

## BEHAVIOR

# Host-Finding, Host-Recognition, and Host-Acceptance Behavior of *Cephalonomia tarsalis* (Hymenoptera: Bethyridae)

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**ABSTRACT** Several bethylids, including various *Cephalonomia* spp., are commonly associated with the stored-commodity environment. These parasitoids are often host-specific and can be important biocontrol agents. Although *C. tarsalis* (Ashmead) reportedly uses several different stored-product beetle hosts, it appears to be primarily associated with the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.). This article reports on some of the sensory modalities that female *C. tarsalis* use to find, recognize, and accept a host, and it provides an ethogram for the behavior of the parasitoid from initial searching until she releases the host in preparation for oviposition. Comparative ethograms for putative alternative hosts of *C. tarsalis* are also given. Vision plays only a limited role in host-finding and -recognition. Chemical cues, primarily borne on the cuticle of the host and perceived through the wasp's antennae, as well as movement by the host once contacted, are major host-recognition cues used by the parasitoid. Analysis of the ethograms indicates that a complex behavioral repertoire is used by *C. tarsalis* in the sequence of searching for the host, host-location, -recognition, -stinging, and host-feeding before oviposition occurs. The putative alternative hosts studied showed markedly truncated ethograms (which never include stinging) compared with the ethogram with the sawtoothed grain beetle, indicating that the parasitoid would not be likely to use these alternative hosts under normal situations.

**KEY WORDS** Bethyridae, behavioral ecology, ectoparasitoid, biological control, kairomone, parasitoid

THE PRIMITIVE ACULEATE family Bethyridae uses small larvae as hosts (primarily Coleoptera and Lepidoptera), and these hosts live in cryptic situations such as in the soil, plant stems, wood, seeds, cases, or rolled leaves (Evans 1964). Bethyrids subdue their hosts by multiple stinging and lay 1 to several eggs externally. The resulting bethylid larvae develop as ectoparasitoids, dropping off the exhausted remains of their host to pupate gregariously in silk cocoons. Males normally emerge before females and often inseminate their sisters or even their mothers in some cases (Evans 1964). Some bethylids are gregarious, have complex polymorphisms, and exhibit subsocial behavior (Evans 1964, Casale 1991).

Several bethylids are parasitoids of beetles infesting the stored grain environment, and can be important biocontrol agents (Gahan 1931, Kearns 1934, Powell 1938, Flinn and Hagstrum 1995, Flinn et al. 1996). The major species found in the stored grain ecosystem represent the genera *Cephalonomia*, *Holepyris*, and *Laelius* (Evans 1964, Gordh and Móczár 1990). The literature suggests that some of these parasitoids will attack a number of hosts, whereas others are thought to be more host-specific. As part of a larger program

to evaluate parasitoids as biological control agents for the stored grain environment, we have been studying the chemical and physiological ecology of several bethylids, including *C. tarsalis* (Ashmead), whose nominal host is the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae). Basic life history data has been previously published for *C. tarsalis* (Powell 1938), but the behavioral mechanisms by which this parasitoid locates, recognizes, and accepts its host(s) remain largely unknown. The objective of this study was to characterize these behavioral mechanisms and to examine behavioral responses of *C. tarsalis* to alleged alternative hosts.

## Materials and Methods

Stocks of *C. tarsalis*, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst), and *Plodia interpunctella* (Hübner) were collected from farm-stored wheat in Kansas and have been in culture for several years. All *C. tarsalis* used for experiments, unless noted otherwise, were newly emerged to 5-d-old females that had been reared on *O. surinamensis* in wheat, in total darkness, at 30°C and 45–65% RH. These females were mated but had no exposure to host larvae as adults. Before testing, each wasp was placed individually in a screen-topped 4-ml vial containing a drop of 50% honey-water and held for 2 d at the same conditions as above.

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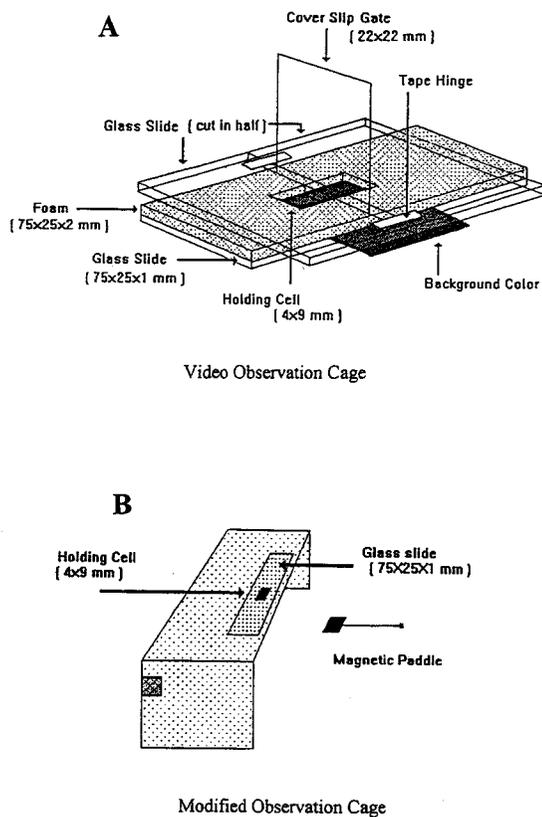


Fig. 1. Observation cages used for video recordings. 1A was used for normal recordings. 1B was for recording with the moveable paddle underneath the cage.

*O. surinamensis* was reared on rolled oats with 3% brewer's yeast; *C. ferrugineus* on unbleached wheat flour with 6% brewer's yeast and 1% stabilized wheat germ; *T. castaneum* on whole wheat flour with 5% brewer's yeast; and *P. interpunctella* on a semisynthetic diet (McGaughey and Beeman 1988). All host cultures were held under the same environmental conditions as the parasitoids. Host larvae were taken directly from stock cultures at the time of testing. Fourth instars of *O. surinamensis* and *C. ferrugineus*, and 2nd instars of *T. castaneum* and *P. interpunctella* were used for assays. The 2nd instars were the same size as the 4th instars.

