Argentine Ant (Hymenoptera: Formicidae) Trail Pheromone Enhances Consumption of Liquid Sucrose Solution

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ABSTRACT We investigated whether the Argentine ant, *Linepithema humile* (Mayr), trail pheromone, Z9–16:Ald, could enhance recruitment to and consumption of liquid sucrose solutions. All tests were done as paired comparisons with a 10% sucrose solution as food. In the laboratory, mixing 20 μl of a 10-μg/ml solution of the pheromone with 50 μl of the 10% sucrose solution increased the number of ants feeding by >150%. In a field test, we combined the trail pheromone with a 10% sucrose solution in 50-ml vials. These vials were covered with a plastic membrane that has 1.5-mm-diameter holes punched uniformly across its surface. Ants could drink from the holes after the vials were inverted. For half of the vials, 1 μg of the pheromone was put onto the plastic membrane before the vials were filled with a 10% sucrose solution. The remaining vials had no pheromone on the plastic membrane. After 4 h we measured the consumption in each vial. Bait consumption with the pheromone was enhanced by 29%. In a 2nd series of tests, vials were left outside for 24 h. The consumption rate was 33% higher with the pheromone compared with the controls that didn't have pheromone.

KEY WORDS *Linepithema humile,* Argentine ant, trail pheromone, bait

WILSON AND PAVAN (1959) and Robertson et al. (1980) showed that artificial trails from ventral organs (Pavan's gland) of the Argentine ant, *Linepithema humile* (Mayr), elicited trail following by workers of this species. Cavill et al. (1979, 1980) identified Z9–16:Ald as the behaviorally active compound in these glands. Van Vorhis Key et al. (1981) and Van Vorhis Key and Baker (1982) measured response rates to the pheromone and showed that Argentine ants will follow synthetic trails. They demonstrated that airborne trail pheromone could be detected and followed. Van Vorhis Key and Baker (1986) concluded that Z9–16:Ald is the most important component of the trail pheromone, although other synergists are possible. The synthetic form was 100 times less active than the natural compound (Van Vorhis Key and Baker 1982).

Van Vorhis Key et al. (1981) showed that ants could follow an airborne trail up to 6 mm away from its source. Furthermore, trail following diminished as the pheromone concentration increased beyond 1 ant equivalent of extract per 50 cm of trail. These results suggest that at higher concentrations the pheromone may be repellent. They also showed that on filter paper, activity declined to half the original level after ≈4 h and was almost gone completely after 8 h. Workers would follow trails longer if they had a previous encounter with a recruiting ant (Van Vorhis Key and Baker 1982).

Given these findings, we address the question of whether the synthetic trail pheromone can increase worker recruitment to liquid food sources, thereby enhancing consumption rates. Rather than attempting to describe which aspects of recruitment behavior are involved, we are using the word "recruitment" generically to refer to an increase in the number of feeding ants. This study was not designed to elucidate the mechanisms responsible for the observed behavior. An inexpensive form of the pheromone is commercially available, facilitating experimentation. As baiting technology advances, the use of natural products to enhance baits is a fertile area for research.

Materials and Methods

Argentine ant colonies were kept in the laboratory in plaster of paris nests inside a plastic shoe box (36 by 30 by13 cm). Each colony contained several thousand workers and several queens. They were fed a diet of 10% sucrose water and insects. The nest box was connected by a flexible plastic rod (4 mm diameter) to another plastic tray (44 by 56 by 12 cm) that served as a foraging area. Both the box and tray were coated with Fluon (Imperial Chemical Industries Americas, Bayonne, NJ) to prevent ant escapes. We did all laboratory pheromone tests on glass slides placed in the foraging box. Thus, foragers had to cross back over the connecting bridge to recruit workers. The slides were thoroughly cleaned with soap and water and rinsed with acetone between trials. Ants were provided water but no food for 1 d before tests.

The synthetic trail pheromone Z9–16:Ald was ordered from Aldrich, Milwaukee, WI. The pheromone has the appearance of a viscous oil at room tempera-

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ture. We dissolved it in hexane and then prepared serial dilutions, as described below.

