Oviposition Behavior of *Pheropsophus aequinoctialis* L. (Coleoptera: Carabidae): A Natural Enemy of *Scapteriscus* Mole Crickets (Orthoptera: Gryllotalpidae)

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In the southeastern United States, South American Scapteriscus *mole crickets are serious pests of turf and pasture grasses and vegetable seedlings. The larval stage of* Pheropsophus aequinoctialis *L. is a specialist predator of* Scapteriscus *mole cricket eggs and is currently under evaluation as a potential biological control agent. The objective of this study was to understand the oviposition behavior of* P. aequinoctialis. *The results indicated that in two-choice substrate oviposition arenas, female* P. aequinoctialis *significantly preferred to lay eggs in sand with mole cricket tunnels compared with artificially created tunnels or sand without tunnels. Physical tunnel presence influenced oviposition depth, but was not the only factor influencing oviposition. The reproductive strategy and behavior of* P. aequinoctialis *is discussed in relation to its specialized life history and to other carabid beetles displaying close host associations.*

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INTRODUCTION

The oviposition site is an important determinant of larval success for most ground beetles and many other holometabolous insects because of limited mobility and the unprotected nature of the larval stage (Thiele, 1977; Lövei and Sunderland, 1996). Compared to generalist feeders, the oviposition site of specialist predators and parasitoids is one of the most important factors influencing larval development because their host range is so restricted. In these species, host-oriented searching behavior by the adults may have evolved to increase the chances for successful larval development. The occurrence of host searching behavior in combination with learning is a common behavioral trait of many specialist hymenopterous parasitoids and has been studied extensively due to its relevance to control and interest of biological control specialists and interest to ecologists. Weseloh (1981) and Vinson (1984) summarized the stepwise fashion of host detection by parasitoid wasps and determined that host finding behavior generally proceeds through habitat location, host location within a habitat, and host acceptance. Orientation behavior toward an intended host involves the detection of a combination of chemical, visual, and acoustic stimuli in a hierarchal fashion, where olfaction usually precedes and stimulates further visual detection (Weseloh, 1981). The final steps involved in host location entail the detection of short-range chemical cues, usually emitted by the host, which elicit intensive searching behavior leading to the final discovery of the host.

Erwin (1979) reported three tribes within the Carabidae which exhibit specialist ectoparasitoid or predatory behavior in at least one life stage, typically the larval stage. Acknowledged examples occur in the tribes Peleciini (Salt, 1928; Liebherr and Ball, 1990), Lebiini (Silvestri, 1905; Chaboussou, 1939; Lindroth, 1954; Erwin and Erwin, 1976), and Brachinini (Wickham, 1894; Dimmock and Knab, 1904; Habu and Sadanaga, 1965; Erwin, 1967, 1970; Juliano, 1984, 1985). Within these tribes, no thorough investigations have directly considered host detection and oviposition behavior; only *in situ* observational accounts have been recorded. A species of South American bombardier beetle, *Pheropsophus aequinoctialis* L. (Coleoptera: Carabidae: Brachinini), is currently under evaluation for its potential to control *Scapteriscus* mole crickets, which are pests of turf and pasture grasses and vegetable seedlings in the southeastern United States. Previous observations on the life history traits of *P. aequinoctialis* suggest that the larval stage is a specialist predator of *Scapteriscus* mole cricket eggs (JHF, unpublished data), but oviposition behavior is poorly understood. Release and further establishment of *P. aequinoctialis* could potentially provide a substantial reduction in the number of emerging nymphs and

ultimately lower costly pesticide applications intended to control this life stage. In addition, *P. aequinoctialis* could supplement other biological control agents introduced since 1980 (Frank and Parkman, 1999).

