



# Adaptations to biotic and abiotic stress: *Macaranga*-ant plants optimize investment in biotic defence

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## Abstract

Obligate ant plants (myrmecophytes) in the genus *Macaranga* produce energy- and nutrient-rich food bodies (FBs) to nourish mutualistic ants which live inside the plants. These defend their host against biotic stress caused by herbivores and pathogens. Facultative, 'myrmecophilic' interactions are based on the provision of FBs and/or extrafloral nectar (EFN) to defending insects that are attracted from the vicinity. FB production by the myrmecophyte, *M. triloba*, was limited by soil nutrient content under field conditions and was regulated according to the presence or absence of an ant colony. However, increased FB production promoted growth of the ant colonies living in the plants. Ant colony size is an important defensive trait and is negatively correlated to a plant's leaf damage. Similar regulatory patterns occurred in the EFN production of the myrmecophilic *M. tanarius*. Nectar accumulation resulting from the absence of consumers strongly decreased nectar flow, which increased again when consumers had access to the plant. EFN flow could be induced via the octadecanoid pathway. Leaf damage increased levels of endogenous jasmonic acid (JA), and both leaf damage and exogenous JA application increased EFN flow. Higher numbers of nectary visiting insects and lower numbers of herbivores were present on JA-treated plants. In the long run, this decreased leaf damage significantly. Ant food production is controlled by different regulatory mechanisms which ensure that costs are only

incurred when counterbalanced by defensive effects of mutualistic insects.

Key words: Ant plant, anti-herbivore defence, mutualism, myrmecophyte, tropics.

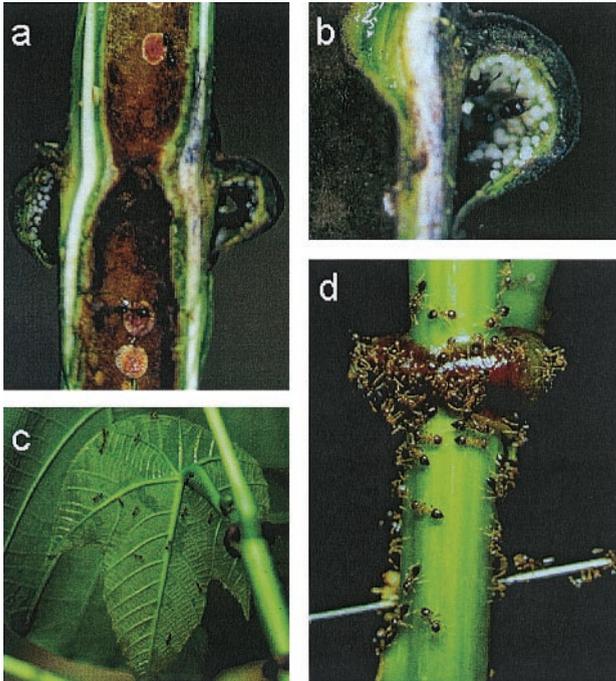
## Introduction

Many tropical plants of different taxonomic groups have evolved mutualisms with ants (Beattie, 1985; Buckley, 1982; Hölldobler and Wilson, 1990; McKey and Davidson, 1993). In most cases, ants are used as an indirect (Price *et al.*, 1980), 'biotic' defence mechanism. Obligate 'ant plants' (myrmecophytes) of the genus *Macaranga* (Euphorbiaceae) house specific ant colonies in their hollow shoots (Fig. 1a). The ants feed on so-called food bodies (FBs, Fig. 1b), cellular structures which are produced by the plant and which contain large amounts of lipids, proteins and carbohydrates (see below, and Fiala and Maschwitz, 1992a, b; Heil *et al.*, 1998). The ants (mostly belonging to the genus *Crematogaster*) live symbiotically in specific *Macaranga* trees (Fiala *et al.*, 1999; Fiala and Maschwitz, 1990). They patrol the plant surface (Fig. 1c, d), remove all kinds of foreign material, and thereby defend their host against various forms of biotic stress caused by herbivores, by competing and parasitic plants, and by fungal pathogens (Fiala *et al.*, 1994, 1989; and below). Other forms of ant-plant interaction in the genus *Macaranga* are less specific. 'Myrmecophilic' plants do not house species-specific ant

