Bait-preference Tests for the Argentine Ant (Hymenoptera: Formicidae)

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ABSTRACT In choice feeding tests, Argentine ant, *Iridomyrmex humilis* (Mayr), workers preferred 25% honey water or sucrose water over granulated brown sugar or other solid foods with high protein content such as tuna meal. In the field in competition with the preferred natural food, honeydew, workers fed consistently on sucrose water and honey water over a period of 15 days and exhibited a significant preference for sucrose water. Various emulsifiers and ethanol used to suspend toxicants in the sucrose water did not by themselves significantly deter feeding. Workers readily fed upon, and were killed by, the sucrose water bait containing 10 or 100 ppm Avermectin.

THE ARGENTINE ANT, *Iridomyrmex humilis* (Mayr), has steadily displaced many native species in human-disturbed habitats since its introduction into the United States ca. 1891 (Erickson 1971). Since its appearance in California ca. 1905, the ant's unchecked presence in citrus groves has caused the failure of biological control in the integrated management of honeydew-producing pests, such as scales, aphids, and mealybugs. The workers' incessant honeydew-gathering activities interfere with oviposition by parasites and feeding by predators (Flanders 1943, 1958, DeBach et al. 1951). However, in groves where various controls have been used to suppress ant populations, biological control has proven to be quite effective and has been a cornerstone in successful integrated pest management (IPM) on citrus. Over the years, the most successful techniques for ant control have been mechanical barriers on the trunks, water-filled ditches around entire groves, and, most recently, chlordane or dieldrin granules under the trees (Musgrove and Carman 1965). Cancellation of the registration of chlorinated hydrocarbon insecticides for use in ant control on citrus in 1979 has threatened to upset successful citrus IPM programs. Growers in California have experienced increased damaging outbreaks of homopterans accompanied by Argentine ant infestations.

We initiated a study to develop a toxic food bait that would be competitive with the ants' preferred food, honeydew, throughout the season. The toxicant ideally would be slow-acting, so that amounts high enough to cause mortality in the multiple queens of this species would be carried back to the nest and distributed by foraging workers. This paper reports the results of our initial experiments in developing the food-bait portion of the toxic bait, plus our initial findings on colony mortality following the addition of one potential toxicant, Avermectin.

Materials and Methods

Laboratory Colonies. Workers, queens, and brood were collected in soil and leaf litter in Riverside, Calif., and allowed to form nests in plastic petri dishes filled with hardened, moist plaster-of-paris. One nest containing brood, workers, and several queens was placed in each of 24 open plastic laboratory colony boxes. Colony size ranged from ca. 300–1,000 workers and could be assessed by observing the ants through the red cellophane-covered lids of the dishes. Ants could move freely into and out of their nest through holes in the lid, but were confined to colony boxes by a coating of Fluon AD1 (liquid teflon) (Northeast Chemical Co. Inc., Woonsocket, R.I.) on the inner sides of each box. Colonies were provided with water ad lib. Between periods of testing, ants were regularly supplied with several milliliters of dilute sucrose water and also with dead noctuid moths and larvae as a protein source.

Choice Tests. Prospective food baits were placed in random linear order on thin glass plates (0.1 by 4 by 15 cm). The liquid baits were kept in place by a circle drawn with a wax pencil (Fig. 1). Solid baits also were encircled. A different plate was used for each colony, and plates were washed with detergent and rinsed with acetone after testing. Liquid foods were presented as 0.5-ml drops and solid foods were placed so as to yield a circumference similar to that of the drops (ca. 6 cm). Food, but not water, was withheld from each colony for 2 days before the initiation of each experiment, whereupon one glass plate with an array of food baits was placed at the far end of a colony box, ca. 15 cm from the nest. The number of ants feeding on each food item was counted 15 min after introduction of the plate. In all experiments, the mean number of workers feeding was transformed to $x + 0.5$ and subjected to a two-way analysis of

Table 1. Food preferences of workers of the Argentine ant, *Iridomyrmex humilis* (Mayr), in choice tests

