

Agonistic Behavior of Argentine Ants to Scales and Scale Parasitoids and Their Cuticular Extracts

by

Dong-Hwan Choe & Michael K. Rust¹

ABSTRACT

The Argentine ant, *Linepithema humile* (Mayr), is an economically important tramp ant species that displaces native ants and other arthropods in agricultural, urban and natural settings. Even though Argentine ants are frequently described as aggressive, little research has been conducted on their agonistic behavior towards homopterans and parasitoids. An ethogram of the responses to scales and parasitoids was constructed that included antennating, lunging, opening mandibles and grabbing. *Linepithema humile* workers did not respond aggressively towards brown soft scale, *Coccus hesperidum* L., after they had tended them. They readily attacked the parasitoid, *Metaphycus flavus* (Howard), killing 33% of them within 1 h. The cuticular extracts of scales, aphids, and parasitoids applied to dead fruit flies triggered agonistic responses.

Keywords: agonistic behavior, cuticular extracts, *Linepithema humile*, *Coccus hesperidum*, *Metaphycus flavus*

INTRODUCTION

The Argentine ant, *Linepithema humile* (Mayr), is one of the most important invasive ant species in agricultural, urban, and natural environments with Mediterranean climates worldwide (Vega & Rust 2001). In agricultural and urban settings, *L. humile* maintains a trophobiotic relationship with various kinds of honeydew-producing homopterans such as brown soft scale, *Coccus hesperidum* L., black scale, *Saissetia oleae* (Olivier) (Markin 1967, Vega & Rust 2001), citrus mealybug, *Planococcus citri* (Risso), woolly white fly, *Aleurothrixus floccosus* (Maskell), and several aphid species (Smith 1965, Moreno *et al.* 1987, Thompson 1990).

Ants collect honeydew from homopterans, and defend them from parasitoids and predators. Argentine ants interfere with the activities of natural

¹Department of Entomology, University of California, Riverside, Riverside, CA 92521-0314

enemies of scale insects either by direct attack or incidental disturbance, inhibiting oviposition or feeding by parasitoids (Gullan 1997). The aggressiveness of ants towards predators and parasitoids depends on their innate aggressiveness (Buckley & Gullan 1991), distance of the scales from the ant nest (Way 1963), or availability of other food for the ants (Bartlett 1961). Behavioral responses of Argentine ants toward various kinds of scale parasitoids have been documented by Bartlett (1961), who showed that *L. humile* aggressively chased the scale parasitoid, *Metaphycus flavus* (Howard). However, in his study the agonistic behavior was not analyzed in detail but only ranked as low, medium, or high.

Ants use a wide variety of chemical signals and cues when interacting nestmates and other species. In some ant species, cuticular hydrocarbons play an important role in nestmate and species recognition. For example, *Lasius niger* L., which tends the black bean aphid, *Aphis fabae cirsiacanthoidis* Schrank, does not attack the aphid parasitoid, *Lysiphlebus cardui* (Marshall), because the parasitoid mimics the cuticular hydrocarbon profile of its aphid host (Liepert & Dettner 1996). *Paralipsis enervis* (Nees), a parasitoid of the root-feeding aphid, *Anoecia corni* Koch, mimics the chemical cues of its host to also avoid aggression by *L. niger* (Völkl *et al.* 1996).

However, the responses toward some cuticular chemical profiles are not necessarily fixed. *L. niger* is known to switch from tending to attacking aphids (Sakata 1994), and there are several reports of ant species preying on the homopterans formerly tended by them (Gullan 1997). *L. humile* is known to respond aggressively to and attack former nestmates reared on different insect prey, suggesting that diet altered the chemical cues on their cuticle (Liang & Silverman 2000).

In the present study, we examined how the invasive ant *L. humile* responded to different insects which they might frequently encounter in a citrus grove. Initially, we determined the behavioral responses of *L. humile* to brown soft scale, *C. hesperudum*, and its parasitoid, *Metaphycus flavus* (Howard). We also examined the agonistic responses of *L. humile* to the brown sowthistle aphid, *Uroleucon sonchi* (L.), which produces small quantities of honeydew and is not tended by *L. humile*. To determine whether cuticular components are responsible for ants discriminating between different insect species, differ-

ent cuticular extracts were applied to fruit flies and the behavioral responses of *L. humile* were recorded.

MATERIALS AND METHODS

Collecting and Maintaining Ants

Argentine ants were collected from the biological control grove on the University of California, Riverside campus. These citrus trees have never been treated with pesticides and they support large populations of homopterans, especially brown soft scales. Ant nests were dug up from the base of trees and transported to a laboratory chamber where they were extracted from the soil using a procedure described by Hooper-Bui and Rust (2000).

The inner walls of the plastic colony boxes (26.5 by 30 by 10 cm, Spectrum Containers Inc., Evansville, IN) were coated with fluoropolymer resin to prevent ants from escaping. Each box was provisioned with one polystyrene weighing dish (7 cm, Fisher, Tustin, CA) filled with water, and an 'ant condominium.' Ant condominiums were constructed using a procedure described by Soepronon and Rust (2004). This box served to house the ant colony.

Another plastic box (26.5 by 30 by 10 cm) with coated inside walls was connected to the colony box by a 50 cm Tygon tube (0.6 cm diam) to serve as a 'foraging arena.' To make the connection, a hole was drilled in the end of each box located in the middle of the bottom edge and fitted with a plastic micropipette tip using hot glue (Glue Sticks, Ace Hardware Corp., Oak Brook, IL). The micropipette tips were cut to permit ants access and connected with the Tygon tube (0.6 cm diam.). The foraging arena was provisioned with two polystyrene weighing dishes, one filled with 25% sugar water, and the other with pieces of fresh American cockroach, *Periplaneta americana* (L.).

The colony was maintained in the laboratory for several months before testing. The colony consisted of 10 to 20 queens, brood, and 2,000 to 5,000 workers.

