Behaviors of Female *Eretmocerus* sp. nr. *californicus* (Hymenoptera: Aphelinidae) Attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae) on Sweet Potato

DAVID H. HEADRICK, THOMAS S. BELLOWES, JR., AND THOMAS M. PERRING

Department of Entomology, University of California, Riverside,
Riverside, CA 92521

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ABSTRACT Behaviors of *Eretmocerus* sp. nr. *californicus* Howard females on *Bemisia argentifolii* Bellows & Perring infesting sweet potato, *Ipomoea batatas* (L.) Lam, were described and quantified. Walking speeds of up to 1.3 mm/s were calculated for females searching for host whitefly nymphs on sweet potato leaves. Females encountered all host stages during searching with approximately the same relative frequency as their relative abundance (average of 17.03% of hosts available were encountered). Females also arrested and antennated all of the host stages with the same relative frequency as their encounter rate (62.8%). Females showed a clear and significant preference for probing second instars over all other stages. Of the hosts probed, females chose all stages for oviposition with the same relative frequency. Successful exertion of the ovipositor under a host nymph occurred after initial probes 12 times and after repeated probing attempts 15 times. Oviposition occurred under 13.5% of the hosts assessed by antennation; however, 20 of the 27 (74%) nymphs under which the ovipositor was exerted received an egg. Females spent 41% of the total time in searching, host assessment, probing, and oviposition; the remainder of the time (59%) was spent host feeding, grooming, and resting.

KEY WORDS *Eretmocerus*, *Bemisia*, behavior

THE SILVERLEAF WHITEFLY, *Bemisia argentifolii* Bellows & Perring (Bellows et al. 1994), was identified in California in the fall of 1990 (Perring et al. 1991). In response, research was undertaken to survey crop, weed, and native plant species in the Imperial Valley for indigenous entomophagous natural enemies attacking *B. argentifolii*. In the survey, we found that one of the principal species of parasitic Hymenoptera attacking *B. argentifolii* was *Eretmocerus* sp. nr. *californicus* Howard. Percentage parasitism, however, varied greatly among plant species (T.S.B., unpublished data). Collection records and descriptions of parasitism of *Bemisia* spp. from around the world indicate differences in guild structure and parasitism intensity depending on the species of host plant sampled (Azab et al. 1969; Gerling 1983, 1986, 1990; Onillon 1990). One potential cause for variation in percentage parasitism is different searching effectiveness by the parasites on different host-plant species. This has been addressed experimentally for the aphelinid *Encarsia formosa* Gahan attacking the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (van Lenteren et al. 1980, Hua et al. 1987, van Lenteren & Woets 1988; cf. Dowell 1990).

Comparative studies describing *Eretmocerus* spp. searching behavior on different host-plant species have not been reported previously. A study

on the effects of two different host-plant species, each infested with *Bemisia tabaci* (Gennadius), on the fitness of two populations of *Eretmocerus* sp. was conducted. The two populations, an arrhenotokous one from southern California and a thelytokous one from Hawaii, showed significant differences in the preimaginal development rate, survival, and fertility between host-plant species (Powell & Bellows 1992).

Research reported in this article is part of a larger study that describes searching and ovipositional behaviors of female *E. sp. nr. californicus* and that quantifies the extent to which these behaviors are influenced by five morphologically and taxonomically diverse plant species. Herein we present descriptions of search behaviors of *E. sp. nr. californicus* females, together with the results of the behavioral analyses, on sweet potato, *Ipomoea batatas* (L.) Lam. (Convolvulaceae), a plant with glabrous leaves, which serves as the baseline for comparison among four other species that will be reported separately.

Materials and Methods

Colonies of silverleaf whitefly and *E. sp. nr. californicus* were maintained on sweet potato at 24 ± 1°C and 60% RH with a 14:10 (L:D) h photo-

period. Noninfested sweet potato plants were maintained in greenhouses at 16–30°C and ≈60% RH. Silverleaf whitefly colonies were obtained from naturally occurring populations in the Imperial Valley collected from broccoli, melon, and cotton in the fall of 1990 and summer of 1991. *E. sp. nr. californicus* colonies were initiated for this study with individuals reared from samples of *Lantana camara* L. infested with *B. argentifolii*. Collections were made on 1 March 1993 at Riverview Cemetery, Western Avenue, 3 km N of Highway 86, Brawley, Imperial County, California. The parasitoid population is arrhenotokous, with a sex ratio of ≈1:1, and is probably the same population reported earlier from *B. tabaci* from the same area (Powell & Bellows 1992). Voucher specimens of the population used in this study have been curated and are in the care of T.S.B.

