Chemoecology 14:45–52 (2004)
0937–7409/04/010045–8

DOI 10.1007/s00049-003-0257-x

CHEMOECOLOGY

Main nutrient compounds in food bodies of Mexican *Acacia* ant-plants

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Summary. Myrmecophytic plants use obligate ant mutualists as a constitutive indirect defence mechanism. These plants often produce cellular food bodies (FBs) to nourish their resident ants. Lipids, proteins, and even highly specialised compounds such as glycogen have been reported from FBs, but detailed chemical analyses of FB composition have so far been presented only for Southeast Asian *Macaranga* and Central American *Piper* myrmecophytes. Here we report the chemical composition of FBs of five myrmecophytic *Acacia* (Fabaceae) species from Mexico using HPLC (carbohydrates and proteins) and GC-MS (lipids). Feeding experiments revealed no hints on any use of external food sources by the inhabiting *Pseudomyrmex* ants. These ants obviously rely completely on FBs and extrafloral nectar provided by their hosts. The total content of nutrients in *Acacia* FBs was 15–25 % of FB dry mass, being much lower than in *Macaranga* or *Piper* FBs. Proteins were dominating (8–14 % dm) in *Acacia* FBs and thus were present in higher amounts than in *Macaranga* FBs, yet in lower amounts than in *Piper*. Lipids contributed 1–9 % of dry mass, showing a lower proportion than in FBs of *Macaranga* or *Piper*. Carbohydrates made up 3–11 % dm, reaching in most *Acacia* species the same range as observed in *Macaranga* and in *Piper* FBs. Water content was 18–24 % of FB fresh mass, and structural tissue obviously made up a much higher proportion in *Acacia* FBs than in *Macaranga* or *Piper* FBs. Both characters might represent an adaptation to producing FBs unprotected at the leaf tips under dry conditions. *Acacia* FBs contain all amino acids and all fatty acids that are considered essential for insects, and their contents of lipids and proteins are higher than in the leaves from which they are ontogenetically derived. This indicates a putatively adaptive enrichment of nutritionally valuable compounds in structures functioning as ant-food.

Key words. *Acacia chiapensis – Acacia collinsii – Acacia cornigera – Acacia globulifera – Acacia hindsii* – ant-plant interaction – indirect defence – mutualism – myrmecophytism – *Pseudomyrmex* – swollen-thorn-acacias

Introduction

Myrmecophytism belongs to the most specialised forms in the wide spectrum of mutualistic plant-insect relationships. Plants of different taxonomic groups and growing in all major tropical regions house ant colonies, which act as a constitutive indirect defence (Buckley 1982; Beattie 1985; Hölldobler & Wilson 1990; Huxley & Cutler 1991; Davidson & McKey 1993; Heil & McKey 2003). One of the first myrmecophytic systems described in detail is that of several *Acacia* (Mimosoideae, Fabaceae) species and *Psyeudomyrmex* ants in Central America (Belt 1874; Janzen 1966, 1967, 1974). Other examples of obligate ant-plants are species in the genera *Macaranga* (Euphorbiaceae) in Southeast Asia (Fiala *et al.* 1989; Heil *et al.* 2001a; Linsenmair *et al.* 2001) and *Piper* (Piperaceae) in Central America (Risch 1982; Letourneau 1998; Dyer *et al.* 2001).

Myrmecophytic systems are widespread and ecologically well described, but the chemical ecology of the various interactions among ants and plants is still poorly understood. Ants inhabiting *Macaranga* or Central American *Acacia* myrmecophytes are generally believed to make no use of attacked arthropods or other potential prey as an additional food source, but only discard them from the plant (Janzen 1974; Fiala & Maschwitz 1990). Although a nitrogen flow from ants to plants has been reported for *Cecropia* (Sagers *et al.* 2000) and *Piper* (Fischer *et al.* 2003), even in the latter system the flow of nitrogen from the ant to the plant appeared quantitatively low as compared to the nutrient flow from the plant to the ant (Fischer *et al.* 2002, 2003). That food rewards produced by the host plant can indeed be a limiting factor for the ants is underlined by the observation that *Macaranga triloba* plants producing more FBs were inhabited by larger ant colonies (Heil *et al.* 2001b). Most cases of protective ant-plant interactions are characterised by a flow of nutrients from the plant to the ant rather than vice-versa (Heil & McKey 2003), the composition of these rewards thus forms a central issue in the chemical ecology of ant-plants.

