and understory leaves. All analyses were conducted in R 1.1.463 (R Development Core Team 2018).

**Results**

Chlorophyll content differed between gap and understory leaves – understory leaves had a higher chlorophyll content (Figure 1; \( p < 0.05 \)). In terms of photosynthesis rate, gap leaves had a much higher fluctuation in the rate of photosynthesis in response to changing light levels when compared to understory leaves. Leaves from both gap and understory habitats also had different photosynthetic rate at different light levels (Figure 2). Gap leaves also respire at low light levels, and that was not observed in the understory leaves, and that was also consistent with the total carbon fixed as understory leaves had assimilated a much higher amount of carbon (Figure 3; \( p < 0.05 \)).

For photosynthetic induction rate, there was an average of approximately 1.5 s difference between the leaves from the two environments. However, this was not found to be a
significant variation in the time taken to reach 50% of maximum rate of photosynthesis between the gap and understorey leaves (Table 1; Figure 4; \( p > 0.05 \))

Figure 1: Chlorophyll density (mg/m\(^2\)) of Prunus polystachya in different light environments. Error bars represent standard deviation; \( p < 0.01 \).

Figure 2: Photosynthesis rate over time with changing light intensities; three high and low light cycles, low light: 10 \( \mu \text{mol m}^{-2}\text{s}^{-1} \), high light: 1,500, 500 and 1300 \( \mu \text{mol m}^{-2}\text{s}^{-1} \) in that order.
Figure 3: Total carbon fixed by gap and understorey leaves in the three burst light cycles (30 seconds of high light and 30 seconds of low light). Error bars represent standard deviation ($p = 0.048$).

Table 1: Time taken for each sample leaf to reach 50% of maximum rate of photosynthesis. Standard deviation next to the averages ($p = 0.088$).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gap 1</th>
<th>Gap 2</th>
<th>Gap 3</th>
<th>Under 1</th>
<th>Under 2</th>
<th>Under 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to reach 50% (s)</td>
<td>13</td>
<td>4</td>
<td>7</td>
<td>12</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Average (s)</td>
<td>8 (SD 4.58)</td>
<td>9.67 (SD 2.08)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4: Average time taken for leaves in gap and understorey to reach 50% of maximum rate of photosynthesis ($p = 0.088$).
Discussion
This study aimed to investigate how gap and understory leaves of *P. polystachya* differ in their photosynthetic characteristics. The following hypotheses were proposed: 1) understory leaves will have a higher chlorophyll density; 2) understory leaves will have a higher carbon fixation overall; and 3) understory leaves will have a faster photosynthetic induction rate when compared to leaves from the gap environment. The data collected were consistent with hypotheses one and two. However, hypothesis three was rejected as understory leaves did not have a faster photosynthetic induction rate.

Chlorophyll density
The results obtained were consistent with the hypothesis that leaves from the understory contained a higher chlorophyll content compared to leaves from gaps (Figure 1; $p < 0.05$). This is also supported by past findings (Brand 1997; Lichtenthaler *et al.* 2007). Shade leaves need a higher chlorophyll concentration, particularly chlorophyll b, in order to maximise the little sun they get (Lüttge 2014). This result suggests that the samples collected were from different light environments, as they have adapted to their respective light conditions.

Carbon assimilation
The hypothesis that understory leaves would fix more carbon was demonstrated through the summation of all the recorded data points of the rate of photosynthesis (Figure 3; $p < 0.05$). Although gap leaves have a higher maximum photosynthesis rate, as they are exposed to more sunlight throughout the day, the steeper gradients also suggest a faster induction rate (Figure 2). However, as sun leaves are exposed to a higher light intensity overall, their photosystems would require more energy for maintenance (Lüttge 2014). Therefore, in Figure 2 the gap leaves also show a negative photosynthetic rate during low light bursts, as they utilised that time to repair and maintain their photosynthesis machinery at a cost of using up CO$_2$. The results in Figure 2 do not indicate that understory leaves do not respire, it just was not observed in those light bursts.

Photosynthetic induction
The idea that gap leaves have a faster photosynthetic induction rate than understory leaves was suggested by Figure 2, as they had steeper slopes when reaching their maximum rate of photosynthesis. This was suggested after the calculation of time taken to reach 50% of maximum photosynthesis by each leaf. Gap leaves were approximately 1.5 seconds faster to reach the 50% mark than shade leaves; however, the induction rates were not significant ($p = 0.088$). We can conclude that in the case of *P. polystachya*, the hypothesis of understory leaves having a faster photosynthetic induction rate is rejected.

