Leaf Damage and Traits of Dipterocarp Seedlings in a Lowland Rain Forest in Peninsular Malaysia

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ABSTRACT We examined the relationships between leaf damage caused by insects and pathogens and three leaf traits (leaf toughness, total phenol contents, and nitrogen contents) of five dipterocarp species in a lowland rain forest in peninsular Malaysia. The measurements were made on all leaves of 9-month-old dipterocarp seedlings that emerged at mast fruiting in September 1996. Means of leaf damage levels were significantly different among the species. Percentages of heavily damaged leaves (damaged leaf area > 50%) varied from 5.0% in *Shorea multiflora* to 13.8% in *S. pauciflora*. Mean leaf damage levels were not correlated with the three leaf traits across species, but percentages of heavily damaged leaves showed a significant negative correlation with the total phenol contents in leaves. This result suggests that phenolics may not necessarily reduce the average amounts of leaf damage, but may suppress heavy leaf feeding by herbivores.

Key words: Dipterocarpaceae / herbivory / lowland rain forest / Malaysia / Pasoh / defense / seedlings

Dipterocarp trees dominate lowland tropical rain forests in Southeast Asia (Symington, 1943) and are important for the international hardwood timber trade (Appanah & Weinland, 1993).

Seedling establishment is the most vulnerable stage during the life history of plants (Harper, 1977). Furthermore, it has been reported that tropical plants have more leaf damages than temperate plants, even though the former tend to be better defended than the latter (Coley & Aide, 1991). Most of dipterocarp seedlings in the understory either do not grow or grow only a few centimeters a year once their first few leaves have expanded (Liew & Wong, 1973; Whitmore, 1984; Manokaran & Swaine, 1994). Therefore, it is expected that herbivores could stunt or sometimes kill dipterocarp seedlings (Kachi *et al.*, 1995).

Plant species differ in both the amounts and types of leaf defenses against herbivores (Coley & Barone, 1996). Phenolics act as effective defensive compounds against diverse herbivores by forming tannins, which decrease the availability of nitrogen to the herbivores (Feeny, 1970, 1976; Rhoades & Cates, 1976; Rhoades, 1979; Fleck & Tomback, 1996). Some dipterocarp seedlings are known to contain condensed tannins and other phenolics in their leaves (Bate-Smith & Whitmore, 1959; Becker, 1981). Some alkaloids are more toxic than phenolics, but they were not detected in leaves of several

dipterocarp seedlings (Becker, 1981; Razak *et al.*, 1982). It has been reported that leaf toughness plays a major role in physical resistance to herbivores, pathogens, and other physical damage to limit the chewing capacity of insect herbivores (Beck, 1965; Schowalter *et al.*, 1986; Choong *et al.*, 1992). Nutritional contents may also be partially shaped by selection caused by herbivores and pathogens (Moran & Hamilton, 1980). A low plant nitrogen content reduces insect growth and preference (Mattson, 1980; Coley & Barone, 1996; Hartley & Jones, 1997). Although differences in these leaf traits among species may explain specific differences in the rates of herbivory under natural conditions, many studies could not detect clear relationships between leaf damage and traits under natural conditions (Coley, 1983; Lowman & Box, 1983; Folgarait & Davidson, 1994).

Mast fruiting in 1996 resulted in synchronous dispersal and germination of tremendous amounts of dipterocarp seeds at one time, and made it possible to compare leaf damage and traits among same-aged seedlings of dipterocarp species. In this study, relationships between the levels and patterns of leaf damage and leaf traits (toughness, total phenol contents, and nitrogen contents) were examined by using cross-species comparisons for seedlings of five dipterocarp species.

MATERIALS AND METHODS

The study was done in Pasoh Forest Reserve (2° 59' N, 102° 18' E) in the state of Negeri Sembilan, approximately 70 km southeast of Kuala Lumpur, Malaysia. Annual precipitation is approximately 1800 mm, with two distinct peaks in April - May and November - December (Malaysian Meteorological Service). The total area of the reserve is 2450 ha. Our field study site was located in a 2-ha permanent ecological plot near the entrance to the reserve. One hundred subplots (1 m × 1 m) were set regularly in the 2-ha plot, which contains seedlings of eight dipterocarp species. The forest contains stands at various stages of maturity from canopy gaps to mature stands topped by emergent trees with heights of 50 to 60 m. Gaps are common from place to place in the forest. The proportion of newly opened gaps in the 2-ha plot was about 4% during 1992 and 1994, and increased to 8.8% in 1997 (Yasuda, 1998).

All seedlings growing in the subplots were tagged in September 1996. The seedlings were ca. 9-months old and between 5 and 20 cm tall at the census in May 1997. Densities of five dipterocarp seedlings in the subplots ranged from 0 to 52 m⁻² (mean 2.04) and the mean density of all seedlings in the plots were 6.49 m⁻².