Behaviors of *E. sp. nr. californicus* females were recorded on videotape for subsequent description and quantification. The video camera used was a Javelin JE3362, the videorecorder was a Gyr time-lapse TLC2051-232, and the monitor was a NEC PM1271A. The camera was mounted on a Zeiss dissecting microscope with Greenough optics. A Javelin JLUX 150 fiber optic light was used for illumination. Video recordings were made at 40× magnification. *E. sp. nr. californicus* females, <24 h after eclosion, were allowed to mate and were exposed to hosts for oviposition and feeding continuously for 24–48 h before each trial. A single female was then placed on the surface of an excised leaf bearing a known number of whitefly host stages placed adaxial side down in a glass, 10-cm diameter petri dish. The densities of hosts on the leaf surface ranged from 3.5 to 14 per square centimeter. After exposure to the leaf, the female had the choice of either remaining to search or flying away. All females initially remained and began searching. Video recording began, and each trial lasted for up to 1 h per female; all trials were conducted between 1000 and 1600 hours. The behaviors of nine females were recorded, yielding a total recording time of 382.8 min. After each trial, the presence or absence of parasite eggs was verified microscopically by examining each nymph probed by a female. Video recordings were then analyzed. Each behavior was listed for each female in sequence, and the duration of each behavior in seconds was recorded. Walking speeds of females were determined by placing acetate sheets over the video monitor, tracing the path of a female, and timing the duration of the walking episode. The relative distance of the path was measured, and the absolute distance in mm was calculated using a stage micrometer.

Behavioral data were entered into a spreadsheet software program and collated for statistical analysis in SAS (SAS Institute 1987). Means are based on total frequencies over all trials, unless otherwise noted. Differences among mean durations of behaviors were examined by analysis of variance

Fig. 1. Ethogram for the main behavioral pathway of *E. sp. nr. californicus* attacking *B. argentifolii* on sweet potato.

(ANOVA) of the original data and also of log-transformed data (because the data were skewed); in all cases the conclusions from the analyses were the same for both untransformed and transformed data. Differences among frequencies were examined by chi-square analysis. All means are from the original (not transformed) data and are reported with their associated standard error (SEM).

Specimens for scanning electron microscopy were fixed in 2% aqueous osmium tetroxide for 24 h, washed in double-distilled water twice, 5 min each, dehydrated in an increasing series of acidulated EtOH, critical-point dried, mounted on stubs, sputter-coated with a gold-palladium alloy, and examined on a scanning electron microscope (JEOL JSM C35) in the Department of Nematology, University of California, Riverside. Micrographs were prepared with Polaroid 55P/N film at 15 kV accelerating voltage.

Results

Ethograms for *Eretmocerus sp. nr. californicus* females were developed from the recorded behavioral sequences. Females typically exhibited behaviors in the following order after being introduced to a leaf: walking on the leaf surface, encountering a host, assessing that host with antennation, probing the host for oviposition or host feeding, then the process may be repeated (Fig. 1). Complexity within this behavioral pathway occurred through intervening behaviors such as grooming, resting, or resumption of walking. Within the general pathway, frequencies of behavioral sequences were re-

Fig. 2. Ethogram for behaviors leading to oviposition of *E. sp. nr. californicus* attacking *B. argentifolii* on sweet potato. Arrows indicate subsequent behavioral events, and the associated numbers indicate the frequency of observation, summed over all trials. The main behavioral pathway begins at the top left, in boldface, and moves down the left margin. The number associated with each behavior type is the frequency of observation collated over all trials. The behavioral sequences for host reassessment and repeated probes are indicated by the dashed lines and arrows. Host-feeding behaviors were not included in this ethogram and thus account for the slight differences between the summed behaviors leading into and out of a main behavioral pathway category. A, antennation; CA, circling antennation; D, disengagement; G, grooming; HE, host encounter; OHE, oblivious host encounter; P₁, initial probe; P_R, repeat probe; R, resting; SF, surface feeding (=nonhost feeding); W, walking.

