Preovipositional Behaviors of *Tiphia pygidialis* and *Tiphia vernalis* (Hymenoptera: Tiphiidae), Parasitoids of White Grubs (Coleoptera: Scarabaeidae)

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ABSTRACT   The events leading to oviposition by *Tiphia pygidialis* Allen and *Tiphia vernalis* Rohwer on their host *Cyclocephala* spp. and *Popillia japonica* Newman grubs, respectively, were compared and quantified using videotaped recordings. Mean ($\pm$SE) duration of the oviposition process was 49.3 $\pm$ 2.9 min for *T. pygidialis* and 31.5 $\pm$ 2.2 min for *T. vernalis*. Behaviors included stinging, moving soil from around grubs, host kneading, host examination, host feeding, and scraping of the grub's cuticle. Sequence of behavioral events was translated into a first-order (Markovian) contingency table to construct flow charts of the oviposition process for both species. *T. pygidialis* spent more time kneading and scraping the cuticle of grubs than did *T. vernalis*, whereas *T. vernalis* performed more stinging and soil-moving events. These behaviors most likely serve to move the grubs into a suitable position for oviposition. Scraping of the host's cuticle where an egg was to be laid took more time than any other behavior. Compared with nonparasitized grubs, cuticle from parasitized grubs contained numerous grooves filled with cement used to anchor the parasitoid egg. Scanning electron microscopy revealed peg-like structures, similar in size to the grooves made in the cuticle of parasitized grubs, on the ovipositor sheath of female wasps. These structures are thought to be used in making the grooves in the cuticle, whose postulated function is to increase the surface area of the host cuticle to facilitate egg attachment. This is the first study to implicate such use of the ovipositor sheath by a parasitoid.

KEY WORDS   *Popillia japonica*, *Cyclocephala* spp., ovipositor sheath sensilla, host scraping, venom

WASPS OF THE GENUS *Tiphia* (Hymenoptera: Tiphiidae) are the predominant group of parasitoids attacking white grubs (Coleoptera: Scarabaeidae) in the soil (Clausen 1940). More than 80 species of *Tiphia* occur in North America (Krombein et al. 1979). *Tiphia* usually have one generation per year, emerging when second- or third-instar grubs are available for oviposition. They are host specific, parasitizing a few closely related species, usually within the same genus (Jaynes and Gardner 1924, Rogers and Potter 2002). To parasitize a grub, these wasps fly to an area where hosts are present and burrow into the soil. Once underground, the wasps locate their root-feeding hosts using species-specific kairomones present in residual body odor trails and frass (Rogers and Potter 2002), and then parasitize the host, attaching an egg in a location that is specific for that species of wasp (Clausen et al. 1927). The wasp larva develops as an ectoparasitoid on its host, with development to the cocoon stage taking ≈21 d (Gardner and Parker 1940, Rogers and Potter 2004).

Two species of *Tiphia* commonly attack turf-infesting white grubs in Kentucky. *Tiphia pygidialis* Allen is a native wasp that parasitizes third-instar masked chafer (*Cyclocephala* spp.) grubs from mid-August through early October. *Tiphia vernalis* Rohwer, which is native to Japan, Korea, and China, was first released in the United States in 1924 for biological control of the Japanese beetle, *Popillia japonica* Newman (Clausen 1978). Since its introduction, *T. vernalis* has spread to many parts of the United States where *P. japonica* has become established (Clausen 1978). In Kentucky, adults of *T. vernalis* are active from mid-April through mid-June, ovipositing on overwintered third-instar *P. japonica*. We have found both *Tiphia* species to be abundant at our study sites, parasitizing up to 58% of their respective host grubs in a given stand of turf (Rogers and Potter 2004).

The process of ovipositing on hosts while in the soil has been described for only one species of *Tiphia*, *T. popilliavora*, which was imported and released into the United States during the 1920s for control of *P. japonica* (Clausen et al. 1927, Clausen 1978). Moreover, no previous studies have examined the method by which eggs of *Tiphia* spp. are attached to their soil-dwelling hosts. In this work, we describe and compare the oviposition process of *T. pygidialis* and

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T. vernalis on their respective host species based upon videotaped oviposition events. We also investigated the manner by which these wasps secure eggs to their soil-dwelling hosts.