**Video Recording.** The behavior of experimental organisms was recorded using a Panasonic digital Hi-8 video camera, model WV-CP4100 (Panasonic Broadcast and Television Systems, Secaucus, NJ) attached to a Wild M8 stereomicroscope (Wild Heerbrugg, Heerbrugg, Switzerland) and a Sony digital Hi-8 video cassette recorder, model EV-S7000 (Sony, Park Ridge, NJ). Recording tapes were Sony HMP120 Hi-8 format. Images were viewed on a Sony KV-27V10 Trinitron stereo color television. Lighting was provided by a fiber optic microscope continuous ring light, which gave an intensity of 40,000 lx ( $\pm 10\%$ ) at the level of the insects. Insects were viewed in an observation cage (Fig. 1) constructed from 2 glass microscope slides,

separated by a comparably sized 2-mm thick piece of foam. A cell (9 by 4 mm) was cut from the center of the foam, and a piece of light blue paper was cut to fit under the foam as a background for filming and to provide purchase for the insects. The bottom slide, foam, and background were held together with double-sided tape. The top slide was cut in half widthwise and the pieces were placed side by side and attached with 2 tape hinges to form a set of hatch doors. A slit was cut in the foam across the center of the cell, just wide enough to hold a glass cover slip (22 by 22 mm). The slit was cut at a slight angle, away from the side to be filmed, to prevent interference by the cover slip gate. The hatch doors were then centered on the foam and the cover slip gate put in place to form 2 cells. The microscope magnification was adjusted so that one side of the cell filled the field of view. To record behavior, a parasitoid was put in one side of the cage and a host larva in the other side. The insects were allowed to acclimate for 5 min. The video recording was started, the gate carefully lifted, and the parasitoid allowed to enter the cell holding the host larva. The gate was then closed, and unless otherwise noted, behavior was recorded for 2 min.

**Construction of Ethograms.** The interaction of the parasitoid and host in the observation cage was observed from the initiation of searching by the parasitoid through the resting behavior. Detailed analysis of the sequence of behaviors using frame-by-frame (30 frames per second) video analysis allowed the identification of 18 discrete behaviors, which were then used for quantitative analysis. Behavioral sequences were recorded for individual parasitoids: frequencies associated with transitions from one behavior to another were tabulated and translated into a 1st-order transition probability matrix using established methods (Fagen and Young 1978), with the modification of Charlton and Cardé (1990) that provides equal weighting to the individual behaviors in the consolidated matrix. Self transitions and impossible transitions were left as blanks. These probabilities were used to generate ethograms to summarize the interaction of *C. tarsalis* with its normal host, *O. surinamensis* (presented in 2 sections, with the 1st section derived from the observation of 25 wasps and the 2nd section derived from the observation of 12 wasps), and the interactions of the parasitoid with 3 other stored grain pests: *Cryptolestes ferrugineus* (potential host in the same family as the normal host), *Tribolium castaneum* (potential host in the same Order but different family) and *Plodia interpunctella* (potential host in a different Order).

**Sensory Modality Experiments.** Female parasites were held in individual 4-ml vials with a drop of 50% honey-water for 24 h in the incubator before being anesthetized on ice and manipulated as indicated below. They were then held another 24 h in the incubator before bioassays were performed by placing the wasp in the observation cage, presenting her with a host larva, and filming the resulting behavior. Controls consisted of sham operated wasps held under identical conditions. The results of both negative and positive

experiments were analyzed by replaying the tape, noting which wasps stung the hosts, and how long it took for the wasp to sting the host.

**Test of Required Visual Input.** The eyes of female wasps were completely coated with nail polish (Zia white ultragloss nail color, toluene and formaldehyde free, Lea Cosmetics, Port Washington, NY) and allowed to dry for 15 min before removing the wasp from the ice. Controls in this case consisted of treated wasps that successfully groomed the white paint from their eyes.

**Test of Required Chemosensory Input Via Antennae.** Both antennae, except for the basal segments, of the female wasp were removed using a razor blade.

**Test of Required Chemosensory Input Via Maxillary Palps.** Both maxillary palps were scraped away using a small knife constructed from a razor blade.

**Test of Required Movement by Host.** Beetle larvae were killed by freezing for 24 h, thawed, air-dried on filter paper for a few minutes, and presented to the wasp. Controls consisted of live untreated larvae. Because this experiment was a negative test (no movement), a positive test was also conducted. In the positive test the beetle larvae were killed by freezing for 24 h and 30 min before testing, each larva was impaled longitudinally as close to the ventral surface as possible with a size 0.1 minuten pin. The exposed end of the pin was clipped off and the impaled larvae were then returned to the freezer until test time. A modified observation cage (Fig. 1B) was used for the bioassay. To make the cage, a cell (9 by 4 mm) was cut out of a piece of cardboard, and a floor was formed by taping a colored piece of paper underneath it for contrast. A glass microscope slide was placed on top. The cage was raised up on a cardboard platform for ease of movement underneath it. To conduct the positive movement bioassay, a frozen, impaled beetle larva was removed from the freezer, allowed to thaw and dry on a piece of filter paper for  $\approx 5$  min, and placed in the cell of the observation cage. One 0–3 d mated female *C. tarsalis* that had been held for 24–48 h with 50% honey water, but without exposure to host larvae, was introduced into the observation cage and the cell was quickly covered with the glass slide. A magnetic paddle constructed from a flat magnet (4 by 6 mm) placed on the end of an insect pin was then placed under the cell and rotated in a circular motion, causing the impaled beetle larva to move around the cell. The response of the parasitoid was video recorded for 2 min or until the wasp stung the host. Frozen, impaled larvae that were not moved with the paddle served as controls.

