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Changes in Leaf Characteristics with Tree Age in *Dryobalanops beccarii* Dyer in a Restored Forest of Sarawak, Malaysia

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Abstract: Study on leaf properties of an endangered dipterocarp tree species, *Dryobalanops beccarii*, was conducted in a restored forest. The study focused on the morphology, nutrient concentration and gas exchange in well-developed leaves of *D. beccarii* at different ontogenetic phases. The hypothesis tested that leaf physiological and morphological changes are related to the nutrient concentration level of *D. beccarii* leaves in each ontogenetic phase. Seedling leaves were small and thin, whereas adult tree leaves were the large and thick. The stomata density per unit was abundant in adult tree leaves as compared to seedlings and saplings leaves. The net photosynthetic rate (P_n), transpiration rate (A) and stomatal conductance (g_s) was increased from the seedling phase until the sapling phase, but decreased as the *D. beccarii* reached the mature phase. Scarce stomata density in seedling leaves contributed to low P_n , A and g_s , while sapling leaves recorded opposite results due to abundant stomata density accompanied by highest accumulation of nitrogen (N) content per unit leaf area. The low concentration of nitrogen (N), phosphorus (P) and magnesium (Mg) detracted the gas exchange performance. Hence, changes in leaf morphology and physiology of *D. beccarii* were affected by the differences in resource used at each tree ontogenetic phase.

Keywords: *Dryobalanops beccarii*, Leaf Morphology and Physiology, Leaf Nutritional Status, Ontogenetic Phase

Introduction

The morphological and biological changes of a tree become more complex as the tree grows and aged. In various environment conditions across each ontogenetic phase, the tree increases in size and develops more multipurpose structures (Coste *et al.*, 2009). The changes in traits was experienced by trees in order to maximize fitness in an unpredictable environment (Bazzaz, 1991). The development of tree structures greatly affects the flexibility of leaves morphology and physiology properties. Leaves are of essential importance to a tree, representing their ability to generate power and act as the sensor of the tree towards changes and adaptation in various conditions. Leaf shapes, sizes and durability may absolutely differ as the tree age (Kerstiens, 1996b).

The changes in leaf traits influence the gas exchange performance. The flattened surface gather energy

efficiently from sunlight and the underside absorb CO_2 and release O_2 . The thickness of the leaf is optimally minimized, to ease gases diffusion process. On the exterior, leaf has epidermal cell layers that secrete waxy and impermeable cuticle for protection against dehydration (Kerstiens, 1996a). The internal parts of the leaves such as the number of stomata and chlorophyll are directly affecting the gas performance rate. The distribution of stomata vary among leaves depending on the exposure to the environment condition. The abundant number of stomata may increase the photosynthesis, stomatal conductance and transpiration rate (Lawson and Blatt, 2014). However, as the tree aged, the stomatal conductance, photosynthetic rate and transpiration rate vary due to leaves exposure to unpredictable environment conditions in the rainforest and complex tree structures (Day *et al.*, 2001; Hubbard *et al.*, 2001; Yoder *et al.*, 1994).

Apart from leaves morphology, the macronutrient

mobilization process between leaves is vital in determining the increase and decrease of leaves physiology performance. The mobility of each macronutrient differs depending on the degree of deficiency in the leaves (Avice and Etienne, 2014; Fischer, 2008). The mobile macronutrients such as N, P, K and Mg are readily translocated from old leaves to new growth, leading to deficiencies in older leaves. While immobile macronutrient, Ca, do not appear to be retranslocated from old leaves to new growth under any circumstances causing the deficiency symptoms to occur generally in young growing areas of the tree (Maillard *et al.*, 2015; Ohe *et al.*, 2005).

Current study involved a dipterocarp tree species, *Dryobalanops beccarii* Dyer. This species can be found in the south-east of Johore, Peninsular Malaysia and Borneo Island and locally known as kapur merah, kapur bukit and keladan. In the natural forest, this species is a large emergent tree, up to 65 m tall and usually can be found on shallow leached soils of mixed dipterocarp forests (Ashton, 2004). In Sarawak, *D. beccarii* is labeled as a vulnerable species, however, nationally and globally, this species is categorized as an endangered species (Ashton, 2004; Julia *et al.*, 2014).

This study was conducted to understand the leaves morphology and physiology responses in a restored forest. The chronosequence method by sequencing observations at different tree growth development phase of *D. beccarii* was applied in multi-cohort stands in the Universiti Putra Malaysia (UPM)–Mitsubishi forest restoration area in Bintulu, Sarawak (Fukami and Wardle, 2005). Hence, the aim of this study was to observe the changes in leaf properties of *D. beccarii* in four different groups of tree stand ages.