Test insects

Fruit fly, *Drosophila melanogaster* Meigen, cultures were maintained on standard artificial diet. Adult flies were collected from the culture jars just prior to testing. Cultures of a scale parasitoid, *M. flavus*, were obtained from the insectary and quarantine facility at the University of California, Riverside.

The parasitoid and brown soft scale *C. hesperidum* populations had been maintained on excised *Yucca* sp. (Agavaceae) leaves for several generations (Bernal *et al.* 1999). Brown sowthistle aphids were collected from local plants. This species is mostly restricted to plants belonging to the tribe Lactuceae in the family Asteraceae (Compositae), especially sowthistle, *Sonchus oleraceus* L. (Carver 1999).

Interspecific interactions among ant, homopteran, and parasitoid

Behavioral interactions were observed in an arena designed for maintaining parasitoids, a yucca leaf infested with *C. hesperidum*, and foraging *L. humile* (Fig. 1). The arena consisted of an inverted transparent plastic cylindrical

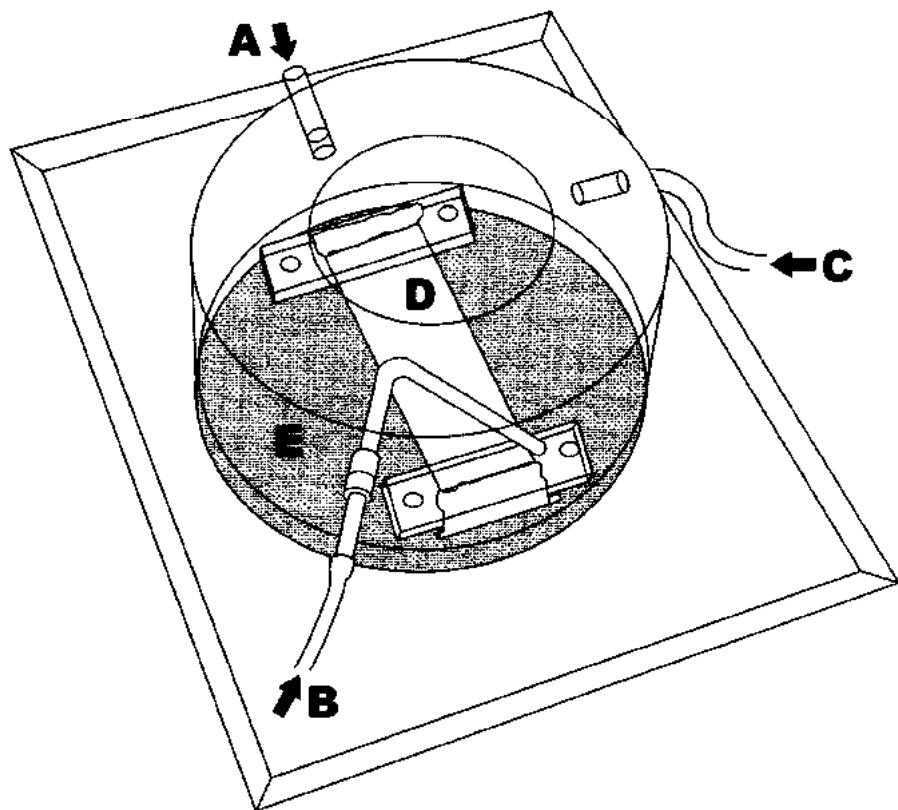


Fig. 1. The experimental arena for studying ant interactions. Two holes on the side of the arena were used to introduce *M. flavus* (A) or Argentine ants (B) into the arena containing a scale-infested yucca leaf (D). The other hole (C) was connected with a small air pump for air circulation. A water reservoir (E) under the floor of arena provided water for maintaining the yucca leaf.

container cover (19 cm diam by 1.5 cm high) attached to a plastic tray (26.5 by 30 cm) with hot glue. The space (1.5 cm high) between the cover and tray served as a water reservoir. Two slits (each 7 cm long) were made along the edge of the reservoir top. One end of a piece of dental wick protruded through each slit into the water reservoir, and the other end was placed between two plastic mounts (9 by 2.5 by 0.3 cm). The wicks provided water to the yucca leaf held between the plastic mounts. The water reservoir and yucca leaf were covered with a cylindrical plastic container (19 cm diam by 8 cm high) with the inside wall coated with fluoropolymer resin to prevent the ants and wasps from escaping. A hole (9 cm diam) was made in the center of the container top, and covered with a clear glass plate (10 cm diam) to permit observation and filming. Two additional small holes (1 cm diam) were also made in the top for air circulation and sealed with a fine nylon mesh to prevent the wasps from escaping. Three small holes (0.9 cm diam) were made on the side of plastic cylinder about 120° apart. Two of them were used for introducing ants and wasps, and the other one was used for air circulation. To introduce the wasps, a small glass vial containing the insects was attached to the cylinder over the hole. To introduce ants, the colony box was connected with the arena using Tygon vinyl tube (0.6 cm diam). To prevent moisture from accumulating inside the arena and obscuring observation, a small air pump (Whisper 600, Willinger Bros. Inc., Fort Lee, NJ) was connected to the arena with Tygon tube (0.6 diam.).

Ants were allowed to forage in the arena and began tending the brown soft scales on the yucca leaf. After 1 h, the interactions between the ants and scales were recorded. After the ants began tending scales, six female *M. flavus* were released into the arena. Within about 10 min, some of the wasps arrived on the yucca leaf and began host-searching behavior. The entire interaction between the ants and wasps was recorded using a digital camcorder (XL-1, Canon Inc., Lake Success, NY) equipped with a 100 mm macro lens. The recorded image was repeatedly observed on a separate television monitor by using the slow-motion and stop-action features of the camcorder.

Interactions between ants and aphids (*U. sonchi*) were observed by introducing a sowthistle stem infested with the aphids into the foraging arena. The sowthistle plant was maintained by placing the stem into a small glass vial filled with water. The gap between the stem and the vial edge was sealed

with Parafilm (American National Can Co., Menasha, WI). Thus, the foraging ants had easy access to the aphids via the vial surface and stem. The entire interaction between the ants and aphids was observed until the ants no longer climbed the stem.

