

Martin Heil · Brigitte Fiala · Ulrich Maschwitz
K. Eduard Linsenmair

On benefits of indirect defence: short- and long-term studies of antiherbivore protection via mutualistic ants

Received: 13 March 2000 / Accepted: 6 September 2000 / Published online: 3 November 2000
© Springer-Verlag 2000

Abstract Many plants are defended indirectly by mutualistic animals. In this field study, we investigated the efficacy of indirect antiherbivore defence in symbiotic and non-symbiotic ant-plant associations in three *Macaranga* species. We tested whether obligate interactions are more effective than facultative ones by comparing ant-free plants, or parts of plants, with untreated controls. All three species gained significant protection from the ants' presence. The efficacy of defence was higher in the obligate associations represented by *M. triloba* and *M. hosei* than in the facultative interaction (*M. tanarius*). After 40 days of ant exclusion, missing leaf area amounted to 1.7% in *M. hosei* (compared to 0.2% in untreated, ant-defended controls), 2.6% in *M. triloba* (controls 1.2%) and 4.2% in *M. tanarius* (controls 3.2%). In a long-term study of *M. triloba* and *M. hosei*, ant protection was orders of magnitude higher than in the short-term results. Short-term experiments obviously are unsuited to obtaining a realistic picture of the long-term efficacy of antiherbivore defence. Within 1 year, ant-free plants lost, on average, between 70% (*M. hosei*) and 80% (*M. triloba*) of their total leaf area. Both species appear to require their mutualistic ants for survival. Defence via symbiotic ants is obviously a very effective form of antiherbivore protection. Ants are highly mobile and defend preferentially young, vulnerable leaves and shoot tips, and they fulfil several functions which normally have to be provided by different chemical substances. This may be a general benefit of indirect plant defence, which makes

use of “animal-specific” traits rather than intrinsic plant properties.

Keywords Ant-plant · Biotic defence · *Macaranga* · Mutualism · Herbivory

Introduction

Most field studies on antiherbivore defence suffer from the fact that a low damage level may result either from a low herbivore pressure or from an effective defence. These factors are difficult to separate experimentally, since most kinds of antiherbivore defence cannot be removed selectively from the plants. However, plants have evolved different mechanisms of defence. Direct defences (acting via toxins, digestibility reducers, repellents, or by morphological properties) can be distinguished from indirect mechanisms (also called “extrinsic” defence by Price 1986). These use interactions via the third trophic level, i.e., plants emphasise the suppressive effects of predators or parasitoids that attack herbivorous arthropods (Price et al. 1980; Baldwin and Preston 1999; Vet 1999). For example, “ant-plants” are predominantly defended by mutualistic ants. Ants represent a defence mechanism that can easily be removed from the plant. Such systems offer the opportunity to create plants with reduced defence under field conditions (McKey 1988).

Two strategies can be distinguished within defensive ant-plant interactions. Myrmecophytic plants (for example, in the genera *Acacia*, *Cecropia*, *Leonardoxa* and *Macaranga*) offer nesting space (so-called domatia) as well as ant food (extrafloral nectar or food bodies) which is specifically adapted to nourish ants (Heil et al. 1998). These plants are permanently inhabited by specialised ant colonies which protect their hosts, predominantly against herbivores and climbers (for reviews, see Buckley 1982; Beattie 1985; Davidson and McKey 1993). The mutualistic hypothesis (Belt 1874) has been supported by several studies comparing plants that were naturally ant-free with inhabited ones (e.g., Janzen 1972;

M. Heil (✉) · B. Fiala · K.E. Linsenmair
Zoologie III, Theodor-Boveri-Institut, Biozentrum, Am Hubland,
97074 Würzburg, Germany

U. Maschwitz
Zoologisches Institut der J.W. Goethe-Universität,
Siesmayerstr. 70, 60054 Frankfurt, Germany

Present address:

M. Heil, Centre d'Ecologie Fonctionnelle et Evolutive (CEFE, CNRS),
Route de Mende, 34293 Montpellier, Cédex 5, France,
e-mail: Martin_Heil@hotmail.com
Tel.: +49-931-8884378, Fax: +49-931-8884352

Letourneau 1983; McKey 1984; Fiala et al. 1989; Fowler 1993), and by experimental studies including the artificial removal of ants (Janzen 1967; Schupp 1986; Vasconcelos 1991; Fiala et al. 1994; Fonseca 1994; Gaume et al. 1997; Letourneau 1998; Gaume and McKey 1999). Myrmecophilic plants, in contrast, just attract ants from the vicinity by offering food, and they gain protection resulting from these ants' foraging behaviour. Especially in the case of plants with extrafloral nectaries, many studies have demonstrated the protective function of the attracted ants (for reviews, see Buckley 1982; Koptur 1992).

Though both phenomena are widespread and well-known, few studies have directly compared the outcomes of facultative, myrmecophilic with obligate, myrmecophytic ant-plant associations. These strategies differ in the plants' investments (Heil 1998; Heil et al. 1998) and in the specificity of the interaction (Fiala and Maschwitz 1990). Moreover, knowledge of the long-term effects of mutualistic ants in general is scarce. Several studies have demonstrated that short-term studies measuring only missing leaf area or leaf area loss over a short time span may not be suitable for estimating the herbivores' effects on plant fitness. Coley (1982), Lowman (1984, 1992) and Filip et al. (1995) demonstrated that the "real" leaf damage over the whole lifetime of the leaf can be much higher than that estimated from short-term experiments. Furthermore, defence may not be restricted to avoidance of leaf area loss.

We therefore compared the results of short-term experiments on leaf damage with those of long-term studies. Three *Macaranga* species were chosen that have evolved different types of interactions with ants, and two species were studied at three sites. We assumed that defence, despite being "indirect" in the sense of Price et al. (1980) or Baldwin and Preston (1999), should be more effective in the myrmecophytic interactions represented by *M. triloba* and *M. hosei* than in the myrmecophilic *M. tanarius*.

Materials and methods

Study site and species

The *Macaranga* species chosen for this study represent different types of interaction with ants: *M. triloba* (Bl.) Muell. Arg. is an obligate myrmecophyte, while *M. tanarius* (L.) Muell. Arg. has evolved only myrmecophilic interactions. *Macaranga hosei* King. ex Hook changes its strategy from myrmecophilic to myrmecophytic during its early ontogeny. For detailed information on the ecology of these species see Fiala and Maschwitz (1992a, 1992b) and Fiala et al. (1994, 1999). Species were determined according to Whitmore (1973), but *M. triloba* as described there probably must be re-named as *M. bancana* (T.C. Whitmore and S.J. Davies, personal communication; see also Slik 1998).

