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Relationship between leaf flushing phenology and defensive traits of canopy trees of five dipterocarp species in a tropical rain forest

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ABSTRACT While the canopy layer shows the highest productivity in forests, it also has the highest herbivore population density. How do canopy trees cope with attack from herbivores under such conditions? We investigated the relationship between leaf flushing phenology, defensive and photosynthetic traits, such as leaf flushing frequency, the number of emerging leaves per flushing term, the leaf life span, total phenolic and condensed tannin contents, leaf toughness, leaf nitrogen content, the photosynthetic rate at light saturation (\(P_{\text{max}}\)) and leaf mass per area (LMA), in canopy trees of five dipterocarp species in a Malaysian tropical rain forest. Interspecific variations were clearly observed in leaf flushing frequency, ranging from occasional to continuous patterns. The total phenolic content significantly increased with leaf flushing frequency. Leaf toughness showed no correlation with leaf flushing phenology, but species with occasional leaf flushing had greater leaf toughness than those with continuous leaf production. There was a negative correlation between leaf toughness and tannin content, and a positive correlation between the former and \(P_{\text{max}}\). In addition, the leaf herbivory rate significantly increased with a larger number of emerging leaves per term and a higher comprehensive index (PC1) from PCA analysis using leaf flushing frequency and the number of leaves. Therefore, our results suggest that tropical canopy species have various defensive strategies against herbivore attack by regulating the intensity of chemical, physical and phenological defenses; species with high leaf flushing frequency have more chemically defended leaves, while those with low leaf flushing frequency have tougher leaves with higher photosynthetic abilities.

Key words: herbivory rate, Malaysia, leaf toughness, phenolics, tannin

INTRODUCTION In forest ecosystems, photosynthesis occurs mainly in the upper canopy layer (Ellsworth and Reich 1993, Rijkers and Bonger 2000, Evans and Pooter 2001, Kenzo et al. 2015), because it receives direct sunlight (Yoda 1974, Thomas et al. 2006). This layer has the highest productivity in forests by using its large resource gains for leaves, flowers, fruits and so on (Parker 1995). However, herbivore population density in the canopy layer also becomes the highest in forests, due to its rich and attractive resources for their food (Basset et al. 1992, Basset 2001, Kato et al. 1995, Novotny and Basset 2005, Wardhaugh 2014). How do canopy trees cope with attack from herbivores under such conditions?

It is well known that trees have responded to herbivore pressure by the evolution of their physical and chemical defenses (Coley 1983, Matsuqi et al. 2004, Agrawal and Fishbein 2006). Leaf toughness, typical of physical defense, is one of the major deterrents against various herbivores (Coley 1983, Aide 1988, Agrawal and Fishbein 2006, Caldwell et al. 2016). Since new leaves are not provided with toughness because they become tougher with maturity, most damage to leaves occurs when they are still at a young and immature stage (Coley 1983, Coley and Kursar 1996, Kursar and Coley 2003). In fact, Coley and Kursar (1996) reported that more than 70 percent of the life time damage of each leaf occurred during a period of a few weeks after it had started to develop in tropical shade tolerant trees. In contrast, many trees produce secondary metabolites, represented by phenolics and tannin (Coley and Barone 1996, Hättenschwiler and Vitousek 2000), for chemical defense, which can be effected by just leaf sprouts (Coley and Barone 1996, Brenes-Arguedas et al. 2006).

Leaf flushing phenology may also affect the behavior of herbivores in tropical rain forests, in which the climate is suitable for herbivores all year round (Whitmore 1990, Corlett 2009). It is known that trees in tropical rain forests have several leaf flushing patterns; Hatta and Darnaedi (2005) reported that many trees in the tropical rainforests showed irregular simultaneous flushing within the crown
and species, though some species show continued leaf flushing. Osada et al. (2005) also reported that leaf flushing, i.e. the frequency of leaf emergence (FLE) and the number of emerging leaves per leaf emergence event (NLE), varied according to the canopy species in a Malaysian tropical rain forest. Some researchers have suggested that many trees in the lowland tropical rainforest of Southeast Asia show synchronous leaf flushing at community level (Koriba 1958, Ichie et al. 2004, Itioka and Yamauti 2004, Kishimoto-Yamada et al. 2009). It is considered that such simultaneous and synchronous leaf flushing might decrease herbivory pressure by satiating them (Aide 1988, Lamarr et al. 2014). Although these trees with intermittent leaf flushing may not need plenty resources for chemical defense and can allocate them for their growth (Aide 1988), they may also have high risk of losing all new leaves when they abort synchronous flushing or the satiation of herbivores together with the same and/or other species. In contrast, since species with continuous leaf flushing always suffer the risk of attack from herbivores on their new leaves, they may have to provide leaf sprouts with some specific chemical and/or physical defenses against those herbivores.