Laboratory Tests. We prepared serial dilutions of the trail pheromone varying by 1 order of magnitude over the range of 0.01–100 μg of pheromone per milliliter of hexane solution. We put a 20-μl drop of each solution onto a depression slide and mixed it with 50 μl of 10% sucrose solution. After the hexane evaporated, the treated slide and a 2nd slide with only 10% sucrose solution were placed side by side in the foraging areas of 3 Argentine ant colonies. Every 3 min during a 30-min period we counted the number of ants feeding at the sucrose solution on each slide. In all, 21 paired choice tests were run over the 5 pheromone test-concentrations.

As a control we also ran 9 paired choice tests where 1 slide contained 50 μl of a 10% sucrose solution and the adjacent slide contained 20 μl of hexane (without the pheromone) plus 50 μl of the same sucrose solution.

We did statistical tests at each pheromone concentration by using a mixed model analysis of variance (ANOVA) where each of the 3 colonies represents a block (the random effect). Each block consisted of 10 paired measurements taken over the 30-min test period. The treatment effect was tested using interaction as the error term (Systat 1997).

Field Tests. Field tests were conducted during October and November 1997 at a citrus grove on the University of California-Riverside campus. We selected trees with active ant trails going up the trunks. The 10% sucrose solution was poured into 50-ml vials. Using the results of the laboratory tests as a guide, we pipetted 100 μl of a 10-μg/ml hexane solution onto a plastic membrane that contained evenly spaced 1.5-mm holes across its surface (Vispore Polymer Fabrics #6607, Tredegar Film Products, Richmond, VA). After the hexane evaporated, these membranes were placed over the vials and held in place by a cap that had its center removed to expose the membrane. When the vials were inverted, ants could drink from the small holes in the membrane. One control and 1 pheromone vial were taped side by side to each of 23 trees for ≈4 h, beginning at 1200 hours (PST). In a 2nd series of trials, 12 pairs of vials were left outside for ≈24 h. Consumption was measured by weighing vials before and after the tests and converting to milliliters. We evaluated the results for significance using the paired *t*-test (Systat 1997).

We also did 11 paired comparisons as a hexane control by repeating the above experiment (8 trials were done over 4 h and 3 trials over 24 h) and applying just hexane on 1 of the membranes instead of a mixture of hexane and pheromone. The results were evaluated with the paired *t*-test.

Results

Laboratory Tests. Fig. 1 defines and illustrates the percentage of feeding enhancement (recruitment) over the experimental range of pheromone concentrations. Both the 10-μg/ml and the 1-μg/ml concentrations had a significantly higher number of feeding ants ($F = 45.7$; df = 1, 3; $P = 0.007$ and $F = 10.8$; df = 1, 4; $P = 0.03$, respectively) than the controls. The pheromone ceased to attract significantly more workers at 1 order of magnitude above the optimal concentration of 10 μg/ml. Although the controls and treatments at the lower concentrations were not significantly different, as a result of their small sample sizes, even the lowest pheromone concentration showed some enhanced recruitment. The photograph inserted in Fig. 1 shows the enhanced recruiting effect of the pheromone when mixed with the sucrose solution. The ants did not significantly prefer the hexane sucrose solution control over the plain sucrose solution ($F = 0.68$; df = 1, 8; $P = 0.43$).