The study was carried out at the Ulu Gombak Field Studies Centre (UFSC) in Selangor, Peninsular Malaysia. The vicinity of the Studies Centre is covered by a secondary forest approximately 30 years old. Plants were chosen from four sites:

1. Site A: *roadside A*. This road crosses the forest at the UFSC. The roadway is about 6 m wide; the road verge between road-

way and forest edge is about 4 m wide and is mown regularly. At this site, several different *Macaranga* species can be found under the trees forming the forest edge, but *M. tanarius* clearly dominates the most isolated parts.

2. Site B: *logging path*, an approximately 3-m-wide trail in the forest that begins at the road (site A). The investigated plants were all growing less than 3 km from site A. Mainly myrmecophytic *Macaranga* species grow at this site, most of them in large clusters.
3. Site C: *isolated plants* growing along small paths and in gaps in the forest crossed by the logging path, about 5–10 km from the road (site A). We included only plants that were growing at least 100 m from the nearest conspecific.
4. Site D: *roadside D*. A road in the Genting Highlands; its main site characteristics are comparable to site A. However, this site is about 35 km away from the UFSC and at an elevation of 530 m above sea level (a.s.l.) (compared to 250 m a.s.l. at the UFSC).

Short-term experiments on herbivory

Preparatory experiments: the method of selective ant exclusion

Preparatory experiments were conducted from January to March 1994 to establish the method of selective ant exclusion and to check for detectable detrimental effects of Spruzit (Neudorff, Emmerthal, Germany), a neurotoxic pyrethroid insecticide used to remove ants from the experimental branches or plants. From five *M. triloba* plants, three branches per tree were chosen and their leaves were marked individually and divided into four age classes (age class 3, mature leaves which were already fully expanded and hardened; age class 2, unfolded but not yet hardened leaves; age class 1, leaves which were still unfolding; age class 0, leaves that emerged during the experiment).

The branches were treated in three ways:

1. *Treatment A*: untreated control.
2. *Treatment B*: ants in treatment B branches were poisoned by injecting a 1% solution of Spruzit into the domatia. The surviving ants of the colony were prevented from entering these branches by the application of a ring of sticky resin (Tangletrap, Tanglefoot Corp., Grand Rapids, Mich., USA) around the base of the branch and by injecting Tangletrap into the branch to block the domatium (Fig. 1).
3. *Treatment C*: in treatment C branches, ants were poisoned, holes were bored and Tangletrap was applied as in treatment A, but 3 days after the injection of Spruzit the surviving ants were allowed to enter these branches again by covering the Tangletrap ring with earth and small twigs.

The same treatments were conducted in 15 unbranched saplings arranged in five groups of three plants each (one devoted to treatment A, B and C, respectively). In these saplings, treatment C plants were connected to untreated, inhabited ones serving as a "source" for living ants which colonised them within a few days. The plants were monitored weekly for ant activity and occasionally for the presence of herbivorous insects. In treatment B, if ants were still present on the treated branches, ant removal and Spruzit injection were repeated once. After 40 days, leaves were harvested and dried to measure damage levels.

Measuring herbivory

Leaf damage was measured for each leaf using the computer program DIAS (Delta-T Devices, Cambridge, UK) and was expressed as percentage of missing leaf area. Leaves do not shrink by more than 3% during drying, so data from dried leaves give a reliable estimate of missing leaf area (M. Heil, unpublished work). Since leaves were marked individually and plants were visually monitored weekly, several leaves that were destroyed completely by herbivores or leaf suckers were observed: these leaves were included in the results with a damage level of 100%.

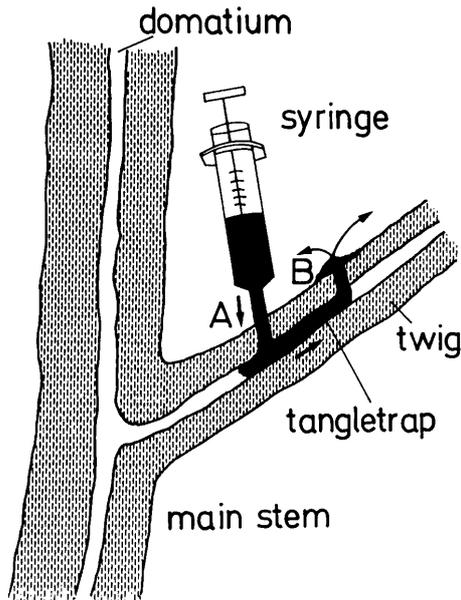


Fig. 1 Selective ant exclusion from experimental branches by injection of Tangletrap. To prevent surviving ants from the untreated plant parts entering the poisoned experimental branches, two holes were drilled into these branches about 5 cm apart, and Tangletrap was injected into one hole until it came out of the other

Experimental design

For the short-term experiment (August–October 1994), 28 *M. triloba* and 19 *M. hosei* trees and saplings were selected from sites B–D. Since *M. tanarius* is restricted to the roadside, it was not possible to compare different sites and all 36 individuals originated from site A. Overall, there were seven parts to the experiment (ST1–ST7) which used different numbers of branch pairs (bps): ST1: *M. triloba*, site B, 27 bps; ST2: *M. hosei*, site B, 31 bps; ST3: *M. tanarius*, site A, 49 bps; ST4: *M. triloba*, site C, 13 bps; ST5: *M. hosei*, site C, 6 bps; ST6: *M. triloba*, site D, 20 bps; ST7: *M. hosei*, site D, 12 bps. Each two neighbouring branches were regarded as a pair, and one branch per pair was selected randomly as the experimental branch. Leaves of experimental and control branches were assigned to age classes, and all young leaves (age classes 1 and 2) and the five youngest of the mature leaves were marked individually. Ants were removed from the experimental branches as described in treatment B in the preparatory experiments (Fig. 1), and for the next 40 days, plants were monitored weekly for ant activity. All pairs in which the experimental branch was recolonised by ants more than once were excluded from the experiment. After 40 days, all marked leaves and the younger ones which had emerged during the experiment (age class 0) were harvested from the remaining pairs of branches, dried, and their leaf damage was measured as described above.

Data analysis

Data on leaf area losses were, in general, not normally distributed. In addition to a high number of small values, a small number of high values up to 100% can occur. Furthermore, intrinsic patterns of leaf development mean that on each twig the four age classes may be represented by very different numbers of leaves. For these reasons, multifactorial ANOVA was not possible.

Box-whisker plots were chosen to present all data on herbivory. In the preliminary experiment, data were log-transformed to achieve normal distributions ($P > 0.10$, Kolmogorov–Smirnov test), and a unifactorial one-way ANOVA with a Scheffé post hoc multi-

ple comparison was conducted within each leaf age class, separately for unbranched saplings and branched twigs.

