Behavioral Changes in Japanese Beetle and Masked Chafer Grubs (Coleoptera: Scarabaeidae) After Parasitism by Tiphiid Wasps (Hymenoptera: Tiphiidae)

M. E. ROGERS, T. J. COLE,[1, 2] S. B. RAMASWAMY,[2] AND D. A. POTTER[3]

Department of Entomology, S-225 Agricultural Science Building N, University of Kentucky, Lexington, KY 40546–0091

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ABSTRACT We studied effects of parasitism by two *Tiphia* species (Hymenoptera: Tiphiidae) on burrowing behavior of their respective scarabaeid hosts and tested the hypothesis that parasitoid alteration of host hormones accounts for the observed behavioral change. In turf field-plots, third-instar masked chafer grubs, *Cyclocephala* sp., parasitized by native *Tiphia pygidialis* Allen burrowed to depths of 12–16 cm within 21 d, whereas nonparasitized grubs remained in the upper 4 cm of soil. Grubs bearing an egg or early-instar *Tiphia* larva were found relatively close to the surface, whereas those with a fourth- or fifth-instar parasitoid were deeper. Experiments in soil-filled, vertical observation chambers confirmed downward movement by parasitized *Cyclocephala*, and similar response in third-instar Japanese beetle, *Popillia japonica* Newman, parasitized by *Tiphia vernalis* Rohwer. Removal of the *Tiphia* egg from masked chafers was followed by initial downward movement of grubs and then a gradual ascent, suggesting that host behavior is affected both by the venomous sting and feeding by the wasp larva. Significant but inconsistent differences were found in juvenile hormone-III (JH III) titers in hemolymph taken from nonparasitized grubs or grubs parasitized for 1 or 14 d. Topical application of JH III and methoprene acid resulted in grubs moving deeper into the soil, whereas injection of 20-hydroxyecdysone did not affect grub behavior. Failure to dig deeply enough in soil when sampling for parasitized scarab grubs or *Tiphia* cocoons likely will result in underestimation of parasitism rates.

KEY WORDS *Tiphia pygidialis*, *Tiphia vernalis*, *Popillia japonica*, *Cyclocephala* sp., Scarabaeidae, soil insects

PARASITISM OFTEN RESULTS in altered behavior and physiology of affected hosts (Moore and Gotelli 1990, Thompson and Kavaliers 1994, Adamo 1997). In insects, such changes may include increased or decreased food intake or movement, cessation or prolongation of feeding, stimulation or inhibition of wandering behavior, and accelerated or delayed development (Lawrence 1986, Thompson and Kavaliers 1994, Adamo 1997). These responses may be mediated by parasitism-induced hormonal changes, particularly in juvenile hormone and ecdysone levels (Beckage and Riddiford 1982, Bollenbacher 1988, Thompson and Kavaliers 1994). Parasitized individuals may also move to microhabitats where they normally would not be present, at least at that time. For example, parasitized individuals of the aphid *Macrosiphum euphorbiae* (Thomas) spend more time on adaxial leaf surfaces than on leaf undersides where nonparasitized counterparts are found (Brodeur and McNeil 1989). Similarly, *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae) infected with polyhedrosis virus move to the top of food plants, whereas noninfected larvae remain lower in the plant canopy (Goulson 1997). Awareness of such behavioral changes can be important when sampling insect populations to estimate parasitism rates or when assessing the impact of biological control.

Most studies concerning parasitism-induced changes in host behavior have dealt with endoparasitoids and caterpillars on plants. The current study examined such interactions for two species of root-feeding scarabaeid larvae (Coleoptera: Scarabaeidae) and their tiphiid ectoparasites in the soil.

Wasps of the genus *Tiphia* (Hymenoptera: Tiphiidae) are the primary parasitoids attacking scarabaeid larvae, or white grubs (Clausen 1940). More than 80 species of *Tiphia* occur in North America (Krombein et al. 1979). Two species commonly parasitize turf-infesting white grubs in Kentucky. *Tiphia pygidialis* Allen is a native species that attacks grubs of northern and southern masked chafers (*Cyclocephala borealis*

[1] Current address: Centre for Applied Entomology and Parasitology, School of Life Sciences, Keele University, Keele, Staffordshire ST5 5BG, United Kingdom.

[2] Department of Entomology, Kansas State University, Manhattan, KS 66506.

[3] E-mail: dapotter@uky.edu, to whom correspondence should be addressed.