**Trail-Following by Female Wasps.** Mated bioassay females were isolated at 0–3 d postemergence and held in individual vials for 48 h with a drop of 50% honey water. A single sawtoothed grain beetle larva was added to each vial and the female wasp was allowed to sting it. The host was removed before the wasp could host feed. These host-experienced wasps were then held for an additional 24 h before being used in the trail-following bioassay. Preliminary experiments had shown that using host-experienced fe-

males provided more consistent behavioral responses than trials using host-naive females, as was the case for trail-following responses of *Cephalonomia waterstoni* Gahan (Howard and Flinn 1990). Host trails were obtained by allowing 10 *O. surinamensis* larvae to walk for 3 h in the trail-laying apparatus described in Howard and Flinn (1990), the only change being that the circular path on which the beetles walked was lightly marked with a number 3 pencil every centimeter. The beetle larvae and rings were removed, and a single *C. tarsalis* female, treated as described above, was introduced into the center of the filter paper (which had been placed in a petri dish and covered with a petri dish lid to exclude air currents), and monitored with videotaping for evidence of trail-following behavior for 2 min. The wasp was then removed, and another wasp added to the center of the bioassay chamber. Five wasps were tested sequentially ( $n = 3$  trails). Trail-following was considered to have occurred if the female turned upon contacting the circle the hosts had walked on and followed the path the beetles had traversed while intensely antennating and walking more slowly, using the stereotypical zigzag walking behavior of trail-following insects (Howard and Flinn 1990). Single frame video analysis was then used to calculate the total distance walked by the parasite on the deposited beetle trail. Control experiments consisted of testing the parasites in chambers in which no beetles had been allowed to walk. No female wasp was used for  $>1$  bioassay.

**Statistical Analyses.** All analyses were conducted using the personal computer software program Statgraphics Plus (Statgraphics 1997).

**Voucher Specimens.** Voucher specimens of *C. tarsalis* (Lot No. 71) were deposited in the Museum of Entomological and Prairie Arthropod Research, Kansas State University, Manhattan, KS.

## Results

Eighteen discrete behavioral categories were recognized in the interaction of *C. tarsalis* females and their hosts. These categories and their descriptions are as follows.

**Search.** Movement of wasp in response to its environment apart from direct interaction with host. Consists of walking, running, antennae-waving or touching surroundings. Usually ultimately leads to host location by either random processes or directed ones.

**Move Away.** Any movement by the wasp away from the host after the host has been contacted. Consists of walking or running, moving away as a defensive behavior, being pushed away or thrown off by host.

**Bite.** Wasp grabs host with its mandibles and successfully pierces the host integument preparatory to stinging.

**Antennal Tip.** Touching host with 1 or both tips of the antennae, either 1 time or several times in a row, uninterrupted by any other behavior.

**Antennal Side.** Same as antennal tip except the sides of the antennae are used for touching.

**Head/Mouth.** Moving head or mouth close to host and touching without biting.

**Climb On.** Climbing up on host larvae in either a parallel or perpendicular orientation.

**Sting.** Curling of abdomen around host and inserting sting into host.

**Turn.** Change in body direction of wasp by at least 90 degrees in response to host movement.

**Pause.** A momentary cessation of all obvious behavior except for antennal waving.

**Attempted Bite.** Wasp attempts to grab larvae with its mandibles, but fails to do so.

**Groom.** Self-grooming by the wasp.

**Catatonic.** The wasp's state after she has either attempted to or has stung the host. The wasp continues to hold on with its mandibles, but ceases all other movement, including antennal waving. This state continues until the host's movements have slowed to almost complete cessation, or until after several seconds of no change in the host's movement, when the wasp begins to move again.

**Chew.** When the wasp has its mandibles embedded in a paralyzed host and appears to be extracting fluids from the host. There is little movement except for slight rhythmic movement of the wasp's head and mandibles, with an occasional pumping motion of her abdomen.

**Pull.** When a wasp grabs the host larvae with her mandibles and pulls (or pushes) it on the substrate.

**Rub.** Wasp rubs host larvae with the tip of her abdomen.

**Touch.** Includes both antennal tip and antennal side touching.

**Rest.** Wasp tilts head downward against substratum and ceases all movement except for an occasional abdominal pumping motion or twitch. This lasts for at least 30 s.

The ethogram detailing the behavioral interactions of *C. tarsalis* females with their normal host, *O. surinamensis*, is presented in Fig. 2. Diagrammatic drawings of key behaviors are shown in Fig. 3. The most typical behavioral sequence involved searching for the host, followed by antennal contact, climbing onto the host, biting it, and stinging it. This was followed by 15–30 s of catatonic immobility until the host's movement ceased, after which the wasp pulled the host on the substrate for a short distance, climbed back onto it, rubbed and chewed it. The wasp then moved away, and began grooming and resting. It is clear, however, from an examination of the ethogram that many alternative behavioral transitions can and do occur. Comparative ethograms were produced for 3 other potential hosts and are presented in Fig. 4. In all cases the behavioral sequence stops before biting of the host, and never was any attempt at stinging noted for these putative alternative hosts. The mean conditional probabilities used to generate these ethograms are found in Tables 1–4 of Appendix 1.

