

Food body production in *Macaranga triloba* (Euphorbiaceae): a plant investment in anti-herbivore defence via symbiotic ant partners

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Summary

1 Quantifying the costs is the first step necessary for assessing the net value of any plant trait, and the costs of defence mechanisms in particular are largely unknown.

2 Several species of the important south-east Asian pioneer tree genus *Macaranga* (Euphorbiaceae) possess hollow stems that harbour ant colonies that act as a 'biotic' anti-herbivore defence. In *Macaranga triloba*, ants are nourished by food bodies (FBs) that are produced on the abaxial surfaces of recurved stipules. We estimated the costs arising from this kind of anti-herbivore defence by following FB production.

3 FB production of 36 different-sized plants was estimated in the field by comparing even-aged stipules with and without access for ants.

4 FB dry mass production amounted to about 5% of daily above-ground biomass production in unbranched saplings. When the chemical composition of FB and leaf tissue was taken into account, this represented about 9% of the above-ground tissue construction costs. In energetic terms, unbranched saplings invested 0.6–5% of their total assimilation in the FBs.

5 The relative investment in anti-herbivore defence arising from FB production decreased hyperbolically with increasing plant size. However, a linear relationship was found between FB production and plant size. Thus, in spite of the plants' decreasing relative investment with increasing size, a continually increasing food supply was provided for the ant colonies.

6 A second study was conducted to investigate whether FB production is influenced quantitatively by the presence of symbiotic ants. Ant-inhabited plants produced up to 35 times (mean 8 times) more FBs than similar-sized but ant-free ones. This difference resulted mainly from lower stipule numbers in ant-free plants. FB production of a whole plant therefore seems to be regulated to a high degree via stipule longevity.

7 Since the ants protect their host-plant very effectively, nourishing specialized mutualistic ants by FBs must be considered a rather expensive, but nevertheless highly beneficial, strategy of anti-herbivore defence, but regulation ensures that FB production is maintained at high rates only when ants are present.

Keywords: ant–plant interaction, costs of 'biotic' defence, mutualism, myrmecophytism, regulation of metabolic costs

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Introduction

Although understanding the function and evolution of a particular mutualism requires the quantification of costs arising from the production of 'rewards' or

the provision of 'services' by one of the partners (Bronstein 1994), few previous studies have been devoted to this issue. Mutualistic ant–plant interactions, such as when ants are nourished by the plant function as a so-called 'biotic' defence mechanism,

provide a system in which the costs and benefits may be quantified relatively easily (McKey 1988).

Throughout the wet tropics, many different symbiotic ant–plant interactions exist, and most of them are believed to be mutualistic (see reviews by Buckley 1982; Beattie 1985; Davidson & McKey 1993). In south-east Asia, a considerable number of species of the pioneer tree genus *Macaranga* (Euphorbiaceae) are obligate ant-plants and live in close association with certain ant and coccid species in a three-partner system. The hollow internodes (domatia) of the obligate myrmecophyte *Macaranga triloba* (Bl.) Muell. Arg. are predominantly inhabited by one specialized ant species of the genus *Crematogaster* (subgenus *Decacrema*) (Fiala & Maschwitz 1992a; Fiala *et al.* 1994). The worker ants protect their plant very effectively against herbivores and climbers (Fiala *et al.* 1989).

In myrmecophytic *Macaranga*, the ants feed exclusively on food bodies (FBs) produced directly by the plants and on honeydew supplied indirectly via scale insects kept in the interior of the domatia (Fiala & Maschwitz 1990, 1992b). In general, myrmecophytic plants differ interspecifically in the kinds of resources they offer to the ants as well as in the rates at which they supply these resources. For example, the investment in defence due to the production of ant-FBs varies according to life-history characteristics as well as to environmental circumstances (Davidson & Fisher 1991; Folgarait & Davidson 1994, 1995).

FBs belong to a large group of plant emergences, collectively named pearl bodies, which often contain relatively large amounts of lipids, proteins and carbohydrates (O'Dowd 1982). Those of myrmecophytic *Macaranga* species are characterized by especially high lipid contents (Heil *et al.*, in press) and their production should therefore incur considerable metabolic costs for the plant. FB production rates have so far been studied in three genera: *Piper* (Risch & Rickson 1981), *Ochroma* (O'Dowd 1980) and *Cecropia* (Folgarait & Davidson 1994, 1995; Folgarait *et al.* 1994). Since the chemical composition of most FBs is still unknown, a quantification of production costs is impossible for the vast majority of myrmecophytes.

In contrast to the synthesis of secondary metabolites, which often have a variety of different functions (Herms & Mattson 1992), FB production seems to represent a plant trait evolved exclusively for defence. The question of whether anti-herbivore defence may be costly is still largely unresolved (Gershenzon 1994a; Fineblum & Rausher 1995). This remains true for chemical defence (e.g. Gershenzon 1994b; Bryant & Julkunen-Tiitto 1995; Steinberg 1995) as well as for other types of defence, e.g. mechanical properties. Studies on the costs of secondary metabolites have been carried out mainly by comparing the growth rates or seed set of plants that produce different amounts of a given substance (e.g. Vrieling 1991; Fineblum & Rausher 1995; Sagers & Coley 1995;

Steinberg 1995). These results, however, indicate relative differences rather than provide absolute values. Data on metabolic costs of synthesis and on concentrations present in plant tissue are available for several secondary plant compounds, but due to the confusing information on turnover rates, calculating the metabolic costs of defence is usually impossible (Gershenzon 1994a). Quantifying the FB production of a myrmecophyte therefore provides an opportunity for estimating the metabolic costs of one distinct type of anti-herbivore defence based on data on biomass production and energy allocation.

The ecological and evolutionary relevance of a particular metabolic cost may be inferred if the cost is regulated with respect to the actual demand. Indeed, *Cecropia* populations growing over evolutionary timespans in ant-free habitats seem to lose the ability to produce FBs (Janzen 1973; Rickson 1977). Evidence for short-term regulation of FB production was provided by studies in the genera *Piper* and *Cecropia*. Risch & Rickson (1981) reported large differences in FB production rates between inhabited and uninhabited domatia of *Piper cenocladum*, and Folgarait *et al.* (1994) demonstrated that FB productivity of ant-free *Cecropia* saplings depended on the intensity with which FBs were artificially removed. Furthermore, Folgarait & Davidson (1994, 1995) found influences of nutrient supply and light intensity on FB production rates of *Cecropia*. Together, these results demonstrate that FB production by myrmecophytic species can be influenced by several abiotic and biotic factors.

In a series of field studies, we investigated (i) the production rates of FBs in *Macaranga triloba* trees and saplings growing under natural conditions and (ii) possible influences of the presence of mutualistic *Crematogaster* ants on the rate of FB production. Such data are among those essential for cost–benefit analyses of myrmecophytic systems in which, on average, the summed benefits would always be expected to exceed the costs for each partner.

Materials and methods

STUDY AREA AND MATERIAL

The studies were carried out during four field stays, February–April 1994, February–March 1995, September–October 1995 and February–April 1996, near the Ulu Gombak Field Studies Centre of the University of Malaya in Selangor, Peninsular Malaysia. The surrounding area is covered by secondary forest containing at least nine species of *Macaranga*. More than 90% of the individuals of *M. triloba*, one of the more common species, are inhabited by ants (Fiala *et al.* 1994; $n > 5000$, personal observations by the authors).

