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Herbivory of tropical rain forest tree seedlings correlates with future mortality

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Abstract. Tree seedlings in tropical rain forests are subject to both damage from natural enemies and intense interspecific competition. This leads to a trade-off in investment between defense and growth, and it is likely that tree species specialized to particular habitats tailor this balance to correspond with local resource availability. It has also been suggested that differential herbivore impacts among tree species may drive habitat segregation, favoring species adapted to particular resource conditions. In order to test these predictions, a reciprocal transplant experiment in Sabah, Malaysia, was established with seedlings of five species of Dipterocarpaceae. These were specialized to either alluvial (*Hopea nervosa*, *Parashorea tomentella*) or sandstone soils (*Shorea multiflora*, *H. beccariana*), or were locally absent (*S. fallax*). A total of 3000 seedlings were planted in paired gap and understory plots in five sites on alluvial and sandstone soils. Half of all seedlings were fertilized. Seedling growth and mortality were recorded in regular samples over 3.5 years, and rates of insect herbivore damage were estimated from censuses of foliar tissue loss on marked mature leaves and available young leaves. Greater herbivory rates on mature leaves had no measurable effects on seedling growth but were associated with a significantly increased likelihood of mortality during the following year. In contrast, new-leaf herbivory rates correlated with neither growth nor mortality. There were no indications of differential impacts of herbivory among the five species, nor between experimental treatments. Herbivory was not shown to influence segregation of species between soil types, although it may contribute toward differential survival among light habitats. Natural rates of damage were substantially lower than have been shown to influence tree seedling growth and mortality in previous manipulative studies.

Key words: Dipterocarpaceae; growth rates; habitat specialization; herbivory; insect herbivores; mortality; rain forest; reciprocal transplant; seedling; Sepilok Forest Reserve, East Sabah, Malaysia.

INTRODUCTION

In natural communities, plants face the dual pressures of competition and attack by natural enemies. These challenges are often heightened by the privations of resource limitation, forcing plants to adopt strategic responses to balance the competing demands of growth and defense (Herms and Mattson 1992). A trade-off occurs since defenses enhance survival but also incur costs, thereby reducing growth and competitive ability. The balance struck by each species may be finely attuned to a specific set of resource conditions, equalizing fitness between species within heterogeneous environments (Chesson 1991). This process has been implicated as a mechanism by which plant species are able to coexist within communities.

The fundamental assumptions of this theory are that variation in the allocation of resources to defense influences the damage caused by natural enemies and that this in turn influences plant growth and survival. In tropical rain forests the conflict between plants and herbivores is especially intense, with between 10% and 40% of the foliage consumed each year by herbivores (Lowman 1984, Coley and Aide 1991, Coley and Barone 1996). In contrast to temperate forests, defoliation is principally caused by insects (Coley and Barone 1996). Even small amounts of foliar damage can have substantial effects on photosynthetic production by leaves (Zangerl et al. 2002), resulting in retarded growth. This in turn will increase the time a seedling spends below the canopy, where it is subject to other risk factors and greater resource competition (Jackson and Bach 1999, Sullivan 2003).

Local conditions can also influence the magnitude of herbivore impacts. Understory plants in tropical wet forests typically receive only 1–2% of full daylight, which is close to the light compensation point for net

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whole-plant carbon balance (Chazdon 1988). Tissue loss in low-light conditions may therefore be more detrimental than for plants in full sunlight, where a higher rate of leaf production can compensate for losses (Coley 1983). Similar predictions can be made for seedlings in soils in which growth is limited by nutrient availability, whether due to natural variation or artificial manipulation (i.e., fertilization; Fine et al. 2004).

The impact of herbivory on growth and survival varies markedly between groups of plants (Hawkes and Sullivan 2001), and this can be influential in determining competitive outcomes between species (Pearson et al. 2003, Massey et al. 2006). In the heterogeneous environment of a tropical rain forest, divided by boundaries between varying soil types and light conditions, this may contribute to habitat partitioning among tree species (Fine et al. 2004, 2006). Specialist species may suffer increased impacts of herbivory when exposed to unfamiliar conditions to which they are relatively poorly adapted, whether a different light environment (Coley 1983) or an alien soil type (e.g., Fine et al. 2004).

Nevertheless, gaps remain in our understanding. The influential study by Fine et al. (2004) examined distinct communities on strongly contrasting soils, but it remains to be demonstrated that such effects are widespread. In Southeast Asia, where lowland forests are dominated by a single family of canopy trees, the Dipterocarpaceae, there is significant evidence of fine-scale habitat partitioning among tree species along gradients of topography and soil conditions (Ashton 1968, Russo et al. 2005, Paoli et al. 2006). The mechanisms underlying this remain obscure, and the potential importance of a growth–survival trade-off mediated by herbivory in generating high beta diversity has yet to be investigated.

Sepilok Forest Reserve in East Sabah, Malaysia, is an ideal study site for addressing these questions. The canopy is dominated by trees in the Dipterocarpaceae, with distinct floristic associations of dipterocarp forest on alluvial (15–30 m above sea level [asl]) and sandstone soils (up to 337 m asl; Fox 1973, Nilus 2004). The two soil types differ markedly in fertility and nutrient cycling (Dent et al. 2006), with alluvial forest soils having more than double the concentration of nitrate and total nitrogen and more than fourfold greater total phosphorus concentrations, although extractable phosphorus concentrations are similar (Appendix A; Baltzer et al. 2005). Crucially, there is clear evidence that resource availability influences seedling physiology and growth between the soil types (Baltzer et al. 2005). Previous work has demonstrated interspecific variation in rates of foliar herbivory on dipterocarp seedlings (Eichhorn et al. 2006) linked to concentrations of phenolic defenses (Eichhorn et al. 2007). We found a given species received remarkably consistent rates of damage over time, regardless of light or nutrient status and with only minor differences between soil types (Eichhorn et al. 2006). However, the impact of this herbivory on growth and survival would be expected to differ according to the resources available to the seedlings. It is possible that the impacts of herbivory may provide a selective barrier preventing the successful establishment of specialized seedlings on an alien soil type.

