Coprophagy Facilitates Horizontal Transmission of Bait Among Cockroaches (Dictyoptera: Blattellidae)

ROBERT J. KOPANIC, JR. AND COBY SCHAL

Department of Entomology, North Carolina State University, Box 7613, Raleigh, NC 27695–7613

Environ. Entomol. 28(3): 431–438 (1999)

ABSTRACT Baits offer several advantages over other insecticide formulations in the control of populations of the German cockroach, *Blattella germanica* (L.). However, they may fail to target certain life stages that feed only sparingly. Recently we have demonstrated that 1st instars are significantly more vulnerable to insecticidal baits when adults are present. By preventing adults or nymphs from eating bait we now conclude that adults translocate insecticide bait to the shelter, thus facilitating a horizontal transfer of the insecticide to nymphs. By tracking bait movement with a tracer dye, we show that nymphs take up adult-delivered bait via coprophagy. An alternative hypothesis, that adults delivered novel food odors to nymphs thereby stimulating them to forage and eat bait, was experimentally rejected. Analysis of time-lapse video records showed that 1st instars foraged sparingly compared with 2nd instars and adults, indicating that direct ingestion of a remotely placed bait accounted for little, if any, mortality in 1st instars. The magnitude of coprophagy in 1st instars was related to the proximity of the food to their aggregation site; nymphs ate significantly more adult feces when food was far from the shelter. We conclude that aggregating 1st instars are relatively sedentary, and that they depend on conspecific foragers to deliver widely dispersed food. Innovative baiting strategies should therefore maximize forager-mediated translocation and delivery of slow-acting bait insecticides to inaccessible cockroach aggregations.

KEY WORDS *Blattella germanica*, coprophagy, horizontal toxicant transfer, bait

BAIT FORMULATIONS THAT target urban pests have become increasingly popular among consumers and commercial pest control professionals. Among the factors contributing to and supporting this trend are (1) increased public awareness of the negative consequences of broadcast pesticide applications; (2) increased consumer demand for products that are less toxic, less volatile, relatively more contained, and environmentally benign; (3) recent reports of excellent efficacy of baits; and (4) the proliferation of bait formulation types and active ingredients (see Schal and Hamilton 1990, Reierson 1995). Efforts to improve bait delivery systems and bait efficacy have been significantly hampered by fragmented and incomplete understanding of the habits and foraging behavior of target pests. Termites, ants, and cockroaches are particularly susceptible to baiting because of their clustered distributions and the fact that foragers return to a nest or aggregation.

To be effective, baits must be located and consumed by the target insect. In addition to their intrinsic qualities (e.g., attraction, palatability, texture) the efficacy of baits may be severely constrained by the spatial distribution of the pest population and by the behavior of certain life stages that either forage minimally or feed sparsely. The bait may be uniformly distributed to enhance the probability of contact by widely dispersed insects, as in the case of grasshopper control (Mukerji et al. 1981, Johnson and Henry 1987). However, a severe limitation of effective cockroach control is the clustered and at times, unpredictable location of the insects. Even more significant is the limited feeding and foraging exhibited by certain life stages, a factor that can hinder timely suppression of the population. In the German cockroach, *Blattella germanica* (L.), gravid females and young nymphs forage and feed significantly less than other stages (Sommer 1975, Cochran 1983, Bret and Ross 1985, Silverman 1986, Hamilton and Schal 1988, Cloarec and Rivault 1991, DeMark et al. 1993, Schal et al. 1997). The progeny of gravid females that escape insecticide applications may represent a failure of the treatment and provide the foundation for future population expansion. The early instars represent a significant proportion of the total population under normal conditions (Sherron et al. 1982, Ross et al. 1984) and failure to target them with baits can significantly diminish the efficacy of the bait.

Clearly, an effective approach is to place baits in close proximity to known pest aggregations. However, the aggregations are often located in inaccessible deep crevices and voids. A strategy long employed in the control of social insects, such as ants and termites, is to offer the bait to foragers who in turn deliver the insecticide to the colony (Deneubourg et al. 1990, Su and Scheffrahn 1991, Klotz et al. 1997). More sedentary stages are then targeted through contact, trophallaxis, coprophagy, and necrophagy. Silverman et al. (1991) first demonstrated horizontal toxicant transfer in *B. germanica* using radiolabeled hydramethylnon. They employed a sequential design in which "donors" (adult males and females) defecated onto petri dishes

0046-225X/99/0431–0438$02.00/0

and "receivers" (1st- and 2nd-instar nymphs) were subsequently assayed for labeled hydramethylnon. Silverman et al. (1991) also demonstrated that ingested hydramethylnon was passed into the feces, whereas topically applied hydramethylnon was not. Horizontal transfer of toxicants was also demonstrated in laboratory and field populations of *B. germanica* in which adult foragers delivered bait to shelters and hence to aggregating nymphs therein (Kopanic and Schal 1997). In mixed populations of adults and either 1st or 2nd instars, adult mortality was 100% and nymphal mortality was >95%. Physically preventing 1st instars from ingesting a bait 150 cm from the shelter did not diminish nymph mortality, suggesting that adults translocated the bait (Kopanic and Schal 1997). Moreover, when only adults were excluded from the bait, 1st- and 2nd-instar mortality declined significantly to <5 and 60%, respectively. These results suggested that nymphal mortality caused by the ingestion of bait could be distinguished from mortality caused by horizontal toxicant transfer. Nevertheless, although these results suggested the occurrence of coprophagy, Kopanic and Schal (1997) did not ascertain how nymphs obtained toxicant from foraging adults.

