Acceptance and Intake of Gel and Liquid Sucrose Compositions by the Argentine Ant (Hymenoptera: Formicidae)

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ABSTRACT Liquids and gels are common delivery forms used in commercial ant baits, but the relative effectiveness of each is unknown. We compared the feeding responses of the Argentine ant, *Linepithema humile* (Mayr), to liquid and gel compositions of sucrose. In choice assays, more workers were counted on gel than liquid; however, substantially more liquid was consumed. Because workers could stand on the gel, more workers could feed simultaneously on the gel. The feeding bouts of individual workers, however, were much less efficient at extracting sucrose in gel form. Workers fed eightfold longer on the gel, yet removed fivefold less sucrose than workers feeding on liquid. This potential bias should be considered during attraction and palatability studies that use physically different bait compositions. When the toxicant fipronil was added to the compositions, a greater proportion of the colony died after workers had fed on liquid than gel baits. This finding suggests that liquid formulations may provide more effective control of Argentine ants due to the greater speed and abundance in which it is ingested.

KEY WORDS *Linepithema humile*, Argentine ant, ants, bait, liquid, gel

IN ITS INTRODUCED range, the Argentine ant, *Linepithema humile* (Mayr) (formerly known as *Iridomyrmex humilis*), is an important urban and agricultural pest (Markin 1970, Knight and Rust 1990) that has had dramatic effects on terrestrial ecosystems by displacing native ants and other arthropods (Markin 1970, Ward 1987, Holway 1998). This ant produces large multiple-queen colonies that lack clear boundaries because of a general absence of intraspecific aggression (Suarez et al. 1999, Tsutsui et al. 2000), which contributes to its invasive success (Holway et al. 1998). Also, the large, diffuse colony structure of *L. humile* makes nests difficult to locate and treat.

Efficacy with toxic baits does not require the precise location of the nest; however, an acceptable bait base and incorporation of a nonrepellent, delayed-action toxicant is considered paramount to effective control. Commercial baits containing hydramethylnon, sulfluramid, or boric acid have provided some control of Argentine ant field populations (Forschler and Evans 1994, Krushelnycky and Reimer 1998, Klotz et al. 2000).

Honeydew produced by homopterans is an important food source for *L. humile* (Smith and Armitage 1931, Flanders 1951). Homopteran excretions may contain very high sugar concentrations (Gray 1952); therefore, liquid baits with relatively high levels of sugar are typically favored by *L. humile* (Baker et al. 1985, Klotz et al. 1998). Although liquid formulations would appear to be a preferred bait delivery composition, loss of material and environmental contamination due to spillage might negate any efficacy advantages. A possible compromise, which maintains the

desired sugar concentration yet avoids spillage problems, is to formulate the sugar solution, toxicant, and other desired inert ingredients in a gelatin matrix. Insecticide gel bait compositions are commercially available for ant control, yet it is not known how this delivery form compares with a liquid composition.

The objective of this study was to compare the response of Argentine ants to liquid and gel sucrose compositions. We measured worker visitation rate, food removal, feeding duration, and the effects of incorporating a toxicant in each composition. Despite higher ant counts on the gel, the liquid composition performed better in the more relevant criteria impacting control.

Materials and Methods

Argentine ants used in our studies were obtained from a single large field colony from Pleasanton, CA, by using the soil-free method of Silverman and Nsimba (2000). These ants were maintained in the laboratory housed in plastic petri dishes (9 cm in diameter) filled with casting plaster to a depth of 2 cm and affixed to the lid of a 200-ml glass jar. A 13-mm-diameter hole was drilled through the dish and jar lid and a piece of plastic tubing (20 mm in length) was glued to the hole. The petri dish-jar lid was fitted to a jar with water, and a length of cotton dental wick was inserted through the plastic sleeve. The plastic sleeve allowed the sealed plaster chamber housing the ants to maintain a high humidity without the plaster becoming saturated. Ants were maintained at 24 ± 3°C and 50 ± 15% RH and fed 25% sucrose, artificial diet (Nonacs and Dill

