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Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant

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Abstract Tropical plants of different genera defend themselves via symbiotic ant colonies, which are housed and often nourished by their host plant. Many studies deal with the defensive effects of the ants, but none has linked the plants' investment in this type of defence to the size and defensive efficacy of the symbiotic ant colony. We show here that ant-food production by the obligate myrmecophyte, *Macaranga triloba*, is limited by nutrient supply. The colony size of the ants in untreated plants (which had not been affected by experiments in advance of colony collection and determination of food body production) was significantly correlated with the amount of food produced by their hosts, and the plants' level of leaf damage was significantly and negatively correlated with the number of inhabiting ant workers. Our study provides the first field data that show that nutrient availability can directly influence a myrmecophyte's investment in its ants. Further studies are needed to evaluate whether soil nutrient contents in general can be a factor that limits the ability of myrmecophytes to defend themselves indirectly by nourishing symbiotic ants.

Keywords Antiherbivore defence · *Macaranga triloba* · Mutualism · Myrmecophytism · Tropics

Introduction

Understanding the ecology and evolution of any plant defence mechanism requires information on both the resulting benefits and on external and internal limiting factors such as resource availability (Bryant et al. 1983; Coley et al. 1985) or intrinsic constraints that lead to a metabolic competition between e.g. growth and defence (Herms and Mattson 1992). Many studies have shown, for different forms of direct defence, that nutrient supply may be a limiting factor (for recent studies see e.g. Wilkens et al. 1996; Baldwin et al. 1998; Stout et al. 1998; for overviews of plant resource allocation to defence see Bloom et al. 1985; Bazzaz et al. 1987; Zangerl and Bazzaz 1992; Bazzaz and Grace 1997), but few comparable studies have been conducted for indirect defence, i.e. defence mechanisms that are mediated by interactions including the actions of enemies of the plants' enemies (Price et al. 1980; Dicke 1999; Vet 1999).

A widespread form of indirect defence comprises mutualistic interactions with ants that defend plants against herbivores and encroaching vegetation (Buckley 1982; Beattie 1985; Hölldobler and Wilson 1990; Davidson and McKey 1993). The most specialised cases can be observed in the tropics, where so-called myrmecophytic plants of different genera house and nourish specific ant colonies, which defend them effectively against damage caused by herbivorous arthropods and fungal pathogens (Heil et al., in press, and references therein). Though many studies have clearly shown the positive effect of these interactions, only a few have addressed factors that may limit the plants' investment in their ant defenders or the efficacy of defending ants (but see Duarte-Rocha and Godoy-Bergallo 1992; Folgarait and Davidson 1994, 1995).

This study provides for the first time field data that combine information on these different aspects for a single plant species. Several properties make the myrmecophyte *Macaranga triloba* (Bl.) Muell. Arg (Euphorbiaceae) an optimal species for this study. *M. triloba* and its ants live in an obligate symbiosis, with the ants being totally dependent on food and nesting space provided by their host plant (Fiala and Maschwitz 1990), while the host plant relies on the defence provided by the ants (Heil et al., in press, and references therein). All branches and the main stem of a *M. triloba* plant are hollow and form domatia which are inhabited by the ants (Fiala and

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Maschwitz 1992a). The ants collect food bodies (FBs) which are produced under specialised, recurved stipules (Fiala and Maschwitz 1992b; Heil et al. 1998). Food bodies of *M. triloba* contain high amounts of proteins and thus nitrogen (Heil 1998; Heil et al. 1998), they therefore represent an important nitrogen and energy cost to the plants (Heil et al. 1997). Since there are strong correlations between the number of stipule pairs, the FBs present on, and the FB production of a given plant (Heil et al. 1997; Heil 1998), simply counting FBs allows one to estimate a plant's FB production.

Three main questions were addressed by our study:

1. Is FB production limited by soil nutrient contents?
2. Is ant colony size in *M. triloba* related to FB production?
3. Is the damage level of a host plant related to the size of its resident ant colony?

Materials and methods

The study was conducted during two field stays in West Malaysia. For detailed information on the study site and species see Heil et al. (1997; in press) and references therein.

Nutrient availability and food body production

To investigate the effect of increased nutrient supply on FB production, 15 *M. triloba* plants were selected in a forest near the Ulu Gombak Field Study Centre in Selangor, West Malaysia, in March and April 1998. Their total leaf area was determined by measuring length and width of all leaves and multiplying their product with the slope of a regression line based on data derived from 100 leaves (r^2>0.97; Heil et al. 1997).