In our current study we demonstrate coprophagy in 1st- and 2nd-instar *B. germanica* and examine how starvation and distance between the shelter and a food resource affect the level of coprophagy. We experimentally reject an alternative hypothesis that novel odors, flavors, or both brought back to the shelter by foraging adults stimulate small sedentary nymphs to forage. Analysis of video records of bait visits show that 1st instars visit baits infrequently compared with 2nd instars or adult insects. Furthermore, 1st-instar mortality is low when adults are prevented from feeding on the bait. Lastly, high 1st-instar mortality evident when adults feed on the bait results from coprophagy and not from enhanced foraging of nymphs.

Materials and Methods

Insects. The *B. germanica* used were an insecticide-susceptible laboratory strain originally obtained from the American Cyanamid (Princeton, NJ) stock. Insects were reared at 27°C, under variable ambient humidity, and a photoperiod of 12:12 (L:D) h, and were provided water and Purina Rat Chow #5012 (Purina Mills, St. Louis, MO) ad libitum. Food pellets were ground in a Waring blender and then sieved to a fine powder. Stejskal (1997) demonstrated that adult males deposit more fecal pellets than nonpregnant adult females. Our preliminary results (not shown) differ from these findings; therefore, we used adult females to generate dye-laden feces. Insects used were selected so that assays were conducted during the peak feeding stage of adult females (Cochran 1983, Hamilton and Schal 1988) and nymphs (Valles et al. 1996, Young and Schal 1997). For all assays, females were collected within 12 h of eclosion from synchronous cultures, whereas 1st and 2nd instars were collected within 12 h of hatching or ecdysis, respectively.

Insecticide Bait. MAXFORCE Roach Killer bait gel (2.15% hydramethylnon; The Clorox Company, Pleasanton, CA) was purchased from a local distributor. Hydramethylnon, an amidinohydrazone, is known to have delayed toxicant activity (Silverman et al. 1991). Identical baits that lacked hydramethylnon were obtained from The Clorox Company.

Tracking of Dyed Food and Feces. To quantify the degree of coprophagy in nymphs, adult females were fed 5% Solvent Green 3 (Alizarin Cyanine Green, Aldrich, Milwaukee, WI), a lipid-soluble dye, in rat chow. Insects and feces were extracted by a modified Bligh and Dyer (1959) procedure that separates an organic solvent phase (containing the dye) from a methanolic-water phase. Macerated samples were ultrasonicated for 1 min (model KT40 Micro Ultrasonic Cell Disrupter; Kontes, NJ) in 1.5-ml microcentrifuge tubes containing 50 μl distilled H_2O and 150 μl MeOH. After the addition of 400 μl isooctane and vigorous vortexing, the samples were centrifuged 12 min at 7,000 $\times$ *g*. The absorbance of an aliquot of the isooctane phase was determined at 590 nm in a microplate spectrophotometer (Bio-Tek, Winooski, VT). The absorbance of an isooctane blank was subtracted from all reported absorbance values.

Coprophagy as the Mechanism of Horizontal Transfer. Coprophagy-based transfer of insecticide can occur only if food and the ingested insecticide pass through the donor's alimentary canal before the donor insect dies. We determined the rate of passage of dyed food through the digestive tract of adult day 0 females that had been starved for 1 scotophase (12 h) and then fed dyed rat chow for 4 h. Twenty females were then grouped in plastic petri plates (150 by 25 mm) and provisioned with water and normal rat chow. Two females were removed from each of 5 replicate dishes at 4- to 12-h intervals after the start of the assay. Extraction of samples and quantification of their dye content was conducted as described above.

To document coprophagy and to measure the passage of ingested feces through nymphs, 25 newly eclosed adult female "donors" were used to generate dyed feces. After being starved for 1 scotophase, females were fed dyed rat chow for 4 h, and then allowed to defecate for 24 h in clean dishes each containing a single tube of water. The adults were then replaced with 35 first instars. One group of nymphs was continuously exposed to dyed feces, and another group was transferred after 4 h to dishes containing undyed adult feces. Nymphs in both treatments were sampled (5 nymphs per sample) 4, 8, 12, 20, and 24 h after the start of their exposure to dyed feces. Five replicates of each treatment were conducted simultaneously.

To determine what fraction of the ingested diet consists of feces, food intake was determined gravimetrically. The mass of feces ingested was estimated by comparing whole body extracts of nymphs that fed on dyed feces to a standard absorbance curve of weighed dyed fecal pellets. Food intake was determined by placing 100 first or 50 second instars that had just ecdysed into rectangular Plexiglas cages (13 by 18 by 9 cm) with food and water available ad libitum.

Food and insects were weighed at 24-h intervals for the first 3 d of the stadium to determine mean food intake per insect. Experiments with 1st and 2nd instars were replicated 5 times each.

Effects of Starvation and the Distance Between Food and Shelter on Coprophagy. All experiments were conducted under controlled environmental conditions identical to the rearing regime. Rectangular Plexiglas cages (120 by 30 by 15 cm high) described in Kopanic and Schal (1997) were used in these assays. A section of cardboard egg carton placed in one corner of each cage served as a shelter. To ensure that introduced cockroaches would remain in this shelter rather than aggregate elsewhere in the cage, we placed the egg carton in a cockroach colony for 7 d; this procedure results in the deposition of aggregation pheromone on the egg carton (Ishii 1970). Fecal particles were removed before the shelter was used. Each cage housed 20 adult females and 50 first or 2nd instars. Two cotton-stoppered water vials were placed near the shelter. Four treatments were used to examine the effects of food availability and proximity to the shelter on coprophagy: (1) Food near (2 cm) the shelter; (2) Food far (125–150 cm) from the shelter; (3) No food for 24 h; and (4) No food for 48 h. Two stainless steel planchets packed with finely ground rat chow were placed either 2 cm from the shelter, 1 near each wall, or in the diagonal corner of the cage opposite the shelter (125–150 cm). We hypothesized that as the distance between the shelter and the food increased, 1st instars would rely more on feces as an alternative food source. A treatment block consisted of 4 cages and this arrangement was replicated 6 times.