Chemical extracts

Cuticular extracts of *M. flavus*, fruit flies, brown sowthistle aphids, and brown soft scales were prepared by first killing the insects in a freezer at -50°C overnight. The fruit flies were first washed in distilled water before freezing them to remove any artificial diet adhering to their cuticle. The insects (50 wasps, 10 fruit flies, 10 aphids, and 10 scales) were placed in separate clean 1.5-ml microcentrifuge tubes containing 100 μl hexane. The tube was shaken gently by hand for 1 min, and the hexane supernatant was transferred to a clean tube. Extracts were used in the behavioral bioassays within 10 min.

Arena bioassay

The aggressive behavior was categorized by observing *L. humile's* behavior toward dead fruit flies in the foraging arena. Observations were started when the first ant encountered the fruit fly, and terminated when a foraging ant picked up the fruit fly. An ethogram was constructed based on different behavioral categories observed and possible responses to each category. The ethogram was designed to show the sequential progression of behaviors and the likelihood of these occurring within an observed population (Nurindah *et al.* 1999).

The responses of worker ants to different cuticular extracts were determined by coating dead fruit flies with extracts and exposing them to ants in the foraging arena. Fruit flies were killed by freezing them in a freezer for 5 min at -50°C . Then they were washed with hexane to remove their cuticular lipids and allowed to dry for 10 min. Approximately 8 μl of extract was applied to the pre-washed fruit fly with a 10 μl capillary tube (Drummond Scientific Company, Broomall, PA) and the hexane was allowed to evaporate before additional extract was applied. This amount represented one insect equivalent for the fruit fly, brown sowthistle aphid, and scale, and five insect equivalents for *M. flavus*.

Using clean forceps, a single treated fruit fly was placed on an ant foraging trail in the arena. The entire behavioral response was recorded using the digital camcorder from the first encounter of the foraging ant and treated fruit fly until the fruit fly was removed from its original place by the ant. The final place where the fruit fly was carried was noted. For each extract, 10 fruit flies were treated and tested.

Data analysis

We assessed the effect of different extracts on ant behavior by comparing the proportions of specific responses to whole encounters (i.e. proportion of aggression to total encounters) as well as comparing the first-order transitions (Slater 1973, Hagler *et al.* 2004) from a preceding behavioral sequence to a succeeding behavior (i.e. the transition from initial aggression to successful grabbing). Because the numbers of encounters were highly variable, the arena bioassay data were pooled for each extract. For the pooled data, chi-square tests were used to test differences in the proportions of each behavioral category and the transitions from one behavior to the other. Yates' correction for continuity for small sample size was applied to the binary comparison data to minimize the value of the χ^2 calculated statistic, resulting in a more conservative chi-square test (Zar 1999). The specific transition rates from one behavioral sequence to the other within each trial were analyzed with a one-way ANOVA. Means were compared with the Tukey's honestly significant difference test (HSD).

RESULTS

Ant-scale interactions

Within 1 h after introducing ants into the arena, *L. humile* began tending the brown soft scales. Foraging ants showed typical 'scale tending behaviors' such as palpitating or caressing the scales around their anal plates for several seconds with their antennae to induce scales to excrete honeydew (Gullan 1997), a behavior referred to as solicitation (Hölldobler & Wilson 1990).

The presence of tending ants changed the manner in which *C. hesperidum* excreted honeydew. The honeydew droplet was held stationary between the splayed anal ring setae until ants consumed it rather than being propelled

away by a sudden withdrawal of the anus and bunching of the setae (Gullan 1997). The anal ring setae were withdrawn only after the honeydew was consumed by the ants.

No aggression of Argentine ant foragers towards scales was observed when the ants were tending them. Ants did not flare their mandibles when soliciting the scales.

Ant-parasitoid interactions

After locating a host scale, the female *M. flavus* parasitoid stopped moving and began host examination behavior, climbing onto the host scale and antennating it. During this exploratory phase, the parasitoid often encountered ants collecting honeydew on the yucca leaf. When an ant's antennae touched the wasp's body, the ant immediately responded aggressively by pursuing and trying to grab the parasitoid with its mandibles. In some encounters, the wasp managed to escape by flying away. In others, the wasp was seized by the ant and killed. After handling the wasp, the ant carried it to the nest (Fig. 2). Ants captured 33% of the wasps within 1 h.

Scale-searching *M. flavus* were sensitive to the close approach of ants, and they were easily distracted by nearby ants, causing them to run or fly away.

Ant-aphid interactions

When an ant encountered a *U. sonchi* aphid on a sowthistle stem, both the ant and the aphid displayed mutual aggression. The ant opened its mandibles, and displayed alerted behavior. In most cases, the ants encountered the front of the aphids because most *U. sonchi* were facing downward on the stem. When the ant encountered the aphid, the aphid displayed 'defensive' behavior by raising and waving the tip of its abdomen. Most of the ants retreated, but some attempted to seize the aphid. When the aphid was seized by an ant, it secreted droplets from the tips of its cornicles, and bent its abdomen forward to wipe the droplets on the ant (Fig. 3). These lipid droplets coalesced on the ant rapidly crystallizing to form a hard waxy plaque (Edwards 1966). Ants were unable to remove their antennae, legs, and mandibles from the wax once they contacted it. Consequently, the ants were not able to recruit other ants to the sowthistle and aphids.