We hypothesized that (1) increased rates of herbivory would have negative impacts on seedling growth and survival and that these impacts would (2) be greater in understory light conditions compared to gap sites and (3) in conditions of greater nutrient limitation, either on sandstone soils compared to alluvial soils or in the absence of fertilization. Finally, we predicted that (4) patterns of reduced growth and survival, driven by herbivory, would play a part in segregation of species between forest habitats at the seedling stage.

These hypotheses were investigated in a reciprocal transplant experiment in which four locally habitat-restricted dipterocarp species and one nonlocal species were grown in experimental plots. Herbivory rates were recorded over 24 months and growth and mortality were recorded over 36 months in gap and understory sites on two distinct soil types. This design allowed us to determine the mechanistic association between local resource availability, herbivory, and impacts on plant growth and survival.

METHODS

Study site and species

Sepilok Forest Reserve, Malaysia (5°10′ N, 117°56′ E), is a 4294-ha forest fragment of dipterocarp-dominated lowland tropical rain forest. The experiment incorporated five dipterocarp species native to the region: *Hopea nervosa* King, *Parashorea tomentella* (Sym.) Meijer, *Shorea fallax* Meijer, *S. multiflora* (Burck) Sym., and *H. beccariana* Burck. For details of seedling collection and storage conditions prior to transplantation, see Eichhorn et al. (2006). All are shade-tolerant, non-pioneer species, capable of germination, establishment, and survival beneath the forest canopy (Swaine and Whitmore 1988). Within Sepilok Forest Reserve, *H. nervosa* and *P. tomentella* are considered to be alluvial soil specialists and *S. multiflora* and *H. beccariana* to be sandstone specialists as they do not occur in any of three fully enumerated 4-ha plots on the soil type to which they are not native (Nilus 2004), and they were not encountered on a nonnative soil type during incidental surveys. *Shorea fallax* is not present in Sepilok but is generally found on alluvial soils in the Danum Valley Conservation Area. It is therefore not possible to definitively associate it with either soil type in Sepilok.

Experimental design

In a reciprocal transplant experiment, we planted seedlings of these edaphic specialist species on both soil types present in Sepilok Forest Reserve. Ten sites were chosen by the availability of recent natural treefall gaps (range 400–600 m^2), five in alluvial lowland forest and five on sandstone ridges (see Plate 1). A plot was sited centrally within each gap, and a paired understory plot with approximately the same slope and aspect was established within 50 m of the gap edge. In gaps, all dead wood and plants above 50 cm in height were removed, with one plot requiring removal of overhanging branches. No clearing took place in the understory.

Seedlings were planted in March 2000 in a randomized block design to counter the potential effects of microclimatic gradients. There were 15 blocks per plot, each containing 10 randomly ordered seedlings comprising two of each species, one of which was fertilized. Seedling heights were recorded, and an initial ANOVA confirmed there were no significant differences in height between sites or treatments. Mean heights at planting for each species were: *H. nervosa*, 32.8 cm; *P. tomentella*, 40.0 cm; *S. fallax*, 30.1 cm; *S. multiflora*, 35.0 cm; and *H. beccariana*, 38.1 cm. Gap seedlings were planted in a 75-cm grid at least 1.5 m from gap edges, with the dimensions of the grid determined by gap shape. The dimensions of the paired understory plots were identical, although seedling placement was permitted a leeway of 50 cm to avoid planting directly beneath another understory plant. All seedlings were numbered with a metal tag tied to the base. Following an exceptional drought in April 2000, replacement seedlings of identical height were planted until June 2000 to counter the high establishment mortality. No further replacement or maintenance took place apart from periodic clearing of gap plots to prevent liana overgrowth.

A slow-release complete fertilizer (Agroblen, Scotts Australia, Baulkham Hills, New South Wales, Australia; 16N:8P:9K:3Mg plus trace elements) was applied twice per year (10–12 August 2000, 4–8 March 2001, 25–29 September 2001, 9–13 March 2002). All seedlings were trenched using a dining fork to ~5 cm depth and 5 cm from the base, with half of seedlings receiving 12 g of fertilizer before the soil was replaced. All efforts were made to leave visible roots intact, although some disruption was inevitable. Fertilized seedlings showed no increase in foliar nutrient concentrations (Dent 2004).

Growth and mortality

Censuses of all seedlings were conducted eight times over the first 42 months of the experiment. Basal stem diameter was measured using calipers at 10 cm above the soil surface. Perpendicular height was measured from the soil surface to the highest live apical bud. Seedlings with no live leaves or buds were recorded as dead.

Relative growth rates per seedling (RGR) were calculated as

$$\mathrm{RGR} = [\ln Q_2 - \ln Q_1]/[t_2 - t_1].$$

where Q_1 and Q_2 are the basal stem diameters or heights at time t_1 and t_2, respectively.

Herbivory rates

Surveys of herbivore damage began four months after planting. Each full survey took approximately two months, and all measurements were nondestructive. Five seedlings from each species and fertilizer treatment were chosen randomly in each plot, excluding replacements planted after March 2000, giving a total of 1000 seedlings. In the initial survey, leaves were sampled from the second-highest branch with secondary thickening upwards or all stem leaves for seedlings without branches. Seedlings with fewer than four leaves were replaced. Leaves were individually numbered on the underside with an indelible marker pen. Senescent or heavily damaged leaves (due to action other than herbivory) were excluded. Subsequent surveys included additional leaves on previously surveyed branches and typically added two branches: the highest new branch with secondary thickening and a second branch halfway through the additional growth, with a minimum of five leaves. The majority of gap plants produced new leaves between herbivory surveys, though the production rate was lower on sandstone soils and very low in the understory.