Early observations indicated that FBs can have high nutritive values: The storage carbohydrate glycogen, generally known only from animal tissue, was found in 'Müllerian bodies' of *Cecropia peltata* (Rickson 1971). 'Pearl bodies', the second class of ant-rewards produced by myrmecophytic *Cecropia* species, were reported to be rich in proteins and amino acids (Folgarait & Davidson 1994, 1995). Janzen (1974) assumed high contents of proteins, lipids and carbohydrates in *Acacia* FBs. Histological staining methods confirmed high lipid contents in FBs of the myrmecophytes *Acacia cornigera* and *Macaranga triloba* (Rickson 1975, 1980) and of the non-myrmecophyte

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Ochroma pyramidale (O'Dowd 1980). However, most these studies reported qualitative rather than quantitative data and dealt with one or a few rather than the whole set of main nutrient compounds.

Here we focus on the three main nutrient classes in plant-derived cellular ant rewards. Ant-acacias (also called "swollen thorn" acacias) occur both in Africa and in Central America, and apparently all acacia-ants live in enlarged, hollow stipular thorns and feed on extrafloral nectar secreted by foliar nectaries as a source of carbohydrates and water (Janzen 1974; Young *et al.* 1997; Raine *et al.* 2002). However, food bodies (FBs, "Beltian bodies", see Fig. 1) are produced only by the Central American ant-acacias (Janzen 1974). These FBs are modified leaflet tips (Rickson 1969), which are harvested by the ants and fed to their larvae (Janzen 1974). The acacia-ants form a group of ten closely related species described as *Pseudomyrmex ferrugineus* – group (Ward 1993). Each plant species can be inhabited by different ant species, ants of the *P. ferrugineus* group and their host acacias have obviously experienced diffuse coevolution rather than strict cospeciation (Ward 1993). Even individual ant colonies can occupy *Acacia* plants of different species (pers. observations by MH). The ants continuously patrol the surface of their host plant and protect it from herbivores, climbers, and competing vegetation. Ant-acacias depend on this protection and grow poorly in the absence of their ant partner (Brown 1960; Janzen 1966, 1967).

To our knowledge, detailed quantitative analyses of FB tissue contents are available only for *Macaranga* (Heil *et al.* 1998) and *Piper* myrmecophytes (Fischer *et al.* 2002). Here we present information on carbohydrates, proteins, and lipids in FBs of five myrmecophytic *Acacia* species. In addition we report feeding experiments testing whether external food sources are used by the resident *Pseudomyrmex* ants.

Fig. 1 Food bodies of *Acacia collinsii*. The food bodies (FBs, or "Beltian bodies") of myrmecophytic *Acacia* species are modified leaflet tips produced at every young, unfolding leaf of an inhabited plant (Δ). FBs are collected by the ants and carried into the hollow thorns, where they most probably are fed to the ants' larvae. Extrafloral nectar is secreted by enlarged nectaries (↑) on the petiole

Materials and methods

Plant material and study sites

Food bodies and leaves of five mymecophytic *Acacia* (Mimosoideae, Fabaceae) species were collected in Mexico in March and April 2000. Material from *A. hindsii* Benth., *A. cornigera* (L.) Willendow, *A. globulifera* Safford and *A. chiapensis* Safford was collected at different sites in the Isthmus of Tehuantepec (state of Oaxaca), and samples of *A. collinsii* Safford were obtained from two sites near Coba, peninsula Yucatan (state of Quintana Roo). All sites were extensively used pastures or similarly structured, open secondary shrublands. All used plants were shrubs 1.5–2.5 m high and grew in the full sun. Further selection criteria were a good general shape of the plant and average ant activities. Plants being damaged above average and therewith obviously poorly defended by ants were avoided, as were plants with exceptionally high ant activities. Species were determined following Janzen (1974) and Seigler & Ebinger (1995) and by comparison with specimens held at the Herbario MEXU at UNAM (Mexico City). Voucher specimens are held by M. Heil and are deposited at the Herbario MEXU.

Feeding experiments

Feeding experiments were conducted on ants inhabiting *A. chiapensis, A. cornigera, A. hindsii,* and *A. collinsii*. Three shrubs per species were selected, and resident ants were offered small items (length 1–2 mm, therewith being in the range of food bodies) of four different types of materials, covering a range from likely suitable to likely unsuitable. Materials were dead plant material (occurring naturally, and very likely being unsuitable as a food source), boiled egg (a common bait for generalist ants), parts of small insects (mostly flies and grasshoppers from the direct vicinity, thus likely occurring as natural 'invadors' on the respective *Acacia* shrub), and food bodies (of the same species, yet derived from another individual than the one investigated). All items were presented on the rachis or blade of young, FB-producing leaves (ten replicates per type of item and shrub). Ant behaviour was observed for the following 5 min, and six different types of behaviour were distinguished: (a) not found, (b) ignored (physical contact by at least one ant, yet not removed), (c) carried (FB taken up by an ant and then carried to another part of the plant), (d) removed (actively discarded from the plant), (e) collected (carried into a domatium, i.e., thorn), (f) attacked (visibly attacked by biting and stinging). In case that several different behaviours took place (e.g., resident ants first attacking an insect and then discarding the dead insect from the plant), the last defined behaviour was used for evaluation.