Fig. 3. Diagram of *E. sp. nr. californicus* female assessing a host nymph by circling antennation.

corded (Fig. 2). The main behavioral pathway is shown from top to bottom on the left. Arrows are associated with the horizontal lines to the right of each of the main pathway components and indicate the frequencies of behaviors that led to or were followed by the behavior on the left. For example, walking (W) was followed by grooming 24 times, grooming was followed by walking 33 times, antennation led to walking 46 times, and so on. Continuing down the main pathway on the left, walking led to host encounters (HE) 82% of the time. Host encounters led to arrestment and subsequent antennation (CA) 49 times; thus 34% of all host encounters led to recognition and assessment of a host. Circling antennation (CA) led to an initial probe (P_i) 30% of the time. Initial probes led to successful exertion of the ovipositor under the host nymph 33% of the time. If an initial probe did not lead to exertion of the ovipositor, reassessment of the host occurred 11 times (Fig. 2, far right vertical arrow). Assessment by circling antennation followed by probing was an oft-repeated sequence, with the female typically moving from one side of the host to the other before successful exertion of the ovipositor. Host encounters, followed by circling antennation, led to probing the vasiform orifice for host feeding on five nymphs in all trials by three of the females. During three host-feeding episodes, the feeding wounds were reprobated (P_r) a total of 39 times for all nymphs, with two fourth instars having 27 reprobating events.

All behaviors were analyzed to determine whether two factors, (1) host nymphal stage or (2) preceding or subsequent behaviors, had any impact on their frequency or duration.

Walking Speed. In this study all of the females remained active on the leaf surface for the duration of each trial. Walking speeds by *E. sp. nr. californicus* on sweet potato leaves averaged 1.08 ± 0.104 mm/s ($n = 20$; range, 0.91–1.32).

Host Encounters. Searching females walked and asynchronously drummed the apices of their antennae on the surface of the leaf. When a female came into contact with a host, one of two events

Fig. 4. Mean duration of circling antennation based on subsequent behaviors in all trials and on all nymphal instars.

took place. Either the female antennated the host further or females continued searching without any observable change in behavior to suggest recognition of the host. These *oblivious host encounters* occurred a total of 52 times (36%) over all trials (Fig. 2, OHE). Antennation of the host involved continued drumming of the apices of the antennae asynchronously on the dorsum of the host. Antennation was followed either by abandonment of the host (walking) or by continued assessment of the host by antennation in a highly stereotypic pattern referred to as circling antennation (Fig. 2, CA, see below). The frequency of host encounters leading to circling antennation was 49 of 146 (34%), and frequency of host encounters leading to antennation without circling was 46 of 146 (31%) over all trials.

Antennation. Circling antennation consisted of a female standing centrally upon the dorsum of larger whitefly nymphal instars or over smaller instars and, while rotating, antennating the submarginal perimeter of the host, counter-turning (reversing direction of rotation) frequently along the lateral margins between the anterolateral tracheal folds and the vasiform orifice (Fig. 3).

The duration of circling antennation was recorded from the initial host contact up to any subsequent behavior (e.g., probing). The circling antennation behavior typically began immediately upon encountering a host, and any preliminary antennation could not be distinguished. Circling antennation led to abandonment 13 (12%) times, with a mean duration of 16.3 ± 4.8 s (range, 2.0–70.0 s) (Fig. 4). Circling antennation led to initial probes 32 (30%) times, with a mean duration of 19.3 ± 2.6 s (range, 3.0–68.0 s) (Fig. 4); these two durations of circling antennation were not significantly different ($F = 0.35$, observed significance level (OSL) = 0.5598). After the initial probe, reassessment of the host by circling antennation before another probe occurred 46 (44%) times, and these

durations averaged 10.8 ± 0.8 s (range, 4.0–27.0 s) (Fig. 4), significantly less than the mean duration of the circling antennation events leading to the initial probe ($F = 16.38$, OSL = 0.0001). There were no significant differences detected in mean durations calculated among host stages for circling antennation, irrespective of the subsequent behavior.