Four herbivory surveys were conducted (June–August 2000, February–March 2001, September–October 2001, April–May 2002), allowing herbivory rates to be calculated over three time intervals. Where death or abscission reduced the number of leaves on seedlings to fewer than four, alternative plants were substituted to replace them, although measurements continued on any remaining marked leaves. In some understory plots it was not possible to find five seedlings with four or more leaves, and therefore all suitable leaves on all seedlings were sampled.

Herbivory was determined using a practiced estimate of lamina damage (0%, <2%, 2–5%, 5–10%, 10–25%, 25–50%, 50–75%, 75–100%) with accuracy confirmed on a subsample of leaves using a grid scale. Periodic repeatability checks (at least one entire plot per survey) found that leaves obtained identical scores in >99% of cases. Damage included all loss of potential photosynthetic tissue, even if not entirely consumed by herbivores, and therefore includes leaves rolled or sewn together. Many leaves were disfigured by damage during development and their potential leaf area was considered to be the same as their closest neighboring entire leaves with the total damage estimated as the difference between potential and actual leaf area. Leaves that were absent in repeat surveys were not counted as herbivory unless the petiole was still attached, as the majority of abscised leaves could be attributed to natural patterns of leaf turnover and senescence (Dent 2004).

The first survey was used as a baseline. Rates of herbivory on mature leaves were calculated from the difference in damage levels on individual leaves between surveys using the midpoints of the percentage damage classes. The increase in damage was divided by the number of months (to nearest half month) between surveys on each plot and means calculated for each seedling. For new leaves the mean rate of herbivory was assumed to be equal to the recorded proportion of damage in a given survey divided by half the number of months since the previous survey to account for their continuous production. This assumes both an even rate of growth and of herbivory between surveys. For further details see Eichhorn et al. (2006).

Statistical analysis

As growth and herbivory survey intervals were not precisely matched, seedling sizes were estimated at the time of each herbivory survey by linear interpolation and used to estimate mean RGR over the course of each herbivory survey interval. The mortality of seedlings over the period following each herbivory survey (13 months) was used to correlate prior herbivory rates with future mortality. The period was chosen to ensure comparability between survey periods given the timing of mortality censuses.

Statistical analysis was conducted on two response variables: growth rates and mortality. Analysis followed a linear mixed-model procedure (Pinheiro and Bates 2000) in R version 2.8.1 (R Foundation for Statistical Computing, Vienna, Austria) utilizing the "lmer" function (Bates and Sarkar 2007). Mortality was analyzed using a binomial error structure. Herbivory rate and initial height were fitted as covariates. An initial model assessed all main effects (species, soil, light, fertilizer, initial height, herbivory rate) and the two-way interactions between each main effect and herbivory. In order to test for evidence of herbivory-induced segregation of species between soil types or light habitats, we assessed the three-way interactions between species, soil, and herbivory and species, light, and herbivory. Interactions between main effects other than herbivory were excluded since the analysis was concerned primarily with the responses of the seedlings to herbivory. Full analyses were conducted on mature leaves, and parallel analyses were conducted on young leaves where sufficient data were available. Since their abundance was generally much lower and the rate calculations were based on several assumptions, the analysis is not presented here in full, and reference to them is only made where the models differed notably from those obtained through analysis of mature leaves. Model simplification followed Crawley (2005). Survey period was included as a repeated measure and plot number as a random effect to account for variability in plot characteristics. In addition, each survey period was analyzed independently to check for repeatability.

RESULTS

Over the duration of the herbivory surveys, the seedlings grew in height by 1.74–3.48 cm/month among species. Mature-leaf herbivory rates varied from 0.07% to 0.99% leaf area/month between species and treatments, and new leaves varied from 1.7% to 6.82% leaf area/month. Mature- and new-leaf herbivory rates were positively correlated on individual seedlings ($P < 0.01$ in each survey interval). Between 57% and 89% of the seedlings of each species were still alive 12 months after the herbivory measurements had concluded. Differences between species in relative growth rate (RGR), survival, and herbivory in gap and understory plots are illustrated in Fig. 1; for further details of the effects of experimental treatments see Appendix B.

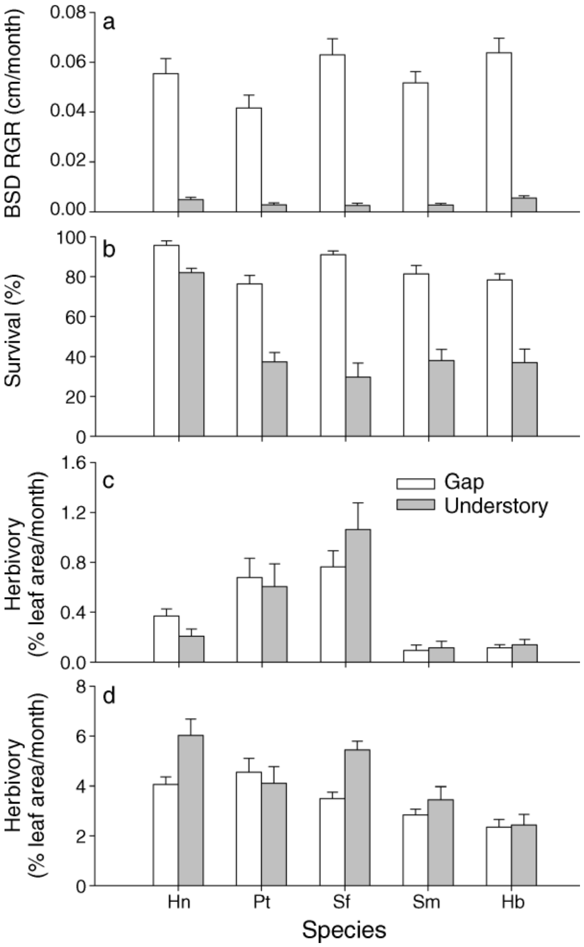

FIG. 1. (a) Relative growth rates (RGR) per month in basal stem diameter (BSD), (b) percentage survival at the end of the study period, and (c) mature-leaf and (d) developing-leaf herbivory rates per month for five species of dipterocarp seedlings in gap and understory plots in Sepilok Forest Reserve, Malaysia. Species abbreviations are: Hn, *Hopea nervosa*; Pt, *Parashorea tomentella*; Sf, *Shorea fallax*; Sm, *Shorea multiflora*; Hb, *Hopea beccariana*. Values are means per plot + SE averaged across other treatments.