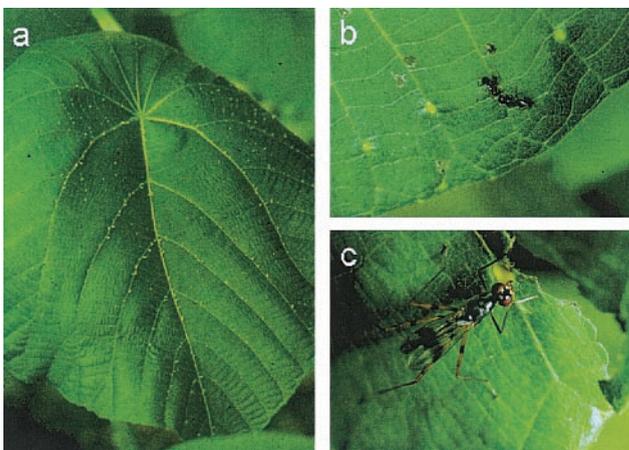
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colonies. However, they produce FBs (Fig. 2a) and extrafloral nectar (EFN) on their leaf blades (Fiala and Maschwitz, 1991). Insects such as ants (Fig. 2b), wasps and flies (Fig. 2c) from the vicinity are attracted to these rewards and defend the plants facultatively against herbivores (Koptur, 1992).



**Fig. 1.** The obligate myrmecophyte *M. triloba*. Obligate myrmecophytes are inhabited by mutualistic ants living in domatia (a) formed by the hollow twigs. The inhabiting *Crematogaster* ant are nourished by food bodies (FBs) emerging from the lower surface of stipules which are recurved backwards and clasp the stem tightly (b). The ants patrol the younger leaves preferably (c), but can concentrate quickly on sites actually requiring defence (d) as simulated here by fixing a termite bait with a needle on a young shoot. All photographs by M Heil.

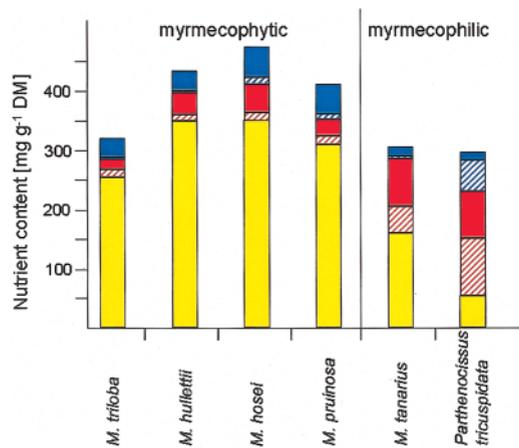


**Fig. 2.** Facultative insect-plant interactions in the myrmecophilic *M. tanarius*. FBs (a) and extrafloral nectaries (b, c) are located openly on the leaf blades. Extrafloral nectar is removed by different insects such as ants (b), wasps, and flies (c). Photographs by B Fiala (a) and M Heil (b, c).

Both strategies enable these plants to cope with the severe biotic stress resulting from high herbivore and pathogen pressure which is characteristic for many secondary forests. In the tropics, nutrients are often limiting for plant growth and reproduction, this holds true especially for the heavily degraded soils occurring in many open, secondary systems. FB production consumes important resources such as nitrogen and energy (see below), and EFN contains carbohydrates and amino acids and thus consumes resources as well. Mechanisms controlling the investment in ant rewards should therefore provide selective advantages. The present paper reviews recently discovered mechanisms regulating the production of FBs and EFN. Two species were chosen. *M. triloba* (Bl.) Muell. Arg. represents the obligate myrmecophytes, while *M. tanarius* (L.) Muell. Arg. has evolved the facultative, myrmecophilic strategy. Most experiments were conducted under field conditions at the plants' natural growing sites on Peninsular Malaysia (see Fiala *et al.*, 1989; Heil, 1998; Heil *et al.*, 2001b for descriptions of the study sites).