Food body analysis

Food bodies and leaves were stored in 100 % ethanol and freeze dried before analysis. Analysis of carbohydrates and proteins were done as described previously (Heil *et al.* 1998). For analysis of

carbohydrates, FB tissue was homogenized by grinding, and about 10 mg dry mass (dm) were subsequently extracted twice for 30 min in a total of 1.5 ml water. After centrifugation, the supernatant was cleared by micro-membrane filtration (Spartan 30/B filters, 0.45 µm, Schleicher & Schuell, Germany) and stored at –20°C until use. The residue was washed twice with water and then hydrolysed (1h at 95°C in 1.5 ml 1 M HCl). After centrifugation and membrane filtration sugars were separated by isocratic (0.1 M NaOH) HPLC on an anion exchange column, and quantified by pulsed amperometric detection (DIONEX Series 4500 Chromatography System, Dionex, Idstein, Germany).

For the analysis of soluble amino acids and proteins, about 40 mg of ground tissue was extracted with 1.5 ml water for 30 min. After centrifugation and membrane filtration, soluble amino acids were directly determined in the supernatant. The residue was washed twice with water and then hydrolysed (4 h at 100°C in 1 ml 6 M HCl). After centrifugation and membrane filtration amino acids were separated and measured with an Amino Acid Analyzer LC 5001 (Biotronic, Maintal, Germany).

For the analysis of lipids, 30 mg dry FBs were extracted in 3 ml $CHCl_3$ (1 h at 70°C) to which 500 µl of an internal standard (10.9 mg tetracosane in 10 ml $CHCl_3$) had been added. After extraction, samples were filtrated. 100 µl of the crude extract were silylated. Samples were evaporated and 10 µl filtered pyridine and 10 µl BSTFA (bis(Trimethylsilyl)trifluoro-acetamide) was added. After reaction (30 min at 70°C) samples were re-dissolved in 200 µl $CHCl_3$ and used for GC-MS analysis at this concentration. Another part of the samples was subjected to transesterification in order to identify fatty acid components of di- and triglycerides. 300 µl of crude extracts were evaporated and 100 µl $CHCl_3$ and 500 µl BF_3-methanol was added. After reaction (24 h at 70°C) samples were re-dissolved in $CHCl_3$, washed two times with water and then silylated. GC-analysis was conducted with a Hewlett Packart 5890 Series II (column: DB-1HT, J&W Scientific, Folsom, CA, USA) with N_2 as carrier gas, while a mass selective detector HP5971 A was used for compound identification via mass spectrometry.

Three to eight independent samples were collected, each single sample pooling FBs from at least three plant individuals. Detailed sample sizes are shown in Tables 1 and 2 and in Figure 3. For each sample, two parallel analyses were conducted. Therefore, a mean was calculated from these parallels for each detected substance to obtain the results for one distinct sample, and further calculations were based on these results. The composition of a 'typical FB' for each species was determined by calculating the mean for each substance from all respective samples.

Results

Feeding experiments

Pseudomyrmex ants inhabiting *Acacia* myrmecophytes distinguished among different items offered to them as putative food sources. In the vast majority of cases, items of straw, boiled egg, and insect parts were discarded from the plant, while food bodies were identified as suitable food source and carried into hollow thorns (Fig. 2). In only one case, a single FB was discarded from the plant by an ant inhabiting an *A. cornigera* shrub, while workers of the eleven remaining colonies carried all ten FBs offered into the domatia. In contrast, only one piece of insect and two pieces of boiled egg were carried into a hollow thorn, while the majority of these types of material and all items of straw were removed from the respective plants (Fig. 2).

Chemical composition of food bodies

Total amounts of carbohydrates, proteins and lipids in FBs of the *Acacia* species ranged from 160 to 240 mg g^{-1} of total dry mass (dm).