Assessment of the host without circling consisted of asynchronously drumming the encountered host with the apices of the antennae upon the dorsal integument. This type of antennation led to abandonment in 46 (31%) occurrences (Fig. 2) and averaged 3.4 ± 0.4 s in duration (range, 1.0–14.0 s). Antennation of the host without circling led to initial probes 4 (11%) times, and these durations averaged 8.0 ± 0.9 s (range, 6.0–10.0 s), significantly longer than antennations leading to abandonment ($F = 10.68$, OSL = 0.0020). Antennation of the host without circling led to repeat probes 13 (22%) times, and these durations averaged 6.1 ± 0.7 s (range, 2.0–11.0 s); this mean was not significantly different from mean times of antennations leading to an initial probe ($F = 1.48$, OSL = 0.2426). There were no significant differences detected in mean durations calculated among host stages for antennation.

Probing and Disengagement. The initiation of probing behavior began with a female stepping off the host onto the leaf surface, facing away from the center of the host and flexing the metasoma upward to expose the appendicular ovipositor. The wings raised $\approx 45^\circ$ above the dorsum and slightly pronated concurrently with metasomal flexure. The apex of the ovipositor was slightly exerted and retracted repeatedly as a female probed against the margin of the host body, apparently attempting to find a gap between the host and the leaf surface. The ovipositor was observed to be flexible and bent with the pressure of exertion.

If a suitable gap between the leaf surface and the host was found, the ovipositor was then fully exerted underneath the whitefly nymph (27 of the 36 hosts probed). The female generally remained quiescent during the time the ovipositor was exerted under the nymph, but slight rhythmic movements of the body were observed just after the female gained purchase and then before disengagement.

Disengagement involved a combination of actions that began when the ovipositor was retracted after a variable period of time and females began asynchronously rubbing the hind tarsi against the host and leaf surface. The wings were lowered into their resting position flat over the dorsum. Subsequent to disengagement, females groomed ($n = 13$) or walked away from the host nymph ($n = 12$). Females were not observed to return to any host they had previously probed; thus no superparasitism was recorded.

If a suitable gap between the host and leaf surface was not found during the initial probing, the

Fig. 5. Scanning electron micrograph of a second-instar nymph on sweet potato. Arrow indicates the ring of marginal wax associated with *B. argentifolii* nymphs.

female then either abandoned the host ($n = 5$) or returned to antennation of the host's margin ($n = 11$). If circling antennation of the same host took place after an initial probe, it was always followed by another probing attempt (Fig. 2, dashed vertical line leading from CA to P_R), either on the same side as the initial probe or on the side opposite. Repeated probes occurred up to 15 times on a single host per female. Females irregularly alternated between sides during repeated probing events until a suitable gap was accessed ($n = 15$ successes) or the host was abandoned ($n = 3$). The margins of the host bodies were nearly contiguous with the leaf surface on this host plant, and the nymphs were encircled by a ring of marginal wax (Fig. 5). Both of these factors may have affected the ability of the females to locate a suitable place for ovipositor extension and thus contributed to the occurrence of repeated probes.

The durations of initial and repeat probes were analyzed (Fig. 6). Probing duration was defined as the time from initial engagement of the ovipositor on the margin of the host until subsequent antennation or abandonment. Some probes included successful exertion of the ovipositor beneath the host nymph; thus the durations of probes that resulted in oviposition also include the time for egg deposition. Initial or repeat probes that led to oviposition averaged 92.7 ± 20.2 s ($n = 9$; range, 17.0–216 s) and 151.7 ± 24.8 s ($n = 11$; range, 51.0–324.0 s), respectively, with no significant difference. Initial and repeat probes that did not result in oviposition averaged 53.0 ± 18.0 s ($n = 27$; range, 1.0–415.0 s) and 23.0 ± 5.0 s ($n = 27$; range, 3.0–183.0 s), respectively, also with no significant difference. Durations of initial probes leading to an egg being laid were not significantly different from those of initial probes not involving oviposition; however, the mean difference of 39.63 s probably represents the time spent depositing an egg. Repeat probes leading to oviposition were significantly longer than repeat probes that did not (t

Fig. 6. Mean duration of initial and repeated probing events over all trials on sweet potato leaves, and mean duration of initial and repeated probing events based on successful oviposition over all trials on sweet potato leaves.

