# Nutrient allocation of *Macaranga triloba* ant plants to growth, photosynthesis and indirect defence

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**Summary**

**1.** Allocation patterns are an important aspect of plant life strategies. In particular, many hypotheses on plant antiherbivore defence – such as the growth differentiation balance hypothesis and the resource availability hypothesis – concern constraints on resource allocation to defence, and assume defence to be limited by nutrient supply under most, yet not all, conditions.

**2.** Field-grown *Macaranga triloba* saplings were fertilized, and temporal and spatial patterns in the response of growth, photosynthesis and investment in biotic defence against herbivores and pathogens were measured simultaneously. *Macaranga triloba* produces food bodies to nourish mutualistic ants which protect their hosts against herbivores and pathogens. Food body production rate is quantitatively related to ant colony structure, and the latter is correlated with defensive efficacy. Food body production can therefore serve as a measure of the plant's investment in defence.

**3.** Food body production responded quickly, and on all food body-producing stipules, to increased nutrient supply. In contrast, photosynthetic capacity responded much more slowly, to a smaller degree, and only in leaves that emerged after the onset of fertilization. No significant effects on plant growth were seen.

**4.** Our results show that food body production by *M. triloba* is regulated directly by nutrient availability. There was no evidence that increased nutrient supply was preferably allocated to growth and/or photosynthesis, which forms a central assumption of most hypotheses on plant antiherbivore defence.

**5.** Phenotypic plasticity and the ability to make use of short pulses in nutrient supply are of particular importance in pioneer plants inhabiting rapidly changing habitats. New theoretical frameworks are required for the inclusion of phenotypically plastic defence traits in the theory of plant antiherbivore defence.

*Key-words*: Ant–plant mutualism, biotic defence, growth differentiation balance hypothesis, resource availability hypothesis, tropics

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## Introduction

Much effort has been devoted to unravelling the influence of nutrient availability on three important plant traits: growth, photosynthesis and defence (Bazzaz & Grace 1997; Bazzaz *et al*. 1987). Several hypothesis have dealt with the question of how plants should defend themselves in habitats that differ in resource availability.

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Most of these hypotheses assume defence to be costly, and to be negatively correlated with plant growth and fitness under enemy-free conditions (Simms & Rausher 1987). Hypothesis such as the resource availability hypothesis (Bryant *et al*. 1985; Coley *et al*. 1985) and the growth differentiation balance hypothesis (Herms & Mattson 1992; Loomis 1953) assume that resources in general will be allocated preferentially to growth-relevant processes such as cell division and photosynthesis. Defence, in contrast, will be preferred only under conditions characterized by lightly limited nutrient supply, as these conditions limit growth more than photosynthesis and thus free up assimilates for secondary metabolism (Herms & Mattson 1992).

As yet, the many theoretical and empirical studies addressing this issue (Bloom *et al.* 1985; Edwards 1989; Kytö *et al.* 1996 for reviews; Bryant *et al.* 1983; Coley *et al.* 1985; Herms & Mattson 1992; Loreau & de Mazancourt 1999 for theoretical studies) have not yielded a clear picture. Many studies have reported a negative correlation of growth rates with investment to defence (Coley 1988; Coley *et al.* 1985; Howlett & Davidson 2001; Niemann *et al.* 1992; Sagers & Coley 1995), but temporal and spatial patterns in the response to enhanced nutrient supply were seldom studied simultaneously for growth, photosynthesis and defence investment. This knowledge is required in order to decide whether the central assumption of preferred allocation to growth and photosynthesis holds true. Furthermore, comparison of different studies is complicated by severe difficulties in quantifying the true cost of plants' investment in chemical defence (Gershenzon 1994; Moore 1998).

'Ant plants' are increasingly being used as model systems to study different aspects of plant antiherbivore defences. Many myrmecophytes (obligate ant plants) house specific ants (Beattie 1985; Bronstein 1998; Buckley 1982; Hölldobler & Wilson 1990; McKey & Davidson 1993). Myrmecophytic plants such as *Macaranga triloba* (Bl.) Muell. Arg. (Euphorbiaceae) house symbiotic ant colonies in their hollow shoots. The ant workers use food bodies provided by the host plant to feed queen and larvae (Fiala & Maschwitz 1992a; Fiala & Maschwitz 1992b) and defend their hosts against different attackers such as herbivores, shoot-borers and pathogenic fungi (Heil *et al.* 1999; Heil *et al.* 2001a). Food bodies contain large amounts of lipids and proteins (Heil *et al.* 1998). Food body production requires up to 5% of the plant's total energy budget and up to 7·5% of the proteins that are invested in above-ground biomass production – it can therefore be regarded as an 'expensive' form of defence (Heil *et al.* 1997). Food body production at the plant's natural growing site is limited by soil nutrient content, and strong correlations between food body production, ant colony size and leaf damage indicate that the indirect defence of *M. triloba* is limited by soil nutrient availability (Heil *et al.* 2001b). Yet, as food bodies are used for the brood, a time lag is to be expected between changes in the investment in defence (a plant's food body production) and responses in the expression of defence (number of ant workers available on this plant). Comparable systems have already been used for the quantification of investment in defence (Folgarait & Davidson 1994; Folgarait & Davidson 1995; Heil *et al.* 1997; Moore 1998).