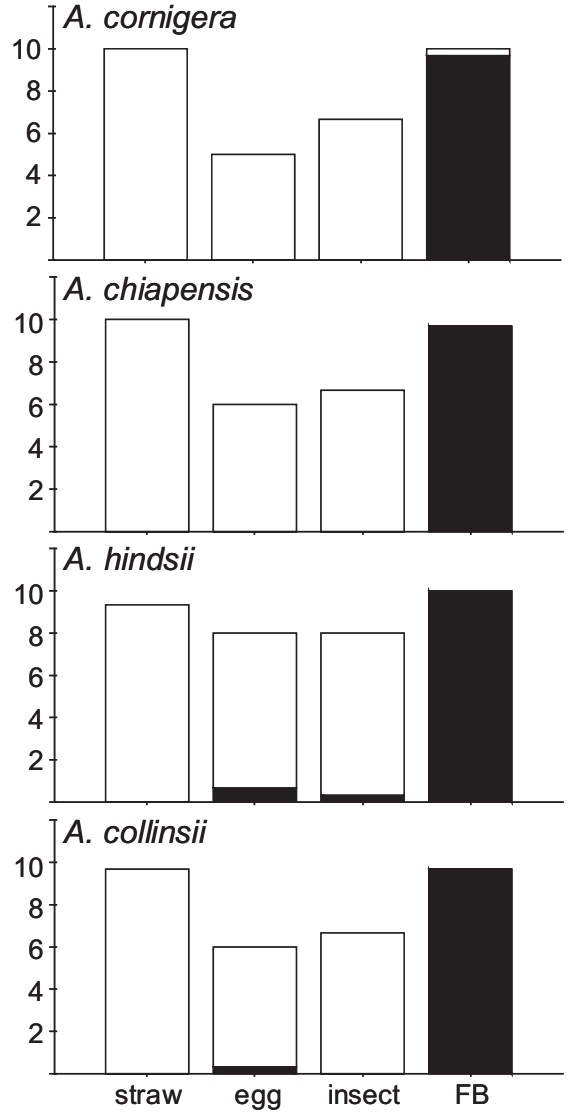

Fig. 2 Behavioural responses of *Pseudomyrmex* ants to different items offered as putative external food sources. The average number of items offered (n = 10 per material type and ant colony) that were removed (□) or accepted (■) by the three ant colonies tested per plant species are given separately for the four *Acacia* species

Proteins and free amino acids formed the largest fraction of analysed nutrients in four of the five examined species. Contents ranged from 77 mg g^{-1} dry mass in *Acacia collinsii* to 139 mg g^{-1} dry mass in *A. cornigera* (Fig. 3). 74–88 % of this fraction consisted of insoluble polymers (proteins) with aspartate, serine, glutamate, glycine, alanine, leucine, lysine and arginine being the main compounds (Table 1). Only 12–26 % were soluble amino acids. Aspartate dominated the fraction of soluble amino acids in all species. The total protein fraction was therefore dominated by nine of 37 analysed amino acids, which made up two thirds of this fraction (Table 1).

Total carbohydrate content was some 30 (27–36) mg g^{-1} dm in four of the examined species and 112 mg g^{-1} dm in *Acacia collinsii* (Fig. 3, Table 1). Sixteen different sugars

Table 1 Main compounds of nutrient fractions in *Acacia* food bodies. The average composition of main nutrients in FB dry matter of five *Acacia* species are given as mean ± SD in mg g^{-1} dry mass. Sample numbers (n) differ among carbohydrates, proteins, and lipids, and appear separately at the top of each substance class

