

Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*

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Summary

1. Many plants produce extrafloral nectar (EFN) to nourish ants and other animals which defend them against herbivores. We aimed to find reasons for the high variability in amounts of EFN produced by most plant species. We investigated the influence of several biotic and abiotic factors (time of day, leaf age, nectar removal and leaf damage) on secretion rates of EFN in the common south-east Asian pioneer tree species, *Macaranga tanarius* (L.) Muell. Arg.

2. In most experiments leaves were washed with pure water and bagged in nets to protect them against nectar-collecting insects, and nectar was collected and quantified 24 h later. Six soluble sugars and up to eight amino acids were detected in nectar samples derived from untreated, field-grown plants. Total amounts of soluble substances varied more than the relative composition of EFN.

3. Nectar secretion rates were highest on young, expanded leaves. A diurnal pattern with a secretion peak in the first 2 h after dusk was detected in the field. Nectar removal had a positive effect and its accumulation a negative effect on further EFN production. Artificial leaf damage (punching leaves with a needle or removing parts of the leaf blade with scissors) led to a significant induction of EFN production for the next 3 days.

4. Extrafloral nectar of *M. tanarius* was secreted in complex patterns influenced by different biotic and abiotic factors; its production appeared to be adapted temporally and spatially in order to ensure optimal use of invested resources.

Key-words: ant–plant interaction, Malaysia, mutualism, myrmecophily, plant–animal interaction

Functional Ecology (2000) **14**, 749–757

Introduction

Extrafloral (or extranuptial) nectaries are nectar secreting glands that are mostly located outside the flowers and are not involved in pollination (Bentley 1977a; Koptur 1992). They are known from many plants of at least 66 families (Elias 1983; Koptur 1992). Their ecological function has been controversial for decades, but many studies have shown that extrafloral nectar (EFN) can play an important role in a plant's defences against herbivores (for overviews see Buckley 1982; Koptur 1992). Given a choice, ants forage preferentially on plants bearing extrafloral nectaries (Barton 1986; Koptur, Rico-Gray & Palacios-Rios 1998; Oliveira, da Silva & Martins 1987), and several ant and wasp species directly defend the nectary-bearing plant parts against other insects (O'Dowd 1979). Both behaviours lead to fewer herbivorous insects on plants with extrafloral nectaries and thus to less damage by herbivores (Barton 1986; Koptur 1984; Koptur *et al.* 1998; O'Dowd 1979; Pickett & Clark 1979; Stephenson 1982). This

can result in increased fruit set (Barton 1986; Bentley 1977a; Oliveira *et al.* 1999; Stephenson 1982).

Plants that produce EFN differ in the quality and quantity of secreted nectar. Many studies on ecological and physiological aspects of EFN secretion have suffered from this high within- and between-plant variability, which is poorly understood. Our goal was to investigate whether different biotic and abiotic factors have a predictable influence on EFN secretion rates of the common south-east Asian pioneer tree *Macaranga tanarius* (L.) Muell. Arg. (Euphorbiaceae). *Macaranga tanarius* was chosen for this study as it is a well known and widespread tropical pioneer tree, and many previous studies have established the method of EFN measurements for this plant (Fiala & Maschwitz 1991; Fiala *et al.* 1994; Heil 1998). The extrafloral nectaries in this species are located on the upper parts of the leaf blade and can easily be manipulated. *Macaranga tanarius* shows no obvious specialization with respect to EFN secretion, and thus is probably representative of general patterns in EFN production.

We tested whether and to what extent selected biotic and abiotic factors contribute to the temporal and spatial patterns of EFN production by *M. tanarius*. We measured nectar volume and concentration to calculate amounts of soluble solids, and analysed the main components of EFN. Some experiments were conducted under field conditions, others in the greenhouse. Different experiments focused on the influence of leaf age, time of day, nectar accumulation or consumption and leaf damage on rates and patterns of EFN production. To our knowledge no comparative studies on secretion patterns of other EFN-producing plant species have been reported to date. The results reported so far by different authors on various plant species do, however, indicate that main traits of EFN secretion appear to be common for different plants. Detailed results obtained from one species can thus provide important hints on what to investigate in other species.

Materials and methods

STUDY SITE AND SPECIES

Macaranga tanarius plants were used in all experiments. Information on myrmecophilic ant-plant interactions within the genus *Macaranga* is provided by Fiala & Maschwitz (1991), Fiala *et al.* (1994) and Heil (1998). We concentrated on nectaries located on the leaf blade. Other glands occur on the leaf margin, but these were never found to secrete detectable amounts of sugar in the field (M. Heil, unpublished results).