= 8.245, OSL < 10^{-15}). Probe times for each category (initial and repeat probes, both with and without oviposition) were examined for differences in duration among host nymphal stages, and in all cases no significant differences among host stages were found.

Oviposition. Eggs were deposited near the center of the host irrespective of nymphal stage. All first-instar nymphs were lifted from the leaf surface as a result of the action of the ovipositor during probing. Probes that resulted in full exertion of the ovipositor beneath the host did not necessarily indicate oviposition. All nymphs under which females fully exerted their ovipositors were examined microscopically for verification of egg deposition. Oviposition occurred under 20 of 27 (74%) nymphs under which ovipositor exertion occurred. The probes that averaged the shortest in duration were those that resulted in no egg being laid (Fig. 6).

Oviposition by *E. sp. nr. californicus* females also was analyzed for frequency and host nymphal stage effects. In the nine 1-h trials, three of the females laid no eggs, and the maximum for one female was seven eggs. The total number of eggs laid (20) per host assessed by antennation (93) was 21.0% in these laboratory trials.

The frequencies of various oviposition behaviors among the different nymphal stages were examined. First-instar nymphs represented 8.6% of the total stages present in all the trials, second instars represented 12.2%, third instars 17.7%, and fourth-instar nymphs made up 61.5% of all stages. Of the nymphs available, 13.3% of the first instars, 19.8% of the second instars, 9.7% of the third instars, and 19.1% of the fourth instars were encountered by searching female wasps (Fig. 7a). Of the 13.3% first-instar nymphs encountered, 90% of those were subsequently antennated, whereas

61.9, 60.0, and 60.8% of the second, third, and fourth instars, respectively, were subsequently antennated (Fig. 7b). Of the first instars antennated, 33.3% were then probed with the ovipositor, 92.3% of the second instars antennated were subsequently probed, and 55.6 and 25.8% of third and fourth instars, respectively, were probed (Fig. 7c). Of the first instars probed, 66.7% ultimately had an egg laid under them, and 58.3, 80.0, and 43.75% of second-, third-, and fourth-instar nymphs, respectively, had eggs laid under them (Fig. 7d). The differences that occurred among the frequency of nymphal stages available and the frequency of them being encountered by a female searching were significant ($\chi^2 = 8.72$, $df = 3$, OSL = 0.033); third instars were found slightly less frequently than expected (Fig. 7a), although we found no biological basis for this difference. The average proportion of stages encountered during this study was 17.03%. There were no stage-related differences between the frequency of host stage encountered and the frequency of that stage being antennated (mean = 62.8%, $\chi^2 = 3.40$, $df = 3$, OSL = 0.334) (Fig. 7b). Of the nymphs antennated, there was a significantly higher frequency of second instars probed than any other stage ($\chi^2 = 21.28$, $df = 3$, OSL = 0.00009); there were no significant differences among first-, third-, and fourth-instar nymphs ($\chi^2 = 3.37$, $df = 2$, OSL = 0.19). Finally, of nymphs probed with the ovipositor, no stage-related differences were detected among instars that ultimately received an egg (mean = 55.6%, $\chi^2 = 2.3$, $df = 3$, OSL = 0.51). Thus, females encountered all host stages during searching with approximately the same frequency as their relative abundance. Females also arrested and antennated all host stages with the same relative frequency as their encounter rate. However, of the hosts antennated, there was a clear and significant preference among females to probe second-instar nymphs over all other stages. Of the hosts probed, females chose all stages for oviposition with the same relative frequency.