Materials and Methods

Study site

The study was conducted in the UPM–Mitsubishi forest restoration area in UPM Bintulu Sarawak Campus, Sarawak, Malaysia (3°12'N, 103°04'E). The site is situated 60–110 m above sea level with a mean annual rainfall of 2327 mm. The soil in the area is acidic and classified as Isohyperthermic Typic Dystropept known as the Nyalau soil series (Ng *et al.*, 2012). The forest area covers approximately 47.5 ha.

Universiti Putra Malaysia and the Yokohama University of Japan had conducted a research partnership in 1990 with financial support from the Mitsubishi Corporation, Japan to restore abandoned or degraded area (Ng *et al.*, 2012). This project adopted Prof. Akira Miyawaki's ecological technique (Miyawaki, 1999). A

collection of indigenous tree species from the nearby natural forests had been used to create a multiple species native forest. Selection of tree species was based on vegetation analyses of nearby areas. Seedlings were densely planted with three plants m⁻² and covered with thick mulch after planting without fertilization.

In this study, four different aged group stands were selected, i.e., newly planted-5 year old, 6-10 year old, 11-15 year old and more than 16 year old. The study plots were established where *D. beccarii* was previously planted i.e., P20, plot of 20 years old (established in 1992) located in Phase II and P1, P9 and P14, three plots of 1, 9 and 14 year old respectively (established in 2011, 2003 and 1998) located in Phase IV of the restoration project area. Size of each plot was approximately 30×10 m. In each study plot, four seedlings, saplings and mature trees of *D. beccarii* were selected for measurement. Assessment of tree growth performance was executed every six months for 18 months.

Study of Leaf

The study was performed twice (Mac–June 2012 and July–September 2013) and was carried out in the laboratory. The length and width of leaves were measured. The leaf area was measured using LI-3100C Area Meter (LI-COR Biosciences, Lincoln, NE). The leaves were cut into 1×1 cm in order to get images of the cross section, adaxial and abaxial surfaces using an Olympus Fe-320 digital camera. The stomata density and leaf thickness were determined using NIS-Elements Advanced Research Microscope Imaging Software.

Leaf physiology involved measurement of photosynthetic rate, transpiration rate and stomatal conductance. The leaf gas exchange measurement was conducted during a clear day, between 7.30 and 11.30 am using LCpro⁺ Portable Photosynthesis System (ADC BioScientific, Hoddesdon, Herts). The measurement of the leaf gas exchange was divided into ex-situ and in-situ method. The in-situ method was applied on the sapling in P1 where only eight leaves were used for the measurement. The ex-situ was applied for leaves from trees in P9, P14 and P20, where the gas exchange was measured from freshly cut stems. Four fully expanded leaves from each canopy level were selected for the measurement.

The chemical analysis of the leaf was carried out through the wet digestion method. The nutrient elements assessed were N, P, K, Mg and Ca. The leaves were oven-dried at 70°C until constant weight was attained and grounded afterward. A 0.2 g of grounded leaves and 5 ml concentrated H₂SO₄ was added in each digestion tube. The samples were heated in the digester. The temperature of the digester was set to increase slowly from 100°C for one h and to 350°C for three h. The samples were left to cool down at room temperature before distilled water was added up to 100 ml. The solution was analyzed using Atomic

Absorption Spectrophotometer (AAS Analyst 800, Perkin Elmer Instruments, Norwalk, CT) for Mg, Ca and K and AutoAnalyzer 3 HR (Seal Analytical, Park Gate, Hants) for N and P.

Data Analysis

Differences between the mean values were evaluated using a one-way analysis of variance (ANOVA). Comparison among the means was performed using the Tukey's test and t-Test, calculated at $P < 0.05$. Growth models were used in order to determine the most fitted and accurate model on each leaf properties related with age. The models were tested via Linear and Non-Linear (NLIN) procedure. Statistical analysis was performed using SAS for Windows Version 9.13.

Results

Leaf morphology

The leaf morphological parameters increased with age of *D. beccarii*. The leaf area increased slowly as the tree aged. The relationship between leaf area and the stand age was significant ($P < 0.05$), with $R^2 = 0.99$ (Fig. 1). Leaf area and width in the upper canopy of P14 and P20 was significantly larger ($P < 0.05$) than P1 and P9 (Table 1). Significant difference ($P < 0.05$) was observed between early and final assessment, where leaf width at the beginning was significantly wider ($P < 0.05$) than the final measurement (Table 1). Similar as the leaf area, leaf width also increased with age (Fig. 1). However, since significant different was observed between assessments, two different growth models were developed to show relationship between stand age and leaf width. Both models were significant ($P < 0.05$), with $R^2 > 0.94$ (Fig. 1).