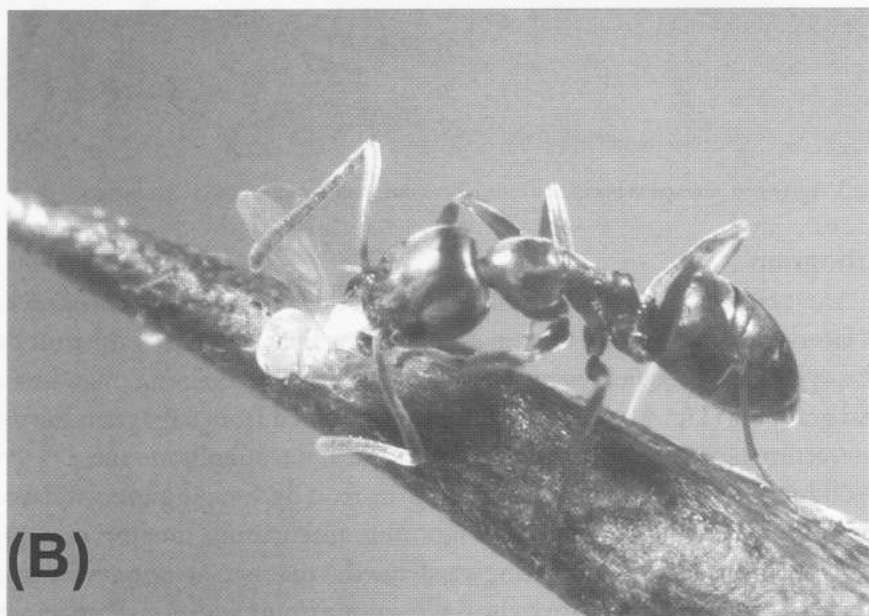
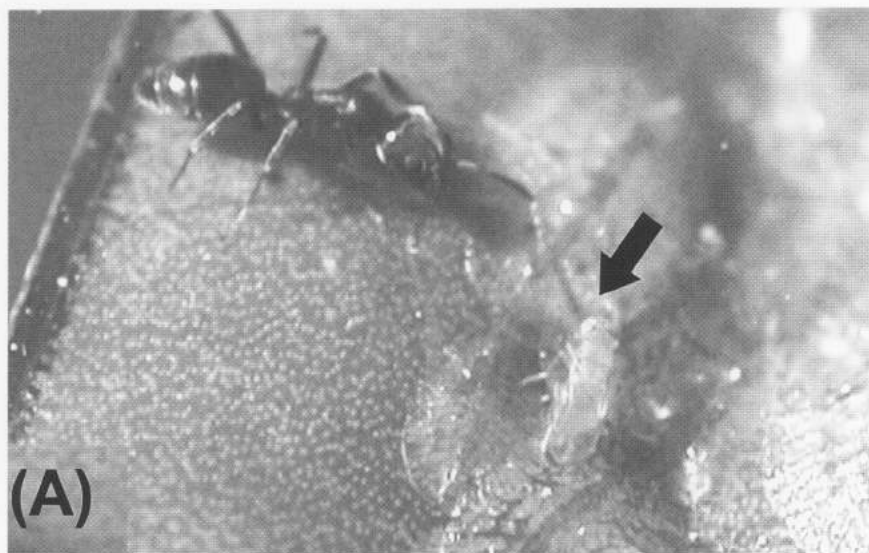


Fig. 2. The interaction between Argentine ant foragers and *Metaphycus flavus*. (A) Argentine ant antennating *Metaphycus flavus* wasp (arrowed) in the experimental arena. When its antennae touch the wasp's surface, the ant immediately lunges towards the wasp with open mandibles. (B) Grabbing behavior — an Argentine ant has successfully caught a parasitoid and is handling the wasp with her mandibles.

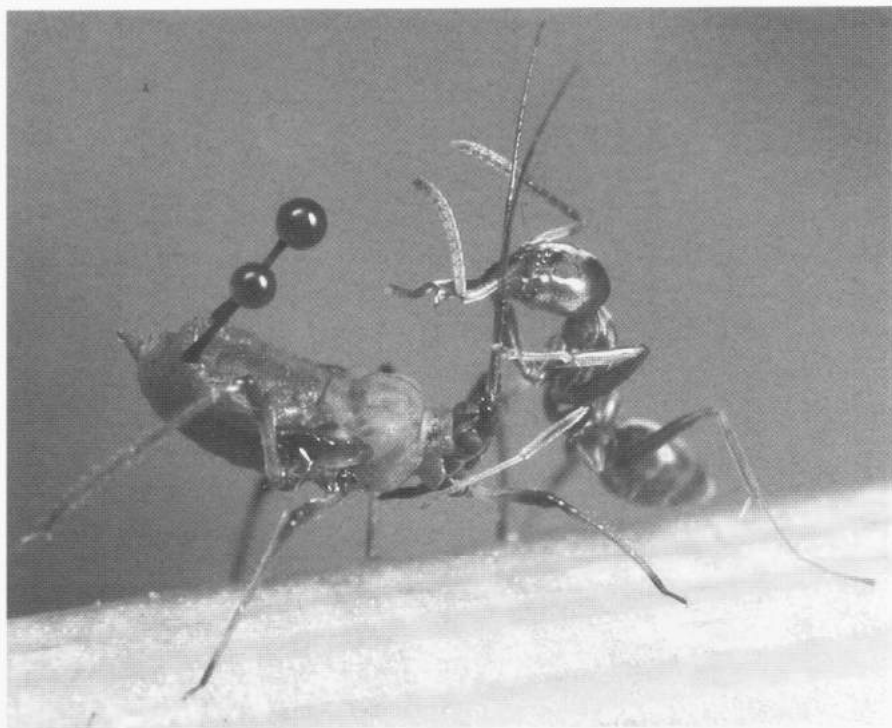


Fig. 3. Aggressive interaction between a brown sowthistle aphid and Argentine ant forager.