FBs (called 'Beccarian bodies' by Rickson 1980) are produced on the abaxial surfaces of the pair of

stipules that grow at the base of each internode. Tiny, but structurally similar, FBs are produced in very low numbers on the leaves (Fiala & Maschwitz 1992b) but were not included in this study since they make up less than 1% of the FB mass (M. Heil *et al.*, unpublished observations). We also excluded FBs found on the bracts of flowering plants and focused our experiment on the Beccarian bodies that are produced continuously during vegetative growth. Because the stipules curve down and backwards and clasp the stem tightly, the FB-producing surface forms part of the inner wall of an enclosed space largely secluded from the outside. A survey of FB production therefore requires the removal of the stipules.

NUMBERS OF FOOD BODIES IN STIPULE PAIRS

In order to quantify the number of FBs present under each stipule of a pair at a particular time, we collected 138 pairs of stipules from eight small plants (six unbranched and two branched saplings, heights *c.* 2 m) during February–April 1994 and counted the FBs under each member of the pair.

FOOD BODY PRODUCTION

Because of similar FB numbers beneath the two stipules of a pair (see the Results) we were able to use one stipule as a control for the effect of excluding ants from harvesting any FBs beneath the sister stipule. A total of 20 plants was selected during February and March 1995: 10 unbranched saplings with heights of 1.30–2.65 m (size class A), and 10 larger, already branched trees with heights of 4.65–7.30 m (size class B). All plants were inhabited by ants and were in good general condition (the degree of herbivory was not above average, there was no damage due to lack of water, soil nutrients or light, and no diseases such as chlorosis, viral or fungal infections were visible). The main shoot of both branched and unbranched plants, as well as three side shoots of the branched plants (the oldest, the youngest and one intermediate-aged twig), were selected and the lengths of both main shoot and twigs, and the total number of stipules, were recorded.

Pairs of stipules were marked, beginning with the youngest already recurved one. Three to 11 pairs of stipules were present on each of the twigs, and six to 14 pairs on the main shoots. One (control) stipule was removed from each of the three youngest pairs and one from every second of the remaining pairs (since stipules border on each other without any space between, selective ant exclusion from one member of a pair is impossible). The FBs of control stipules were collected, divided into two size classes by optical criteria, and counted. Class 1 included all the small, immature FBs (and comprised a continuum of small to intermediate sizes), and class 2 the fully developed ones. However, the two classes were easily distinguished by size and colour. This division was

necessary because, in spite of the high variability within size class 1, the biomass was much lower in these immature FBs (Rickson 1980) than in the fully developed ones (see the Results).

Ants were excluded from the remaining (= experimental) stipules of the chosen pairs by applying a ring of sticky resin (Tangletrap[®], Tanglefoot Corp., CA, USA). After 1 week, these stipules were removed and the FBs were counted. The difference between the numbers found under the experimental stipule after ant exclusion and the control stipule should represent the weekly FB production of the experimental stipule in question under no-harvesting conditions.

FOOD BODY DRY WEIGHT

To determine FB dry weight, FBs were collected from several plants, divided into the two size classes, and counted. The samples were then stored in ethanol; later, they were freeze-dried and weighed. For each FB size class, four samples were taken, each pooled from several plants. The samples for size class 1 comprised 755–18 003 (mean 10 508) small FBs, the samples for size class 2 contained 909–3081 (mean 1711) large FBs. Mean values were used to convert numbers of FBs produced to productivity as mg stipule⁻¹ week⁻¹ and mg plant⁻¹ week⁻¹.

FOOD BODY PRODUCTION IN RELATION TO LEAF AREA AND PLANT GROWTH

In September and October of 1995, another 16 *M. triloba* plants were selected for measurement of FB production as described above. These plants ranged in height from 0.65 m to 5.40 m, and included two plants smaller than the previously defined class A, and four class A and four class B plants. The remaining six fell between the two size classes. The total leaf area of each plant was estimated by measuring length and width of all leaves (Fig. 1). This simplified method was possible since the area of a leaf is linearly correlated with the product of its length and width. Total FB production per unit leaf area could then be calculated.

The relative investment in FB production compared to the total above-ground biomass increment was studied in unbranched (class A) plants over a 19-day period. Due to pronounced differences in growth rates between main stems and twigs as well as between different aged twigs, branched (class B) plants had to be excluded from the measurements of above-ground growth. Growth was measured as increase in internode length and leaf area. More than 95% of the total increase in shoot length resulted from growth in the three youngest internodes (mean from 20 plants measured over 6 weeks = 95.9%, SD = 3.9; M. Heil, unpublished data). The fourth youngest leaf of an *M. triloba* twig had reached its final size and weight (on more than 300 twigs of more than 80 plants studied, only five leaves growing at fourth position had not

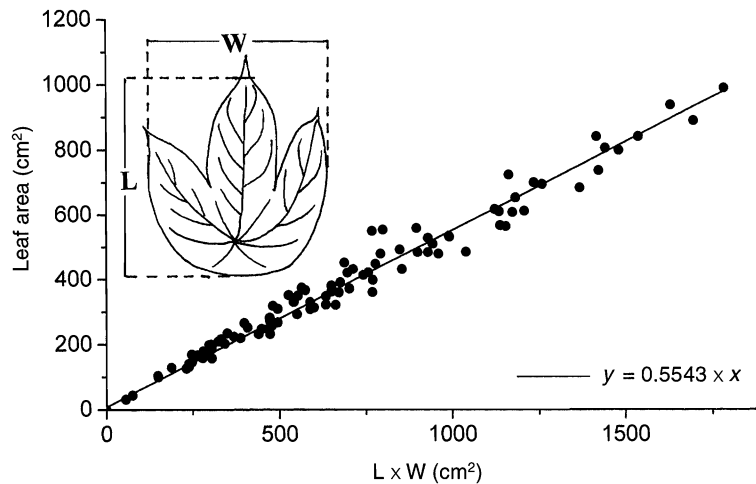


Fig. 1 Estimation of leaf area in *M. triloba*. Length (*L*) and width (*W*) of 100 *M. triloba* leaves were measured as indicated in the line drawing; leaf area was measured with a leaf area meter, and a regression was calculated relating the measured area to the product of length and width ($r^2 = 0.97$).

yet achieved their final size; M. Heil, unpublished data). Therefore, the lengths of the three youngest internodes as well as the lengths and widths of the four youngest leaves were measured. Both the length and width of a leaf and the length of an internode correlated significantly with their respective mass (results of Pearson correlation test: correlation coefficient $c = 0.96$, $P < 0.001$, for the correlation of leaf dry weight and product of leaf length and width, $n = 33$ leaves from seven plants; and $c = 0.92$, $P < 0.001$, for the correlation of internode dry weight and length, $n = 27$ internodes from seven plants). Thus, an increase in mass was easily estimated by measuring the increase in size of leaves and internodes. For better comparison with FB production rates, both the leaves and the internodes included in the growth measurements were dried over silica gel, and all growth data were calculated as dry matter increment.

FOOD BODY COMPOSITION AND CONSTRUCTION COSTS

Total construction costs of a tissue can be calculated from data on its chemical composition by following the method of Penning de Vries *et al.* (1974). These authors listed net costs for the biochemical synthesis of the most common molecules and presented equations for calculating construction costs in terms of glucose equivalents. The main nutrient contents of FBs of several *Macaranga* species, including *M. triloba*, have been analysed (Heil *et al.*, in press) and were used to estimate the minimum construction costs of FB tissue. For comparison, data on the composition of leaf tissue were also used.

These analyses cover only the main nutrients. Thus, for a very conservative estimate of minimum construction costs of both FB and leaf/shoot tissue we assumed that the rest of the tissue consisted of the 'cheapest' substance possible, namely non-poly-

merized glucose. Further, we ignored costs arising from transport within or between cells and from the form in which nitrogen is supplied to the plant (namely as NO_3^- or NH_4^+). The minimal construction costs for both types of tissue estimated in this way were related to the mean dry matter increment per day.