Food bait[a]	Mean no. feeding ± SD[b,c]	Additive[d]	Mean no. feeding ± SD[b,e]	Additive[f]	Mean no. feeding ± SD[b,e]
25% Honey water	37.9a ± 17.6	Peptic peptone	3.1c ± 3.4	Sucrose water alone	21.2a ± 12.0
Undiluted honey	7.0b ± 4.6	Gelatin peptone	3.4c ± 2.7	Triton-X	1.3b ± 2.0
4:1 Tuna meal : honey	1.0c ± 0.8	Indole peptone	2.2c ± 2.2	Tween-80	20.2a ± 15.4
4:2:1 Corn meal : yeast : honey	1.1c ± 1.0	Casein hydrolysate (enzymatic)	3.5c ± 2.8	HEC	20.0a ± 11.2
Tuna meal	0.1c ± 0.2	Tryptic peptone	3.1c ± 2.8	CMC	20.5a ± 13.2
4:1 Corn meal : yeast	0.0c ± 0.0	Dried egg white	19.7a ± 11.6		
		Sucrose water alone	13.1b ± 6.4		

[a] During the course of the experiment, colonies were preconditioned in turn to each of the food baits for the 2 days preceding the choice tests.
[b] Means within a column having no letters in common are significantly different ($P \leq 0.05$; analysis of variance followed by Duncan's [1951] multiple range test).
[c] Mean of 12 replicates.
[d] Additives added as 5% solutions to 25% sucrose water.
[e] Mean of 144 replicates.
[f] Additives added to 25% sucrose water as solutions (down column) of 0, 1, 1, 0.1, and 2%, respectively. HEC is hydroxyethyl cellulose; CMC is carboxymethyl cellulose.

variance followed by Duncan's (1951) multiple range test.

We first tested the feeding responses of the ants to undiluted honey (purified citrus honey, Sue Bee, Sioux City, Iowa); 25% honey–water solution; a 4:1 mixture of tuna meal and honey; a 4:2:1 mixture of corn meal, yeast, and honey; tuna meal alone; and a 4:1 corn meal and yeast mixture. Tuna meal was obtained from the Zoecon Corp. (Palo Alto, Calif.) and sieved through a no. 20 mesh screen to remove large particles. Twelve colonies were preconditioned to one of the six food items by feeding them ad lib for 2 days before they were presented with a choice between all six foods. After 15 min, the number of ants feeding on each food item was counted, the array was removed and the colony was allowed to feed ad lib for 2 days on another of the foods in the array, after which their food preference was measured again. This procedure was continued until each colony's food preferences had been measured after ants had been preconditioned to each of the food items and to water alone. The same regimen was then followed to measure the ants' responses to 50, 25, and 10%

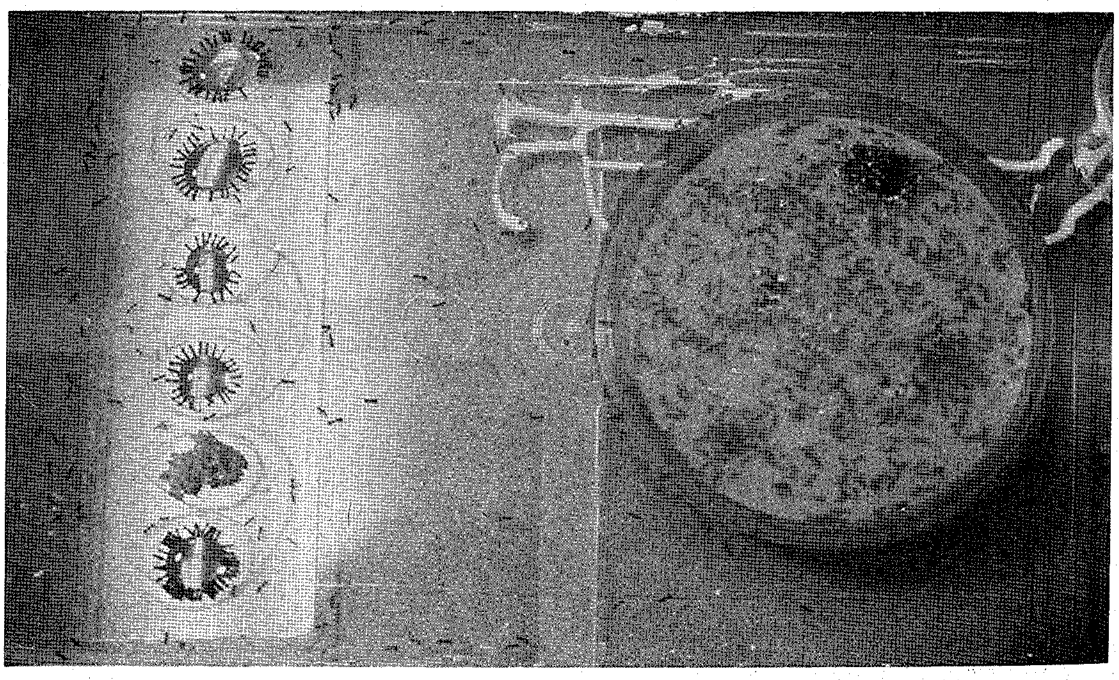

Fig. 1. Typical choice-test array of food baits in a single colony box. Plaster-of-paris petri dish nest is at right and water source is just visible at upper right. Liquid baits are surrounded by waxed pencil circles; 25% sucrose water is at bottom and granular brown sugar is second from the bottom.