**Costs of food body and extrafloral nectar production**

Ants associated with *Macaranga* myrmecophytes do not use any of the material which they find on their host plants as food (Fiala and Maschwitz, 1990). Even insect eggs and small larvae are only removed and thrown off the plant surface. The ants therefore seem to rely fully on the FBs provided by their host plants. In contrast, FBs produced by myrmecophilic species are only a kind of bait for opportunistic ants and other insects living in the vicinity of the plants. These are not likely to rely solely on food produced by one single plant species. Analyses of main nutrient classes in FBs were conducted with HPLC (carbohydrates and proteins) and GC-MS (lipids). They demonstrated that *Macaranga* FBs meet these different requirements (Heil *et al.*, 1998). FBs produced by obligate myrmecophytes were rich in lipids and proteins and had a comparably high N/C-ratio (Fig. 3). FBs produced by the myrmecophilic *M. tanarius* had lower contents of lipids and much lower contents of proteins, while the contents of carbohydrates were higher than in FBs of myrmecophytes (Heil *et al.*, 1998). These trends were even more obvious when regarding FBs of other myrmecophilic species belonging to the family of Vitaceae (Heil *et al.*, 1998). Further characteristic differences between FBs from myrmecophytic and myrmecophilic species occurred with respect to the ratio of soluble to polymeric carbohydrates and the ratio of free amino acids to proteins. In both classes of main nutrients, the relative content of monomeric or dimeric, soluble components was much higher in FBs of myrmecophilic species



**Fig. 3.** Chemical composition of FBs. Main nutrient contents in FBs of four myrmecophytic and two myrmecophilic plant species are given in  $\text{mg g}^{-1}$  dry mass separately for carbohydrates (red bars), proteins (blue bars) and lipids (yellow bars). Contents of soluble, monomeric (sugars and free amino acids: stripped) and of polymeric compounds (starch and proteins: filled) are given separately for both carbohydrates and proteins. (Data from Heil, 1998; Heil *et al.*, 1998.)

(Fig. 3). It is likely that these soluble components are easier to detect chemotactically by feeding insects than polymeric carbohydrates or proteins (Heil *et al.*, 1998). Taste seems to be much more important in FBs which are used by foraging insects from the vicinity, while a high nutritive value should be selected for in FBs which serve as the main food source for ant colonies living in myrmecophytic species. Finally, water contents of fresh FBs also differed between the two functional groups and were much higher in FBs from myrmecophilic species (Heil *et al.*, 1998). Containing a high proportion of soluble sugars and amino acids dissolved in large quantities of water, FBs of myrmecophilic species seem to resemble some kind of 'packed' extrafloral nectar (EFN). Thus, these FBs mimic a widespread kind of plant-derived food resource which is used by many different ant species (Heil *et al.*, 1998; Koptur, 1992).

Due to their high contents of lipids and proteins, production of FBs by the myrmecophyte, *M. triloba*, consumes a considerable amount of resources. Both FB production and overall above-ground biomass production were quantified for unbranched *M. triloba* saplings, and daily courses of photosynthetic rate (net rate of photosynthetic  $\text{CO}_2$  uptake) were measured for the same plants. To estimate the plants' total energy budget under field conditions, a  $\text{CO}_2/\text{H}_2\text{O}$ -porometer system (CQP 130; Walz, Effeltrich, Germany, see Heil *et al.*, 1997; Zotz *et al.*, 1995 for details) was used. FB production amounted to about 5% of total above-ground biomass production. Based on the chemical composition of FBs and shoot and leaf material it could be calculated that about 30% of the total lipids, 7% of the proteins and amino acids and 2% of the carbohydrates invested into the overall above-ground growth were diverted to FB

production (Heil *et al.*, 1997). Based on the construction costs of the different types of tissue (see Penning de Vries *et al.*, 1974 for the chosen method to calculate construction costs and Griffin, 1994, for a discussion of these methods), FB production consumed about 5% of the plants' total energy budget as estimated from photosynthetic net assimilation (Heil *et al.*, 1997).

Parallel measurements and calculations were conducted for the FB and EFN production and photosynthesis of similar-sized saplings of the myrmecophilic *M. tanarius*. Due to the lower contents of lipids and proteins, construction costs of FBs are lower (Heil, 1998). FB and EFN production by *M. tanarius* consumed about 4% of the plants total energy budget (Heil, 1998).