Ethogram

Ants reacted aggressively toward dead fruit flies. In aggressive encounters, *L. humile* workers opened their mandibles, attacking and seizing the fruit fly. The aggressive behaviors were categorized as lunging, opening mandibles, and grabbing (Fig. 4). The ethogram was modified from a diagram for the interaction between the root aphid parasitoid and trophobiotic ant, *L. niger* (Völkl *et al.* 1996). When encountering the fruit fly, foraging ants displayed one of the following five behaviors: ignoring, antennating, lunging, opening mandibles, and grabbing. Ignoring was defined as the ant making physical contact with the fruit fly but otherwise not resulting in any additional behavioral sequences and walking away. This could occur at any time in the sequence. Antennating consisted of the ant making physical contact by tapping the fruit fly at least twice with the antennae. The antennating behavior resulted in the ants ignoring the fruit fly or initiating the following three aggressive

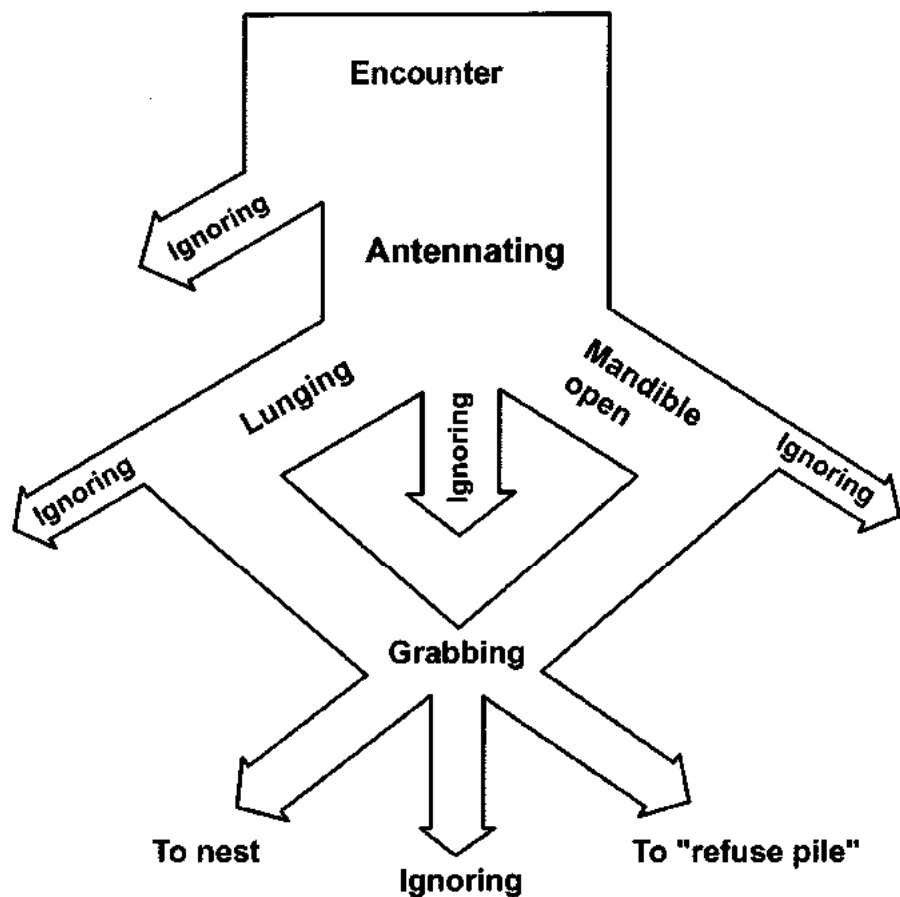


Fig. 4. A flow chart of behavioral responses of foraging Argentine ants towards a dead fruit fly.

behaviors. Lunging consisted of the ant running toward the fruit fly with mandibles open, usually resulting in a quick capture. Opening mandibles consisted of the ant opening its mandibles without lunging, usually resulting in a slow capture. Grabbing consisted of the ant seizing the fruit fly with its mandibles after which the fly was removed, dropped or ignored.

Handling and carrying behavior

When foraging ants found the fruit fly, they began to manipulate it. The handling behavior was characterized by antennating, then grabbing and mutilating the fruit fly's wings. When the ant carried the fruit fly in its mandibles,

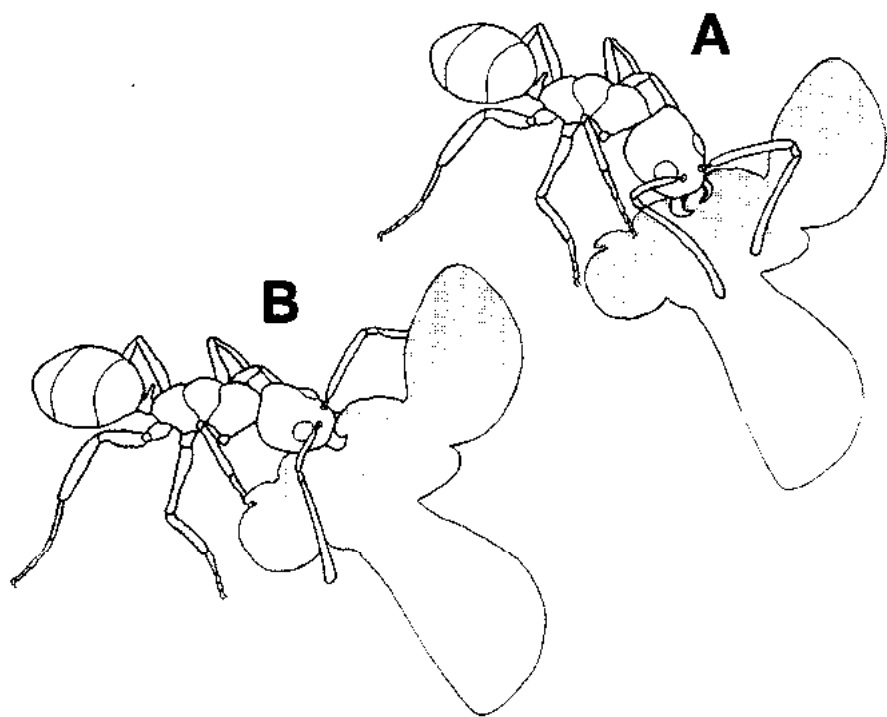


Fig. 5. Two different types of antennation. (A) 'prey antennating behavior' and (B) 'trail antennating behavior.'

the ant began antennating the trail instead of the fruit fly. We refer to this as 'trail antennating behavior' (Fig. 5A). This behavior was distinguished from the handling behavior in at least two respects: (1) the worker stopped antennating the fruit fly and started antennating the arena floor while holding the fruit fly in its mandibles, and (2) this wider antennating motion inhibited other ants from aggregating around the fruit fly.

