Photosynthetic capacity and water use efficiency of *Argyrodendron peralatum* leaves across a vertical gradient

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Abstract

A simple model of rainforest resource allocation is the Big Leaf Theory model. Rainforest ecosystems distribute resources to the canopy in order to maximise the benefits of increased irradiance exposure. *Argyrodendron peralatum* is an Australian tree species, endemic to Far North Queensland. Through analysis of photosynthetic capacity and stomatal capacity, which also allows analysis of water use efficiency, across understorey, mid-canopy and top-canopy levels, it was shown that *A. peralatum* follows the Big Leaf Theory model; performing these functions at higher rates at the top-canopy level.

Key Words: Big Leaf Theory, resource allocation, water use efficiency

Introduction

Tropical rainforest ecosystems are sites of extreme diversity, responsible for 59 per cent of global forest vegetation (Dixon *et al.* 1994). The rainforests of Far North Queensland contain a high number of endemic plants, including a large Gondwanan element and the world's highest concentration of ancient angiosperms (Wet Tropics Management Authority 2014). It is important to understand how these rainforest systems function, in order to predict their responses to changing climates and the potential role of these rainforest systems as carbon sinks (Dixon *et al.* 1994).

Rainforest ecosystems contain many microenvironments relating to vertical canopy position and sun exposure (Pearcy 1987). Plants adapt to these microenvironments with individuals adjusting their photosynthetic capacity to the light environment in which they develop, through phenotypic plasticity (Leakey *et al.* 2003; Meir *et al.* 2002). Photosynthetic capacity is linked to resource allocation, especially that of nitrogen and water. One model of rainforest resource allocation is the Big Leaf Theory (BLT). This model suggests that rainforests act like one big leaf, allocating resources to the canopy position in order to maximise photosynthetic processes where photosynthetic photon flux density (PPFD) is the highest, and thus a single photosynthetic rate can be applied to all canopy leaves with minimal adjustment (Sellers *et al.* 1992 in Carswell *et al.* 2000; Field 1983; Friend 2001).

Photosynthetic capacity varies across vertical position, with understorey plants producing more shade leaves than canopy leaves; plants acclimatise to changes in sun

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exposure due to growth or canopy gaps opening by restructuring photosynthetic apparatus or producing more sun-leaves (Lüttge 2008). Canopy-level leaves are usually associated with higher photosynthetic capacity as a result of higher PPFD exposure (Carswell *et al.* 2000). Photosynthetic capacity is linked to stomatal conductance, which relates to a chloroplast's ratio of CO_2 to oxygen, which affects the biochemistry of photosynthesis; higher photosynthetic capacity is associated with higher stomatal conductance (Easlon *et al.* 2015). Photosynthetic capacity and stomatal conductance are further linked through their contribution to water use efficiency (WUE). WUE is a measure of the efficiency of how water is used to assimilate carbon (Tambussi *et al.* 2007) and is calculated by examining the relationship between photosynthetic capacity (A) and stomatal conductance (g_s) (Equation 1).

Photosynthetic capacity, stomatal conductance and water use efficiency rely on essential nutrients such as nitrogen, and thus act as an indicator of how plants are allocating resources. According to the BLT of resource allocation in rainforests, these functions would be highest in top-level canopy leaves. These leaves experience higher PPFD exposure, allowing greater benefits to be drawn from resource allocation to these leaves.

This paper focuses on *Argyrodendron peralatum*, an evergreen tree species endemic to north-east Queensland (Commonwealth Scientific and Industrial Research Organisation 2010) and characterised by trifoliate leaves, with a pale yellow abaxial surface and a typical diameter increase of 3 mm/year (Cernusak pers. comm. 2017).

Hypothesis

This paper aims to determine if a relationship exists between *A. peralatum* leaves' canopy position and photosynthetic capacity, stomatal conductance, and water use efficiency. According to the BLT model, we would expect that *A. peralatum* has increased photosynthetic capacity, stomatal conductance and WUE with height increases. This research is intended to assess the applicability of the BLT model in the rainforests of Far North Queensland as the basis for further research.

Method

Field location and sample selections

All samples were collected from the vicinity of the canopy crane at the James Cook University's (JCU) Daintree Rainforest Observatory (DRO). Understorey samples were collected in the period 28 to 30 June 2017 from saplings easily accessed from the walking track. Canopy samples were collected in the same period using the canopy crane.