Scapteriscus mole crickets spend most of their lives in underground horizontal tunnel networks. Off from these tunnels females dig vertical tunnels to deposit an egg clutch 9–30 cm underground in small, ovoid chambers (Forrest, 1985). Due to the buried and inconspicuous nature of the egg chamber and the necessity of mole cricket eggs to *P. aequinoctialis* larvae, we suggest that directed adult searching and oviposition behavior may have developed in *P. aequinoctialis* females to increase larval survival. In their oviposition work with *Carabus clatratus* L., Huk and Kühne (1999) suggested that the adaptation of directed oviposition behavior should only evolve when there is a detrimental effect on offspring fitness in the absence of such behavior. *P. aequinoctialis* larvae develop through hypermetamorphosis, where first instars are mobile triungulins and succeeding instars stationary. The high mobility of the first instar is an adaptation for searching, but due to their small size and the nature of their host, adult oviposition site is still likely to be important to their survival. Lake (2000) tested the attraction of gravid females to mole cricket eggs in a Y-tube olfactometer, but failed to determine a response and neglected to test first instars. There is a possibility that mole cricket tunnels signify to female beetles that mole cricket eggs are in the vicinity and beetles might lay their eggs in or around these tunnels. Newly emerged triungulins may then respond to a kairomone emitted from the eggs. As stated by Weseloh (1981) for parasitoid wasps, the final host location step typically is influenced by short-range chemical cues. In the case of *P. aequinoctialis* adults, these short-range cues may be semiochemicals associated with mole cricket-excavated tunnels.

The objective of this study was to determine whether the presence of tunnels influenced beetle oviposition and to determine whether female beetles directed oviposition toward mole cricket tunnels. The observations from this study, combined with others regarding the reproductive ability of *P. aequinoctialis*, are discussed in relation to its specialized larval habits.

MATERIALS AND METHODS

Insect Culture

The shortwinged mole cricket, *Scapteriscus abbreviatus* Scudder, with original stock from southeastern Florida, has been maintained in continuous culture in the Entomology and Nematology Department of the University of Florida since the 1980s (S. A. Wineriter and JHF, unpublished

data). A constant supply of eggs is necessary to maintain a stock of the bombardier beetle *Pheropsophus aequinoctialis*, which was obtained from Bolivia in the early 1990s and is cultured under quarantine conditions (JHF and R. C. Hemenway, unpublished data) because a permit has not yet been issued for its release pending completion of host-specificity trials. The work described here was one aspect of research into the host-specificity of the beetle.

Oviposition Arenas

In this study, gravid *P. aequinoctialis* were released into two-choice oviposition arenas with sand containing tunnels created by female *S. abbreviatus*, sand with artificially created tunnels, and sand without tunnels. Ovipositional substrates were constructed in sand held within Plexiglas® sandwiches. The sandwiches were constructed out of two 30 cm × 30 cm × 0.5 cm Plexiglas® sheets. Three Plexiglas® strips, each measuring 1 cm × 29.5 cm × 0.5 cm, were hot glued around three edges of one sheet so that when the sheets were combined to make the sandwich, there was a width of 1 cm separating the sheets with the top edge still open (but closed with a strip of Plexiglas® during each test). Plexiglas® sheets were combined with four, 5 cm binder clips placed at each corner of the sandwich. The total volume within the sandwich measured 29.5 cm × 29 cm × 1 cm or 855.5 cm^3. The oviposition arena was created by adding a volume of 696 cm^3 (24 cm × 29 cm × 1 cm) of moist, autoclaved builder's sand into the sandwich so the final height of sand equaled 24 cm. This height of sand was chosen so that after oviposition the sand could be horizontally partitioned into six, 4 cm intervals to determine the depth at which eggs were deposited. Black construction paper, measuring 24.5 cm × 30 cm, was attached to the outside of the sandwich and held at the sand surface by the clips. The paper was used to darken the oviposition arena, to simulate being underground when the mole cricket or beetle burrowed into the sand.