In the short-term experiment on herbivory, the statistical analysis of the results followed the pairwise experimental arrangement. A median was calculated for all leaves of a distinct branch which belonged to the same age class, and the two medians of the same-aged leaves of control and experimental branch forming an experimental pair were used as pairs in the Wilcoxon test for matched pairs. Since statistical analyses were conducted only within single age classes and were based directly on comparisons between leaves produced by neighbouring branches of the same tree, variations due to site effects or due to differences between individual trees did not influence the results. These data pairs consequently fulfil the requirements of the Wilcoxon test for matched pairs (H. Klinger, personal communication, see also Wilcoxon 1945; Sachs 1992). All statistical evaluations were conducted with SPSS for Windows 8.0 (SPSS Inc., Chicago, Ill., USA).

Long-term study in plant growth

Preparatory experiments

To check for any long-term effects of Spruzit on plant growth, 16 *M. triloba* saplings inhabited by ant colonies were protected against herbivorous insects by nylon-mesh field cages. Height and total leaf area were measured. Plants were arranged in pairs according to these two parameters, and one plant per pair was chosen randomly as the experimental plant. Over 2 months, a 5% solution of Spruzit (5 times the concentration used in the real experiment) was injected once per week into the domatia of the experimental plants, while the control plants received injections of pure water. At the end of the 2 months, plants were checked for visible effects (e.g., chlorosis, wilting leaves) and were measured again.

Experimental design and estimation of total plant leaf area

Two series of long-term experiments were conducted using different sets of plants, all growing along the logging path or in the adjacent forest (site B in the short-term experiments). The first experiment was carried out from March to August 1994 on 20 pairs of *M. triloba* with heights of 1.2–3.6 m. In the second experiment (September 1994–September 1995), 20 pairs of *M. triloba* and 15 pairs of *M. hosei* with heights of 1.0–5.3 m were used. In both *Macaranga* species, the area of a single leaf can be estimated by measuring its length and width and multiplying their product with the slope of a regression line based on data derived from 100 leaves. Both regressions have a $r^2 > 0.97$ (see Fig. 1 in Heil et al. 1997). To quantify the actual leaf area of whole trees, we measured length and width of the five youngest and every other of the older leaves from all branches and the main stem. Additionally, the age and actual damage level of these leaves were estimated, and their area was corrected by the missing leaf area. The areas of those leaves which were not measured directly were estimated as the mean of the two adjacent ones.

The initial total leaf area of a plant was estimated as the sum of all individual leaf areas, and trees were arranged in pairs according to height, total leaf area, and site: Trees forming a pair did not grow more than 30 m apart from each other and did not differ by more than 10% in their total leaf area. One tree per pair was chosen randomly as the experimental tree and ants were poisoned by injecting a 1% solution of Spruzit, while pure water was injected into the control tree. For the next 6 weeks, plants were monitored weekly for ant activity. In six *M. triloba* and in five *M. hosei* plants, Spruzit injection was repeated once since ants were still alive in some parts of the plants. During the 1-year experiment, monitoring as well as estimation of total leaf area was repeated after 6 months as described above. At this time, one or two twigs of each of four *M. triloba* and of three *M. hosei* plants were recolonised by ants and had to be poisoned a second time.

At the end of the experiment, monitoring was repeated, and all pairs whose experimental plant had been inhabited by ants more than once were excluded from the experiment. From the remaining

pairs, final total leaf area was estimated as described above, and a relative growth in total leaf area (ΔA) was calculated by relating the increase in total leaf area to the plants' initial value. If total leaf area of a plant increased, e.g., from 3,000 to 4,500 cm², ΔA of this plant would be 50%. If there was a loss of leaf area, ΔA could have values of down to -100%.

Results

Preparatory experiments

In neither preliminary experiment was there any visible influence of Spruzit on plant features such as leaf colour or viability. In the 40-day experiment, we found no significant difference in leaf damage between ant-protected controls (treatment A) and poisoned, but recolonised (treatment C) branches or plants within any leaf age class ($P > 0.05$ in all cases). We consequently exclude any detectable influence of Spruzit on *Macaranga* plants for this special experimental design. On the other hand, herbivory was significantly higher ($P < 0.01$) on the poisoned branches or plants (treatment B) as compared to the controls for the leaf age classes 0–2 in the unbranched saplings, and for age classes 0 and 1 in the branched trees. (all tests: unifactorial ANOVA within single age classes, Scheffé post hoc test, $n=5$ cases per treatment in each group; Fig. 2.)

In the 2-month experiment, concentration of Spruzit was 5 times higher and frequency of application was also much higher than in the long-term experiment. Nevertheless, there was no significant difference between experimental and control plants with respect to shoot growth and total leaf area (Mann-Whitney U -test: $P > 0.05$, $n=8$ plants each), and no visible effects such as chlorosis or wilting leaves were evident.

Short-term experiments on herbivory

Damage to leaves on ant-free branches was higher than on control branches in all three species investigated and at most, but not all, experimental sites. These differences were significant in all groups except the isolated *M. triloba* and *M. hosei* trees (Tables 1, 2, Fig. 3). Taking data from the different sites and all leaf age classes together, ant-free leaves of *M. triloba* had an average damage level of 2.6% after 40 days of ant exclusion (median, $n=318$ leaves). In untreated, and therefore protected, control leaves, on average only 1.2% of the area was missing ($n=312$). In *M. hosei* and *M. tanarius*, values for experimental leaves were 1.7% ($n=334$) and 4.2% ($n=401$), respectively, while the medians of the missing area of control leaves amounted to 0.2% ($n=335$) in *M. hosei* and 3.2% ($n=357$) in *M. tanarius*.

The ants' protective effect (estimated as difference between ant-free and control leaves) was much higher for younger leaves (Fig. 3, Table 1). When only those leaves which had emerged during the experiment (age class 0) are considered, leaf damage in *M. triloba* amounted to