The leaf length increased with time and the relationship was significant ($P < 0.05$), with $R^2 = 0.99$ (Fig. 1). The leaf length in both upper and lower canopy in P20 was significantly longer ($P < 0.05$) than other stands (Table 1). Leaf thickness in P1 was the lowest among the tree stands (Table 1). The thickness of leaf increased slowly with time and their relationship was significant ($P < 0.05$), with $R^2 = 0.99$ (Fig. 1). Stomata density was the highest in the lower canopy of P14 and significantly higher ($P < 0.05$) than P20 for both early and final assessments. There was significant difference ($P < 0.05$) between the assessments, where the initial measurements recorded higher stomata density than the final measurement (Table 1). The relationship between stomata density and stand age was significant for both initial and final measurement $R^2 = 0.99$ (Fig. 1). Both relationships showed that stomata density increased when stand age.

Leaf physiology

The rate of photosynthesis and transpiration as well as stomatal conductance were initially increased but

decreased later when the stand got older (Fig. 2). Initial measurement recorded the lowest photosynthetic rate in the upper canopy in P1 ($2.12 \mu\text{mol m}^{-2} \text{s}^{-1}$), while in the upper canopy in P9 recorded the highest photosynthetic rate in final measurement ($3.50 \mu\text{mol m}^{-2} \text{s}^{-1}$). Significant difference ($P < 0.05$) was observed between assessment period where lower photosynthetic values were recorded in the final assessment of P14 and the upper canopy of P20 (Table 2). The relationship between photosynthesis rate and stand age in both measurements were significant, with $R^2 = 0.99$ (initial) and $R^2 = 0.63$ (final) (Fig. 2).

Transpiration rate in the initial measurement showed that P20 at the upper canopy was the lowest (Table 2). Similar to photosynthesis rate, the relationship between transpiration rate and stand age was significant, with $R^2 = 0.65$ (Fig. 2). Similar trend as photosynthetic and transpiration rate, after increasing to the maximum at the age of nine year, stomatal conductance value started to reduce thereafter (Fig. 2). In the initial measurement, comparison between stand age showed that stomatal conductance of the upper canopy of P1 and P20 was significantly lower ($P < 0.05$) than P9 (Table 2).

Leaf nutrient concentration

The concentration of N decreased as stand aged and the relationship between leaf N and stand age was significant, with $R^2 = 0.99$ (Fig. 3). In both initial and final assessments, the upper canopy of P9 recorded the highest N. Significantly higher ($P < 0.05$) N was found in the initial assessment of P1 than the final assessment (Table 3). The reduction of 59.6% in the upper canopy of P1 was significantly lower ($P < 0.05$) than other stands (Table 3). The concentration of P showed similar reduction trend as leaf N (Fig. 3). Leaf P was similar (0.94–1.12%) for all stands and canopy levels throughout the assessment period.

The concentration of leaf K increased slowly over time and their relationship was significant with $R^2 = 0.99$ (Fig. 3). The lower canopy of P14 recorded almost twice the amount of K found in P20 (Table 3). Significant difference ($P < 0.05$) was also found in K between the upper and lower canopies of P20 in the final measurement. In the upper canopy, significantly higher ($P < 0.05$) P was recorded in P14 as compared to P1 and P9 (Table 3). In the initial measurement, leaf Mg showed a decrease trend over time (Fig. 3), where P20 was significantly lower ($P < 0.05$) than P1 and P9 (Table 3). The relationship between leaf Mg and stand age was high with $R^2 = 0.82$ (Fig. 3). The Ca concentration increased with time and their relationship was significant with $R^2 = 0.68$ (Fig. 3). In the final measurement, P20 recorded the highest Ca concentration (Table 3). Significant difference ($P < 0.05$) was observed between the two assessments with the initial assessment recording lower Ca concentration than the final assessment for P20 (Table 3).

