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Honeydew flicking by treehoppers provides cues to potential tending ants

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Abstract. The honeydew-producing treehopper, *Guayaquila xiphias*, is frequently tended by ants on shrubs of *Didymopanax vinosum* in the Brazilian savannah. Field experiments showed that the flicking of accumulated honeydew by untended treehoppers provides cues to ground-dwelling ants. Upon finding scattered honeydew droplets on the ground, the ants climb onto the host plant and start tending activity. Honeydew-soaked filter papers placed beneath unoccupied host plants induced significantly more ants to climb onto the plant than water-soaked papers. Because predation and parasitism on *G. xiphias* can be severe at early stages, and tending ants protect the homopterans against predators and parasitoids, the capacity to attract ants early in life can be crucial for treehopper survival.

Phloem-feeding Homoptera (primarily aphids, membracids and scales) are commonly tended by ants that harvest their energy-rich honeydew (Auclair 1963; Way 1963). Homopteran honeydew is generally regarded as an ant attractant (Sudd 1987), and tending ants can provide a range of benefits to homopterans, including protection from natural enemies (Buckley 1987a, b, and citations therein). Ant colonies may simultaneously tend many homopteran aggregations, and intra- and interspecific competition for the services of ants can adversely affect homopteran fitness through reduced tending levels (Cushman & Addicott 1989; Cushman & Whitham 1991). Predation pressure on homopteran aggregations can be severe at early developmental stages, and ant tending can greatly increase the number of surviving nymphs (Bristow 1983; Cushman & Whitham 1989). Therefore, the capacity to attract ants early in life can be crucial for the survival of ant-tended homopterans, especially under low ant densities (Cushman & Addicott 1989).

When untended by ants, many homopterans flick away the accumulated honeydew either with the hind legs or caudae or by contracting the rectum or entire abdomen (Hölldobler & Wilson 1990). The flicking of accumulated honeydew may reduce fungal attack to the homopterans and adjacent plant parts (Beattie 1985; Buckley 1987a, b, and citations therein).

The honeydew-producing treehopper, *Guayaquila xiphias* (Membracidae), is frequently tended by ants in the cerrado woodland of Brazil. Del-Claro (1995) identified 21 ant species tending *G. xiphias* aggregations, and used ant-exclusion field experiments to show that tending ants protect homopteran nymphs from predators such as salticid spiders and syrphid flies, and egg masses from parasitoid wasps. We have suggested (Del-Claro & Oliveira 1993) that honeydew droplets that fall on lower leaves and ground from *G. xiphias* aggregations could serve as cues to potential tending ants. This behaviour pattern would be particularly important for the survival of developing brood by minimizing mortality due to predation and parasitism at early stages (Del-Claro 1995). In the present paper we tested whether honeydew flicking by nymphs of *G. xiphias* increased the probability that ground-dwelling ants climbed onto the host plant and tended the treehoppers.

METHODS

We conducted field work in an area of cerrado vegetation near Mogi-Guaçu, State of São Paulo,

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southeast Brazil. We tagged 10 experimental pairs of *Didymopanax vinosum* (Araliaceae), an abundant shrub in the study area that frequently hosts aggregations of *G. xiphias* near the apical meristem of the principal stem. Plants within a pair were approximately the same height (1–2 m tall) and at least 4 m apart from each other, had similar numbers of leaves and stems, and were in the same phenological state. Experimental plants were not occupied by homopterans or ants, and did not present any traces of recent use by *G. xiphias* (such as feeding marks on the stem, or spots of dried honeydew and associated fungi). We randomly designated each shrub in a given pair as a treatment or control plant by the flip of a coin. We introduced a group of 5 nymphs of *G. xiphias* (3rd–4th instar) near the apical meristem of the principal stem of treatment plants. No manipulation was performed on control plants. Experimental *G. xiphias* were obtained from aggregations occurring on non-tagged shrubs of *D. vinosum*. Introduced nymphs remained aggregated, and we allowed an establishment period of 40 min before we began to take data. Behavioural data on membracids and ant newcomers were simultaneously gathered for both plants of a pair from 0900 to 1400 hours. For 1 min, at 1-h intervals, we counted both the number of ants inside a 0.6-m diameter circle on the ground (plant in center) and the number of ants climbing onto the plant. We sampled all plant pairs on 9 May 1993.

A final experimental series aimed to test whether honeydew-soaked filter paper would induce ground-dwelling ants to climb onto homopteran-free host plants. Filter papers were soaked with three honeydew droplets obtained directly from ant-excluded *G. xiphias* aggregations. We tagged 10 additional experimental plant pairs similar to the ones described above. In each plant pair we placed four pieces (ca 1.0 × 2.0 cm) of honeydew-soaked filter paper beneath the treatment plant, and four pieces of water-soaked (three droplets) filter paper beneath the control plant. Filter papers were distributed within a 0.6-m diameter circle on the ground (plant in center). For 1 h, at 15-min intervals, we counted the number of ants visiting the filter papers on the ground (snapshot records), as well as the number of ants climbing up the trunk of each plant of an experimental pair. We simultaneously gathered data for both plants of a pair. All plant pairs were sampled on 3 February 1995 from 0900 to 1400 hours.