Materials and Methods

Collection of Wasps and Grubs. Adult female *T. pygidialis* and *T. vernalis* were collected from golf courses in central Kentucky. *T. pygidialis* were collected during late August by spraying 1-m^2 plots of turfgrass with a 10% sugar water solution to thoroughly wet the grass blades. A hand-held vacuum (BioQuip Products, Gardena, CA) was used to collect wasps attracted to the sprays. *T. vernalis*, which feed on honeydew secretions of aphids, scale insects, and other homopterans (Clausen et al. 1927), were collected during May in a similar manner, except that sugar sprays were applied to the foliage of trees bordering areas of turf. Wasps were separated by sex, and females were placed individually into 118-ml plastic cups (Solo, Highland Park, IL) half filled with moist autoclaved soil. A film canister lid containing a piece of dental wick soaked in a 10% sugar water solution was placed on the surface of the soil as a food source. Containers with wasps were held at room temperature (22−24°C) and light regime 14L:10D until used in experiments. Voucher specimens were deposited in the University of Kentucky Insect Collection (Lexington, KY).

Third-instar *Cyclocephala* spp. and *P. japonica* were collected from stands of predominantly Kentucky bluegrass, *Poa pratensis* L., on golf courses and sod farms in central Kentucky. *Cyclocephala* spp. in Kentucky include a mix of southern and northern masked chafers, *C. lurida* Bland and *C. borealis* Arrow, respectively, grubs of which are morphologically indistinguishable. Both species are suitable as hosts for *T. pygidialis* (Rogers and Potter 2004). For experiments with *T. vernalis*, postoverwintered third-instar *P. japonica* were collected during wasp flight in early May. For tests with *T. pygidialis*, third-instar *Cyclocephala* spp. were collected in late August and September.

Preovipositional Behaviors. An observation chamber was constructed to view the preovipositional behaviors of the wasps while in the soil. Two panes of glass (bottom, 27 × 30 cm; top, 20 × 20 cm), positioned horizontally, were separated by an outline of modeling clay. The gap between the glass panes was 0.75 cm, slightly more than the width of a grub. The chamber was filled with autoclaved, moist sifted soil. A 10-cm-long piece of flexible plastic tubing was inserted through a break in the modeling clay at one end of the observation chamber for introduction of wasps. A Panasonic GP-KR222 color video camera (Matsushita Communication Industrial, Japan), positioned 0.3 m above the observation chamber, was used to record each oviposition event. Recordings were made under low-light conditions with only a red bulb as a light source. For each oviposition event recorded, one host grub was placed into the soil-filled observation cham-

ber and a single female wasp was introduced into the chamber through the plastic tubing. Ten separate oviposition events were recorded for both *T. pygidialis* ovipositing on *Cyclocephala* sp. hosts and *T. vernalis* parasitizing *P. japonica*. Later, the recordings were played back for viewing on a monitor. Sequence and duration of behaviors before oviposition were transcribed onto paper for each event recorded. Duration and number of times each behavior was performed by *T. pygidialis* or *T. vernalis* were compared using two-sample *t* tests (Analytical Software 2000). These data are reported in this study as means ± SE. The sequence of behavioral acts for both species was translated into a first-order (Markovian) contingency table (Fagen and Young 1978). This table gives the frequencies of transitions from a given behavior to all the behaviors that could follow it. Expected values in each cell of the contingency table were calculated by χ^2 methods, and transitions greater than the expected frequency were used to construct a flow chart of the preovipositional behaviors for each wasp species.

Host-Scraping Behavior. Before oviposition, both species of *Tiphia* were observed to use the ovipositor sheaths, protruding from the end of the abdomen, to perform extensive scraping of the host's cuticle in the area in which the egg would be deposited. Sections of cuticle (0.2 mm^2) were dissected from 10 parasitized third-instar *P. japonica* and 10 parasitized third-instar *Cyclocephala* sp. grubs where the egg was attached. Cross sections of grub cuticle from the same area were also taken from 10 nonparasitized *P. japonica* and *Cyclocephala* sp. grubs to compare with those from parasitized grubs. Sections were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 2 h, transferred to 1% osmium tetroxide in 0.1 M cacodylate buffer for 2 h at 4°C, and then gradually dried in alcohol and acetone and infiltrated with Spurr's medium overnight at 4°C. Samples were then embedded in Spurr's medium for 8 h at 70°C. Cross sections (1 μm thick) were cut with an ultramicrotome, slide mounted, stained, and then examined using light microscopy.

The portion of the ovipositor sheaths visible during the scraping behavior was examined for presence of structures that might be used to alter the grub's cuticle during host scraping. Ten *T. pygidialis* and 10 *T. vernalis* females were sonicated in a bath of distilled water and Triton X-100 soap (Sigma-Aldrich, St. Louis, MO). Abdomens were then removed from the wasps, fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, gradually dehydrated in alcohol and acetone, and dried. Specimens were then sputter-coated with silver and observed using a Hitachi S-800 (Hitachi, Tokyo, Japan) field emission-scanning electron microscope.