In the present study we investigated nutrient allocation in *M. triloba* under natural field conditions in West Malaysia. We quantified growth, photosynthesis and food body production under normal and enriched nutrient conditions in order to test one of the most important assumptions in theory on plant antiherbivore defence: that resources are preferentially allocated to growth and photosynthesis.

## Materials and methods

### STUDY SITE AND SPECIES

*Macaranga triloba* grows as small trees mainly in secondary forests in South-East Asia. Plants were identified after Whitmore (1967); Whitmore (1973). However, the species probably should be renamed *M. bancana* (T. C. Whitmore and S. J. Davies, personal communication; see also Slik 1998). *Macaranga triloba* produces food bodies and houses one ant colony throughout its lifetime (Fiala & Maschwitz 1992b). Food bodies are produced under the lower surface of recurved stipules inserting pairwise beneath the petioles at rates depending on stipule age (Heil *et al.* 1997). The study was conducted during March/April and September/October 1998 at the Ulu Gombak Field Studies Center in Selangor, Peninsular Malaysia. The area of this field station is covered by a secondary forest that had been logged about 30 years ago.

### GENERAL DESIGN AND FOOD BODY PRODUCTION

Food body production by *M. triloba* plants depends on plant size and on the presence of an ant colony (Heil *et al.* 1997). To exclude size effects, only young, unbranched saplings were used that were inhabited by ant colonies. All plants used in these experiments grew in a habitat representing the species' preferred site, along a small trail (3 m wide) crossing a secondary forest. Plants were measured at the beginning of the experiments, and arranged in pairs with respect to leaf number, stipule number, plant height and total leaf area in order to guarantee equal representation of different plant sizes in the various experimental conditions.

The direct survey of food body numbers is not possible because food body-producing stipules are recurved down and backwards and tightly clasp the stem, hiding the food bodies (Figure 1c of Heil *et al.* 1998). Food body production was therefore quantified by an indirect method, as described earlier (Heil *et al.* 1997). Briefly, uninfluenced plants possess similar amounts of food bodies under the two stipules of a pair. One randomly selected stipule was removed from the first three and every other of the older stipule pairs, and food bodies present under this stipule were counted. Before counting, food bodies were divided into two size classes with a mean DM of ≈9 µg (small) and ≈23 µg (large) (Heil *et al.* 1997). Ants were excluded from the remaining stipules by applying a sticky resin (Tangletrap, Tanglefoot Corp., USA) around the stipule. These stipules were removed 1 week later to count food bodies and calculate food body DM. The difference in the amount of food bodies among stipules forming one pair and collected before and after 1 week of exclusion was interpreted as the weekly food body production of a given stipule.

### EXPERIMENT 1: GROWTH AND FOOD BODY PRODUCTION

In March and April 1998, 15 *M. triloba* plants were selected in the field for preliminary experiments on the quantitative effects of fertilization on growth and food body production, and to compare the putative effects of different commercially available fertilizers. Their initial total leaf area was estimated by measuring the length and width of all leaves, and calculating their area based on a regression equation derived from a sample of 100 leaves of known leaf area ($r^2 > 0.97$; Heil *et al.* 1997). The development of leaf area was taken as a measure for plant growth. Plants were assigned to five groups, each consisting of three plants which differed by <10% in total leaf area and number of stipule pairs. Within each group the plants were assigned randomly to three treatments (fertilizers 1 and 2, and untreated control). Two commercial fertilizers differing in N : P : K ratio and amounts of trace elements were used in this experiment (fertilizer 1, Westcott's garden fertilizer, SPGS Agriculture and Research Division, Petaling Jaya, West Malaysia; fertilizer 2, Organic Yellow plant food, Gardenic, Melaka, West Malaysia). Fertilizer 1 contained on average (g kg$^{-1}$) 127 N, 37 P, 158 K, 26 Ca, 18 S, 13 Mg, 3.3 C, 1.8 Na, and (mg kg$^{-1}$) 960 Fe, 240 B, 230 Mn, 213 Al, 150 Zn. Fertilizer 2 contained on average (g kg$^{-1}$) 120 N, 33 P, 110 K, 90 S, 49 Ca, 48 C, 9.4 Mg, 3.8 Al, 3.0 Na, and (mg kg$^{-1}$) 208 Mn, 251 Zn, 0.3 B (results of analyses of two samples per fertilizer). The fertilizer was applied directly onto the soil surrounding the stems of experimental plants. Plants <1 m high received 15 g; those 1–1.5 m, 25 g; and the largest plants (1.5–2 m), 30 g fertilizer per week. Due to unusually low rainfall, all plants were watered daily throughout the experiment ($\approx 2$ l day$^{-1}$). After 6 weeks the total leaf area of all plants was determined again, and growth was calculated as relative growth rate (RGR) by relating the newly produced leaf area to the initial total leaf area of the respective plant. Food body production was measured as described above.