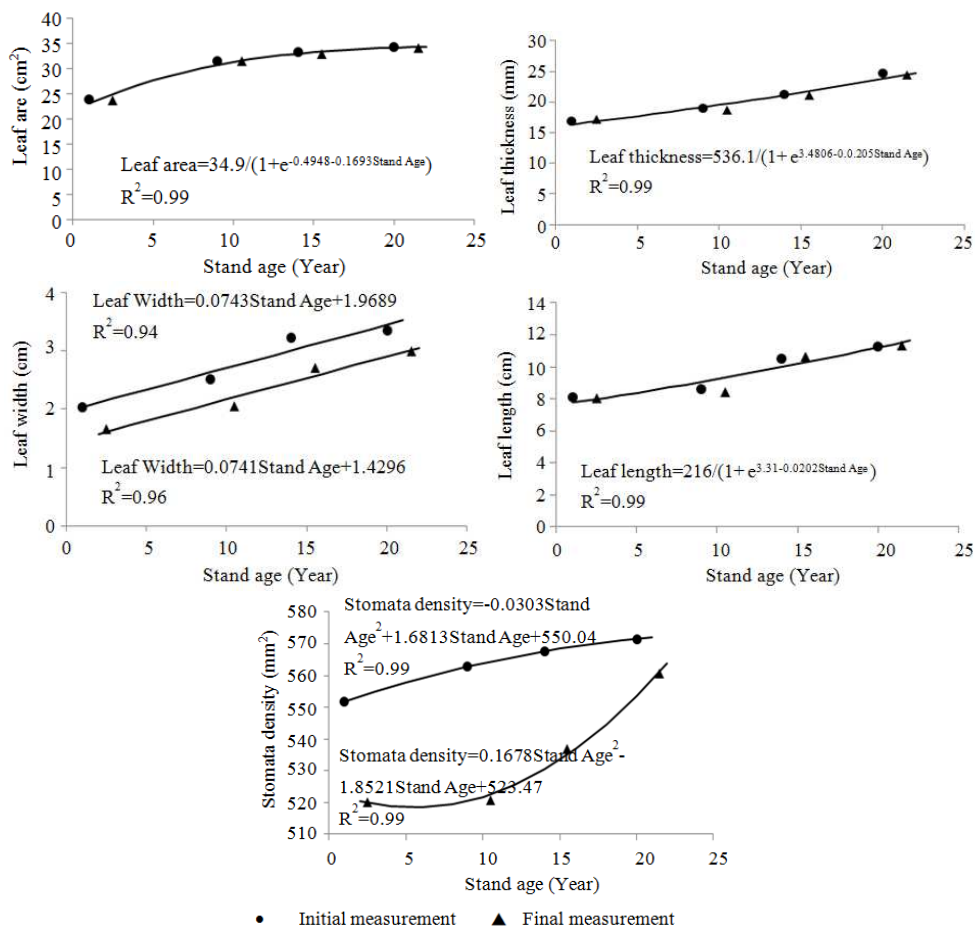


Fig. 1. Relationships between *Dryobalanops beccarii* leaf parameters and stand age

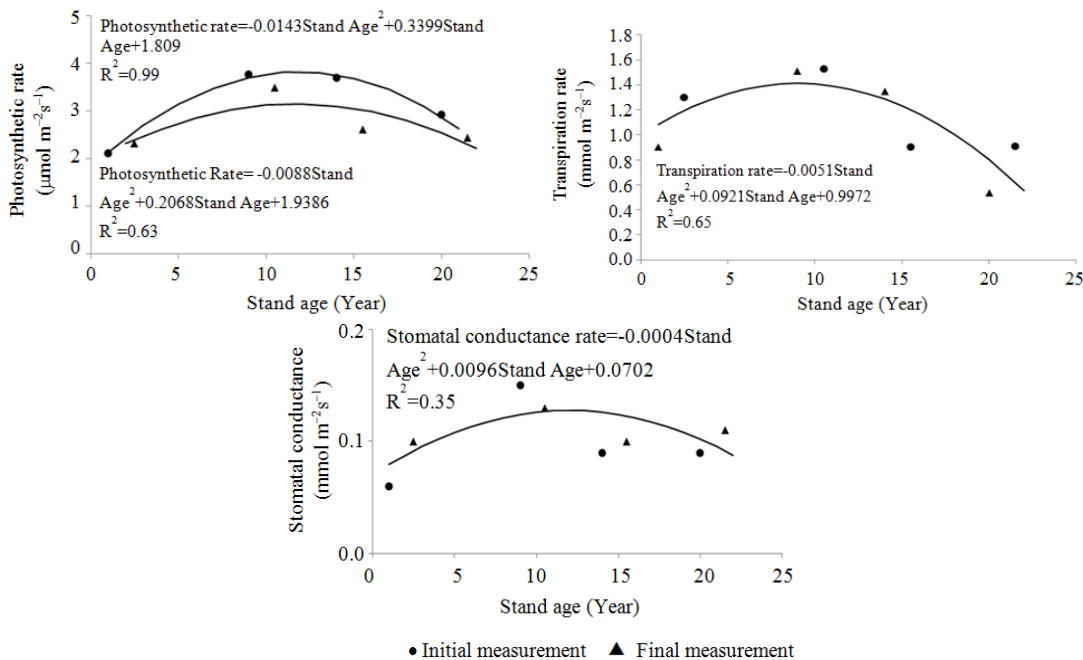


Fig. 2. Relationships between leaf physiology and *Dryobalanops beccarii* stand age