To quantify the degree of coprophagy in nymphs, newly eclosed adult females were starved for 1 scotophase (2000–0800 hours) and then fed dyed rat chow for 4 h. Four hours after the beginning of the photophase adults and nymphs were placed for 2 h in a small cage that contained an aggregation pheromone-laden shelter. The shelter with insects clinging to it was then gently moved into the large assay cage. After 20 h, all cockroaches were collected in glass tubes and immediately frozen at −80°C. Samples for spectrophotometric analysis included individual adult females, groups of 5 first or second instars, and fecal samples.

Coprophagy in Field Populations. A similar design was used in vacant apartments to demonstrate that coprophagy occurs under field conditions that do not restrict foraging. Experiments were conducted in vacant apartments of the Raleigh Housing Authority (Raleigh, NC) that had been abandoned for 3 yr and were devoid of live cockroaches. Rooms measuring 2.4 by 3.6 m were cleared of debris and the floors cleaned with a mild detergent. The general design and procedures were similar to the large-cage laboratory assays. Twenty 1-d-old adult females were starved for a single scotophase, fed dyed rat chow, and placed along with 50 first or second instars, into an egg carton shelter in the corner of a room. Water vials were positioned adjacent to either side of the shelter and rat chow, when provided, was placed either near (2 cm) or far (150 cm) from the shelter, on either side. Cockroaches were released at 1400 hours and collected 20 h later. The 3 treatments (no food, rat chow near, and rat chow far) were blocked and replicated 3 times each with 1st and 2nd instars.

Mechanism of Horizontal Transfer: Tests of Alternative Hypotheses. In the cage and field experiments our design is inherently asymmetrical: The bait (with insecticide) is farther than the rat chow from the shelter. Thus, cockroaches that forage near the shelter may not encounter the bait. The linkage observed between neonate and adult mortality (Kopanic and Schal 1997) might therefore result from a coupling of their foraging activities, not from horizontal transfer of the bait. An alternative to coprophagous behavior is the hypothesis that adults that fed on bait might stimulate nymphs to forage. To test the hypothesis that novel odors obtained by foraging adults stimulate nymphs to forage in search of the odors, we conducted a double exclusion assay using 2 feeding stations symmetrically equidistant (120 cm) from the shelter. One station contained a nontoxic bait (MAXFORCE gel without hydramethylnon) and permitted adults access but not nymphs (an oil moat prevented nymph entry; see Kopanic and Schal 1997). The 2nd station contained a toxic bait (MAXFORCE gel with hydramethylnon) and permitted nymphs access but not adults (a 2-mm mesh screen prevented adult entry). Each assay cage contained 20 adult females and 50 first or second instars. After 48 h the baits were removed and adult and nymph mortality was recorded 24 h later. In another set of experiments the bait was kept in place for 96 h, removed, and adult and nymph mortality was recorded 48 h later. This treatment was conducted to determine if exposure time would directly affect the amount of nymph mortality. In these assays, adults could deliver insecticide-free bait to the shelter and, if nymphs were stimulated to forage, nymphal mortality would increase because they had direct access to the toxic bait. Three replicates were conducted for both 1st and 2nd instars.

Time-Lapse Video Analysis of Foraging Activity. Differences in foraging between nymphs and adults were directly examined by continuous video recording of all visits to baits in the large-cage exclusion assays over a 48-h period. Moreover, placement of a screen over the bait that excluded adults allowed us to directly test the conjecture that adult contact with the bait might induce nymphs to forage. Visits to baits were monitored with an infra-red sensitive ultricon video camera (RCA TC1005) with a 16- to 160-mm auto iris and remote control zoom lens. The camera was connected to a time-lapse video recorder (Panasonic AG-6050, Secaucus, NJ) providing ≈1.25 images per second. Video records were analyzed frame-by-frame where necessary to distinguish among the several insects that may have been visiting a bait simultaneously. A visit was defined as contact with the screen covering the bait because in some treatments adults were excluded from the bait. Cage designs were the same as those described in Kopanic and Schal (1997). Cages with a small mesh screen and large mesh

screen were videotaped simultaneously and replicated 3 times.

Data Analysis. The passage of dyed food through adult females and the passage of ingested feces through nymphs were analyzed using the Kruskal–Wallis nonparametric single-factor analysis of variance (ANOVA). The Mann–Whitney *U* test was then used to identify significant differences among treatments (StatView 1998). Assays to determine what effect starvation and food proximity to shelter had on coprophagy were analyzed using a single-factor ANOVA. The Games–Howell test was then used to identify significant differences among treatments for 1st and 2nd instars (StatView 1998). Comparisons of food intake and of mortality in the novel food odor assays were analyzed using the Student *t*-test.

The frequencies of visits to baits, obtained from video records, were analyzed with the sign test, a nonparametric paired-sample test (Zar 1996). This test permitted hourly comparison of visits during the scotophase between or within replicates. Only the 1st scotophase was considered because this was the peak period of activity. In all statistical procedures, results were considered significant when $P < 0.05$.