Arrow and *Cyclocephala lurida* Bland, respectively) from August to October (Rogers and Potter 2002). *Tiphia vernalis* Rohwer, a native of Japan, was introduced into the Eastern United States during the 1920s for biological control of the Japanese beetle, *Popillia japonica* Newman (Fleming 1968). Active from May to early June, it often is locally abundant in central Kentucky wherever *P. japonica* grubs are found (M.E.R. and D.A.P., unpublished data).

Once in the soil, female *Tiphia* wasps find their respective hosts using species-specific kairomones present in grub body odor trails and in host frass (Rogers and Potter 2002). Both *T. pygidialis* and *T. vernalis* attack primarily third instars. Once a suitable grub is found, the wasp stings it, causing temporary paralysis. An egg is then laid externally on the host, in a species-specific location. *T. pygidialis* attaches its egg dorsally between the second and third thoracic segment of *Cyclocephala* spp., whereas *T. vernalis* attaches its egg ventrally between the third thoracic and first abdominal segment of *P. japonica.* Once the egg hatches, the wasp larva feeds externally on its host, devouring all but the sclerotized portions. The fifth-instar *Tiphia* then spins a cocoon in the soil in which it overwinters, emerging as an adult the following year.

Turf-infesting scarabaeid grubs burrow down, typically 10–20 cm deep, to pupate or overwinter, but they feed on roots just under the soil surface during other larval stages (Potter 1998). While sampling for *P. japonica* or *Cyclocephala* in spring or autumn, respectively (e.g., Rogers and Potter 2002), we observed that parasitized grubs tended to be found deeper in the soil than expected. We therefore tested the hypothesis that parasitism by *T. pygidialis* and *T. vernalis* induces premature downward burrowing of their respective hosts, and that alteration of host hormones after parasitism accounts for the observed behavioral change.

Materials and Methods

Collection and Handling of Parasitoids and Grubs. Female *T. pygidialis* and *T. vernalis* were collected from golf courses in central Kentucky. *Tiphia pygidialis* was 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, Gardena, CA) was used to collect wasps attracted to the sprays. *T. vernalis,* which feeds on honeydew secretions of aphids, scale insects, and other homopterans (Clausen and King 1927), was collected during May in a similar manner, except that sugar sprays were applied to the foliage of trees bordering areas of turf.

Host grubs were collected from beneath turf at local golf courses and sod farms. In Kentucky, both *C. lurida* and *C. borealis* are abundant. These species are closely related, and they even share the same sex pheromone (Haynes and Potter 1995). Because their larvae cannot be separated by known morphological characteristics, we refer to them as *Cyclocephala* spp. grubs. For tests with *T. pygidialis,* third-instar *Cyclocephala* spp. were collected in late August and September. For experiments with *T. vernalis,* postoverwintered third-instar *P. japonica* were collected during wasp flight in early May. Grubs were held in plastic containers (26.5 × 19.5 × 10 cm) containing a 1:1 mixture of autoclaved soil and peat moss at room temperature (22–24°C) until used in experiments. To obtain grubs bearing a parasitoid egg of known age, female wasps 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 10% sugar water was placed on the soil surface as food for the wasps. One third-instar host grub was introduced, and a lid was placed on the cup to prevent the wasp from escaping. Containers with wasps were held at room temperature and light regimen of 14:10 (L:D) h. Parasitized grubs containing a newly laid (≤1 d old) egg were removed from the containers after 24 h and used in the following experiments.

Depth of Parasitized Versus Nonparasitized *Cyclocephala* spp. Grubs in the Field. Twelve PVC rings (39.0 cm diameter × 10.2 cm height) were driven completely into Kentucky bluegrass, *Poa pratensis* L., turf at the University of Kentucky Spindletop research farm near Lexington. On 13 September 2001, each enclosure was provisioned with 10 nonparasitized third-instar *Cyclocephala* spp. and 10 parasitized third-instar *Cyclocephala* spp. bearing a *T. pygidialis* egg that had been laid within the past 24 h. Grubs were placed on the surface in the center of each ring and allowed to burrow into the turf. At 3, 7, 14, and 21 d after beginning the experiment, three rings were destructively sampled to a depth of 30 cm. Depth of all parasitized and nonparasitized grubs and the instar of the developing *Tiphia* larva present on parasitized grubs recovered at different depths were recorded. Instar determination was based on *T. pygidialis* head capsule measurements previously determined in a rearing study (M.E.R. and D.A.P., unpublished data). Depth of parasitized versus nonparasitized grubs was compared within sample dates by a two-sample *t*-test (Analytical Software 2000). Depths of grubs bearing a *Tiphia* egg or various instars was compared by one-way ANOVA (Analytical Software 2000) followed by means separation using Tukey's honestly significant difference (HSD) test (Analytical Software 2000).