Feeding, Grooming, Resting. Feeding was divided into two categories, surface feeding and host feeding. Surface feeding involved arrestment at and antennation of a potential food or water source, then extending the antennae away from the face and lowering the mouthparts to the food or water source. Further antennation of the food or water source occurred sporadically during feeding episodes. Surface feeding events averaged 90.5 ± 43.7 s ($n = 17$; range, 2.0–579.0 s). Females also were observed to feed upon accumulated honeydew or water droplets condensed on the upper surfaces of nymphs; this, however, was not host feeding. Host feeding involved assessment of the host by antennation, orienting anteriorly along the midline of the host body, turning 180° toward the vasiform orifice of the host, stepping forward, and engaging the ovipositor in the vasiform orifice. The female exerted the appendicular ovipositor against

Fig. 7. Frequencies, represented as a percentage, of behavioral events leading to oviposition by nymphal instar (columns for each instar with different letters differed in frequency with an OSL ≤ 0.05 ; weighted averages are included). (a) Of the numbers available, those that were encountered; (b) of those encountered, those that were antennated; (c) of those antennated, those that were probed; (d) of those probed, those that received an egg.

the crevices of the vasiform orifice, especially against and underneath the operculum (Fig. 8). When the ovipositor engaged an area of the integument soft enough to pierce, typically under the operculum, then the ovipositor was fully exerted

Fig. 8. Diagram of probing the vasiform orifice of *B. argentifolii* for subsequent host feeding by *E. sp. nr. californicus*. Arrow indicates direction of wing loft.

and retracted repeatedly into the body of the host. The process averaged 108.8 ± 20.9 s ($n = 39$; range, 8.0–516.0 s) in duration (Table 1). Upon completion, the ovipositor was retracted and the female typically groomed. Before feeding, the female again mounted the nymph, antennated the host dorsum in a circling pattern, lined up facing the vasiform orifice, extended her antennae away from her face, and placed her mouthparts onto the droplet of host haemolymph exuding from the wound. If the flow of haemolymph diminished, then the probing process was repeated, followed again by antennation and feeding. Over all trials, five host nymphs were used by three females for host feeding (Table 1). Of these, seven occurred with first-instar nymphs, one with a third instar, and eight with fourth-instar nymphs; second-instar nymphs were not fed upon. Of the first-instar nymphs that were encountered, a larger proportion was subsequently fed upon than occurred for other stages ($\chi^2 = 40.0$, $df = 3$, OSL = 0.0001). Host-feeding durations on first instars averaged 144.6 ± 43.8 s ($n = 7$; range, 1.0–306.0 s) and on fourth instars averaged 172.2 ± 155.1 s ($n = 8$; range, 1.0–1,255.0 s); the longest feeding event was 2,128.0 s on a single third-instar nymph.

Table 1. Host-feeding frequencies and their duration

Event	Frequency	Duration, s		
		Mean	SEM	Range
Probing the vasiform orifice	39	108.85	20.92	8.0–516.0
Host feeding at the puncture wound	16	282.37	145.25	1.0–2128.0

Grooming was observed most frequently following an ovipositional episode, but could occur at any time and could be sustained during other activities such as antennation or walking. Grooming involved use of the fore legs to clean the anterior portion of the thorax, the head, and antennae. The hind legs were used to clean the wings, metasoma, and ovipositor. The fore legs were rubbed together for cleaning and together cleaned the middle legs; the hind legs were rubbed together for cleaning. During disengagement (retraction of the ovipositor from beneath a host), females began asynchronously rubbing the hind tarsi against the host and leaf surface. As the ovipositor was removed from beneath the host, the hind tarsi were then used to groom asynchronously the still partially exerted ovipositor and often continued with grooming of the metasoma and the remainder of the body. However, grooming did not always follow disengagement of the ovipositor because some females immediately walked away and continued searching for hosts. Grooming episodes averaged 32.1 ± 5.8 s ($n = 59$; range, 1.0–219.0 s).

Resting was observed rarely during the trials ($n = 12$), but some females were recorded resting up to 10 min in a given episode. The mean duration of resting events in all trials was 119.9 ± 51.4 s ($n = 12$; range, 5.0–587.0 s).

Overall Time Budget. Forty-one percent of the total time was spent in walking (7%), antennation (12%), and probing (22%); the remaining time was

spent host feeding, grooming, and resting (Fig. 9). Host feeding was the most time-consuming activity, accounting for 39% of the total time. However, this behavior was observed for only three females; for one of these, host feeding took place over the entire hour-long trial. The percentage of the total host feeding time for this one event was calculated (Fig. 9, crosshatch portion of bar). Grooming and resting combined for 20% of the total time budget for all females (Fig. 9).