Results

Preovipositional Behaviors. Both *T. pygidialis* and *T. vernalis* performed six distinct behaviors before completion of the oviposition process.
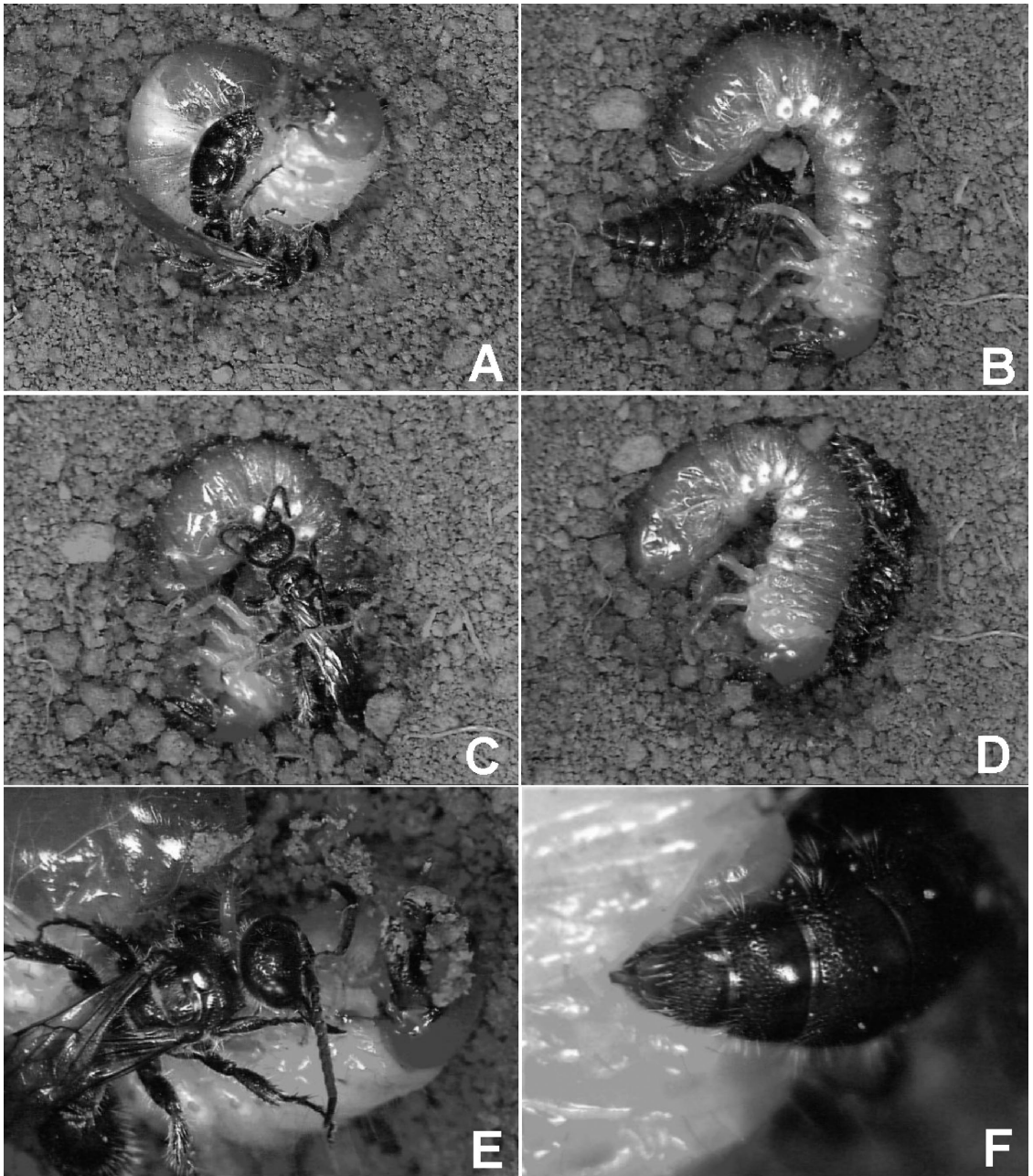
Fig. 1. Behavioral acts performed by *T. pygidialis* ovipositing on third-instar *Cyclocephala* sp. (A) Host stinging. (B) Moving soil from around grub. (C) Host kneading of abdominal segments with mandibles. (D) Female wasp kneading host from abdomen to head capsule while pushing grub into C-shape position. (E) Wasp wounding and host feeding on leg of grub. (F) Ovipositor sheath visible during scraping of host cuticle.