### EXPERIMENT 2: FOOD BODY PRODUCTION AND PHOTOSYNTHESIS

The second experiment was conducted in September 1998 in order to determine responses in food body production and photosynthetic capacity to increased nutrient supply. The total leaf area of 40 field-grown plants was measured as before, and plants were arranged in pairs based on total leaf area and number of stipules. Four pairs each were assigned to five groups, each covering the whole size range of plants that were used. One randomly chosen plant per pair was assigned to the fertilization treatment, while the other plant was used as control. Fertilized plants received the same fertilizer amounts as described above of a 1 : 1 (weight) mixture of fertilizers 1 and 2 twice a week. As this experiment was conducted during the rainy season,

watering was not necessary. At the beginning of the experiment and weekly thereafter, one of the five plant groups was selected randomly for quantification of food body production (see above) and measurement of photosynthetic capacity. Photosynthetic $O_2$ production was measured using a Hansatech LD2 Leaf Disc Electrode (Hansatech Ltd, King's Lynn, UK). Disks (10 cm$^2$) of the youngest fully expanded leaf and a middle-aged, non-senescent leaf of each plant were enclosed in the cuvette. Measurements of leaves of two plants of a pair were conducted immediately after each other. Photosynthesis was induced at photon flux densities of $\approx 450$ μmol m$^{-2}$ s$^{-1}$ (until a constant rate of $O_2$ production occurred for at least 2 min), and $O_2$ production was then measured at 1000 and 1800 μmol m$^{-2}$ s$^{-1}$. After each change in photon flux density, the cuvette was purged with air containing $\approx 5\%$ $CO_2$ and saturated $H_2O$ vapour. The maximum $O_2$ evolution measured for a leaf was regarded as its photosynthetic capacity.

### EXPERIMENT 3: SHORT-TERM EFFECTS ON FOOD BODY PRODUCTION

A third field experiment was conducted to detect fast effects of fertilization on food body production. Weekly food body production rates could not be determined. However, previous studies had demonstrated that the number of food bodies present under a stipule at a given time, and the productivity of this stipule, are highly correlated (Heil *et al.* 1997). Thirty unbranched *M. triloba* plants were selected in October 1998. These plants were measured and assigned to five groups each consisting of three pairs, as described above. From each pair, one plant was selected randomly and was fertilized as in the previous experiment. Fertilized and control plants were watered daily with at least 2 l water per plant. The five plant groups were randomly assigned to different sampling dates (at the beginning of the experiment and every other day thereafter). At each harvest, food bodies under all stipules of the respective plants were counted.

### DATA ANALYSIS

Statistical tests followed Cohen (1998); Sachs (1992); Sokal & Rohlf (1981). Differently aged stipules were included in all experiments. Therefore repeated-measures ANOVA (GLM procedure in SPSS; SPSS Inc., Chicago, IL) with stipule age (represented by its position on the stem, with position 1 indicating the youngest) as within-subject factor was used for main data analyses. As repeated-measures ANOVA requires balanced sample sizes, these tests were based on subsamples of the stipule positions present on all plants involved in the respective experiment.

Different plants were measured at different times, so sampling date represented a between-subject factor. Fertilization was a between-subject factor in all experimental designs. Prior to all ANOVA procedures, the main

assumption of sphericity was tested with Mauchly's test. In case of significant deviations from sphericity, analysis was based on adjustments estimated by the Huynh–Feldt epsilon. *Post hoc* tests were conducted to localize the sources of significant differences. These tests follow the specific experimental designs as closely as possible. All *t*-tests conducted in this study are based on different data sets and thus do not require a Bon-ferroni correction. All statistics were calculated with SPSS for Windows 8·0.