	A. cornigera	A. chiapensis	A. hindsii	A. globulifera	A. collinsii
Soluble carbohydrates	**(n = 8)**	**(n = 7)**	**(n = 5)**	**(n = 4)**	**(n = 8)**
glucose	3.31 ± 1.20	6.40 ± 2.40	2.97 + 0.92	5.76 ± 0.72	9.05 ± 3.54
fructose	3.37 ± 1.38	8.69 ± 3.45	1.82 + 0.74	5.74 ± 1.34	4.78 ± 3.96
unknown sugar C	3.97 ± 2.34	1.24 ± 0.79	2.29 + 2.01	1.03 ± 0.54	1.04 ± 0.44
sucrose	1.84 ± 1.85	6.72 ± 4.53	1.84 + 1.59	2.58 ± 2.06	79.34 ± 24.99
Polysaccharides	**(n = 8)**	**(n = 7)**	**(n = 5)**	**(n = 4)**	**(n = 8)**
arabinose	8.38 ± 2.87	8.57 ± 2.10	10.24 + 3.32	8.49 ± 1.20	9.03 ± 1.95
glucose	2.83 ± 1.87	3.26 ± 1.74	3.95 + 1.00	2.09 ± 0.88	2.72 ± 0.43
Soluble amino acids	**(n = 7)**	**(n = 7)**	**(n = 6)**	**(n = 5)**	**(n = 8)**
aspartic acid	1.02 ± 0.54	0.82 ± 0.37	1.00 ± 0.48	1.93 ± 2.22	0.90 ± 0.53
serine	3.26 ± 1.70	0.83 ± 0.57	1.34 ± 0.82	0.69 ± 0.16	0.50 ± 0.16
asparagine	6.59 ± 2.94	2.39 ± 0.91	10.83 ± 7.03	7.38 ± 2.77	3.13 ± 1.57
glutamine	2.45 ± 0.86	1.38 ± 0.68	2.50 ± 0.96	1.81 ± 0.48	2.02 ± 0.77
alanine	0.59 ± 0.35	0.41 ± 0.25	0.38 ± 0.13	0.75 ± 0.22	0.92 ± 0.49
leucine	0.33 ± 0.31	0.21 ± 0.15	0.43 ± 0.24	0.36 ± 0.12	0.36 ± 0.24
lysine	2.19 ± 2.32	0.47 ± 0.22	1.02 ± 0.41	1.74 ± 1.06	0.60 ± 0.22
arginine	1.21 ± 0.95	0.45 ± 0.30	1.65 ± 0.69	1.36 ± 0.64	0.83 ± 0.46
Proteins	**(n = 7)**	**(n = 7)**	**(n = 6)**	**(n = 5)**	**(n = 8)**
aspartic acid	12.36 ± 3.14	12.11 ± 1.96	11.33 ± 1.68	11.14 ± 2.28	8.53 ± 3.04
serine	7.93 ± 1.98	6.29 ± 1.15	5.76 ± 0.86	5.84 ± 1.38	3.56 ± 1.00
glutamic acid	11.21 ± 1.53	10.62 ± 1.35	10.00 ± 1.42	10.13 ± 1.09	6.72 ± 1.62
glycine	7.51 ± 1.48	7.10 ± 1.18	6.20 ± 0.80	5.75 ± 1.39	4.95 ± 1.27
alanine	7.31 ± 1.90	5.87 ± 0.73	6.03 ± 1.01	5.60 ± 1.11	3.64 ± 0.99
leucine	7.85 ± 2.16	7.37 ± 1.87	5.54 ± 2.36	6.39 ± 1.01	3.77 ± 0.79
lysine	8.01 ± 2.33	6.50 ± 1.47	6.12 ± 1.41	6.47 ± 1.34	3.58 ± 0.82
arginine	5.57 ± 1.34	5.47 ± 1.16	4.70 ± 0.63	5.22 ± 0.77	2.65 ± 0.61
Lipids	**(n = 5)**	**(n = 6)**	**(n = 5)**	**(n = 4)**	**(n = 4)**
palmitic acid	0.52 ± 0.26	1.90 ± 0.94	2.18 ± 2.15	0.49 ± 0.13	0.67 ± 0.76
linolenic acid	0.35 ± 0.20	2.52 ± 2.09	1.17 ± 0.97	0.38 ± 0.35	0.51 ± 0.62
linoleic acid	1.96 ± 1.59	4.33 ± 4.78	4.59 ± 3.99	2.53 ± 1.83	2.21 ± 2.73
oleic acid	3.72 ± 3.83	46.50 ± 34.75	24.05 ± 21.16	5.18 ± 3.66	4.44 ± 5.89
stearic acid	0.77 ± 0.48	7.31 ± 5.76	3.40 ± 2.14	1.12 ± 0.49	0.63 ± 0.74

and sugar alcohols (mannitol, glucose, fructose, arabinose, sucrose, maltose and 10 unidentified sugars) could be detected. Soluble sugars made up 44–88 % of total fraction of carbohydrates, with the highest rate (88 %) occurring in *A. collinsii*. In the other four species the proportions of soluble and polymeric carbohydrates were almost balanced (Fig. 3). Four sugars (glucose, fructose, sucrose and an undetermined 'sugar C') in general dominated the carbohydrates, while soluble sucrose was the dominating compound in *A. collinsii* (70 % of total carbohydrates). Polysaccharides made up 12–56 % and consisted mainly of the monomers arabinose and glucose (Table 1). Arabinose formed the main part of polysaccharide fraction (65–74%) in three of the *Acacia* species, while in *A. chiapensis* the amount of glucose was slightly higher.

The lipid fraction made up 10–12 mg g^{-1} dm in FBs of *A. cornigera*, *A. globulifera*, and *A. collinsii*. In *A. hindsii* and *A. chiapensis,* the lipid fraction was higher (46 mg g^{-1} and 91 mg g^{-1}) (Figure 3). The largest part of the lipid fraction consisted of free and bound fatty acids, with palmitic acid, linolenic acid, linoleic acid, oleic acid, and stearic acid as main compounds (Table 1).

Water content could be analysed in three of the five *Acacia* species. Relative water content in FBs was 18 % (*A. cornigera*) to 24 % of fresh weight (*A. chiapensis* and *A. hindsii*) (Table 2).

Chemical composition of leaves

The contents of lipids and proteins in leaves were lower than in FBs: Within all five species, the contents of soluble amino acids and proteins were significantly higher in FBs than in leaves, as was the content of lipids in three of the five species (Fig. 3). In contrast, the contents of polysaccharides were significantly higher in leaves of all species than in the respective FBs, and leaves of three of five species had contents of soluble carbohydrates that differed significantly from the FBs.