It is estimated that sun flecks provide up to 80 per cent of the light that an understory plant receives, contributing 30–60 per cent of the total daily carbon gain for shade plants (Pearcy 1987; Way and Pearcy 2012). For that reason, it is generally assumed that understory plants will have a faster induction rate to maximise the sunlight they receive (Küppers and Schneider 1993; Yanhong *et al.* 1994). However, it has been suggested in previous research that some species have a similar induction rate in the sun and shade.
individuals (Rijkers et al. 2000), or even that sun individuals have a faster photosynthetic induction rate (Han et al. 1999; Tausz et al. 2005; Bai et al. 2008). Currently, too few studies have been done on *P. polystachya* to determine whether this is the case or not.

Another possible explanation for the lack of significant difference between the results could be related to the stomatal opening of the leaves. As suggested by Valladares et al. (1997), there is a significant correlation between the rate of photosynthetic induction and the initial stomatal conductance in low light. During their study, the samples were collected from the trees at different times of the day, so each branch had a different period of time in the bucket before the measurements were taken. All of these factors could potentially affect the stomatal opening of the leaves before the measurements of photosynthetic induction.

Sample size and equipment constraint could have also affected the results. Due to time constraints, we were only able to collect a small sample pool. Also, we were not able to sample above a height of ~5 m, which eliminated that portion of individuals whose lowest branch was higher than this. This may have led to the elimination of older trees, so plant age may need to be a factor for consideration also.

**Future directions and broader ecological context**

In the case of *P. polystachya*, it would be worthwhile to further study the species’ photosynthetic characteristics, as they occur in both primary and secondary forests, they would be a good model species. Also, there has been a distinct lack of studies on this species, and as this study has shown, there are still a lot of gaps to be filled. Furthermore, as different species would react differently in their own environments, it would also be important to investigate other species and see how they differ to *P. polystachya*.

The photosynthesis taking place within leaves is one of the most crucial factors for plant growth and survival. However, the success of the whole plant also depends on a range of other plant systems, such as root systems and water uptake ability by different species, how leaves come together to form a structure, effective canopy, and even the interaction between other plants and herbivores might all determine a species success in their habitat (Givnish 1988; Steinbrenner et al. 2011).

Secondary forests are a much higher light environment than primary forests, and with increased light there will be more photosynthesis and carbon gain overall, leading to greater growth rate and energy produced. At the moment *P. polystachya* is increasing in abundance in the secondary forests of Singapore (Wong 1987), showing that they are adapting to the changing physical conditions. By looking at plant functional traits we can determine why different species excel under different conditions. With that information, we will be able to determine the species composition in both primary and secondary forests and the potential species turnover rate.

In terms of conservation, we will need to adopt a more general approach rather than looking at individual species. This is especially important as climate change is currently happening all around us, and it is heavily affecting not just plants in the rainforests, but biodiversity all around our world (Araújo and Rahbek 2006; Bellard et al. 2012). With climate change our rainforests will experience an increase in temperature, increasing atmospheric CO₂ concentration, more variable rainfall pattern and an increased
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frequency of fire and storm (Pawson *et al.* 2013). By looking at the species composition of whole forests we can see which species are better at dealing with these changing abiotic conditions and are more likely to succeed in the future. This would not only allow us to determine which species we should prioritise when manually restoring secondary forests that have been disturbed, but also which species in the primary forests are most likely to be under threat as the environmental conditions change and therefore should be protected.

**Conclusion**

*Prunus polystachya* does demonstrate some plasticity in photosynthesis characteristics between gap and understorey environments. Individuals in low light environments showed a higher chlorophyll density in order to maximise the little sunlight they receive over the course of the day. Understorey leaves also assimilated a higher amount of carbon when compared to gap leaves, probably due to the fact that gap leaves are exposed to higher radiation and therefore will need to expend more carbon for the maintenance and repair of their photosystems. Even though our third hypothesis of understorey leaves having a faster photosynthesis induction rate was rejected, there are a few explanations that should be taken into account for the non-significant results. These results could potentially contribute to the determination of species success in their environment and, ultimately, mapping of species composition of primary and secondary forests. Through species composition and prediction of success we will be able to predict the species turnover rate between primary and secondary forests, and therefore play a role in the conservation and regeneration of rainforests in South-East Asia, which are currently under threat due to human activity and climate change.

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**References**


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