Growth and herbivory

Relative growth rate of seedlings as measured by basal stem diameter varied significantly between species, with lower growth rates on sandstone soils relative to alluvial, in understory habitats relative to gaps, of seedlings with greater initial height, and with an overall reduction over the course of the experiment (Table 1). The model was not improved by the inclusion of herbivory rate ($\Delta d = 1.134$, df = 1, $P = 0.287$) nor by fertilizer treatment ($\Delta d =$

TABLE 1. Summary of linear mixed-effects ANCOVA model for relative growth rate (RGR) in basal stem diameter of dipterocarp tree seedlings in the reciprocal transplant experiment.

Effect	Estimate	SE	Δ*d*	df	*P*
Intercept	0.091	0.004			
Species					
Hopea nervosa	−0.007	0.002			
Parashorea tomentella	−0.011	0.002			
Shorea fallax	−0.005	0.002			
Shorea multiflora	−0.01	0.002			
Hopea beccariana	0		46.580	4	<0.001
Soil, sandstone	−0.017	0.002	21.300	1	<0.001
Understory light	−0.052	0.001	1538.800	1	<0.001
Survey	−0.005	0.001	41.688	1	<0.001
Initial height	>−0.001	>−0.001	16.869	1	<0.001

Notes: Parameter estimates are given as deviations from the intercept; SE is the standard error of the estimate. The Δ*d* column gives the change in deviance on removal of each term. The study was conducted in Sepilok Forest Reserve, Malaysia, a 4294-ha forest fragment of dipterocarp-dominated lowland tropical rain forest.

1.920, df = 1, $P = 0.166$). There were no significant interactions between species, soil, and herbivory ($\Delta d = 6.700$, df = 4, $P = 0.153$), nor species, light, and herbivory ($\Delta d = 1.407$, df = 4, $P = 0.843$), suggesting that herbivore-induced impacts were not differentially affecting the performance of the five species among light or soil treatments.

On splitting the data set into the three survey periods, the model structure remained identical throughout, apart from the addition of a significant interaction between soil and herbivory during the first period ($\Delta d = 4.468$, df = 1, $P = 0.035$). This effect resulted from a greater reduction in growth rate with increasing herbivory on sandstone relative to alluvial soils, but was absent in the second and third census periods and had limited explanatory power. The effect of initial height ceased to be significant in the third period ($\Delta d = 0.561$, df = 1, $P = 0.454$). Results for height RGR (not presented) were qualitatively identical to those for basal stem diameter RGR, indicating that both measures of growth responded in a comparable fashion, although the interaction between herbivory and soil type in the first census period was nonsignificant for seedling height.

Results for the model incorporating new leaf herbivory were qualitatively similar for basal stem diameter RGR, and although an interaction between new-leaf herbivory rates and soil type was present throughout the study period ($\Delta d = 9.211$, df = 1, $P = 0.002$), the direction of the effect suggested reduced growth of damaged seedlings on alluvial soils relative to sandstone. Once again there was no main effect of herbivory rate ($\Delta d = 0.333$, df = 1, $P = 0.564$). Analysis of height RGR yielded a qualitatively identical model.

Survival and herbivory

Mortality rates varied significantly between species, and there was significantly greater mortality in the understory relative to gaps. Rates also diminished over the course of the experiment and were lower for seedlings of greater initial height (Table 2). There was a significant effect of mature-leaf herbivory on seedling mortality, indicating that greater herbivory rates were strongly associated with a reduced likelihood of a given seedling surviving until the following year (Table 2, Fig. 2). The explanatory power of the model was not improved by the addition of soil type ($\Delta d = 1.283$, df = 1, $P = 0.257$). Nonsignificant indications were found for

TABLE 2. Summary of binomial linear mixed-effects ANCOVA model for mortality of dipterocarp tree seedlings in the reciprocal transplant experiment.

Effect	Estimate	SE	Δ*d*	df	*P*
Intercept	−2.331	0.368			
Species					
Hopea nervosa	−1.990	0.236			
Parashorea tomentella	−0.581	0.180			
Shorea fallax	−0.507	0.190			
Shorea multiflora	−0.321	0.175			
Hopea beccariana	0		98.205	4	<0.001
Understory light	2.501	0.162	360.500	1	<0.001
Herbivory rate	21.781	4.053	28.278	1	<0.001
Initial height	−0.019	0.008	5.813	1	0.016

Note: Parameter estimates are given as deviations from the intercept; SE is the standard error of the estimate. The Δ*d* column gives the change in deviance on removal of each term.

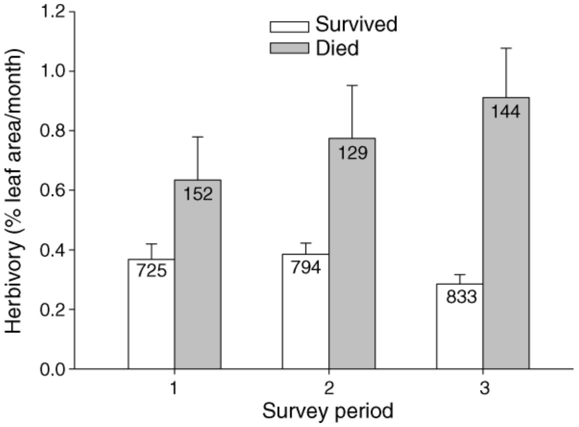

FIG. 2. Mature-leaf herbivory rates on seedlings in each of three survey periods split between those that survived or died in the following 13-month period. Values are means per plot + SE averaged across all species and treatments. Numbers of seedlings are shown at the top of each bar; these represent the total number of assessed seedlings remaining at the end of each herbivory survey. Survey periods were: 1, July 2000–February 2001; 2, February–September 2001; 3, September 2001–April 2002 (see *Methods: Herbivory rates*).