An analysis of statistical power was used to calculate the probability that effects such as those in food body production (calculated as effect size index *d*, the difference between the two means divided by the standard deviation; Buchner *et al.* 1997; Cohen 1998) would have been detected in the respective test on photosynthetic capacity. This type of *post hoc* power analysis has been recommended by Greenwood (1993); Thomas (1997); Thomas & Juanes (1996). Analyses of statistical power were conducted with the program G*POWER (Buchner *et al.* 1997; Erdfelder *et al.* 1996).

## Results

### EXPERIMENT 1: GROWTH AND FOOD BODY PRODUCTION

Treating *M. triloba* plants with fertilizer 2 increased RGR compared both with controls and with plants treated with fertilizer 1 [leaf RGR (% per week) of controls, 0·57%; fertilizer 1, 0·39%; fertilizer 2, 1·06%]; however, these effects were not significant (unifactorial ANOVA comparing effects of controls and fertilizers 1 and 2 on RGR: df = 2, $F = 0.904$, $P > 0.10$, $n = 5$ plants per treatment). In contrast, both fertilizers significantly increased rates of food body production of the same plant individuals (Heil *et al.* 2001b).

### EXPERIMENT 2: FOOD BODY PRODUCTION AND PHOTOSYNTHESIS

Fertilized and control plants did not differ in food body production or photosynthesis at the beginning of the experiment (week 0, Tables 1 and 2; Figs 1 and 2). Stipule age, as well as fertilization and sampling date (week), contributed significantly to the total variance in food body production rates ($P < 0.001$ for all variables; Table 1). One week after the onset of fertilization, all stipules of fertilized plants had already produced more food bodies than did control stipules, on average (Fig. 1). At the end of the experiment, stipules of fertilized plants produced on average ≈4·3 mg food bodies (DM) per stipule per week (mean, $n = 25$ stipule pairs from four plants), while unfertilized controls produced on average only 1·7 mg. Paired *t*-tests comparing whole plants revealed significant differences between treated and untreated plants at all harvests but the first (Fig. 2).

Stipule position, fertilization and time of harvest (week) all significantly affected food body production rates (Table 1). Significant stipule × week and fertilization × week interactions occurred as both mean food body production rates, and patterns of dependency of food body production on stipule age, changed considerably over the experimental time-span, especially in fertilized plants (Figs 1 and 3). Evaluations conducted separately for each week indicate that food body production of whole plants was increased significantly by fertilization after

**Table 1.** Temporal patterns in effects of fertilization on food body production in *Macaranga triloba* (experiment 2). (a) Results of repeated-measures ANOVA on stipule position as within-subject factor and fertilization and week as between-subject factor, $n = 4$ for each stipule position–treatment–day combination. Fertilization tested against unfertilized controls. (b) Results of repeated-measures ANOVAs conducted separately for each week for stipule positions 1, 2, 3, 5 and 7. Only values for between-subject effects (fertilization) are shown; $n = 40$ stipules from eight plants (four fertilized and four controls) for each sampling date. NS = $P > 0.05$

| | SS | df | F | P |
|---|---|---|---|---|
| (a) Within-subject effects | | | | |
| Stipule | 14·343 | 5 | 5·661 | <0·001 |
| Stipule × fertilization | 1·366 | 5 | 0·539 | NS |
| Stipule × week | 19·046 | 20 | 1·879 | 0·030 |
| Stipule × fertilization × week | 6·630 | 20 | 0·654 | NS |
| Error | 30·403 | 60 | | |
| Between-subject effects | | | | |
| Fertilization | 46·138 | 1 | 25·055 | <0·001 |
| Week | 132·667 | 4 | 18·011 | <0·001 |
| Fertilization × week | 31·554 | 4 | 4·284 | 0·022 |
| Error | 22·098 | 12 | | |
| (b) Week | | | | |
| 0 | 0·024 | 1 | 0·044 | NS |
| 1 | 4·096 | 1 | 1·571 | NS |
| 2 | 13·156 | 1 | 13·367 | 0·011 |
| 3 | 8·556 | 1 | 33·197 | 0·001 |
| 4 | 118·198 | 1 | 20·563 | 0·004 |