There may be a trade-off interaction between leaf flushing phenology, leaf defensive traits and photosynthetic traits even of canopy trees in tropical rain forests. The trade-off relation between leaf defensive and photosynthetic traits is well known in comparing a wide range of species, from pioneer to climax species, in forest ecosystems along forest succession; pioneer species have higher photosynthetic ability and thus can grow faster, while climax species have higher defensive ability (Endara and Coley 2011). If tropical canopy species show large interspecific variations in leaf flushing phenology, they may also show different strategies for leaf defense and photosynthesis to adjust to the tropical canopy environment under high herbivore pressure.

In this study, we tested our hypothesis about a trade-off interaction between leaf flushing phenology, leaf defensive traits and photosynthetic traits of canopy species and about large interspecific variations in tropical rain forests in Southeast Asia; species with continuous leaf flushing may have a higher leaf chemical content and thus lower photosynthetic ability, while those with intermittent flushing may gain leaf toughness faster, have a lower chemical content and thus have higher photosynthetic ability. We investigated in detail time series changes in leaf flushing phenology, leaf defensive and photosynthetic traits, such as FLE, NLE, estimated the leaf life spans, total phenolics and condensed tannin contents, leaf toughness, leaf nitrogen content, photosynthetic rates at light saturation ($P_{max}$), and leaf mass per area (LMA), in canopy and emergent trees of five dipterocarp species in a Malaysian tropical rainforest.

### MATERIALS AND METHODS

#### Study sites

Our study was conducted at the Canopy Biology Plot (8 ha: 200 × 400 m) and the Crane Plot (4 ha: 200 × 200 m) in Lambir Hills National Park, Sarawak, Malaysia (4° 20′ N, 113° 50′ E, 150–250 m a.s.l.; Inoue et al. 1995, Sakai et al. 2002). The vegetation of the study site is of a typical lowland mixed dipterocarp forest (Ashton and Hall 1992). The area has a humid tropical climate with a little seasonal change in rainfall and temperature (Kato et al. 1995). Annual precipitation at the Crane Plot averaged 2600 mm from 2000 to 2009, ranging from 2191 mm to 2944 mm, and the average temperature in the study sites was 25.8°C from 2000 to 2009, ranging from 25.7°C to 26.1°C (Kume et al. 2011). We accessed the tree canopy using a canopy observation system, including tree towers and aerial walkways at the Canopy Biology Plot (Inoue et al. 1995; Yamoto et al. 1996) and an 85-m canopy crane at the Crane Plot (Sakai et al. 2002). The canopy crane system allowed access to the entire crown of each emergent tree (Sakai et al. 2002; Ozanne et al. 2003).

#### Study species

Five dipterocarp species, *Dipterocarpus globosus* Vesque (DG), *Dipterocarpus pachyphyllus* Meijer (DP), *Dryobalanops aromatica* Gaertn.f (DrA), *Shorea beccariana* Burck (SB) and *Shorea fallax* Meijer (SF), were selected for this study. All the species are evergreen and dominate the canopy and emergent layers in the study sites (Table 1). DG, DrA and SB are distributed over sandy soil and, DP and SF over clay soil (Itoh 1995, Hirai et al. 1997, Hiromi et al. 2012, Lee et al. 2002, Ashton 2004) (Table 1). But the distribution of these dipterocarp species is unrelated to soil pH (Lee et al. 2002).

#### Leaf flushing phenology

A phenological observation was made 11 times from July 2007 to November 2008 at two-week to four-month intervals. Ten sun-exposed shoots without branching per
individual were marked randomly in the outer portion of each crown in July 2007. We measured the shoot length from the marked scar to the base of each terminal bud, and the number of attached, new and fallen leaves was counted each. The leaf life span was calculated with the following equation;

\[
\text{Leaf life span} = \frac{N}{b}
\]

where N is the cumulated number of leaves and b is the daily number of new leaves produced during the observation period (Southwood et al. 1986, Navas et al. 2003). We also calculated FLE and NLE per year from the number of leaf flushing times in the whole observation term (16 months).