COSTS OF FOOD BODY PRODUCTION IN RELATION TO TOTAL ASSIMILATION

To estimate energy costs of FB production in relation to a plant's total energy budget, FB production and gas exchange were studied on a set of nine unbranched *M. triloba* plants during April 1996. FB production was measured as described above for all stipule pairs of each plant. Simultaneously, leaf gas exchange measurements were conducted using a $\text{CO}_2/\text{H}_2\text{O}$ -porometer system (CQP 130; Walz, Effeltrich, Germany) operating in an open-flow mode, as described previously (Zotz *et al.* 1995). Net CO_2 exchange was determined at about hourly intervals from shortly before dawn until shortly after dusk. This frequency of data acquisition allows reliable estimates of 24-h carbon budgets, $A_{24\text{h}}$ (Zotz & Winter 1996). In smaller plants with up to five leaves, CO_2 exchange was studied on all leaves, while in larger plants only the youngest three totally unfolded and every second of the older leaves were included. The CO_2 exchange of the remaining leaves was assumed to be the average of the two adjacent ones. The diel carbon budget was obtained by multiplying $A_{24\text{h}}$ of each leaf with its estimated area. The sum of all leaf carbon budgets of a plant yielded the total plant net assimilation. Energy costs of FB production per day were calculated separately for each plant, following Penning de Vries *et al.* (1974), as construction costs in mol glucose equivalents per day, and then further converted to mol CO_2 day $^{-1}$.

EFFECTS OF PRESENCE OR ABSENCE OF ANTS ON FOOD BODY PRODUCTION

This experiment was carried out from February to April 1996. In the natural habitat, the chances of unprotected *M. triloba* plants surviving for more than a year are close to zero due to herbivore damage (M. Heil, unpublished data). The ant-free plants required for this experiment were therefore cultivated in a greenhouse built with fine-meshed net (mesh diameter 1.5 mm) to exclude herbivores. Ant-free seedlings were collected from the forest. To make growing conditions in the greenhouse as similar as possible to those at the natural site, soil was taken from the forest site to prepare the soil in the greenhouse. The ant-free plants were grown under a net and planted into soil without using pots. Thus, they probably received the same amount of rainfall and experienced almost the same nutrient conditions as the inhabited ones. Moreover, to minimize effects of transplantation, the ant-free plants were cultivated for more than 5 months in the greenhouse before the experiment was started. For comparison, we used ant-inhabited plants growing at the site from which the ant-free ones had been collected.

Twelve pairs of unbranched saplings were selected with respect to similarity in height, leaf number and total leaf area, but not stipule number, since ant-free plants in general had fewer stipules (M. Heil *et al.*, personal observations; see 'Ant inhabitation and stipule number'). Each pair consisted of one inhabited plant growing at the field site and one ant-free plant growing in the greenhouse. The ant-free plants were 0.30–0.90 m tall, the inhabited plants were 0.25–0.95 m. Leaf number and stipule number were registered and total leaf area was estimated as described above; the greatest differences within a pair amounted to 0.30 m in height and about 6% in total leaf area. FB production was measured for all stipule pairs.

Light intensities were measured using a luxmeter during one cloudy and one sunny day. Measurements were conducted every 1.5 h for all plants on the same day. For each plant, light intensity was measured directly over the highest, the lowest and one intermediate leaf, twice with an interval of about 10–15 min. The mean of the two measurements was calculated to even out short-term fluctuations due to sunflecks. The curves calculated from these 9–10 data points were integrated separately for the three measuring heights for each plant over the whole day. Finally, the results of all three integrations were summed to estimate the total amount of light received by each plant.

To determine possible differences in nutrient supply, equal-sized parts from all leaves of the studied plants were collected after the experiment and dried over silica gel. After return to Germany, samples were ground, dried for 48 h at 70°C and analysed for N, P, K and S, using a CHN analyser (CHN -O- Rapid;

Elementar, Hanau, Germany) and an elemental analyser (ICP Spectrometer JY Plus; Instruments SA GmbH, Grasbrunn, Germany).

ANT INHABITATION AND STIPULE NUMBER

Field observations suggested that stipule numbers depend on ant habitation. To quantify this phenomenon, 10 plants with and 14 without ants were cultivated in a greenhouse in Germany. Seedlings 10–20 cm high were collected from the field site in April and November 1995. At both times, some of the seedlings contained at least one ant queen, and some already had workers active on the plant surface, while other plants were completely ant-free. The seedlings were planted into pots and then cultivated in a glasshouse without supplemental light under typical warmhouse conditions. All plants were grown in the same substrate and watered and fertilized simultaneously, and they were grouped without regard to the presence or absence of ants. Numbers of leaves and stipule pairs were counted twice, in May and December 1996. At the censuses, the plants had been growing under the same conditions for at least 6 and 13 months, respectively.

In addition to the greenhouse experiment, a survey of plants growing naturally with and without ants was conducted at the study site in Malaysia. Due to the high mortality of uninhabited plants, only 10 ant-free saplings could be found. Leaf and stipule numbers from 33 similar-sized but inhabited plants were compared to those of uninhabited plants.

Results

NUMBERS OF FOOD BODIES ON STIPULE PAIRS

Our first survey of non-treated plants revealed that very similar numbers of FBs were present under the two stipules of a pair at a particular time; the mean difference in total FB dry weight between stipule 1 and stipule 2 amounted to 127 µg or 14%. Total dry weight of FBs as well as number of small or number of large FBs were correlated highly significantly between the two stipules of a pair ($P < 0.001$ for all three variables, Pearson correlation test, $n = 138$ stipule pairs). A *t*-test for paired variables revealed no significant differences between the two stipules of a pair ($P > 0.50$ for total FB dry weight and number of small FBs, $P > 0.05$ for number of large FBs, $n = 138$ pairs). The regression analysis for the expected linear relationship (dry mass beneath stipule 1 = dry mass beneath stipule 2) revealed a coefficient of determination (r^2) of 0.72.

However, number of FBs and therefore FB dry weight depended significantly on stipule age ($P < 0.001$, Kruskal–Wallis test for the 12 age categories). The largest number of FBs was found under the second and third youngest pairs. Older ones

showed continually decreasing numbers with increasing age (Table 1).

FOOD BODY PRODUCTION

The mean FB dry weight was 9.29 μg for small and 23.25 μg for large FBs. All subsequent calculations concerning FB production are based on these values. Overall, class A plants produced more FBs per stipule than did class B plants. This effect was reflected in higher productivity in class A stipules of all ages except position 1 (Fig. 2), and was significant for stipule numbers 2, 3, 5 and 9 ($P < 0.05$, *U*-test). Stipule age affected FB production similarly in both plant size classes (Fig. 2), and the pattern of productivity corresponded well with that found for the numbers of FBs present under different-aged stipules at a given time (Table 1).

FB production of entire plants was calculated to be 19.0–73.9 mg (median 37.7) dry weight week⁻¹ for unbranched size class A plants, and 87.9–340.9 mg week⁻¹ (median 168.8) in size class B plants. The decreased productivity per stipule was therefore more than counterbalanced by the increased size. When all 20 plants were considered, there was a significant negative correlation between productivity per stipule and total number of stipules ($P < 0.01$, Spearman rank correlation test). This relationship is best described by a hyperbolic regression (Fig. 3).