Results and Discussion

Coprophagy Effects the Horizontal Transfer of Bait. Our experimental results support the hypothesis that coprophagy is the primary mechanism by which nymphs obtain toxicant from foraging adults. Moreover, the availability and proximity of other food to nymphs affects the level of coprophagy in both laboratory and field populations of *B. germanica.* To validate the passage of toxicant from foraging adults to small nymphs we tracked the flow of a tracer dye that was incorporated into rat chow and fed to "donor" insects. We first determined how long after feeding adult female donors could produce labeled feces by measuring the rate of passage of dyed food through the digestive tract of adult females. Females were starved for 1 scotophase (12 h) and then fed dyed rat chow for 4 h. In all cases, the exterior of each female was washed to remove extraneous dye before females were homogenized and the ingested dye extracted. In general, the amount of recovered dye decreased over time, but loss of dye (i.e., defecation) occurred in 3 different phases over the course of 48 h (Kruskal–Wallis test; $P < 0.001$, df = 9, $H = 76.838$) (Fig. 1). Females were maximally labeled in the first 8 h after they fed on dyed food. Afterward, between 12 and 28 h, there was a significant sharp drop by ≈50% in dye content. A significant decline to a plateau followed between 32 and 48 h (Fig. 1). These data suggest that a significant amount of the ingested bait is defecated by 12 h after it is ingested. This implies that for horizontal transfer of bait to occur via coprophagy, the forager must remain alive for at least 12 h after it ingests the toxic bait. It is nonetheless important to note that commercial baits, especially liquid gel baits, may be processed more quickly than rat chow (R.J.K., unpublished data).

To confirm that nymphs ingested adult feces and to measure the rate of passage of adult feces through

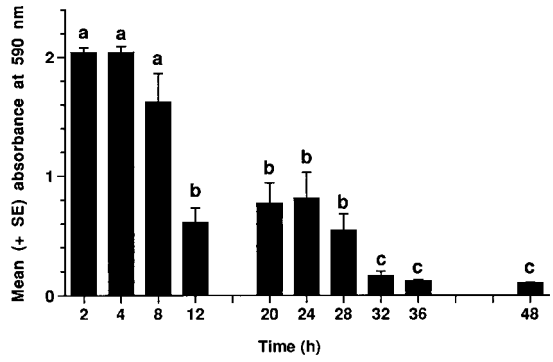

Fig. 1. Time course of dye elimination by adult female *B. germanica.* Females were starved for 1 scotophase (12 h) and were then provided with 5% Solvent Green 3 dye in rat chow for 4 h. Two females were randomly sampled from each dish at each period. Whole bodies were externally rinsed and then homogenized. Solvent Green 3 was extracted from each sample. Bars represent mean absorbance per insect ± SE ($n = 5$). Different letters above bars represent a significant difference among means as determined with the Mann–Whitney *U* test.

nymphs, we placed 35 first or second instars either continuously or for only 4 h in petri dishes containing female-generated dyed feces. First instars ingested significant amounts of adult feces in the absence of food, resulting in a monotonic increase in dye within the nymphs for up to 12 h (Fig. 2A). Even when given

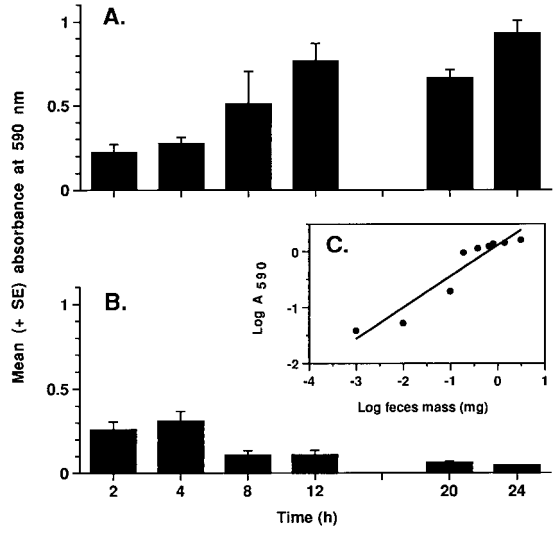

Fig. 2. Time course of dye uptake in 1st-instar *B. germanica.* (A) After 12 h starvation, nymphs were continuously exposed to dyed feces. (B) After 12 h starvation, nymphs were given 4 h access to dyed feces and then switched to undyed feces. Bars represent the means ± SE of 6 replicates (5 insects per sample at each sample time). (C) Relationship between the mass of dyed adult feces and its dye content. $n = 3$ per determination. The equation for the line is $y = 0.556$ [SE = 0.068]$x + 0.112$ [SE = 0.087] ($r^2 = 0.906$; 9 observations; $P < 0.001$).

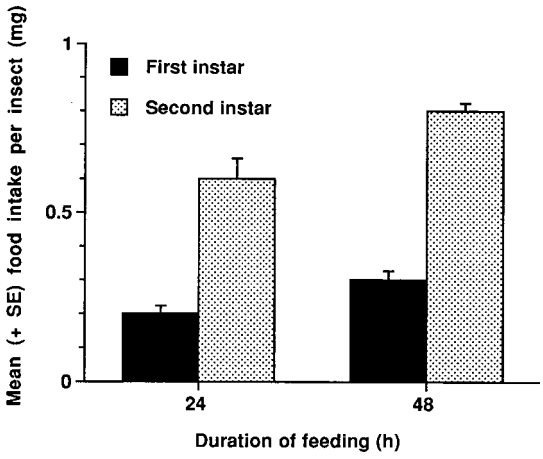

Fig. 3. Rat chow intake in 1st- and 2nd-instar *B. germanica.* Nymphs (100 first or 50 second instars) that had just ecdysed were provided rat chow and water ad libitum. Intake was determined by weighing food after 24 and 48 h. Bars represent the means ± SE of 5 replicates.

