

Spatiotemporal patterns in indirect defence of a South-East Asian ant-plant support the optimal defence hypothesis

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(Accepted 2 October 2003)

Abstract: The optimal defence hypothesis predicts that plant parts characterized by a high value and/or a high risk of being attacked should exhibit the highest level of defence. We tested this hypothesis with *Macaranga bancana* ant-plants, which are protected efficiently by resident, mutualistic ants from herbivores, parasites and encroaching vegetation. Because cost-effective defence of the host by ants increases ant fitness, selection should act on ant behaviour to produce patterns of distribution of defence as predicted for direct chemical defence traits. Termites and pieces of tape were equally distributed over the uppermost ten leaves and over the leaf-bearing part of the stems (with termites mimicking a transient herbivore, while tape mimics a long-term stress caused by a climber or plant parasite). This arrangement allowed a separation of putative coevolutionary adaptations in the ants' behaviour from other potential sources of spatial patterns in ant defence, such as differences in herbivore pressure, in the vulnerability of different herbivores, or in direct plant defences. Ant activity dropped rapidly at termite baits, but remained high at tapes for at least 5 h, thereby demonstrating adaptive differential responses to the differences between the two stressors. Most importantly, ants preferentially defended young leaves and shoot parts. The temporal and spatial patterns of ant attention to exogenous stressors thus were clearly adaptive, varying with likely costs and benefits of defence as formulated in the optimal defence theory.

Key Words: ant-plant interaction, anti-herbivore defence, *Crematogaster*, *Macaranga bancana*, *Macaranga triloba*, mutualism, myrmecophytism

INTRODUCTION

Herbivore pressure is assumed to have significant fitness consequences, which vary depending on the part of a plant that is attacked. The optimal defence hypothesis (ODH) was originally formulated by McKey (1974) and was later extended (McKey 1979, Rhoades 1979) to answer the question: In what regions of the plant should the limited quantity of defensive compounds be concentrated? Costs of defence are defined as a reduction of plant fitness resulting from the expression of defensive traits under enemy-free conditions (Simms & Fritz 1990). Such costs might be caused, for example, by the allocation of limited resources to defensive traits (Herms & Mattson 1992), they limit a plant's overall metabolic investment in defensive traits. Therefore, it is not possible to maximally

defend all plant parts all the time. In the ODH it is assumed that adaptive strategies must underlie temporal and spatial patterns in allocation to defence, and it is predicted that defences should be most intensive in the most valuable and most vulnerable parts of a plant (McKey 1974, 1979; Rhoades 1979).

Although the ODH appears to be widely accepted, empirical tests have been plagued by several difficulties. (1) The assumption that defence is costly must be met for the defensive trait in question; (2) the spatial and temporal distribution of the defensive trait must be determined; and (3) the trait must have a defined defensive effect that can be quantified, preferably under realistic field conditions. The last prerequisite is particularly difficult to meet, since many defence traits serve several functions, thus being subject to different selective forces. Moreover, a low level of herbivory seen on a plant or a part of a plant under field conditions can result either from an effective defence or from limited herbivore pressure.

We used the ant-plant, *Macaranga bancana*, to test predictions of the ODH. *Macaranga bancana* is an obligate

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myrmecophyte that houses and nourishes specialized *Crematogaster* ants. The ants live in domatia (hollow twigs and shoots, see Fiala & Maschwitz 1992) and collect so-called food bodies, modified trichomes with high contents of lipid and protein that are specifically adapted to meet the ants' nutritional requirements (Heil *et al.* 1998). The ants effectively protect their host plant from herbivores (Fiala *et al.* 1989, Heil *et al.* 2001a), shoot borers and pathogenic fungi (Heil *et al.* 1999). The *M. bancana* – *Crematogaster* system fulfils all requirements needed for a test of ODH. (1) Providing nutrition for ants consumes limited resources (Heil *et al.* 2001b, 2002) and thus imposes relevant costs to the host plant (Heil *et al.* 1997). (2) The ants represent an effective defensive trait (Heil *et al.* 2001a). (3) The protective efficacy of the ants is correlated with their number (Heil *et al.* 2001b), the distribution of ants thus gives a direct estimate of the spatial distribution of defences.