Leaf traits

Another ten shoots per individual were also marked in the outer portion of each crown to track changes in various leaf traits from the beginning of leaf flushing in July 2007.

We collected ten leaves around 5, 10 and 20 days after their sprouting and also ten fully-expanded and apparently non-senescing leaves from each of the ten marked shoots per individual. We then measured their leaf area by scanning the leaf images with LIA32 ver.0.376 (Kazukiyo Yamamoto http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32). The leaf toughness of each collected leaf was estimated at the mean from three punch tests per unit fracture length with a digital penetrometer (model RX-1: Aikoh, Osaka, Japan) (Katabuchi et al. 2012). We then dried these leaf samples with silica gel for two weeks and ground to a fine powder using Wonder Blender (Osaka Chemical, Japan) for leaf chemical analysis.

The concentrations of the total phenolics and condensed tannin in mature (fully expanded) and immature leaves were quantified by their extraction with 50 percent methanol and determined by the Folin-Ciocalteu (for the total phenolics) and proanthocyanidin methods (for condensed tannin) respectively (Julkunen-Tiitto 1985, Waterman and Mole 1994) by using a spectrophotometer (Hitachi, Tokyo, Japan, Model U-1800). The standards for the assays were gallic acid for the total phenolics and cyanidin chloride for condensed tannin.

The nitrogen concentrations of mature and immature leaves were measured by using a C/N analyzer (Shimadzu, Kyoto, Japan, Sumigraph NC-900). Nitrogen peaks were identified by using acetanilide as a standard. Then, changes in leaf traits, such as leaf toughness and the total phenolics, condensed tannin and nitrogen contents, were approximated by using the equation, \( y = a + b \times \log(X) \), for each species. The days taken to reach 95 percent of the mature leaf value were calculated from this equation.

The net photosynthetic rate at light saturation \((P_{\text{max}})\) of each mature leaf was measured in March 2009. Measurements were made between 0800 h and 1000 h in order to avoid the midday depression in photosynthesis (Ishida et al. 1996, Kenzo et al. 2003). Three leaves at the top of the crown of each tree were chosen randomly and used for measurements. \(P_{\text{max}}\) was measured by using a portable open gas exchange measurement system (Li-Cor, NE, USA, LI-6400) at a leaf temperature of 30°C with CO\(_2\) concentrations of 360 ppm. The light intensity was controlled at 1500 \(\mu\) mol photon m\(^{-2}\) s\(^{-1}\) by an internal LED light source (Li-Cor, NE, USA, LI-640B).

Leaf herbivory rates were measured in 100 mature leaves per individual in November 2008. The damage of each of the leaves was estimated at 0, 2.5, 15, 37.5 or 75 percent by visual observation.

Statistical analysis

The correlation between FLE and NLE was tested by using Spearman’s rank correlation coefficient. To acquire the comprehensive knowledge of leaf flushing characteristics among the tropical canopy species studied, a principal component analysis (PCA) was made by using the FLE and NLE. The first principal component (PC1) explained 91 percent of the variation in FLE and NLE. We then analyzed the correlation of the investigated leaf traits with FLE, NLE and PC1 by using Pearson’s correlation coefficient. One-way analysis of variance (ANOVA) with Tukey’s HSD test was done to compare the different parameters of the

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### Table 1. Tree species, codes, the number of individuals, mean tree height (± SD) and preferable soil conditions.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Code</th>
<th>n</th>
<th>Height (m)</th>
<th>Preferable soil condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipterocarpaceae</td>
<td><em>Dipterocarpus</em></td>
<td><em>globosus</em></td>
<td>DG</td>
<td>3</td>
<td>41 ± 3.3</td>
<td>Sandy (Itoh et al. 1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>pachyphyllus</em></td>
<td>DP</td>
<td>3</td>
<td>55 ± 2.6</td>
<td>Clay-rich (Ishizuka 1998, Hiromi et al. 2012)</td>
</tr>
<tr>
<td></td>
<td><em>Dryobalanops</em></td>
<td><em>aromatica</em></td>
<td>DrA</td>
<td>3</td>
<td>48 ± 3.5</td>
<td>Sandy (Itoh 1995)</td>
</tr>
<tr>
<td></td>
<td><em>Shorea</em></td>
<td><em>beccariana</em></td>
<td>SB</td>
<td>3</td>
<td>49 ± 3.9</td>
<td>Sandy (Ashton 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>fallax</em></td>
<td>SF</td>
<td>3</td>
<td>35 ± 2.2</td>
<td>Sandy and sandy clay (Ashton 2004)</td>
</tr>
</tbody>
</table>
species. For all the statistical analyses, we used the R version 3.3.1 (R Development Core Team 2016).