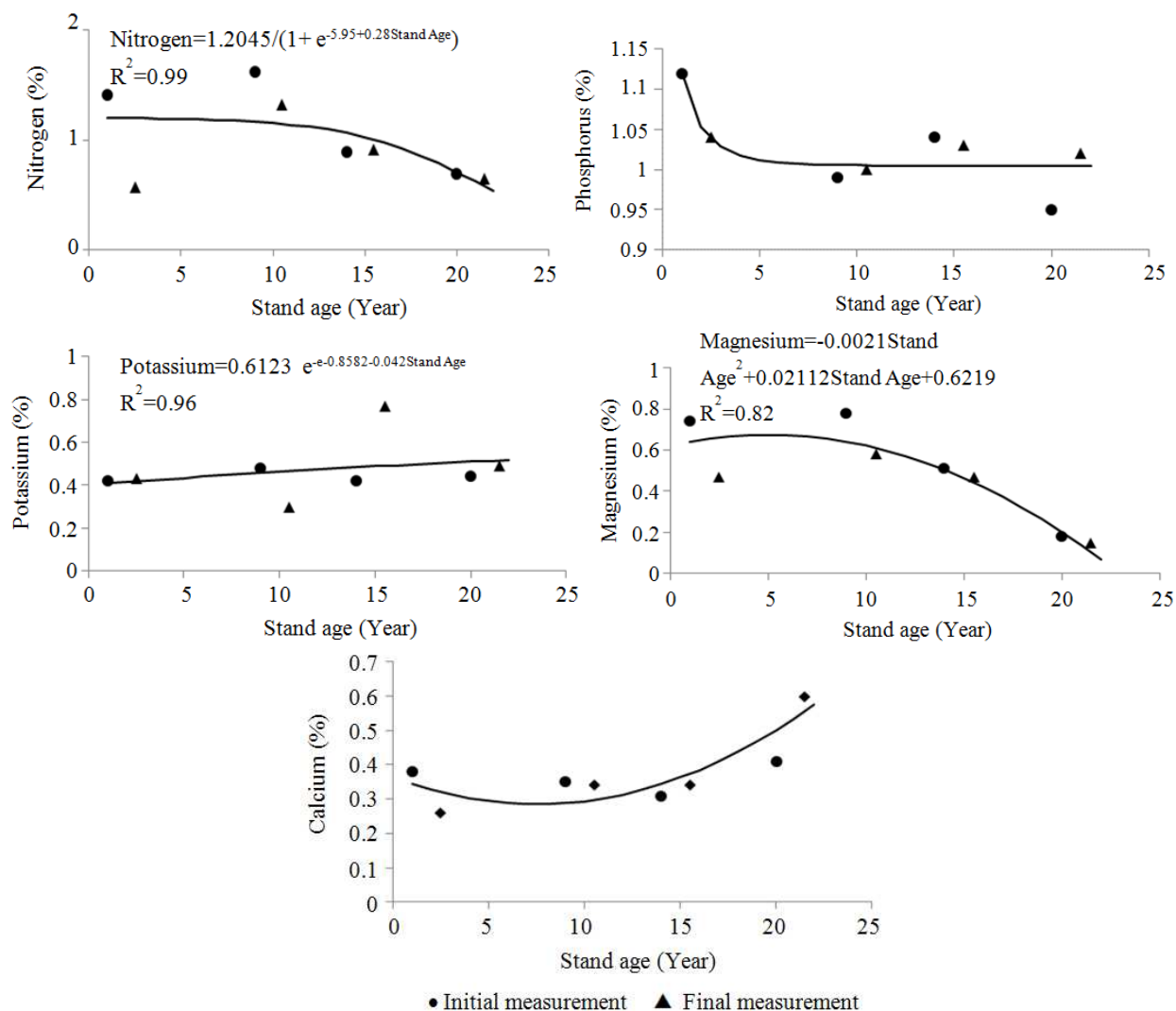


Fig. 3. Relationships between leaf nutrient content and *Dryobalanops beccarii* stand age