Stinging. Upon making antennal contact with the host, the female wasp climbs onto the dorsal region of the grub's thorax and wraps her abdomen around to the ventral side of the grub's body. Wasps were then observed to sting the grub in the area of the thoracic ganglia (Fig. 1A). During this time, the grub violently responds to the attack by attempting to bite the wasp. Once the wasp's stinger is inserted, the grub may react by rubbing the end of its abdomen against the stinger, occasionally dislodging it. If dislodged, the wasp reinserts the stinger until the host is paralyzed. During the entire oviposition process, up to five separate stinging acts may be performed (Fig. 3), each directed at the thoracic ganglia and lasting ≈30 s. From the point of the initial paralysis-inducing sting, grubs were observed to regain movement in ≈1 h.
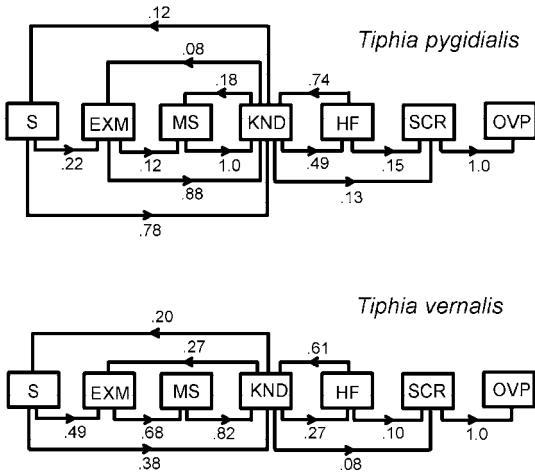
Fig. 2. Flow charts of the behavioral activities of (upper chart) *T. pygidialis* ovipositing on *Cyclocephala* sp. grubs, and (lower chart) *T. vernalis* ovipositing on *P. japonica* grubs. Numbers associated with arrows are the conditional probabilities of an indicated transition. S, stinging; EXM, examining host; MS, moving soil from around host; KND, kneading host; HF, host feeding; SCR, scraping host with ovipositor sheaths; OVP, oviposition.

Host Examination. The wasp walks over and around the body of the paralyzed grub, antennating both the grub and the surrounding soil.

Moving Soil. The wasp moves soil away from the grub and creates a cell that facilitates handling of the grub before oviposition (Fig. 1B). Using her forelegs, the wasp digs soil from around the body of the grub, passing it back toward her abdomen using her mid and hind legs. The wasp then pushes soil away with her abdomen.

Host Kneading. The wasp uses her large sickle-shaped mandibles to firmly grasp each body segment of the grub and exert pressure at that spot by pressing her head down against the grub's body (Fig. 1C). This kneading is performed one body segment at a time, beginning at the thoracic region of the grub, with the wasp working its way to the posterior region of the grub's abdomen and then back to the host's head capsule. As the wasp moves along the body of the grub, kneading it with her mandibles, she uses her abdomen to push against the segments that she recently kneaded. Pushing with the abdomen, coupled with the kneading and associated downward pressure exerted by the wasp's head, she moves the grub into a C-shape position (Fig. 1D).

Host Feeding. During the oviposition process, the wasps were always observed to feed on the paralyzed grubs by using their mouthparts to pierce the grub's body and imbibe hemolymph exuding from the wound. Host feeding occurs either on the area of the grub's thorax near the head capsule, at the base of one leg (Fig. 1E), or at the joint of a leg segment, the latter of which may completely sever the leg. Host-feeding acts performed near the head capsule are typically shorter in average
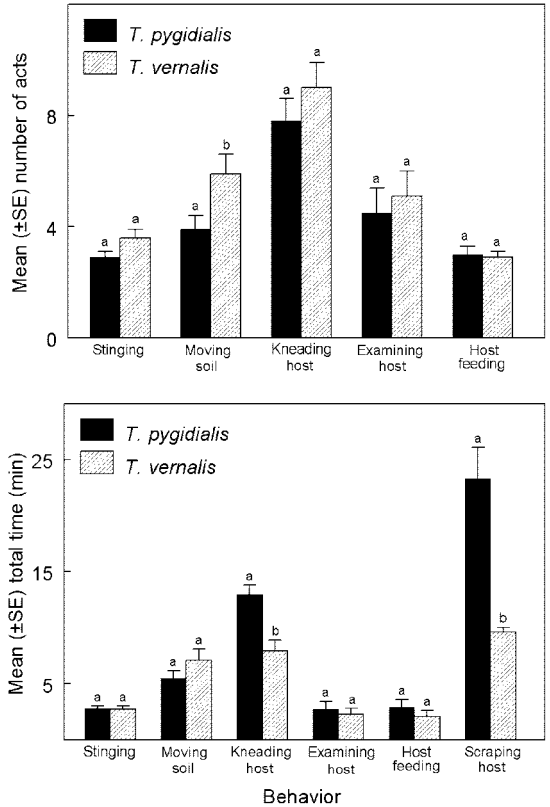