Response to cuticular extracts

Fruit flies coated with *M. flavus* extract resulted in a greater proportion (76.1%) of aggressive encounters (i.e. lunging and opening mandibles) than did the fruit flies coated with fruit fly extract (59.2%) or *U. sonchi* extract (69.2%) (Fig. 6). Ants were significantly more aggressive to the *M. flavus* and scale extracts than they were towards the fruit fly extract (*M. flavus* extract – fruit fly extract: $\chi^2 = 6.03$, $df = 1$, $P = 0.01$; *C. hesperidum* extract – fruit

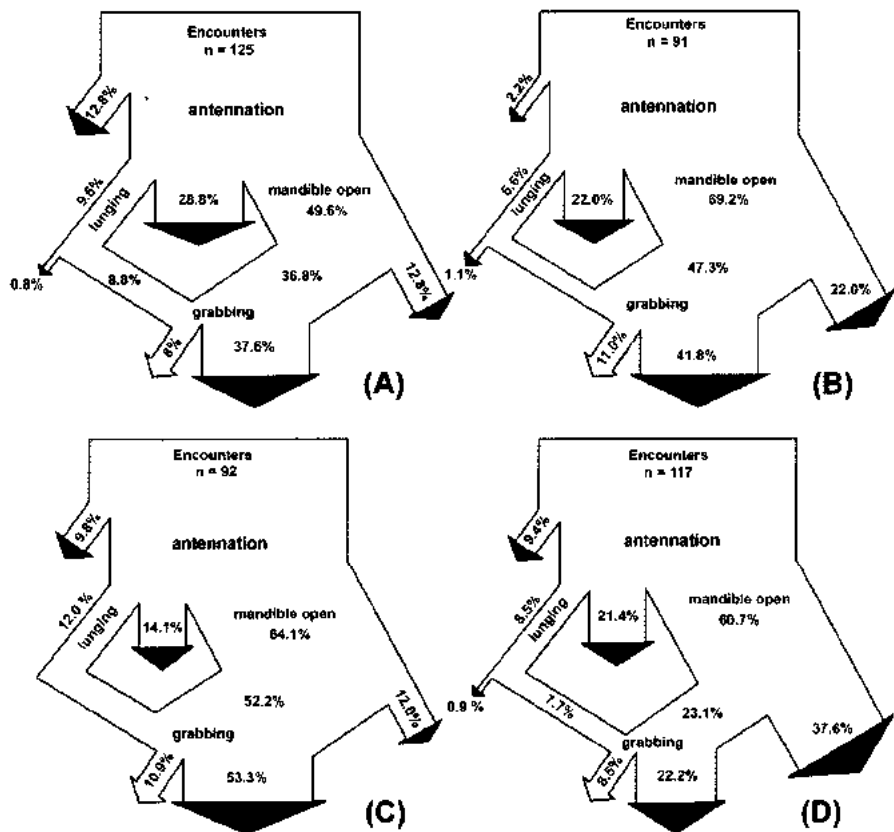


Fig. 6. The ethograms of a laboratory colony of Argentine ants toward fruit flies treated with different insect extracts: (A) fruit fly extract, (B) scale extract, (C) *M. flavus* extract, and (D) *U. sonchi* extract. The shaded arrows represent the proportion of ants that ignored treated fruit flies resulting in no succeeding behavioral sequences.

fly extract: $\chi^2 = 5.78$, $df = 1$, $P = 0.01$). However, there was no significant difference between the responses towards the untreated fruit fly and *U. sonchi* extracts ($\chi^2 = 2.22$, $df = 1$, $P = 0.14$).

U. sonchi extract deterred workers from grabbing the fruit fly). When exposed to *U. sonchi* extract, the percentage of encounters transitioning from initial aggression to ignoring (i.e. even though ants initially showed aggression such as lunging or opening mandibles towards the treated fruit fly, they failed to grab it) was greater than it was for *M. flavus*, fruit fly, and scale extracts [*U. sonchi*, 55.6% (45/81); *M. flavus*, 15.7% (11/70); fruit fly, 23.0% (17/74);

Table 1. Transition rates (mean \pm SEM) from the preceding behavioral sequences to aggression or successful grabbing of fruit flies treated with different extracts. Each value stands for the frequency of the behavioral response from the preceding level.

| Behavioral response | Transition rate (mean \pm SEM) ^a | | | |
|--------------------------------------|---|-------------------|-------------------|----------------------|
| | <i>M. flavus</i> | Fruit fly | <i>U. sonchi</i> | <i>C. hesperidum</i> |
| Aggression after encounter | 0.78 \pm 0.006a | 0.67 \pm 0.090a | 0.73 \pm 0.043a | 0.81 \pm 0.051a |
| Successful grabbing after aggression | 0.81 \pm 0.054a | 0.82 \pm 0.064a | 0.47 \pm 0.097b | 0.71 \pm 0.074ab |

^aMeans in a row followed by different letters are different (ANOVA, Tukey's HSD, $P < 0.05$)