In the understorey, seven *A. peralatum* specimens were located and sampled. Multiple leaves were selected from some specimens, resulting in a total of 10 understorey samples. Six mid-canopy and six top-canopy leaves were collected from four *A. peralatum* individuals, which were identified using the DRO database crane plot map. In total, 22 samples were collected, including understorey, mid-canopy and top-canopy samples.

Leaf samples were chosen on the basis of their condition, prioritising those with minimal damage and discolouration, and an effort was made to select the youngest fully expanded leaf. Understorey leaves were sampled while still attached to the plant. Midand top-canopy samples were taken from branches cut and placed in buckets of water before being brought to the ground. These samples remained in buckets of water overnight, and stems recut under water immediately prior to testing in order to minimise the occurrence of embolism.

Photosynthetic capacity

Using a Li-Cor 6400XT (LI-COR Inc., Lincoln, NE, USA) portable photosynthesis system, leaves were exposed to 1,500 μmol m^{-2} s^{-1} of light, 400 parts per million (ppm) of carbon dioxide (CO_2), and a flow rate of 500 μmol s^{-1}. A light intensity of 1,500 μmol m^{-2} s^{-1} is higher than understorey leaves would normally be exposed to, and was considered sufficient to saturate both understorey and canopy leaves (Doley *et al.* 1988). Leaves were surface dried prior to testing. The Li-Cor 6400's graphic display was used to observe the photosynthetic and stomatal conductance rates of each leaf; once these graphs appeared to stabilise, indicating photosynthetic capacity was reached, the infrared gas analyser was matched to ensure accurate CO_2 readings, and the data logged. Samples were then removed from the Li-6400 chamber, and removed from the sapling or branch.

Leaf thickness

To further explore leaf differences along a vertical gradient, and to ensure the relationship between photosynthetic capacity and leaf thickness was taken into account, leaf thickness was measured using a micrometer. The thickness measurement was taken on the lamina, between veins, to ensure an accurate reading. Measurements were recorded to the nearest 0.05 mm.

Statistical analysis

To analyse difference between the photosynthetic capacity of each vertical position, least square means and a Tukey's honestly significant difference test (HSD) were used. An analysis of variance (ANOVA) was used to examine stomatal conductance across different levels, and a HSD was used to analyse the relationship between stomatal conductance and photosynthetic capacity across different levels in order to understand WUE. For the purposes of this paper, WUE is defined as the CO_2 assimilated, as indicated by photosynthetic capacity (A), divided by transpired water, indicated by stomatal conductance (g_s) of a leaf sample, as in Equation 1 (Cernusak pers. comm. 2017).
Equation 1

$$WUE = \frac{A\ (\mu mol/m^2/s)}{\mathrm{g_s}\ (mol/m^2/s)}$$

Results

Samples from the top-canopy position had the highest maximum photosynthetic capacity (A_{max}; Figure 1). followed by mid-canopy position had the second highest A_{max}. The A_{max} of the understorey was significantly lower than the mid-canopy (p = 0.0043), and the A_{max} of the mid-canopy was significantly lower than the top-canopy (p = 0.0398).

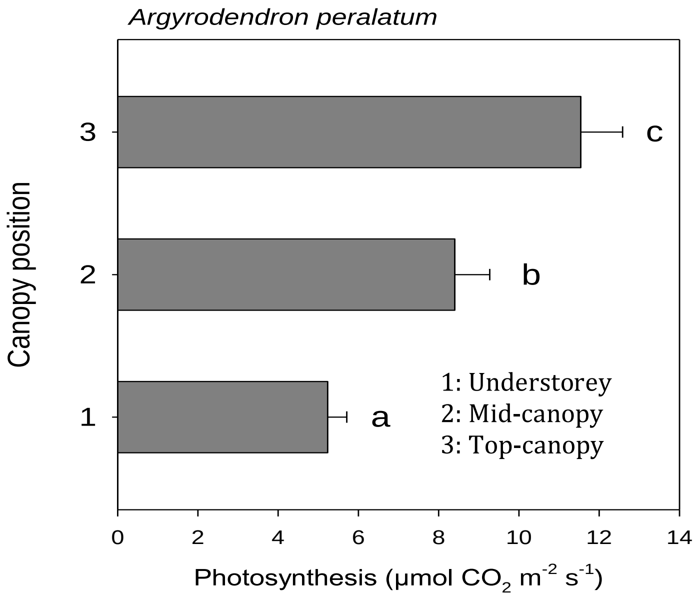

Figure 1: Mean Photosynthetic Capacity across differing canopy positions, with letters indicating significant difference.