### Benefits of defence

Defence via housed or attracted insects is indirect (Price *et al.*, 1980) and thus might be expected to have only a low efficiency. Indeed, ant-exclusion experiments demonstrated that the defence resulting from facultative, myrmecophilic interactions is less efficient than defence provided by symbiotic *Crematogaster* plant ants living in myrmecophytic species (Fiala *et al.*, 1994, 1989). Recent field experiments were based on the comparison of damage suffered by leaves situated on ant-defended twigs to the damage suffered by leaves on twigs of the same plants from which ants were kept off experimentally. Neighbouring twigs were selected and were treated as matched pairs with one twig per pair being randomly selected for ant removal. The results were therefore not biased by any site effects or genetic differences between experimental plants (Heil *et al.*, 2001b). After 6 weeks, leaves on *M. triloba* twigs on which ants still were present had lost on average 1.2% of their total area (median,  $n=411$ ), while leaf damage on ant-free twigs of the same plants amounted to 2.6% missing area (median,  $n=412$ ). The highest efficacy of defence became apparent on young leaves that had newly developed during the experimental time span (1.4% missing leaf area on defended leaves compared to 4.2% for ant-free leaves,  $n=57$  and 40, respectively). In *M. tanarius*, the effect of defence was less pronounced. On average, defended leaves lost 3.2% leaf area ( $n=294$ ), while leaves to which ants had no access lost 4.2% ( $n=362$ ). Values for newly developed leaves were 2.1% ( $n=95$ ) and 3.9% ( $n=117$ ), respectively (all data from Heil *et al.*, 2001b). Based on Wilcoxon tests for matched pairs, all differences between damage levels of leaves on treated and control twigs were highly significant ( $P<0.001$  in all four cases mentioned).

Long-term studies conducted over the time span of one year demonstrated that short-term experiments are not suitable to estimate the 'real' effect of a given defence (Heil *et al.*, 2001b). A study comparing inhabited

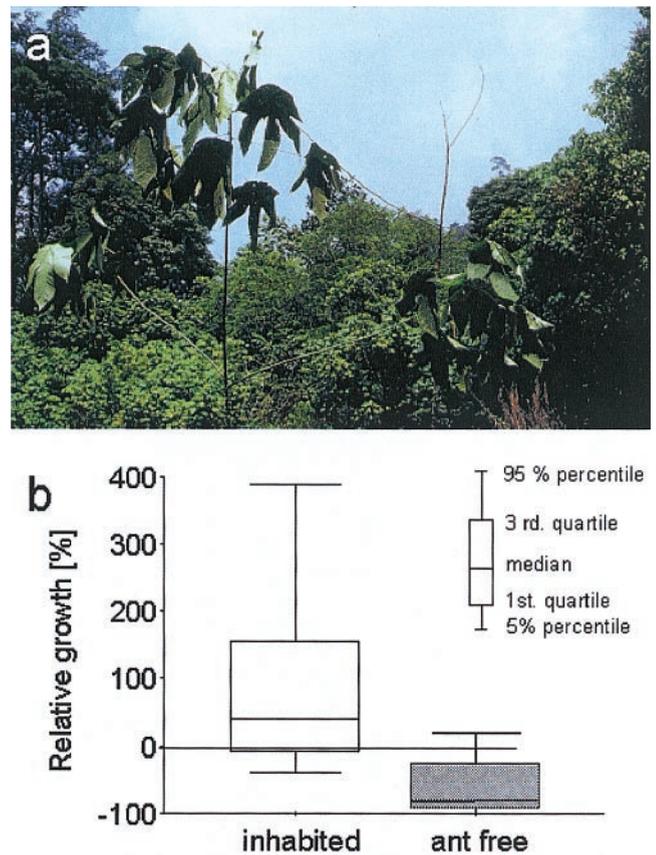
*M. triloba* trees to similar-sized ones which had been deprived from their ants experimentally for one year revealed that ant-free plants lost, on average, about 80% of their total leaf area (mean,  $n=16$ ), while total leaf area of inhabited trees increased by about 40% (mean,  $n=16$ ) within the same time span (Fig. 4).