Table 2. Food preferences of workers of the Argentine ant after 2 days of continuous access (preconditioning) to baits before testing

Precondition	Mean no. feeding on baits ± SD					
	50% Honey water	25% Honey water	10% Honey water	25% Sucrose water	25% Brown sugar water	Brown sugar
Water	31.8a ± 15.2	22.2b ± 10.6	13.5c ± 6.5	11.8c ± 3.9	13.8c ± 6.2	0.9d ± 1.2
50% Honey water	1.2c ± 2.0	1.8c ± 0.9	1.8c ± 1.8	11.5a ± 3.2	9.1b ± 3.1	0.2d ± 0.6
25% Honey water	0.0c ± 0.0	0.2c ± 0.4	0.0c ± 0.0	6.6a ± 3.0	1.0b ± 1.4	1.5b ± 1.2
25% Sucrose water	0.3c ± 0.7	0.7c ± 1.2	0.5c ± 0.7	4.3a ± 1.8	2.3b ± 1.2	0.3c ± 0.5
25% Brown sugar water	2.4c ± 4.6	3.3bc ± 4.5	4.1bc ± 7.3	10.3a ± 4.9	4.5b ± 4.9	0.3d ± 0.8
Brown sugar	36.4a ± 15.2	32.8a ± 12.6	26.1b ± 8.3	16.8c ± 4.4	23.0b ± 7.5	0.0d ± 0.0

Baits were presented in choice tests to 12 different colonies. Means in the same row having no letters in common are significantly different ($P \leq 0.05$; analysis of variance followed by Duncan's [1951] multiple range test; $n = 12$ replicates)

honey water, 25% brown sugar water, undiluted brown sugar, and 25% sucrose (refined sugar) water.

Responses of colonies in the field over a period of time to both sucrose water and honey water were measured in an ant-inhabited citrus grove on the Riverside campus of the University of California. A 10-ml amount of each of 25% sucrose water and 25% honey water, respectively, was placed in covered petri dishes having four access holes (0.5 cm diam) in the sides. Each pair of dishes was placed on a paper-covered tray at the base of each of five trees near ant trails that led to and up the tree trunks. Fresh solutions were introduced daily to each petri dish for the 15-day period that each pair of dishes was observed. Thirty minutes after the introduction of fresh food solutions, ants feeding at each dish at each tree were counted. To measure consumption, the volume of liquid in each dish was measured daily. Dishes at which no feeding was allowed were used to monitor loss due to evaporation alone, and this value was subtracted from the volume lost at feeder dishes.

Various proteinaceous additives to 25% sucrose water were tested in the laboratory to see if they enhanced the feeding response compared to that of sucrose water alone. These included several concentrations of casein hydrolysate, and 5% solutions or suspensions of dried egg white or five peptones (indole, tryptic, generic, peptic, and gelatin). They were presented to each of 12 colonies twice a day for 6 consecutive days, and, as before, the ants feeding on the solutions were counted 15 min later. The baits were removed immediately after counting the ants.

Addition of Emulsifiers. Most potential toxicants that could be used for Argentine ant control do not readily form an aqueous solution. In anticipation of the need to dissolve or suspend a toxicant in the sugar water-based bait, we tested the workers' feeding response to various emulsifiers mixed with the sugar water. We also tested their response to ethanol mixed with some of the more favorable emulsifiers in sugar water because ethanol might be needed to help the toxicant form a solution. Choice tests were conducted in colony boxes as described previously. Counts of feeding ants were taken 15 min after introduction of the food bait.

Addition of Toxicant. Ants representing three laboratory colonies were allowed to feed on one of two concentrations of Avermectin, 10 or 100 ppm in 25% sucrose water, with or without egg white, during a 30-min exposure daily to the bait. The numbers of workers feeding at the solutions were measured 15 min after introduction to each of the 12 colonies. All dead workers and queens were counted and removed from each colony daily. Cumulative daily mortality of workers and queens as a fraction of the total colony size was calculated for each colony.