Fig. 3. (Upper graph) Number (mean $\pm$ SE) of behavioral acts performed by *T. pygidialis* and *T. vernalis* during each oviposition bout ($n = 10$). (Lower graph) Total time (mean $\pm$ SE) that wasps performed each behavior during the oviposition process. For each behavior, means with the same letter do not differ significantly (two-sample t test; $P > 0.05$).

duration than those taking place on or near leg segments (12 ± 6 vs 126 ± 30 s, respectively).

Host Scraping. Immediately preceding oviposition, the wasp uses the sheaths of the ovipositor to scrape the portion of the grub's cuticle where the egg will be attached (Fig. 1F). Scraping is performed dorsally between the second and third thoracic segments by *T. pygidialis*, whereas *T. vernalis* scrapes the host's cuticle ventrally between the third thoracic and first abdominal segments.

The sequence of acts leading to oviposition by *T. pygidialis* on *Cyclocephala* spp. and *T. vernalis* on *P. japonica*, including the conditional probabilities of behavioral transitions, is shown in Fig. 2. After stinging the host, *T. pygidialis* and *T. vernalis* transitioned either to host examination (22 and 49%, respectively) or host kneading (78 and 38%, respectively). If the host was first examined, *T. pygidialis* then began host kneading (88%), followed by periods of host examination or moving soil from around the grub. *T. vernalis* usually transitioned from host examination to removing soil from around the grub (68%), followed by kneading behavior. After periods of host kneading, both species also transitioned to host-feeding behavior
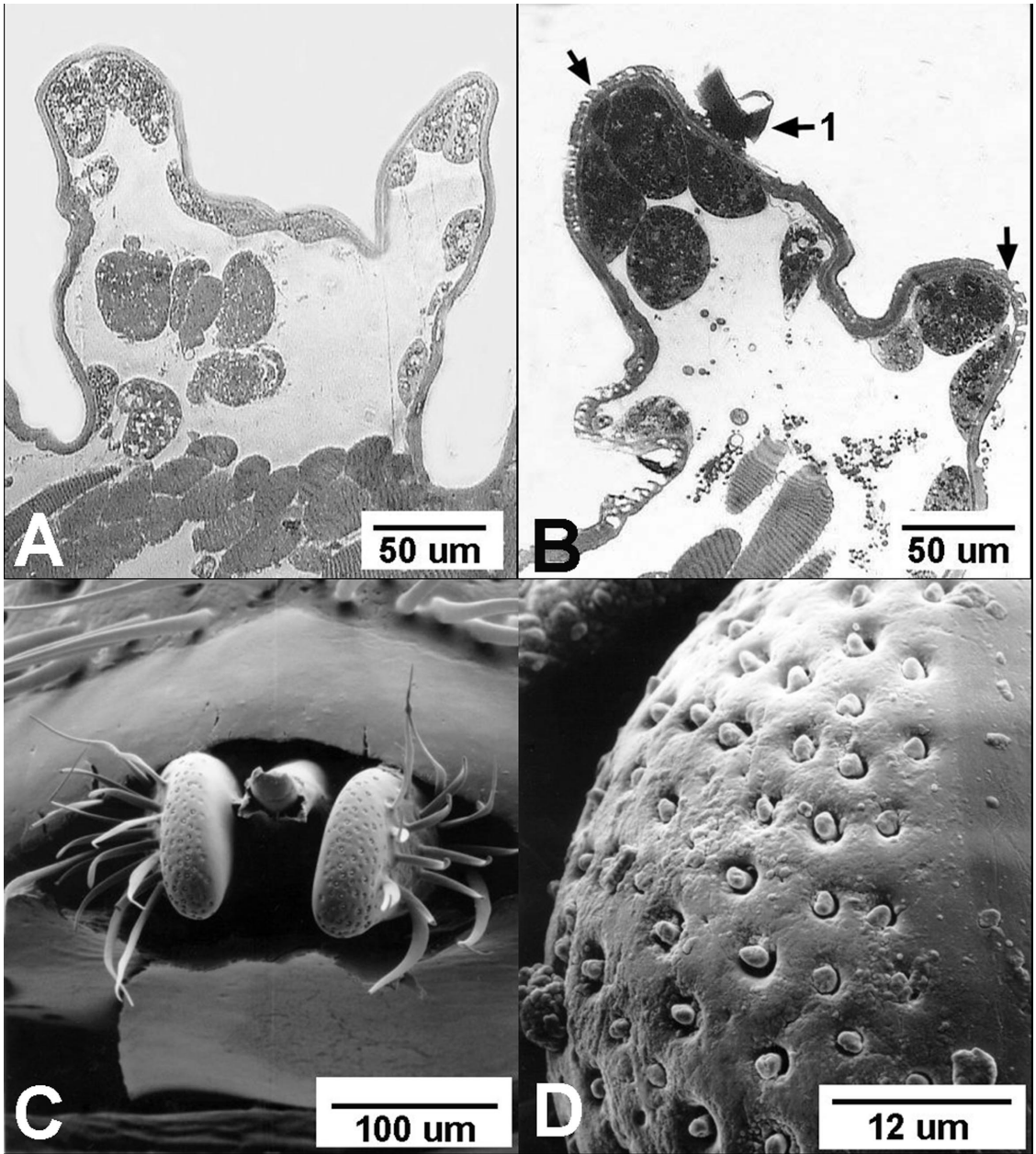
Fig. 4. (A) Cross section (×250) of thoracic segment of nonparasitized *Cyclocephala* sp. (B) Cross section (×250) of thoracic segment of parasitized *Cyclocephala* sp. with remains of parasitoid egg. Arrow 1 points to remains of parasitoid egg and adhesive substance, and other arrows point to mechanical damage to outer layers of cuticle. (C) SEM (×300) showing structures on the ovipositor sheath of *T. pygidialis*. (D) SEM (×2500) of peg-like structures on ovipositor sheath of *T. pygidialis*.