1988, modified by addition of 1% carrageenan) and German cockroaches, *Blattella germanica* (L.), ad libitum. For all experiments, except the final one examining toxicant transfer, ants ($\approx$1,000 workers, 1,000 brood, five queens) were held in 5-cm-diameter plastic petri dish nests, similar to the 9-cm-diameter nest described above. Individual nests were placed in 25 by 20-cm plastic trays coated with Fluon (DuPont, Wilmington, DE) and the ants were fed as described above before the beginning of the experiments. Gel compositions were made by mixing the gelling agent at a level of 1% in 25% sucrose and then heating to a boil. The gel was dispensed and then allowed to cool before being presented to the ants. In a preliminary experiment comparing several gelling agents, we determined that kappa carrageenan received the most visits by *L. humile.* Consequently, kappa carrageenan was selected as the gelling agent for all experiments.

Worker Counts on Liquid and Gel Compositions. We determined the number of ants feeding on gel and liquid sucrose compositions in a binary choice assay. Ants were deprived of food for 0, 1, or 2 d before being presented with 100 μl of each composition dispensed 3 cm apart on a plastic card (25 cm^2). The number of workers on each composition was counted every 10 min for 60 min, with the average of these six counts used in the analysis. Eight replicates were performed.

Consumption of Liquid and Gel Compositions. We determined the mass of liquid and gel sucrose compositions consumed by Argentine ants. Compositions were dispensed in culture tubes (50 by 6 mm in diameter). Each treatment pair was weighed and then placed in a 50-ml centrifuge tube. Water loss due to evaporation was reduced in the treatments by placing a moist cotton plug in the bottom of the centrifuge tube and also covering the tube and allowing ants access through a 5-mm-diameter hole in the lid. Water-loss controls also were treated in a similar manner, with ants excluded from these compositions. Compositions were reweighed each day for 5 d, with weight loss corrected for evaporation. No other food was provided during the experiment. Eight treatment and four water-loss control replicates were performed.

Time on Sucrose Compositions. We determined the time an individual worker ant spent at each of the compositions before returning to the colony. One hundred microliters of either gel or liquid sucrose was presented 10 cm from the nest. A single worker was observed and the time recorded from the moment it began feeding to when it left the composition to return to the colony. Twenty replicates were performed for each treatment.

Worker Weight Gain After Consumption of Sucrose Compositions. We compared the relative quantity of gel and liquid retrieved by *L. humile* workers. Colonies were deprived of food for 1 d, and then 50 workers outside the nest were placed in a preweighed 50-ml fluon-coated centrifuge tube. The tube was weighed and the workers were released. This was repeated another two times to get an average group worker weight before feeding. Ants were then provided either gel or liquid composition. Workers re-

turning to the nest that showed evidence of feeding, i.e., expansion of the gaster and appearance of intersegmental membrane (which was apparent in both treatments), were collected in groups of 50 and weighed. Each composition was evaluated against two different colony fragments by using three weighed worker groups (six replicates per treatment).

Effect of Toxicant Delivered in Gel Versus Liquid Composition. We determined the effect of the insecticide fipronil incorporated in liquid and gel sucrose compositions on the mortality of *L. humile* workers and queens. Five hundred workers and one queen were placed in each of six fluon-coated plastic trays (25 by 20 cm). A covered plastic petri dish (5 cm in diameter) with a water-moistened cotton dental wick was provided as a nesting site. Ants were deprived of food for 24 h. Thirty workers (donors) from each tray were collected and placed in an open petri dish (5 cm in diameter) that was coated with fluon on both inside and outside vertical surfaces to prevent escape and prevent entry of other workers. The toxicant fed to these ants was prepared by adding 5 μl of a 1% acetone solution to 10 ml of heated gel or liquid sucrose to yield a final fipronil concentration of 5 $\times$ 10^{-4}%. Three replicates for each composition were performed. The donor ants were allowed to feed on the fipronil-sucrose composition for 5 min, and then they were released with the remaining unfed (recipient) ants. Trophallaxis between fed and unfed workers was observed. Sucrose (25%) was provided 1 h after the donor and recipient ants were mixed. Worker and queen mortality were assessed after 24 and 72 h. Gel and liquid compositions without fipronil were included as controls.

Statistical Analysis. A one-way analysis of variance (ANOVA) was performed on log transformed data in the study comparing the time on sucrose compositions. The general linear models procedure was used to perform ANOVAs on the original data in all other experiments (MINITAB 2000).