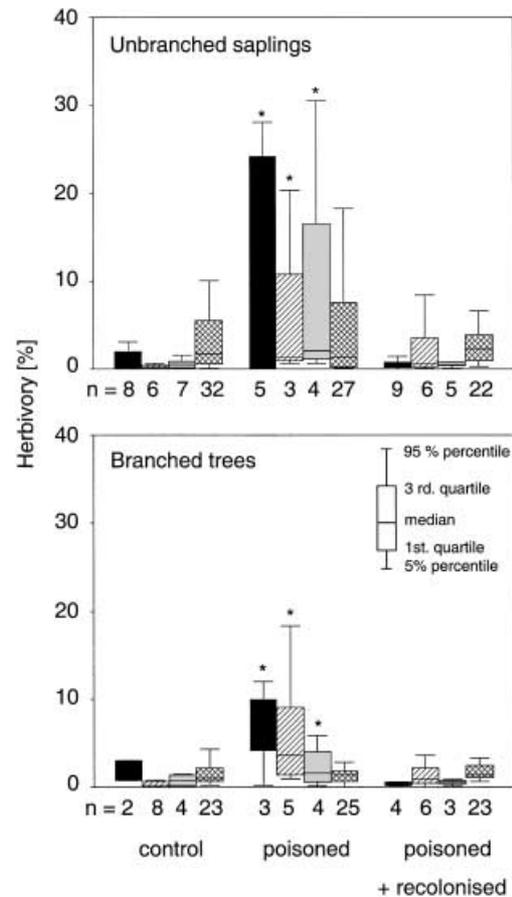


Fig. 2 Preparatory study on herbivory. Leaf area losses after 40 days for three different treatments in relation to leaf age class (solid bars class 0, shaded bars class 1, dotted bars class 2, hatched bars class 3). Sample sizes (n) appear below the bars. Five unbranched saplings were used per treatment in the first part of the experiment, and three branches from each of five plants in the second part. Asterisks indicate significant differences within single leaf age classes between leaves on twigs in which defending ants had been poisoned and the other two treatments ($P < 0.05$, unifactorial ANOVA and Scheffé post hoc test). See insert for explanation of box-whisker plots

1.4% in control and 4.2% in ant-free leaves (median of age class 0 leaves, $n=44$ and 31, respectively). In *M. hosei*, these values were 0.2% and 3.3% ($n=66$ and 66), and in *M. tanarius* 2.4% and 3.8% ($n=114$ and 133). Differences between treated and control leaves were significant ($P < 0.05$) for age class 0 leaves in all cases where Wilcoxon tests could be conducted (first column in Table 2), while leaves of age classes 2 and 3 showed no significant effect ($P > 0.05$) in *M. tanarius* plants (site A) and in *M. triloba* plants from site C (Table 2, last two columns). Age class 2 leaves of *M. triloba* further had no significant increase in herbivory at site B; the same was true for those of isolated *M. hosei* plants. Since sample numbers were much higher for older leaves, this disparity cannot result from different sample sizes.

The effect of ant defence varied strongly between the *Macaranga* species. At site A, the missing area of ant-free leaves of both *M. triloba* and *M. hosei* was at least

Table 1 Results of short-term experiments on herbivory. Medians, first and third quartiles of damage level (% missing leaf area after 40 days of ant exclusion) for controls (0) and ant-free branches (1) separately for all leaves and for the distinct leaf age classes (n values represent the number of leaves in each distinct group from which the medians and quartiles were calculated)

Part of experiment		Leaf age class							
		0		1		2		3	
		Treatment							
		0	1	0	1	0	1	0	1
ST 1 (<i>Macaranga triloba</i> , site B)	n (leaves)	26	15	25	30	24	19	82	91
	1st Quartile	0.3	2.1	0.2	0.8	0.5	0.8	0.7	0.9
	Median	1.3	4.0	0.5	2.1	0.8	1.8	1.4	2.2
	3rd Quartile	3.1	4.4	1.3	3.7	1.8	4.4	3.9	3.8
ST 2 (<i>M. hosei</i> , site B)	n (leaves)	52	57	35	38	39	43	117	116
	1st Quartile	0.0	1.1	0.0	0.7	0.1	0.3	0.1	0.3
	Median	0.2	3.3	0.1	2.5	0.3	1.6	0.4	1.5
	3rd Quartile	0.4	8.5	1.1	6.5	0.9	4.3	1.4	4.6
ST 3 (<i>M. tanarius</i> , site A)	n (leaves)	114	133	59	66	53	55	130	145
	1st Quartile	0.6	1.3	0.7	2.2	1.0	1.3	1.3	3.0
	Median	2.1	3.9	2.0	5.3	2.6	3.0	3.4	5.5
	3rd Quartile	4.2	14.9	4.6	9.8	5.5	11.0	6.8	10.7
ST 4 (<i>M. triloba</i> , site C)	n (leaves)	7	4	8	8	8	9	34	39
	1st Quartile	1.5	9.3	0.2	1.5	0.9	2.4	1.7	0.9
	Median	2.6	16.6	0.5	4.2	1.5	3.1	2.5	1.9
	3rd Quartile	3.4	18.9	19.1	18.9	4.4	6.4	6.1	6.3
ST 5 (<i>M. hosei</i> , site C)	n (leaves)	1	4	2	3	2	2	10	9
	1st Quartile	0.5	1.3	1.1	0.2	2.1	0.6	0.1	0.1
	Median	0.5	1.9	1.2	0.2	3.2	1.1	0.3	1.4
	3rd Quartile	0.5	3.7	1.3	1.4	4.2	1.6	1.0	2.4
ST 6 (<i>M. triloba</i> , site D)	n (leaves)	11	11	17	20	15	14	54	58
	1st Quartile	1.2	2.4	0.3	1.6	0.4	1.9	0.8	1.4
	Median	2.8	3.8	0.6	3.0	0.8	4.1	1.6	4.1
	3rd Quartile	5.0	6.5	3.6	4.3	1.4	16.9	3.2	11.5
ST 7 (<i>M. hosei</i> , site D)	n (leaves)	13	5	11	10	15	14	39	33
	1st Quartile	0.0	0.9	0.0	2.0	0.0	0.1	0.0	0.1
	Median	0.2	1.2	0.1	3.2	0.2	0.3	0.1	0.3
	3rd Quartile	0.8	37.1	0.4	5.4	0.6	2.4	0.4	1.2

Table 2 Results of Wilcoxon pair tests for matched pairs (P values, numbers of tested pairs in parentheses) in different parts (ST 1–7) in the short-term experiment on herbivory. No Wilcoxon test was conducted (– instead of P values) when sample numbers were too small ($n < 6$, see Sachs 1992)

Part of experiment (see Table 1)	Leaf age class			
	0	1	2	3
ST 1	0.007 (12)	0.016 (21)	0.092 (18)	0.043 (21)
ST 2	<0.001 (18)	0.002 (24)	0.001 (26)	0.008 (26)
ST 3	0.021 (25)	<0.001 (25)	0.230 (25)	0.147 (25)
ST 4	– (4)	0.384 (9)	0.069 (10)	0.084 (10)
ST 5	– (1)	– (2)	– (2)	– (2)
ST 6	0.046 (11)	0.027 (16)	<0.001 (18)	0.002 (18)
ST 7	– (5)	0.014 (6)	0.086 (7)	0.014 (7)

twice as high compared to protected control leaves. This remained true for all leaf age classes. In contrast, the missing area of *M. tanarius* leaves increased only from 2.1 to 3.9% in the youngest and from 3.4 to 5.5% in mature leaves when ants were kept off the plants (Table 1). On average, the ants' protective efficacy (as estimated by the relative increase in leaf damage in experimental compared to control leaves) amounted to 1.2 in *M. triloba*, 7.5 in *M. hosei* and 0.3 in *M. tanarius*, while the respective values calculated separately for the youngest leaves were 2.0, 15.5 and 0.5, respectively.