effects of fertilizer treatment ($\Delta d = 2.754$, df = 1, $P = 0.097$) and survey period ($\Delta d = 2.879$, df = 1, $P = 0.089$). There was no significant interaction between species, soil, and herbivory ($\Delta d = 2.515$, df = 4, $P = 0.642$), suggesting that variation in herbivory rate does not contribute to habitat segregation through differential survival of seedlings among species on each soil type. However, a significant interaction between species, light, and herbivory suggests that this might occur with respect to gap and understory treatments ($\Delta d = 13.678$, df = 4, $P = 0.008$; Fig. 3). Four out of five species suffered greater mortality when damaged by herbivores in the understory, with *H. beccariana* being the exception. No significant interactions occurred between any other main effect and herbivory rate, although there was a nonsignificant indication of a possible interaction between light conditions and herbivory rate, with greater herbivore-induced mortality in the understory ($\Delta d = 3.021$, df = 1, $P = 0.082$).

Models partitioned between the three survey periods produced qualitatively identical results, and the effect of herbivory rate did not vary significantly between survey periods ($\Delta d = 1.248$, df = 1, $P = 0.264$). Within each of the three periods, seedlings with higher rates of mature-leaf herbivory were more likely to die subsequently (Fig. 2).

Analysis of new-leaf herbivory rates produced broadly similar results, although there was a significant interaction between species and new-leaf herbivory rates ($\Delta d = 13.384$, df = 4, $P = 0.010$) with damaged *H. beccariana* seedlings showing a reduced likelihood of subsequent mortality. In contrast to mature-leaf herbivory rates, there was no overall effect of new-leaf herbivory rates on mortality ($\Delta d = 1.966$, df = 1, $P = 0.161$).

DISCUSSION

Growth and herbivory

This experiment showed no indication that herbivory of mature leaves correlated with the relative growth rates of seedlings, measured either by basal stem diameter or height increments. Basal stem diameter is a more accurate measure of dipterocarp seedling biomass than height (Turner 1990), which can be overly influenced by minor shoot damage. Relative growth

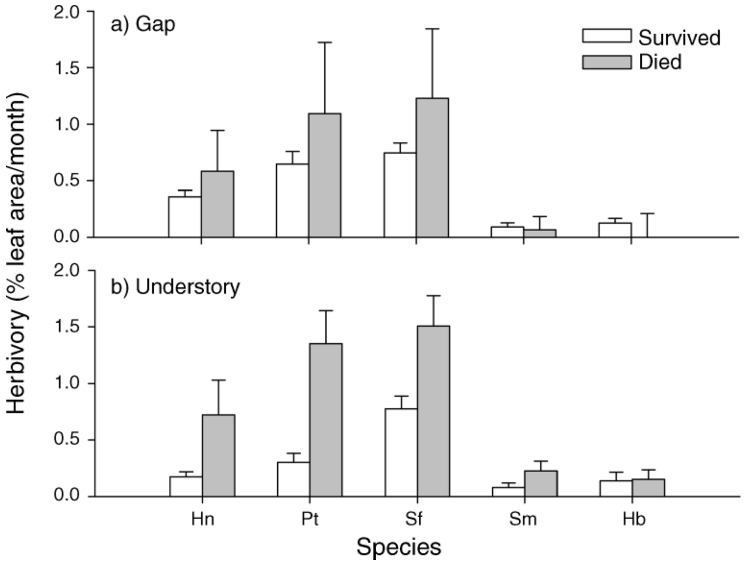

FIG. 3. Mature-leaf herbivory rates on seedlings in (a) gap and (b) understory plots split between those that survived or died in the following 13-month period. Species abbreviations are: Hn, *Hopea nervosa*; Pt, *Parashorea tomentella*; Sf, *Shorea fallax*; Sm, *Shorea multiflora*; Hb, *Hopea beccariana*. Values are means per plot + SE averaged across all other treatments.

PLATE 1. Experimental seedlings in a natural forest gap on a sandstone ridge in Sepilok Forest Reserve, Malaysia. The seedling in the foreground (*Shorea fallax*) shows evidence of Lepidopteran herbivory on its upper leaves. Photo credit: Jake L. Snaddon.

rates varied between species, soil types, and light conditions and decreased over time (Fig. 1, Table 1), but the effect of herbivory rate did not vary significantly across any of these factors. The only exception was a weak interaction with soil type in the first survey period, indicating a minor growth reduction due to herbivory for seedlings on sandstone soils relative to alluvial ones. Since this did not occur later in the experiment, it may reflect greater vulnerability of seedlings at early stages of establishment, although its absence from the height RGR model cautions against overinterpretation. We have no information on belowground parts, and therefore all conclusions pertain exclusively to aboveground growth.

Our initial predictions of variable impacts of herbivory on growth between treatments were not borne out by the analysis. No consistent interactions were found that would support greater impacts of herbivory in conditions of either light or nutrient limitation. Rates of herbivory on mature leaves were low, ranging from 0.07% to 0.8% leaf area/month among species (Eichhorn et al. 2006; see Appendix B), but are consistent with values reported elsewhere (Coley and Barone 1996, Howlett and Davidson 2001). Previous work has demonstrated that herbivory rates in this system do not vary consistently between soil types, light environments, and fertilizer treatments, although variability between species is high (Eichhorn et al. 2006). In gap plots, it seems plausible that the seedlings are capable of compensating for tissue loss to herbivory by increased growth. In the understory, however, equivalent herbivory rates coupled with low growth rates may ultimately reduce the survival of seedlings on longer timescales. The rise in standing herbivore damage in understory plots relative to the gaps over two years indicated that shaded plants are not compensating for foliar loss to the same extent (Eichhorn 2003), probably due to reduced leaf turnover, but this was not reflected in reduced growth relative to undamaged plants in the understory.