Results

Worker Counts on Liquid and Gel Compositions. The number of worker *L. humile* counted on both the liquid and gel compositions was relatively low when the colonies were deprived of food for up to 1 d (Fig. 1). However, these counts increased approximately fourfold when colonies were starved for 2 d. Ants completely covered the perimeter of the liquid droplet, whereas feeding on the gel occurred both at the edge and on top. Significantly more ants were counted on gel than liquid when starved for 2 d ($P = 0.008$; df = 1, 7; $F = 13.37$).

Consumption of Liquid and Gel Compositions. Initially, there were little differences in the levels of liquid and gel consumed by *L. humile* (Fig. 2). However, by day 5, workers consumed approximately twice as much sucrose liquid than sucrose gel ($P = 0.015$; df = 1, 7; $F = 10.14$).
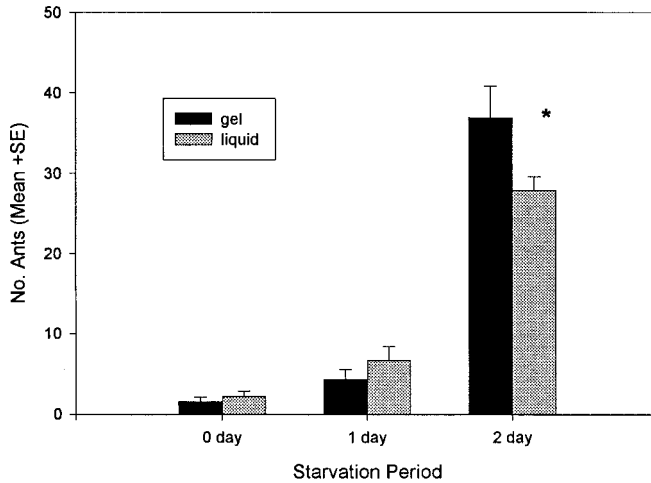
Fig. 1. Counts of *L. humile* workers on gel and liquid sucrose compositions after various periods of colony starvation. *, significant difference between treatments ($P < 0.05$; ANOVA).

Effect of Sucrose Composition on Residence Time and Worker Weight Gain. Argentine ants fed more than eight times longer on gel (842.4 ± 597.84 s) versus liquid (104.6 ± 55.05 s) sucrose ($P = 0.001$; df = 1,38; $F = 69.8$). Despite the increased time on the gel, worker cohort weight was 3.2 ± 1.17 mg after a visit to the gel compared with 15.2 ± 1.47 mg after a visit to liquid sucrose ($P = 0.035$; df = 1, 8; $F = 324.0$).

Effect of Toxicant Delivered in Gel Versus Liquid Compositions. We recorded significantly higher mortality in worker cohorts exposed to liquid versus gel compositions of equivalent fipronil concentrations. The total mortality of workers fed liquid fipronil was greater than those fed gel fipronil for 24 h ($P = 0.017$; df = 1, 8; $F = 8.97$) and 72 h ($P = 0.005$; df = 1, 8; $F = 15.01$) after toxicant exposure (Fig. 3). The number of workers affected that received toxicant, presumably via trophallaxis, 72 h after exposure to donors was 2.3-fold above the workers fed fipronil gel directly and 5.9-fold above workers fed fipronil liquid directly. None of the queens in either treatment were killed within 72 h. The mortality of ants fed sucrose compositions without fipronil was negligible (gel: 24 h, 1%; 72 h, 4% and liquid: 24 h, 1%; 72 h, 3%).