Besides the defence against folivores, protection from shoot-boring insects and pathogenic fungi adds significantly to the overall effect of symbiotic *Crematogaster* ants associated with *M. triloba* (Heil *et al.*, 1999). Measurements of chitinase activity, a plant trait which has been shown to play an important role in the plants own, direct defence against pathogenic fungi (Iseli *et al.*, 1996; Jackson and Taylor, 1996; Sahai and Manocha, 1993), revealed very low activities in myrmecophytic *Macaranga* species (Heil *et al.*, 1999). This probably represents a general trait of ant plants, since Mexican *Acacia* myrmecophytes showed comparably low chitinase activities when compared to related species exhibiting no obligate mutualism with defending ants (Heil *et al.*, 2000c). In *M. triloba*, the inhabiting ants can have a direct protective effect against pathogenic fungi (Heil *et al.*, 1999), a result which is supported by similar findings on ant plants of the genus *Piper* (Letourneau, 1998).

The high efficacy of ant defence results from both the ants' mobility and low specificity. Ants can concentrate very quickly on those parts of the plant surface which actually require defence (Fig. 1d), and they are effective against many types of insects, climbers, and pathogens. They fulfil several functions for which otherwise a variety of different chemical substances would be necessary. This may be the most important general benefit of indirect plant defence, which makes use of 'animal-specific' traits rather than of intrinsic plant properties (Price *et al.*, 1980). Ants in the obligate myrmecophytic interactions are adapted much closer to their individual host and are much more dependent upon it. The higher efficacy of their interactions as compared to those in myrmecophilic relationships therefore seems to be an adaptive trait.

### Regulation of FB production

FB production represents a comparably costly defence (see above, and Heil *et al.*, 1997). In general, so-called 'allocation costs' are assumed to occur whenever defence consumes limited resources which then cannot be used for other vitally important functions such as growth and reproduction (Simms and Fritz, 1990; Simms and Rausher, 1987; Herms and Mattson 1992). Therefore, every 'costly' defensive trait should reduce the plants' fitness when expressed under conditions not requiring defence. This model can be used to understand the evolution of inducible defences (Heil, 1999, 2000) and explains why the induction of defence can cause a reduction of



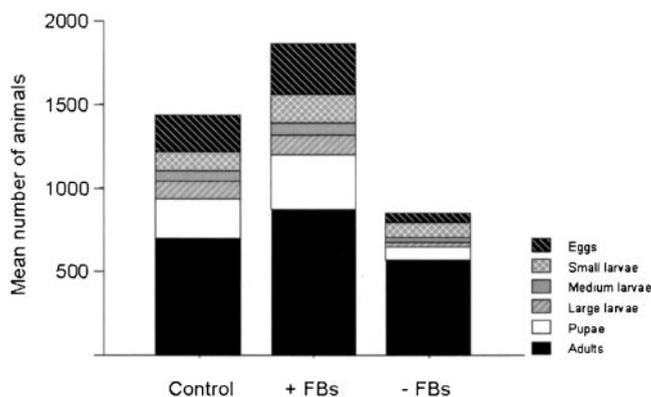
**Fig. 4.** Protective efficacy of *Crematogaster* ants living on the myrmecophyte *M. triloba*. (a) A pair of plants after 1 year of ant exclusion from the right plant. The left plant remained untreated as a control for the same time span. Both plants had very similar total leaf area and did not differ significantly in their leaf damage at the beginning of the experiment. (b) Average change in total leaf area (see insert for explanation of box plots) of 16 plants each which had been subjected to 'ant-free' (dark box) or to 'protected control' (open box) treatment. The difference between ant-free and control plants was highly significant ( $P < 0.001$ , Wilcoxon test for matched pairs). (Data from Heil *et al.*, 2001b; photograph by M Heil.)

reproductive success when occurring under enemy-free conditions (Agrawal *et al.*, 1999; Baldwin, 1998; Heil *et al.*, 2000b).