Parasitism Effects on Behavior of *P. japonica* and *Cyclocephala* spp. Grubs in Soil Microcosms. The effect of the developing *Tiphia* larva on its host's behavior was also examined in the laboratory. Ten observation chambers resembling an ant "farm" were constructed to allow nondestructive, repeated measurements of movement by parasitized and nonparasitized grubs over time. The observation chambers consisted of two panes of Plexiglas (61 cm length × 30 cm height × 0.5 cm thick) separated at both ends by wooden dividers (1.8 cm width × 33 cm height × 1.2 cm thick). Each observation chamber was vertically subdivided into four separate, equal-sized compartments by placing three additional wooden dividers between the panes, 12.5 cm apart and from each end.

Holes were drilled through both the Plexiglas and wood dividers, and 5-cm-long bolts were passed through the holes and secured with wing nuts to hold the observation chamber together. Once the panes of Plexiglas were secured to the dividers, the chamber was attached to a wooden base (81 cm length × 3.7 cm height × 8.3 cm width) having five square holes (1.2 cm width × 1.8 cm length × 3.0 cm depth) positioned to line up with the wooden dividers, which extended 3 cm past the bottom of the chamber, to help secure the chamber to the base. Once assembled, each observation chamber was filled with autoclaved, moistened, sifted soil. As soil was being poured, it was gently tamped using a wooden dowel. Plugs of greenhouse-grown *P. pratensis* were placed into the top of the observation chambers and allowed to root to provide food for the grubs. Each compartment in the observation chambers was irrigated with 5 ml of water every 3 d to keep the grass alive while maintaining uniform soil moisture conditions.

In the first set of experiments, two parasitized grubs bearing a newly laid *Tiphia* egg and two nonparasitized grubs were introduced into the top of each of the 10 observation chambers. Each grub was placed into a separate compartment. The Plexiglas portion of each chamber was then wrapped with black velvet cloth to provide dark soil conditions. Chambers were placed into a growth chamber and held at a constant temperature of 22°C. Every 3 d over the next 21 d, the cloth was removed and the depth of each grub was measured. Separate experiments were conducted with *Cyclocephala* spp. (autumn 2000) and *P. japonica* grubs (spring 2001). Depth of grubs was analyzed for main effects of treatment (parasitized versus nonparasitized), date, and treatment by date interaction using univariate ANOVA for repeated measures (SAS Institute 1996); *t*-tests were used to make comparisons within each observation date (Analytical Software 2000).

Based on results of the aforementioned tests, another experiment was conducted to determine the extent downward movement of parasitized grubs resulted from venom from the wasp's sting as opposed to response to the externally feeding parasitoid larva. Parasitized and nonparasitized *Cyclocephala* spp. grubs were placed into the observation chambers as before except that the egg was removed from each parasitized grub. Depth of nonparasitized grubs versus parasitized grubs from which the egg had been removed was compared over the next 21 d as before. Repeated measures ANOVA was used to analyze differences in grub depth over time; *t*-tests were used to make comparisons within each observation date.

Hormone Analysis and Manipulations. We tested the hypothesis that parasitism by *Tiphia* results in altered juvenile hormone levels. Analyses were conducted with *Cyclocephala* spp. grubs collected in autumn 2000 and 2001 and with *P. japonica* grubs collected in spring 2001. Hemolymph was sampled from nonparasitized grubs and from hosts that had been parasitized by their respective *Tiphia* species for 1 or 14 d. Ten microliters of hemolymph were collected into micropipettes from the cut legs of each of 10 CO_2-anesthetized grubs at the same stage of parasitism and transferred to a chilled glass vial containing 250 μl acetonitrile and 250 μl of 2% $NaCl_2$. Samples were kept −80°C until analysis. Three replicates were obtained for each treatment. Titers of juvenile hormone were determined using gas chromatography-mass spectrometry (GC-MS) techniques as described in Shu et al. (1997) and compared by one-way ANOVA followed by means separation using Tukey's HSD test (Analytical Software 2000).