Results

I. humilis workers preferred liquid, sugary food over all other types of food we offered them. In

Table 3. Mean proportion of workers of the Argentine ant (calculated from the total population of each colony) feeding on Avermectin-laced sucrose water with and without dried egg white (EW) in the laboratory, demonstrating lack of feeding deterrency of Avermectin in the bait

	Mean proportion feeding ± SD				
	25% Sucrose water	10 ppm Avermectin	100 ppm Avermectin	10 ppm Avermectin + EW	100 ppm Avermectin + EW
Day 1 ($n = 3$)	0.06a ± 0.03	0.04a ± 0.02	0.06a ± 0.03	0.05a ± 0.02	0.08a ± 0.02
Days 2–5 ($n = 12$)	0.03b ± 0.01	0.01c ± 0.01	0.00d ± 0.00	0.06a ± 0.02	0.01cd ± 0.01

n = Three colonies per treatment. All Avermectin treatments also contained CMC (carboxymethyl cellulose) and ethanol to suspend the toxin. Workers were given only 30 min of feeding on each day, and the number feeding at the end of this time was counted. High mortality to the 100-ppm treatments after day 1 caused a severe reduction in feeding. Means in the same row having no letters in common are significantly different ($P \leq 0.05$; analysis of variance followed by Duncan's [1951] multiple range test)

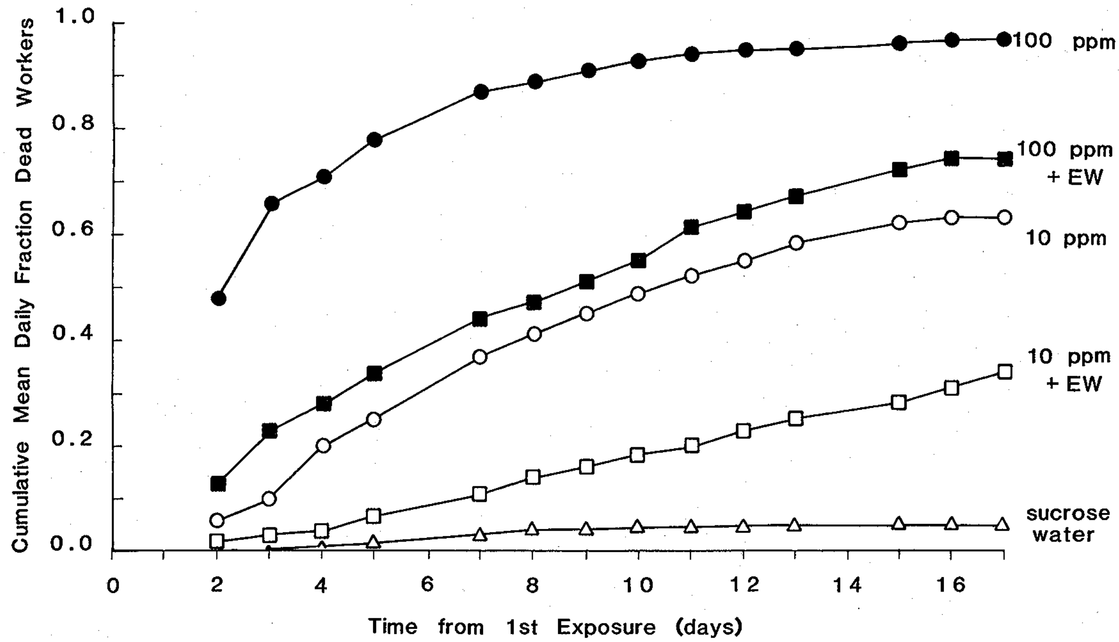

Fig. 2. Mean cumulative mortality of workers after feeding on sucrose water containing 10 or 100 ppm Avermectin with or without 5% egg white added. Workers were allowed to feed on the toxic bait for only 30 min each day. Mortality is expressed as a fraction of the total colonies' sizes as measured on the first day of the experiment by counting the number of living workers in the colony boxes through the clear red cellophane lids on the petri dish nests. On subsequent days, dead workers were counted in the same way.

the first choice test, during the course of the entire experiment, workers fed upon 25% honey water significantly more frequently than upon the more concentrated honey and more solid, proteinaceous foods, with and without honey added (Table 1, Fig. 1). This preference occurred regardless of whether or not the workers had been satiated with honey water for 2 days before the test. A mean of 11.1 workers visited the honey water in this case, compared to a mean of less than 0.5 for all other treatments.