However, this also leads to the prediction that investments in indirect defences, such as the production of FBs or EFN, should be regulated by the plants in order to avoid superfluous costs. Fertilization studies conducted under field conditions revealed that FB production by *M. triloba* is limited by soil nutrient content (Heil *et al.*, 2001a) and thus is likely to cause relevant allocation costs. On average, FB production by fertilized *M. triloba* plants was more than three times higher than by untreated controls (4-week field experiment). Significantly higher amounts of FBs were already present on fertilized *M. triloba* plants 2 d after the onset of fertilization (M Heil, unpublished data). In comparison to the untreated controls, *M. triloba* saplings which had been

fertilized for 4 weeks to increase FB production contained higher numbers of eggs, larvae, pupae, and of adult ants, while colonies in plants from which FBs had been removed showed the reverse pattern (Fig. 5). These treatments had highly significant effects on ant colony structure and size. The effects of different treatments affected all developmental stages, but the small larvae significantly (Table 1). FB supply is obviously a main factor determining the size and structure of inhabiting ant colonies. Ant colony size is an important defensive trait, since the number of adult workers living in a plant is negatively correlated to the plant's leaf damage (Heil *et al.*, 2001a).

According to these results, FB production consumes important and limited resources but is positively correlated to the plants' defensive ability. It was thus likely to be regulated 'actively' by the plant itself. Indeed, ant-free *M. triloba* plants, grown in nylon-mesh field cages to prevent them from herbivore damage, showed much lower rates of FB production than similar-sized inhabited plants that had been cultivated under otherwise identical conditions (Heil *et al.*, 1997). The same held true for



**Fig. 5.** Dependence of ant colony size on FB production by the myrmecophyte, *M. triloba*. Mean numbers of ants present 30 d after the beginning of the field experiment are given separately for adult ants, pupae, larvae, and eggs. Plants were either subjected to a fertilization treatment (+FBs), to a complete removal of FB producing stipules (-FBs) or were left untreated as controls. Sample size is 30 plants per treatment. See Table 1 for results of ANOVA tests.

plants grown in a greenhouse in Germany. Negative effects of missing defence on plant performance and FB production were thus not likely to bias these patterns (Heil, 1998; Heil *et al.*, 1997). Obviously, FB production is upregulated in the presence of an inhabiting ant colony. It still remains to determine whether FB removal or the presence of a specific ant colony are triggering this response.

## Regulation of EFN production

Similar adaptive patterns were found in the EFN production by *M. tanarius*. Field experiments based on the experimental protection of nectaries from nectar-consuming insects revealed a decrease in EFN secretion when nectar accumulated on the nectaries, while EFN flow increased again when consumers had access to the plants for 1 d (Heil *et al.*, 2000a). When EFN was collected with microcapillaries from plants cultivated under greenhouse conditions, repeated EFN removals (8 EFN removals in 24 h conducted every 3 h) led to a higher average amount of nectar produced per day than one single nectar removal after 24 h (Heil *et al.*, 2000a). While the influence of chemical signals produced by nectar-consuming insects cannot yet be excluded, the latter result shows that the frequency of EFN removal alone can have some regulatory effect on the amount of nectar produced. Similar patterns have been reported for the secretion of floral nectar (Pyke, 1991) and thus might represent a general trait preventing plants from spending resources for insect rewards which are not likely to be consumed.

Several studies have indicated that EFN secretion or amino acid concentrations in EFN may increase in response to herbivory (Koptur, 1989; Smith *et al.*, 1990; Stephenson, 1982; Swift and Lanza, 1993), and that this response does not require herbivore-specific elicitors (Heil *et al.*, 2000a; Wäckers and Wunderlin, 1999). It has therefore been discussed whether or not EFN can be considered as an induced defence (Agrawal and Rutter, 1998). However, most of these studies suffered from

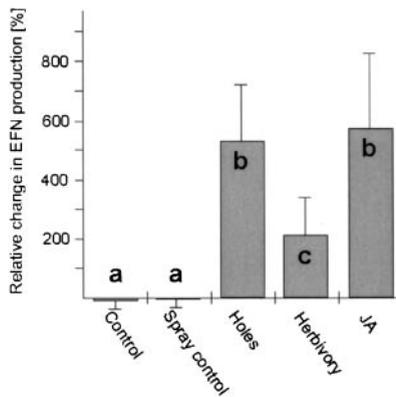
**Table 1.** Effects of increased or decreased FB production on colony structure

After analysis of main effects (repeated measures ANOVA: SS = 4417570, d.f. = 2,  $F = 14.846$ ,  $P < 0.001$  for a significant effect of treatment on numbers of ants living inside the plants with developmental stage as within-subject variable), effects of treatments were tested by univariate ANOVA separately for each developmental stage. Different letters given for fertilized (+), control (C) or stipule-free (-) plants indicate significant differences (LSD *post hoc* tests) between single treatments.