The field experiments were conducted at the Ulu Gombak Field Studies Centre (UFSC) in Selangor, Peninsular Malaysia. All plants were unbranched saplings 1–2 m tall. Several seedlings were transported to Würzburg and were cultivated in a greenhouse (12 h night/day, 20 °C, 90% RH/28 °C, 60% RH). Plants received natural light supplemented with additional 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 4 h daily. They were watered daily and fertilized twice a week using a standard fertilizer solution. To quantify the plants' leaf areas we measured the length and width of all single leaves and multiplied their product by the slope of a regression line based on the area data from 100 leaves ($r^2 > 0.99$; Heil 1998).

NECTAR COLLECTION AND QUANTIFICATION

In the field, nectar had to be protected against rain and nectar-collecting insects. We therefore pruned all vegetation that had contact with the experimental plants and applied a ring of sticky resin (Tangletrap®, Tanglefoot Corp., USA) around the plants' stems to keep foraging ants off the plant. All leaves were washed with pure water to remove accumulated EFN, and leaves were then bagged into nets (mesh size 0.5 mm) to protect them against both flying insects and rain. In most cases, EFN was measured 24 h later. In the greenhouse studies with no nectar-collecting insects

present, leaves were washed with water 24 h before measurements, but were not bagged.

All nectar collections (apart from those focused on short-term secretion patterns) were conducted in the first 3 h after dawn (between 08 : 00 and 11 : 00 h). Extrafloral nectar was removed with 5 μl micropipettes (graduated by 1 μl divisions) that allowed a direct quantification of nectar volume. Nectar concentration was measured immediately upon its removal as concentration of soluble solids with a portable, temperature-compensated refractometer (ATAGO hand refractometer, L. Kübler, Karlsruhe, Germany). To remove EFN quantitatively, 5 μl pure water was then applied on all nectaries. The resulting solution was removed and measured as described above, and the whole procedure was repeated up to five times until it had concentrations <1%. Values from all collections conducted for the nectaries on one leaf were summed to quantify a leaf's overall production of solid EFN compounds; see Stephenson (1982) and Pyke (1991) for details concerning this method.

CHEMICAL COMPOSITION

Nectar samples for chemical analyses were collected from seven plants in the field (March 1994), absorbed on Whatman No. 1 chromatography paper (Baker & Baker 1976; Baker, Opler & Baker 1978), and dried immediately with silica gel. These dry nectar compounds were later dissolved in distilled water and cleared by micromembrane filtration (Spartan 30/B filters, 0.45 μm , Schleicher & Schuell, Germany). Three parallel measurements per sample were conducted. Sugars were separated by isocratic (0.1 N NaOH) HPLC on an anion-exchange column and quantified by pulsed amperometric detection (Dionex Series 4500 Chromatography System, Dionex, Idstein, Germany). Amino acids were separated and measured with an Amino Acid Analyser LC 5001 (Biotronic, Maintal, Germany). Results of analyses were related to the initial volume of nectar collected in order to calculate EFN concentrations.

TIME COURSE OF EFN SECRETION

Nectar was collected repeatedly over 24 h from two groups of plants in the field to determine whether EFN production rates depended on the time of day. The first plant group was examined over 3 days starting on 15 October 1995, at the beginning of the rainy season. Four plants were protected with nets and Tangletrap, and leaves were washed on the first day at 07 : 00 h. Nectar was collected at 11 : 00, 15 : 00, 19 : 00, 23 : 00 and 07 : 00 h on the next day. This temporal pattern of measurements was continued for another 2 days, and mean hourly nectar production rates were calculated for the elapsed 4 or 8 h. A similar study was conducted for three other plants on 20 March 1996 (dry season) with collection intervals of 3 or 6 h.

LEAF AGE AND EFN SECRETION

The intensity of different forms of mechanical and chemical defence depends strongly on leaf developmental stage. To prove whether EFN production depends on leaf age, the production rates of all leaves of 17 plants were measured in the field in March 1994. Prior to nectar collection, leaves were divided into leaf age classes by visual criteria: class 1 represented the young, still unfolding leaves; classes 2 and 3 those which were already mostly (2) or totally (3) unfolded, but not yet hardened. Classes 4–6 comprised all fully unfolded and hardened leaves (divided into three groups according to their insertion sequence at the stem), and class 7 senescent leaves. These leaf age classes differ from each other with respect to N and C concentrations, photosynthetic rates and food body production rates (Heil 1998 and unpublished results).