The hypothesis that juvenile hormone or 20-hydroxyecdysone titer might directly cause parasitized grubs to move deeper into the soil was investigated using the previously described observation chambers. In one set of experiments, juvenile hormone-III (JH III; 96% purity; Sigma-Aldrich, St. Louis, MO) (1 μg per grub in 10 μl acetone) was applied topically to the dorsal surface of the thorax of 12, third-instar *Cyclocephala* spp., which were then placed into the observation chambers. Additional sets of grubs receiving only 10 μl acetone or no treatment were also placed into separate compartments of the observation chambers. Grub depths were measured 6, 12, 24, 48, 72, and 96 h after treatment. A similar experiment was conducted using methoprene acid (98% purity; Sigma-Aldrich), an analog of JH III that is not degraded as rapidly in vivo as JH III. Methoprene acid (1 μg in 10 μl acetone) was applied topically to 12 third-instar *Cyclocephala* spp. grubs. A second group of grubs was treated with 10 μl of acetone only, and a third group received no treatment.

The alternative hypothesis that elevated ecdysone levels are associated with the downward movement of parasitized grubs was investigated by injecting third instar *Cyclocephala* spp. with 1 μg of 96% pure 20-hydroxyecdyone (Sigma-Aldrich) in 10 μl of 0.01 M phosphate-buffered saline solution. Injections were used because 20-hydroxyecdysone does not readily transverse the insect cuticle. Three sets of controls, including grubs injected with 10 μl of phosphate-buffered saline solution, grubs receiving only a needle prick, and untreated grubs were also placed into the observation chambers. Grub depths were measured at 6, 12, 24, 48, 72, and 96 h. Within each of the above experiments, repeated measures ANOVA was used to compare depth of grubs across all observation periods, as before. Data also were analyzed by one-way ANOVA within each observation period, followed by a one-tailed Dunnett's procedure (Steel and Torrie 1960) to compare depths of treated grubs against the controls.

Results

Depth of Parasitized Versus Nonparasitized *Cyclocephala* spp. Grubs in the Field. *Cyclocephala* spp. grubs parasitized by *T. pygidialis* moved significantly deeper into the soil than did nonparasitized grubs (Fig. 1). There were also significant differences in grub depth based on the instar of *Tiphia* present on a host ($F = 44.1$; df = 5, 85; $P < 0.01$). Grubs bearing

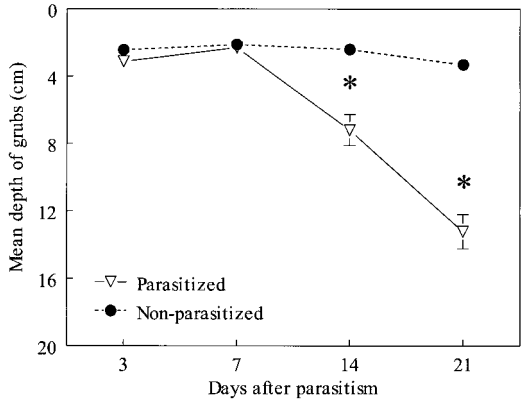

Fig. 1. Location of *Cyclocephala* spp. newly parasitized by *Tiphia pygidialis*, compared with nonparasitized grubs, after introduction into turfgrass field plots. Values are means ± SE for 60 grubs per sample date. Some SEs were too small to be depicted graphically. Means marked with an asterisk are significantly different (two-sample *t*-test, $P < 0.05$).

third-, fourth-, or fifth-instar parasitoid larvae were found significantly deeper in the soil than were parasitized grubs bearing an egg or early-instar *Tiphia* (Tukey's HSD; $P < 0.005$, Fig. 2).

Parasitism Effects on Behavior of *P. japonica* and *Cyclocephala* spp. Grubs in Soil Microcosms. Nonparasitized grubs of both species remained in the root zone near the top of the observation chambers, whereas grubs parasitized by their respective *Tiphia* species burrowed deeper into the soil (Fig. 3). For *Cyclocephala* spp. grubs parasitized by *T. pygidialis*, there were significant main effects for parasitism ($F = 56.0$; df = 1, 36; $P < 0.0001$), time ($F = 7.94$; df = 6, 216; $P < 0.0001$), and their interaction ($F = 10.2$; df = 6, 216; $P < 0.0001$). A similar phenomenon occurred with *P. japonica* grubs parasitized by *T. vernalis*. Depth of those grubs was significantly affected by parasitism ($F = 10.8$; df = 1, 29; $P < 0.01$) and time ($F = 12.4$; df = 6, 174; $P < 0.0001$), with significant interaction ($F = 8.06$; df = 6, 174; $P < 0.0001$). For each species, the difference in depth of parasitized versus nonparasitized grubs was also significant within each sample date (*t*-tests, $P < 0.05$). Notably, this included the 3-d observation at which time *Tiphia* eggs, not larvae, were present on parasitized hosts.