Developmental stage	Sum of squares	d.f.	<i>F</i>	<i>P</i>	+	C	-
Adult	4 273 739	2	8.927	<0.001	a	b	b
Pupae	1 114 809	2	26.861	<0.001	a	b	c
Large larvae	176 396	2	19.541	<0.001	a	a	b
Medium larvae	54 488	2	4.921	0.009	a	a	b
Small larvae	185 948	2	1.768	ns	a	a	a
Eggs	1 172 363	2	10.728	<0.001	a	b	c

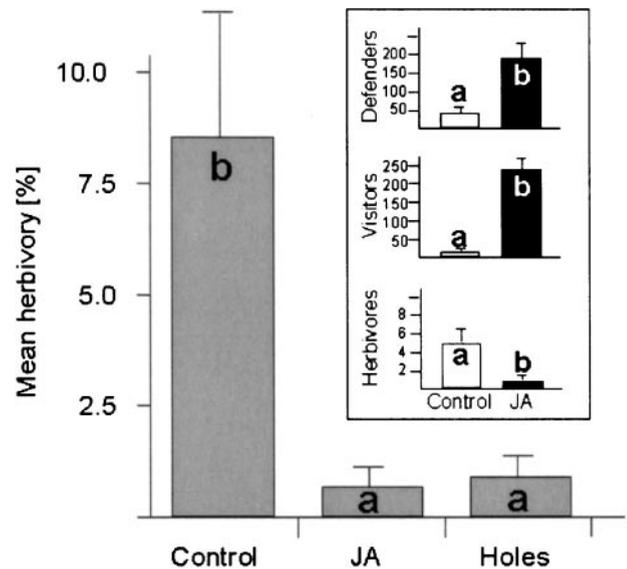
methodological problems (discussed in Heil *et al.*, 2000a). Moreover, no information was available on the underlying signalling pathway, and no study had focused on the effects of induced EFN production on nectary-visiting insects and herbivores.

Recently, a first data set answering these questions has been obtained from field studies on *M. tanarius*. Secretion rates of treated experimental and untreated control plants were compared to reference values measured in advance of the experiments on the same individual plants (Heil *et al.*, 2001c). EFN production was quantified as the amount of soluble solids produced per 24 h. Although the concentration of nectar can change quickly once it has been secreted (Corbet *et al.*, 1979), the relative composition of EFN of *M. tanarius* remains relatively constant even under field conditions. Combined measurements of nectar volume and concentration thus allow a reliable quantification of the amounts of secreted substances (method described and discussed in Heil *et al.*, 2000a). All nectaries present on four identically treated leaves of each of 10 plants per treatment were included in these experiments. Herbivory, artificially damaging leaves with a needle, and exogenous jasmonic acid (JA) application all increased nectar flow significantly, while controls by spraying of the solvent used to apply JA (water) and zero-controls (no treatment) elicited no response (Fig. 6; repeated measures ANOVA for the effect of treatments with leaf number as the within-subject variable:  $F_{(4,196)} = 9.849, P < 0.001$ ). The response of EFN flow to



**Fig. 6.** Induction of EFN secretion by *M. tanarius* via the octadecanoid pathway. The relative change in EFN flow 24 h after treatment is given as mean + standard deviation (sample size = 10 plants per treatment). Treatments were as follows. ‘Herbivory’: about 1.6% of the leaf area removed by three grasshoppers (*Xenocantops humulis*, Serville 1839) being placed on the experimental leaves. ‘Holes’: about 2% of leaf area damaged by punching the leaves 100 times with a needle. ‘JA’ 2.5 ml of a 1 mM aqueous solution of JA sprayed on the leaf surface. ‘Spray control’: 2.5 ml of water sprayed on the leaf surface. ‘Control’: zero control consisting of plants subjected to no other treatment than nectar removal itself. Different letters indicate significant differences between single treatments ( $P < 0.05$ , *post hoc* tests with Fishers’s least significant difference (LSD) following repeated measures ANOVA on treatment as the between-subject variable and leaf number as the within-subject variable). (Redrawn from Heil *et al.*, 2001c.)