RESULTS

Leaf flushing phenology

Species-specific leaf flushing phenology was observed in five dipterocarp species (Fig. 1, Table 2). DrA showed continuous branch development, a high FLE, and a small NLE (Fig. 1, Fig. 2, Table 2). In contrast, SB and SF showed only about two flushing terms during the observation period and high NLE (Fig. 1, Fig. 2, Table 2). In addition, the lower-FLE species, SB and SF, flushed synchronously with the higher-FLE species, DrA, DP and DG (Fig. 1). There was a negative relationship between FLE and NLE (Fig. 2, $P = 0.054$). The leaf life span significantly differed according to the species; longer in SB and shorter in DrA, DG and SF. However, there was no

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Fig. 1. Shoot growth of 5 dipterocarp species. The point of 100% on the vertical axis as maximum shoot length in the observation term. Arrows mean the day new leaves were observed on more than 50% of the shoots of each species.
Table 2. Each species’ frequency of leaf emergence (FLE), the number of emerging leaves per leaf emergence term (NLE), investigated mature leaf traits and average of 95 percent of mature leaf toughness (Day95% mature) (mean ± SD). Different letters are significant differences (*P<0.05).

<table>
<thead>
<tr>
<th>Trait</th>
<th>DG</th>
<th>DP</th>
<th>DrA</th>
<th>SB</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLE (time per year)</td>
<td>2.67 ± 0.58a</td>
<td>5.51 ± 0.23b</td>
<td>7.15 ± 0.48b</td>
<td>1.93 ± 0.12b</td>
<td>2.00 ± 0.000</td>
</tr>
<tr>
<td>NLE (leaf number)</td>
<td>2.90 ± 0.36d</td>
<td>1.00 ± 0.00f</td>
<td>1.00 ± 0.00f</td>
<td>3.00 ± 0.18f</td>
<td>5.76 ± 0.46d</td>
</tr>
<tr>
<td>Leaf life span (days)</td>
<td>430.50 ± 15.76</td>
<td>474.51 ± 11.52</td>
<td>380.25 ± 9.07</td>
<td>552.48 ± 22.48</td>
<td>407.10 ± 19.37d</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>1.49 ± 0.54f</td>
<td>0.81 ± 0.13hi</td>
<td>0.21 ± 0.02i</td>
<td>1.50 ± 0.57hi</td>
<td>1.24 ± 0.27hi</td>
</tr>
<tr>
<td>Area (cm²)</td>
<td>60.60 ± 13.00c</td>
<td>36.31 ± 3.37f</td>
<td>11.24 ± 2.03f</td>
<td>55.18 ± 5.14d</td>
<td>56.36 ± 17.86c</td>
</tr>
<tr>
<td>LMA (g/m²)</td>
<td>245.66 ± 73.67</td>
<td>223.37 ± 19.94</td>
<td>192.89 ± 49.44</td>
<td>268.01 ± 81.85</td>
<td>237.16 ± 107.15</td>
</tr>
<tr>
<td>Toughness (M Pa)</td>
<td>1.43 ± 0.23f</td>
<td>1.12 ± 0.06e</td>
<td>1.49 ± 0.19f</td>
<td>2.98 ± 0.09f</td>
<td>3.20 ± 0.04e</td>
</tr>
<tr>
<td>Day95% mature</td>
<td>16.45 ± 7.68</td>
<td>18.65 ± 0.36</td>
<td>21.50 ± 0.57</td>
<td>15.21 ± 1.68</td>
<td>14.60 ± 0.65</td>
</tr>
<tr>
<td>Phenolics conc. (mg/g)</td>
<td>110.99 ± 3.06c</td>
<td>126.30 ± 9.29d</td>
<td>208.90 ± 1.31d</td>
<td>61.11 ± 6.16c</td>
<td>58.22 ± 7.67c</td>
</tr>
<tr>
<td>Tannin conc. (mg/g)</td>
<td>13.60 ± 2.7a</td>
<td>11.08 ± 1.41b</td>
<td>11.32 ± 0.47b</td>
<td>7.72 ± 1.13c</td>
<td>3.53 ± 0.31b</td>
</tr>
<tr>
<td>Nitrogen conc. (mg/g)</td>
<td>15.00 ± 0.38c</td>
<td>14.39 ± 2.28c</td>
<td>12.03 ± 0.25c</td>
<td>17.10 ± 0.29c</td>
<td>20.78 ± 1.40c</td>
</tr>
<tr>
<td>$P_{max}$ (µmol/m²/s)</td>
<td>14.60 ± 0.23d</td>
<td>9.88 ± 0.29e</td>
<td>12.00 ± 0.54d</td>
<td>17.69 ± 0.41c</td>
<td>16.69 ± 0.90d</td>
</tr>
<tr>
<td>Herbivory rate (%)</td>
<td>4.84 ± 1.03d</td>
<td>4.12 ± 0.64e</td>
<td>2.79 ± 0.07b</td>
<td>5.18 ± 0.43b</td>
<td>8.22 ± 3.55b</td>
</tr>
</tbody>
</table>