NECTAR ACCUMULATION AND CONSUMPTION

Accumulation of floral nectar can suppress further nectar secretion (Gill 1988; Pyke 1991). To determine whether this holds true for EFN secretion of *M. tanarius*, three plants growing in the field were washed and protected against all insects, and EFN production measured once every 24 h for 4 days. On the fifth day nets were removed and plants were connected to the surrounding vegetation for 24 h. During this period ants as well as other insects visited the nectaries (personal observation). Afterwards, EFN was removed by washing the leaves which then were bagged in nets again to measure EFN production 24 h later. This temporal pattern of EFN accumulation and removal was repeated twice. To control for environmental influences and long-term patterns in EFN secretion, four more plants growing in the vicinity of the experimental plants were protected against insects for 1 week, and EFN secretion was measured once a day.

In a second study, nectar production rates of 13 plants in the greenhouse were measured once by a single nectar collection 24 h after washing the plants. Over the next 5 days, EFN production rates were measured again for the same plants by collecting EFN at 3 h intervals for 24 h. Total EFN production per 24 h was calculated and compared between the two collection designs.

LEAF DAMAGE

Extrafloral nectar has been thought to be induced by herbivory. Natural or artificial leaf damage should therefore increase EFN secretion rates. We applied two different forms of artificial leaf damage to test this possibility for *M. tanarius*. In March and April 1998, a total of 60 plants in the field were assigned to 20 groups of three plants, all characterized by total

leaf areas differing by less than 10%. Before the experiment the plants' daily EFN production rates were measured from the four youngest of the totally unfolded leaves. Plants were assigned randomly to one of three treatments. In treatment A, ≈40% of the blades of leaves 2–4 were removed with scissors. In treatment B, the blades of leaves 2–4 were punctured 100 times using a needle. These damage levels were chosen to resemble severe natural damage caused by beetles, grasshoppers and stick insects, which can be seen on *M. tanarius* plants from which defending ants are removed for several weeks (personal observation). In treatment C (control) all leaves remained untreated, as did the youngest leaves (class 1) in all treatments. Immediately after damaging, leaves were washed and bagged, and EFN production was measured 24 h later. Nets were removed for 1 day and then replaced on the plants to measure EFN production again.

DATA ANALYSIS

Statistical tests followed Sokal & Rohlf (1981) and Sachs (1992). Several leaves from most plants were used in the experiments, and in some studies the same plants were measured on several days. Repeated-measures ANOVA designs (GLM procedure in SPSS), with leaf position and day of collection as within-subject variables, were used for data evaluation whenever possible. As the data were not normally distributed and homogeneity of variances and sphericity could not be assumed in most cases, corrections according to Huyn-Feldt's epsilon were conducted, and *post hoc* tests followed Games-Howell. Statistical calculations were conducted with SPSS for Windows 8.0 (SPSS Inc., Chicago, IL, USA).

Results

CHEMICAL COMPOSITION

Six soluble sugars and eight amino acids were detected in EFN samples (Table 1). Fructose, glucose and sucrose dominated the sugar fraction, while methionine, serine and glycine dominated the fraction of soluble amino acids (Table 1). Total soluble solids differed by up to 17-fold in sugar concentration and >30-fold with respect to amino acids. The largest amounts detected were 66 g sugars l⁻¹ and 0.6 g amino acids l⁻¹. These were measured in a nectar sample from the same plant (Table 1). The relative composition of nectars differed less than the absolute concentrations. For example, the relative proportion of the dominant sugar, fructose, varied only between 43 and 56%, and the relative proportion of the dominant amino acid, methionine, varied approximately twofold, from 14 to 28% (Table 1). Nectar production thus varied with respect to absolute production rates (or concentrations of nectar present *in situ*) more than with regard to the relative chemical composition of nectar.