Plants were assigned to five groups, each consisting of three plants that differed by less than 10% in total leaf area and number of stipule pairs. Within each group, the plants were assigned randomly to three treatments (fertiliser 1, or 2, and untreated control). Commercial fertilisers were used (fertiliser 1: Westcott's garden fertiliser, SPGS Agriculture and Research Division, Petaling Jaya, West Malaysia; fertiliser 2: Organic Yellow plant-food, Gardenic, Melaka, West Malaysia). We took two samples each of fertilisers and analysed their composition. Fertiliser 1 contained on average 12.68% N, 3.66% P, 15.82% K, 26,015 ppm Ca, 18,160 ppm S, 13,060 ppm Mg, 3,315 ppm C, 1,755 ppm Na, 958 ppm Fe, 235 ppm B, 229 ppm Mn, 213 ppm Al, and 147 ppm Z. Fertiliser 2 contained on average 12.07% N, 3.23% P, 11.14% K, 90,025 ppm S, 48,630 ppm Ca, 48,035 ppm C, 9,399 ppm Mg, 3,761 ppm Al, 3,023 ppm Na, 208 ppm Mn, 251 ppm Zn, and 26 ppm B. Depending on their size, the experimental plants were treated once per week with fertiliser which was applied directly onto the soil surrounding the stems. Plants of less than 1 m height received 15 g, those of height between 1 m and 1.5 m 25 g, and the largest plants (up to 2 m high) 30 g fertiliser per week. Since little rain fell during the experimental period, we watered all plants throughout the experiment with at least 2 l water per plant and day applied once per day before noon.

Six weeks later, FB production was estimated by an indirect method (see Heil et al. 1997). Briefly, similar amounts of FBs are present under the two stipules of a pair. We therefore removed one randomly selected stipule from each of the the first three and every other pair of the older stipule pairs (numbers 5, 7, 9, and 11 according to their insertion at the stem, see Heil et al. 1997 for details) and counted FBs. Ants were excluded from the remaining stipules by applying a sticky resin (Tangletrap, Tanglefoot Corp., USA) around the stipule. After 1 week, these stipules were removed, the FBs were counted and their dry mass was calculated by multiplying FB number and average FB weight. The difference between the first and the second FB mass represents the weekly FB production of the stipules in question. FB production rates of the remaining stipule pairs were estimated as the mean of the two adjacent ones.

Food body production, ant colony size and herbivory

To investigate relationships between FB production, ant colony size, and the actual level of leaf damage, a further study was conducted in August and September 1999. Thirty plants were selected that were growing at a forest edge along a roadside between Seremban and Kuala Pilah, Negeri Sembilan, West Malaysia. To exclude the influence of plant size on ant colony size (M. Heil and H.-P. Heckroth, unpublished work; cf. Fonseca 1993), only young, unbranched saplings with heights from 1.0 m to 1.5 m were used. For all plants, FBs present under the stipules were counted to estimate the plants' current rate of FB production (Heil et al. 1997). Ant colonies were completely removed from the plants and stored in alcohol to count numbers of adult workers, pupae, and larvae. All leaves from all plants were collected and dried to measure the plants' actual leaf area as well as the actual damage level (in % missing leaf area, see Heil et al. (in press) for a detailed description of this method).

Results

Nutrient availability and food body production

Application of both fertilisers increased FB productivity two- to threefold (Fig. 1). When data from all stipules were pooled, stipules of control plants had an average productivity of 1.5 mg FBs stipule^{-1} week^{-1} (mean, n=30 stipules from five plants), while those of plants with fertiliser 1 produced 3.2 mg FBs stipule^{-1} week^{-1} (n=36 stipules) and those with fertiliser 2 produced 4.4 mg FBs sti-

Table 1 Results of repeated-measures ANOVA on stipule position as within-subject factor and treatment (fertilisation) as between-subject factor. Sample size=5 for each stipule position-treatment combination. Analysis was based on data stipule positions 1, 2, 3, 5 and 7, since several plants had no more than seven stipule pairs

	Source	SS	*df*	*F*	*P*
Within-subject effects	Stipule	37.716	5	5.768	<0.001
	Stipule×Fertilisation	21.967	10	1.680	n.s.
	Error	58.850	45		
Between-subject effects	Fertilisation	104.606	2	16.126	<0.001
	Error	29.191	9		