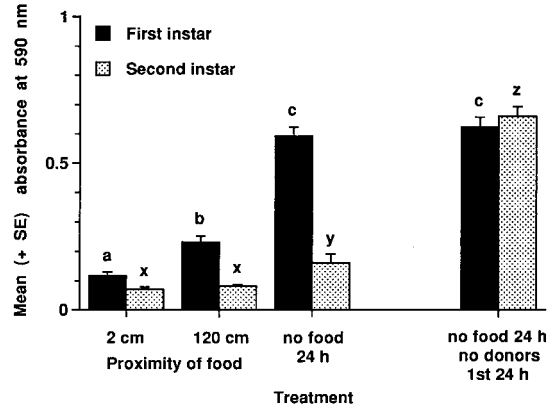

Fig. 4. The relationship between food availability and coprophagy in 1st and 2nd instars in large cage bioassays. The distance between the food and shelter and availability of food were varied while nymphs had access to dyed adult feces. In the first 2 sets of treatments, food was 2 and 120 cm from the shelter, respectively. In the 3rd treatment there was no food present. In the 4th treatment there was no food present and adult donor insects were added 24 h after start of assay instead of simultaneously with 1st and 2nd instars. Bars represent the means ± SE of 60 measurements over 6 replicates. Different letters represent significant differences among treatments for 1st or 2nd instars (ANOVA; Games–Howell test).

a short (4-h) exposure to dyed feces and subsequently offered undyed adult feces, 1st instars ingested significant amounts of dyed feces (Fig. 2B). Interestingly, a decline in whole body (ingested) dye occurred between 4 and 8 h, earlier than in adult females ingesting dyed rat chow (Fig. 1). The results from continuous exposure assays suggest that, in the absence of food, 1st instars readily ingest adult feces. A linear relationship between absorbance at 590 nm and the mass of dyed adult feces is depicted in Fig. 2C. Extrapolation from this relationship showed that in the first 4 h, a 1st instar ingested ≈0.063 mg of adult feces and after 24 h contained dye equivalent to that contained in ≈0.646 mg of adult feces. The mass (mean ± SE) of an adult female fecal pellet is 0.205 ± 0.0175 mg (n = 20). Direct gravimetric determinations of food intake indicated that each 1st instar ingested 0.211 ± 0.024 mg of rat chow in 24 h (Fig. 3), suggesting that when food is not available nymphs ingest 3-fold more fecal material by mass than diet. Consumption of this amount of feces supports results showing that coprophagy in 1st instars is adaptive, significantly increasing their survivorship in the absence of other food (R.J.K., unpublished data).

Coprophagy Affected by Food Availability and its Proximity to the Shelter. To elucidate the effects of starvation and proximity of food on nymphal coprophagy we fed adult females dyed rat chow (to label their feces) and varied the distance between undyed rat chow and the shelter. Based on assays in smaller cages (Fig. 2), we hypothesized that coprophagy would be higher in starved nymphs and even more predominant when the distance between the shelter and the food was increased.

In all cases the dyed feces of adult females were maximally labeled ($A_{590} \geq 2.00$; data not shown). Coprophagy increased significantly in both 1st (ANOVA; $P < 0.001$, df = 3, $F = 95.414$) and 2nd ($P < 0.001$, df = 3, $F = 156.880$) instars as the food was placed farther from the shelter (Fig. 4), suggesting a propensity of nymphs, especially 1st instars, to remain near the shelter. Interestingly, despite their larger mass (2 times) and greater food intake (3 times) (Fig. 3), 2nd instars ingested significantly less adult feces than 1st instars in the first 24 h. For example, when food was 120 cm from the shelter, 1st instars ingested nearly 3 times more feces than 2nd instars (Student t-test; $P < 0.001$, df = 97, $t = 6.618$) (Fig. 4).

The most striking increase in coprophagy occurred in starved nymphs. A comparison of coprophagy by starved 1st instars in large (Fig. 4) and small cages (Fig. 2A) indicated almost identical A_{590} values (0.593 and 0.766, respectively), supporting a switch to coprophagy under both conditions. The significant increase in coprophagy in starved 1st instars compared with treatments where nymphs must forage 120 cm supports the idea that nymphs use coprophagy more when other food resources are distant (Kopanic and Schal 1997).

Although coprophagy increased in starved 2nd instars, the latter still ingested significantly less dyed feces than did starved 1st instars (Student t-test; $P < 0.001$, df = 94, $t = 10.236$) (Fig. 4). We hypothesized that 2nd instars, perhaps because of their greater mass, might be more tolerant of starvation and therefore resort to coprophagy only after a longer period of starvation. When neither food nor feces were available for 24 h and only dyed adult feces was provided during the next 24 h, nymphs readily ingested feces and 2nd instars ingested significantly more after 48 h

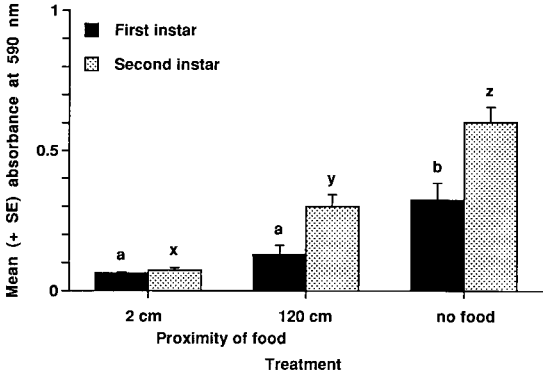

Fig. 5. The relationship between food availability and coprophagy in 1st and 2nd instars in field bioassays. The distance between the food and shelter and availability of food were varied while nymphs had access to dyed adult feces. In the first 2 sets of treatments, food was 2 and 120 cm from the shelter, respectively. In the 3rd treatment there was no food present. Bars represent the means ± SE of 30 measurements over 3 replicates. Different letters represent significant differences between comparisons of treatments among 1st or 2nd instars (ANOVA; Games–Howell test).