Table 1. Concentrations (mean and standard deviation, SD, and minima and maxima) of single soluble sugars and amino acids as well as relative sugar and amino acid concentrations in extrafloral nectar from seven *M. tanarius* plants in the field

	Absolute concentration*				Relative concentration (% of total)			
	mean	SD	min	max	mean	SD	min	max
Sugars								
Fructose	64.4	41.5	11.3	132.6	50.7	6.0	55.9	43.0
Glucose	44.2	34.0	6.4	105.6	32.0	1.8	31.7	34.3
Sucrose	23.6	23.3	1.6	66.4	14.4	7.1	7.9	21.6
Mannitol	0.6	0.6	0.5	1.9	0.5	0.8	2.5	0.6
Sugar 'F' (unidentified)	2.3	2.1	0.3	1.3	2.1	1.7	1.5	0.4
Arabinose	0.4	0.3	0.1	0.3	0.3	0.2	0.5	0.1
Total (g l ⁻¹)	28.5	21.2	3.9	66.4				
Amino acids								
Methionine	380	248	47	784	25	10	28	14
Serine	395	400	45	1217	27	7	27	22
Glycine	311	284	36	878	18	5	28	16
Cysteine	222	147	21	441	14	5	18	8
Ornithine	342	679	0	1798	11	16	0	33
Isoleucine	65	114	0	0	6	10	0	0
Alanine	93	141	17	386	4	6	10	7
Phenylalanine	23	62	0	0	1	3	0	0
Total (mg l ⁻¹)	200	168	19	637				

*Sugars, mmol l⁻¹; amino acids, μmol l⁻¹.

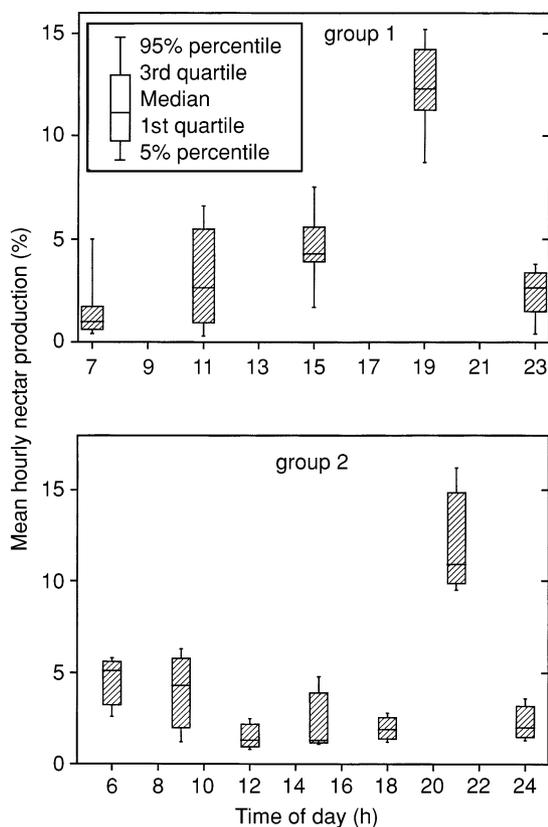


Fig. 1. Diurnal patterns of extrafloral nectar (EFN) production. Mean hourly nectar production rates (as a percentage of total daily EFN production) were calculated for each plant from data obtained at various collection times. Group 1, production measured on 15–18 October 1995, *n* = four plants each measured two times. Group 2, production measured on 20 March 1996, *n* = three plants.

Table 2. Influence of time of day on EFN production in the field. Repeated-measures ANOVA for mean relative nectar production (percentage EFN production per hour, cf. Figure 1) with time of nectar collection as within-subject factor. Five collections per day were conducted for the first set of four plants, seven collections were conducted for the three plants in the second field study

Plant group	Source	SS	df	<i>F</i>	<i>P</i>
1 (field, <i>n</i> = 4)	Time of day	246.342	4	11.464	<0.001
	Error	64.464	12		
2 (field, <i>n</i> = 3)	Time of day	247.888	6	8.468	0.001
	Error	58.550	12		

TIME COURSE OF EFN SECRETION

The two field studies revealed similar diurnal patterns in relative EFN secretion (Fig. 1). Relative secretion remained rather constant over most of the day but increased sharply at dusk. Time of day was a significant source of variation of mean hourly nectar secretion rates (Table 2).

LEAF AGE

Nectar secretion rates depended strongly on leaf age. Most nectar was produced by totally unfolded, but not yet hardened young leaves (age class 3; Fig. 2). The leaves of classes 2–4 effectively produced all the EFN in *M. tanarius*, while no EFN was found on any of the leaves of age classes 1, 6 and 7 (Fig. 2).