FOOD BODY PRODUCTION IN RELATION TO LEAF AREA

A similar range of production rates was observed in the 16 plants surveyed in September–October (19.7–231.0 mg FBs plant⁻¹ week⁻¹). The four plants representing size class A showed production rates from 33.8 to 73.6 mg FBs plant⁻¹ week⁻¹, and the four plants representing class B 86.1–231.0 mg FBs plant⁻¹ week⁻¹.

When the production rate of these plants was expressed per stipule per week and plotted against the total number of stipule pairs, the hyperbolic curve estimated from the first 20 plants described the new data quite well, although the plants surveyed during September–October seemed to have slightly lower productivities (Fig. 3). Since the number of stipule pairs is strongly correlated with total leaf area in *M. triloba*, a similar relationship was found between production rate per unit leaf area and total leaf area, while total FB production increased linearly with total leaf area (data not shown).

FOOD BODY PRODUCTION IN RELATION TO TOTAL ABOVE-GROUND GROWTH

Total above-ground dry matter increment of unbranched saplings was 0.88–3.13 g during the surveyed period of 19 days (median 1.93 g, $n = 7$ plants). This

corresponded to an average increase of 100 mg day⁻¹. The FB production of unbranched plants was about 5.39 mg day⁻¹ (median), and thus FBs constituted on average about 5.4% of the above-ground dry biomass production for unbranched saplings.

Comparative analyses of leaves and FBs showed much higher contents of fat and proteins in the FBs (Heil *et al.*, in press). This implies that the metabolic costs of FB production are considerably higher than suggested by a simple comparison of dry biomass. Assuming only slightly lower construction costs for shoot than for leaf tissue (Griffin 1994), we considered similar contents of fats, proteins and carbohydrates in the tissue of young leaves and not yet hardened shoot tissue. Based on chemical composition data, it can be estimated for unbranched plants that about 30% of the total lipids, 7% of the proteins and amino acids and 2% of the carbohydrates needed for total above-ground growth were diverted to FB production.

According to the equations presented by Penning de Vries *et al.* (1974), the construction of 1 g dry FB tissue required about 10.2 mM glucose, compared to only about 6.4 mM glucose needed for construction of 1 g dry leaf tissue. When growth rates were taken into account, shoot growth of unbranched plants consumed about 640 μM glucose day⁻¹, and FB production added about 60 μM glucose day⁻¹. Thus, FB production made up on average 8.6% of the energy expenditure in glucose equivalents for above-ground tissue construction of unbranched *M. triloba* saplings.

ENERGY COSTS OF FB PRODUCTION IN RELATION TO TOTAL ASSIMILATION

The nine *M. triloba* plants had total leaf areas from 590 to 11 100 cm², FB production was 3.76–10.62 mg day⁻¹ (dry mass), so that total construction costs of FBs amounted to 130.4–584.4 μM CO₂ day⁻¹ plant⁻¹.

The 24-h carbon budget of individual leaves ranged from -23.7 up to 133.8 mmol m⁻² day⁻¹ ($n = 44$ leaves). The estimated net CO₂ gain of entire plants was 6.7–63.3 mmol CO₂ day⁻¹ plant⁻¹ ($n = 9$ plants). The relative energy investment in the FBs thus amounted to between 0.6 and 5.0% of a plant's total assimilation. A regression analysis relating relative energy investment to total plant leaf area resulted in a hyperbolic curve strongly resembling the one found for the relation between FB production per stipule and plant size (Fig. 4).

EFFECT OF PRESENCE OR ABSENCE OF INHABITING ANTS

Several abiotic site factors and morphological plant traits were measured to check for differences between inhabited and ant-free plants (Table 2). Paired *t*-tests revealed no significant differences for total leaf area or leaf numbers between the two groups ($P > 0.10$).

Table 1 Food body numbers on stipule pairs. The survey of FB numbers beneath different-aged stipules was conducted on a total of 138 stipule pairs from six unbranched and two branched *M. triloba* plants. Stipule age categories are represented by the stipules' order of insertion at the shoot, number 0 represents the youngest stipule being not yet recurved, number 1 represents the next youngest stipule which in all cases was already totally recurved. Numbers of small and large FBs as well as total FB dry weight are given separately for the two stipules forming a pair. Since data are normally distributed (Kolmogorov-Smirnov test: $P > 0.10$ for all three variables), means \pm standard deviation are given for all variables. Numbers of stipules (n) are given for the various classes of stipule age

n	Age category of stipule pairs											
	0	1	2	3	4	5	6	7	8	9	10	11
	14	14	14	14	14	14	13	14	10	8	6	3
Small FBs												
1	0 \pm 0	26 \pm 40	108 \pm 43	99 \pm 38	89 \pm 27	77 \pm 29	71 \pm 22	58 \pm 29	59 \pm 33	69 \pm 25	53 \pm 40	53 \pm 12
2	0 \pm 0	23 \pm 37	107 \pm 39	100 \pm 30	90 \pm 29	87 \pm 42	75 \pm 28	59 \pm 22	59 \pm 42	52 \pm 22	54 \pm 33	70 \pm 11
Large FBs												
1	0 \pm 0	0 \pm 0	9 \pm 10	9 \pm 10	7 \pm 8	12 \pm 13	9 \pm 6	5 \pm 5	4 \pm 4	6 \pm 6	2 \pm 1	1 \pm 0
2	0 \pm 0	0 \pm 0	7 \pm 8	9 \pm 9	7 \pm 7	8 \pm 10	9 \pm 7	6 \pm 5	3 \pm 3	3 \pm 4	1 \pm 1	1 \pm 0
FB dry weight (mg)												
1	0.00 \pm 0.00	0.24 \pm 0.38	1.21 \pm 0.47	1.13 \pm 0.42	1.00 \pm 0.30	1.00 \pm 0.40	0.87 \pm 0.24	0.66 \pm 0.27	0.63 \pm 0.31	0.78 \pm 0.29	0.56 \pm 0.38	0.49 \pm 0.11
2	0.00 \pm 0.00	0.22 \pm 0.35	1.16 \pm 0.40	1.12 \pm 0.35	1.00 \pm 0.29	0.98 \pm 0.49	0.89 \pm 0.29	0.70 \pm 0.23	0.60 \pm 0.41	0.53 \pm 0.18	0.52 \pm 0.31	0.68 \pm 0.11

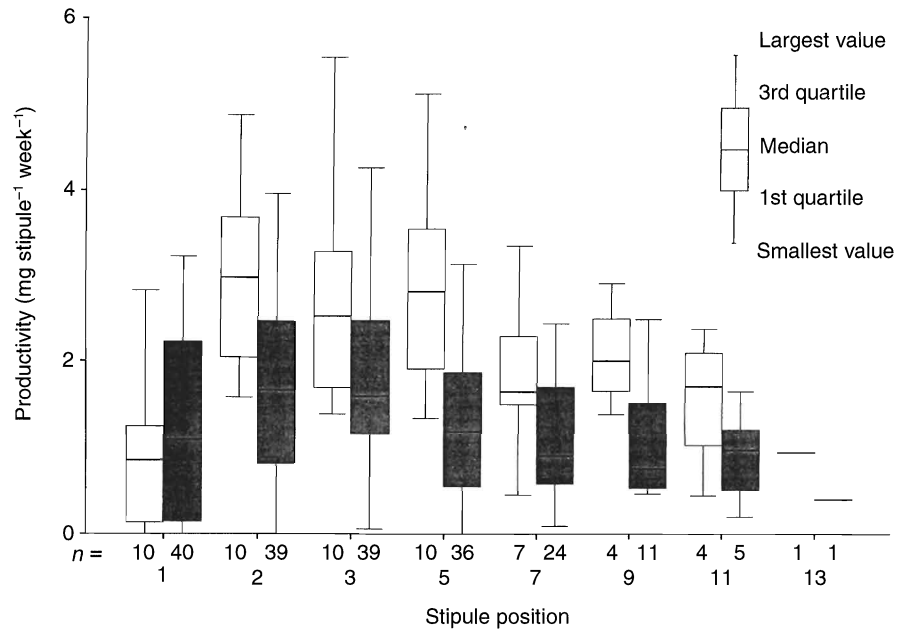


Fig. 2 Dependence of the food body productivity on stipule age. Stipule positions represent stipule age, e.g. stipule number 1 is the youngest totally recurred one. FB productivity is given separately for plant size classes A (open boxes) and B (dark boxes). Sample size (n) could be as large as 40 in size class B because four twigs were included from each of the 10 plants belonging to this size class. Productivity is expressed as milligrams FB dry mass per stipule per week.

