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 Pseudomyrmex 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...
Ochroma pyramidale (O’Dowd 1980). However, most of 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.

Materials and methods

Plant material and study sites

Food bodies and leaves of five myrmecophytic Acacia (Mimosoideae, Fabaceae) species were collected in Mexico in March and April 2000. Material from A. hindii 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. collinsi 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. chiaensis, A. cornigera, A. hindii, and A. collinsi. 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 ‘invaders’ 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

Fig. 1 Food bodies of Acacia collinsi. 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.