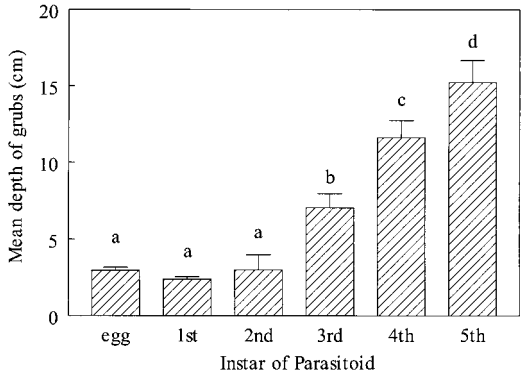

Fig. 2. Depth (mean ± SE) in the soil under turfgrass field plots where *Cyclocephala* spp. grubs bearing each of five *Tiphia pygidialis* larval instars were found ($n = 29$ for grubs with eggs; $n = 22$, 4, 18, 11, and 16 for grubs with first to fifth instar *Tiphia*, respectively). Means with the same letter do not differ significantly (Tukey's HSD, $P > 0.05$).

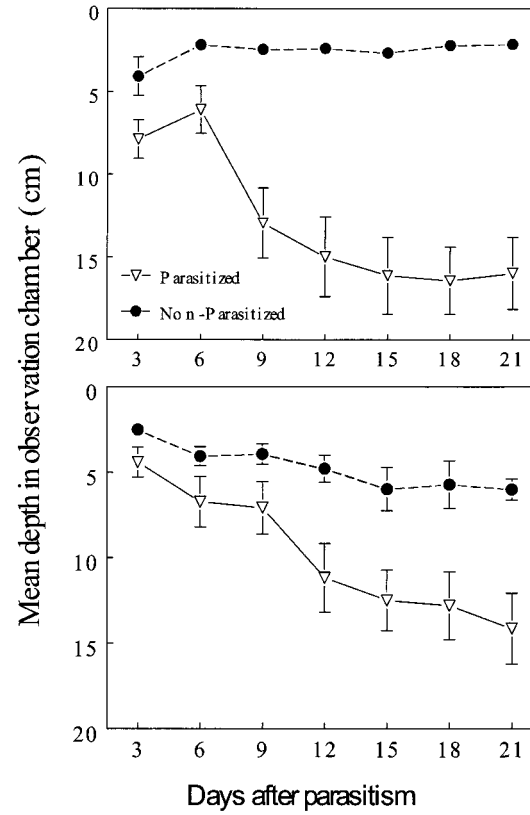

Fig. 3. Location of grubs placed into observation chambers after being parasitized by *Tiphia* compared with nonparasitized grubs. (Top) *Cyclocephala* spp. parasitized by *T. pygidialis*. (Bottom) *Popillia japonica* parasitized by *T. vernalis*. Values are means ± SE for 40 grubs per date. Some SEs were too small to be depicted graphically.

When the *Tiphia* egg was removed from parasitized *Cyclocephala* spp. before the grubs were placed in the observation chambers, there was no overall difference over time between depths of grubs from which eggs had been removed versus nonparasitized hosts ($F = 3.31$; df = 1, 30; $P > 0.05$, ANOVA for repeated measures, Fig. 4). Grubs from which the parasitoid egg had been removed were, however, significantly deeper in the soil at the first observation period, 3 d after parasitism (Fig. 4).

Hormone Analysis and Manipulations. Hemolymph JH III titers were 10-fold higher in *P. japonica* grubs on which *T. vernalis* had recently oviposited than in nonparasitized grubs ($F = 12.5$, df = 2, 6; $P < 0.01$; Table

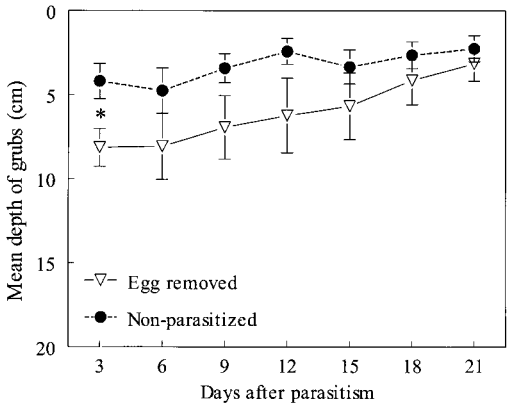

Fig. 4. Depth in observation chambers of nonparasitized *Cyclocephala* spp. grubs, compared with grubs parasitized by *Tiphia pygidialis*, but from which the parasitoid egg was removed. Values are means ± SE for 40 grubs per date. Means marked with an asterisk are significantly deeper than the control (*t*-test, $P < 0.05$).