Table 1. Leaf morphological characteristics of *Dryobalanops beccarii*

Age		P1		P9		P14		P20	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Leaf Area (cm ²)	Initial	24.30±0.53 ^{cA}	31.37±0.38 ^{bA}	33.18±0.69 ^{1aA}	33.52±0.34 ^{1aA}	34.81±0.59 ^{1aA}	34.19±0.36 ^{1aA}	34.81±0.59 ^{1aA}	34.19±0.36 ^{1aA}
	Final	23.95±0.55 ^{cA}	31.40±0.40 ^{bA}	32.99±0.56 ^{1aA}	33.15±0.31 ^{1aA}	34.34±0.62 ^{1aA}	34.02±0.49 ^{1aA}	34.34±0.62 ^{1aA}	34.02±0.49 ^{1aA}
Leaf Width (cm)	Initial	2.05±0.04 ^{cA}	2.50±0.07 ^{bA}	2.87±0.17 ^{1aA}	3.49±0.16 ^{1aA}	3.10±0.12 ^{1aA}	3.58±0.16 ^{1aA}	3.10±0.12 ^{1aA}	3.58±0.16 ^{1aA}
	Final	1.66±0.07 ^{cB}	2.05±0.11 ^{bB}	2.45±0.09 ^{1aB}	3.03±0.13 ^{1aB}	2.49±0.11 ^{1aB}	3.36±0.12 ^{1aB}	2.49±0.11 ^{1aB}	3.36±0.12 ^{1aB}
Leaf Length (cm)	Initial	8.16±0.10 ^{dA}	8.59±0.06 ^{cA}	10.44±0.27 ^{1bA}	10.34±0.40 ^{1bA}	10.81±0.20 ^{1aA}	11.53±0.25 ^{1aA}	10.81±0.20 ^{1aA}	11.53±0.25 ^{1aA}
	Final	8.03±0.10 ^{dA}	8.42±0.07 ^{cA}	10.02±0.15 ^{1bA}	11.11±0.18 ^{1bA}	10.63±0.21 ^{1aA}	11.70±0.22 ^{1aA}	10.63±0.21 ^{1aA}	11.70±0.22 ^{1aA}
Leaf Thickness (mm)	Initial	17.13±0.28 ^{dA}	19.01±0.22 ^{cA}	21.85±0.30 ^{1bA}	20.95±0.44 ^{1bA}	24.91±0.38 ^{1aA}	24.39±0.44 ^{1aA}	24.91±0.38 ^{1aA}	24.39±0.44 ^{1aA}
	Final	17.68±0.55 ^{dA}	18.62±0.24 ^{cA}	21.48±0.32 ^{1bA}	20.29±0.46 ^{1bA}	24.57±0.47 ^{1aA}	24.46±1.68 ^{1aA}	24.57±0.47 ^{1aA}	24.46±1.68 ^{1aA}
Stomata Density (mm ⁻²)	Initial	556.4±20.6 ^{abA}	567.4±11.6 ^{abA}	530.2±18.0 ^{2bA}	611.1±41.4 ^{1aA}	594.8±18.5 ^{1aA}	583.9±19.2 ^{1bA}	594.8±18.5 ^{1aA}	583.9±19.2 ^{1bA}
	Final	520.7±21.3 ^{abB}	523.0±11.8 ^{abB}	502.7±19.3 ^{2bB}	568.7±11.0 ^{1aB}	561.6±22.8 ^{1aB}	570.8±19.2 ^{1aB}	561.6±22.8 ^{1aB}	570.8±19.2 ^{1aB}

Means with standard deviation represented by different superscript lowercase alphabets indicates significant differences between different ages in the upper canopy (P1, P9, P14, P20) by Tukey's test at p<0.05 and the lower canopy (P14 and P20) by t-Test.

Means with standard deviation represented by different superscript numbers indicates significant differences between canopy levels (P14 and P20) by t-Test.

Means with standard deviation represented by different superscript uppercase alphabets indicates significant differences between assessment dates (initial and final) by t-Test.

Table 2. Leaf physiological characteristics of *Dryobalanops beccarii*

Age	Canopy level	Photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		Transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$)		Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)	
		Initial	Final	Initial	Final	Initial	Final
P1	Upper	2.12±0.49 ^{ba}	2.32±0.60 ^{ba}	0.93±0.57 ^{aA}	1.31±1.36 ^{aA}	0.06±0.02 ^{ba}	0.10±0.05 ^{aA}
P9	Upper	3.78±0.55 ^{aA}	3.50±0.91 ^{aA}	1.51±0.31 ^{aA}	1.53±0.46 ^{aA}	0.17±0.07 ^{aA}	0.13±0.07 ^{aA}
P14	Upper	3.96±0.54 ^{1aA}	2.68±0.17 ^{1bB}	1.59±0.50 ^{1aA}	1.03±0.41 ^{1aA}	0.10±0.05 ^{1abA}	0.08±0.07 ^{1aA}
	Lower	3.35±0.95 ^{1aA}	2.56±0.61 ^{1aB}	1.11±0.48 ^{1aA}	0.77±0.44 ^{1aA}	0.08±0.06 ^{1aA}	0.12±0.13 ^{1aA}
P20	Upper	3.26±1.12 ^{1aA}	2.49±0.61 ^{1bB}	0.46±0.23 ^{1bA}	0.97±0.42 ^{1aA}	0.07±0.05 ^{1bA}	0.13±0.08 ^{1aA}
	Lower	2.59±1.83 ^{1aA}	2.40±0.85 ^{1aA}	0.61±0.29 ^{1aA}	0.85±0.40 ^{1aA}	0.11±0.08 ^{1aA}	0.09±0.05 ^{1aA}

Means with standard deviation represented by different superscript lowercase alphabets indicates significant differences between different ages in the upper canopy (P1, P9, P14, P20) by Tukey's test at $p < 0.05$ and the lower canopy (P14 and P20) by t-Test.

Means with standard deviation represented by different superscript numbers indicates significant differences between canopy levels (P14 and P20) by t-Test.