WUE varied between understorey and canopy leaves. The raw mean of understorey WUE was 66.63; mid-level canopy was 127.293; top-level canopy was 116.618. No difference was found between the WUE of mid-canopy and top-canopy samples ($p = 0.925$), although the understorey WUE was different from the canopy positions. Figure 2 shows the photosynthetic capacity and stomatal conductance of each sample, placed on a scatter plot with a line of best fit; canopy data was combined as there was no difference in WUE between canopy levels. There are two distinct lines of best fit, illustrating the difference in groupings.

Understorey leaves were the thinnest, with a mean of 0.22 ± 0.03 mm. Mid-canopy leaves had a raw mean of 0.36 ±0.04 mm, and top-canopy leaves had a raw mean of 0.34 ± 0.01 mm.

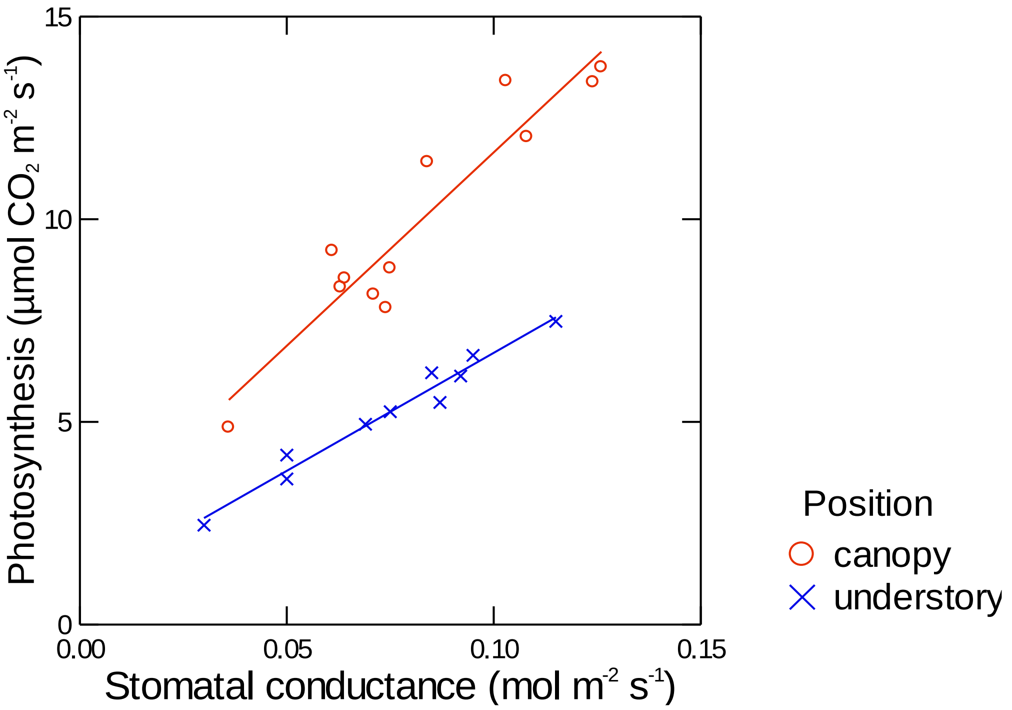

Figure 2: Difference in water use efficiency rates between canopy and understorey samples.

Discussion

The results of this study support the BLT of rainforest resource allocation among species in the rainforests of tropical Far North Queensland. Photosynthetic capacity and WUE of *A. peralatum* increased with height. Analysis of gas exchange data showed a significant difference in these functions as undertaken by understorey and canopy-level plants, as well as a significant difference in some of these functions between the mid- and top-canopy samples. *A. peralatum* distributed resources as hypothesised, suggesting that the BLT can be applied to the rainforests of Far North Queensland.