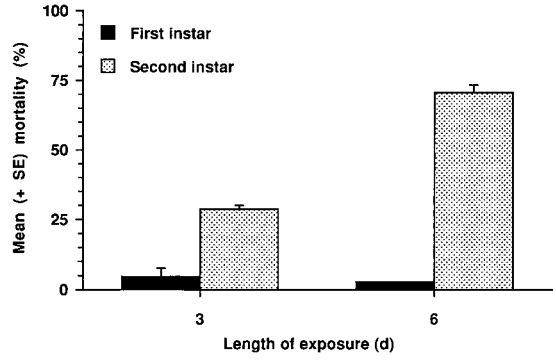

Fig. 6. Mortality of 1st- and 2nd-instar *B. germanica* in large cage bioassays to determine if adults could stimulate nymphs to forage to a remote bait. Adults fed on a novel bait lacking an insecticide, whereas nymphs had access to the same bait, but with an insecticide. Bars represent the means ± SE of 3 replicates.

than after 24 h of starvation ($P < 0.001$, df = 97, t = 11.403). These data clearly demonstrate that small nymphs engage in coprophagy and also support the hypothesis that 1st instars rely more on the feces of foraging insects than do 2nd instars. It thus appears that younger nymphs ingest more feces near the shelter as food becomes more scarce. Older nymphs may be more tolerant of starvation and coprophagy fulfills a smaller fraction of their dietary needs.

Coprophagy in Field Bioassays. As in the large cage assays, nymphal coprophagy increased in field bioassays as the distance between the aggregation and food increased (ANOVA; $P < 0.001$, df = 2, $F = 16.098$); 2nd instars: $P < 0.001$, df = 2, $F = 49.640$) (Fig. 5). The greatest amount of coprophagy occurred when nymphs were starved. The apparently lower intake of feces in apartments compared with the laboratory assays might be attributable to presence of other food in the apartments, lower night-time temperatures in the field, and a wider dispersion of adult feces than in large-cage laboratory bioassays. Interestingly, 2nd instars contained more dye than 1st instars, suggesting that 2nd instars were more effective than 1st instars at locating feces dispersed over a larger area, that some cannibalism and necrophagy by 2nd instars might occur in the field, and that 1st instars tended to remain within or near the shelter and therefore encountered less dyed feces. Nevertheless, these data again support the idea that coprophagy in nymphs plays a significant role in the horizontal dissemination of bait by foraging insects, and supports the notion that baits should be placed near cockroach aggregations if they are to effectively target smaller nymphs.

Adult Foragers Do Not Induce Nymphs to Forage Greater Distances. Although these results, together with earlier observations (Silverman et al. 1991, Kopanic and Schal 1997), strongly implicate coprophagous behavior in nymphal mortality, they do not preclude other mechanisms that may account for nymphs obtaining dyed food or toxic baits. To test the hypothesis that adults deliver odors or bait to the shelter that in turn stimulate nymphs to forage and hence obtain active ingredient on their own, we conducted a double exclusion assay using 2 feeding stations adjacent to each other and equidistant (120 cm) from the shelter. When adults foraged on a nontoxic bait and nymphs could gain access to an adjoining toxic bait, 1st-instar mortality remained low (4.7 and 5.0% in 3 and 6 d exposure, respectively) and constant ($t = 1.180$, df = 4, $P = 0.304$) (Fig. 6). It thus appears that foraging by 1st instars was not stimulated by the novel odors or bait ingredients delivered to them by the adult females.

By contrast, 2nd instar mortality increased significantly ($P = 0.001$, df = 4, $t = 14.087$) from 28.7 ± 1.3% in 3 d to 70.7 ± 2.7% in 6 d of exposure to the baits (Fig. 6). In similar experiments without the nontoxic baits, Kopanic and Schal (1997) had comparable results for 2nd instars, indicating that more extensive foraging and greater mortality were independent of adult female activity. Substantially greater mobility and dietary needs of 2nd instars (Fig. 3) presumably result in greater locomotor activity, as in other insects (Bernays and Simpson 1982), thus increasing the encounter rate with the toxic bait. Interestingly, although adult females were physically prevented from eating the toxic bait, 8.3 ± 6.0% females died in the 6-d treatment with 2nd instars (data not shown). Adult mortality could be caused by antennal contact with bait, or possibly by a reversal of the horizontal transfer pathway in which some adults contacted insecticide that was delivered to the shelter by foraging 2nd instars.

Video recording of baits provided a direct means of resolving whether or not nymphs foraged more when adults fed on bait. In cages where adults were excluded from the bait (i.e., small-mesh screen), nymph and adult visits to the bait persisted during the 2nd