Means with standard deviation represented by different superscript uppercase alphabets indicates significant differences between assessment dates (initial and final) by t-Test

Table 3. Leaf nutrient properties of *Dryobalanops beccarii*

Age	Canopy level		P1	P9	P14		P20	
			Upper	Upper	Upper	Lower	Upper	Lower
Nitrogen (%)	Initial		1.41±3.05 ^{aA}	1.32±0.80 ^{aA}	0.92±0.21 ^{1aA}	0.86±0.27 ^{1aA}	0.64±0.16 ^{1aA}	0.73±0.06 ^{1aA}
	Final		0.57±0.71 ^{aB}	1.32±0.80 ^{aA}	0.97±0.56 ^{1aA}	0.84±0.24 ^{1aA}	0.64±0.16 ^{1aA}	0.65±0.16 ^{1aA}
Phosphorus (%)	Initial		1.12±0.06 ^{aA}	0.99±0.14 ^{aA}	1.05±0.01 ^{1aA}	1.03±0.02 ^{1aA}	0.96±0.17 ^{1aA}	0.94±0.06 ^{1aA}
	Final		1.04±0.12 ^{aA}	1.00±0.13 ^{aA}	1.02±0.03 ^{1aA}	1.04±0.02 ^{1aA}	1.04±0.02 ^{1aA}	1.00±0.02 ^{1aA}
Potassium (%)	Initial		0.42±0.25 ^{aA}	0.48±0.43 ^{aA}	0.41±0.30 ^{1aA}	0.43±0.19 ^{1aA}	0.46±0.25 ^{1aA}	0.41±0.20 ^{1aA}
	Final		0.43±0.30 ^{ba}	0.30±0.11 ^{ba}	0.80±0.24 ^{1aA}	0.74±0.35 ^{1aA}	0.58±0.18 ^{1abA}	0.39±0.12 ^{2bA}
Magnesium (%)	Initial		0.78±0.02 ^{aA}	0.74±0.14 ^{aA}	0.40±0.10 ^{1abA}	0.62±0.03 ^{1aA}	0.12±0.08 ^{1bA}	0.23±0.10 ^{1bA}
	Final		0.47±0.02 ^{abA}	0.58±0.02 ^{aA}	0.32±0.01 ^{1abA}	0.61±0.02 ^{1aA}	0.11±0.02 ^{1bA}	0.18±0.34 ^{1bA}
Calcium (%)	Initial		0.39±0.01 ^{aA}	0.37±0.03 ^{aA}	0.32±0.04 ^{1aA}	0.30±0.01 ^{1aA}	0.40±0.01 ^{1aA}	0.42±0.06 ^{1aA}
	Final		0.24±0.03 ^{ba}	0.29±0.04 ^{ba}	0.38±0.17 ^{1bA}	0.31±0.10 ^{1bA}	0.61±0.49 ^{1abA}	0.60±1.70 ^{1abA}

Means with standard deviation represented by different superscript lowercase alphabets indicates significant differences between different ages in the upper canopy (P1, P9, P14, P20) by Tukey's test at $p < 0.05$ and the lower canopy (P14 and P20) by t-Test.

Means with standard deviation represented by different superscript numbers indicates significant differences between canopy levels (P14 and P20) by t-Test.

Means with standard deviation represented by different superscript uppercase alphabets indicates significant differences between assessment dates (initial and final) by t-Test.

Discussion

As tree grow taller with age, the tree tend to develop large size leaves when exposed to the combination of mesic condition and high solar radiation (Givnish and Vermeij, 1976). Matured trees are expected to develop larger leaves than juvenile trees (Martínez-Garza and Howe, 2005). Thus, leaves of matured *D. beccarii* showed greater length, width, thickness and leaf area than leaves of juvenile *D. beccarii*. The comparison of leaf size between the canopy levels showed no significant difference found, neither in terms of leaf area, length, width, nor thickness. This was in contrast to the understanding that leaf in the upper canopy (sun leaves) is commonly smaller but thicker than leaf in the lower canopy (shade leaves) (Givnish, 1988; Keller, 2015; Pallardy, 2008).

Stomata density is influenced by atmospheric CO₂ concentration. The CO₂ concentration is different at every elevation or canopy height, where it is low at high canopy level and vice versa (Buchmann *et al.*, 1996).

There is usually a reduction in stomatal density accompanied with increasing CO₂ (Woodward and Kelly, 1995). Hence, higher stomata density in matured *D. beccarii* as compared to juvenile *D. beccarii* may due to lower CO₂ concentration at the higher canopy level. Stomata density is also affected by the radiation exposure and water loss. The size of *D. beccarii* leaves during the seedling phase was small than other phases. The leaf form has strong association with the sapling architectural form (King and Maindonald, 1999), of which the seedling and sapling of *D. beccarii* is classified as plagiotropic with simple sun leaves (Turner, 2001). Sun leaves have higher stomata density while shade leaves showed the opposite trend (Givnish, 1988; Keller, 2015; Pallardy, 2008). However, current results was found to be contradicted with other findings, where the stomata density was fewer in sun leaves than in shade leaves of P14.