In *M. triloba*, unprotected leaves suffered most from herbivory in the Genting Highlands (site D) and less at the logging path (site B), while in *M. hosei*, absolute values were highest at site B and lowest at site D. However, in both species protected leaves suffered most from herbivory when growing on isolated plants (site C), although no differences in ant activity were observed on those plants (M. Heil, personal observations). No significant differences between protected and control leaves could be detected in *M. triloba* trees from site C (Table 2), since protected leaves also tended to show

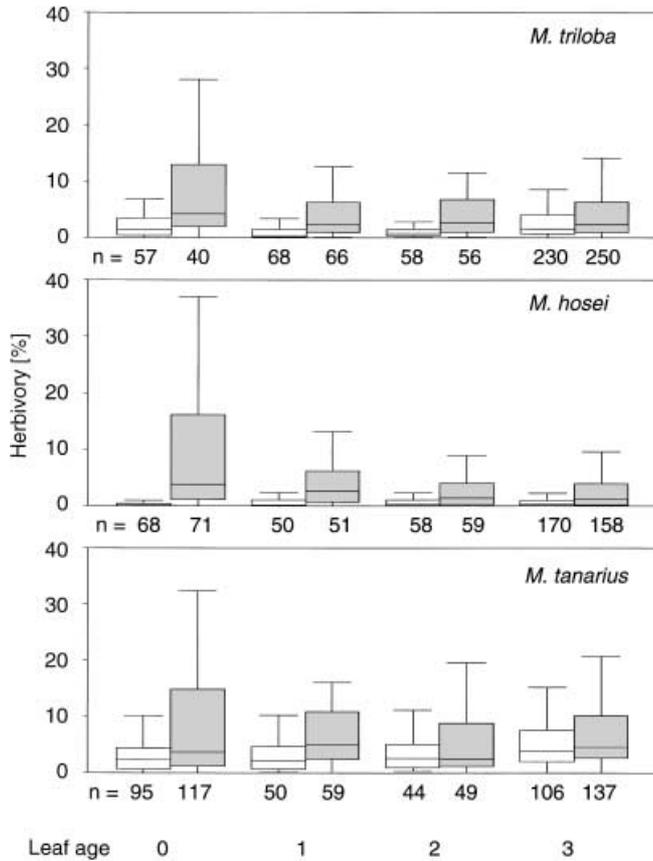


Fig. 3 Herbivory and ant defence in relation to leaf age. Herbivory (percent leaf area loss after 40 days) of leaves on ant-free (filled bars) and control (open bars) branches is given separately for the four leaf age classes. Leaves from all sites are combined for each species. Sample sizes (n) of leaves appear below the bars

higher damage levels than at the other sites. Consequently, the protective efficacy of ants was highest in the Genting Highlands (site D) and lowest in the isolated plants (site C) for both species. In spite of these differences, there were at least tendencies towards a protective effect at all sites, since control leaves had lower damage levels in all but three single comparisons. Only in isolated plants (site C) did some single leaf age classes show a reversed pattern (Table 1), and none of these was significant (Table 2). Since sample sizes were too different between the single leaf age classes and sites, no further statistical analyses on effects of these two factors could be conducted.

Long-term study in plant growth

In the 5-month study of *M. triloba*, the relative growth rate in total leaf area, ΔA , was significantly higher in the control group than in the plants that had been experimentally deprived of ants ($P < 0.001$, Wilcoxon pair test, $n = 19$ plant pairs). While 13 control plants showed an increase in total leaf area, all but one of the experimental plants had suffered from a decrease in leaf area, with

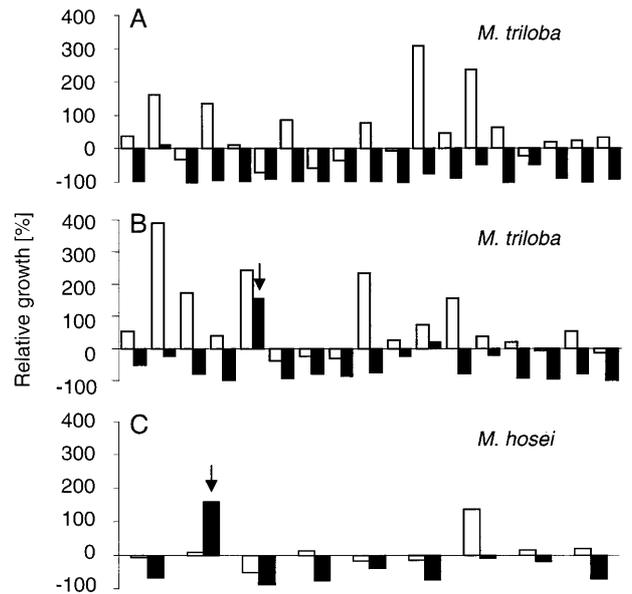


Fig. 4A–C Relative plant growth in the long-term study. Plant growth is calculated as relative change in total leaf area (ΔA) experienced by ant-free (filled bars) and by control plants (open bars). Values of the two plants forming a pair are plotted side by side. **A** Preparatory experiment on *Macaranga triloba* ($n = 19$ plant pairs). **B** 1-year study on *M. triloba* ($n = 17$ plant pairs). **C** 1-year experiment on *M. hosei*, only those pairs are shown in which both plants were still living at the end of the experiment ($n = 9$). Arrows indicate experimental plants which had been recolonised by ants during the study

several plants having lost nearly all their leaves (see Fig. 4A).

Similar effects were found in both species in the 1-year experiment (see Fig. 4B, C): In *M. triloba*, 17 of 20 plant pairs had survived this 1-year period. Only two experimental plants showed an increase in total leaf area, and one of these had been recolonised by ants during the experiment (see Fig. 4 B). The median of ΔA values was -78.8% in this plant group. On the other hand, 12 of the 17 control plants had a positive ΔA (median: $+40.0\%$), leading to a highly significant difference between these two plant groups ($P < 0.001$, Wilcoxon test, $n = 17$ pairs).

In *M. hosei*, the effect was less obvious due to low survivorship in both the control and the experimental group. Since the reason for the plants' death could not be detected for all plants (several had simply been cut by the local Orang Asli people), analysis of results was conducted only for those plant pairs where both plants survived (Fig. 4C). ΔA was 2.6% in the control group compared to -69.5% in the ant-free experimental group (medians, $n = 9$ plants each; $P < 0.01$, $n = 9$ plant pairs, Wilcoxon test).