Understorey leaves were visibly larger, darker green and thinner than top-canopy leaves. Understorey leaves were approximately twice the size of top-canopy samples. This is likely to provide an increased surface for photosynthesis, as the capacity of the leaf’s photosynthetic structures is lower in the understorey due to reduced light exposure; in order to maximise use of sun flecks, understorey plants develop larger leaves. The darker pigmentation is also related to photosynthetic efficiency – darker colouring indicates denser chlorophyll, suggesting the plant is maximising its response to limited light exposure. Despite these characteristics, photosynthetic capacity was recorded as being highest in the top-canopy, and higher in the mid-canopy than in the understorey; higher PPFD exposure has allowed these leaves to develop a higher photosynthetic capacity. The difference between canopy levels suggests that fully developed *A. peralatum* trees still channel resources to their uppermost leaves: those found in the top-canopy position. This fits the BLT model of resource allocation, with individual trees within a tropical rainforest ecosystem allocating resources in the same manner. Canopy leaves are thicker than understorey leaves as they are exposed to higher irradiance, and therefore need more protection for internal structures.

Using the Li-6400, leaf samples were exposed to 1,500 µmol m^{-2} s^{-1} of light. This is roughly equivalent to three-quarters of full sunlight (Cernusak 2017), allowing samples to quickly reach light saturation point, or photosynthetic capacity. Most understorey leaves would not usually be exposed to this level of irradiance, existing instead on lower intensity light from gap openings and sun flecks. It is possible that the samples were adversely affected by this sudden, intense exposure, having not adapted to or developed in a high irradiance environment; this could have affected our findings. Future work should allow understorey leaves sufficient time to equilibrate. However, the significant difference between understorey and mid-canopy photosynthetic capacity suggests that any errors in data logging have not influenced our analysis detrimentally – as an indication of where most of *A. peralatum*'s photosynthesis occurs, our data remains valid.

The increase in WUE among samples taken from higher vertical positions may be attributable to hydraulic pressure. The understorey saplings were less woody and dense, with much thinner and younger stems; both mid- and top-canopy samples came from thick, woody branches. Increased trunk diameter likely indicates a greater volume of xylem, which contributes to higher WUE. The older, more developed trunk and branches of plants, which had reached canopy level possibly, contained more lignin and cellulose, further contributing to xylem structure and function. Leaves higher along the vertical gradient were also, obviously, placing pressure on the plant to transport water further and higher. Canopy level samples were removed from branches that had recently been removed from the tree itself and placed in water. This may have triggered a trauma response; however, the immediate placement in water and recutting under water prior to measuring the gas-exchange may have solved this issue.

There were no notable outliers in the dataset, despite data being collected by separate groups across different days. This suggests that the method was sound. The weather and sampling-time did vary, both across days and on individual days – this may potentially have influenced the photosynthetic capacity, due to varying daytime exposure to PPFD, but given the data appears unaffected and samples were treated to the same 1,500 µmol m^{-2} s^{-1} of light within the LI-COR chamber, it does not appear to have unduly affected the results. Increased sample size would contribute to this study, reducing concern about faulty sample collection and increasing the accuracy of the data analysis. Similar studies in other tree species would also contribute to an understanding of the mechanisms and applicability of BLT to Far North Tropical Queensland rainforests.

Samples taken from multiple *A. peralatum* individuals displayed the resource allocation expected under a BLT model. This study has offered an indication of the applicability of BLT, but in order to further understand how plant species in Far North Queensland, as well as the rainforests itself, distribute resources across a vertical gradient, more research is needed. Research should focus on widening the scope of understanding, sampling more species native to Far North Queensland rainforests and collecting samples from across the rainforest, expanding from the DRO crane plot. This sampling should look at the functions studied in this paper, in conjunction with more detailed nutritional analyses of where resources such as nitrogen are allocated.

Conclusion

This study shows that there is a relationship between the vertical position of *A. peralatum* leaves and their photosynthetic capacity, stomatal conductance and water use efficiency. Top-canopy leaf samples have a higher maximum photosynthetic capacity than mid-canopy samples, which in turn have a higher capacity than understorey leaf samples. Stomatal conductance was highest in the top-canopy samples, although it was not significantly different from mid-canopy stomatal conductance. Stomatal conductance was significantly lower in understorey samples. Water use efficiency was higher in the canopy than in the understorey, and there was no significant difference between top and mid-canopy levels. *A. peralatum* follows the Big Leaf Theory model of resource allocation, with individuals (and, by extension, the rainforest ecosystem) allocating resources to the canopy in order to maximise access to higher Photosynthetic Photon Flux Density available to canopy leaves, in turn maximising energy producing functions.

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