C. hesperidum, 30.4% (21/69)] (Fig. 6). Thus, the actual percentages of encounters transitioning from initial aggression to successful grabbing were significantly different between *U. sonchi* and fruit fly extracts ($\chi^2 = 15.78$, $df = 1$, $P < 0.001$). However, there were no significant differences in the transition rate from initial aggression to successful grabbing among the *M. flavus*, scale, and fruit fly extracts (*M. flavus* extract vs. fruit fly extract: $\chi^2 = 0.79$, $df = 1$, $P = 0.37$; *C. hesperidum* extract vs. fruit fly extract: $\chi^2 = 0.67$, $df = 1$, $P = 0.41$). For the cases in which the fruit fly was carried away in subsequent encounters (e.g. trials with at least two ant encounters), the overall ANOVA indicated that great proportion of ants exhibiting initial aggression (lunging or opening mandibles) actually failed to grab or seize the fruit fly when it was coated with *U. sonchi* extract. The number of encounters transitioning from initial aggression to successful grabbing was significantly smaller for fruit flies treated with *U. sonchi* than those for fruit flies coated with *M. flavus* or fruit fly extract, but not significantly different with that for fruit flies coated with scale extract ($F = 4.63$; $df = 3, 29$; $P = 0.009$) (Table 1, Fig. 7). However, the transition rates from encounter to aggression were not significantly different among the extracts ($F = 0.94$; $df = 3, 29$; $P = 0.43$) (Table 1).

DISCUSSION

Linepithema humile workers do not respond aggressively towards brown soft scales, *C. hesperidum* after they have tended them, whereas they readily attack the scale parasitoid *M. flavus*. Our observations of the behavioral interactions among *L. humile*, *C. hesperidum*, and *M. flavus* are consistent

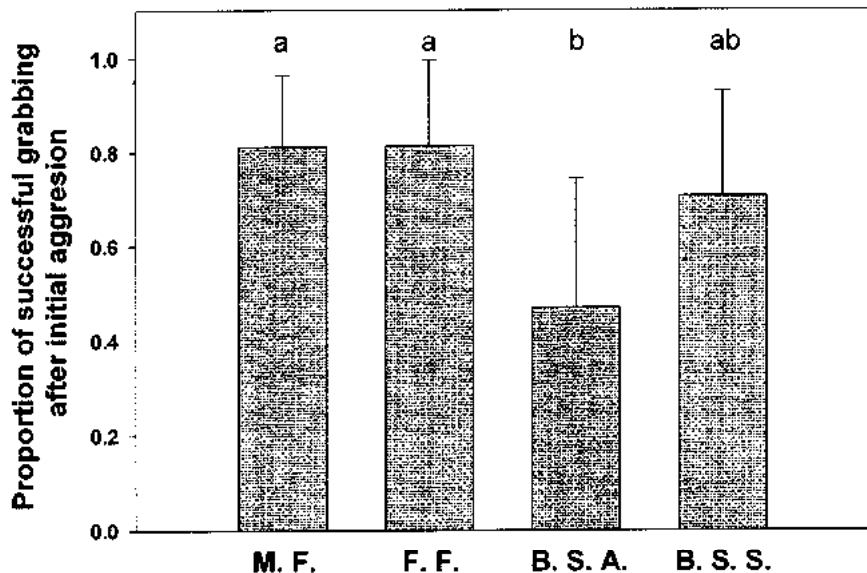


Fig. 7. The transition rates from initial aggression to successful grabbing extract-treated flies by laboratory colony ants. M.F. = *Metaphycus flavus* extract, F.F. = fruit fly extract, B.S.A. = brown sowthistle aphid extract, and B.S.S. = brown soft scale extract. Bars with the same letter are not significantly different (Tukey's HSD, $P < 0.05$)

with the reported trophobiotic relationship. Predation of scale insects has been reported among several ant species involved in certain obligate ant-plant mutualisms, but there is no indication of significant levels of predation by the invasive *L. humile* upon soft scale pests (Gullan 1997). Our observations of aggressive behavior of *L. humile* towards *M. flavus* support Bartlett's (1961) findings in which he suggested aggression of *L. humile* towards *M. flavus* in the ant nest. However, Bartlett (1961) reported that the successful capture of *M. flavus* by *L. humile* was a rare event because wasp's searching and oviposition behavior was easily disturbed by nearby moving objects (e.g. scale-tending ants), and wasps jumped or flew away when disturbed. In our trial, 33% of the wasps introduced into the arena were successfully captured by ants and carried to the ant nest within 1 h after introduction. Thus, the capture of *M. flavus* by tending ants may occur relatively frequently at least in a confined environment (e.g. scale colony between the leaves). In some field studies, considerable indication of predation of a scale parasitoid (*Aphytis* sp.) by *L. humile* was reported (Heimpel *et al.* 1997).

To initiate trophobiosis, ants and aphids need adequate interspecific communication based on tactile, chemical, and other cues (Hölldobler & Wilson 1990). Several aphid species, such as *Aphis spiraeicola* Patch (Klotz *et al.* 2004) and *Aphis nerii* Boyer de Fonscolombe (Bristow 1991), are tended by *L. humile*. In these cases, the honeydew production is induced by the antennating movement of the ants, and the aphids stop ejecting the honeydew (Hölldobler & Wilson 1990). In contrast, when *L. humile* foragers encountered the aphid, *U. sonchi* in the arena, the encounter resulted in mutual aggression. Foraging ants readily recognized these aphids as being 'foreign' and opened their mandibles in an alerted posture while the aphids displayed defensive behaviors (i.e. production of cornicle secretion, raising abdomens) once the antennae of *L. humile* touched them.

The cornicle secretions of *U. sonchi* act as an effective defense against *L. humile*. It is known that some myrmecophilous aphids such as *Aphis fabae* (Scop.) use cornicle secretions as an effective defense against small predators such as the parasitoid *Aphidius* sp. (Edwards 1966). Nault *et al.* (1976) suggested that the cornicle defensive substances of myrmecophilous aphids alert both the host ants and conspecific aphids, consequently letting the ants quickly attack foreign invaders. *Uroleucon sonchi*, unlike myrmecophilous aphids which usually have poorly developed cornicles (Hölldobler & Wilson 1990), have well developed, long cornicles. The cornicle secretions of *U. sonchi* probably defend them against parasitoids and predators, accounting for the incompatibility with Argentine ants.