Gas exchange is influenced by leaf properties and environmental factors such as light, temperature, nutrition and water availability (Aighewi and Ekanayake,

2004; Xie and Luo, 2003). Since the photosynthetic rate, transpiration rate and stomatal conductance was positively correlated with each other, the reduction of one of these parameters will be followed by reduction of the others two in order to maintain a functional homeostasis (Evans and Poorter, 2001; Saliendra *et al.*, 1995; Yoder *et al.*, 1994). Seedling of *D. beccarii* recorded lowest leaf area, length, thickness and width. These characteristic enhance the adaptability of seedlings to prevent dehydration in open area, although it may contributed to low efficiency of photosynthesis, transpiration and stomatal conductance rate (Chang and Miller, 2004; Greer and Halligan, 2001). Saplings of *D. beccarii* in lesser stand density recorded considerably high photosynthetic rate, transpiration rates and stomatal conductance. In less dense stand, there were substantial gaps between the saplings, allowing the leaves surface to be exposed directly to the environmental factors such as high irradiance and wind (Lowman *et al.*, 2012). The exposure of leaves to high light intensity, temperature and wind speed increases the transpiration rate, of which led to high photosynthesis and transpiration rate (Kirkham, 2000; Toole and Toole, 2002).

The saplings (P14) in high stand density and matured *D. beccarii* showed reduction of transpiration and photosynthetic rates and stomatal conductance. In high density stand where the saplings grew too close to each other as well as in matured *D. beccarii* stand where there were massive canopy size and root system, led to overlapping canopies and limited space causing the saplings to compete for limited resources. The stomatal conductance decreased due to the limitation of CO₂ as the CO₂ mitigation in dense stand was higher than sparse or open stand (Del Pozo *et al.*, 2007). Meanwhile, the competition to absorb water in the soil through overlapping root system (Manoli *et al.*, 2014) directly reduce water availability for transpiration (Prieto *et al.*, 2012). The rate of photosynthesis declined due to sunlight obstructed by the canopy layer through self-shading or being shaded by nearby trees (Gundel *et al.*, 2014; Manoli *et al.*, 2014; Ng *et al.*, 2016).

The concentration of N, P and Mg of *D. beccarii* was slowly reduced from seedling to mature phase. Unlike N and Mg, P was decreased tremendously during seedling phase and become almost plateau in sapling and mature phase. The N, P and Mg are key nutrients that influence a number of important leaf traits, which vary in the use and allocation between leaves. These N, P and Mg are highly mobile and always being remobilized in young leaves (Avice and Etienne, 2014; Fischer, 2008; Mediavilla and Escudero, 2003), causing deficiency symptom in old leaves. The inadequate supply of N, P and Mg in soil may reduce the N, P and Mg in the leaves over time. The supply of nutrients from soil was highly dependent on the decomposition of accumulated organic matter since there was no fertilizer application in the study plots. The cation that competes with Mg in uptake, such as K, also cause leaf Mg to decrease (Mengel *et al.*,

2012). Leaf K and Ca were increased as *D. beccarii* aged. The increased of K maybe related to the accumulation of K in the leaf as leaf aged and only being mobilized to young expanding leaves under conditions of K deficiency (Lin and Yeh, 2008). The leaf Ca possessed high in mobility, causing the Ca to accumulate in the leaf as the leaf aged (Suárez, 2010).

Conclusion

Leaf size increased with stand age although the leaf width declined. The leaf morphology was found to be significantly correlated with the tree morphology parameters, except for leaf width and canopy diameter. The sun leaf was similar to the shade leaf. The stomata density reduced during yearly assessment in each stand age. The photosynthetic rate, transpiration rate and stomatal conductance showed similar pattern where these three parameters was low in the freshly emerged saplings, increased rapidly until *D. beccarii* aged approximately 9-14 year old and decreased once it reached the mature phase. The transpiration rate was positively affected by the leaf thickness and length as well as leaf width. The negative correlation of N, P and K may have caused the deficit of photosynthesis rate, transpiration rate and stomatal conductance.

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Author's Contributions

Emilla Yastari LAPOK: Involved in all experiments, field work, coordinated data analyses and contributed to the scientific writing of the manuscript.

ONG Kian Huat: Project leader, involved in the supervision of the research, field work, data analyses, editing and proofreading of the manuscript.

John Keen CHUBO: Involved in the supervision of the research and editing of the manuscript.

Patricia KING Jie Hung: Involved in the supervision of the research.

Ethics

The authors declare no conflicts of interest and this article is original and contains unpublished material in accordance with the ethical standards specified by the OnLine Journal of Biological Sciences.

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