n.s. *P*>0.05

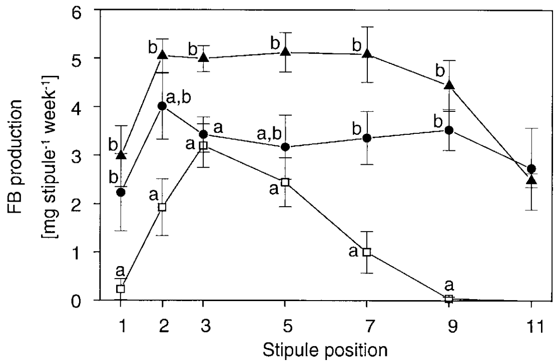

Fig. 1 Food body (*FB*) production in *Macaranga triloba* after different fertilisation treatments. Mean FB production rates (mg dry mass stipule^{-1} week^{-1}) of different aged stipules are plotted separately for plants treated with the two fertilisers (*filled circles* fertiliser 1, *filled triangles* fertiliser 2) and for untreated controls (*open squares*). Five plants were subjected to each treatment. Stipule age is indicated by stipule position, with position 1 representing the youngest one. Significant differences ($P<0.05$) between fertilisation treatments at particular stipule positions are indicated by *different letters* (Scheffé post hoc tests conducted separately for stipule positions 1–9 following a repeated-measures ANOVA on stipule position as within-subject factor and treatment as between-subject factor, $n=5$ for each stipule position-treatment combination)

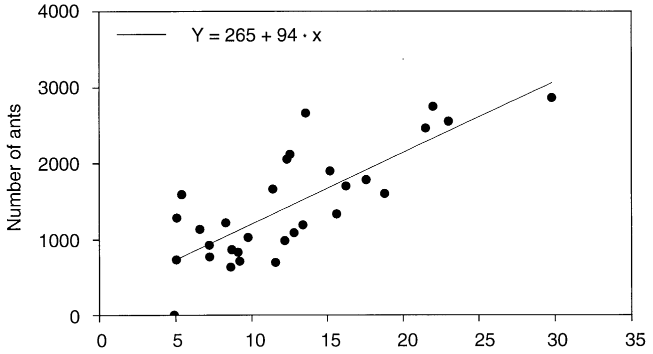

Fig. 2 Relation between FB supply and ant colony size in *M. triloba*. Total amounts of food bodies present on 30 untreated, field grown plants (mg dry mass per plant) are related to the total number of ants (including larvae, pupae, and adult workers) inhabiting these plants ($r^2=0.62$)

pule^{-1} week^{-1} ($n=35$ stipules). Both stipule position and fertilisation had a significant effect on FB production ($P<0.001$ in both cases, Table 1). Though interaction was not significant (Table 1), the effect of fertilisation was most pronounced at the youngest and oldest stipule pairs which are characterised by low production rates in the uninfluenced state (Fig. 1).

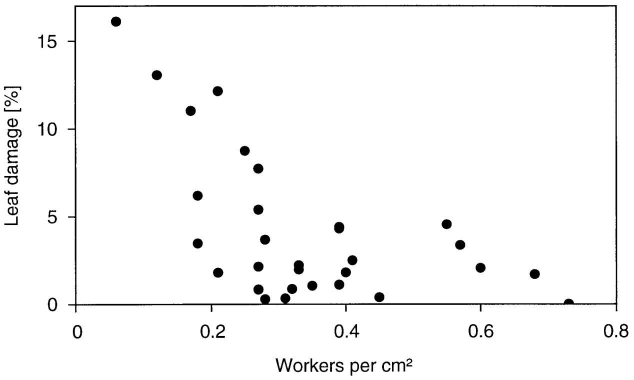

Fig. 3 Relation between number of adult workers and leaf damage of untreated *M. triloba* plants. Numbers of adult ant workers are related to total leaf area and expressed in workers cm^{-2}, the damage level is given in percent of missing leaf area (correlation coefficient=–0.54, $P=0.001$, $n=30$, Spearman rank test)

Food body production, ant colony size and herbivory

Though influences of plant height and total leaf area on ant colony size were successfully excluded ($P>0.10$ for both variables, Spearman rank correlation test, $n=30$), both total numbers of ants and amounts of ant-food present on the plants varied at least six-fold (Fig. 2). Ant colony size was significantly and positively correlated with the amounts of FBs, and this holds true for the numbers of larvae and pupae as well as for numbers of adult workers (Table 2). A linear regression explained 62% of the total variance in numbers of ants (all brood and adults summed up) present in the various plants (Fig. 2).

To calculate the number of ants available to defend a given leaf area, the number of adult workers was related to the respective plant's total leaf area. The plants' leaf damage level was significantly and negatively correlated with the number of workers per square centimeter (Fig. 3), while leaf damage had no detectable influence on amounts of FBs present on the plants ($P>0.10$, $n=30$, Spearman rank test).