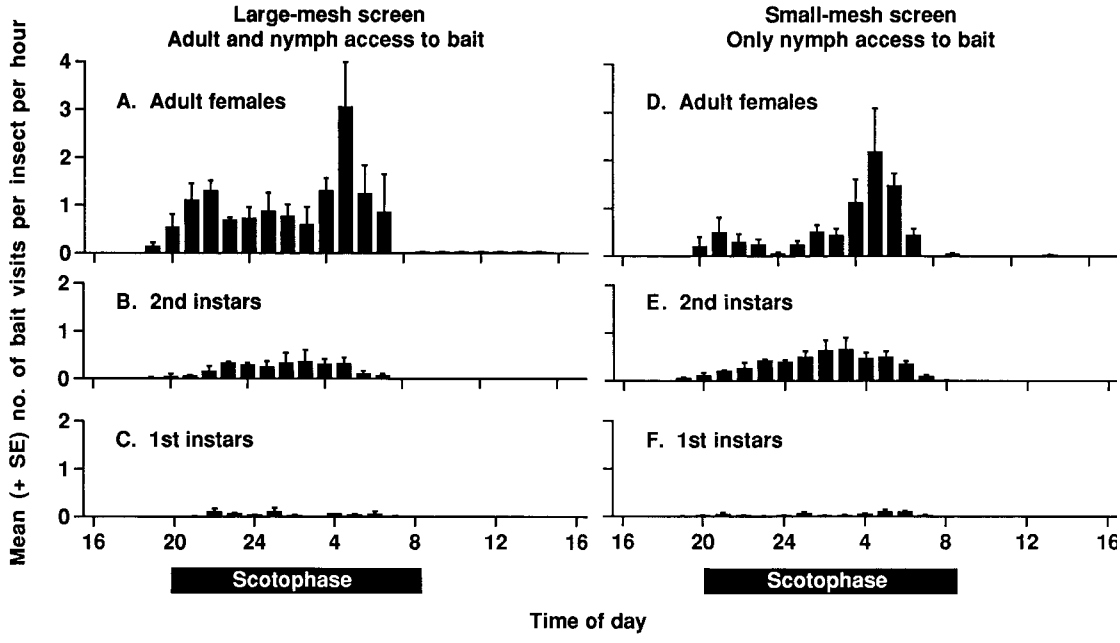

Fig. 7. Hourly visits to baits by adults (A, D), 2nd instars (B, E), and 1st instars (C, F) of *B. germanica* in the large cage bioassay. Only the first 24 of a 48-h experiment is presented for both large-mesh screen (A, B, C) and small-mesh screen (D, E, F) treatments. Large-mesh screens over the bait permitted both adults and nymphs access to the bait, whereas small-mesh screens permitted only nymphs access to the bait. Bars represent the mean number of visits per hour per insect ± SE $n = 3$.

scotophase because all adults survived this treatment and mortality was minimal in 1st instars (Kopanic and Schal 1997). However, when adults could feed on the toxic bait (i.e., large-mesh screen type) a dramatic decline in adult bait contacts occurred during the 2nd scotophase. Therefore, the frequency of visits to the baits was measured only during the first 24 h of the assay. Adults and nymphs in all assays exhibited clear diel patterns of activity, with feeding peaks occurring ≈2–4 h before lights-on (Fig. 7). Neonates, unlike adults, seldom visited baits 120 cm from their shelter and the activity of the nymphs appeared independent of whether adults had access to the bait. There was no difference in visitation to baits between 1st instars in small- and large-mesh screen assays throughout the scotophase (Sign test; $P > 0.05$; 36 comparisons). These results support the hypothesis that, at least when food is nearby, neonates do not venture far from the shelter.

Assays with 2nd instars revealed similar diel patterns of activity. Interestingly, 2nd instars visited the bait significantly more (Sign test; $P < 0.001$) when adult females were denied access to the bait (Fig. 7), contrary to the hypothesis that adults somehow facilitate the foraging activities of small nymphs. However, 2nd instars, although only 6 d older than 1st instars, were significantly more active than 1st instars in both large-mesh and small-mesh screen exclusion assays over the course of the entire scotophase (Sign tests; $P < 0.001$ for both comparisons).

These results corroborate the conclusion that intrinsic differences in shelter-fidelity and foraging ranges between 1st and 2nd instars influence their relative vulnerability to baits and to horizontal transfer of bait ingredients. Young, less mobile nymphs are the most difficult to reach with conventional insecticides under normal conditions (Bret and Ross 1983, Ross et al. 1984). Ross et al. (1984) demonstrated that in experimental field populations early instars remained within known harborage sites and trapping studies demonstrated that they were seldom captured. Laboratory experiments also suggested strong within-harborage aggregation of early instars (Bret and Ross 1983). In the field, young nymphs (1st and 2nd instars) foraged less frequently and with 'less efficiency' than older nymphs (Cloarec and Rivault 1991) and 2nd-instar *B. germanica* were less mobile than 5th instars in a small research arena (DeMark et al. 1993). Adult-mediated translocation of insecticide bait to the shelter contributed much more to mortality of 1st than 2nd instars (Kopanic and Schal 1997). These findings, coupled with the time-lapse video results, clearly show that 1st instars remain closer to their shelter, forage less frequently than older insects, engage more readily in coprophagy, and are therefore most affected by horizontally translocated insecticide.

In conclusion, we have demonstrated that coprophagy in nymphs contributes significantly to their vulnerability to translocated baits. In both laboratory and field trials, nymphal coprophagy increased as the distance between the aggregation and food increased; maximal levels coprophagy occurred in starved nymphs. These results are consistent with our conclusion that mortality in 1st instars was largely attrib-

utable to toxic bait being delivered to the aggregation in the feces of foragers (Kopanic and Schal 1997). We also supported this conclusion in 1 experiment by rejecting an alternative explanation that bait ingredients might stimulate 1st instars to forage. Coprophagous exposure of nymphs to insecticide baits can thus be an effective means to deliver insecticides to hidden aggregations of *B. germanica* provided the active ingredient permits the foraging insect to return to an aggregation before it succumbs to the insecticide. This approach will be less effective with fast-acting insecticide baits that fail to traverse the alimentary tract before the forager dies.

Acknowledgments

We thank R. L. Brandenburg, G. L. Holbrook, R. J. Kuhr, C. Nalepa, and H. P. Young for critical reviews of earlier drafts of the manuscript. We also thank the Clorox Company for the gift of hydramethylnon and baits free of hydramethylnon. This study was supported in part by the Blanton J. Whitmire Endowment at North Carolina State University, a grant from the W. M. Keck Foundation in support of a Behavioral Biology Program at North Carolina State University, a Research Excellence Award from Orkin Pest Control, the Steritech Group, and an Urban Entomology Scholarship from the North Carolina Pest Control Association.