Because there are few seedlings of the three dipterocarp species, five dipterocarp species (Dipterocarpaceae) were chosen for the study; *Dipterocarpus cornutus* Dyer, *D. sublamellatus* Foxw., *Shorea leprosula* Miq., *S. multiflora* Sym., and *S. pauciflora* King. *Shorea leprosula* has been described as a light-demanding species (Symington, 1943; Appanah and Weinland, 1993), whereas *S. multiflora* is shade-tolerant. All species except *S. multiflora* are distributed mainly in lowland forests. *Shorea multiflora* is also distributed at higher elevations on hill ridges (Symington, 1943; Turner, 1990; Appanah and Weinland, 1993).

Leaf damage caused by insect herbivores and pathogens were visually estimated for individual leaves of all seedlings growing in the 100 subplots. Few seedlings shed old leaves under shade. Damage to individual leaves was estimated by using four damage categories: no damage (0%); light (< 5%); moderate (5% - 50%) and heavy (> 50%).

Ten seedlings of each species were sampled randomly in the forest near the 2-ha plot under canopy. The average height and number of intact leaves of these sampled seedlings were not significantly different from those of seedlings used for monitoring leaf damage. Leaf toughness was measured for fresh leaves by using a digital force gauge (FGC-0.5 Shimpo, Japan), which indicates the force (in newtons) necessary to punch a rod 0.5 mm in diameter through the leaf (Sands & Brancatini, 1991). Twenty leaves of each species were perforated three times, and the mean of the three measurements was used for statistical analyses.

The sampled seedlings were dried at 80°C for 3 days in an electric oven before leaf dry weight was measured. The dried leaves were powdered for measurements of the total phenol and nitrogen contents. The powdered samples from 9 - 12 leaves were mixed to a single lot to obtain appropriate amounts of samples for chemical analyses. Extraction and centrifugation were repeated four times, and all extracts were combined for each leaf sample. Total phenol contents in the extracts were measured by Folin-Denis assay (Waterman and Mole, 1994). Nitrogen and carbon contents were determined by using a C/N Analyzer (Sumigraph NC-90, Sumitomo Chemical Industries, Japan). These procedures were repeated three times.

All statistical analyses were done with StatView J-4.5 (Abacus Concepts, Inc., Berkeley, CA, USA). Frequencies of damaged leaves were tested by using G-test. Variations in the number of leaves, leaf toughness, and total phenol and nitrogen contents among species were tested by using one-way ANOVA. Multiple comparisons of these variables among species were made by Scheffé's post-hoc test. We calculated Pearson's correlations between the levels of leaf damage and the three leaf traits to determine which traits were associated with the extent of leaf herbivory.

RESULTS

Means of leaf damage levels varied significantly among species, and ranged from 10.6 (*D. sublamellatus*) to 19.3 (*D. cornutus*) ($F = 2.52$, $df = 4, 198$, $P < 0.05$; Table 1). Variations in the percentages of heavily damaged leaves were significantly greater than those of lightly and moderately damaged leaves (Table 1).

The three leaf traits differed significantly among species (Table 2). *Shorea multiflora* had significantly higher total leaf phenol contents than *S. leprosula* ($F = 70.4$, $df = 4, 10$, $P < 0.0001$; Table 2). *Shorea leprosula* had significantly softer leaves ($F = 29.5$, $df = 4, 93$, $P < 0.0001$; Table 2) and higher levels of leaf nitrogen ($F = 9.3$, $df = 4, 10$, $P < 0.01$; Table 2) than the other species.

Means of leaf damage levels and percentages of lightly or moderately damaged leaves were not significantly correlated with the leaf toughness nor with leaf phenol or nitrogen contents. On the other hand, the total phenol contents in leaves showed a significant negative correlation with the percentages of heavily damaged leaves ($N = 5$, $r = -0.92$, $P = 0.02$; Fig. 1a), while the leaf toughness and leaf nitrogen contents were not significantly correlated with the percentages of heavily damaged leaves (Fig. 1b, c).

Although no significant relationships between the three measured leaf traits and the means of leaf damage levels were detected, we found a significant correlation between the leaf phenol contents and the percentages of heavily damaged leaves.

Fig. 1. Correlations between the percentages of leaves showing more than 50% damage and three leaf traits. Each point indicates an individual species. Abbreviations for the species name are as follows: Dc (*Dipterocarpus cornutus*), Ds (*D. sublamellatus*), SI (*Shorea leprosula*), Sm (*S. multiflora*), Sp (*S. pauciflora*). Only the total phenol contents in leaves were significantly correlated with the percentages of damaged leaves ($N = 5$, $r = -0.92$, $P = 0.02$).

Table 1. Comparisons of the number of leaves, mean leaf damage levels and percentages of damaged leaves in five dipterocarp species. Means $\pm$ standard deviations are shown.