**Table 2.** Temporal patterns in fertilization effects on photosynthesis in *Macaranga triloba* (experiment 2). (a) Results of repeated-measures ANOVA on leaf age as within-subject factor and treatment and week as between-subject factor, $n = 4$ for each leaf–treatment–day combination. (b) Results of repeated-measures ANOVAs conducted separately for each week. Only data for between-subject effects (fertilization) are shown. $n = 16$ leaves from eight plants (four fertilized and four controls) for each sampling date. NS = $P > 0.05$

| | SS | df | F | P |
|---|---|---|---|---|
| (a) Within-subject effects | | | | |
| Leaf | 143·552 | 1 | 9·693 | 0·007 |
| Leaf × fertilization | 10·084 | 1 | 0·681 | NS |
| Leaf × week | 17·014 | 5 | 0·230 | NS |
| Leaf × fertilization × week | 30·626 | 4 | 0·517 | NS |
| Error | 236·954 | 16 | | |
| Between-subject effects | | | | |
| Fertilization | 28·633 | 1 | 1·249 | NS |
| Week | 78·278 | 5 | 3·414 | 0·027 |
| Fertilization × week | 7·568 | 4 | 0·330 | NS |
| Error | 22·927 | 16 | | |
| (b) Week | | | | |
| 0 | 0·661 | 1 | 0·181 | NS |
| 1 | 14·251 | 1 | 0·292 | NS |
| 2 | 0·562 | 1 | 0·143 | NS |
| 3 | 35·708 | 1 | 2·002 | NS |
| 4 | 39·054 | 1 | 158·112 | <0·001 |
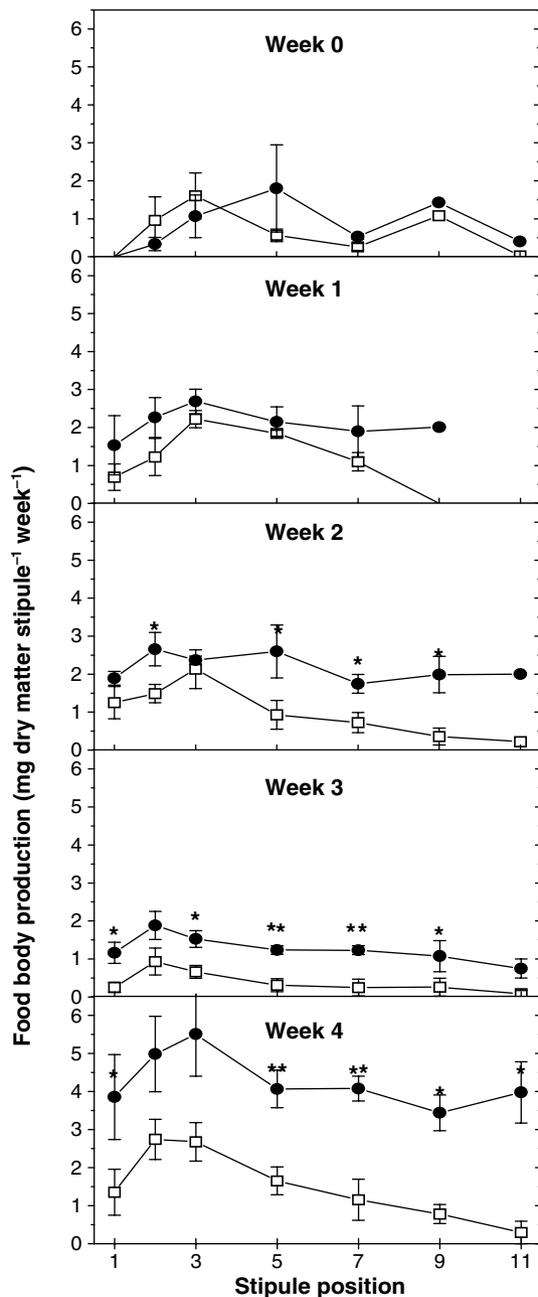
**Fig. 1.** Temporal patterns in fertilization effects on food body production in *Macaranga triloba*. Mean food body production rates (mg DM per stipule per week) of differently aged stipules are plotted separately for the plants observed at the beginning of the experiment (week 0) and after 1–4 weeks. Sample size, four fertilized and four control plants per week. Asterisks indicate significant fertilization effects at single stipule portion (*, $P < 0.05$; **, for $P < 0.01$ according to *t*-tests for independent samples). □, Controls; ■, fertilized plants; error bars, SE.

2 weeks (Table 1). Tests comparing production rates at single stipule positions revealed the first significant effects at this time (Fig. 1). Stipules at all positions but 2 and 3 showed significant fertilization effects at the end of the experiment.