Table 3. Pearson’s correlation $r$ values between the investigated leaf traits and the frequency of leaf emergence (FLE), the number of emerging leaves per leaf emergence term (NLE) and PC1 of principal component analysis using FLE and NLE. * and ** indicate a significant correlation at $P<0.05$ and $P<0.01$, respectively.

<table>
<thead>
<tr>
<th>Trait</th>
<th>FLE ($r$)</th>
<th>NLE ($r$)</th>
<th>PC1 ($r$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf life span</td>
<td>-0.211</td>
<td>-0.259</td>
<td>-0.025</td>
</tr>
<tr>
<td>Dry weight</td>
<td>-0.952*</td>
<td>0.616</td>
<td>0.824</td>
</tr>
<tr>
<td>Area</td>
<td>-0.957*</td>
<td>0.706</td>
<td>0.874</td>
</tr>
<tr>
<td>LMA</td>
<td>-0.908*</td>
<td>0.507</td>
<td>0.744</td>
</tr>
<tr>
<td>Toughness</td>
<td>-0.720</td>
<td>0.820</td>
<td>0.809</td>
</tr>
<tr>
<td>Day95% mature</td>
<td>0.980**</td>
<td>-0.840</td>
<td>-0.960*</td>
</tr>
<tr>
<td>Phenolics conc.</td>
<td>0.938*</td>
<td>-0.770</td>
<td>-0.897*</td>
</tr>
<tr>
<td>Tannin conc.</td>
<td>0.495</td>
<td>-0.776</td>
<td>-0.668</td>
</tr>
<tr>
<td>Nitrogen conc.</td>
<td>-0.825</td>
<td>0.944*</td>
<td>0.935*</td>
</tr>
<tr>
<td>$P_{max}$</td>
<td>-0.859</td>
<td>0.783</td>
<td>0.863*</td>
</tr>
<tr>
<td>Herbivory rate</td>
<td>-0.788</td>
<td>0.965**</td>
<td>0.921*</td>
</tr>
</tbody>
</table>

**Leaf flushing phenology and defensive traits in dipterocarp canopy species**

Leaf flushing phenology and defensive traits in dipterocarp canopy species

Leaf defensive traits

Leaf toughness was significantly greater in SB and SF than in DG, DP and DrA (Table 2). However, there is no interspecific variation in the number of days taken to reach 95 percent toughness of the mature leaf between the five study species (Table 2, Appendix 1). In contrast, the concentrations of the total phenolics and condensed tannin were significantly lower in SB and SF and higher in DG, DP and DrA (Table 2). Almost all the study species showed negative slopes with leaf maturing in the total phenolics and condensed tannin concentrations (Appendix 2, 3). The total phenolics concentrations in their mature leaves were significantly related to FLE and PC1 (Table 3), and condensed tannin content to leaf toughness (Table 4).