Altogether, proteins, carbohydrates, and lipids made up 110–150 mg g^{-1} dm. Proteins and amino acids made up 31–46 mg g^{-1} dm of leaf tissue. Although in particular the content of proteins was much lower in leaves than in FBs (Table 2, Fig. 3), the relative composition of proteins and amino acids was quite similar in both types of tissue (data not shown). In contrast to FBs, leaves of four species contained more carbohydrates than food bodies (55–93 mg g^{-1} dm). Only in *A. collinsii* sugar content of leaves was lower than in food bodies (Fig. 3). Lipids made up 5–9 mg g^{-1} dm

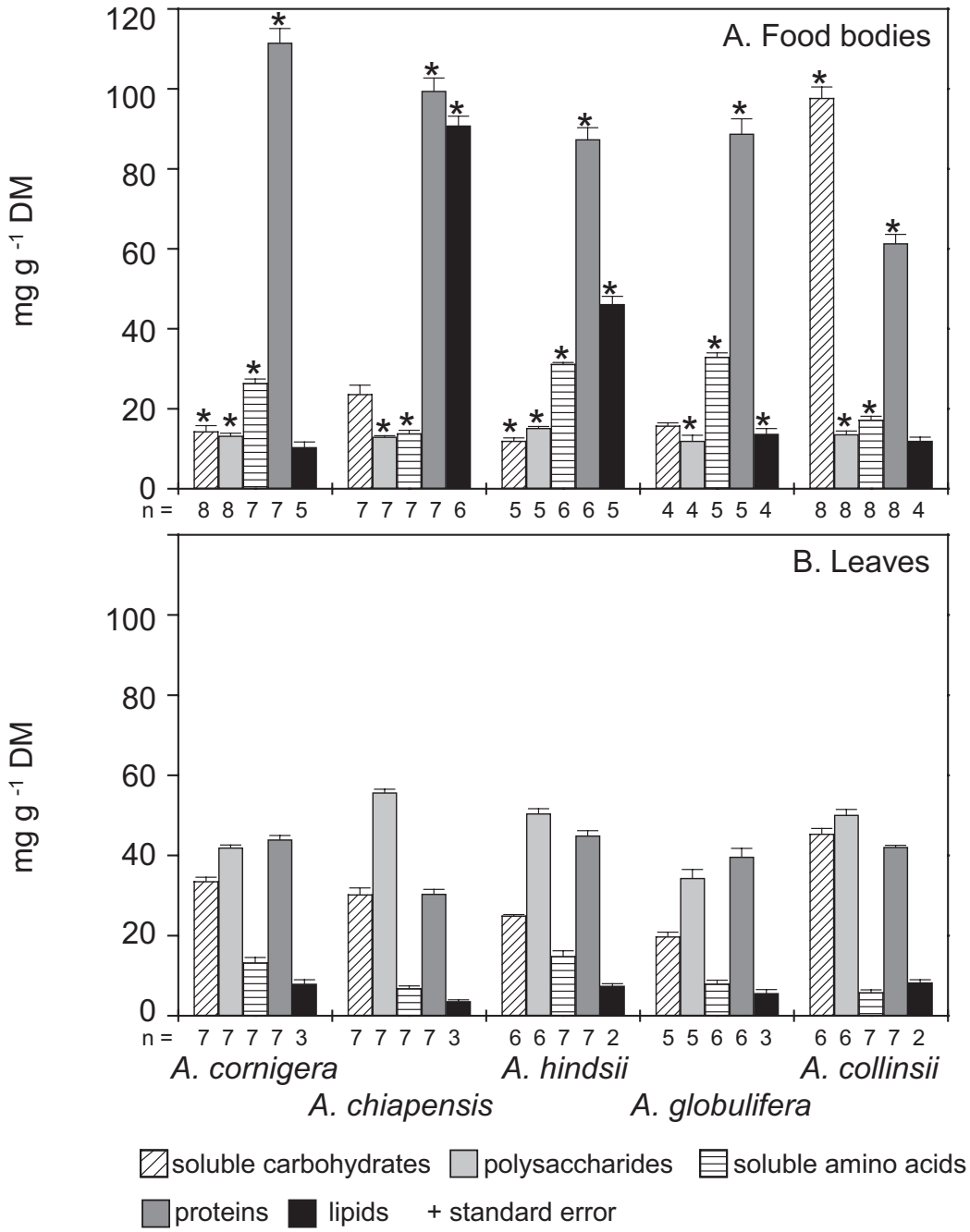

Fig. 3 Contents of main nutrient classes in food bodies (**A**) and leaves (**B**) of five myrmecophytic *Acacia* species. Values are given in mg g^{-1} dry mass. Sample numbers appear below the bars. Asterisks (*) indicate that contents of individual substance classes differ significantly ($p < 0.05$ according to t-test) between food bodies and leaves of the same species

in leaves, while water content was higher than in FB tissue (36–44 % of fresh weight) (Table 2).