Photosynthesis responded more slowly and less strongly to fertilization. Significant differences in
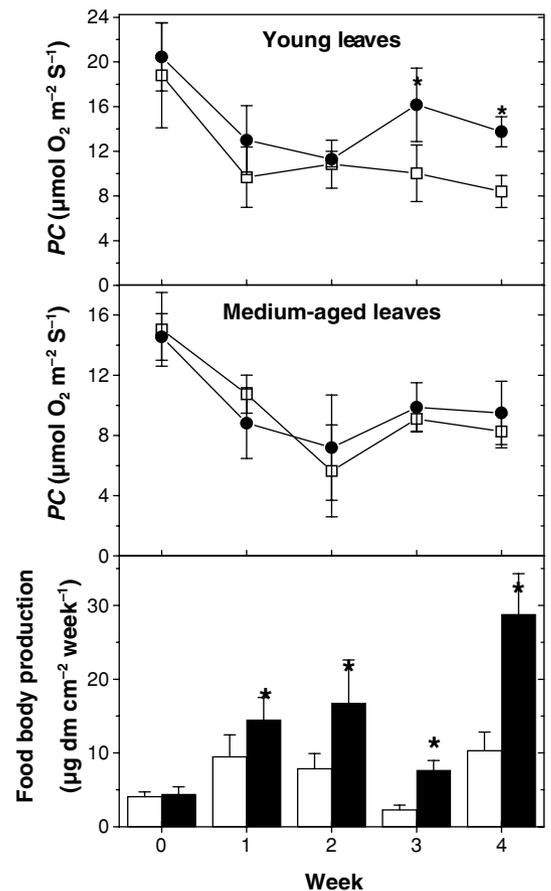


**Fig. 2.** Effect of fertilization on photosynthetic capacity (PC) and food body production by *Macaranga triloba*. Photosynthetic capacity was measured on each four treated and four control plants at the beginning of the experiment (week 0) and after 1–4 weeks. From each plant, one young and one medium-aged leaf were included. Asterisks indicate significant differences between fertilized and control plants at single leaf positions and sampling dates ($P < 0.05$, paired *t*-tests in which values from leaves of the same age class derived from the plants forming a pair were used as matched pair in the test, $n = 4$ leaf pairs per test). Food body production rates were measured on the same plants as photosynthetic capacity, and were calculated for whole plants (µg food body DM produced $cm^{-2}$ leaf area $week^{-1}$). Significant differences are indicated by asterisks ($P < 0.05$, paired *t*-tests based on data from whole plants, $n = 4$ pairs per test). See Fig. 1 for symbols.

photosynthetic capacity were found only for young leaves after 3 and 4 weeks (Fig. 2). On average, young leaves of fertilized plants had a photosynthetic capacity of ≈14 µmol $O_2$ $m^{-2}$ $s^{-1}$ at the end of the experiment, while comparable leaves of unfertilized controls reached only 8·6 µmol $O_2$ $m^{-2}$ $s^{-1}$. Leaf age and week had significant effects on photosynthetic capacity (Table 2). *Post hoc* tests for distinct sampling dates showed a significant fertilization effect only at the end of the experiment (Table 2). Paired *t*-tests comparing fertilized and control leaves separately for both leaf ages revealed no significant fertilization effects for the medium-aged leaves throughout the experimental period (Fig. 2). Effect sizes such as those in food body production would have been found to be significant, with a probability of 78%
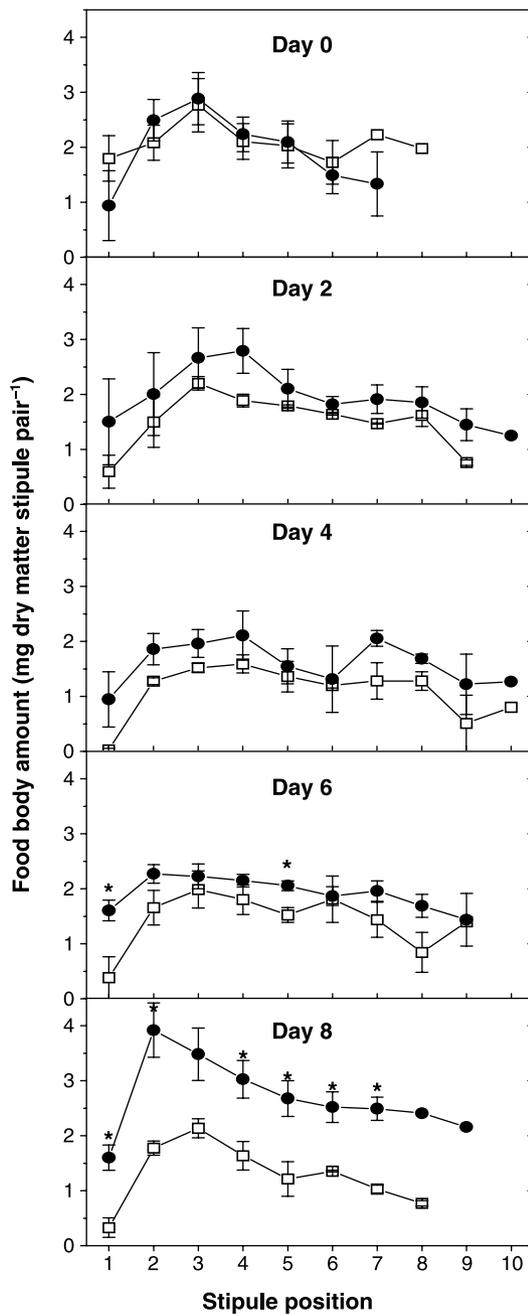
**Fig. 3.** Short-term response to fertilization of food body production by *Macaranga triloba*. Mean amounts of food bodies (mg DM) present under stipules at different positions are given separately for days 0, 2, 4, 6 and 8, respectively, after the beginning of fertilization. Asterisks mark significant differences between fertilized and control plants ($P < 0.05$ according to *t*-tests) at distinct stipule positions; $n = 3$ plants per day and treatment. See Fig. 1 for symbols.