leaf damage could be suppressed by the application of phenidone, an inhibitor of endogenous JA synthesis (Heil *et al.*, 2001c). Under laboratory conditions, artificial damage strongly enhanced endogenous JA concentrations which were quantified by GC-Trace-MS according to the methods described previously (Baldwin *et al.*, 1997; Koch *et al.*, 1999). Quantitative dose–response relationships were found between the increase in nectar production and both the intensity of leaf damage and the amounts of exogenously applied JA, and a similar relationship occurred between the amount of endogenously produced JA and the intensity of leaf damage (Heil *et al.*, 2001c). The numbers of defending insects appearing on the leaves under field conditions increased very soon after inducing EFN flow by the exogenous application of JA, while the numbers of herbivores decreased in the same experimental design (Fig. 7). In a 6-week study, the repeated induction of EFN flow by JA application or artificially damaging leaves resulted in a



**Fig. 7.** Effects of induced EFN secretion by the myrmecophilic *M. tanarius*. Levels of herbivore damage (percentage missing leaf area, mean + standard deviation) after a 6-week period are given for the three youngest leaves of each of 15 plants per treatment. These leaves had newly emerged during the experimental time span. While ‘Control’ plants remained untreated, EFN flow was induced on the other plants every 4 d either chemically by exogenous JA application (‘JA’), or by punching the leaves 100 times with a needle (‘Holes’). Inset: Numbers of insects (mean of 10 plants + SD) counted within 24 h after inducing EFN flow chemically by exogenous JA application. Numbers of ‘defenders’, nectary-visitors’ and of ‘herbivores’ appearing on the leaf blades were summed for the 12 single counts conducted for each plant. Open bars: untreated controls, dark bars: JA-induced. Sample size = 50 leaves (5 of each inserting on the same of 10 plants) per treatment, insects were counted every 2 h for 1 min per leaf and per census. Different letters appearing above or in bars indicate highly significant ( $P < 0.001$ ) differences between treatments (*post hoc* tests with Fishers’s least significant difference following repeated measures ANOVA in case of the experiment of herbivory, Mann-Whitney U tests for insect numbers). (All data from Heil *et al.*, 2001c.)

10-fold reduction in herbivory as compared to untreated controls (Fig. 7). These results demonstrate that EFN production represents a further mechanism for induced, indirect plant defences that are mediated via the octadecanoid signal transduction cascade.

### Conclusions and directions for further research

Many regulatory processes are controlling the production of ant food (FBs) or liquid ant rewards (EFN) by myrmecophytic and myrmecophilic *Macaranga* species. For example, EFN flow increases in response to leaf damage. This response is mediated via the octadecanoid pathway and can be elicited by mechanical leaf damage. By contrast, the factors regulating EFN secretion by *M. tanarius* and FB production by *M. triloba* according to the presence or absence of the respective consumers still have to be identified. Although very successful with respect to their defensive efficacy under field conditions, the strategies to nourish or attract ants or other insects which then act as indirect defensive agents seem to be costly in terms of investment of limited resources. All the mechanisms described can be interpreted as adaptations ensuring that the respective investments occur only under conditions actually requiring defence (EFN) or in the presence of defensive animals which consume the provided food (FBs and EFN). Few comparable studies have been conducted so far. A dependency of FB production by myrmecophytic *Cecropia* species on light and nutrient availability has been reported (Folgarait and Davidson, 1994, 1995), and a dependency of FB production on the presence of a symbiotic ant colony has been reported for *Piper* (Risch and Rickson, 1981). Comparative studies on different genera of ant plants would help to decide whether the regulatory mechanisms as described here are general traits of specialized ant plants or rather specific adaptations of the genus *Macaranga*.

In general, it is still under discussion whether the costs of defensive traits occur at all, and whether they play an important role in the evolution of defence. The present results indicate that these costs do indeed occur in the investigated system, and that several mechanisms have evolved to control and thereby reduce these costs whenever possible. Studies on other forms of defence support these findings (Fagerström, 1989; Gershenzon, 1994; Niemann *et al.*, 1992; Sagers and Coley, 1995; Simms and Rausher, 1989). Further studies on different plant species and different forms of defence should be conducted to determine whether defence in general causes allocation costs, whether and under which conditions these translate into fitness costs, and how these costs influence the plants' life histories and ecological properties.

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