Discussion

We examined the main nutrient composition in food bodies (FBs) of Mexican *Acacia* myrmecophytes and checked whether the *Pseudomyrmex* ants obligately inhabiting these plants are likely to make also use of external, not plant-derived food sources. Feeding experiments revealed no hints to a use of external food sources by these ants. Even potentially suitable materials such as boiled egg and freshly killed insects were regularly discarded from the plant, while FBs were identified as a suitable food source (Fig. 2). The general suitability of the experimental design chosen here is underlined by the fact that foreign ants eventually inhabiting *Acacia chiapensis* shrubs and probably parasitising the system (Janzen 1975; Gaume & McKey 1999) collected FBs as well as pieces of boiled egg and dead insects and carried them into the domatia (M. Heil & R. Krüger, personal observations). This leads to the interpretation that

Table 2 Average contents of total carbohydrates, total proteins, total lipids, and unidentified rest in food bodies and leaves of three *Acacia* species. Values are given as mean ± SD in mg g^{-1} fresh mass. Bold numbers in brackets give sample numbers

	Total carbohydrates	Total proteins	Total lipids	Total water content	Rest
Food bodies					
A. cornigera	21.74 ± 7.34 **(8)**	113.61 ± 22.21**(7)**	8.14 ± 13.63 **(5)**	180.20 ± 61.10 **(3)**	676.31
A. chiapensis	27.56 ± 3.68 **(7)**	84.24 ± 11.70 **(7)**	68.64 ± 7.23 **(6)**	242.10 ± 68.30 **(3)**	577.46
A. hindsii	21.39 ± 6.18 **(5)**	90.89 ± 12.81 **(6)**	35.04 ± 41.55 **(5)**	234.60 ± 111.10 **(3)**	618.07
Leaves					
A. cornigera	47.81 ± 17.75 **(7)**	38.44 ± 5.49 **(7)**	5.33 ± 2.18 **(3)**	356.05 ± 18.78 **(3)**	552.37
A. chiapensis	52.45 ± 13.16 **(7)**	23.23 ± 9.41**(7)**	2.84 ± 0.77 **(3)**	390.82 ± 3.73 **(4)**	530.65
A. hindsii	42.44 ± 13.49**(6)**	34.37 ± 6.61**(7)**	4.76 ± 1.80 **(2)**	444.21 ± 2.55 **(3)**	474.22

Pseudomyrmex mutualists regularly inhabiting *Acacia* myrmecophytes rely on the food sources that are produced by their host plant.

Central American *Acacia* myrmecophytes provide their resident ants with extrafloral nectar and cellular food bodies (Fig. 1). Based on the results of our feeding experiment (Fig. 2), it must be assumed that these directly plant-derived food sources form a complete diet for the resident ant colonies. The contents of – nutritionally highly valuable – proteins and lipids were much higher in FBs of all five species than in the leaves from which they are ontogenetically derived (Fig. 3). Depending on the species, lipids in *Acacia* FBs made up 1–10 % of total dry mass (leaves: 0.5–0.8 %), while proteins and amino acids contributed 8–14 % dm (leaves 3–6 %). Only the contents of carbohydrates were lower in FBs (3–11 % dm) than in leaves (5–9 %) of four of the five species investigated. The complete fraction of amino acids and proteins in FBs was at least twice as high as in the leaves of the respective species, with the amount of polymeric proteins being more than five times higher (Table 2, Fig. 3). FB production can be limited by soil nutrient content (Folgarait & Davidson 1995; Heil *et al.* 2001b, 2002). Proteins, being rich in nitrogen, are likely among the components that make production of FBs metabolically 'expensive'. A significant enrichment of these compounds in FB tissue of *Acacia* plants thus clearly points to functional adaptations.

The suitability of FBs as ant food becomes further obvious when regarding particularly functional compounds. All of the ten amino acids usually considered essential for insects (glycine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophane, and valine, see Hagen *et al.* 1984; Chen 1985; Gewecke 1995; Barbehenn *et al.* 1999) are present in the FBs of all five examined *Acacia* species (data not shown). Some polyunsaturated fatty acids are necessary for insects as accessory growth factors and for a normal development. For hymenopterans, mainly linoleic acid and linolenic acid are considered as essential (Hagen *et al.* 1984; Barbehenn *et al.* 1999). *Acacia* FBs provide these essential fatty acids: three of five main components of lipid fraction were the unsaturated fatty acids oleic acid, linoleic acid, and linolenic acid, which together made up 48–65 % of lipid fraction (Table 1) and thus were present in much higher amounts than in leaf tissue. FBs obviously provide their consumers with a valuable diet being rich in essential compounds.