EXPERIMENT 3: SHORT-TERM EFFECTS ON FOOD BODY PRODUCTION

Larger mean amounts of food bodies were present under all stipules of fertilized plants, as compared to controls,

**Table 3.** Results of power analysis on food body productivity data in *Macaranga triloba* (experiment 2). *Post hoc* power was calculated for paired *t*-tests that had been conducted separately for each week on food body productivity of whole plants. $d$ = Effect size for food body productivity of the respective week; $n$ = number of samples. See Fig. 2 for respective data and results of paired *t*-tests

| Week | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| $d$ | 0·06 | 1·60 | 2·80 | 2·33 |
| $n$ | 4 | 4 | 4 | 4 |
| df | 3 | 3 | 3 | 3 |
| Power $(1–\beta)$ | 0·06 | 0·78 | 0·99 | 0·96 |

as early as 2 days after the onset of fertilization in the short-term experiment (Fig. 3). Stipule position and fertilization contributed significantly to overall variance in food body amounts ($P < 0.001$ for position; $P = 0.003$ for fertilization; Table 4), while no significant interactions between the factors were detected. Plants did not differ significantly from each other with respect to sampling date, and no significant interactions between stipule age or fertilization and day of harvest occurred (Table 4). Significant fertilization effects on food body production of whole plants occurred 4, 6 and 8 days after the beginning of fertilization (Table 4), and significant differences at single stipule positions occurred after 6 days (see Fig. 3). After 8 days, on average 2·8 mg food bodies were present under stipules of fertilized plants (mean, $n = 21$ stipule pairs of three plants), compared to 1·3 mg in the untreated controls.

## Discussion

To our knowledge, this study is the first to reveal that a plant's first measurable response to increased nutrient availability can be increased production of defence compounds, rather than increased photosynthesis or growth. The production of ant food (food bodies) responded strongly and rapidly to nutrient supply (Tables 1 and 4; Figs 1–3). Food body production increased on young and old plant parts (Figs 1 and 3). In contrast, photosynthesis responded to a lesser degree (Table 2; Fig. 2), and the positive response was restricted to young leaves (Fig. 2). Growth (relative increase in total leaf area) showed no significant response during the experiments. Effect sizes as large as those found in food body production would also have been detected with high probabilities in the data on photosynthesis (Table 3; see Hayes & Steidl 1997; Thomas 1997 for a brief introduction to this type of power analysis). Therefore the lack of significant effects cannot simply result from small sample sizes, but rather points to small magnitudes of effects.

Food body production responded significantly to fertilization within 4 days (Table 4), and first effects were visible after only 2 days (Fig. 3). In contrast, significant effects on photosynthetic capacity appeared after 3 weeks at the earliest, and were restricted to

after 2 weeks and with probabilities of 99 and 96%, respectively, after 3 and 4 weeks (Table 3).

**Table 4.** Short-term effects of fertilization on food body production in *Macaranga triloba* (experiment 3). (a) Results of repeated-measures ANOVA on stipule position as within-subject factor and treatment and day as between-subject factor, $n = 3$ for each stipule position–treatment–day combination. (b) Results of repeated-measures ANOVAS conducted separately for each day for stipule positions 1–6. Only values for between-subject effects (fertilization) are shown. $n = 36$ stipules from six plants (three fertilized and three controls) for each sampling date. NS = $P > 0.05$