All field data were taken under warm and humid climate conditions. Data were analysed by repeated measures ANOVA, because ant counts at different times are not independent of each other.

RESULTS

Honeydew Flicking by Treehoppers and Ant Behaviour

Honeydew secretion by nymphs of *G. xiphias* was conspicuous from the beginning of our observations. Within the first hour, flicked honeydew was already detectable on lower leaves and on the ground beneath treatment plants (up to $\simeq 0.3$ m away from trunk). We recorded 10 ant species collecting honeydew from isolated droplets or through tending the membracids: *Camponotus* (4 species), *Brachymyrmex*, *Ectatomma*, *Pseudomyrmex*, *Zacryptocerus*, *Crematogaster* and *Pheidole* (1 species each).

Upon finding a honeydew droplet on the ground, the alerted ant typically began to search around the immediate vicinity, eventually climbing onto the host plant. The ant inspected any additional honeydew droplets that it found on the leaves while climbing up the plant, and eventually found the nymph aggregation as it reached the apical meristem. After collecting some honeydew from the nymphs, the ant would descend the plant and walk directly to her nest. Soon afterwards (10–15 min) a group of workers left the nest, climbed up the plant, and began to tend the membracids. We recorded this behavioural sequence twice for the ant *Ectatomma edentatum*, and once each for *Camponotus crassus*, *C. rufipes* and *Zacryptocerus pusillus*.

Ant Visitation to Host Plants versus Treehopper Presence

Host plants with *G. xiphias* attracted more ground-dwelling ants than plants lacking treehoppers (Fig. 1a, b). More ants were found beneath host plants with treehoppers than beneath plants lacking treehoppers (two-way ANOVA, $F_{1,18} = 5.88$, $P = 0.026$), with no significant interaction between sampling time and

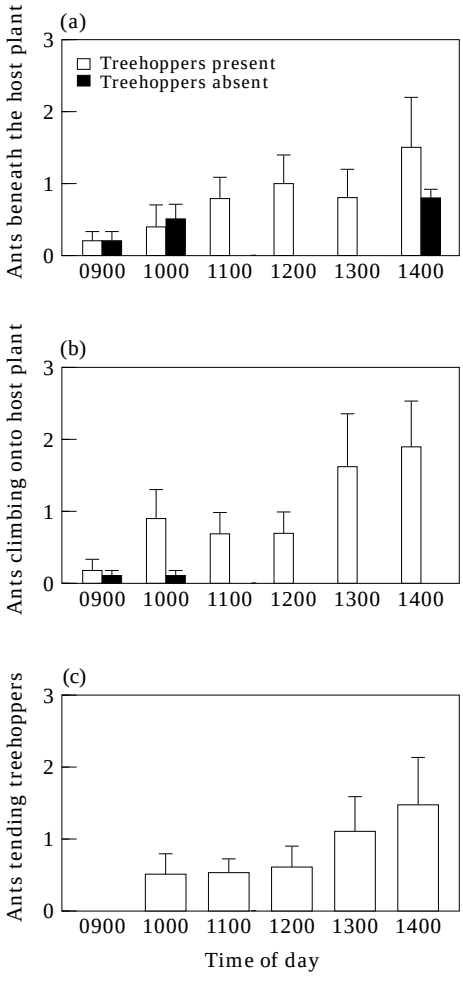

Figure 1. Ant activity in the immediate vicinity and on the foliage of *Didymopanax vinosum* with and without treehoppers, *Guayaquila xiphias*. (a) Number of ants inside a 0.6-m diameter circle beneath the plant, (b) number of ants climbing up the plants, (c) number of ants engaged in tending activity. Values are means + 1 SE; $N = 10$ plants per experimental group (see text).

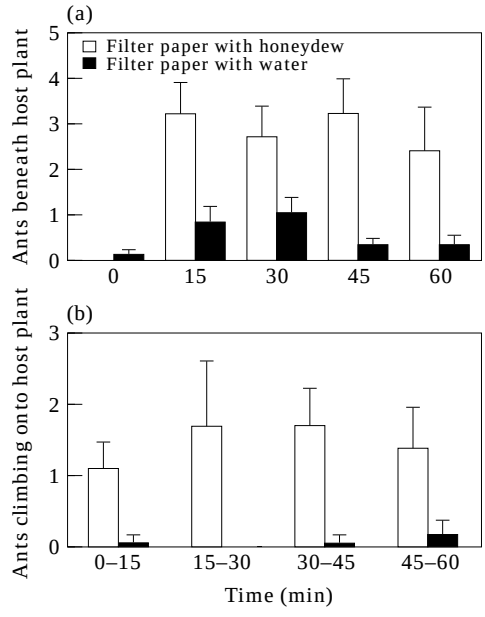

Figure 2. Ant activity in the immediate vicinity and on the foliage of homopteran-free shrubs of *Didymopanax vinosum*. (a) Number of ants visiting pieces of filter paper (with honeydew or water) inside a 0.6-m diameter circle beneath the plant, (b) number of ants climbing up the plants. Values are means + 1 SE; $N = 10$ plants per experimental group (see text).

treatment ($F_{5,90} = 1.39$, $P = 0.23$; Fig. 1a). The number of ants climbing onto host plants also differed significantly between plants with and without treehoppers (two-way ANOVA, $F_{1,18} = 28.03$, $P = 0.004$). We found a significant interaction between sampling time and treatment ($F_{5,90} = 2.44$, $P = 0.04$), indicating recruitment by the ants to the host plant with treehoppers (Fig. 1b). In fact, after climbing onto the host plants, the number of ants engaged in tending activities on homopteran aggregations increased with time (one-way ANOVA, $F_{5,45} = 2.44$, $P = 0.04$; Fig. 1c).