Protective ant-plant mutualisms have repeatedly been used to test different theories on antiherbivore defence (for an overview see Heil & McKey 2003). A central assumption of these and the present study is that obligately plant-inhabiting ants are limited by resources provided by their host, and that they gain a fitness benefit when increasing their host's fitness, thus acting as a true 'defence' *sensu* Karban & Baldwin (1997). Final empirical evidence for these assumptions is missing for all ant-plant mutualisms, but several facts indicate that ants will obtain short-term benefits from living in a vigorous host plant. A limitation of plant-ants by nesting space has already been demonstrated (Fonseca 1993). Moreover, obligately plant-inhabiting ants such as the *Crematogaster* ants in *M. bancana* usually appear to receive all their food from their host plant and cannot survive outside a living host tree (Fiala & Maschwitz 1990), and their colony size can be increased by host plants producing more food bodies (Heil *et al.* 2001b, Itino *et al.* 2001). Ant-free *M. bancana* trees suffered from an average reduction by c. 80% in total leaf area and a dramatic dying of shoot tips and even whole branches after 1 y (Heil *et al.* 2001a). This reduction in photosynthetic area and available nesting space should have dramatic negative effects on resident ants, thus it is obvious that resident *Crematogaster* ants benefit from effectively protecting their host.

Herbivory to stems may be even more costly to the plant than destruction of the leaves that they bear. Protection from stem herbivores has been reported for *Pheidole* inhabiting Costa Rican *Piper* myrmecophytes (Letourneau 1998), for *Camponotus* in *Endospermum labios* in Papua New Guinea (Letourneau & Barbosa 1999), and for *Crematogaster* in *Macaranga bancana* in West Malaysia (Heil *et al.* 2001a). A major part of our experiment thus focused on the protection of shoots. In order to avoid influences from differences in the nature of attacking enemies, artificial mimics of plant enemies were chosen.

We used living termites fixed with a needle to mimic herbivores feeding on the plant (i.e. foreign insects currently causing a wound site), and textile tapes to mimic encroaching or epiphytic plants (i.e. foreign objects that are present for a longer time span and that do not wound the plant). The use of termites all from the same colony and of pieces of the same tape, placed at the same time on the youngest leaves or internodes, allowed us to separate adaptive patterns in the ants' response to enemies from all other putative influences (such as, for example, chemical or behavioural differences among attackers) on the spatiotemporal patterns of defence by ants.

METHODS

Plant material

Macaranga bancana (Miq.) Müll. Arg. grows as small trees mainly in secondary forests in South-East Asia. Plants were determined after Davies (2001), however, this species is referred to as *M. triloba* in earlier publications (Whitmore 1967, 1973). In Peninsular Malaysia, the plants are obligately inhabited by specific *Crematogaster* (*Decracrema*) ants (morphospecies no. 4, see Feldhaar *et al.* 2000, 2003 and Fiala *et al.* 1989 for detailed descriptions of the biology and taxonomy of the ants). In general, more than 99% of all plants > 10 cm are inhabited by ants, and the ants have never been observed to nest outside their *Macaranga* host trees (B. Fiala & H. Feldhaar, *pers. comm.*, and *pers. obs.* by MH). The present study was conducted in an abandoned rubber plantation close to Tamping in the state of Selangor, West Malaysia. This site was particularly rich in young *M. bancana* saplings. Unbranched plants 1.5–2.0 m high were selected, and were randomly assigned in groups of ten to the three different treatments. All experiments started in the morning (c. 10h00).

Preliminary experiments

Preliminary experiments were conducted to select suitable 'enemy mimics'. It was not possible to use living herbivores. The ants' responses differed strongly among different types of herbivores, and it was not possible to find enough herbivores belonging to a single species. Termites only placed on the plant surface elicited a reproducible response of the ants, yet the number of ants recruited to a termite was low as compared with that recruited to feeding herbivores. It turned out that the ants' behaviour and their quantitative response to a feeding (and vulnerable) herbivore was satisfyingly mimicked by a termite fixed with a needle (and therewith close to a wounding site).

Similarly, the ants' behaviour towards encroaching vegetation or any other material placed on the plant

surface differed depending on chemical and physical properties of the different materials. The typical behaviour to large pieces of foreign material that cannot be removed by a single worker is characterized by a comparably slow recruitment resulting in long-term activity of ants trying to remove the foreign material. It was convincingly elicited by placing textile tape on the plant surface (*pers. obs.* by DF and MH).

Experimental design

Small pieces (*c.* 2 cm²) of textile tape were placed on the ten uppermost (*i.e.* youngest) internodes in the 'tape' treatment, since the stem bears leaves only in this part. In the 'termite' treatment, termites (*Globitermes sulphureus*: Isoptera, Termitidae, determined following Bordereau *et al.* 1997 and Tho 1992) were fixed with a needle at the same internode positions of a further set of ten plants. Ants being present on the affected side of these internodes were counted after different time spans (at *t* = 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 300 min after fixing the baits in the 'termite' treatment, and at *t* = 10, 20, 30, 60, 90, 120, 150, 180, 210 and 300 min in the 'tape' treatment). On a third set of ten plants, pieces of tape were placed in the centre of the lower surfaces of the ten youngest totally unfolded leaves, and ants appearing on these surfaces were counted after the same time intervals as stated above. These data were used to define spatiotemporal patterns in the ants' response to the two different types of artificial enemies.