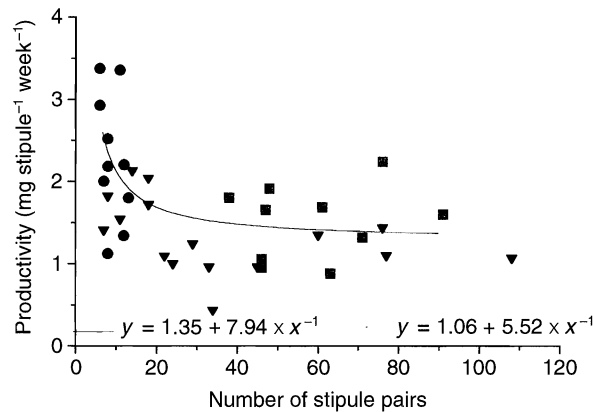


Fig. 3 Dependence of food body productivity on plant size ($n = 36$ plants). Productivity is expressed in mg FB dry mass per stipule per week for each plant; a mean per stipule was calculated from all measured stipules of each plant. Productivity decreased with plant size, which is indicated as the total number of stipule pairs. The curve estimated from the 20 plants of size class A (circles) and B (squares) is very similar to the one calculated from the additional 16 plants selected to cover the whole range of sizes (triangles). To indicate this, the hyperbolic regression of productivity against number of stipules is given for the first 20 plants (unbroken line, $r = 0.60$) and separately for the last 16 plants (dotted line, $r = 0.49$).

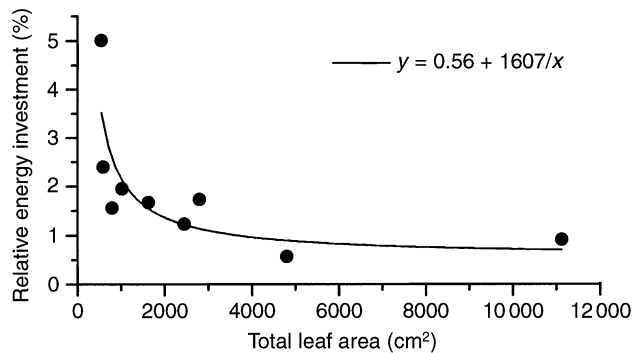


Fig. 4 Energy costs of FB production in relation to total assimilation ($n = 9$ plants). Construction costs of FB production were calculated in mol CO_2 per day per plant and related to the net CO_2 assimilation (assessed by CO_2 exchange measurements) of the same plant ($r^2 = 0.62$ for the hyperbolic regression). Relative energy investment is calculated as a percentage of the total assimilation and plotted against total leaf area.

Table 2 Morphometric parameters, light perception and leaf nutrient contents characterizing the plants included in the experiment on the presence or absence of symbiotic ants. Means and ranges (min, max = minimal and maximal values) are given separately for both inhabited control plants (con) and ant-free experimental plants (exp). Numbers of leaves and stipule pairs were counted. Total leaf area and light perception were calculated as described in the Materials and Methods; day 1 was cloudy, day 2 sunny. Since most data are normally distributed (Kolmogorov–Smirnov test: $P > 0.10$ for all variables, but light day 2 in the experimental plant group, $P > 0.08$ for this variable), P -values were calculated according to paired t -test, $n = 12$ plants for all means and 12 pairs for the t -test

Plant group	Stipule number		Leaf number		Leaf area (cm ²)		Light (day 1)		Light (day 2)		N [%]		P (p.p.m.)		K (p.p.m.)		S (p.p.m.)	
	con	exp	con	exp	con	exp	con	exp	con	exp	con	exp	con	exp	con	exp	con	exp
Min	3	1	4	3	148	159	207	608	318	1393	2.23	2.26	714	1280	10023	9645	2453	2655
Mean	5.6	2.9	6.3	7.0	1488	1491	463	849	1124	1646	2.70	2.66	1477	1662	11899	10977	3338	3271
Max	8	5	12	10	3811	4156	1314	1023	3051	2011	3.17	3.19	2241	2609	14051	12605	4514	3901
P	<0.001		>0.10		>0.10		0.001		0.037		>0.10		>0.10		>0.10		>0.10	

No significant differences were found for N, P, K and S in dry leaf material. However, the ant-free plants received significantly more light than did the inhabited ones during both the sunny and the cloudy day. Light perception was not correlated with plant size (measured as entire plant leaf area) during both the cloudy and the sunny day in both plant groups, nor were N, K and S contents in the leaves ($P > 0.05$, Pearson correlation test). Only leaf P content was significantly negatively correlated with total leaf area ($0.01 < P < 0.05$).

FB production of ant-inhabited plants and ant-free ones differed significantly. All stipules of each plant were included in the measurement of FB production. Since the number of stipules varied within the plant pairs, comparisons could be based either on (i) total FB production per plant per unit leaf area, or on (ii) FB productivity per stipule.

FB production was much higher in the inhabited plants. Within all 12 pairs, the inhabited plant produced more FBs, both on a whole plant basis (Fig. 5a) and on a unit leaf area basis (Fig. 6). The difference between the two plant groups was significant ($P < 0.01$ for both variables, Wilcoxon pair test).

The plants without ants had about half the number of stipules as the inhabited plants ($P < 0.001$, Wilcoxon pair test, data in Table 2). Thus, a less striking result was found for the productivity per stipule (Fig. 5b). Overall, inhabited plants once again showed higher productivity [mean 1.82 ± 0.67 mg FBs stipule⁻¹ week⁻¹ (range 1.17–3.36), compared with 1.04 ± 0.62 mg stipule⁻¹ week⁻¹ (range 0.22–2.13) for ant-free plants]. This difference was significant ($0.01 < P < 0.05$, Wilcoxon pair test, $n = 12$ pairs), although in four pairs FB production per stipule was higher in the uninhabited plant.

The correlation between FB productivity per unit leaf area and entire plant leaf area was negative and highly significant for the ant-inhabited plants ($P < 0.001$, Spearman rank correlation). This relation was best described by a hyperbolic regression ($r^2 = 0.88$; Fig. 6); the curve strongly resembled the one found for the relation between productivity per stipule and total stipule number (Fig. 3). On the other hand, no significant correlation could be found for the ant-free ones ($P > 0.10$, $r^2 = 0.08$ for the linear regression indicated in Fig. 6). Light intensity and plant size were not significantly correlated on either the sunny or the cloudy day ($P > 0.10$).