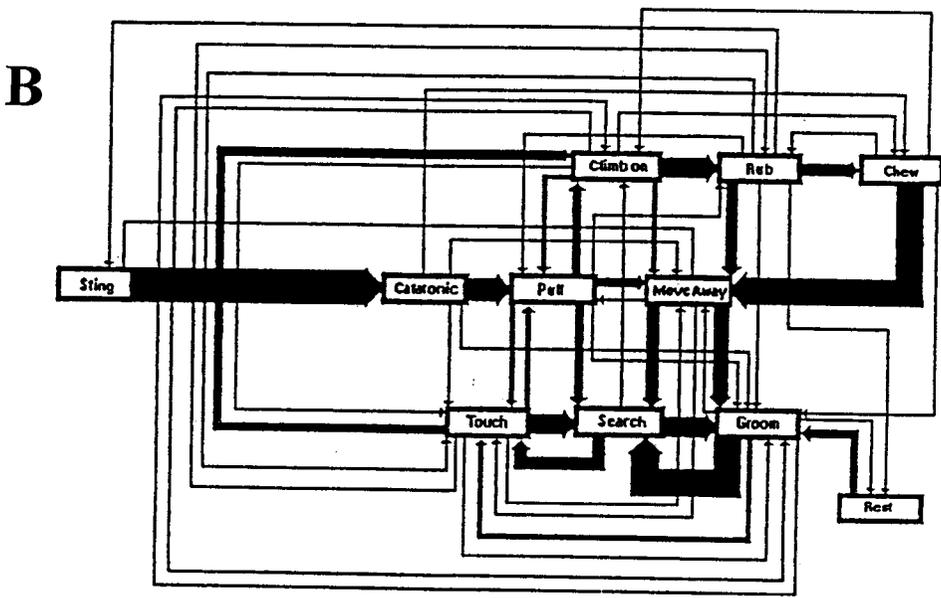
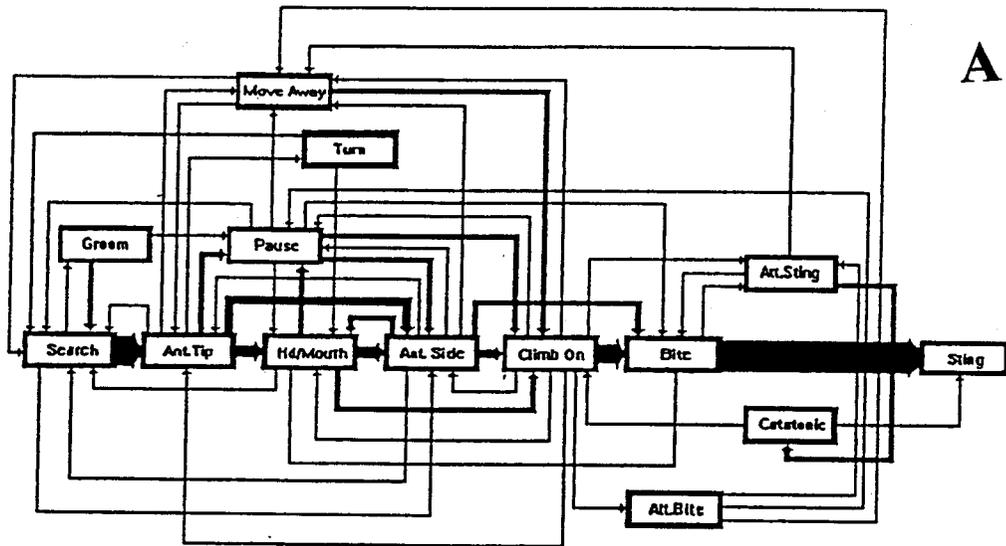
Several experiments were conducted to ascertain some of the sensory modalities used by *C. tarsalis* females in locating, recognizing, and accepting a host. Blinded females and females without maxillary palps

found and stung hosts as readily as did control females. In the blinding experiment, 14 of 20 treated females stung the hosts on average 23 s after introduction to the cell, whereas 9 of 12 sham-operated control females stung the host on average within 19 s (Mann-Whitney *W* test comparing medians,  $W = 74.0$ ,  $P = 0.508$ , not significant). Similarly, in the palp removal experiment, 15 of 15 treated females found and stung the hosts on average within 12 s, whereas 11 of 15 of the sham-operated control females took on average 29 s ( $W = 54.0$ ,  $P = 0.145$ , not significant). However, although all 15 of 15 females without antennae found the host within the 2-min trial (apparently by chance), none of them showed any evidence of having recognized the presence of the host, and none of them attempted to sting the host. Sham-operated controls (11 of 15) found the hosts on average within 27 s and successfully stung them. When the host was a freshly frozen and thawed immobile one (no movement test), none of the 15 females attempted to sting it, whereas 13 out of 14 females stung the live control larvae on average within 18 s. When, however, the dead host was presented as a moving object (positive movement test), the parasitoid then did grab it, and, in 12 of 15 trials, successfully stung on average within 35 s; in the nonmoving sham-operated control group only 1 of 6 larvae was stung (2-sided Kolmogorov-Smirnov test = 1.725,  $P = 0.005$ ).

The trail-following responses of the female parasitoids clearly indicated that they perceived some chemical cue that had been left on the substrate by the host beetle larvae. Wasps exposed to the beetle kairomone on the pencil circle followed the trail for 6 cm on average during the 2-min bioassay, whereas the control group followed the pencil circle on average for only 0.3 cm ( $W = 130.5$ ,  $P = 0.002$ ). Wasps exposed to the kairomone showed trail-following behavior, stopping immediately upon contacting the trail, then turning onto the trail, following it in a zigzag manner, while constantly drumming the paper with their antennae. The wasps often left the trail to explore the area nearby for a few seconds, then returned to the trail and continued trail following. The control wasps did not display these behaviors.