Statistical analysis

Individual plants were censused at different times, and different leaf or internode positions were censused at each time on each individual plant. Data analysis therefore was conducted by repeated-measures ANOVA to test for significant effects of organ position, or time, on the number of ants present at the enemy mimics. Ten plants had been used per treatment, and both time and organ position were used as within-subject variable. The different treatments were conducted on different days and using different plants (and – therefore – ant colonies), they were therefore analysed independently instead of using 'treatment' as a between-subject variable. 'Control' data on ant activity on plants without enemy mimics are included in the results. They are, however, omitted from the statistical analyses since untreated plants have been studied within 2 h before the start of the experiments, yet not at an exactly defined time before placing the enemy mimics. Untransformed data (assumption of sphericity tested in all cases) were analysed using the GLM procedure of SPSS (Version 10.1).

Table 1. Results of repeated-measures ANOVA on the effects of time and organ position on the number of ants present are presented for termites placed on internodes, tapes on internodes, and tapes on leaves. *n* = 10 plant individuals per treatment.

		Source	SS	<i>df</i>	<i>F</i>	<i>P</i>
Termite (Internodes)	Time		1806	13	4.74	< 0.001
	error		1904	65		
	Internode (position)		3527	9	7.43	< 0.001
	error		2372	45		
	Time × Internode		1635	117	1.14	NS
	error		7162	585		
Tape (Internodes)	Time		2304	9	7.78	< 0.001
	error		1490	45		
	Internode (position)		9806	9	6.93	< 0.001
	error		7076	45		
	Time × Internode		1831	81	2.27	< 0.001
	error		4026	405		
Tape (Leaves)	Time		228	9	0.37	NS
	error		2446	36		
	Leaf (position)		8149	9	17.6	< 0.001
	error		1855	36		
	Time × Leaf		821	81	0.60	NS
	error		5497	324		

RESULTS

Resident *Crematogaster* ants quickly detected both tapes and termites and actively removed both materials. Termites in general were successfully removed within 2 h, while it usually took *c.* 10 d for ants to remove the pieces of tape used in this experiment (MH, *pers. obs.*). The ants showed an effective recruiting system and reached maximum average numbers of 14 ants on one side of a particular internode (Figure 1). Both time and internode position had significant effects on the number of ants present at a given time on a given internode or leaf (Table 1). In all treatments, highest ant numbers were reached on the youngest internodes and leaves (Figure 2). However, the spatiotemporal pattern of the response clearly differed between the two types of artificial enemies and the two types of plant organs challenged in this study. On internodes, ants recruited much faster to termites than to tapes and reached highest numbers in the 'termite' treatment already after 15–30 min (Figure 2a), while numbers of ants recruited to tapes increased over more than 2 h (Figure 2b). At termites, the ant number then decreased, yet it remained continuously higher than at the beginning of the experiment on the young internodes, while it dropped to the level of the initial values on older internodes. In contrast, maximum numbers of ants at tapes remained more or less stable even after 5 h (Figure 2b). The spatial pattern of ants at termites was only slightly affected by time (no significant time × internode position interaction in the 'termite' treatment), while the spatial pattern in the 'tape' treatment, which was characterized by very high

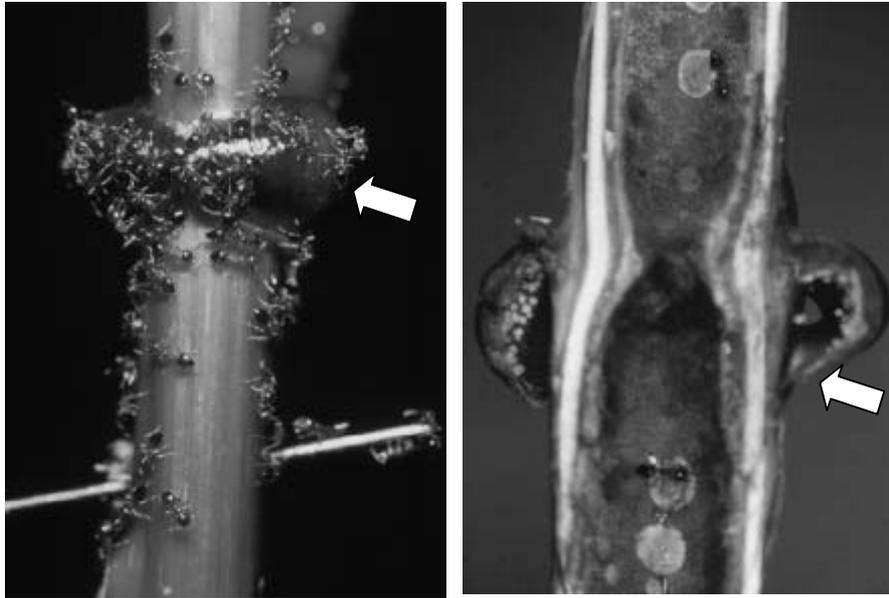


Figure 1. Ants recruited to a termite bait placed with a needle on an internode of *M. bancana*. White arrow: stipule (food bodies are produced on the inner surface). Right panel: Longitudinal cut, showing the domatium inside the twig, scale insects used by the ants as additional food source, and the food bodies produced under the stipule (white arrow).

numbers of ants appearing only on young internodes, developed over the observational time span, leading to a significant time \times internode interaction (Table 1).