References Cited

Bernays, E. A., and S. J. Simpson. 1982. Control of food intake. Adv. Insect Physiol. 16: 59–118.

Bret, B. L., and M. H. Ross. 1983. Influence of adult females on within-shelter distribution patterns of *Blattella germanica* (Dictyoptera: Blattellidae). Ann. Entomol. Soc. Am. 76: 847–852.

1985. A laboratory study of German cockroach dispersal (Dictyoptera: Blattellidae) Proc. Entomol. Soc. Wash. 87: 448–455.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 39: 911–917.

Cloarec, A., and C. Rivault. 1991. Age-related changes in foraging in the German cockroach (Dictyoptera: Blattellidae). J. Insect Behav. 4: 661–673.

Cochran, D. G. 1983. Food and water consumption during the reproductive cycle of female German cockroaches. Entomol. Exp. Appl. 34: 51–57.

DeMark, J. J., T. Kuczek, and G. W. Bennett. 1993. Laboratory analysis of the foraging efficiency of nymphal German cockroaches (Dictyoptera: Blattellidae) between resource sites in an experimental arena. J. Econ. Entomol. 86: 372–378.

Deneubourg, J. L., S. Aron, S. Goss, and J. M. Pasteels. 1990. The self-organizing exploratory pattern of the Argentine ant. J. Insect Behav. 3: 159–168.

Hamilton, R., and C. Schal. 1988. Effects of dietary protein levels on reproduction and consumption in the German cockroach (Dictyoptera: Blattellidae). Ann. Entomol. Soc. Am. 81: 969–976.

Ishii, S. 1970. An aggregation pheromone of the German cockroach, *Blattella germanica* (L.) 2. Species specificity of the pheromone. Appl. Entomol. Zool. 5: 33–41.

Johnson, D. L., and J. E. Henry. 1987. Low rates of insecticides and *Nosema locustae* (Microsporidia: Nosematidae) on baits applied to roadsides for grasshopper (Orthoptera: Acrididae) control. J. Econ. Entomol. 80: 685–689.

Klotz, J. H., K. M. Vail, and D. F. Williams. 1997. Liquid boric acid bait for control of structural infestations of Pharaoh Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 90: 523–526.

Kopanic, R. J., Jr., and C. Schal. 1997. Relative significance of direct ingestion and adult-mediated translocation of bait to German cockroach nymphs (Dictyoptera: Blattellidae). J. Econ. Entomol. 90: 1073–1079.

Mukerji, M. K., A. B. Ewen, C. H. Craig, and R. J. Ford. 1981. Evaluation of insecticide-treated bran baits for grasshopper control in Saskatchewan (Orthoptera: Acrididae). Can. Entomol. 113: 705–710.

Reierson, D. A. 1995. Baits for German cockroach control, pp. 231–265. *In* M. K. Rust, J. M. Owen, and D. A. Reierson [eds.], Understanding and controlling the German cockroach. Oxford University Press, New York.

Ross, M. H., B. L. Bret, and C. B. Keil. 1984. Population growth and behavior of *Blattella germanica* (L.) (Orthoptera: Blattellidae) in experimentally established shipboard infestations. Ann. Entomol. Soc. Am. 77: 740–752.

Schal, C., and R. L. Hamilton. 1990. Integrated suppression of synanthropic cockroaches. Annu. Rev. Entomol. 35: 521–551.

Schal, C., G. L. Holbrook, J.A.S. Bachmann, and V. L. Sevala. 1997. Reproductive biology of the German cockroach, *Blattella germanica:* Juvenile hormone as a pleiotropic master regulator. Arch. Insect Biochem. Physiol. 34: 405–426.

Sherron, D. A., C. G. Wright, M. H. Ross, and M. H. Farrier. 1982. Density, fecundity, homogeneity and embryonic development of German cockroach (*Blattella germanica* (L.) populations in kitchens of varying degrees of sanitation. Proc. Entomol. Soc. Wash. 84: 376–390.

Silverman, J. 1986. Adult German cockroach (Orthoptera: Blattellidae) feeding and drinking behavior as a function of density and harborage-to-resource distance. Environ. Entomol. 15: 198–204.

Silverman, J., G. I. Vitale, and T. J. Shapas. 1991. Hydramethylnon uptake by *Blattella germanica* (Orthoptera: Blattellidae) by coprophagy. J. Econ. Entomol. 84: 176–180.

Sommer, S. H. 1975. Experimental investigation of the circadian locomotor activity of *Blattella germanica* (Dictyoptera: Blattellidae). Biol. Zentralbl. 94: 451–467.

Su, N. Y., and R. H. Scheffrahn. 1991. Laboratory evaluation of two slow-acting toxicants against Formosan and eastern subterranean termites. J. Econ. Entomol. 84: 170–175.

StatView. 1998. Version 5.0 Power PC. SAS Institute, Cary, NC.

Stejskal, V. 1997. Distribution of faeces of the German cockroach, *Blattella germanica*, in a new refuge. Entomol. Exp. Appl. 84: 201–205.

Valles, S. M., C. A. Strong, and P. G. Koehler. 1996. Inter- and intra-instar food consumption in the German cockroach, *Blattella germanica.* Entomol. Exp. Appl. 79: 171–178.

Young, H. P., and C. Schal. 1997. Cuticular hydrocarbon synthesis in relation to feeding and developmental stage in nymphs of *Blattella germanica* (Dictyoptera: Blattellidae). Ann. Entomol. Soc. Am. 90: 656–663.

Zar, J. H. 1996. Biostatistical analysis, 3rd ed. Prentice-Hall, Englewood Cliffs, NJ.

Received for publication 8 July 1998; accepted 17 February 1999.