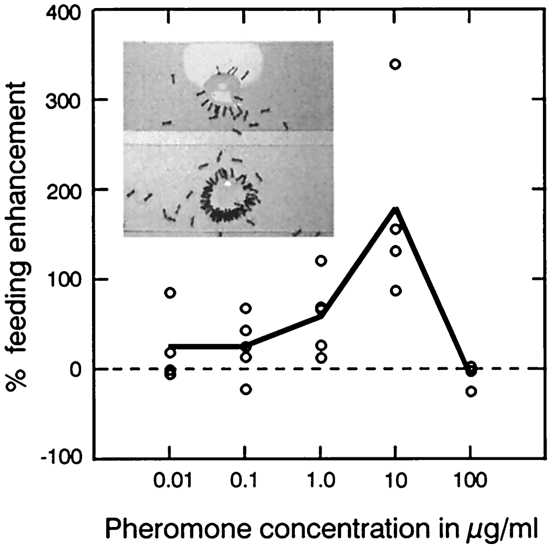

Fig. 1. Dose–response curve for the Argentine ant trail pheromone. Results were obtained for 3 laboratory colonies. More than 3 points for any concentration show that a colony was tested more than once. Twenty microliters of each pheromone concentration was mixed with 50 μl of a 10% sucrose solution. We define the percentage feeding enhancement as the [([number of ants on the treatment] − [number of ants on the control])/(number of ants on the control)] × 100. Each symbol represents the mean value of a 30-min paired comparison test where ants could drink either plain sucrose solution or from an adjacent sucrose solution that had been mixed with the pheromone. The inserted photograph shows ants drinking at 2 drops of the sucrose solution on glass slides. The drop with more ants had the optimal pheromone concentration of 10 μg/ml of hexane mixed with the sucrose solution. The heavier recruitment to the pheromone–sucrose solution is evident in the photograph.

Field Tests with Liquids. Within minutes of taping vials to the trees more ants were observed on the pheromone-treated plastic membrane. The same was true after 4 and 24 h. Many ants started to drink immediately upon encountering the sucrose solution. In the 4-h trials, mean consumption rate increased 29%. For the 24-h field trial there was a 33% increase in the consumption rate (Table 1). The 24-h trial had a lower consumption rate overall than the 4-h trial

Table 1. Statistics for the field tests with the Argentine ant trail pheromone

Treatment	Mean consumption, ml/h	Mean difference ± SD difference, ml	*t*	df	*P*
4 h without pheromone	0.584	0.168 ± 0.201	4.0	22	0.001
4 h with pheromone in hexane	0.752				
24 h without pheromone	0.166	0.054 ± 0.055	3.5	11	0.005
24 h with pheromone in hexane	0.221				
Control: hexane without pheromone	0.846	0.012 ± 0.141	0.3	10	0.790 NS
Control: neither hexane nor pheromone	0.835				

NS, not significant.

because they ran overnight; in the fall, nighttime temperatures are low enough to reduce foraging. In the controls investigating the effect of hexane alone, consumption of sucrose solution did not differ significantly among the paired vials (Table 1).

Discussion

We report on a successful and economically feasible way to enhance Argentine ant recruitment to liquids by combining them with pheromones. Hölldobler and Wilson (1990) tabulated 63 ant species known to produce trail pheromones; 16 of the pheromones have been identified, yet few attempts have been made to combine these compounds with food for possible use as bait. Robinson et al. (1982) tested several leafcutter ant species [*Atta sexdens rubropilosa* Forel, *Atta cephalotes* (L.), and *Acromyrmex octospinosus* (Reich)] by adding their trail pheromone to soybean, *Glycine max* (L.) Merr., baits. Only 1 of their trials with 1 species (*A. octospinosus*) showed increased bait collection in the field, but the increase was not consistent. One apparent problem was that if another attractant, such as sugar, was already on the bait, the effect of the pheromone was lessened. Robinson et al. (1982) concluded that addition of the trail pheromone would not be a cost-effective bait treatment for these ants.

Another attempt to enhance baits with trail pheromones was made by Vilela and Howse (1988) with the leafcutting ant *A. sexdens rubropilosa.* They used whole abdominal extracts applied to vermiculite particles and found an increase in the number of particles removed from their original location, although most were not taken to the nest. However, in field tests with the extracts applied to citrus-pulp bait, removal rates were not improved. Vilela and Howse (1988) found that although the pheromone attracts ants, it does not necessarily increase bait removal.

Other pheromones also can have attractive properties. In red imported fire ants, *Solenopsis invicta* Buren, Vander Meer et al. (1980) showed that the poison sac was the source of a queen pheromone. Lofgren et al. (1983) showed that this pheromone attracts workers when applied to rubber septa or when carried by an airstream into an olfactometer. It has not yet been shown whether these attractants can be used successfully in ant baits.

In Argentine ants, Baker et al. (1985) showed that workers preferred 25% honey or sucrose water over granulated sugar or other solid food containing proteins. Our results indicate that the trail pheromone, if on or near a desirable food (e.g., the sucrose solution), increases the number of feeding ants. Given the negligible cost of the pheromone (≈$16/100 mg), it could be included with liquid baits designed for Argentine ants.

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