

The Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae: Dolichoderinae) is sensitive to semiochemicals involved in the spacing behaviour in the bird cherry-oat aphid *Rhopalosiphum padi* (Sternorrhyncha: Aphididae)

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Abstract. The behavioural response of an aphid-tending ant, the Argentine ant *Linepithema humile*, to semiochemicals related with spacing behaviour in the aphid *Rhopalosiphum padi* (L.) was evaluated. The compounds involved were 6-methyl-5-hepten-2-one (MHO), 6-methyl-5-hepten-2-ol (MHOH), and 2-tridecanone (2-T). Hexane solutions of these semiochemicals either singly or mixed, and extracts obtained by trapping volatile compounds from wheat seedlings either alone or infested with two different densities of aphids were used as stimuli in olfactometric bioassays. Ants showed attraction to volatile extracts from plants infested at both densities, and slightly preferred volatile compounds from lightly over heavily infested plants in a choice test. On the other hand, while a dose-dependent repellence response was elicited by the naturally-occurring mixture of MHO, MHOH and 2-T, single compounds did not elicit significant responses in olfactometric bioassays. The function of these semiochemicals as indicators of quality of the aphid colonies as trophic resource to ants is discussed.

INTRODUCTION

Ants and aphids are associated in a mutualistic relationship. Ants collect and ingest honeydew from aphids feeding on plants and, in return, protect them against their natural enemies, and also prevent fungal attack as a consequence of honeydew collection (Way, 1963). However, ants may prey on aphids under some circumstances (Sudd, 1987). When the mutualistic relationship is facultative, ants may exploit it in an opportunistic way. Ants search plants for aphid colonies and, once detected, they return to the nest laying a trail of recruitment pheromone. Exploitation of the discovered aphid colony will follow the recruitment of worker ants (Sudd, 1987). In cases where the relationship with ants is obligate for aphid survival, the aphid is less mobile and the connection lasts longer (Sudd, 1987).

The widely known use of chemicals for communication between conspecifics (Hölldobler & Wilson, 1990; Pickett et al., 1992) and between different species (Budenberg et al., 1992; Feener et al., 1996) has also been reported for the ant-aphid system. Thus, aphid honeydew acts as an attractant for ants (Ewart & Metcalf, 1956; Buckley, 1987). Furthermore, ants respond to the aphid alarm pheromone showing a protective behaviour of aphids (Nault et al., 1976).

Dependency of the ant-aphid interaction on the density of the aphid colony has been observed. Thus, increases in predation by ants (Sakata, 1994; 1995) and decreases in the

benefit accrued from attendance behaviour by ants (Breton & Addicott, 1992) have been related to increases in density of aphid colonies.

Given that ants are sensitive to aphid semiochemicals, and that the outcome of the ant-aphid interaction is affected by aphid colony density (Addicott, 1979), it is possible that ants are able to respond to semiochemicals related with aphid colony status. Recently, Quiroz et al. (1997) identified four volatile compounds [6-methyl-5-hepten-2-one, (+)- and (-)-6-methyl-5-hepten-2-ol] in 3 : 1 ratio, and 2-tridecanone) emitted from wheat seedlings heavily infested with the aphid *Rhopalosiphum padi* (L.) at cca 9 aphids/cm². Since these compounds were repellent to aphids (Quiroz et al., 1997), they could be considered as a mechanism of regulation of colony density to prevent overpopulation.

Although ant attendance of *R. padi* and other cereal aphids in the field is largely prevented by culturing practices (but see Vinson & Scarborough, 1991), it occurs in experimental fields with no major soil manipulation, as well as in wild grasses (Gianoli, pers. observ.), where an important phase of *R. padi* life cycle takes place (Wiktelius, 1987). The aim of this work was to evaluate the behavioural response of a common aphid-tending ant, the Argentine ant, *Linepithema humile* (Mayr) [= *Iridomyrmex humilis* (Mayr); Shattuck, 1992] (Formicidae: Dolichoderinae), towards the volatile compounds involved in the aphid spacing behaviour of the aphid, *R. padi*.

MATERIAL AND METHODS

Ants

L. humile workers were collected between March and July (autumn) from natural nests in the campus of the Faculty of Sciences, University of Chile (Santiago, Chile). The ants were tested in olfactometric bioassays immediately after collected.

Aphids

Adult aphids of *R. padi* collected from grass fields in Santiago were used to start aphid colonies. Aphids were kept on oat (*Avena sativa* L.) in growth rooms (18–22°C and 16L : 8D photoperiod). Viviparous apterae were used to infest plants utilised for the trapping of volatile compounds.