The response of ants to tapes on leaves differed from the response to tapes on internodes in two ways. First, the number of ants depended on leaf position even more directly than on internode position and dropped very rapidly with increasing leaf age: old leaves received the lowest ant attendance among all bait–organ–position combinations tested (Figure 2c). Second, the highest numbers of ants on leaves were present after 10–20 min. The numbers then dropped slightly, but remained rather stable over the next 5 h. Leaf position had a highly significant effect on number of ants present at a particular piece of tape, while ‘time’ had no significant effect on ants present on tapes placed on leaf blades, and no significant time \times leaf position interaction could be detected in this case (Table 1).

DISCUSSION

We used ants that are specialized to defend their particular host plant and artificial mimics of plant enemies in order to investigate whether these ants’ defensive efforts are adapted to meet the predictions of the optimal defence hypothesis ODH (McKey 1974, 1979). Which parts of *M. bancana* plants are most valuable (and/or most vulnerable) and thus should be most intensively defended? The value of an individual leaf depends on the investment to build up this leaf and on its current and expected

metabolic contribution (Harper 1989). In *M. bancana* plants, leaves at position 2–4 contribute most to photosynthetic carbon fixation (Figure 3) and have an additional value in terms of expected future assimilation. On average *c.* 75% of all ants attacking tape on leaves were concentrated on the four youngest leaves (Figure 2c). The high intensity of defence by ants of the youngest leaves can be explained by their vulnerability (Coley 1983, Coley & Barone 1996) and by their value in terms of future carbon fixation and of resources already allocated to these leaves (Harper 1989). In contrast to leaves, the value of internodes is defined mainly by their leaf-supporting function and thus should not differ strongly among different-aged internodes, or it should even increase with internode age. However, the ODH is based on both the value and the vulnerability of different plant organs, and observations of ant-free *M. bancana* plants demonstrate that young (not yet lignified) internodes are subject to intensive attack by animals (in particular shoot borers) and pathogens (Heil *et al.* 1999, 2001a). Older, lignified internodes are seldom attacked and thus do not require defence by ants.

Spatiotemporal patterns in ant recruitment to the chosen enemy mimics were consistent with these predictions of the ODH: internode and leaf position significantly affected the number of ants present at a given time on a given plant part. Higher ant numbers were present on young than on old internodes and on young as compared with old leaves (Table 1). On the stems, ants recruited faster to termite baits (consisting of a wound caused by the needle and the presence of a foreign insect