In addition to the direct effects of leaf-chewing herbivores, severe damage was caused by shoot borers and pathogenic fungi, which killed whole twigs and, occasionally, also younger, still soft-wooden parts of the main shoot. Of the 17 ant-free *M. triloba* trees which survived the 1-year experiment 12 lost more than 50% of their total leaf area (Fig. 4); and nearly two-thirds (51 of 78) of

these trees' twigs died as a result of shoot borer activity and fungal infections. In *M. hosei*, 10 of 15 ant-free plants suffered from correspondingly severe damage. On these trees, on average 80% of the twigs and six of the ten shoot tips died during the experimental period. In contrast, a single twig was destroyed by shoot borer activity and pathogenic fungi on one ant-defended *M. triloba* control plant, and no shoot borer activity or serious fungal infections was observed on *M. hosei* control trees.

Discussion

The chosen insecticide, Spruzit, had no detectable effect on plant vitality and growth (Heil 1998) and did not affect our measure of herbivory, the missing leaf area (Fig. 2). The method of selective ant exclusion from distinct branches (Fig. 1) allows direct comparisons within individual trees. These results are not influenced by variance among plants caused by e.g. site and/or genetic differences. Therefore, we consider that our data give a quantitative estimate of the effects of the ants' presence and function in plant defence.

In the short-term study, leaves on ant-free twigs suffered more from herbivory than protected leaves (Table 1). This pattern occurred in 25 of 28 single comparisons made within leaves belonging to the same age class, species, and site. Of 22 possible, independent statistical tests within these single groups, 15 were significant ($P < 0.05$, see Table 2). The ants' protective effect differed between the three plant species depending on their special type of ant-plant interaction. When ants were excluded, the two obligate myrmecophytic species suffered from a much higher relative increase in leaf damage than did the myrmecophilic *M. tanarius*, which is visited by ants only facultatively (Fig. 3).

As in the case of *Piper* (Letourneau 1998), our short-term studies led to an underestimation of the long-term effects of ant defence. From the increase in leaf damage observed in the 40-day experiment one would hardly conclude that plants depend vitally on the ants' protective function, as was shown in the 1-year study (Fig. 4). Differences between data on leaf area losses derived from discrete sampling studies and data from studies that took into account the whole leaf life span have been reported (Coley 1982; Lowman 1984, 1992; Filip et al. 1995). However, in these studies the underestimation of herbivore-caused damage in discrete sampling was mostly due to overlooking completely eaten leaves. This does not apply to our study, since we could include these leaves. In both myrmecophytic *Macaranga* species studied, secondary effects such as pathogen infection contributed greatly to the damage detected in the 1-year study.

Ants as antiherbivore defence and plant fitness

The damage level in our short-term experiments was quite low. Even unprotected young leaves suffered from

lower leaf damage than expected according to the results of Coley and Barone (1996) for mean herbivore damage in young leaves of tropical forest trees. However, Coley (1983) has reported similar degrees of herbivory for another genus of ant-plants, *Cecropia*. In our long term studies, ant-free *Macaranga* plants suffered from a significant increase in both leaf damage and shoot damage, which made further survival of these plants very unlikely. Comparably severe effects of herbivory on ant-free myrmecophytes has been reported by Janzen (1967, 1974) for *Acacia* and Janzen (1972) for *Barteria*, by Vasconcelos (1991) for *Maieta*, and by McKey (1984) for *Leonardoxa*. Ferguson et al. (1995) reported significantly higher rates of mortality in ant-free *Cecropia* seedlings. Ants strongly decrease negative effects of herbivore attack and thus clearly represent an effective defence (*sensu* Karban and Baldwin 1997).

Direct observations of ant-free plants demonstrated that, besides folivores, insects feeding on shoot tissue were an important source of plant damage. Furthermore, ant-free plants suffered severely from pathogenic fungi. Recent studies have demonstrated that *Macaranga* plants have low chitinase activities and, thus, are not effectively defended against fungal pathogens, while the ants perform a direct and active defence against pathogenic fungi (Heil 1998; Heil et al. 1999). Correspondingly, Letourneau (1998) reported that, besides shoot borers, pathogenic fungi can have strong detrimental effects on ant-free myrmecophytic *Piper* plants. The importance of an effective defence against shoot borers was further demonstrated for *Endospermum* ant-plants (Letourneau and Barbosa 1999). In *Macaranga*, detrimental effects of pathogenic fungi or shoot borers become fully obvious only after a time span covering at least half a year. In the long run, the ants' protective function against these factors may be of much greater importance than is defence against folivores. More studies are needed to obtain an estimate of the relative importance of the ants' specific defensive actions against different attackers such as chewing herbivores, leaf miners, sap suckers, shoot borers, and pathogens.

Ants as an efficient mobile, indirect defence

Provision of food to mutualistic ants involves metabolic costs (O'Dowd 1979, 1980; Heil et al. 1997) and may depend strongly on abiotic factors such as nutrient availability and light (Folgarait and Davidson 1994, 1995; Heil et al., in press). Most theories on plant defence assume a positive relationship between the investment in and the efficacy of defence. This assumption is met, since an increased ant food production can lead to larger ant colonies in *M. triloba* plants (Heil et al., in press), and since larger ant colonies can be better defenders of their hosts (Duarte-Rocha and Godoy-Bergallo 1992; Heil et al., in press).

The optimal defence theory (McKey 1974, 1979; Rhoades 1979) predicts that defence should be mainly

concentrated on “valuable” plant parts such as, e.g., young shoots and leaves. Moreover, young leaves are more attractive to herbivores (Coley 1980; Kursar and Coley 1991; Coley and Barone 1996). In contrast, Herms and Mattson (1992) in their growth-differentiation balance hypothesis argued that young, developing plant parts may be hard to defend due to a metabolic competition between pathways involved in plant growth and differentiation processes such as the synthesis of defence chemicals. A spatial separation of the plant parts where defensive compounds are produced from those which must be defended would allow plants to meet the requirements formulated in the optimal defence theory and simultaneously to circumvent the restrictions as formulated in the growth-differentiation balance hypothesis (Cronin and Hay 1996).