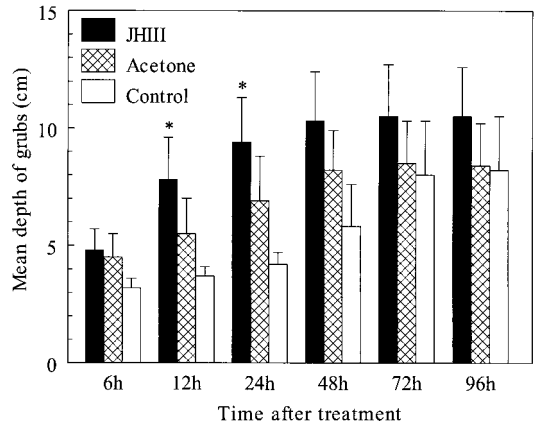

Fig. 5. Depth in observation chambers of nonparasitized *Cyclocephala* spp. grubs treated with topical applications of JH-III, acetone, or no treatment. Values are means ± SE for 36 grubs per observation. Means marked with an asterisk are significantly different than the control (Dunnett test, $P < 0.05$).

1). The difference had disappeared, however, by 14 d after parasitism. *Cyclocephala* spp. also showed some fluctuations in JH III titer after parasitism by *T. pygidialis* in 2000 and when the test was repeated in 2001 ($F = 5.72, 6.74$, respectively; df = 2, 6; $P < 0.05$; Table 1). The pattern, however, was not consistent between the 2 yr.

There was no significant overall difference in depth of JH III–treated, acetone-treated, and nontreated grubs when analyzed across observation periods (ANOVA for repeated measures; $F = 0.16$; df = 3, 35; $P > 0.05$; Fig. 5). Grubs tended to burrow deeper over time ($F = 7.80$; df = 5, 175; $P < 0.001$), but there was no treatment by time interaction ($F = 0.72$; df = 15, 175; $P > 0.05$). There were, however, trends for significant treatment effects after 12 and 24 h ($F = 2.28, 2.76$; df = 2, 33; $P = 0.116, 0.076$, respectively). Grubs treated with JH III were significantly deeper than untreated grubs at the 12- and 24-h observations (one-tailed Dunnett's test; $P < 0.05$).

Similarly, topical application of methoprene acid resulted in significant, initial downward movement of treated grubs at 12 and 24 h. Treatment effects were significant at both times ($F = 5.44, 4.45$; df = 2, 33; $P = 0.009, 0.019$, respectively), with methoprene-treated grubs deeper in the soil than the controls (one-tailed Dunnett's test, $P < 0.05$; Fig. 6). This response waned within 48 h, however, so that there was no experiment-wide difference in depth of methoprene-treated grubs compared with acetone-treated or control grubs (ANOVA for repeated measures; $F = 2.10$; df = 2, 37; $P > 0.05$). The time effect again was significant ($F = 17.1$; df = 5, 185; $P < 0.0001$), but there was no treatment by time interaction ($F = 0.64$; df = 10, 185; $P > 0.05$). There were no significant differences in depth between ecdysone-injected, saline-injected, needle-pricked, or normal grubs (ANOVA for repeated measures; $F = 0.12$; df = 3, 35; $P > 0.05$).

Table 1. JH III titers in hemolymph collected from nonparasitized *Popillia japonica* and *Cyclocephala* sp. grubs, and in hemolymph from grubs of those species parasitized by their respective *Tiphia* species for 1 or 14 d. Data are mean ± SE nanograms JH III per 100 μl hemolymph

Stage of parasitism	*Popillia japonica*	*Cyclocephala* sp.	
	Spring 2001	Autumn 2000	Autumn 2001
Non-parasitized	109.3 ± 9.7a	419.7 ± 87.6ab	98.0 ± 14.3a
1 d	1161.8 ± 334.5b	700.5 ± 187.7a	169.7 ± 36.9ab
14 d	212.6 ± 258.9a	126.8 ± 17.3b	353.3 ± 67.5b

Within columns, means followed by the same letter are not significantly different (Tukey's HSD; $P > 0.05$).