To date, detailed quantitative chemical analyses of FBs have been reported for two other genera of myrmecophytes, *Macaranga* (Heil *et al.* 1998) and *Piper* (Fischer *et al.* 2002). Forming in general a functionally similar system, the *Acacia-Pseudomyrmex* association differs in several aspects from the *Macaranga-Crematogaster* system and the *Piper-Pheidole* association. All these myrmecophytes house their ants in hollow structures and nourish them by FBs. However, FBs in *Macaranga* and *Piper* represent modified uni- or multicellular epidermal trichomes (Rickson 1980; Rickson & Risch 1984), which in most cases occur at protected sites of the plants, e.g., under recurved stipules (*Macaranga*: Fig. 1c in Heil *et al.* 1998), or inside hollow leaf stalks (*Piper*: Rickson & Risch 1984; Fischer *et al.* 2002, 2003). In contrast, *Acacia* FBs are modified leaflet tips (Rickson 1969, 1975) presented openly on young leaves (Fig. 1). Moreover, ants in *Macaranga*, but not in *Acacia* and *Piper*, cultivate scale insects (Ridley 1910; Heckroth *et al.* 1999), whose honeydew (or probably also the scale insects themselves) serves as a source of carbohydrates and amino acids. In contrast, *Acacia* plants provide their ants with extrafloral nectar, while nectaries are missing in myrmecophytic *Macaranga* (Fiala & Maschwitz 1991) and *Piper* species (Table 3).

Redundancies among the different plant-derived food sources (FBs and extrafloral nectar in *Acacia*, FBs and scale insects in *Macaranga*) should have been reduced during evolution. FBs of *Acacia* species provide the ants with much more proteins than carbohydrates (Table 3), therewith obviously counterbalancing the absence of an alternative source of amino acids. Contents of lipids and particularly carbohydrates are low in *Acacia* FBs, yet extrafloral nectar produced by these plants can serve as an additional source of carbohydrates and, thus, energy. Similarly, the higher contents of both lipids and proteins in *Piper* FBs than in *Acacia* or *Macaranga* (Table 3) can be explained by the fact that FBs produced by *Piper* appear to be the only food source of the resident ants, while both other genera provide at least one additional food source (Table 3).

Although several nutrients are clearly enriched in *Acacia* FBs as compared to leaf tissue (see above), the relative composition of food bodies and leaves was more similar in *Acacia* than in *Macaranga* (Table 1, Fig. 3, for *Macaranga* see Heil 1998). Moreover, water content in *Acacia* FBs was much lower (ca. 18 %) than in *Macaranga FBs* (44–69 %), while structural components obviously made up a larger proportion in *Acacia* FBs than in FBs of

Table 3 Chemical composition of FBs and presence of alternative plant-derived food sources provided to resident ant mutualists by three genera of myrmecophytes. The presence (+) or absence (–) of extrafloral nectar (EFN) and trophobionts as well as the range observed in the contents of lipids, proteins and carbohydrates [in mg g^{-1} DM] in FBs of the different species is given for myrmecophytes of the genera *Acacia* (five species), *Macaranga* (four species) and *Piper* (four species). Data on *Acacia* from Figure 3, data on *Macaranga* from Table 2 in (Heil *et al.* 1998) and Figure 3 in (Linsenmair *et al.* 2001), data on *Piper* from Figure 1 in (Fischer *et al.* 2002)

	Acacia	Macaranga	Piper
EFN	+	–	–
trophobionts	–	+	–
total lipids	11–92	250–350	405–486
total proteins	77–138	28–61	166–243
total carbohydrates	26–112	26–63	23–32

the two other genera. These differences may reflect two constraints: *Acacia* FBs are directly derived from the leaves, and they are produced openly at the leaf tips. Probably there are ontogenetical constraints preventing a larger chemical differentiation between FBs and leaf tissue. Moreover, ant-acacias grow in dry habitats and thus have to deal with a strong shortage of water during large parts of the year. While FBs obviously are a highly valuable food source for resident ants, these latter findings demonstrate that they also underlie physiological and ontogenetic constraints.

Conclusions

Food bodies and extrafloral nectar are the only plant-derived food sources of *Pseudomyrmex* ants resident in *Acacia* myrmecophytes. These ants apparently do not make use of external food sources. Physiological constraints appear to affect FB construction and composition of the *Acacia* species investigated in the present study. However, most aspects of FB composition, as well as the similarities and dissimilarities with the composition of *Macaranga* and *Piper* FBs, could most plausibly be explained as adaptations to the FBs' functional role as important ant food.

Acknowledgements

We thank Dr. Mario Sousa and Rafael Torres Colín (Instituto Botanico and Herbario MEXU at UNAM, Mexico City) and Alfredo Saynes-Vasques (Jardin Ethnobotanico Oaxaca) for help with species determination. Comments by Doyle McKey, Graham Stone and C. Kost on earlier versions of the manuscript are gratefully acknowledged. We also are indebted to Eva Wirth, Astrid Boots, and Bianka Pink, who conducted important parts of the analytic routine. Prof. Kaiser, Dr. Jetter and Prof. Riederer (all at University of Würzburg) kindly facilitated these analyses in their laboratories. Financial support by the DFG (TP C8 in SFB 251 and research grants He3169/1–1,2,3,4) and by the Max-Planck-Gesellschaft is gratefully acknowledged.

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Received 10 June 2003; accepted 2 October 2003.