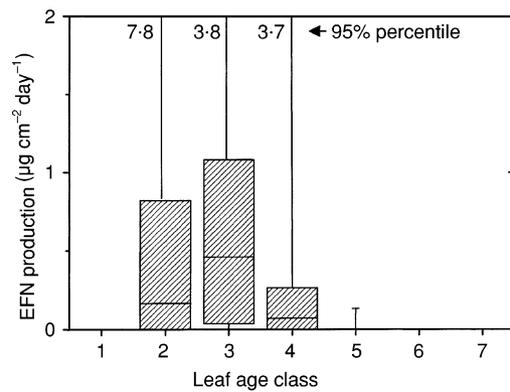


Fig. 2. Dependence of extrafloral nectar (EFN) production on leaf age. The EFN production rates measured after one 24 h interval of nectar accumulation are given separately for the seven leaf age classes ($n = 17$ plants). See Fig. 1 for explanation of box-whisker plots. Three 95% percentile values are given as figures.

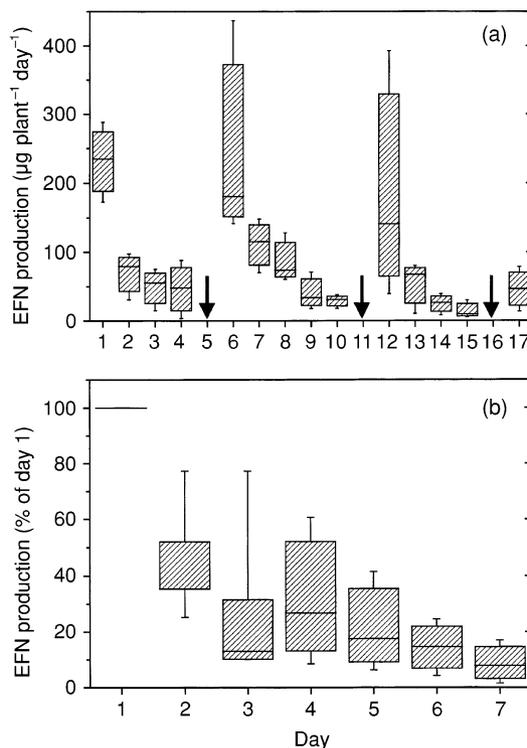


Fig. 3. Changes in extrafloral nectar (EFN) production according to presence or absence of insects. Daily EFN production rates are given for three plants in the field from which insects were kept off for several days. (a) Data (μg sucrose equivalents $\text{plant}^{-1} \text{day}^{-1}$) from three plants to which insects had access on days 5, 11 and 16. Arrows indicate days on which insects had access to the plants for 24 h. (b) Data (as percentage of EFN production rate measured on day 1) from four plants protected against insects for a whole week. See Fig. 1 for explanation of box-whisker plots.

NECTAR ACCUMULATION AND CONSUMPTION

Plants from which nectar was removed daily over several days showed a decrease in nectar production (Fig. 3). After nectar-consuming insects had had access to the three plants of one group for 1 day, nectar production

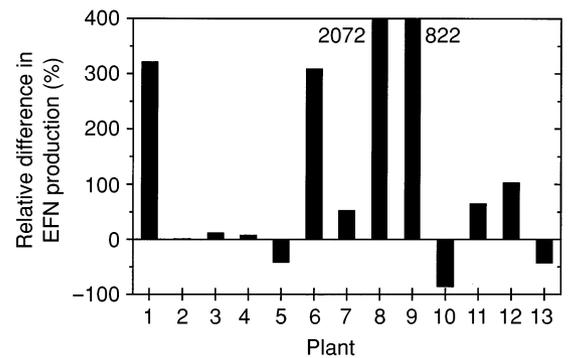


Fig. 4. Change in extrafloral nectar (EFN) production after increased frequency of EFN removal. The EFN was removed from 13 greenhouse-grown plants once after 24 h and (within the next 3–5 days) once again at 3 h intervals, covering a total of 24 h. For each plant, the relative increase in EFN production in the latter treatment compared to the first collection was calculated. Two large out-of-scale values are given as figures.