**Leaf photosynthetic traits**

We found an interspecific difference in leaf nitrogen concentrations and $P_{max}$ (Table 2); SB and SF showed clearly higher values than DP, DG and DrA. Both values were significantly and positively related to NLE and/or PC1 (Table 3) and $P_{max}$ was also to leaf toughness (Table 4). All
the study species showed negative slopes with leaf maturing in leaf nitrogen concentrations (Appendix 4).

Leaf herbivory rate

The leaf herbivory rate was significantly higher in SF and lower in DrA (Table 2). It was significantly related to NLE and PC1 (Table 3) and also to leaf nitrogen concentrations (Table 4).

DISCUSSION

There was a clear difference in leaf flushing phenology between the five dipterocarp canopy species, but their leaf flushing timings synchronized more or less. In addition, leaf flushing phenology was closely related to both leaf defensive and photosynthetic traits. These suggest that the dipterocarp species studied have different interspecific strategies for a defense against herbivores even in similar environments in the canopy layer in a tropical rain forest.

We found a clear interspecific difference in leaf flushing phenology; from twice-a-year to continuous new-leaf production (Fig. 1, Table 2). A high leaf-flushing frequency is generally found in pioneer species, and a low one in climax species (Dungan et al. 2008). But this study showed continuous new-leaf production even in dipterocarp climax species in a tropical rain forest as did previous reports in the Malay Peninsula and Indonesia (Koriba 1958, Osada et al. 2005, Hatta and Darnaedi 2005). Lamarre et al. (2014) suggested that leaf flushing phenology in tropical rain forest trees might be closely related to soil resource availability; some species growing in poor soil under frequent drought stress might be favored to produce a large number of leaves all at once, which might represent lower cost than to produce leaves continuously. However, it seems that the species studied did not reflect any soil resource availability for their leaf flushing phenology, because they bore little relation to soil texture; species distributed over sandy soil, such as DrA, SB and DG, showed various leaf flushing patterns, and the same goes for those distributed over clay soil, such as DP and SF (Itoh 1995, Hirai et al. 1997, Hiromi et al. 2012, Lee et al. 2002, Ashton 2004).

A clear tendency to a negative relation was found between FLE and NLE (Fig. 2). These results support the previous models that suggested the aforementioned trade-off relation (Ackerly 1996, Hikosaka 2003, 2005), and also suggest that each of the dipterocarp canopy species may have a different allocation strategy for leaf development. It is well known that the leaf life span is closely related to leaf flushing phenology; species with continuous leaf production have a shorter leaf life span and those with a large number of produced leaves per term a longer one (Dungan et al. 2008). However, there was no significant correlation between the leaf life span, leaf flushing frequency and the number of produced leaves in the five dipterocarp species (Table 3). Since we could not find any significant correlation between the leaf life span, leaf defensive traits and photosynthetic traits in the study species (Table 4), further research is still needed to clarify determinant factors in the leaf life span in tropical canopy species.

Synchronous leaf flushing by those species may enhance effect on plant defensive ability against herbivores. Several classic studies have shown that a rapid and synchronous flush of leaf production may contribute to plants’ escape from their natural enemies (Feeny 1976, McKey 1975). Even canopy and emergent trees in tropical rain forests in Southeast Asia seem to escape herbivores by adjusting the time for new leaf development among themselves due to climatic factors, such as short-term drought (Ichie et al. 2004, Itoika and Yamauti 2004, Kishimoto-Yamada et al. 2009) and daily insolation (Borchert et al. 2015), as triggers for synchronous leaf development in the tropical environment. Species with a higher leaf flushing frequency such as DrA and DP have higher concentrations of leaf total phenolics, while species with a lower flushing frequency such as SB and SF have