Though new-leaf herbivory rates were an order of magnitude higher than for older leaves (Eichhorn et al. 2006; see Appendix B), new leaves constituted a minority of those present on any given seedling, and it is therefore unsurprising that new-leaf herbivory was not correlated with any reduction in overall growth rates. An interaction between soil type and new-leaf herbivory suggested that it may depress growth on alluvial soils relative to sandstone, a result inconsistent with our hypotheses. However, the overall low rates of leaf production on sandstone soil and the use of a single census to estimate herbivory rates on new leaves mitigate against overinterpretation of this result.

Despite the prevailing assumption that herbivory negatively affects the growth of plants, there is surprisingly little empirical evidence that defoliation has impacts on tropical woody plants grown in natural conditions. For example, studies have found no link between short-term herbivory rates and RGR of Panamanian tree saplings (Coley 1983), natural defoliation rates and growth of Australian tree seedlings (Jackson and Bach 1999), or standing levels of herbivory and the growth and mortality of Neotropical pioneer species (Pearson et al. 2003). Studies that have demonstrated such links (Marquis 1984, Dominguez and Dirzo 1994, Gerhardt 1998, Blundell and Peart 2001) had rates of natural or artificial damage that far exceed those observed in our study. Clear evidence that low or intermittent rates of folivory might have long-term impacts on tree seedling growth has yet to emerge.

Several studies have documented increased growth rates of tropical plants when herbivores are excluded by mesh cages (Sagers and Coley 1995, Fine et al. 2004), which allow experimental manipulation of herbivory, but also exclude all guilds of herbivores so that the impacts of folivores cannot be separately assessed. Most studies showing a strong impact of damage on growth or reproduction of tropical woody plants under natural conditions have considered root, stem, or shoot-tip herbivores (Whitmore and Brown 1996, Bebber et al. 2002, Sullivan 2003) rather than folivores, and it is likely that homopteran stem-feeders will also contribute to the apparent reduced impact of herbivory on growth in exclusion experiments. Homopteran densities were not enumerated in the present study, although observations suggested that their density was substantially greater in the alluvial forest gaps relative to all other plots. This does not correspond to the patterns of herbivory, growth, or mortality. While it remains possible that herbivory in the broader context might be influencing growth of seedlings in our study, it appears that the impact of folivores, the most commonly studied category of herbivores, is negligible.

Survival and herbivory

Herbivory rate was a powerful correlate of future mortality rates (Table 2). In each of the three intervals between herbivory assessments, seedlings that received greater rates of damage were more likely to perish in the following year (Fig. 2). Effects of herbivory on survival rates have been documented previously, but, as with impacts on seedling growth rates, are typically associated with greater rates of damage and smaller seedlings than were considered here (e.g., Clark and Clark 1985, Nunez-Farfan and Dirzo 1991, Nascimento and Hay 1994, Jackson and Bach 1999, Fine et al. 2006). In this study, herbivory rates of less than 1% per month on mature leaves were sufficient to have profound impacts on mortality (Fig. 2). Herbivory on new leaves, in contrast, was not correlated with future mortality.

Although mortality rates also varied between species and were greater in the understory, there was no indication of interactions of these factors with herbivory, suggesting that the impact of herbivory is consistent across all species and treatments. However, there was an indication of a greater impact of herbivory in low-light conditions, and this effect varied between species. *Hopea beccariana* is more capable than the other species of withstanding herbivory in shaded conditions (Fig. 3). Such effects have been inferred by previous studies (Pearson et al. 2003, Massey et al. 2006) and may contribute to partitioning of seedlings between light habitats within the forest. In our system, the effect of herbivory on habitat segregation may be significant with respect to light regime, but not between soil types.

A potential mechanism consistent with our results is that plants already weakened by exogenous stress are subject to greater rates of herbivory, which is not necessarily the primary cause of their later mortality. There are no clear interactions between herbivory rate and other experimental treatments, suggesting that herbivory is not merely acting upon seedlings that are already affected by shortage of light, nutrients, or growth on an unfamiliar soil type. Nevertheless, in the understory, where growth rates are low and few new leaves are produced, variation in the ability of species to cope with cumulative damage to mature leaves may influence their ability to survive (Fig. 3). These effects might influence the outcome of competitive interactions between species in the understory (e.g., Dirzo 1984). It is also possible that herbivory has unmeasured impacts on belowground resource accumulation, which in turn influences their survival, but we have no cause to suspect that this is occurring.

An alternative hypothesis consistent with our general finding that herbivory increases mortality across multiple species and treatments is that herbivory facilitates infection by pathogens, which can have powerful effects on tree seedling mortality (Bell et al. 2006). Unfortunately no quantitative data exist on fungal attack in our experiment, and it was difficult in the field to distinguish fungal necrosis from natural dieback of leaf tissues following damage. Nevertheless, previous work suggests that this may be a plausible mechanism. In a Mexican rain forest, wounding was the primary mechanism of fungal establishment in leaves, with a clear association between herbivore damage and infection (Garcia-Guzman and Dirzo 2001). Other studies have found an association between amounts of standing herbivory and pathogen damage on tree seedlings within forests and at the edges of fragments (Benitez-Malvido et al. 1999, Benitez-Malvido and Lemus-Albor 2001). This explanation implies that pathogen transmission may be a more potent cause of seedling mortality than herbivory itself. In contrast, De Walt et al. (2004) applied herbicides and pesticides to a Neotropical shrub, finding an approximately equal reduction in mortality among treatments. There was an additive combined effect, which implies that in this case herbivore exclusion did not lead to a reduction in pathogen transmission and subsequent mortality.