Table 3. Influence of two forms of leaf damage on EFN production. Sources of variance in the absolute difference of EFN production measured 1 and 3 days after treatment as compared to the control (measured immediately before the treatment) were tested by repeated-measures ANOVA with day of measurement (day) and leaf position (leaf) as within-subject factor and treatment as between-subject factor. No sphericity could be assumed ($P < 0.05$, Mauchly's test), results were therefore corrected based on the Huyn-Feldt epsilon

Source	SS	df	<i>F</i>	<i>P</i>
Within-subject effects				
Leaf	296615.5	2.5	1.504	ns
Leaf \times treatment	718471.7	4.9	1.822	ns
Error (leaf)	10648865.9	132.9		
Day	24783.8	1.0	0.703	ns
Day \times treatment	14449.5	2.0	0.231	ns
Error (day)	1687924.7	54.0		
Leaf \times day	17493.6	2.5	0.325	ns
Leaf \times day \times treatment	70520.4	4.9	0.655	ns
Error (leaf \times day)	2906309.5	133.3		
Between-subject effects				
Treatment	934153.7	2	5.575	0.006
Error	4524040.2	54		

again increased in all three plants. This pattern was repeatable (Fig. 3a). The four other plants to which insects had no access over 1 week failed to reveal any marked increase in EFN production over this time span (Fig. 3b). In the second experiment, plants produced on average 2.5 times more nectar when EFN was removed at 3 h intervals (mean difference compared to one collection after 24 h: 256%, $n = 13$ plants). While 10 plants produced more EFN when nectar was removed repeatedly, only three plants showed the reverse pattern (Fig. 4).

LEAF DAMAGE

Artificial leaf damage was a significant source of variance in EFN production 1 and 3 days after the treatment compared to pretreatment production ($P < 0.01$, Table 3).