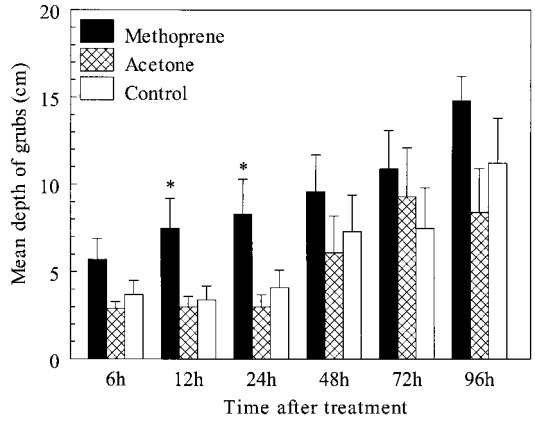

Fig. 6. Depth in observation chambers of nonparasitized *Cyclocephala* spp. grubs treated with topical applications of methoprene, acetone, or no treatment. Values are means ± SE for 36 grubs per observation. Means marked with an asterisk are significantly different than the control (Dunnett test, $P < 0.05$).

Discussion

Our results confirm that grubs parasitized by *Tiphia* spp. are likely to be found deeper in the soil than are nonparasitized grubs and that this phenomenon results from a behavioral change in the host, rather than physical actions by the wasp or its larva. There was significant downward movement of newly parasitized grubs in the observation chambers within 3 d. This is noteworthy because, soon after parasitism, most (80%) of the *Tiphia* eggs had not yet hatched. The initial downward movement of *Cyclocephala* spp. from which the egg was removed, followed by their gradual return to the upper soil, suggests that the burrowing response may be induced by venom injected by the wasp during oviposition and then maintained by feeding and possibly oral secretions by the developing *Tiphia* larva. Absence of similar response to a needle prick (final experiment of hormone manipulations) implies that wounding is probably not the causal agent. Other studies, mainly with parasitoids of caterpillars, have shown that venom from the wasp sting, salivary secretions from ectoparasitic larvae, or both can cause behavioral or developmental changes in a host (Coudron and Brandt 1996, Marris et al. 1996, Doury et al. 1997, Weaver et al. 1997).

Differences in depth of parasitized and nonparasitized *Cyclocephala* sp. grubs were not detected in the field experiment until the third sampling period, 14 d after parasitism, by which time, third-instar *Tiphia* were present on the hosts. One reason for this may be that the soil was much more compacted in the turf plots than in the observation chambers, within which the soil was only lightly tamped with a wooden dowel. Parasitized grubs may take longer to burrow into relatively more compacted soil. Additionally, in the observation chambers, grubs were sandwiched between two panes of Plexiglas, allowing only vertical movements. In the field, initial agitation or other change in grub movement caused by the wasp's sting or venom may result in some lateral movements, as well as moving deeper into the soil. Effect of parasitism of *P. japonica* by *T. vernalis* was not evaluated in the field, but experiments in soil microcosms in the laboratory and observations of parasitized *P. japonica* in the soil during other studies (M.E.R. and D.A.P., unpublished data) suggest a change in behavior similar to that of parasitized *Cyclocephala* grubs.

Induction of downward movement of hosts may be beneficial to *Tiphia* spp. because wasps that encounter a grub bearing an egg laid by a conspecific female will destroy that egg before laying their own (Clausen et al. 1932; M.E.R. and D.A.P., unpublished data). Wasps encountering a host with a *Tiphia* larva, however, will abandon that grub with no attempt to oviposit. Because grubs are patchily distributed (Dalthorp et al. 2000) and parasitism by *Tiphia* spp. tends to be density-dependent (M.E.R. and D.A.P., unpublished data), induction of downward movement by parasitoid oviposition probably would reduce the chance of superparasitism. This behavioral response may also reduce vulnerability of the parasitoid larvae or pupae to other natural enemies. While the invertebrate natural enemies of *Tiphia* spp. have not been studied, they reportedly include certain sphecid, mutillid, and perilampid wasps, rhipiphorid beetles, and bombyliid flies (Clausen et al. 1932). Vertebrate predators such as skunks and raccoons may also have an impact on larval *Tiphia* spp. These mammals often dig up and consume large numbers of scarab larvae that are feeding just below the surface in turf or pasture (Rivers 1977, Potter 1998). In other systems, changes in host behavior may benefit the parasitoid by reducing risks of hyperparasitism (Stamp 1981, Fritz 1982, Brodeur and McNeil 1989), predation (Fritz 1982), or exposure to cold temperatures (Brodeur and McNeil 1989, Muller 1994).