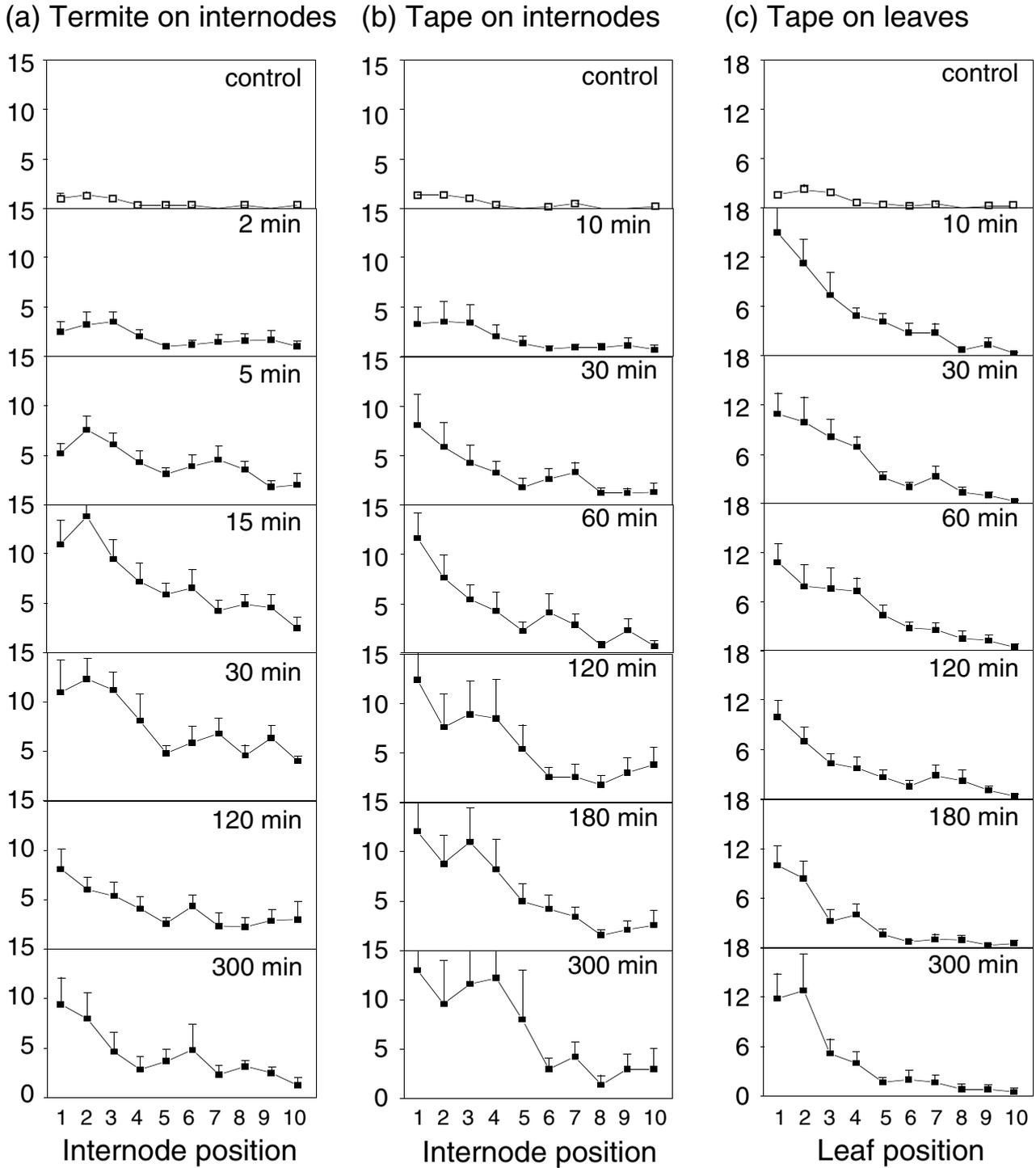


Figure 2. Spatiotemporal patterns in ant recruitment to termite baits (a) and pieces of tape (b) on internodes, and to pieces of tape on leaves (c), of *M. bancana* saplings. Mean ant numbers ($n = 10$ plants each) are presented for different times after placing the enemy mimic on the 10 youngest internodes, or leaves (No. 1 being the youngest, uppermost one). For reasons of clarity only a subset of censuses is displayed. Control: ant activity on untreated plants.

at this site, mimicking a feeding herbivore, see Figures 1, 2a) than to tapes (mimicking a continuously present foreign object such as an encroaching plant, see Figure 2b). This difference indicates that herbivores

currently feeding on a plant might elicit a faster response than do encroaching plants. Among tapes, those placed on leaves recruited ants more rapidly than those on internodes, but only tapes placed on very young leaves

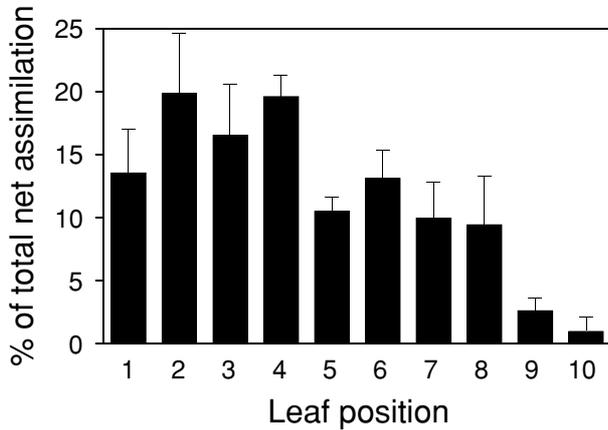


Figure 3. Relative contribution of leaves at different positions to a plant's net carbon gain (in per cent of total CO_2 fixation per 24 h). Gas exchange was measured for six *M. bancana* plants in the vicinity of the Ulu Gombak Field Studies Centre in Selangor, Malaysia, in 1996. We used a $\text{CO}_2/\text{H}_2\text{O}$ porometer system (CQP 130, Walz, Effeltrich, Germany) in an open-flow mode to quantify net CO_2 fixation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) repeatedly (once per leaf and per hour during day time) for the three youngest mature and every other of the older leaves (see Zotz, 2001, for a detailed description of methods). Assimilation rates of the non-measured of the older leaves were estimated as the average of the two adjacent ones, and a whole plant's net assimilation per 24 h was calculated from these data and the areas of the respective leaves (data from Heil 1998).

attracted high numbers of ants (Figure 2c). Owing to the easy removal of the termites, ant numbers decreased quite rapidly at the 'termite' sites (Figure 2a), while difficult-to-remove tapes led to a continuously high number of ants being present (Figure 2b, c). The experimental setup used here is a quite artificial one, since ten enemy mimics were placed at the same time on different parts of a plant. However, simultaneous attacks by enemies of different parts of the plants may happen also under natural conditions. Our data demonstrate that the pattern in defence by ants resulting when several enemies are present at the same time is neither a random one nor does it depend simply on differences among enemies, but rather it is strongly affected by the type and position of the organ under attack.