|  | SS | df | F | P |
|---|---|---|---|---|
| **(a) Within-subject effects** |  |  |  |  |
| Stipule | 37·718 | 5 | 7·544 | <0·001 |
| Stipule × fertilization | 1·713 | 5 | 1·730 | NS |
| Stipule × day | 6·480 | 20 | 1·636 | NS |
| Stipule × fertilization × day | 3·400 | 20 | 0·170 | NS |
| Error | 19·810 | 100 |  |  |
| **Between-subject effects** |  |  |  |  |
| Fertilization | 15·011 | 1 | 11·760 | 0·003 |
| Day | 10·918 | 4 | 2·729 | NS |
| Fertilization × day | 10·551 | 4 | 2·638 | NS |
| Error | 25·529 | 20 |  |  |
| **(b) Day** |  |  |  |  |
| 0 | 0·033 | 1 | 0·015 | NS |
| 2 | 2·673 | 1 | 1·583 | NS |
| 4 | 6·308 | 1 | 8·945 | 0·040 |
| 6 | 2·270 | 1 | 8·945 | 0·040 |
| 8 | 18·677 | 1 | 12·786 | 0·023 |

young leaves (Fig. 2). This was exactly the time when young leaves that had emerged during the experiment could be used for the first time for photosynthetic measurements (M.H., personal observation). Food body production responded strongly to fertilization, especially in older stipules (Figs 1 and 3; Heil *et al.* 2001b). These temporal and anatomical patterns indicate that food body production in *Macaranga* is limited primarily and directly by nutrient supply. Any indirect effects of fertilization, for example due to greater or faster carbon fixation (Bazzaz *et al.* 1987), cannot explain these patterns as photosynthesis showed a slower response than food body production. Only two studies have previously investigated responses of food body production to nutrient supply in a genus other than *Macaranga*. Food body production by six *Cecropia* species (Folgarait & Davidson 1995) and by *Piper cenocladum* (Dyer & Letourneau 1999) responded positively to fertilization. Other defensive investments that appear to be limited by soil nutrient supply are constitutive and induced proteinase inhibitors in *Brassica napus* (Cipollini & Bergelson 2001).

Many studies on nutrient allocation in plants have been conducted in the greenhouse. In contrast, our experiments on *M. triloba* (this study; Heil *et al.* 2001b) demonstrated that food body production – and thus defence – of myrmecophytes are limited by nutrients at the plants' natural growing sites. The South-East Asian *Macaranga* (Euphorbiaceae) and neotropical *Cecropia* (Cecropiaceae) are important pioneers, and respond similarly to nutrient supply by an increased investment in defence. This is somewhat contradictory to the expectation that resources are preferably allocated to growth and photosynthesis, which forms a main

assumption of the resource availability hypothesis (Bryant *et al.* 1985; Coley *et al.* 1985) or the growth differentiation balance hypothesis (Herms & Mattson 1992). These hypotheses predict that plant species growing on nutrient-rich soils should be characterized by high inherent growth rates and small defence investments, and therefore should respond quickly to pulses in resource availability with increased photosynthetic rate and realized growth rate (Coley *et al.* 1985).

Fertilization studies require cautious interpretation, as 'There is no reason to assume that the plant will be infinitely plastic in its response' (Loehle 1996). Yet *M. triloba* plants in our experiments were able to use pulses in soil nutrient availability for rapidly increased allocation to defence. The first theory that explicitly used the chemical composition of defensive chemicals to predict how they should be affected by current resource availability was the C/N balance hypothesis: Bryant *et al.* (1983) predicted that plants in which soil nutrient supply limits growth more than photosynthesis have 'free' carbon to be allocated to defence. *Vice versa*, nutrients are free to be allocated to defence when other factors, such as light, are limiting. Much evidence has been presented against this hypothesis (recently reviewed by Hamilton *et al.* 2001). Most crucially, the C/N balance hypothesis regarded 'N-free' compounds such as phenolics as having no cost in terms of nitrogen, an assumption that is not true according to the biosynthesis of these compounds (Jones & Hartley 1999).

Yet the costs of a given defence can differ greatly among sites differing in their growing conditions. Cost may, for example, increase under low nutrient supply (Bergelson 1994b; Heil *et al.* 2000), or when plants face intraspecific competition (Bergelson 1994a; van Dam & Baldwin 1998). It is this aspect of the C/N balance hypothesis (costs of defence depending on current growing conditions) that should not be ignored.

In our study, it must be remembered that physiologically based, central traits such as growth rate and photosynthetic capacity cannot respond so quickly to increased nutrient supply, due to the necessity to reorganize complex cellular processes. Soil nutrients – under 'normal' growing conditions strongly limiting defence investments by *M. triloba* – were transiently not used up by these processes in the fertilized plants, and therefore could be allocated to food body production at low costs. This might have the beneficial side effects that additional ants are already present when newly produced young leaves are to be protected. Responses to highly flexible pulses in nutrient availability and other changes in growing conditions are of particular importance for pioneer trees such *M. triloba* which inhabit rapidly changing habitats.

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