An alternative hypothesis is that the grub's downward movement represents a defensive response to parasitism. At each molt, the larval *Tiphia* releases its mouthparts, moves forward to free itself from the old exuvium, and then reanchors its mouthparts in the host. Movement of parasitized grubs through the soil sometimes dislodges the egg (Rivers 1977) or the developing ectoparasitic larva, especially during the early instars (Clausen and King 1927). This was not a factor in the observation chambers but we have observed it while rearing *Tiphia* spp. in the laboratory (e.g., Rogers and Potter 2002).

Many scarab larvae overwinter as third instars at depths of 5–25 cm to avoid freezing (McColloch et al. 1928, Hoshikawa et al. 1988). Grubs may also burrow downward in response to heat or drought (Villani and Nyrop 1991). Early observations of *Tiphia* cocoons deep in the soil (McColloch et al. 1928) were attributed to the wasps having oviposited after the grubs had begun their normal descent to overwintering or pupation depth (McColloch et al. 1928, Clausen 1940). The physiological mechanism triggering grubs to burrow deeper for overwintering has not been investigated but is likely a response to declining temperatures (Villani and Nyrop 1991). Possibly, parasitism by *Tiphia* spp. induces the same physiological state of stress, resulting in a similar response. The depths at which hosts with late-instar *T. pygidialis* and resulting cocoons were found in our field study are similar to those at which *Cyclocephala* spp. grubs overwinter in Kentucky. The host's downward movement places the legless *Tiphia* larva deeper in the soil so that when the cocoon is formed, the univoltine parasitoid will overwinter and complete its development at a depth that buffers it from adverse environmental conditions.

Parasitism-induced changes in host physiology and endocrinology are well documented for endoparasitoids (Beckage 1985, Lawrence and Lanzrein 1993, Beckage and Gelman 2001) but have been less commonly reported for ectoparasitoids (Marris et al. 2001). Some ectoparasitoids must also regulate host development to avoid being shed during ecdysis; such manipulation of the endocrine system serves to arrest or slow down host development (Marris et al. 2001). While *T. pygidialis* and *T. vernalis* parasitize third (final)-instar grubs, we nonetheless found significant changes in JH III titers of both *P. japonica* and *Cyclo-*

cephala spp. after parasitism by their respective *Tiphia* species. The pattern differed for the two species, however, and it also was inconsistent when the test with *Cyclocephala* was repeated the following year. The reason for this is not clear. Injection of 20-hydroxyecdysone into third-instar *Cyclocephala* spp. did not alter grub movement patterns. *Cyclocephala* grubs topically treated with JH III and methoprene acid showed only a short-term response, moving deeper into the soil within 12 h. This transitory effect may reflect an indirect action of JH III and methoprene. Further work is needed to clarify whether the parasitism-induced changes in downward movement are mediated through alteration in JH titers. For example, Cole et al. (2002) demonstrated that the alteration in JH titers in parasitized caterpillars is the result of both changes in JH synthesis, release, and degradation in the host and synthesis and release by the parasitoid itself. Similarly, other modulators of neural function and behavior, including biogenic amines and neuropeptides, may play a more direct role. In endoparasitic relationships, several neuropeptides are known to accumulate in the parasitized host (Zitnan et al. 1995), but a paucity of information prevents us from drawing a similar conclusion in ectoparasitoids.

The altered behavior of parasitized grubs should be considered when surveying for the occurrence of *Tiphia* spp. or when sampling to estimate parasitism rates. Early in the seasonal *Tiphia* wasp flight, parasitized grubs bearing eggs or early-instar larvae can be found in the upper 5 cm of soil. However, once *Tiphia* larvae have reached the third instar, parasitized hosts will have moved deeper into the soil than nonparasitized grubs. Because estimates of grub parasitism (e.g., Cappaert and Smitley 2002) are often done by sampling for parasitized grubs and cocoons toward the end of wasp flight or after it has ended, the soil should be examined down to 20 cm or deeper, depending on compaction. Failure to dig deep enough when searching for parasitized grubs or *Tiphia* cocoons will result in underestimation of grub parasitism rates.

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