Many plant-ants patrol and preferentially defend the young leaves. The concentration of protection by ants to young leaves has been reported for *Piper* (Risch 1982), *Acacia* (Madden & Young 1992), *Tachigali* (Fonseca 1994), *Leonardoxa* (Brouat *et al.* 2000, Gaume & McKey 1999, McKey 1984), *Crypteronia* (Moog *et al.* 1998), and *Macaranga* (Heil *et al.* 2001a). In general, plant-ants thus form a defence mechanism that is spatially distributed according to the optimal defence hypothesis.

What are the proximate reasons leading to these (ultimately beneficial) distributions of ant defence? Spatiotemporal patterns in defending ants on a field-grown ant-plant could reflect patterns in different factors such as herbivore pressure or the production of plant-

derived food rewards being concentrated on the most valuable and vulnerable plant parts, rather than intrinsic adaptations in the ants' behaviour.

In our study, the use of artificial mimics allowed us to study patterns in the ants' defensive response independently of patterns in herbivore attack and of differences among enemies. Termite baits have often been used to compare the potential antiherbivore activity of ants on plants with and without extrafloral nectaries (Apple & Feener 2001, Barton 1986, Bentley 1981, Freitas & Oliveira 1996, Oliveira 1997, Oliveira *et al.* 1987) and are generally believed to reveal a realistic impression of ant recruitment patterns. By applying an equal spatial distribution of chemically identical foreign objects, we were able to determine whether ants show an optimal defensive behaviour even when they face different attackers at the same time.

The spatiotemporal activity patterns of *Pseudomyrmex veneficus*-ants inhabiting the myrmecophyte *Acacia hindsii* in Mexico obviously depended to a large degree on the provision of a food resource, extrafloral nectar (Raine *et al.* 2002). In our system, in contrast, the observed patterns were independent of the provisioning of food resources by the host plants, since food bodies are produced on the inner surface of stipules (Figure 1). Highest production rates are reached by stipules 3–5 (Heil *et al.* 1997), corresponding to internodes 4–6 in this study. In contrast, the highest defensive efficacy was observed on internodes 1 and 2. Very high numbers of defending ants were also observed on the lower surfaces of young leaves, although *M. bancana* produces a few tiny pearl bodies on its leaf blades (upper surface only). That obligate plant-ants preferentially patrol the young leaves of their host, although food rewards are either not produced at all or presented at spatially separated parts of the plant, has also been reported for myrmecophytes in the genera *Cecropia* (Downhower 1975) *Barteria* (Janzen 1972), and *Leonardoxa* (McKey 1984). Based on cost-benefit considerations, it has already been predicted that ants obligately inhabiting specialized ant-plants should recruit to those parts of their host that actually require defence without being guided by any short-term changes in the spatiotemporal patterns in resource provisioning by the host plant (Agrawal & Rutter 1998).

How are ants, then, able to distinguish among different-aged leaves, or internodes? Studies on other ant-plants have already demonstrated that chemical cues are likely to be involved in such patterns: *Azteca* ants on *Cecropia* myrmecophytes and *Macaranga*-inhabiting *Crematogaster* ants recruit to plant sap released at wound sites (Agrawal 1998, Fiala & Maschwitz 1990, Fiala *et al.* 1989), a response probably elicited by volatile compounds (Agrawal 1998, Brouat *et al.* 2000) that could differ among leaf age classes (Brouat *et al.* 2000). The most recent observations in this context have shown

that *Allomerus octoarticulatus* ants recruit intensively to damaged young leaves of their host tree, *Hirtella myrmecophila*, while damaged older leaves elicited no detectable response (G. Q. Romero *pers. comm.*). All these observations indicate that obligate plant-ants are behaviourally adapted to protect their host in a way that allows distribution of the limited defensive efforts of the ants in a spatiotemporal pattern as predicted by the optimal defence hypothesis. Relying on ants as an indirect defence mechanism allows the host plant to spatially separate parts where highest investment in defence is expended from parts actually requiring the most intensive defence, thereby fulfilling adaptive requirements as formulated in the optimal defence hypothesis without being affected by physiological 'growth-differentiation' trade-offs (Herms & Mattson 1992).

ACKNOWLEDGEMENTS

The EPU (Economic Planning Unit) gave permits to conduct field studies in Malaysia. We are grateful for many valuable comments by M. Rutter, D. McKey and an anonymous referee on earlier versions of the manuscript. Financial support by the Deutsche Forschungsgemeinschaft (TP C8, SFB 251 and grants He3169/1-1 and /1-2) and the Max-Planck-Gesellschaft, and much logistic assistance provided by the MAS (Malaysian Airline System) is gratefully acknowledged.