or stung the host again. After repeated bouts of these behaviors, both *T. pygidialis* and *T. vernalis* scraped the area of the grub's cuticle where the egg was then laid.

Of the six behaviors leading to oviposition, moving of soil from around the host was performed more often by *T. vernalis* than by *T. pygidialis* (Fig. 3; *t* tests; *t* = 2.3, df = 18, *P* = 0.03). There was also a trend for

T. vernalis to sting their hosts more times than *T. pygidialis* (Fig. 3; *t* tests; *t* = 1.98, df = 18, *P* = 0.06). However, there was no significant difference between the two species in total duration of these acts (Fig. 3; *t* = 1.35, df = 18, *P* = 0.20; *t* = 0.38, df = 18, *P* = 0.71, respectively). There was also no difference in the number of host-kneading acts (*t* = 1.02, df = 18, *P* = 0.32), number of times grubs were examined (*t* = 0.46,

df = 18, P = 0.64), or number of host-feeding acts (Fig. 3, t = 0.25, df = 18, P = 0.88). Of these latter behaviors, *T. pygidialis* spent significantly more time kneading their hosts (Fig. 3; t = 3.96, df = 18, P = 0.0009), and longer scraping the host's cuticle before oviposition (Fig. 3; t = 4.9, df = 18, P = 0.0001) than did *T. vernalis.* Subsequently, there was a significant difference in the overall time to complete the oviposition process for the two species (t = 4.9, df = 18, P = 0.0001). The mean (±SE) time for *T. pygidialis* to complete the oviposition process was 49 ± 2.9 min, whereas oviposition by *T. vernalis* on *P. japonica* lasted 31.5 ± 2.2 min.

Host-Scraping Behavior. Compared with cross sections of cuticle from nonparasitized grubs (Fig. 4A), the surface of the cuticle of parasitized *P. japonica* and *Cyclocephala* spp. was roughened in appearance with numerous small grooves (Fig. 4B). Where the parasitoid egg was attached, the grooves were filled with an adhesive substance.