Effects on soil habitat segregation

This experiment failed to provide evidence that a growth–survival trade-off among dipterocarp seedlings in this system is mediated through herbivory. An interesting comparison may be drawn between our results and the paradigm that shade tolerance in tree seedlings is more directly related to survival than growth (Canham 1989, Kobe et al. 1995); perhaps tolerance of natural enemies is a niche dimension operating in the same fashion. In a parallel study to our own that replicated the design in controlled nursery conditions, the growth–survival trade-off was found to occur in the absence of herbivory (Dent and Burslem 2009), a finding repeated in a comparable study in French Guiana (Baraloto et al. 2006).

Our conclusions contrast with those who have suggested that herbivory may be a force driving soil specialization in rain forest trees (Fine et al. 2004) and in excluding species from particular habitats within their native range (DeWalt et al. 2004). This is despite alluvial soils having markedly greater fertility than sandstone soils (Appendix A). Although the difference in soil fertility was not as pronounced as that between clay and white-sand forests in the study by Fine et al. (2006), there were nevertheless substantial differences in nutrient concentrations experienced by established saplings on the two soil types (Dent et al. 2006). These results imply that herbivory may only drive specialization when differences between habitats are more pronounced, although the small number of comparable studies makes it difficult to quantify the scale of this effect. Soil types in themselves may be the primary drivers of mortality, independent of biotic interactions (Table 1, Appendix B; Baltzer et al. 2005, Dent and Burslem 2009).

Conclusions

Foliar herbivory is associated with greater mortality of tropical rain forest seedlings, but this impact is apparently not mediated through reduced aboveground growth. The effect of herbivory on mortality does not differ between soil types, suggesting that herbivory does not drive habitat partitioning by edaphic specialists. In contrast, for four species out of five, mortality in the shade was sensitive to the amount of herbivory. This interaction may result in segregation of species between gap and understory light conditions. The impacts occurred with natural rates of tissue removal substantially lower than those previously shown to influence mortality. An interaction with pathogens may be a plausible explanation for these observations and merits further investigation.

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LITERATURE CITED

Ashton, P. S. 1968. A manual of the dipterocarp trees of Brunei State and of Sarawak—supplement. Cathay Press, Hong Kong.

Baltzer, J. L., S. C. Thomas, R. Nilus, and D. F. R. P. Burslem. 2005. Edaphic specialization in tropical trees: physiological correlates and responses to reciprocal transplantation. Ecology 86:3063–3077.

Baraloto, C., D. Bonal, and D. E. Goldberg. 2006. Differential seedling growth response to soil resource availability among nine neotropical tree species. Journal of Tropical Ecology 22: 487–497.

Bates, D. M., and D. Sarkar. 2007. lme4: linear mixed-effects models using S4 classes. R package version 0.9975-13. R Foundation for Statistical Computing, Vienna, Austria. ⟨http://cran.r-project.org⟩

Bebber, D., N. Brown, and M. Speight. 2002. Drought and root herbivory in understorey *Parashorea* Kurz (Dipterocarpaceae) seedlings in Borneo. Journal of Tropical Ecology 18: 795–804.

Bell, T., R. P. Freckleton, and O. T. Lewis. 2006. Plant pathogens drive density-dependent seedling mortality in a tropical tree. Ecology Letters 9:569–574.

Benitez-Malvido, J., G. Garcia-Guzman, and I. D. Kossmann-Ferraz. 1999. Leaf-fungal incidence and herbivory on tree seedlings in tropical rainforest fragments: an experimental study. Biological Conservation 91:143–150.

Benitez-Malvido, J., and A. Lemus-Albor. 2001. The seedling community of tropical rain forest edges and its interaction with herbivores and pathogens. Biotropica 37:301–313.

Blundell, A. G., and D. R. Peart. 2001. Growth strategies of a shade-tolerant tropical tree: the interactive effects of canopy gaps and simulated herbivory. Journal of Ecology 89:608–615.

Canham, C. D. 1989. Different responses to gaps among shade-tolerant tree species. Ecology 70:548–550.

Chazdon, R. L. 1988. Sunflecks and their importance to forest understorey plants. Advances in Ecological Research 18:1–63.

Chesson, P. 1991. A need for niches? Trends in Ecology and Evolution 6:26–28.

Clark, D. A., and D. B. Clark. 1985. Seedling dynamics of a tropical tree: impacts of herbivory and meristem damage. Ecology 66:1884–1892.

Coley, P. D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecological Monographs 53:209–233.

Coley, P. D., and T. M. Aide. 1991. A comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. Pages 25–49 *in* P. W. Price, T. M. Lewinsohn, G. W. Fernandes, and W. W. Benson, editors. Plant–animal interactions: evolutionary ecology in tropical and temperate regions. John Wiley and Sons, New York, New York, USA.

Coley, P. D., and J. A. Barone. 1996. Herbivory and plant defenses in tropical forests. Annual Review of Ecology and Systematics 27:305–335.

Crawley, M. J. 2005. Statistics: an introduction using R. John Wiley and Sons, Chichester, UK.

Dent, D. H. 2004. The mechanistic basis of habitat specialisation in dipterocarps. University of Aberdeen, Aberdeen, UK.

Dent, D. H., R. Bagchi, D. Robinson, N. Majalap-Lee, and D. F. R. P. Burslem. 2006. Nutrient fluxes via litterfall and leaf litter decomposition vary across a gradient of soil nutrient supply in a lowland tropical rain forest. Plant and Soil 288:197–215.

Dent, D. H., and D. F. R. P. Burslem. 2009. Performance trade-offs driven by morphological plasticity contribute to habitat specialization of Bornean tree species. Biotropica 41:424–434.

DeWalt, S. J., J. S. Denslow, and K. Ickes. 2004. Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. Ecology 85:471–483.

Dirzo, R. 1984. Herbivory: a phytocentric overview. Pages 141–165 *in* R. Dirzo and J. Sarukhan, editors. Perspectives on plant population ecology. Sinauer, Sunderland, Massachusetts, USA.