LITERATURE CITED

- AGRAWAL, A. A. 1998. Leaf damage and associated cues induce aggressive ant recruitment in a neotropical ant-plant. *Ecology* 79:2100–2112.
- AGRAWAL, A. A. & RUTTER, M. T. 1998. Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos* 83:227–236.
- APPLE, J. L. & FEENER, D. H. 2001. Ant visitation of extrafloral nectaries of *Passiflora*: the effects of nectary attributes and ant behavior on patterns in facultative ant-plant mutualisms. *Oecologia* 127:409–416.
- BARTON, A. M. 1986. Spatial variation in the effect of ants on an extrafloral nectary plant. *Ecology* 67:495–504.
- BENTLEY, B. L. 1981. Ants, extrafloral nectaries, and the vine life-form: an interaction. *Tropical Ecology* 22:127–133.
- BORDEREAU, C., ROBERT, A., VAN TUYEN, V. & PEPPUY, A. 1997. Suicidal defensive behaviour by frontal gland dehiscence in *Globitermes sulphureus* Haviland soldiers (Isoptera). *Insectes Sociaux* 44:289–296.
- BROUAT, C., MCKEY, D., BESSIÈRE, J.-M., PASCAL, L. & HOSSAERT-MCKEY, M. 2000. Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: preliminary results on *Leonardoxa* (Fabaceae: Caesalpinioideae) and *Petalomyrmex* (Formicidae: Formicinae). *Acta Oecologica* 21:349–357.
- COLEY, P. D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* 53:209–233.
- COLEY, P. D. & BARONE, J. A. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics* 27:305–335.
- DAVIES, S. J. 2001. Systematics of *Macaranga* sects. *Pachystemon* and *Pruinosae* (Euphorbiaceae). *Harvard Papers in Botany* 6:371–448.
- DOWNHOWER, J. F. 1975. The distribution of ants on *Cecropia* leaves. *Biotropica* 7:59–62.
- FELDHAAR, H., FIALA, B., BIN HASHIM, R. & MASCHWITZ, U. 2000. Maintaining an ant-plant symbiosis: secondary polygyny in the *Macaranga triloba*–*Crematogaster* sp association. *Naturwissenschaften* 87:408–411.
- FELDHAAR, H., FIALA, B., GADAU, J., MOHAMED, M. & MASCHWITZ, U. 2003. Molecular phylogeny of *Crematogaster* subgenus *Decacrema* ants (Hymenoptera: Formicidae) and the colonization of *Macaranga* (Euphorbiaceae) trees. *Molecular Phylogenetics and Evolution* 27:441–452.
- FIALA, B. & MASCHWITZ, U. 1990. Studies on the south east asian ant-plant association *Crematogaster borneensis*/*Macaranga*: adaptations of the ant partner. *Insectes Sociaux* 37:212–231.
- FIALA, B. & MASCHWITZ, U. 1992. Domatia as most important adaptations in the evolution of myrmecophytes in the paleotropical tree genus *Macaranga* (Euphorbiaceae). *Plant Systematics and Evolution* 180:53–64.
- FIALA, B., MASCHWITZ, U., THO, Y. P. & HELBIG, A. J. 1989. Studies of a South East Asian ant-plant association: protection of *Macaranga* trees by *Crematogaster borneensis*. *Oecologia* 79:463–470.
- FONSECA, C. R. 1993. Nesting space limits colony size of the plant-ant *Pseudomyrmex concolor*. *Oikos* 67:473–482.
- FONSECA, C. R. 1994. Herbivory and the long-lived leaves of an Amazonian ant-tree. *Journal of Ecology* 82:833–842.
- FREITAS, A. V. L. & OLIVEIRA, P. S. 1996. Ants as selective agents on herbivore biology: effects on the behaviour of a non-myrmecophilous butterfly. *Journal of Animal Ecology* 65:205–210.
- 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.
- HARPER, J. L. 1989. The value of a leaf. *Oecologia* 80:53–58.
- HEIL, M. 1998. *Quantitative Kosten-Nutzen-Analyse verschiedener Ameisen-Pflanzen-Assoziationen innerhalb der Gattung Macaranga*. Wissenschaft & Technik Verlag, Berlin. 187 pp.
- HEIL, M. & MCKEY, D. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology and Systematics* 34:425–453.
- HEIL, M., FIALA, B., LINSSENMAIR, K. E., 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. *Journal of Ecology* 85:847–861.