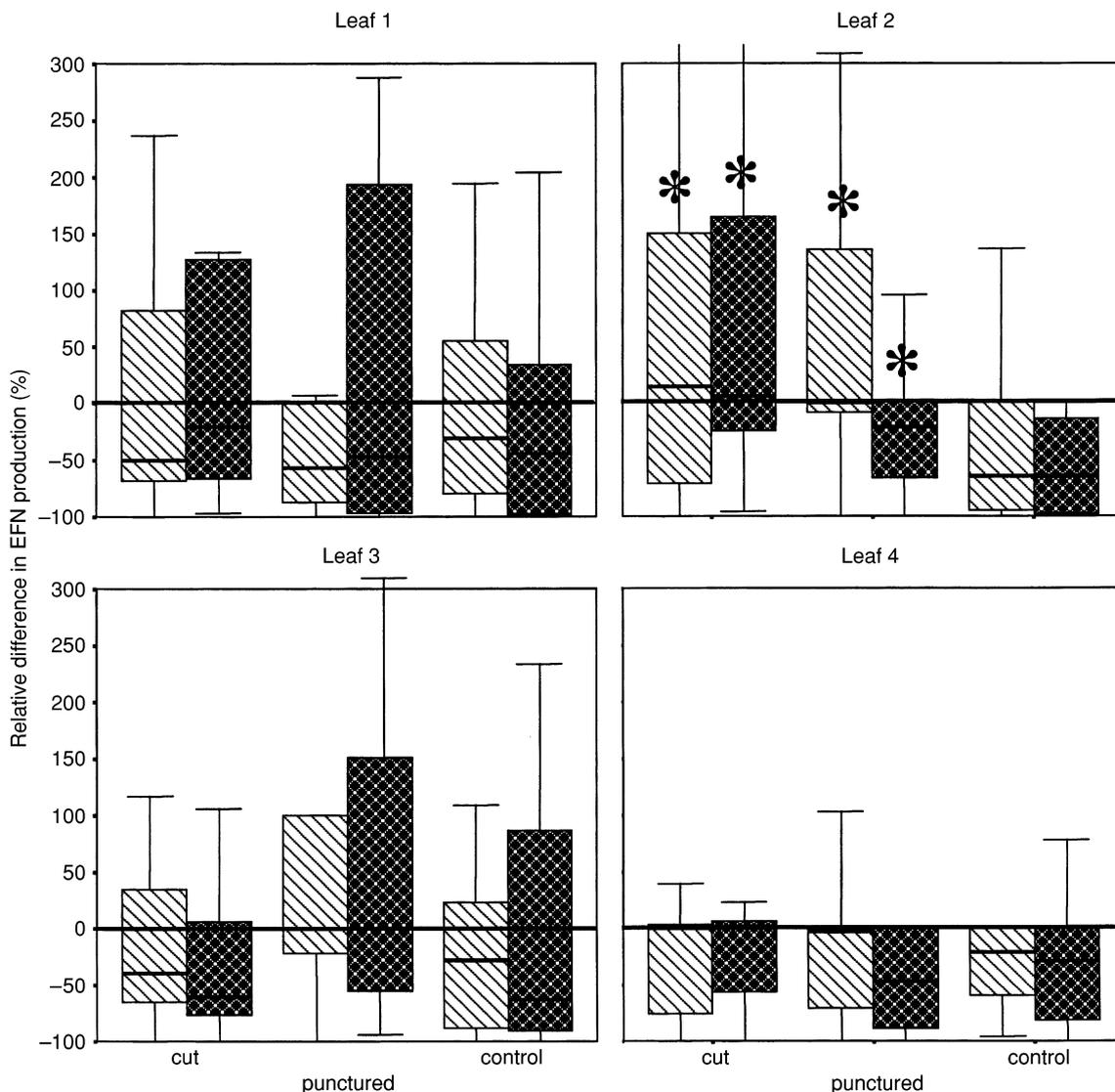


Fig. 5. Effects of artificial leaf damage on extrafloral nectar (EFN) production. The relative change in EFN production on day 1 (grey columns) and day 3 (dark columns) after treatment compared to the pretreatment value was calculated for each leaf (leaves 1–4, with leaf 1 the youngest). ‘Cut’ means that one-third of the leaf blade of leaves 2–4 were removed. Leaves 2–4 in the ‘punctured’ treatment were each punctured with 100 holes. Leaves at position 1 remained untreated on all plants. Asterisks indicate significant differences between treated leaves compared to untreated controls measured on the respective day ($P < 0.05$, according to *post hoc* tests after Games–Howell comparing single treatments to the controls separately for each leaf position – day combination; $n = 20$ for each combination of treatment, leaf position and day). See Fig. 1 for explanation of box-whisker plots.

None of the within-subject factors (leaf position and day of nectar collection) had a significant effect, and no significant interactions between the various factors could be detected (Table 3). *Post hoc* tests were conducted after Games–Howell to compare effects of single treatments separately for each leaf position. They revealed for leaf position 2 that both treatments differed significantly from the controls, but not from each other, on day 1 as well as on day 3 after treatment ($P < 0.05$ for all four comparisons of treated to untreated plants, $n = 20$ in all three groups; Fig. 5). In contrast, no significant effects were detected for the younger, untreated leaves (leaf 1), nor for older treated leaves (leaves 3 and 4), at both times of post-

treatment nectar collection ($P > 0.05$ in all 18 single comparisons; Fig. 5).

Discussion

The efficacy of insects attracted by EFN in plant defence against herbivores has been demonstrated for many plant species (Buckley 1982; Koptur 1992). However, studies that focused on a possible induction of EFN production following herbivory were less convincing (but see Stephenson 1982) because of the very high variances in EFN production rates. Our experiments on *M. tanarius* have demonstrated that EFN production was influenced by several biotic and

abiotic factors, which contribute to its wide variation especially under field conditions.

NECTAR COMPOSITION

The chemical composition of EFN of *M. tanarius* was similar to that reported for other plant species (Baker *et al.* 1978; Galetto & Bernardello 1992; Koptur 1979; Ruffner & Clark 1986; Smith, Lanza & Smith 1990) in that fructose, glucose and sucrose dominated the fraction of soluble sugars (Table 1). Similarly, the dominant amino acids (serine, glycine, cysteine and methionine) are commonly found in extrafloral nectars of many species (Inouye & Inouye 1980; Koptur 1979, 1994). In *M. tanarius*, qualitative EFN composition remained constant compared with the quantitative differences (Table 1). Similar results were reported by Baker & Baker (1977) for floral nectars and by Smith *et al.* (1990) for extrafloral nectar of *Impatiens sultani*. Extrafloral nectar secretion varies with respect to absolute amounts of compounds much more than in the relative proportions of single components. Amounts of total soluble solids, as estimated from combined measurements using hand refractometers and microcapillars, thus appear to provide a suitable measure for biologically relevant changes in EFN secretion.

SPATIAL AND TEMPORAL SECRETION PATTERNS

Extrafloral nectar production by field-grown *M. tanarius* varied diurnally, with most production occurring at dusk (Fig. 1). An increase in EFN production at dusk was reported for *Bixa orellana*, but in *Bixa* high secretion rates persist throughout the night (Bentley 1977b). We interpret the temporal pattern for *M. tanarius* as a potential adaptation to the activities of herbivores, which also show a marked activity peak at dusk and in the first few hours of darkness (M. Heil, A. Hilpert and W. Scholz, unpublished results). Several authors have reported long-term temporal patterns in EFN secretion which were mostly interpreted as an adaptation either to defence-demanding stages of the respective plant parts, or to the occurrence of detrimental herbivores (Bentley 1977a; Ruffner & Clark 1986; Tilman 1978). However, no other data on diurnal patterns have been reported apart from those of Bentley (1977b). Confirmation that the diurnal rhythm of EFN production is really adapted to herbivore activity patterns is therefore not yet possible for any plant species.