Dominguez, C. A., and R. Dirzo. 1994. Effects of defoliation on *Erythroxylum havanense*, a tropical proleptic species. Ecology 75:1896–1902.

Eichhorn, M. P. 2003. Insect herbivory and the regeneration of dipterocarp seedlings in a Malaysian rain forest. Thesis. University of Leeds, Leeds, UK.

Eichhorn, M. P., S. G. Compton, and S. E. Hartley. 2006. Seedling species determines rates of leaf herbivory in a Malaysian rain forest. Journal of Tropical Ecology 22: 513–519.

Eichhorn, M. P., K. C. Fagan, S. G. Compton, D. H. Dent, and S. E. Hartley. 2007. Explaining leaf herbivory rates on tree seedlings in a Malaysian rain forest. Biotropica 39:416–421.

Fine, P. V. A., I. Mesones, and P. D. Coley. 2004. Herbivores promote habitat specialization by trees in Amazonian forests. Science 305:663–665.

Fine, P. V. A., Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. H. Stevens, I. Saaksjarvi, L. C. Schultz, and P. D. Coley. 2006. The growth-defense trade-off and habitat specialization by plants in Amazonian forests. Ecology 87(Supplement):S150–S162.

Fox, J. D. 1973. A handbook to Kabili-Sepilok Forest Reserve. Sabah Forestry Department, Sandakan, Malaysia.

Garcia-Guzman, G., and R. Dirzo. 2001. Patterns of leaf-pathogen infection in the understory of a Mexican rain forest: incidence, spatiotemporal variation, and mechanisms of infection. American Journal of Botany 88:634–645.

Gerhardt, K. 1998. Leaf defoliation of tropical dry forest tree seedlings—implications for survival and growth. Trees: Structure and Function 13:88–95.

Hawkes, C. V., and J. J. Sullivan. 2001. The impact of herbivory on plants in different resource conditions: a meta-analysis. Ecology 82:2045–2058.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants—to grow or defend. Quarterly Review of Biology 67: 283–335.

Howlett, B. E., and D. W. Davidson. 2001. Herbivory on planted dipterocarp seedlings in secondary logged forests and primary forests of Sabah, Malaysia. Journal of Tropical Ecology 17:285–302.

Jackson, R. V., and C. E. Bach. 1999. Effects of herbivory on growth and survival of seedlings of a rainforest tree, *Alphitonia whitei* (Rhamnaceae). Australian Journal of Ecology 24:278–286.

Kobe, R. K., S. W. Pacala, J. A. J. Silanda, and C. D. Canham. 1995. Juvenile tree survivorship as a component of shade tolerance. Ecological Applications 5:517–532.

Lowman, M. D. 1984. An assessment of techniques for measuring herbivory: Is rainforest defoliation more intense than we thought? Biotropica 16:264–268.

Marquis, R. J. 1984. Leaf herbivores decrease fitness of a tropical plant. Science 226:537–539.

Massey, F. P., K. Massey, M. C. Press, and S. E. Hartley. 2006. Neighbourhood composition determines growth, architecture and herbivory in tropical rain forest tree seedlings. Journal of Ecology 94:646–655.

Nascimento, M. T., and J. D. Hay. 1994. The impact of simulated folivory on juveniles of *Metrodorea pubescens* (Rutaceae) in a gallery forest near Brasilia, Federal-District, Brazil. Journal of Tropical Ecology 10:611–620.

Nilus, R. 2004. Effect of edaphic variation on forest structure, dynamics, diversity and regeneration in a lowland tropical rain forest in Borneo. Thesis. University of Aberdeen, Aberdeen, UK.

Nunez-Farfan, J., and R. Dirzo. 1991. Effects of defoliation on the saplings of a gap-colonizing neotropical tree. Journal of Vegetation Science 2:459–464.

Paoli, G. D., L. M. Curran, and D. R. Zak. 2006. Soil nutrients and beta diversity in the Bornean Dipterocarpaceae: evidence for niche partitioning by tropical rain forest trees. Journal of Ecology 94:157–170.

Pearson, T. R. H., D. Burslem, R. E. Goeriz, and J. W. Dalling. 2003. Regeneration niche partitioning in neotropical pioneers: effects of gap size, seasonal drought and herbivory on growth and survival. Oecologia 137:456–465.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer-Verlag, New York, New York, USA.

Russo, S. E., S. J. Davies, D. A. King, and S. Tan. 2005. Soil-related performance variation and distributions of tree species in a Bornean rain forest. Journal of Ecology 93: 879–889.

Sagers, C. L., and P. D. Coley. 1995. Benefits and costs of defense in a neotropical shrub. Ecology 76:1835–1843.

Sullivan, J. J. 2003. Density-dependent shoot-borer herbivory increases the age of first reproduction and mortality of neotropical tree saplings. Oecologia 136:96–106.

Swaine, M. D., and T. C. Whitmore. 1988. On the definition of ecological species groups in tropical rain forests. Vegetatio 75:81–86.

Turner, I. M. 1990. The seedling survivorship and growth of three *Shorea* species in a Malaysian tropical rain forest. Journal of Tropical Ecology 6:469–478.

Whitmore, T. C., and N. D. Brown. 1996. Dipterocarp seedling growth in rain forest canopy gaps during six and a half years. Philosophical Transactions of the Royal Society B 351: 1195–1203.

Zangerl, A. R., J. G. Hamilton, T. J. Miller, A. R. Crofts, K. Oxborough, M. R. Berenbaum, and E. H. de Lucia. 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. Proceedings of the National Academy of Sciences (USA) 99:1088–1091.

APPENDIX A

Environmental characteristics of experimental plots (*Ecological Archives* E091-077-A1).

APPENDIX B

Heights and rates of growth, survival, and herbivory of seedlings in experimental plots (*Ecological Archives* E091-077-A2).