- HEIL, M., FIALA, B., KAISER, W. & LINSENMAIR, K. E. 1998. Chemical contents of *Macaranga* food bodies: adaptations to their role in ant attraction and nutrition. *Functional Ecology* 12:117–122.
- HEIL, M., FIALA, B., BOLLER, T. & LINSENMAIR, K. E. 1999. Reduced chitinase activities in ant plants of the genus *Macaranga*. *Naturwissenschaften* 86:146–149.
- HEIL, M., FIALA, B., MASCHWITZ, U. & LINSENMAIR, K. E. 2001a. On benefits of indirect defence: short- and long-term studies in anti-herbivore protection via mutualistic ants. *Oecologia* 126:395–403.
- HEIL, M., HILPERT, A., FIALA, B. & LINSENMAIR, K. E. 2001b. Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant. *Oecologia* 126:404–408.
- HEIL, M., HILPERT, A., FIALA, B., KAISER, W., BIN HASHIM, R., ZOTZ, G., STROHM, E. & LINSENMAIR, K. E. 2002. Nutrient allocation of *Macaranga triloba* ant plants to growth, photosynthesis, and indirect defence. *Functional Ecology* 16:475–483.
- HERMS, D. A. & MATTSON, W. J. 1992. The dilemma of plants: to grow or to defend. *Quarterly Review of Biology* 67:283–335.
- ITINO, T., ITIOKA, T., HATADA, A. & HAMID, A. A. 2001. Effects of food rewards offered by ant-plant *Macaranga* on the colony size of ants. *Ecological Research* 16:775–786.
- JANZEN, D. H. 1972. Protection of *Barteria* (Passifloraceae) by *Pachysima* ants (Pseudomyrmecinae) in a Nigerian rain forest. *Ecology* 53:885–892.
- KARBAN, R. & BALDWIN, I. T. 1997. *Induced responses to herbivory*. University of Chicago Press, Chicago and London. 319 pp.
- LETOURNEAU, D. K. 1998. Ants, stem-borers, and fungal pathogens: experimental tests of a fitness advantage in *Piper* ant-plants. *Ecology* 79:593–603.
- LETOURNEAU, D. K. & BARBOSA, P. 1999. Ants, stem borers and pubescence in *Endospermum* in Papua New Guinea. *Biotropica* 31:295–302.
- MADDEN, D. & YOUNG, T. P. 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. *American Naturalist* 108:305–320.
- MCKEY, D. 1979. The distribution of secondary compounds within plants. Pp. 55–133 in Rosenthal, G. A. & Janzen, D. H. (eds). *Herbivores: their interactions with secondary plant metabolites*. Academic Press, New York.
- MCKEY, D. 1984. Interaction of the ant-plant *Leonardoxa africana* (Caesalpiniaceae) with its obligate inhabitants in a rainforest in Cameroon. *Biotropica* 16:81–99.
- MOOG, J., DRUDE, T. & MASCHWITZ, U. 1998. Protective function of the plant-ant *Cladomyrma maschwitzi* to its host, *Crypteronia griffithii*, and the dissolution of the mutualism (Hymenoptera: Formicidae). *Sociobiology* 31:105–129.
- OLIVEIRA, P. S. 1997. The ecological function of extrafloral nectaries: herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Functional Ecology* 11:323–330.
- OLIVEIRA, P. S., DA SILVA, A. F. & MARTINS, A. B. 1987. Ant foraging on extrafloral nectaries of *Qualea grandiflora* (Vochysiaceae) in cerrado vegetation: ants as potential antiherbivore agents. *Oecologia* 74:228–230.
- RAINE, N. E., WILLMER, P. & STONE, G. N. 2002. Spatial structuring and floral avoidance behavior prevent ant-pollinator conflict in a Mexican ant-acacia. *Ecology* 83:3086–3096.
- RHOADES, D. F. 1979. Evolution of plant chemical defense against herbivores. Pp. 4–53 in Rosenthal, G. A. & Janzen, D. H. (eds). *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York.
- RISCH, S. 1982. How *Pheidole* ants help *Piper* plants *Brenesia* 19/20:545–548.
- SIMMS, E. L. & FRITZ, R. S. 1990. The ecology and evolution of host-plant resistance to insects. *Trends in Ecology and Evolution* 5:356–360.
- THO, Y. P. 1992. *Termites of Peninsular Malaysia*. Ampang Press, Kuala Lumpur. 224 pp.
- WHITMORE, T. C. 1967. Studies in *Macaranga*, an easy genus of Malayan wayside trees. *Malayan Nature Journal* 20:89–99.
- WHITMORE, T. C. 1973. *Macaranga* Thou. Pp. 105–113 in Whitmore, T. C. (ed.). *Tree flora of Malaya*. Longman, Kuala Lumpur.
- ZOTZ, G. 2001. The relationship between maximum *in situ* rates of CO₂ gas exchange and leaf carbon budgets in tropical gap plants of the genus *Macaranga*. *Flora* 196:364–369.