Plants

Wheat seedlings (*Triticum aestivum* L. cv. Paleta) were grown in a growth chamber (20°C and 12L : 12D photoperiod). Seedlings at growth stage 12 (Zadoks et al., 1974) were used in the trapping of volatile compounds.

Chemicals

6-methyl-5-hepten-2-one (MHO), 6-methyl-5-hepten-2-ol (MHOH), and 2-tridecanone (2-T) were purchased from Aldrich Chem. Co. and diluted with hexane before using in bioassays.

Entrainment of volatile compounds

Volatile compounds were trapped as described by Blight (1990). Air purified by passage through 5Å molecular sieves and activated charcoal was drawn at 1 liter/min during 48 h through two bell jars containing the odour sources. The odour sources comprised: (a) 14 wheat seedlings, (b) 14 wheat seedlings which were infested with a high load of aphids (cca 7–12 aphids/cm²) 24 h before the onset of volatile trapping, and (c) the same as (b), but most of the aphids being removed immediately before the onset of the trapping, resulting in a density of cca 1–2 aphids/cm². Cumulated aphid honeydew present in the trapping system was hence similar between treatments (b) and (c). The volatile compounds were adsorbed onto Porapak Q, washed from the adsorbent with 3 ml hexane, and concentrated to 750 µl under a stream of nitrogen. Aliquots of these extracts were used for olfactometric bioassays.

Olfactometry

The olfactometer consisted of a Petri dish 8.5 cm in diameter and 1.8 cm in height, without cap. Four equidistant holes were made through the walls close to the base and four teflon tubes were inserted into

them. Air previously dried and purified was introduced through the teflon tubes at a total flow rate of 500 ml/min. The arena was divided into five areas consisting of four semicircles (2.5 cm radius) in such a way that their centres were located in each hole and a fifth neutral area was formed by the rest of the arena (Lofgren et al., 1983). A tiny piece of cotton was placed inside the internal tips and impregnated with 10 μ l of either chemicals or extracts. Two of the four arms (tubes) of the olfactometer were assigned to contain the compound to be tested and the remaining two arms contained hexane and were used as controls. Treatment and control arms were placed alternated. The walls were covered with gypsum powder to prevent the escape of ants. A single worker ant was placed in the centre of the arena one minute after the placement of the chemicals to be used as stimuli. One minute later the test started. The position of the test ant in the arena was continuously observed during 10 min and recorded in an ad hoc program (Observer®). The total time spent in each area was exactly calculated by the program. The results were compared using the Wilcoxon matched pairs test. Given that the groups of workers showed a strong aggregation behaviour in previous bioassays, they were evaluated individually. Workers can forage individually for aphid colonies (Sudd, 1987; Schmid-Hempel, 1993) and hence this procedure was deemed accurate. Likewise, the suitability of this experimental design for evaluating ant foraging behaviour was tested by preliminary trials with sugar solutions.

Hexane solutions of the extracts obtained by trapping of volatile compounds, as well as of MHO, MHOH, 2-T, and a mixture of these three chemicals, were used in bioassays. The amounts of MHO, MHOH, 2-T used in olfactometric bioassays were similar to those reported following 24 h of collection of volatile compounds in the same biological system (aphid density on infested plants: cca 9 aphids/cm²) and their proportion in the mixture similar to that previously described (Quiroz, 1996). Since the olfactometric activity on aphids of both the racemic and the 3 : 1 mixture of MHOH enantiomers is similar (Quiroz & Niemeyer, 1998), the experiments were performed only with the racemate. This does not exclude, however, that ants may behave distinctly. Control olfactometry with pure hexane did not show significant activity, thus rendering it suitable as solvent.

RESULTS

Ants showed attraction to volatile extracts obtained from plants infested with aphids at both low and high densities. However, when confronted with a plants infested either with high or low aphid density, ants showed a slight, even though statistically significant, preference for volatile extracts from plants with a low aphid density (Table 1).

TABLE 1. Response of workers of the Argentine ant in an olfactometer to extracts from three sources: (a) wheat plants, (b) wheat plants infested with *R. padi* (cca 7–12 aphids/cm²) plus cumulated honeydew (heavy infestation), and (c) same as (b) but removing most of the aphids just before trapping, leaving a density of cca 1–2 aphids/cm² (light infestation).