Cuticular lipids play an important role in species recognition in ants (Leipert & Dettner 1993, 1996; Völkl *et al.* 1996; Liang & Silverman 2000), and specific hydrocarbon profiles of parasitoid species have been demonstrated to account for different aggression levels of ants towards them (Leipert & Dettner 1993, 1996; Völkl *et al.* 1996). Argentine ant workers responded more aggressively towards fruit flies coated with *M. flavus* cuticular extract than they did to flies coated with fruit fly cuticular extract. The ants' responses towards dead fruit flies coated with *M. flavus* cuticular extract were instantaneous, just like their responses to live *M. flavus*. Other factors such as movement by *M. flavus* may also alert the ant to its presence, but the cuticular extract clearly releases the aggressive attack.

Argentine ant workers were not significantly more aggressive towards fruit flies coated with *U. sonchi* cuticular extract than those coated with fruit fly cuticular extract. However, *U. sonchi* cuticular extract inhibited a significant proportion of *L. humile* foragers from successfully grabbing the treated fruit flies after the initial aggression. This finding was consistent with the behavioral response of *L. humile* to live *U. sonchi* in the arena experiment. One possible explanation is that some components of the cuticular extracts may be distasteful or repellent to *L. humile* workers (Eisner 1994). For example, several alkaloids in cuticular extracts of lepidopteran larvae make an otherwise palatable prey deterrent to *L. humile* foragers (Montllor *et al.* 1991).

Argentine ants were capable of recognizing cuticular components of *C. hesperidum* from their scale-tending experience, and learned not to attack and kill the scales. However, there was also a difference between the aggression of laboratory colony ants towards *C. hesperidum* cuticular extracts and the suppressed aggression in the scale-tending ants towards live *C. hesperidum*. Other tactile factors may be important in suppressing aggression. Sakata (1994) suggested that workers of *L. niger* were less aggressive towards the aphid species which they were tending than they were towards other myrmecophilous aphid species. It was suggested that ants 'memorize' characteristics of the aphid species which they are tending, and use those characteristics for making decisions between predation or tending on other individual aphids they encounter. In addition, Argentine ants have strong site fidelity, repeatedly returning to the same honeydew source from their nest (Fernandes & Rust 2003). Once they arrive at the scales previously tended by them, they began to collect honeydew by inducing scale insects to produce honeydew. Learning the odor cues associated with a food resource can lead to increased effectiveness for locating and harvesting it; learning how to handle a specific food source can greatly increase foraging success (Papaj & Lewis 1993). Learning will allow foragers to exploit new honeydew sources (i.e. different homopteran insects) more efficiently and quickly, which is especially important for an invasive tramp species such as the Argentine ant.

Even though Argentine ants were inherently aggressive toward any foreign cuticular chemical profiles, they were more aggressive to *M. flavus* extracts than they were to the other extracts. The cuticular extracts of brown sowthistle aphids deterred Argentine ants from grabbing. Additional studies

- Hooper-Bui, L.M. & M.K. Rust. 2000. Oral toxicity of abamectin, boric acid, fipronil, and hydramethylnon to laboratory colonies of Argentine ants (Hymenoptera: Formicidae). *J. Econ. Entomol.* 93:858-864.
- Klotz, J.H., M.K. Rust & P. Phillips. 2004. Liquid bait delivery systems for controlling Argentine ants in citrus groves (Hymenoptera: Formicidae). *Sociobiology* 43: 419-427.
- Liang, D. & J. Silverman. 2000. "You are what you eat": Diet modifies cuticular hydrocarbon and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften* 87: 412-416.
- Liepert, C. & K. Dettner. 1993. Recognition of aphid parasitoids by honeydew-collecting ants: the role of cuticular lipids in a chemical mimicry system. *J. Chem. Ecol.* 10: 2143-2153.
- Liepert, C. & K. Dettner. 1996. Role of cuticular hydrocarbons of aphid parasitoids in their relationship to aphid-attending ants. *J. Chem. Ecol.* 22: 695-707.
- Markin, G.P. 1967. Food distribution within colonies of the Argentine ant, *Iridomyrmex humilis* Mayr. Ph.D. Dissertation, University of California, Riverside.
- Montllor, C.B., E.A. Bernays & M.L. Cornelius. 1991. Responses of two hymenoptera predators to surface chemistry of their prey: significance for an alkaloid-sequestering caterpillar. *J. Chem. Ecol.* 17: 391-399.
- Moreno, D.S., P.B. Haney & R.F. Luck. 1987. Chlorpyrifos and diazinon as barriers to Argentine ant (Hymenoptera: Formicidae) foraging on citrus trees. *J. Econ. Entomol.* 80: 208-214.
- Nault, L.R., M.E. Montgomery & W.S. Bowers. 1976. Ant-aphid association: role of aphid alarm pheromone. *Science* 192: 1349-1351.
- Nurindah, B., W. Cribb & G. Gordh. 1999. Effects of physiological condition and experience on oviposition behaviour of *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae) on eggs of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). *Aust. J. Entomol.* 38: 104-114.
- Papaj, D.R. & A.C. Lewis (eds.) 1993. *Insect Learning: Ecological and Evolutionary Perspectives*, Chapman and Hall, New York.
- Sakata, H. 1994. How an ant decides to prey on or attend aphids. *Res. Pop. Ecol.* 36: 45-51.
- Slater, P.J.B. 1973. Describing sequences of behavior. p. 131-153. *In*: P.P.G. Bateson & P.H. Plopper (eds.). *Perspectives in Ethology*, Plenum, NY: Plenum Press.
- Smith, M.R. 1965. House-infesting Ants of the Eastern United States. *USDA Tech. Bull.* No.1326.
- Soeprono, A.M. & M.K. Rust. 2004. Effect of horizontal transfer of barrier insecticides to control Argentine ants (Hymenoptera: Formicidae). *J. Econ. Entomol.* 97: 1675-1681.