Scanning electron micrographs (SEMs) of the distal portion of the abdomen of female wasps showed no external morphological structures that might be used in the scraping behavior to create the characteristic grooves found in the cuticle of parasitized individuals. There were, however, numerous flattened, hair-like, apparent sensory structures on the ovipositor sheath (Fig. 4C) that was observed protruding from the abdomen of wasps during the scraping behavior (Fig. 1F). At ×2,500 magnification, small peg-like structures were observed covering the ventral and distal portions of the ovipositor sheath (Fig. 4D). These structures did not appear to have any pores indicative of a chemosensory function. Measurements of these pegs and of the grooves rubbed in the cuticle of parasitized grubs were similar. Mean (±SE) width and depth of grooves in the cuticle of parasitized grubs were 5.0 ± 2.1 and 1.6 ± 1.1 μm, respectively, whereas the width and height of the pegs were 3.0 ± 0.8 and 1.2 ± 0.5 μm, respectively.

Discussion

Before oviposition, both *T. pygidialis* and *T. vernalis* locate their respective host grubs using species-specific kairomones present in grub frass and residual body odor trails left in the soil (Rogers and Potter 2002). Once a host is located, the sequence of behavioral acts is similar for the two *Tiphia* species (Fig. 3). After grubs were stung, resulting in temporary paralysis, wasps performed repeated bouts of host examination, kneading, and moving soil from around the grub. These acts were punctuated by periods of host feeding or repeated stinging of the grub. Successive kneading acts moved the grubs into a C-shape position. This position most likely facilitates oviposition by making the area of cuticle between the host's body segments (where the egg will be laid) more defined and accessible to the ovipositing female. Once the host was in a suitable position, wasps extensively scraped the cuticle of the grub between the body segments where the egg was then laid. With the exception of removing soil from around the grub, host stinging, examining, kneading, feeding, and scraping behaviors have been described in other, nontiphiid parasitoid species. We suggest that *Tiphia* spp. have adapted these behaviors for parasitism of their edaphic hosts.

Many species of parasitic wasps sting their hosts, causing either temporary or permanent paralysis (Steiner 1986, Quicke 1997). Such paralysis may serve to protect the parasitoid from defensive responses from the larger host and facilitate oviposition events of long duration by subduing a writhing host (Steiner 1986). Sting-induced paralysis serves similar purposes for *Tiphia* spp. Before succumbing to the effects of *Tiphia* venom, grubs react by either rubbing their abdomen against the wasp's abdomen to displace the sting, or biting at the attacking wasp. In previous studies, we have observed wasps losing part of an antenna to this defensive biting response. Such injury may impair the ability of wasps to locate and parasitize subsequent hosts (Rogers and Potter 2002). Additionally, when a wasp's antennae made initial contact with a grub, the grub often responded by attempting to burrow away. The oviposition processes of *T. pygidialis* and *T. vernalis* are lengthy, requiring a mean of 49 and 31.5 min, respectively, to complete. The behaviors performed by *Tiphia* spp. during the oviposition process would not be possible on an actively moving or defensive host.

The paralyzing stings of female *Tiphia* are delivered to the ventral side of the thoracic segments of their hosts, most likely affecting the thoracic ganglia of the central nervous system. Many other parasitoids that paralyze their hosts also sting in this location (Steiner 1986). Despite grubs already being paralyzed, wasps performed multiple stinging acts during the oviposition process with *T. vernalis* usually stinging the host more often than *T. pygidialis* (3.6 vs 2.9 acts; Fig. 3). These additional stings, lasting ≈30 s, were also placed in the ventral aspect of the thorax.

There are several plausible reasons for repeated stinging of already paralyzed hosts. First, such stinging may ensure that the effects of the wasp's venom do not dissipate before completion of oviposition. A grub that recovers from paralysis might pose a threat to the ovipositing female. *P. japonica* grubs are very active compared with those of *Cyclocephala* spp. (unpublished data). This could explain the higher number of stinging acts performed by *T. vernalis* than *T. pygidialis.* Stinging may also serve to further relax the host's muscles, making it easier to move the grub into place for oviposition. After the initial paralyzing sting, grubs removed from wasps before host kneading are relatively limp compared with nonstung grubs, suggesting that venom from the wasp's sting aids in relaxing the muscles of the host (M.E.R., unpublished data). Acts of repeated stinging performed once the grub is already paralyzed are preceded by host kneading, a behavior that most likely serves to make the grub flaccid and easier to move (see below). It is possible that during host kneading, grubs perceived by wasps as being more difficult to knead may be stung to

facilitate the kneading process by further relaxing the muscles of the host.

Repeated stinging by *Tiphia* spp. may also serve to alter the physiology of their host grubs. Parasitoid venom can cause behavioral or developmental changes in a host (Steiner 1986, Coudron and Brandt 1996, Weaver et al. 1997). Indeed, *P. japonica* and *Cyclocephala* spp. parasitized by their respective *Tiphia* sp. exhibit changes in behavior, due in part to venom from the wasp's sting, that may be advantageous to the developing parasitoid larva. The most obvious change is that parasitized grubs move deeper into the soil than nonparasitized grubs (Rogers et al. 2003).