Discussion

In this study, searching behaviors commenced immediately when females were introduced to leaves bearing hosts. During searching on sweet potato leaves, walking speeds averaged 1.08 mm/s for *Eretmocerus* sp. nr. *californicus*, which is comparable to the walking speed of *Encarsia formosa* on similarly glabrous leaves (van Lenteren et al. 1975, van Lenteren & de Ponti 1990). Counterturning during walking was frequent and apparently random as females antennated the substrate. During periods of walking when no hosts were encountered for up to 10 s, females were observed to begin walking along leaf veins, which on sweet potato were more darkly colored than intervein areas. During searching, females walked over at least some host nymphs without displaying any observable arrestment or recognition behaviors. These we referred to as oblivious host encounters. Females were observed to stop and assess an encountered host that was ignored previously. However, females rarely encountered the same host nymph twice.

Antennation behaviors on encountered hosts by *E. sp. nr. californicus* were similar to those of other aphelinid species that attacked hosts with circular bodies. Gerling (1966b) briefly described the antennation behaviors of what was identified as *Eretmocerus californicus* Howard from southern California on *Trialeurodes vaporariorum*. There are a few differences in behaviors noted in his study and this one. Gerling (1966b) described *E. californicus* females mounting the host nymph and walking along its margins while antennating, often stabbing the host several times with the ovipositor. He also noted that females preferred second and third instars for oviposition, and females examined and probed empty whitefly pupal cases. In our study, females rotated while standing centrally on a host during antennation (*B. argentifolii* nymphs generally are smaller than *T. vaporariorum* nymphs of the same stage) and were not observed to probe

Fig. 9. Time budget of *E. sp. nr. californicus* females attacking *B. argentifolii* on sweet potato summed over all trials. Host-feeding percentages were calculated with (solid + crosshatch) and without (solid) the one 2,128-s host-feeding event.

the host during assessment by antennation or to assess empty pupal cases. *Eretmocerus mundus* (Mercet) females antennate in a manner similar to *E. sp. nr. californicus* (i.e., they rotate while standing over the center of a host nymph during antennation of the host's margin [Foltyn & Gerling 1985]).

Other aphelinid species show similar antennation behaviors; however, the details of host assessment differ slightly. *E. formosa* females that attack *T. vaporariorum* also drum their antennae asynchronously upon the substrate while searching (van Lenteren et al. 1980). An *E. formosa* female continues drumming as she mounts an encountered host nymph, walks toward its anterior, and then turns 180°, drumming posteriorly along the midline. As she reaches the posterior margin, she turns 180° to antennate and walk forward again. This process is repeated until the female engages her ovipositor on the dorsum of the host and then pierces the integument for egg deposition; thus, antennation of the margins is not displayed (van Lenteren et al. 1980). Host assessment along the midline of the body of the host nymph also was observed for *Encarsia lutea* (Masi) (Gerling & Foltyn 1987) and *Encarsia luteola* (Howard) (Gerling et al. 1987; D.H.H. & T.S.B., unpublished data).

The actions of *E. sp. nr. californicus* females during antennation of the host before probing the margins or the vasiform orifice suggest that the female is using surface features of the host body as landmarks for alignment. Females displayed counter-turning between the tracheal furrows and the posterior margin that preceded probing the lateral margin for oviposition, whereas counter-turning took place anteriorly between the tracheal furrows before turning 180° to probe the vasiform orifice. Observation under a dissection microscope showed that the apices of the antennae were able to engage the lateralmost margins of the body on fourth-instar nymphs; thus, the wax filaments extruding from the anterolateral and posterior tracheal fold, the vasiform orifice, and other setae may function as landmarks.