## Discussion

The literature on parasitoid host-finding, -recognition, and -acceptance is extensive and has been well-summarized in several recent monographs (van Alphen and Vet 1986, Vet et al. 1995, van Alphen and Jervis 1996, Quicke 1997). Most of this literature, however, deals with parasitoids of field crop or forest pests. Although stored-product insects are found in a diversity of situations, generally hosts located in stored grain commodities are in a darkened environment with little air movement and only modest daily changes in temperature or relative humidity. Furthermore, the commodities are frequently present as extremely large masses (often several tons) and the host insects are present in relatively low levels (the economic threshold is commonly given as 1 insect per



Conditional Transition Probability

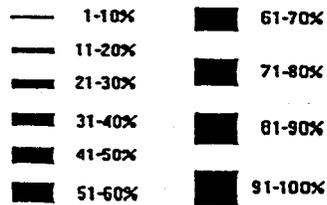


Fig. 2. Ethogram of behavioral interactions between a *C. tarsalis* female and a *O. surinamensis* larva. (A) Interactions from search to sting. (B) Interactions from sting to rest. Width of each line is associated with the transitional probability of the indicated behavioral event. See text for definition of individual behaviors.

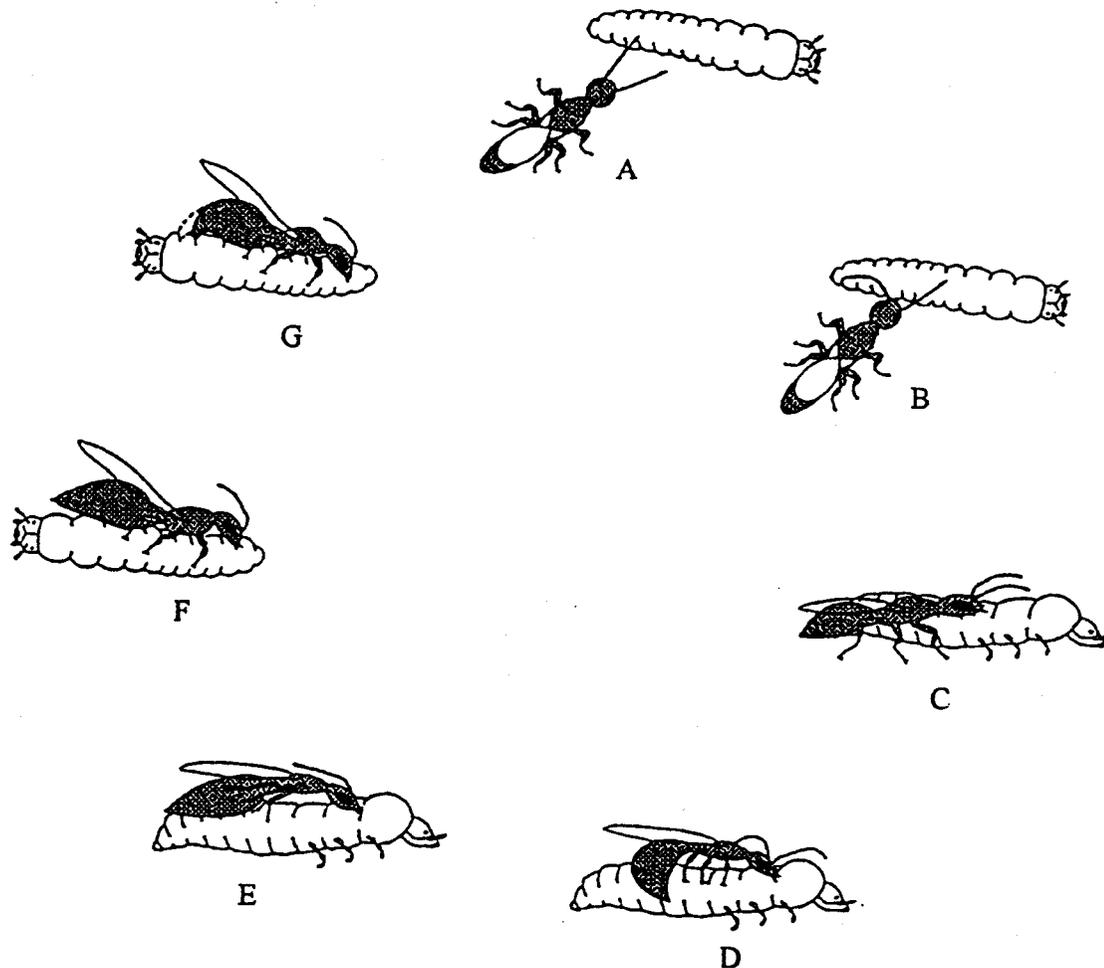


Fig. 3. Diagrammatic sketches of 7 behavioral interactions between *C. tarsalis* and *O. surinamensis* larvae. A, antennal touch; B, antennal side; C, head/mouth; D, sting; E, catatonic; F, chew; G, rub.

kilogram of stored grain) (Hagstrum and Flinn 1995). The problems presented for the parasitoids in finding their hosts are therefore substantial and possibly involve different strategies from those found for the parasitoids of field crop pests.

*Cephalonomia tarsalis* females have well-developed eyes and might be expected to use vision to locate their hosts in the stored commodities, at least when the hosts are near the surface of the stored commodity where some light might be present. Female wasps blinded with fingernail polish, however, found the host as readily as did normal females. One might argue that the polish did not actually blind the wasps. However, our finding that wasps without antennae, but with normal eyes, were unable to find the host, or if they did find it, did not recognize it, argues strongly that for short-range host location, vision is not important. This conclusion is strengthened by the fact that we frequently observed vision-competent parasitoids passing close to moving sawtoothed grain beetle larvae without the parasitoid showing any evidence of rec-

ognizing that the beetle was there. If, however, the wasp's antennae touched the beetle larva, then attack usually was immediate.