ANT-INHABITATION AND STIPULE NUMBER

The comparison of inhabited and ant-free plants cultivated in the greenhouse in Germany revealed a large difference in stipule numbers. All inhabited plants showed nearly equal numbers of leaves and stipule pairs. Ant-free plants, however, had only about a third as many stipule pairs as leaves. After 6 months, the inhabited plants had 5–12 leaves and 4–11 stipule

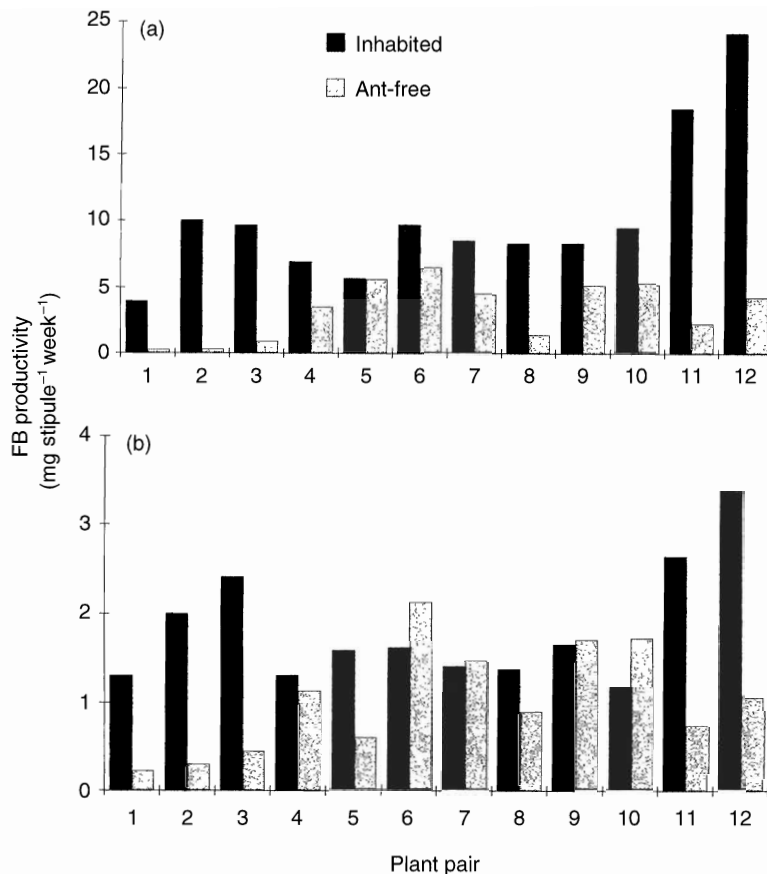


Fig. 5 Effect of ant inhabitation on food body production ($n = 12$ plant pairs). Each plant pair consisted of one inhabited and one ant-free plant of about the same size. The pairs are represented by the same numbers in both (a) and (b); they are arranged according to plant size, with pair number 1 comprising the smallest plants. (a) Total FB production of all stipule pairs of a given plant is shown in milligrams dry FBs per total plant per week. (b) FB productivity (total FB production of a particular plant divided by total stipule number) is given in milligrams dry FBs per stipule per week.

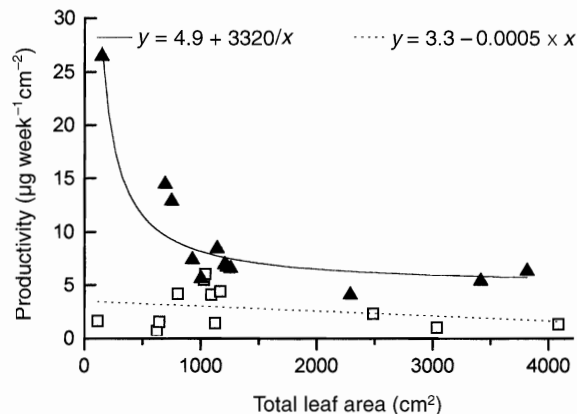


Fig. 6 Dependency of FB productivity on total leaf area in inhabited and ant-free plants. FB productivity is calculated in micrograms dry FBs per cm^2 leaf area per week and plotted against total leaf area as a value representing plant size. Regressions were calculated separately for inhabited plants (triangles, $r^2 = 0.88$, unbroken line), and for ant-free plants (squares, $r^2 = 0.08$, dotted line), $n = 12$ plants in both groups.

pairs, while the ant-free plants had 3–12 leaves and 1–4 stipule pairs. One pair of stipules emerges with every new leaf during plant growth. Thus, all plants had at least one stipule pair at the youngest node and in most cases one more at the second youngest node. However, on ant-free plants the stipules at older nodes had been shed in most cases, while they persisted much longer on inhabited plants.

For better comparisons between the two plant groups, we calculated the ratio of the numbers of leaves to stipule pairs present on each plant. At the first census, inhabited plants showed a leaf/stipule pair ratio of 1.1 (median, $n = 10$ plants), while the ant-free plants had 3.3 times more leaves than stipule pairs (median, $n = 14$). At the second census 7 months later, the ratio was 1.2 (median, $n = 8$) for the

inhabited plants and 3.5 (median, $n = 9$) for the ant-free ones. The differences were highly significant ($P < 0.001$, Mann–Whitney U -test for both censuses).

The survey of young saplings growing under natural conditions with and without ants revealed similar results: inhabited plants had 4–18 leaves and 3–14 stipule pairs resulting in a leaf–stipule pair ratio of about 1.4 (median, range 1.0–2.3, $n = 33$), while uninhabited plants had 5–10 leaves and 1–5 stipule pairs and thus a leaf–stipule pair ratio of 3.0 (median, range 1.4–7.0, $n = 13$). When based on the quotients of leaf and stipule pair number, the difference between the two plant groups was highly significant ($P < 0.001$, Mann–Whitney U -test).

Discussion

Our study shows that production of the FBs used by the symbiotic ants of the myrmecophyte *M. triloba* incurs considerable energy costs. FBs made up 5% of the total biomass increase in unbranched saplings. The FBs contained *c.* 30% of the lipids and 7% of the proteins invested in above-ground tissue construction. Calculations of FB and leaf tissue construction costs according to Penning de Vries *et al.* (1974) led to the estimation that, in unbranched saplings, about 9% of the total energy costs of above-ground growth were involved in FB production, and CO₂ exchange measurements demonstrated that saplings allocated up to 5% of their total assimilation to FB production.

There are a number of reasons why these figures should only be regarded as first estimates of the order of magnitude of the minimum costs resulting from 'biotic' defence in *M. triloba*.

The calculation of FB production is based on the assumption of equal FB numbers beneath the two stipules forming a pair (Table 1). Due to natural variability, this assumption is subject to a mean error of 14% corresponding to a mean difference of 127 μg FB tissue between the two stipules (mean of 138 pairs). However, this would account for 8% of the mean FB production measured after 1 week of ant exclusion (mean production of the 252 stipule pairs of the first set of 20 plants was 1.65 mg). Furthermore, any error resulting from an initial difference between sister stipules should be balanced out due to the random assignment of stipules to the control or experimental treatment.

Above-ground biomass increase was only estimated by measuring the growth in the youngest parts of seven plants. The variability within these plants leads to estimates of FB production ranging from 3.3% to 10.8% of the total above-ground biomass increase. However, relative energy investment was also calculated for a further set of nine plants on which gas exchange measurements were conducted as well as measurements of FB production. These independent data fit the already calculated curve for relative investment in terms of biomass production

and size (compare curves in Fig. 3 and Fig. 4); values ranged from 0.6% to 5% of a plant's total energy assimilation (Fig. 4). Plants cannot invest all the assimilated energy into above-ground growth (Bazzaz *et al.* 1987), and the proportion of energy invested should therefore be higher in relation to above-ground growth than to the total energy budget. Our data are consistent with this prediction and show that different calculation procedures using different sets of plants lead to rather similar estimates of the costs resulting from FB production.