In our study, probing durations did not differ significantly among nymphal instars. Lakin & Bellows (1995) noted that *Eretmocerus* sp. females attacking citrus whitefly, *Dialeurodes citri* (Ashmead), spent more time probing fourth-instar nymphs and hypothesized that this greater time was used in trying to find a suitable place for oviposition beneath these larger nymphs. Probing durations and frequencies have not been reported for other species of *Eretmocerus*. Females of *E. mundus* raised their wings during exertion of the ovipositor in a manner similar to *E. sp. nr. californicus* (Foltyn & Gerling 1985). However, *Eretmocerus* sp. females attacking citrus whitefly did not (Lakin & Bellows 1995), and the position of the wings during probing was not described for *E. californicus* (Gerling 1966b). During exertion of the ovipositor in *E. sp. nr. californicus*, the metasoma is

distorted; this action may in turn compress the megaphragma. The megaphragma serves as an insertion point for large flight muscles and if compressed would involuntarily raise the wings. The muscular action for oviposition in other *Eretmocerus* species may be different.

During disengagement, females of *E. mundus* rubbed their hind tarsi on the host nymph (Foltyn & Gerling 1985) as reported for *E. sp. nr. californicus* herein; however, *Eretmocerus* sp. females did not (Lakin & Bellows 1995). The function of rubbing the hind tarsi on the nymph during disengagement is not clear, although Foltyn & Gerling (1985) suggested that it was for the deposition of an ovipositional deterrent. In our study, rubbing the hind tarsi was noted for all disengagements irrespective of egg deposition. Some character of the host may change after probing because females did not attempt circling antennation or probing on any previously assessed host nymph and no superparasitism was noted in this study. Because all known *Eretmocerus* spp. oviposit externally, no internal chemical transfer to the host is made; thus, the hypothesis for external marking needs further testing. Foltyn & Gerling (1985) and Lakin & Bellows (1995) noted superparasitism in *E. mundus* and *Eretmocerus* sp., respectively.

Our results provide evidence for a probing preference under second instars (Fig. 7). Circumstantial evidence for instar preference in *E. californicus* was noted by Cameel (1969) and Gerling (1966b) based on life-history analyses. In our study, females probed second instars more frequently than other instars irrespective of how many they encountered, which contrasts with the general assumptions that later instars are preferred because they are larger and thus easier to locate (cf. Gerling 1990). We found no distinct, stage-related ovipositional preferences by *E. sp. nr. californicus* females. Preferences for oviposition into later instars were noted for *Eretmocerus* sp. (Lakin & Bellows 1995). Other aphelinids (e.g., *Encarsia formosa* and *Encarsia luteola*) have been reported to prefer later-instar nymphs for oviposition (Nell et al. 1976; D.H.H. & T.S.B., unpublished data), but *Encarsia opulenta* (Silvestri) showed a significant preference for second-instar nymphs of citrus blackfly, *Aleurocanthus woglumi* Ashby (Dowell et al. 1981), and the platygasterid *Amitus hesperidium* Silvestri prefers only first or second instars (Clausen & Berry 1932, Dowell et al. 1981).

There was no discernible difference in searching behaviors leading to oviposition or to host feeding. However, during the 1-h trials females spent most of their time either in ovipositional behavior or in host feeding. Assessment of the host for feeding differed only in mannerism, but not in duration from assessment for oviposition, which contrasts with the findings of van Lenteren et al. (1980) for *E. formosa*. Stage preferences for host feeding by females in our study showed that a larger proportion of first-instar nymphs that were encountered

were fed upon than other stages, and second instars were not fed upon. *E. formosa* (van Alphen et al. 1976, van Lenteren et al. 1980), *Encarsia lahorensis* (Howard) (Viggiani & Mazzone, 1978), *Encarsia pergandiella* (Gerling 1966a) Howard, and *Encarsia transvena* (Timberlake) (Gerling 1983) were reported to show distinct instar preferences for host feeding. Most *Eretmocerus* spp. examined thus far apparently do not (Gerling 1990), except Lakin & Bellows (1995) reported host feeding exclusively on first-instar nymphs by *Eretmocerus* sp. Host searching and oviposition occurred directly before and after host feeding, and no host was observed to be used for both oviposition and host feeding, which is consistent with findings for other aphelinids attacking Aleyrodidae (Nell et al. 1976, van Lenteren et al. 1980, Gerling 1990).

Walking, antennation, and probing compose the behaviors leading to oviposition. Over all trials, these behaviors consumed 41% of the total time budget for females. The remaining time was spent host feeding, grooming, or resting. Host feeding was the single most time-consuming event, but was observed in only three of the nine females.

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