If vision is of little importance, what sensory modality would be most likely to explain host location in this species? We judged the use of a chemical modality to be most likely, although we cannot rule out the possibility of either airborne or substrate-borne sound cues (Hagstrum et al. 1996). For medium to long-range detection of odor sources, the antennae of insects are considered the primary site of chemoreception, and, as noted, removal of the antennae of *C. tarsalis* females severely hampered their ability to locate and recognize the host. Wasps wave their antennae from side-to-side over the substrate in front of them as they walk, and we hypothesized that they might locate their hosts by following a host-produced chemical trail in the same manner that *C. waterstoni* does in locating its host *Cryptolestes ferrugineus* (Howard and Flinn 1990). Indeed, when *C. tarsalis* females are exposed to papers on which sawtoothed

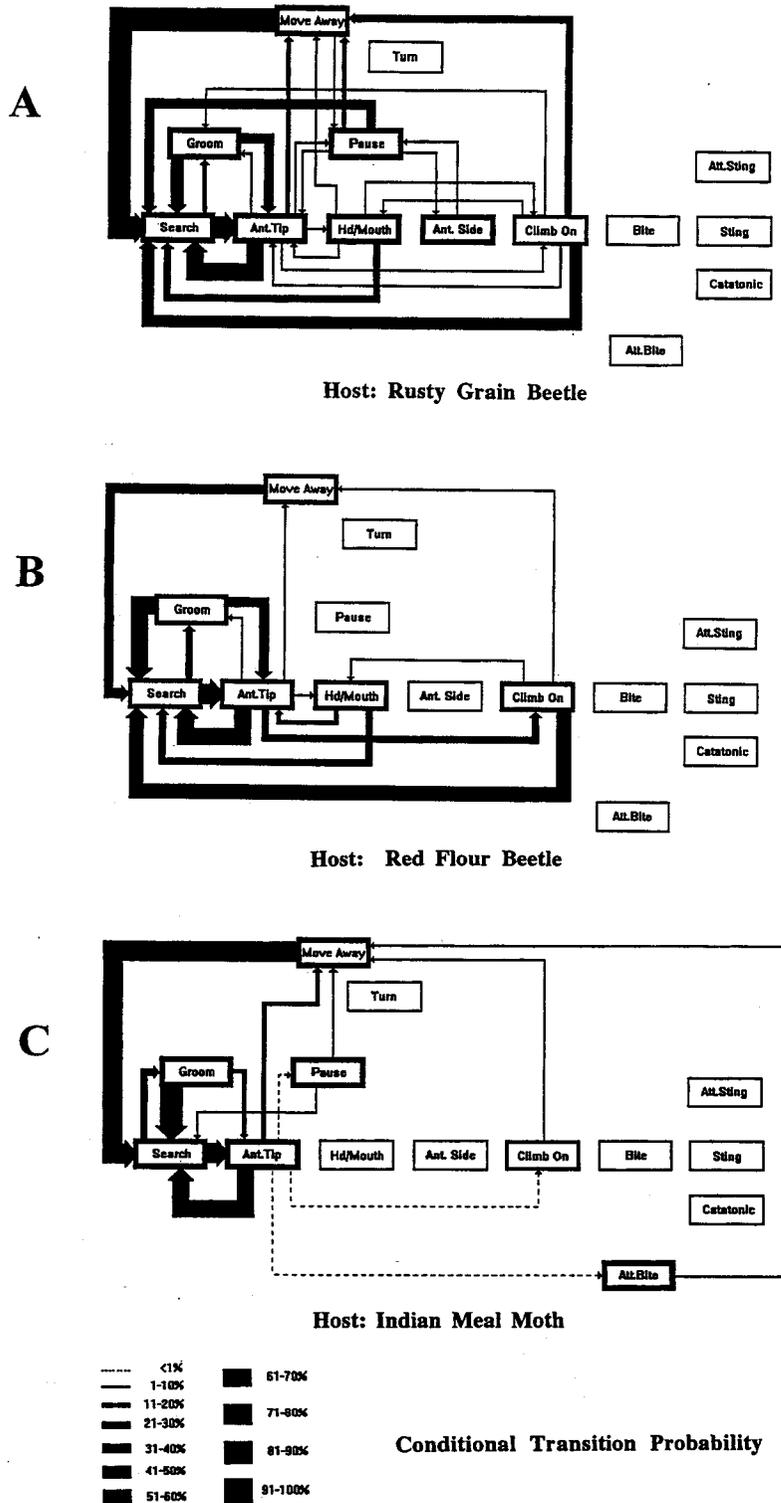


Fig. 4. (A) Ethogram of behavioral interactions between a *Cephalonomia tarsalis* female and a *Cryptolestes ferrugineus* larva. (B) Ethogram of behavioral interactions between a *Cephalonomia tarsalis* female and a *Tribolium castaneum* larva. (C) Ethogram of behavioral interactions between a *Cephalonomia tarsalis* female and a *Plodia interpunctella* larva. Width of each line is associated with the transitional probability of the indicated behavioral event (see Fig. 2). See text for definition of individual behaviors.

grain beetle larvae had walked in a defined path, the parasites followed this same path and did so in the stereotypical manner described above. Although we have not yet examined this trail-following behavior in as great a detail with *C. tarsalis* as we did with *C. waterstoni*, it appears that the sensitivity of *C. tarsalis* to putative sawtoothed grain beetle kairomone is somewhat less than *C. waterstoni* sensitivity to rusty grain beetle kairomone. In the earlier experiments, 8 rusty grain beetle larvae were allowed to deposit their trail kairomone for 30 min, and when *C. waterstoni* females were tested for trail-following stimulation, the wasps spent 53 s out of the 2 min allowed on the trail and followed it for a total distance of 12.5 cm (Howard and Flinn 1990). In comparison, we allowed 10 sawtoothed grain beetle larvae to deposit a putative trail kairomone for 3 h and the *C. tarsalis* females followed that trail for only 6 cm (total time on the trail was not monitored in this instance).