| Leaf life span & Toughness & Phenolics conc. & Tannin conc. & Nitrogen conc. & $P_{\text{max}}$ |
|----------------|----------------|----------------|----------------|----------------|----------------|
| Toughness      & 0.28           & -              & -              & -              & -              |
| Phenolics conc. & -0.56          & -0.74          & -              & -              & -              |
| Tannin conc.   & -0.09           & -0.89*         & 0.63           & -              & -              |
| Nitrogen conc. & 0.25           & 0.87           & -0.91*         & -0.86          & -              |
| $P_{\text{max}}$ & 0.29           & 0.91*          & -0.75          & -0.65          & 0.79           |
| Herbivory      & 0.03           & 0.78           & -0.83          & -0.81          & 0.97**         | 0.71           |
higher values in leaf toughness. Since there was a significant negative correlation between leaf toughness and condensed tannin content (Table 4), our results showed the trade-off relation between physical and chemical defensive traits in the five dipterocarp canopy species as did many previous researches (Hanley and Lamont 2002, Moles et al. 2013, Eichenberg et al. 2015), probably by investment from a finite pool of resources (Read et al. 2009). Species with a lower flushing frequency have a larger number of leaves per leaf-flushing term, and thus less herbivory pressure than those with a higher flushing frequency, because they may decrease herbivory pressure with more new leaves per term by synchronous leaf emergence with many other species (Nilsen et al. 1987, Aide 1991). This advantage may be a less investment in chemical defensive traits. In contrast, species with a higher leaf flushing frequency need higher concentrations of the total phenolics due to more herbivory pressure (Macauley and Fox 1980).

Photosynthetic traits, such as $P_{\text{max}}$ and leaf nitrogen content, were also closely related to leaf flushing phenology in the five dipterocarp canopy species. It is well known that species with a higher defensive chemical content in their leaves show lower growth rates and thus lower photosynthetic ability than those with a lower content (Coley 1983, Endara and Coley 2011). Our results suggest that species with a higher leaf flushing frequency have a higher defensive chemical content, and thus may show lower photosynthetic ability even in the same canopy environment in tropical rain forests, which supports our hypothesis. In addition, Kenzo et al. (2004) indicated that the leaves of SB had thicker palisade-cell layers than those of DRA and DG, and found a positive correlation between palisade-cell-layer thickness and photosynthetic traits in dipterocarp canopy species in a tropical rain forest. Thus, species with a lower leaf flushing frequency might have a higher level of photosynthetic traits to realize quick leaf development (Kursar and Coley 2003) without investment in defensive chemical substances. In contrast, species with a higher leaf flushing frequency may have chemically well-defended leaves at the expense of investment in photosynthetic traits for protection from more herbivory pressure.

The leaf flushing phenology showed a significant correlation with the leaf herbivory rate in the five dipterocarp species. Species with a higher leaf flushing frequency showed lower herbivory rates than those with a lower frequency, suggesting that the former have chemically well-defended leaves, which are thus firmly protected even under much herbivory pressure in the tropical canopy environment. On the other hand, species with a lower flushing frequency showed relatively higher leaf herbivory rates, even though they produced many new leaves in one flushing term of a smaller number of days taken to reach mature leaf toughness. This may imply that species with a low defensive chemical content are easily targeted by herbivores in synchronous leaf flushing. Although rapid leaf maturing is generally thought to be an important defensive trait, because most herbivory occurs in expanding leaves (Coley and Kursar 1996, Kursar and Coley 2003, Coley et al. 2018), our result showed that species with rapid leaf maturing such as SB and SF had higher herbivory rates in their leaf damage. This may cause leaves to require much protein for rapid maturing, and thus attract herbivores demanding their nutrients (Coley and Kursar 1996, Kursar and Coley 2003, Coley et al. 2018). However, the herbivory rate in the dipterocarp canopy species was commonly lower than 10 percent, which was about the same as or lower than that of dipterocarp seedlings in Malaysia (Eichhorn et al. 2007) and canopy trees reported in other temperate and tropical forests (Neves et al. 2010, Lowman 1992). Therefore, it could be suggested that different leaf flushing strategies, observed in five dipterocarp species in a Malaysian tropical rain forest, are successful by having different leaf defensive and photosynthetic traits.

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Leaf flushing phenology and defensive traits in dipterocarp canopy species


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**Appendix 1.** Changes in leaf toughness from sprouting for 5 dipterocarp species. Each species’ mean of mature leaf toughness was plotted at the last point. Arrows indicate each species’ average of 95 percent of mature leaf toughness (Day_{95%mature}, see Table 2). The approximated curve, equation and $r^2$ are shown in each figure.
Appendix 2. Changes in leaf phenolics concentrations from sprouting to maturity. The approximated curve and coefficient of determination ($r^2$) are shown in each figure.
Appendix 3. Changes in leaf tannin concentrations from sprouting to maturity. The approximated curve and coefficient of determination \( r^2 \) are shown in each figure.
Appendix 4. Changes in leaf nitrogen concentrations from sprouting to maturity. The approximated curve and coefficient of determination ($r^2$) are shown in each figure.