During the oviposition process, both *Tiphia* species removed soil from around the grub, creating a cell in the soil, to facilitate the oviposition process. While *T. vernalis* performed more soil-moving acts than *T. pygidialis*, such observed differences may not exist under natural conditions. Grubs form a cell, slightly larger than their body, in the soil, where they feed on the roots of plants growing into the cell (Vittum et al. 1999). However, grubs placed into the soil-filled observation chambers in the laboratory were not given enough time to form such an earthen chamber. Therefore, *Tiphia* encountering grubs under natural conditions may not need to devote as much time to soil-moving behavior.

During the oviposition process, wasps were observed examining the host by walking in circles over and around the grub while antennating it and the surrounding soil. Brunson (1938) demonstrated that *T. popilliavora* can control the sex of its offspring, laying eggs that develop into male wasps on second-instar *P. japonica*, whereas eggs that develop into females are laid on third-instar hosts. While host examination acts were relatively brief, such behavior may be used to gauge the size of the host when determining the sex of offspring to allocate to a host. Other species of parasitoids have been shown to determine host size in similar manners (Sandlan 1979, Schmidt and Smith 1985). Additionally, because host examination always preceded either moving soil from around the host or host kneading, this behavior also most likely serves to evaluate the host's position relative to the surrounding soil during the process of moving it into a C shape before oviposition.

Adults of both *T. pygidialis* and *T. vernalis* were observed host feeding, a behavior previously documented for one other species of *Tiphia, T. popilliavora* (Clausen et al. 1927). Parasitoids host feed to gain proteins and other nutrients for egg development and/or to meet the metabolic needs of the adult female (Quicke 1997). In a related study, host feeding did not increase longevity of *T. pygidialis* (M.E.R. and D.A.P., unpublished data). It therefore most likely serves to nurture maturing eggs.

Host kneading preceded most other behaviors performed during the oviposition process. Host kneading by other species of parasitoids has been hypothesized to provide sensory information on host quality (i.e., instar of development), via differences in the chem-

ical composition of the cuticle (Luft 1996). While it is possible that host kneading by *Tiphia* spp. may also provide such information, our observations suggest that kneading causes the grub to become more flaccid, making it easier to move into a position suitable for oviposition. If that is correct, then it is reasonable to expect that wasps should spend more time kneading larger grubs than smaller ones. Indeed, *Cyclocephala* sp. grubs are about twice as large, by weight, as *P. japonica* grubs (Potter et al. 1996), and *T. pygidialis* spent more time kneading *Cyclocephala* sp. grubs than *T. vernalis* spent kneading *P. japonica* grubs. Females of *T. pygidialis* and *T. vernalis* are similar in size (mean body length = 1.1 ± 0.02 vs 1.2 ± 0.03 cm, respectively; $n = 20$).

Host-scraping behavior was by far the most time-consuming component of the oviposition process for both *Tiphia* species. Scraping or rubbing the host's integument could serve several purposes. One possibility is that the parasitoid could be marking the host to prevent superparasitism (Quicke 1997). This seems unlikely, however, for *Tiphia* spp. because conspecific females that encounter a grub containing an egg readily destroy that egg and then perform all six of the preovipositional behaviors and then lay their own egg (Clausen et al. 1932; M.E.R., unpublished data). Examination of cross sections of cuticle from parasitized grubs revealed extensive mechanical damage, in the form of grooves, to the outer layers of the cuticle where the egg was attached. These grooves were filled with an adhesive substance in the immediate area where the egg was attached, suggesting that the scraping behavior serves to roughen the cuticle, thereby increasing the surface area for the adhesive to anchor the egg to the host. The dimensions of the grooves were similar to the individual peg-like structures found on the ovipositor sheath that was extended during the scraping process, suggesting that these structures are used to prepare the surface of the cuticle for egg attachment. To our knowledge, this is the first study to suggest a role for the structures associated with the ovipositor sheath of a parasitoid (Quicke 1997). Further examination of the ultrastructure of these peg-like structures on the ovipositor sheath is necessary before any sensory function can be ruled out. Regardless of other possible roles of these pegs, host scraping using the ovipositor sheath, and the associated mechanical damage it causes to the grub cuticle, is most likely an adaptation by which *Tiphia* spp. can better secure an egg to their soil-dwelling hosts.

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