The restriction of EFN production to young leaves (Fig. 2) can also be interpreted as a potential adaptive pattern. As in many other plant species (Coley 1983; Coley & Barone 1996; Kursar & Coley 1991, 1992), young, still unfolding leaves of *M. tanarius* suffer most from herbivory, have a high potential value (for example in terms of future assimilation),

and thus require most investment in defence (McKey 1974; Rhoades 1979). It is on these leaves that EFN production is most intensive, although they have not yet reached maximum rates of photosynthetic assimilation (unpublished results). This pattern matches a spatial and temporal distribution of defence investment as predicted by the 'optimal defence theory' (McKey 1974; Rhoades 1979). A comparable dependence of EFN production on leaf age has been reported by O'Dowd (1979).

Extrafloral nectar production in the field declined when nectar accumulated repeatedly on the nectaries over 24 h (Fig. 3a,b). This decrease could result partly from negative influences of artificial nectar removal on further EFN production (F. Wäckers, personal communication; M. Heil *et al.*, unpublished observations). However, production rates increased again on those plants to which insects had had access for 1 day (Fig. 3a). The comparison of nectar collection in 3 h and 24 h intervals revealed greater EFN production rates in the 3 h treatment (Fig. 4). These results indicate that plants reduce EFN production when EFN is not removed, a response previously reported for floral nectar production by Koptur (1983), Gill (1988) and Pyke (1991). Adapting nectar flow to consumption can reduce the plants' investment in 'superfluous' EFN production, and reduces the risk of infections of extrafloral nectaries by a variety of different fungi (M. Heil, unpublished observations).

RESPONSE TO HERBIVORY AND ARTIFICIAL LEAF DAMAGE

Our experiments on artificial leaf damage provide further support for the assumption that EFN may be an inducible defensive response (Fig. 5). Significant effects could be detected only for the youngest of the treated leaves. The response of *M. tanarius* was restricted to those leaves that already showed the highest production rates before the treatment, with no hint of a systemic response, and is comparable to the response of *Passiflora* (Swift & Lanza 1993) or *Gossypium* (Wäckers & Wunderlin 1999). Several studies indicate that EFN production may increase in response to herbivory (Agrawal & Rutter 1998; Koptur 1989; Smith *et al.* 1990; Stephenson 1982), and does not require herbivore-specific elicitors (Wäckers & Wunderlin 1999). However, most studies have suffered from methodological problems, for example from a lack of untreated controls (Wäckers & Wunderlin 1999). In our own experiments, interpretation of the results suffered from the fact that EFN production by most leaves and plants declined during the experiments, probably due to the effects of nectar accumulation.

Conclusions

Extrafloral nectar secretion rates are affected by different biotic and abiotic factors. Differences in EFN

production result from differences in leaf age, time of day or nectar accumulation, all of which should be controlled in experiments on effects of biotic factors such as herbivory. Our findings on the influence of nectar accumulation demonstrate that EFN production rates can increase and decrease in response to the onset of nectar removal or to different removal frequencies. Even groups of plants can show synchronized patterns when they are subjected to no other treatment than nectar collection itself. Therefore controls (untreated plants grown under the same conditions as treated plants) are required before clear statements on any response in the secretion of EFN can be made. Future research should seek data on amounts of soluble solids produced per time interval and per unit leaf area to enable comparisons among studies.

Acknowledgements

We thank Dr Rosli Hashim (University of Malaya) and the Economic Planning Unit (EPU) for permission to conduct field studies in Malaysia. We also thank H. Vogt, A. Hilpert, K. Heil, A. Brunschweiler, E. Wirth and J. Gombert for help in both field and greenhouse studies, F. Thiele and A. Kreiner for cultivating greenhouse plants, and H. Riedel, S.D. Wratten, F. Wäckers and two anonymous referees for helpful comments. We are grateful for financial support by the Deutsche Forschungsgemeinschaft (TP C8, SFB 251). The Malaysian Airline System provided logistical support.

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