Among various types of sugary liquids, workers denied food for 2 days visited honey water more frequently than sucrose water on their initial exposure (Table 2). However, when allowed to feed to satiation on several sugary liquids for 2 days before testing, sucrose water was preferred, even by workers that had fed to satiation on sucrose water for the 2-day period (Table 2). Again, solid foods were not visited as frequently as liquids by workers.

In the field, workers visited both the 25% honey water and 25% sucrose water consistently over a period of 15 days, demonstrating that even in competition with natural honeydew, sugary waters elicit prolonged feeding by these ants. At four of the five trees, the feeder dishes were saturated with ants whenever counts were taken; many workers had to stand on top of their nestmates to drink from the edge of the liquid bait. Over the course of the entire experiment, over twice as many workers preferred the sucrose water compared to honey water (mean = 115 per dish compared with 40.6 per dish) ($P \leq 0.05$; paired t test; $n = 75$). Subtracting for the amount of liquid that evaporated, we estimated that over 160 ml of sucrose water and 73 ml of honey water was imbibed by workers at the five trees. Because of the prolonged higher levels of feeding it evoked, the sucrose water was the food bait base that we chose for the addition of toxicants.

In the laboratory, the addition of various proteinaceous substances to the sucrose water all failed to elevate the levels of feeding in competition with sucrose water, and usually significantly reduced feeding (Table 1). Egg white was the only substance that did not lower the levels of feeding. In fact, this additive significantly elevated the levels of feeding. In a separate experiment, casein hydrolysate in 25% sucrose water reduced feeding compared to sucrose water alone, even when added at a concentration of only 0.3%. Forty percent of the feeding workers fed upon 25% sucrose water alone, whereas only 16, 14, and 10% fed upon sucrose water containing 0.3, 1, and 3% enzymatic casein hydrolysate, respectively.

Tween-80, hydroxyethyl cellulose (HEC), and carboxymethyl cellulose (CMC) at 1, 0.1, and 2% solutions, respectively, all had no effect on workers' feeding response to 25% sugar water (Table 1). Triton-X, on the other hand, caused a drastic suppression of feeding, mainly because it caused

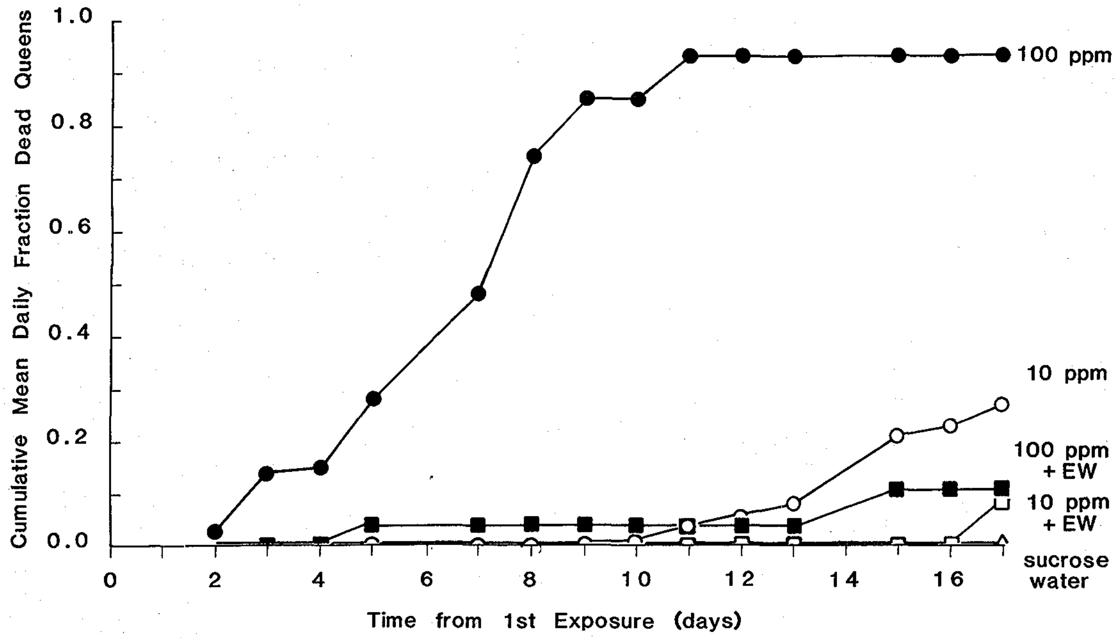

Fig. 3. Mean cumulative mortality of queens after feeding on toxic baits described in Fig. 2 legend. Mortality is expressed as a fraction of the total number of queens in each colony. Details for queens are the same as those for workers in Fig. 2 legend.