Discussion

Although the compositions were equivalent nutritionally, Argentine ants handled liquid sucrose more efficiently than sucrose in gel form. This response was not unexpected considering that *L. humile*, like many ants, feeds naturally on honeydew from Homoptera and on plant exudates, including extrafloral nectaries and therefore is adapted to collecting, transporting, and sharing a liquid diet. According to Markin (1970) 99% of the food entering a nest of *L. humile* is in a liquid
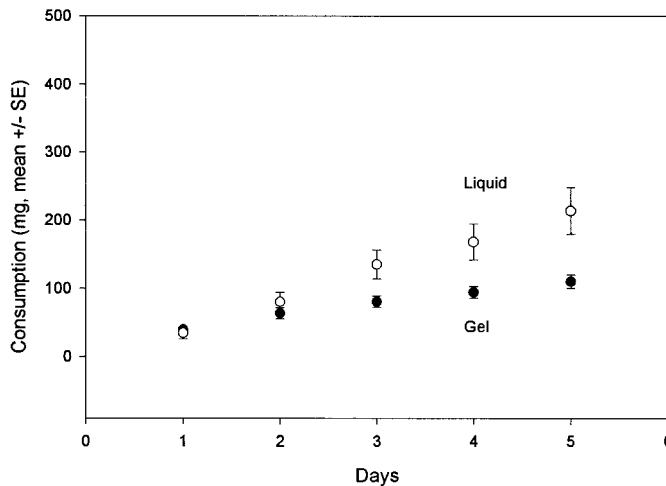

Fig. 2. Consumption of gel and liquid sucrose compositions by *L. humile* through 5 d.
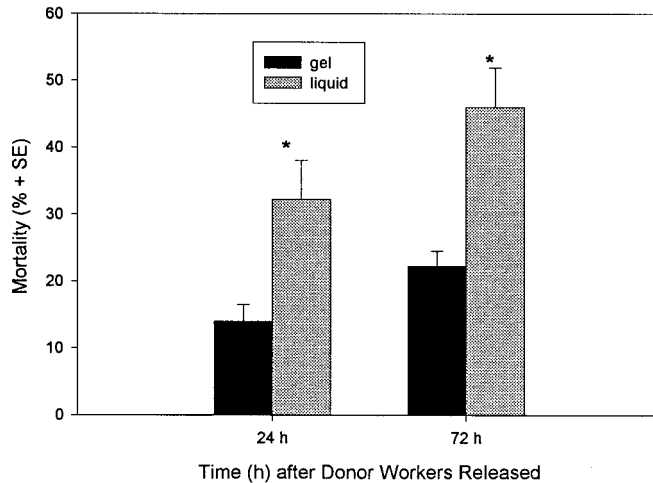
Fig. 3. Total mortality of *L. humile* when 30 of 500 workers were fed either liquid or gel sucrose with $5 \times 10^{-4}\%$ fipronil insecticide. *, significant difference between treatments ($P < 0.05$; ANOVA).

state. Although *L. humile* worker counts on gel were higher, more liquid was consumed. The greater effective surface area of the gel and the reduced ability of workers to extract sucrose from the gel matrix explain this disparity. Workers only fed around the periphery of the liquid, whereas the entire gel matrix was covered with workers, especially after 48 h of prior food deprivation. Despite greater access to gel, workers required substantially more time to remove sucrose bait and retrieved much less sucrose bait during a feeding interval than ants visiting liquid sucrose. Haack and Vinson (1990) reported that solid protein baits required greater handling time and longer visits by *Monomorium pharaonis* L. than liquid sucrose. In addition, a higher degree of trophallactic activity by *M. pharaonis* occurred with liquid sucrose versus solid protein. Although Haack and Vinson (1990) did not investigate the relative contribution of nutrient type (protein or carbohydrate) and food matrix (solid or liquid), our findings with *L. humile* demonstrate the importance of the matrix on food handling efficiency. Furthermore, we determined that a lethal dose of toxicant formulated in liquid sucrose was distributed between substantially more workers than the solid (gel) form, probably because more liquid was consumed. Although up to 46% of the workers died in the bait study, no queens were killed with either formulation. These data contrast with those of Hooper-Bui and Rust (2000) who achieved complete queen mortality with a comparable level of fipronil in 25% liquid sucrose. These queen mortality differences can be attributed to the substantially shorter toxicant exposure time used in our study (5 min versus 24 h).

Although gel bait formulations overcome some of the problems associated with dispensing liquid ant bait, our studies revealed that Argentine ants consumed more sucrose in liquid than in gel form. We suggest therefore that liquid baits should outperform gel baits of similar toxicant and nutrient composition against this species. Furthermore, because relative worker numbers on the two compositions were not consistent with actual consumption levels, we caution against the sole reliance on worker residence at baits as an indicator of bait performance.

Acknowledgments

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