A further bias may result from the fact that FB production was measured for 1 week under no harvesting conditions. However, this should depress the intensity of FB production rather than increase it (Folgarait *et al.* 1994) and thus would lead to a slight underestimation of 'real' FB production rates.

Several additional metabolic costs, such as those arising from the construction and maintenance of stipules and other tissues involved in the production of FBs, were not included. The construction of secretory glands or trichomes and of storage sites has been reported to increase the costs of defence (Gershenzon 1994a). We also assumed the non-analysed part of the tissue to be as 'cheap' as possible in energetic terms.

In summary, our estimates of the cost of FB production are very conservative, possibly underestimating the 'real' values rather than overestimating them.

ENERGY COSTS OF FOOD BODY PRODUCTION

To date, a plant's energy costs accruing from nutrition of mutualistic ants by producing FBs have been estimated in only one single case. O'Dowd (1980) presented data based on bomb calorimetry indicating that the pearl body production of non-myrmecophytic but ant-attracting Balsa (*Ochroma pyramidale*) saplings amounted to about 0.2% of the energy costs of leaf production. The extrafloral nectar production of the same species was estimated to use an additional 1% (O'Dowd 1979). Comparable information for other species is available only for costs of floral (e.g. Southwick 1984; Pyke 1991) and extrafloral nectar production (Bentley 1977).

Not only did we collect data on the amount (numbers and dry mass) of FB production at different growth stages (Figs 2 and 3), but we also studied their chemical composition (Heil *et al.*, in press). In addition, we measured the total leaf area and above-ground growth rates of some plants and the photosynthesis of a second set of plants (Fig. 4) to estimate the relative energetic and metabolic costs incurred for FB production. Since different methods of calculating energy costs lead to rather different results (Griffin 1994), a comparison of our data with those of O'Dowd (1979, 1980) is possible only with caution. However, we suggest that ant nutrition by the myr-

mecophyte *M. triloba* incurs higher costs than ant attraction by the myrmecophilic *Ochroma pyramidale*.

A bigger set of data exists on the construction costs of secondary plant compounds. When actual concentrations are taken into account, cost of defence per gram plant tissue calculated following Penning de Vries *et al.* (1974) comes to 2–307 mg glucose equivalents g^{-1} , with a mean of about 33 mg glucose g^{-1} (SD = 57, $n = 30$, calculated from table 1 in Gershenson 1994a). Food body production costed about 86 mg glucose per gram of above-ground tissue, a value that is higher than all but two of the 30 values listed by Gershenson (1994a). Unfortunately, turnover rates could not be included in these calculations and probably led to underestimates of the real cost of secondary compounds. Since our results may also suffer from a net underestimation rather than an overestimation (see above), this comparison leads to the assumption that nourishing symbiotic ants with FBs may be quite an expensive strategy of anti-herbivore defence.

COST-BENEFIT BALANCE OF DEFENCE VIA FB-MAINTAINED ANTS

Several authors have reported that there were no apparent costs associated with the production of secondary compounds involved in anti-herbivore defence (Simms & Rausher 1987, 1989; Vrieling 1991; Adler *et al.* 1995). Others have found that costs depended on the actual growth or the nutritional or developmental state of the plant, but they were in general rather low (Martens & Boyd 1994; Sagers & Coley 1995; Steinberg 1995).

Mutualism is characterized by a positive benefit–cost balance for all parties involved directly (Douglas 1994). The relatively high costs of FB production nevertheless must pay off for *M. triloba* plants. The degree of herbivory experienced by unprotected *M. triloba* plants was more than twice as high as in plants protected by their symbiotic ants (Fiala *et al.* 1989; M. Heil *et al.*, unpublished data). These differences increased dramatically in long-term experiments. Since damage due to shoot borers and fungal infection added to the crude leaf herbivory, the chances of survival for unprotected plants decreased to almost zero during 1 year (M. Heil *et al.*, unpublished data). Therefore, this ant–plant interaction is obviously beneficial for both ants and plant.

One possible mechanism increasing the ants' protective effect over that of chemical or mechanical defence may result from the ants' mobility (McKey 1984). Herbivore pressure on young leaves is much higher than on mature leaves (reviewed by Coley & Barone 1996). Young leaves are a more valuable food source (Orians & Janzen 1974); however, defence in young leaves is constrained by several intrinsic factors, ranging from the lack of leaf toughness (Kursar & Coley 1992; Turner 1994; Coley & Barone 1996)

to trade-offs between growth and differentiation (Herms & Mattson 1992). Thus, herbivore pressure on young leaves is reduced in several cases by 'escape' strategies such as delayed greening (Kursar & Coley 1992) or synchronous leaf flushes, preferably in times characterized by low herbivore abundance (Aide 1992). Furthermore, transport of defence chemicals seems to be a strategy bypassing the negative trade-offs between growth and differentiation that constrain the defence of young, developing tissues (Cronin & Hay 1996).

Nourishing mutualistic ants with food bodies as an anti-herbivore defence enables myrmecophytic plants to separate production of defence metabolites spatially from developing leaf tissue. This separation effectively eludes the constraints mentioned above; it is clearly possible only because the defence mechanism itself (the ants) is highly mobile. A further factor that may make the defence via ants highly successful is the avoidance of autotoxicity, a problem strongly limiting the effectiveness (or, at least, increasing the costs) of several chemical defences (Fowden & Lea 1974; Gershenson 1994b).

FOOD BODY PRODUCTION IN RELATION TO PLANT SIZE AND ANT COLONY DEVELOPMENT

In spite of the decrease of relative productivity per stipule or unit leaf area with increasing plant size (Figs 3, 4 and 6), the total FB production is linearly related to plant size. The ant colony size grows linearly with the total lengths of the stem and all twigs of a *M. triloba* tree (H.-P. Heckroth & M. Heil, unpublished data). Since the number of stipules is directly and linearly correlated with this total length (M. Heil *et al.*, unpublished data), the total FB production of *M. triloba* parallels the growth of the inhabiting ant colony. The relationships between productivity, plant size and total FB production can be demonstrated in a three-dimensional plot (Fig. 7a): the 'plant's point of view' shows a decrease in the investment in FB production with increasing plant size (Fig. 7b), while the 'ant's point of view' shows a continually growing FB supply (Fig. 7c). To date, it is not known whether this pattern results from a permanent regulation of FB production depending on, for example, the intensity of FB removal by the ants, or if it depends only on the plant's ontogenetic stage and correlated resource allocation to different functions. However, both cases result in FB production rates that seem to fulfil the actual needs of the ants.

REGULATION OF FOOD BODY PRODUCTION BY THE PRESENCE OF INHABITING ANTS

The comparisons of ant-free and inhabited plants support the hypothesis that, in the myrmecophyte *M. triloba*, FB production is significantly raised in the presence of symbiotic ants. Total FB amounts pro-