Patrolling ants in general are concentrated on younger plant parts. This holds true for e.g. *Macaranga* (Fiala and Maschwitz 1990; Fiala et al. 1994), *Acacia* (Madden and Young 1992), *Barteria* (Janzen 1972), *Cecropia* (Downhower 1975), and *Leonardoxa* (McKey 1984). Furthermore, ants can concentrate quickly on plant parts where defence is actually required (Fiala and Maschwitz 1990) and thus may even be regarded as an induced response mechanism (Agrawal 1998; Agrawal and Dubin-Thaler 1999). Correspondingly, our short-term experiments revealed a much higher effect of the ants’ presence on young than on mature leaves (Table 1, Figs. 2, 3). On the other hand, food bodies in the two myrmecophytic *Macaranga* species are produced on specialised stipules, and highest production rates occur on stipules inserting beneath mature rather than beneath young leaves (Heil et al. 1997; Heil et al., in press). By using ants as defenders, plants can therefore effectively separate those parts where defence compounds (here: ant food) are produced from parts which require defence.

The high efficacy of ant defence may result from both the ants’ mobility and rapid “inducibility”, and from their relatively low specificity: ants are effective against many types of insects, climbers and pathogens. The ants in the obligate myrmecophytic interactions are much more closely adapted to their individual host, and many aspects of the overall defence (such as pruning of competing plants and the cleaning behaviour which leads to the removal of all foreign material from the plant surface) are clearly restricted to these associations, since they require that ants can distinguish between their specific host and “other” plants or materials. These behaviours add significantly to defence against insects and may lead to the higher efficacy of these as compared to the myrmecophilic interactions. Ants thereby fulfil several functions which normally have to be provided by a variety of different chemical substances. This may be a general benefit of indirect plant defence, which makes use of animal-specific traits rather than intrinsic plant properties.

Acknowledgements We thank the Economic Planning Unit (EPU) and Dr. Azaræ Hj. Idris (University of Malaya) for permission to work at the Ulu Gombak Field Studies Centre in Malaysia, and H. Gimmler (University of Würzburg) for the equipment for

measuring herbivory. Practical help by Birgit Fuchs, Laura Scott and Claudia Yahya, and statistical advice by H. Klinger (Dept. of Applied Statistics, University of Düsseldorf) are gratefully acknowledged. Many thanks also to Phyllis Coley, University of Utah, to Werner Kaiser, University of Würzburg, and to two anonymous referees for helpful comments on the manuscript. We are grateful for financial support by the Deutsche Forschungsgemeinschaft (Research Grant Li 150/13–1/3, and TP C8 in SFB 251). M.H. was also supported by grants from the DAAD (German Academic Exchange Service) and the Graduiertenkolleg “Arthropodenverhalten” (GRK 200).

References

- Agrawal AA (1998) Leaf damage and associated cues induce aggressive ant recruitment in a neotropical ant-plant. *Ecology* 79:2100–2112
- Agrawal AA, Dubin-Thaler BJ (1999) Induced responses to herbivory in the Neotropical ant-plant association between *Azteca* ants and *Cecropia* trees: response of ants to potential inducing cues. *Behav Ecol Sociobiol* 45:47–54
- Baldwin IT, Preston CA (1999) The eco-physiological complexity of plant responses to insect herbivores. *Planta* 208:137–145
- Beattie AJ (1985) The evolutionary ecology of ant-plant mutualisms. Cambridge University Press, Cambridge
- Belt T (1874) The naturalist in Nicaragua. Dent, London
- Buckley RC (1982) Ant-plant interactions: a world review. In: Buckley RC (ed) Ant-plant interactions in Australia. Junk, The Hague, pp 111–162
- Coley PD (1980) Effects of leaf age and plant life history patterns on herbivory. *Nature* 284:545–546
- Coley PD (1982) Rates of herbivory on different tropical trees. In: Leigh EJJ, Rand AS, Windsor DM (eds) The ecology of a tropical forest. Smithsonian Institution Press, Washington, pp 123–132
- Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol Monogr* 53:123–233
- Coley PD, Barone JA (1996) Herbivory and plant defenses in tropical forests. *Annu Rev Ecol Syst* 27:305–335
- Cronin G, Hay ME (1996) Within-plant variation in seaweed palatability and chemical defenses: optimal defense theory versus the growth-differentiation balance hypothesis. *Oecologia* 105:361–368
- Davidson DW, McKey D (1993) The evolutionary ecology of symbiotic ant-plant relationships. *J Hymenopt Res* 2:13–83
- Downhower JF (1975) The distribution of ants on *Cecropia* leaves. *Biotropica* 7:59–62
- Duarte-Rocha CF, Godoy-Bergallo H (1992) Bigger ant colonies reduce herbivory and herbivore residence time on leaves of an ant-plant: *Azteca muelleri* vs. *Coelomera ruficornis* on *Cecropia pachystachya*. *Oecologia* 91:249–252
- Ferguson BG, Boucher DH, Maribel Pizzi CR (1995) Recruitment and decay of a pulse of *Cecropia* in Nicaraguan rain forest damaged by hurricane Joan: relation to mutualism with *Azteca* ants. *Biotropica* 27:455–460
- Fiala B, Maschwitz U (1990) Studies on the South East Asian ant-plant association *Crematogaster borneensis*/*Macaranga*: adaptations of the ant partner. *Insectes Soc* 37:212–231
- Fiala B, Maschwitz U (1992a) Domatia as most important adaptations in the evolution of myrmecophytes in the paleotropical tree genus *Macaranga* (Euphorbiaceae). *Plant Syst Evol* 180:53–64
- Fiala B, Maschwitz U (1992b) Food bodies and their significance for obligate ant-associations in the tree genus *Macaranga* (Euphorbiaceae). *Bot J Linn Soc* 110:61–75
- Fiala B, Maschwitz U, Tho YP, Helbig AJ (1989) Studies of a South East Asian ant-plant association: protection of *Macaranga* trees by *Crematogaster borneensis*. *Oecologia* 79:463–470
- Fiala B, Grunsky H, Maschwitz U, Linsenmair KE (1994) Diversity of ant-plant interactions: protective efficacy in *Macaranga*