Stimulus	Total time spent Mean \pm SE (min)	n	p*
Plants	3.3 \pm 0.3	10	0.037
Plants lightly infested	4.5 \pm 0.3		
Plants	3.4 \pm 0.3	12	0.028
Plants heavily infested	4.6 \pm 0.3		
Plants lightly infested	4.7 \pm 0.3	12	0.049
Plants heavily infested	3.3 \pm 0.2		

* Wilcoxon matched pairs test.

On the other hand, neither attraction nor repellence was observed in bioassays with single compounds (MHO, MHOH, 2-T). Interestingly, when their mixture at the proportion

naturally-occurring in dense aphid colonies on cereals was used as a stimulus, a non-significant trend to repellency was observed (Table 2). Consequently, dose-response studies were performed with the mixture of compounds. In addition, since MHO has been reported as alarm pheromone of some Dolichoderinae ants (Blum, 1969), this compound was also submitted to dose-response studies. Dose-dependent repellent activity was elicited by the mixture of compounds (Fig. 1); however, ants did not show significant responses to different amounts of MHO (Table 3).

TABLE 2. Response of workers of the Argentine ant in an olfactometer to hexane solutions of volatile compounds found in wheat heavily infested with *R. padi* (Quiroz et al., 1997). The quantities used in these bioassays were those corresponding to 24 h of volatile trapping. (MHO: 6-methyl-5-hepten-2-one; MHOH: 6-methyl-5-hepten-2-ol; 2-T: 2-tridecanone).

Stimulus	Total time spent Mean \pm SE (min)	n	p*
41 ng MHO	3.7 \pm 0.4	18	0.248
Hexane	3.9 \pm 0.4		
13.2 ng 2-T	4.1 \pm 0.5	14	0.331
Hexane	3.2 \pm 0.4		
12 ng MHOH	3.5 \pm 0.4	21	0.741
Hexane	3.8 \pm 0.4		
Mixture**	3.3 \pm 0.4	18	0.078
Hexane	4.5 \pm 0.5		

* Wilcoxon matched pairs test.

** MHO: 41 ng; 2-T: 13.2 ng; MHOH: 12 ng.

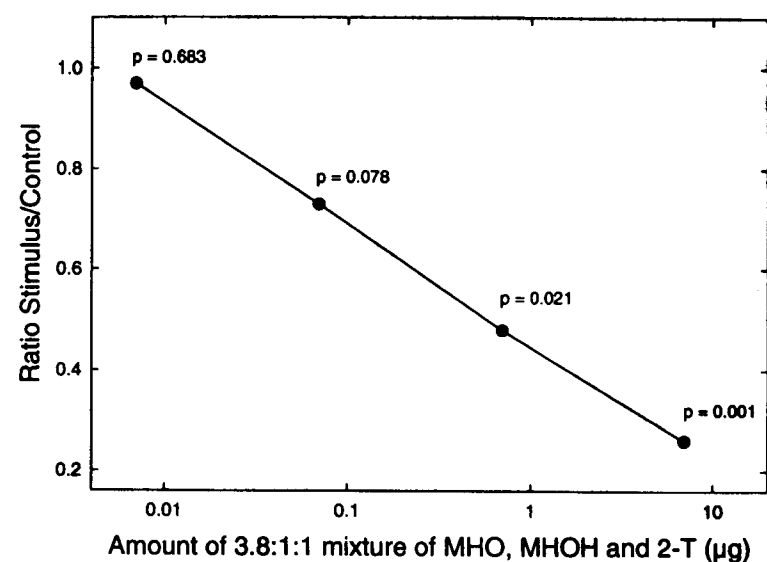


Fig. 1. Dose-response curve of workers of the Argentine ant in an olfactometer to different amounts of a mixture of MHO, MHOH and 2-T in hexane solutions, with hexane as a control. (MHO: 6-methyl-5-hepten-2-one; MHOH: 6-methyl-5-hepten-2-ol; 2-T: 2-tridecanone). The ratio stimulus/control (y-axis) indicates the relationship between the time spent by the ant in the treatment areas and the time spent in the control areas of the olfactometer. Thus, a ratio close to 0 means repellence and a ratio close to 1 means no effect on ant behaviour. Results were compared using the Wilcoxon matched pairs test. The p-values are shown above each point.

TABLE 3. Response of workers of the Argentine ant in an olfactometer to different amounts of 6-methyl-5-hepten-2-one (MHO) in hexane.

Stimulus	Total time spent Mean \pm SE (min)	n	p*
41 ng MHO Hexane	3.7 \pm 0.4 3.9 \pm 0.4	18	0.248
410 ng MHO Hexane	4.8 \pm 0.6 3.7 \pm 0.7	14	0.433
4,100 ng MHO Hexane	2.8 \pm 0.4 5.1 \pm 0.6	17	0.084

* Wilcoxon matched pairs test.