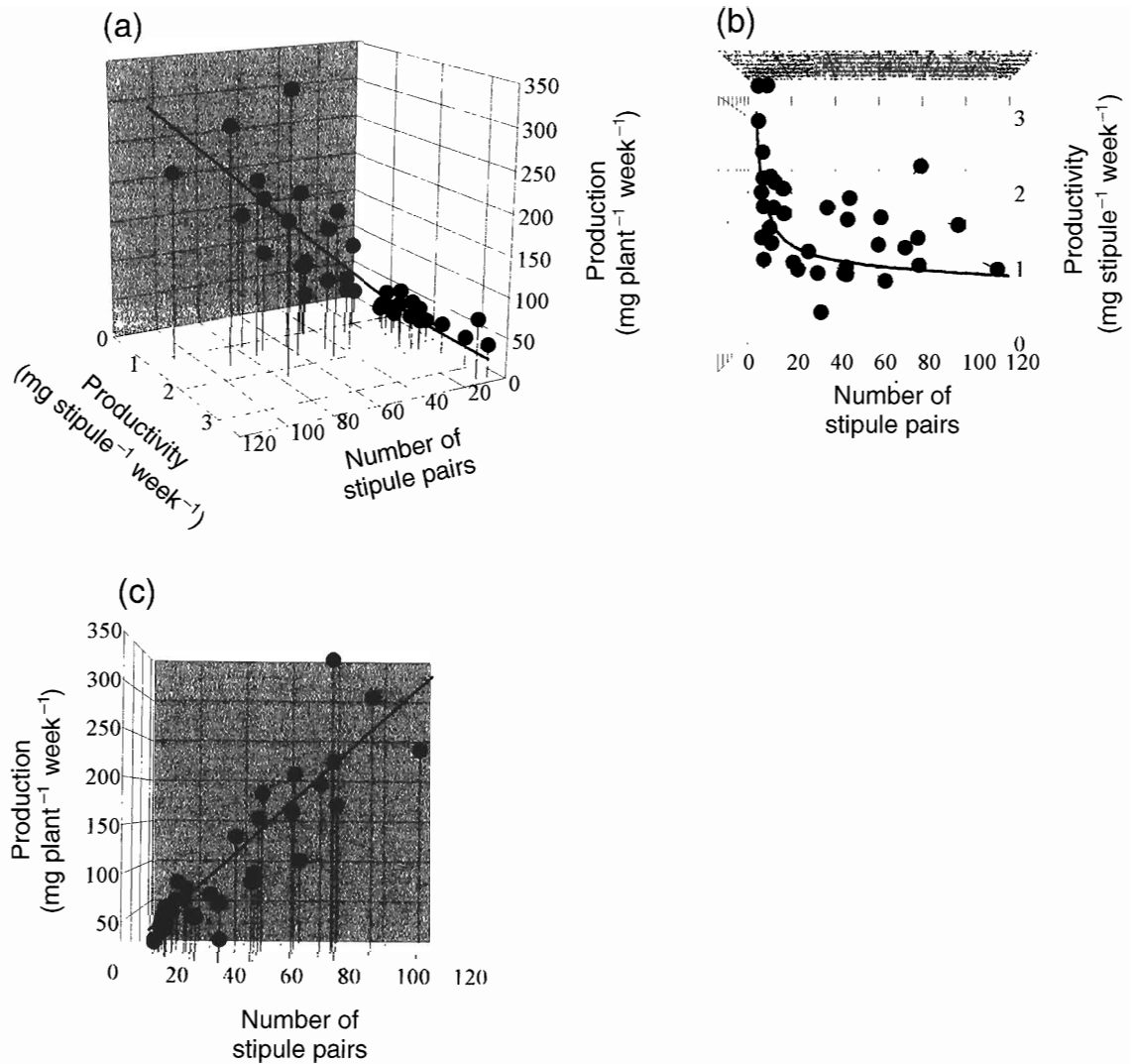


Fig. 7 Relationships between productivity, plant size and total food body production ($n = 36$ plants). Productivity per stipule for varying plant sizes is given in milligrams dry FBs per stipule per week; total FB production of entire plants is calculated as milligrams FB dry mass per week; and plant size is represented by the total number of stipule pairs. For better illustration, the same coordinate system is shown from three different perspectives. (a) An overview; (b) the 'plant's point of view': the projection to the x,y -area shows the relationship between productivity per stipule and plant size; (c) the 'ants' point of view': the projection to the x,z -area indicates the relationship between total FB production and plant size.

duced by ant-inhabited plants were on average about eight times higher than those of ant-free ones (Figs 5a and 6). This difference was caused by lower stipule numbers in ant-free plants rather than by lower FB production rates per stipule (Table 2 and Fig. 5b). Davidson & Fisher (1991) and Folgarait & Davidson (1994, 1995) reported influences of some abiotic site factors on FB production rates of *Cecropia*. Due to methodological constraints, ant-free plants and inhabited plants had to be cultivated and investigated in spatially separated clusters. Among several abiotic factors tested, only light intensity differed significantly between the two plant groups, yet in favour of the ant-free plants. In the absence of photoinhibition, increased light availability should have had a positive effect on FB production (Folgarait & Davidson 1994). Light intensities experienced by ant-free plants, however, did not exceed site-typical values. The differences

in FB production can therefore not be attributed to effects of abiotic factors. Only inhabited plants showed the characteristic dependence of FB production rate on plant size (Fig. 6), while in ant-free plants these variables were not correlated. Light intensity did not correlate with plant size in either inhabited and uninhabited plants, nor did N, K or S content.

This suggests that the presence or absence of ants was the decisive variable causing these highly significant differences between inhabited and ant-free *M. triloba* plants. This claim is substantiated by the results of greenhouse experiments comparing ant-free and inhabited plants. These plants, cultivated for up to 13 months under identical conditions, showed significantly higher stipule numbers in inhabited plants.

Since all plants were in good general condition, particularly in not being infested by herbivores or parasites, a loss of stipules due to destruction by ani-

mals or pathogens can be excluded for both the field and the greenhouse survey. The survey of plants grown naturally with and without ants demonstrated the same dependency of stipule number on the presence of ants, and we thus assume that, in *M. triloba*, total FB production in general can be regulated via the number of stipules. A similar effect can be observed when trees are flowering: *M. triloba* produces large amounts of FBs on its bracts (Whitmore 1973; Fiala & Maschwitz 1992b), while fewer stipules occur on the twigs during this time (M. Heil *et al.*, personal observations).

A loss of FB-producing structures in the absence of mutualistic ants has already been reported for *Cecropia* (Janzen 1973; Rickson 1977; Putz & Holbrook 1988). While *C. peltata* plants grown without ants in the greenhouse have normal numbers of Müllerian bodies producing trichilia (D. Davidson, personal communication), a population recently introduced to Malaysia (where symbiotic *Azteca* ants do not occur) shows a high variability in trichilia numbers (Putz & Holbrook 1988; D. Davidson, personal communication). However, the loss of trichilia in *Cecropia* populations growing without ants over several generations was interpreted as an evolutionary process (Janzen 1973; Rickson 1977). A control of the energy investment in 'biotic' defence via changes in the longevity of the FB producing structures, which becomes effective in time spans of weeks to months, has not been reported before.

Evidence from other *Macaranga* species indicates that different regulating mechanisms occur within this genus. Experiments based on artificial FB removal revealed a dependency of FB production rates on harvesting frequency in the two species *M. hypoleuca* (Reichenb. f. Zoll.) Muell. Arg. and *M. motleyana* ssp. *griffithiana* (Muell. Arg.) Whitmore. No effect of different FB removal intensities was observed in the non-myrmecophyte *M. tanarius* (L.) Muell. Arg. (Menke 1996).

Conclusion

The quantification of costs arising from traits evolved by mutualistic species, especially in adaptation to the requirements of the respective partner, is of fundamental importance for understanding the function and evolution of mutualism (Bronstein 1994). FB production seems to be a plant 'service' established exclusively to enable mutualism with ant defenders. In *M. triloba*, this type of 'biotic' defence causes substantial metabolic costs that decrease quickly when trees become larger and branched. The existence of fitness costs of FB production is further corroborated by the observation of decreased FB production in the absence of ants. High FB production in ant-inhabited plants resulted to a high degree from increased stipule longevity. This indicates that the persistence of FB-producing stipules, which seems to be an important

trait of several myrmecophytic *Macaranga*, may be only a small evolutionary step in the development of highly specialized obligate myrmecophytes in this genus.

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