- species with different degrees of ant association. *Oecologia* 97:186–192
- Fiala B, Jakob A, Maschwitz U, Linsenmair KE (1999) Diversity, evolutionary specialisation and geographic distribution of a mutualistic ant-plant complex: *Macaranga* and *Crematogaster* in South East Asia. *Biol J Linn Soc* 66:305–331
- Filip V, Dirzo R, Maass JM, Sarukhan J (1995) Within- and among-year variation in the levels of herbivory on the foliage of trees from a Mexican deciduous forest. *Biotropica* 27:78–86
- Folgarait PJ, Davidson DW (1994) Antherbivore defenses of myrmecophytic *Cecropia* under different light regimes. *Oikos* 71:305–320
- Folgarait PJ, Davidson DW (1995) Myrmecophytic *Cecropia*: antiherbivore defenses under different nutrient treatments. *Oecologia* 104:189–206
- Fonseca CR (1994) Herbivory and the long-lived leaves of an Amazonian ant-tree. *J Ecol* 82:833–842
- Fowler HG (1993) Herbivory and assemblage structure of myrmecophytous understory plants and their associated ants in the central Amazon. *Insectes Soc* 40:137–145
- Gaume L, McKey D (1999) An ant-plant mutualism and its host-specific parasite: activity rhythms, young leaf patrolling, and effects on herbivores of two specialist plant-ants inhabiting the same myrmecophyte. *Oikos* 84:130–144
- Gaume L, McKey D, Anstett M-C (1997) Benefits conferred by 'timid' ants: active anti-herbivore protection of the rainforest tree *Leonardoxa africana* by the minute ant *Petalomyrmex phylax*. *Oecologia* 112:209–216
- Heil M (1998) Quantitative Kosten-Nutzen-Analyse verschiedener Ameisen-Pflanzen-Assoziationen innerhalb der Gattung *Macaranga*. Wissenschaft und Technik, Berlin
- Heil M, Fiala B, Linsenmair KE, Zotz G, Menke P, Maschwitz U (1997) Food body production in *Macaranga triloba* (Euphorbiaceae): a plant investment in anti-herbivore defence via mutualistic ant partners. *J Ecol* 85:847–861
- Heil M, Fiala B, Kaiser W, Linsenmair KE (1998) Chemical contents of *Macaranga* food bodies: adaptations to their role in ant attraction and nutrition. *Funct Ecol* 12:118–122
- Heil M, Fiala B, Boller T, Linsenmair KE (1999) Reduced chitinase activities in ant plants of the genus *Macaranga*. *Naturwissenschaften* 86:146–149
- Heil M, Hilpert A, Fiala B, Linsenmair KE (in press) Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant. *Oecologia* DOI s004420000534
- Hermes DA, Mattson WJ (1992) The dilemma of plants: to grow or to defend. *Q Rev Biol* 67:283–335
- Janzen DH (1967) Interaction of the bull's-horn acacia (*Acacia cornigera* L.) with an ant inhabitant (*Pseudomyrmex ferruginea* F. Smith) in eastern Mexico. *Kans Univ Sci Bull* 47:315–558
- Janzen DH (1972) Protection of *Barteria* (Passifloraceae) by *Pachysima* ants (Pseudomyrmecinae) in a Nigerian rain forest. *Ecology* 53:885–892
- Janzen DH (1974) Swollen-thorn acacias of Central America (Smithsonian Contributions to Botany 13). Smithsonian Institution Press, Washington, DC
- Karban R, Baldwin IT (1997) Induced responses to herbivory. University of Chicago Press, Chicago
- Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. In: Bernays EA (ed) *Insect-plant interactions*, vol IV. CRC, Boca Raton, pp 81–129
- Kursar TA, Coley PD (1991) Nitrogen content and expansion rate of young leaves of rain forest species: implications for herbivory. *Biotropica* 23:141–150
- Letourneau DK (1983) Passive aggression: an alternative hypothesis for the *Piper-Pheidole* association. *Oecologia* 60:122–126
- Letourneau DK (1998) Ants, stem-borers, and fungal pathogens: experimental tests of a fitness advantage in *Piper* ant-plants. *Ecology* 79:593–603
- Letourneau DK, Barbosa P (1999) Ants, stem borers and pubescence in *Endospermum* in Papua New Guinea. *Biotropica* 31:295–302
- Lowman MD (1984) An assessment of techniques for measuring herbivory: is rainforest defoliation more intense than we thought? *Biotropica* 16:264–268
- Lowman MD (1992) Herbivory in Australian rain forests, with particular reference to the canopies of *Doryphora sassafras* (Monimiaceae). *Biotropica* 24:263–272
- Madden D, Young TP (1992) Symbiotic ants as an alternative defense against giraffe herbivory in spinescent *Acacia drepanolobium*. *Oecologia* 91:235–238
- McKey D (1974) Adaptive patterns in alkaloid physiology. *Am Nat* 108:305–320
- McKey D (1979) The distribution of secondary compounds within plants. In: Rosenthal GA, Janzen DH (eds) *Herbivores: their interactions with secondary plant metabolites*, Academic Press, New York, pp 55–133
- McKey D (1984) Interaction of the ant-plant *Leonardoxa africana* (Caesalpinaceae) with its obligate inhabitants in a rainforest in Cameroon. *Biotropica* 16:81–99
- McKey D (1988) Promising new directions in the study of ant-plant mutualisms. In: Greuter W, Zimmer B (eds) *Proceedings of the XIV International Botanical Congress*. Koeltz, Königstein/Taunus, Germany, pp 335–355
- O'Dowd DJ (1979) Foliar nectar production and ant activity on a neotropical tree, *Ochroma pyramidale*. *Oecologia* 43:233–248
- O'Dowd DJ (1980) Pearl bodies of a neotropical tree, *Ochroma pyramidale*: ecological implications. *Am J Bot* 67:543–549
- Price PW (1986) Ecological aspects of host plant resistance and biological control: interactions among three trophic levels. In: Boethel DJ, Eikenbary RD (eds) *Interactions of plant resistance and parasitoids and predators of insects*, Horwood, Chichester, pp 11–30
- Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu Rev Ecol Syst* 11:41–65
- Rhoades DF (1979) Evolution of plant chemical defense against herbivores. In: Rosenthal GA, Janzen DH (eds) *Herbivores: their interaction with secondary plant metabolites*, Academic Press, New York, pp 4–53
- Sachs L (1992) *Angewandte Statistik*. Springer, Berlin Heidelberg New York
- Schupp EW (1986) *Azteca* protection of *Cecropia*: ant occupation benefits juvenile trees. *Oecologia* 70:379–385
- Slik JWF (1998) A key to the *Macaranga* Thou. and *Mallotus* Lour. (Euphorbiaceae) species of east Kalimantan (Indonesia). *Flora Malesiana Bull* 12:157–178
- Vasconcelos HL (1991) Mutualism between *Maieta guianensis* Aubl., a myrmecophytic melastome, and one of its ant inhabitants: ant protection against insect herbivores. *Oecologia* 87:295–298
- Vet LEM (1999) Evolutionary aspects of plant-carnivore interactions. In: Chadwick DJ, Goode JA (eds) *Insect-plant interactions and induced plant defence* (Novartis Foundation Symposium 223). Wiley, Chichester, pp 3–13
- Whitmore TC (1973) *Macaranga* Thou. In: Whitmore TC (ed) *Tree Flora of Malaya*, Longman, Kuala Lumpur, pp 105–113
- Wilcoxon F (1945) Individual comparisons by ranking methods. *Biometrics* 1:80–83