Honeydew as Cues to Ground-dwelling Ants

The results from the experiments with soaked filter papers confirmed the role of honeydew droplets as an ant attractant. More ants were found visiting honeydew-soaked filter papers beneath treatment plants than water-soaked filter papers beneath control plants (two-way ANOVA, $F_{1,18} = 13.52$, $P = 0.002$), with a significant interaction between sampling time and treatment ($F_{4,72} = 3.53$, $P = 0.011$; Fig. 2a). The number of ants climbing on plants was also significantly greater on treatment plants than on control plants (two-way ANOVA, $F_{1,18} = 15.89$, $P = 0.001$). No significant interaction was found between sampling time and treatment, however, indicating no recruitment activity by ants to either experimental plant class ($F_{3,54} = 0.28$, $P = 0.84$; Fig. 2b). Upon finding the honeydew-soaked filter

papers beneath treatment plants, ground-dwelling ants behaved in much the same way as described above for plants occupied by treehoppers. Although both plants of a pair were free from homopterans, on 13 occasions we observed ants climbing up to the apical meristem of treatment plants. Water-soaked filter papers beneath control plants did not elicit such a response by the ants.

DISCUSSION

Homopteran honeydew consists of a mixture of nutrients, including sugars, amino acids, amides, proteins, minerals and vitamins (Hölldobler & Wilson 1990, and included references). Although the use of honeydew varies widely between different ant species, it can be a major part of the diet of many ants (Buckley 1987a, b; Sudd 1987; Rico-Gray 1993). The occurrence of honeydew droplets on lower foliage and on the ground beneath untended homopterans is well documented (Douglas & Sudd 1980; Beattie 1985; Buckley 1987 a, b; Hölldobler & Wilson 1990), but its ecological consequences for the organisms involved are still unclear. The deposition of melezitose (a trisaccharide found only in homopteran honeydew) on the ground has been suggested to stimulate non-symbiotic nitrogen fixation in the soil to the benefit of the host plant (Owen & Wiegert 1976; Owen 1978). Petelle (1980) discussed data suggesting that fructose, another component of honeydew, increases nitrogen fixation nine-fold more than does melezitose. Kiss (1981), on the other hand, contended that the synthesis of melezitose may have evolved to attract tending ants. Douglas & Sudd (1980) stated that honeydew droplets are unlikely to be attractive to ants, because they have seen uncollected droplets on aphids and on twigs being ignored by attendant ants. More recently, Grier & Vogt (1990) showed that honeydew reaching the soil results in decreased ammonification and nitrification, and reduces above-ground net primary production and nitrogen uptake by trees.

The accumulation of honeydew on the bodies of untended homopterans may result in potentially harmful fungal infections (Beattie 1985; Buckley 1987a, b). Therefore the flicking of honeydew in the absence of ants may be related to avoidance of fungal damage. Irrespective of the primary factor favouring honeydew flicking in homopterans, however, our results show that the occurrence of scattered honeydew on lower leaves and ground incidentally serves to attract potential tending ants.

To our knowledge, the current study is the first experimental demonstration that honeydew flicking by treehoppers provides cues to ground-dwelling ants, which significantly increases the probability that these ants will climb onto the host plant and tend the homopterans. We also previously speculated that such a tactic might also be used by females of *G. xiphias* during the oviposition phase (Del-Claro & Oliveira 1993). In fact, on three different occasions, we observed ants climbing on plants after discovering honeydew droplets flicked by single *G. xiphias* females with egg masses. After finding the female and her brood, the ants immediately began to tend them. Honeydew flicking by *G. xiphias* females may also attract ants to small nymphs.

Attraction of ants through honeydew flicking is obviously advantageous for *G. xiphias* in view of the benefits it receives through ant-tending, including protection from predators and parasitoids (Del-Claro 1995). We have censused more than 100 aggregations of *G. xiphias* in the study area, and we have never observed treehoppers untended by ants (Del-Claro & Oliveira 1993), which may suggest that ant attraction through honeydew flicking is efficient and/or ant availability is high in the cerrado. The outcome of homopteran–ant interactions may depend on several factors, such as the quality of the host plant and ant mutualists, homopteran attractiveness or proximity to ant nests (Cushman & Whitham 1989; Cushman 1991; Cushman & Addicott 1991). Future investigations should clarify how these factors can affect the interaction between *G. xiphias* and ants.

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