Discussion

Many studies based on ant-exclusion experiments demonstrate that "biotic" indirect defence via mutualistic ants can strongly benefit their myrmecophytic host plants (Heil et al., in press, and references therein), but few have focused on factors that may have an influence on ant colony size or on the ants' defensive effect. Duarte-Rocha and Godoy-Bergallo (1992) have demonstrated that the

Table 2 Correlations (Spearman rank test) between the amount of FBs (mg dry weight per plant) and ant colony size in *Macaranga triloba*. Data analysis was carried out separately for the brood (number of pupae and larvae) and for adult workers

Variable 1	Variable 2	Sample size	Correlation coefficient	*P*
FBs (mg per plant)	Brood	30	0.65	<0.001
FBs (mg per plant)	Adult workers	30	0.74	<0.001

colony size of *Azteca* ants in *Cecropia pachystachya* can affect the time that specialised herbivorous beetles spend on feeding on these plants. Consequently, ant colony size was negatively correlated with leaf damage of their host plant (Duarte-Rocha and Godoy-Bergallo 1992). In an attempt to detect factors affecting colony size of *Pseudomyrmex* plant-ants living in leaf domatia of *Tachigali*, Fonseca (1993) found that nesting space was limiting. Myrmecophytic plants thus may be able to increase ant colony size just by providing larger domatia. Light and nutrient availability have been shown to affect ant-food production under greenhouse conditions for different myrmecophytic *Cecropia* species (Folgarait and Davidson 1994, 1995), but no comparable studies have so far been conducted under field conditions, and the effect that an increase in FB production rates may have on ant colony size was unknown.

Our results demonstrate that all these factors interact. Ant-food production increased in response to increased nutrient supply (Fig. 1), the amount of ant-food present on a given plant was significantly correlated with the number of ants living inside (Fig. 2), and the number of ant workers was negatively correlated with the actual level of leaf damage (Fig. 3). This indicates that larger colonies can be better defenders of heir host plants, as reported earlier by Duarte-Rocha and Godoy-Bergallo (1992), and that FB production – and thus defence – by *M. triloba* is limited by soil nutrient content under natural conditions. A direct influence of herbivory on ant-food production could be excluded, at least for the comparably low damage levels that occurred in our data set, and that are typical for inhabited *M. triloba* plants (Heil et al., in press). Folgarait and Davidson (1995) have reported positive effects of nutrient supply on food body production rates of several *Cecropia* species. This suggests that ant-food production in general may depend on soil nutrient contents. Janzen (1974) had already supposed that myrmecophytic *Acacia* species might be restricted to comparatively wet sites, since they are forced to produce young leaves (and therewith ant-food) during the dry season. Nutrient availability seems to be a further factor that determines site selection by myrmecophytic plants.

Interestingly, there was a linear relationship between the number of ant workers and the maximum damage levels, while low damage levels occurred nearly independently of the number of workers (Fig. 3). Ants in *M. triloba* represent a very effective defence mechanism (Heil et al., in press), and plants that invested heavily in FB production and housed a large ant colony (i.e. with more than 0.3 workers available per square centimeter of leaf area) never suffered from high damage levels (of more than 5% missing leaf area, see Fig. 3). However, some of the plants that did not invest much in FB production, and that housed only small ant colonies, “escape” herbivory, probably just by chance, while others with similar low numbers of ants lost high amounts of leaf area (Fig. 3). A given number of ants living in a plant can therefore result in different levels of leaf damage. This could be due to factors such as spatio-temporally varying herbivore pressure or differences in the respective ant colonies’ behaviour, both of which can hardly be controlled by the plant. Variable defensive outcomes of a given investment in defence ensure the development of genetic variability with respect to plant investment in defence. Genotypes adapted to invest heavily in defence may suffer from negative effects on plant growth under enemy-free conditions, while genotypes that invest less may suffer from high damage levels when herbivore pressure is high. Independently of this, the amounts of FBs that a plant can produce should vary between sites differing in soil nutrient contents. Abiotic site factors thus also have an important effect on the outcome of a given defensive strategy. Maintaining high genetic variability can be very important for a pioneer tree species that grows in heterogeneous and fast-changing habitats.

Our conclusions are based on correlations between factors from untreated plants growing under natural conditions, but the underlying causal relations are still unclear. Presumably, the amount of FBs produced by a plant determines the size of its resident ant colony, which then determines the possible efficacy of indirect defence. However, ants could also regulate the FB production rate of their host plant, at least up to a maximum level that is determined by nutrient availability. This view gains some support from the fact that inhabited *M. triloba* plants produce more FBs than do uninhabited ones (Heil et al. 1997). Further, experimental field studies are needed to understand the causal relations that lead to the strong relationships between soil nutrient availability, a plant’s investment in indirect defence via symbiotic ants, the size of the resident ant colony, and the resulting benefit to the plant, which all can be seen in this first, exploratory data set.

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