This apparent reduced response to trail kairomone by *C. tarsalis* could arise from its host's leaving less chemical residues on the substrate, or because *C. tarsalis* is inherently less sensitive to such cues. Larval rusty grain beetles, internal seed feeders, wander extensively before they select a pupation site (Smith 1972) while depositing a potent kairomone (Howard and Flinn 1990) that is readily followed in a species-specific manner by *C. waterstoni*. We have not seen a similar wandering and marking behavior by larvae of the sawtoothed grain beetle, nor have we been able to find any literature describing such behavior, although the adults readily disperse (Surtees 1963). Sawtoothed grain beetle larvae are external feeders, however, and may not migrate to find a pupation site in the same manner as do rusty grain beetle larvae. Nevertheless, sawtoothed grain beetle larvae did leave a chemical residue on the paper substrate, and *C. tarsalis* females followed the resultant path. Rather than mediating a well-defined trail-following response, the kairomone may convey to the wasp that a host larva is nearby and thus trigger general searching in the immediate vicinity. Indeed, in our bioassays the parasitoid often left the trail and wandered nearby for several seconds before commencing to follow the trail again. Although *C. waterstoni* also leaves the trail of its host to wander nearby (Howard and Flinn 1990), our impression is that it does so less often than that observed with *C. tarsalis*. Additional studies are needed to clarify these issues.

Although sawtoothed grain beetle larvae may not be leaving a directional chemical trail that *C. tarsalis* females use, it is clear that the parasitoid is using chemical cues perceived by their antennae to locate and recognize the host beetle. Upon locating the beetle, the wasp briefly antennates it with outstretched antennae and proceeds to grab hold of it and sting it. What sensory structure(s) and cue(s) is the parasite using to initiate this cascade of events? Clearly, the wasp's antennae are necessary, whereas its maxillary and labial palps do not seem to be of importance, because removing the palps does not hinder successful attack. Cuticular-borne chemical cues (possibly cu-

ticular hydrocarbons [Howard 1992, Howard et al. 1995]) probably provide the primary signal that the correct host has been found, but the wasp also requires that the beetle respond to its attack by moving. When we killed the beetle and offered it to the wasp, she investigated it, but did not proceed to biting and stinging. Occasionally, however, she host-fed on the proffered dead host. However, if the dead beetle was moved when the wasp touched it, then the wasp immediately attacked. This selectivity makes sense, because the paralyzed beetle larvae must remain alive during the 4–6 d of larval parasitoid development if the wasps are to pupate successfully. The failure of the wasp to respond to nonmoving larvae could also be a result of its perceiving the offered host as one that had been previously paralyzed.

The suite of behaviors displayed by most *C. tarsalis* females after they contact their normal beetle host is shown in Figs. 2 and 3. The sequence of climbing onto the host, biting it and stinging all occurred very rapidly. If the parasitoid was successful at embedding her mandibles into the cuticle of the host (biting), then she was inevitably successful in stinging and paralyzing the host. Occasionally >1 sting was required to subdue the host. The parasitoid displayed the behavior we categorized as catatonic while waiting for the host to show signs of being paralyzed. This behavior is quite striking and possibly serves to protect the wasp from damage while the beetle is thrashing about. The remaining typical behaviors involved the wasp pulling the paralyzed host around the observation cell (in more natural conditions the wasp would attempt to pull the host to a suitable location to hide it); then the wasp climbs back up on the host, rubs the larva all over with the tip of her abdomen, and then apparently host feeds (chew). Although we were not able to see the transfer of any fluids from the host to the parasitoid during this chewing behavior, we did once see the physical removal of a small piece of larval flesh by the parasitoid, and larvae that were chewed showed evidence of shrinkage. Host-feeding is a common strategy among parasitoids (Heimpel and Collier 1996) and is thought to be important for both somatic maintenance and reproductive development. In addition, Howard (1998) observed that the cuticular hydrocarbon profiles of *C. tarsalis* were dependent on whether the female had host-fed or not. We note that the rubbing behavior of *C. tarsalis* is very similar to a behavior displayed by *C. waterstoni* females while they are preparing their paralyzed host (rusty grain beetle larvae) for oviposition (unpublished data). Because *C. tarsalis* does not oviposit until long after this rubbing behavior, it is possible that she is leaving some sort of a semiochemical mark of unknown function. After host-feeding, the parasitoid leaves the host, grooms herself, and then rests.

Although we terminated behavioral observations at this point, in preliminary experiments the wasp returned 1 or more times for up to several hours later to the hidden host, sometimes moving it to a new location, or host-feeding on it before commencing oviposition (unpublished data). One might expect the para-

sitoid to oviposit soon after hiding the host, but we have never observed this. Rather, the female soon begins searching again for additional hosts, and at least under artificial laboratory conditions (petri dishes), will paralyze and hide several more larvae before she commences oviposition on any of them (unpublished data). Although we do not have direct field observations of this behavior, if it does occur there it would increase the potential efficacy of this parasitoid as a biological control organism, as paralyzed beetle larvae do not recover.