sudden death in workers. Because workers died before they could walk very far away from the solution, no recruitment to this food source occurred, and, thus, visitation was very low (Table 1). Ethanol added to either the CMC or HEC had no effect on workers' feeding. A mean of 23.5 workers ($\pm$12.9 SD) fed upon sucrose water alone, whereas 28.0 ($\pm$6.8) and 20.2 ($\pm$5.2) fed upon CMC plus ethanol and HEC plus ethanol, respectively ($P > 0.05$, analysis of variance followed by Duncan's multiple range test; $n = 6$; $F = 1.13$; df = 7). Because CMC plus ethanol appeared to evoke slightly (but not significantly) higher levels of feeding, CMC was the emulsifier chosen for use with addition of toxicants.

The addition of Avermectin to the 25% sucrose water/CMC/ethanol bait, with or without egg white, did not deter feeding in laboratory colonies, as measured on day 1 of the experiment (Table 3). The feeding response to the 100 ppm Avermectin dose could not be compared after day 1 because of high worker mortality on day 2. The baits containing 10 ppm Avermectin for several days continued to elicit levels of feeding as high as to the sucrose water controls, but there was some indication that worker mortality began to affect food source visitation after ca. 1 week of the experiment.

Even though the colonies were given only one 30-min feeding period each day, worker mortality due to Avermectin was significant and rapid up to the 100 ppm rates (Fig. 2). Significant mortality of queens occurred several days later in the treatment containing 100 ppm Avermectin lacking egg white (Fig. 3), and nearly all workers and queens in the three colonies were dead in ca. 10 days after the first feeding. The mortality from this treatment was even more surprising considering that nearly all the feeding was done on the first day's 30-min feeding period. The toxicant was then passed to enough colony members that, subsequently, the entire colony succumbed. The addition of egg white did not appear to cause as high a level of mortality as did the companion treatments in which egg white was lacking.

Discussion

Argentine ant workers fed more consistently on 25% sucrose water than on honey water in the field in competition with natural honeydew sources. In general, sugary liquids evoked the most consistent feeding response in this species, and are the most logical choice for incorporating toxicants in the future. Becuase 25% sucrose water out-competed honey water in long-term preference tests, we focused on adding toxicants and emulsifiers to it rather than to honey water.

The addition of proteinaceous substances to the sucrose water did not substantially increase feeding by workers and, in most cases, significantly reduced feeding. Moreover, egg white, the one protein source that did not inhibit feeding, appeared to interfere with the toxicity of Avermectin to both workers and queens. Markin (1967) found that sugars and proteins are distributed differentially among *I. humilis* colony members according to their developmental stage and caste, with workers distributing sugars among themselves and proteins to queens. In our experiment, however, it appears that the transfer of toxicant in sucrose water alone among colony members was more likely to produce worker and queen mortality than when protein was also present, although it is not clear why inclusion of protein should interfere with mortality. The egg white portion of the mixture may have preferentially absorbed the Avermectin, thus reducing the effective concentration of toxicant passed to other workers in the sucrose water portion. This does not explain the lower queen mortality to this bait, however, if egg white were preferentially passed to queens. Markin (1967) did find that sugars eventually are passed to queens after a delay of several days, consistent with our results of significant queen mortality to Avermectin in sucrose water alone. Therefore, thus far we see no reason to incorporate protein in the sucrose-water bait.

Workers appeared to be oblivious to the first toxicant, Avermectin, added to the sucrose water plus emulsifier and ethanol. Workers visited and fed upon toxicant-laced baits as readily as on sucrose-water controls, and after the first day, colony mortality began to occur. At the highest dose of Avermectin, 100 ppm, a single 30-min feeding on the first day caused high levels of worker death by the next day. Colonies in the field are sometimes up to several orders of magnitude larger than the ones we tested in the laboratory (Markin 1967), and sustained uptake of bait probably would be needed over a period of several days or weeks to inflict similar levels of mortality in the field.

The sucrose water-based bait appears to have the ability to elicit sustained feeding in the field, and, therefore, holds the most promise in future control programs on citrus.

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