DISCUSSION

Honeydew is attractive to ants and one of the main food sources for the Argentine ant (Ewart & Metcalf, 1956; Markin, 1970). Thus, ant attraction to plants infested with aphids may be due to the cumulated aphid honeydew present on the experimental plants after 72 h of infestation by aphids (24 h for previous infestation plus 48 h for the collection of volatile compounds). Furthermore, results showed a tendency of ants to prefer volatile compounds from plants with a lower aphid load, but a similar amount of cumulated honeydew than on heavily infested plants (Table 1). These results might suggest the presence of substances repellent to ants in the plant with a high density aphid colony, these compounds partially counteracting the attractiveness of honeydew. In order to validate this assumption we evaluated the activity on ant behaviour of the volatile compounds (MHO, MHOH and 2-T) previously identified by Quiroz et al. (1997) from wheat heavily infested with aphids. This does not imply that these compounds are the only components of the extracts likely to affect ant behaviour. Other volatile compounds released from aphid-cereals system (Quiroz, 1996) have been shown to modify insect behaviour (Hardie et al., 1994, and references therein). Nonetheless, those three compounds clearly showed a significant effect on the dynamics of aphid colony in wheat plants (Quiroz et al., 1997). It was hence considered that a follow up of their effect on foraging ants in the same aphid-plant system would facilitate a biological interpretation of the results.

Although MHO, MHOH and 2-T tested individually did not elicit significant responses in ants (Table 2), ants were repelled by their mixture at high concentrations (Fig. 1). This suggests that the repellent activity of the mixture could be responsible for the observed ant preference to plants with a low density of aphids (Table 1).

MHO is reported as alarm pheromone for some *Iridomyrmex* species (Crewe & Blum, 1971) and other Dolichoderinae ants (Blum, 1969). However, in agreement with a previous work on the Argentine ant (Key et al., 1981), a typical alarm behaviour was not observed in our bioassays. This might substantiate early claims on the taxonomic distance of the South American *Iridomyrmex* to their Australian relatives (Crewe & Blum, 1971) as indicated by the transfer of American species from the genus *Iridomyrmex* to other genera such as *Linepithema* (Snelling & Hunt, 1975; Shattuck, 1992).

Chemicals are involved in ant social behaviour, such as recruitment, alarm, caste and nestmate recognition (Hölldobler & Wilson, 1990), as well as in their interactions with

other species. Thus, chemical signals from some species are used by ants as cues for defence, exploitation or predation (Post et al., 1984; Dettner & Liepert, 1994; Völkl et al., 1994). On the other hand, semiochemicals of plant, as well as insect origin, have been shown to disrupt foraging by ants (Henderson & Jeanne, 1989; Howard et al., 1989; Shorey et al., 1992; Sisk et al., 1996). Results obtained in this work lead us to speculate that the Argentine ant may use volatile compounds to know the quality and quantity of its trophic resource. This might be supported by the fact that this ant belongs to the subfamily Dolichoderinae which shows a well-developed habit of attending aphids (Sudd, 1987). Alternatively, the release of these chemicals could be interpreted as a mechanism of ant-avoidance by aphids at aphid densities at which risk of predation by ants increases and benefits of attendance decrease. However, since the source of these compounds (the plant, the aphid, their interaction) is not yet determined (Quiroz et al., 1997), an explanation focused on the ant behaviour is considered more adequate.

At first sight, and assuming adaptive behaviour in ants, our results seem counterintuitive because larger colonies of aphids should be preferred by ants given that they would produce more honeydew. Our results show a slight preference of ants for aphid colonies with low population density. We hypothesise that larger colonies of aphids do not necessarily constitute a high-quality trophic resource. Chemical changes in plants heavily infested by aphids (Miles, 1989a,b; Cabrera et al., 1994) may negatively affect aphid fitness (Dixon, 1985) and hence the quality of the honeydew produced (Buckley, 1987). It has been proposed earlier that the attractiveness of aphids for mutualistic interactions could be related with the quality of their honeydew, this trait being, in turn, related with the quality of the host plant (Cushman, 1991). Thus, these semiochemicals could be used as a signal of possible deterioration of the aphid colony as resource for ants through changes in plant quality or through fungal infestation of the honeydew produced by a large and prolonged aphid infestation. Further experiments evaluating whether ant fitness is actually affected by changes in aphid colony density are necessary to substantiate the proposed hypothesis.

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