Although *C. tarsalis* is normally considered to be an obligate parasitoid of the sawtoothed grain beetle, others have claimed that it is also associated with 3 *Sitophilus* spp. as well as with *Tribolium castaneum* (Gordh and Móczár 1990, p.81). We tested this putative use of alternative hosts by developing ethograms of the response of *C. tarsalis* females to a potential stored-product host in the same order and family (*C. ferrugineus*), the same order, but different family (*T. castaneum*) and a different order (*Plodia interpunctella*). All these potential hosts are external grain feeders, unlike the *Sitophilus* spp., which are all internal grain feeders, and hence in our opinion highly unlikely to be parasitized by *C. tarsalis*. The responses observed to these 3 hosts were graded in terms of phylogenetic similarity to the sawtoothed grain beetle. The wasp entered the cell with the sawtoothed grain beetle more slowly and showed longer pauses than with the 3 putative hosts. Upon contacting the *C. ferrugineus* larva, the wasp paused briefly after touching it with her antennae, and in response, the beetle larva gyrated violently. If a *T. castaneum* larva was touched or walked on by the wasp, it remained motionless until the wasp decamped. When the wasp walked on the larva, she did so in a manner that suggested that she was merely using it as a "stepping stone" to continue searching for a recognizable host. The parasitoid rarely mounted, and in fact appeared repelled by, the *P. interpunctella* larvae, possibly as a consequence of the numerous long setae projecting from the moth larvae. In addition, the moth larvae deposited silk webbing, which tended to entangle the wasps. In summary, the wasp never proceeded beyond a quick and cursory antennal probing of these 3 putative hosts and we did not see any evidence of the wasp recognizing them as suitable subjects for parasitization (Fig. 4). Although we cannot absolutely rule out other host associations for *C. tarsalis*, our data suggests it is likely that this species is essentially host specific in the usual stored product environment.

Additional studies are needed to clarify many of the behavioral mechanisms identified in this article. Although semiochemicals have been implicated in many of these mechanisms (trail-following, host-recognition and host-acceptance in particular), the chemicals need to be isolated and subjected to behavioral testing. Age-related effects also need to be examined, along with the effects of possible learning (Vet et al. 1995). The possibility that the parasitoids are using sound as a cue to locate their hosts is a real one and certainly needs to be tested. And the bioassays need

to be extended to field situations, although that will prove to be a major challenge given the size of the organisms involved. Finally, comparative studies with other bethylids of the stored product environment need to be conducted so that the evolution of these behaviors can be constructed.

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Appendix 1A. Average transitional probabilities for behavioral interactions between *C. tarsalis* females and *O. surinamensis* larvae up to the act of stinging.

	SR	MA	AT	AS	TN	PA	HM	CO	AB	BT	AG	SG	CT	GR
SR	---		.878	.040										.080
MA	.100	---	.060				.120							
AT	.085	.019	---	.258	.087	.113	.345	.013	.003					
AS	.100	.050	.048	---	.008	.070	.128	.228		.108				
TN	.060				---		.100							
PA	.053	.020		.180		---	.020	.113		.053				
HM	.020	.013	.010	.370		.200	---	.167	.100					
CO		.037	.040	.080		.040	.040	---	.073	.580	.020			
AB		.040				.040			---		.060			
BT										---	.060	.940		
AG		.020								.040	---		.100	
SG												---		
CT							.040					.040	---	
GR	.200					.040								---

Behaviors are defined in the text of the article, and abbreviations for rows and columns are as follows: SR, search; MA, move away; AT, antennal tip; AS, antennal side; TN, turn; PA, pause; HM, head/mouth; CO, climb on; AB, attempted bite; BT, bite; AG, attempted sting; SG, sting; CT, catatonic; GR, groom; PU, pull; RB, rub; CW, chew; TH, touch; RT, rest; MA, move away.

Appendix 1B. Average transitional probabilities for behavioral interactions between *C. tarsalis* females and *O. surinamensis* larvae from the act of stinging until final rest. Abbreviations for rows and columns are defined in Appendix 1A.

	SG	CT	PU	CO	RB	CW	MA	TH	SR	GR	RT
SG	---	.917					.083				
CT		---	.667			.083	.083	.083		.083	
PU		.010	---	.266	.016	.006	.253	.115	.207	.044	
CO			.113	---	.585	.038	.202	.042		.020	
RB	.028		.075		---	.334	.401	.047		.060	.057
CW				.083	.083	---	.792			.042	
MA		.006	.042	.012			---	.076	.391	.490	
TH			.141	.212	.020		.037	---	.520	.065	.005
SR			.012	.028				.332	---	.583	
GR			.008	.047			.016	.155	.737	---	.038
RT										.250	---

**Appendix 2.** Average transitional probabilities for behavioral interactions between *C. tarsalis* females and *Cryptolestes ferrugineus* larvae. Abbreviations for rows and columns are defined in *Appendix 1A*.

	SR	MA	AT	AS	TN	PA	HM	CO	AB	BT	AG	SG	CT	GR
SR	---		.879					.006						.114
MA	.833	---			.033									
AT	.695	.139	---		.030	.050	.071							.033
AS				---	.067									
TN					---									
PA	.311	.133	.067	.022		---								
HM	.267	.067	.067				---	.067						
CO	.456	.222	.067				.067	---						.022
AB									---					
BT										---				
AG											---			
SG												---		
CT													---	
GR	.428	.306												---

**Appendix 3.** Average transitional probabilities for behavioral interactions between *C. tarsalis* females and *T. castaneum* larvae.

	SR	MA	AT	AS	TN	PA	HM	CO	AB	BT	AG	SG	CT	GR
SR	---	.717						.004						.279
MA	.313	---												
AT	.641	.035	---				.052	.209						.063
AS				---										
TN					---									
PA						---								
HM	.217		.183				---							
CO	.590	.067					.022	---						
AB									---					
BT										---				
AG											---			
SG												---		
CT													---	
GR	.675	.325												---

Abbreviations for rows and columns are defined in *Appendix 1A*.

**Appendix 4.** Average transitional probabilities for behavioral interactions between *C. tarsalis* females and *P. interpunctella* larvae.

	SR	MA	AT	AS	TN	PA	HM	CO	AB	BT	AG	SG	CT	GR
SR	---		.777											.225
MA	.733	---												
AT	.696	.173	---			.010	.011	.011						
AS				---										
TN					---									
PA	.067	.067				---								
HM							---							
CO		.067						---						
AB		.067							---					
BT										---				
AG											---			
SG												---		
CT													---	
GR	.833	.167												---

Abbreviations for rows and columns are defined in *Appendix 1A*.