Species	N	No. of leaves per seedling	Mean of leaf damage levels	Percentages of damaged leaves		
				Lightly	Moderately	Heavily
<i>Dipterocarpus cornutus</i>	26	3.7 $\pm$ 1.2a	19.3 $\pm$ 13.5	26.8	32.0	12.4
<i>Dipterocarpus sublamellatus</i>	49	3.6 $\pm$ 1.2a	10.6 $\pm$ 10.6	31.4	22.9	5.1
<i>Shorea leprosula</i>	57	3.6 $\pm$ 1.3a	14.6 $\pm$ 13.6	31.7	27.3	10.2
<i>Shorea multiflora</i>	49	2.0 $\pm$ 0.6b	11.5 $\pm$ 13.0	30.0	26.0	5.0
<i>Shorea pauciflora</i>	22	3.6 $\pm$ 1.4a	15.4 $\pm$ 14.3	33.8	22.5	13.8

Table 2. Comparisons of the three leaf characteristics among five dipterocarp species. Means $\pm$ standard deviations are shown. Means that differ significantly (Scheffé's PLSD method: $P < 0.05$) between species have different superscripts.

Species	Leaf toughness (newton)	Total phenol contents (mg/g dry weight)	Nitrogen contents (mg/g dry weight)
<i>Dipterocarpus cornutus</i>	0.163 $\pm$ 0.035 ^{ab}	113.24 $\pm$ 9.16 ^c	21.06 $\pm$ 0.22 ^a
<i>Dipterocarpus sublamellatus</i>	0.158 $\pm$ 0.036 ^a	142.27 $\pm$ 9.46 ^b	19.78 $\pm$ 0.30 ^{ab}
<i>Shorea leprosula</i>	0.082 $\pm$ 0.023 ^{c*}	105.52 $\pm$ 6.17 ^c	20.62 $\pm$ 1.45 ^a
<i>Shorea multiflora</i>	0.180 $\pm$ 0.029 ^a	168.83 $\pm$ 4.65 ^a	17.76 $\pm$ 0.49 ^b
<i>Shorea pauciflora</i>	0.149 $\pm$ 0.024 ^b	78.28 $\pm$ 5.07 ^d	18.58 $\pm$ 0.28 ^b
Number of samples	20	3	3

*: $N = 18$

DISCUSSION

Seedling mortality of *S. leprosula* increased greatly after 100% defoliation, but 25% defoliation had no significant effects on seedling survival of any species (Becker, 1983), implying that light to moderate leaf damage does not critically affect seedling establishment. Therefore, we expect that phenolics in leaves work as a chemical defense to avoid heavy leaf damage. Our results showed that

light to moderate leaf damage occurs irrespective of the level of phenolics in leaves. This result suggests that phenolics may not necessarily reduce the average amounts of leaf damage, but may limit heavy leaf feeding by herbivores. This may explain why many studies failed to detect a significant correlation between phenolic concentrations and plant resistance to herbivores. Phenolics decrease the availability of nutrition to the herbivores and can have adverse effects on the growth and survival of herbivores (Feeny, 1970, 1976; Rhoades & Cates 1976; Rhoades, 1979). Herbivores avoid high-phenolic leaves for better growth and survival (Feeny, 1970, 1976). It is possible that higher leaf phenol contents can reduce the amounts of leaf damage by working as a repellent.

However, other repellents or toxins may be involved in the defense of the dipterocarp seedlings. Becker (1981, 1982, 1983) speculated that insect attacks on three *Shorea* species (*S. acuminata*, *S. leprosula*, and *S. maxwelliana*) may be related to the concentrations of certain kinds of essential oils but not to the amounts of total phenolics. Essential oils are complex mixtures of volatile terpenoids, and may repel or poison herbivores (Harbone, 1977; Morrow & Fox, 1980). Although essential oils of some species may reduce light to moderate damages by working as a qualitative defense, this study did not examine the effects of essential oils. To examine relationships between leaf traits and leaf damage levels, we may need to consider the possible effects of essential oils of these species.

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沼田真也, 可知直毅, 奥田敏統, N. MANOKARAN 半島マレーシア低地熱帯
林に生育するフタバガキ科植物稚樹の葉の食害と防御特性

熱帯樹種の種子及び実生の食害とその防御機構に関する知見を得るために、半島マレーシアの低地自然林に生育するフタバガキ科5種の実生ステージにおける葉の傷害（昆虫及びカビなどの病原体による）と葉の形質（フェノール化合物含量、物理的強度及び窒素含量）との関係を調査した。その結果、野外に生育する実生の葉の平均葉傷害には有意な種間差がみられ、特に全ての着葉のうち50%以上の傷害を受けた葉の割合（重傷害率）は5%（*Shorea multoiflora*）から13.8%（*S. pauciflora*）と大きくばらついた。葉の平均葉傷害とそれぞれの葉の特性の間にみられる種間差を検討するために、相関解析を行ったところ、いずれにおいても有意な相関関係はみられなかった。一方、葉の重傷害率と葉の特性の間について相関解析を行ったところ、葉内フェノール化合物含量との間に有意な負の相関関係がみられた。これは量的防御物質として知られるフェノール物質を葉内に高濃度に蓄積する樹種ほど成熟葉の傷害拡大を回避している可能性を示唆する。