Three separate two-choice oviposition arenas were created as follows. One arena was created with half mole cricket tunnels (MCT) and half no tunnels (NT) (Fig. 1A), another with half MCT and half artificial tunnels (AT) (Fig. 1B), and finally with half AT and half NT (Fig. 1C). The MCT treatment was created as follows. After the sand was added into each sandwich and with the sandwich still open, a 1 cm × 29.5 cm × 0.5 cm vertical strip of sand was removed from the middle to split the arena in half to create two treatments and to make room for a Plexiglas® barrier. A Plexiglas® barrier (1 cm × 29.5 cm × 0.5 cm) was placed into the open area and after closing the sandwich, with black construction paper on the outside, a mated
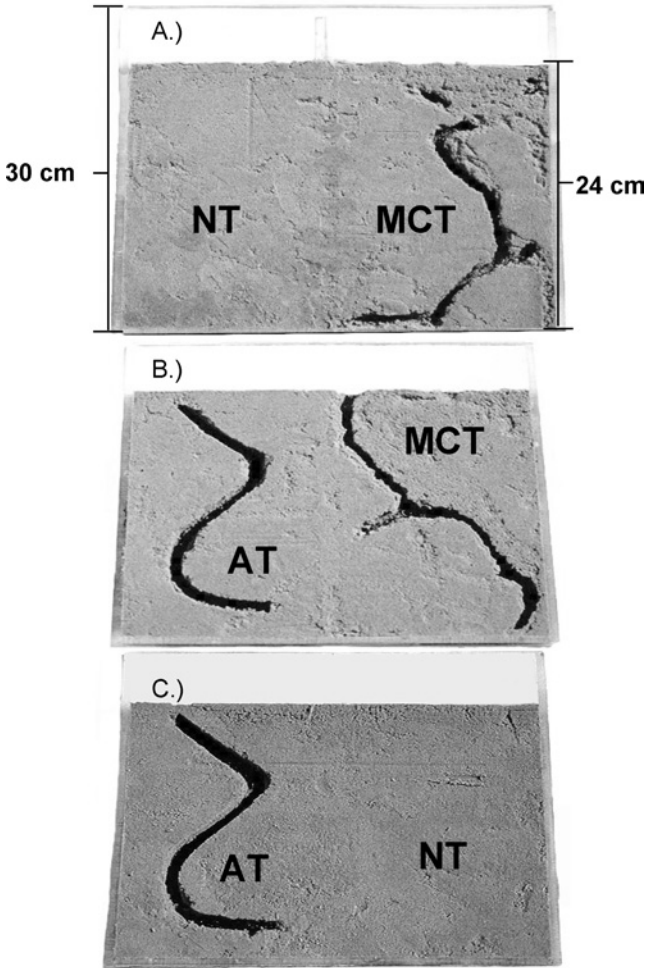
Fig. 1. Choice oviposition arenas with two substrates used in this study. A) Mole cricket tunnel (MCT) versus no tunnel (NT), B) MCT versus artificial tunnel (AT), and C) AT versus NT.

7–10-month-old female *S. abbreviatus* was placed into one-half of the arena. The mole cricket was allowed to create tunnels for 4 days. The sand displaced by the mole cricket was continuously removed throughout the 4 day period so the sand depth was kept at 24 cm. After the fourth day, the sandwich was opened and the mole cricket was carefully removed and detailed drawings of the tunnels were completed. The barrier was removed and

replaced with moist, autoclaved sand to complete the oviposition arena. The sandwich was closed with black construction paper in place, oriented vertically, and ready for the female beetle.

The AT treatment was created to simulate the structure of a mole cricket tunnel, but devoid of any potential chemical cues which might be left by female mole crickets while excavating their tunnels. The dimensions of the artificial tunnel were created from computing the average total depth, average number of turns, and average starting depth of 10 mole cricket tunnels previously created for the MCT treatment. The artificial tunnel was 1 cm wide throughout its length, started 1 cm down from the top of the sand, contained two turns, and ended at a depth of 22 cm. From these measurements, a cardboard tunnel template was created so that tunnels had similar dimensions in each replicate. The top left-hand corner of the tunnel template was placed 1 cm below the sand surface and 3 cm in from the left-hand side of the sand edge. The tunnel was created in the sand by outlining the cardboard template with a sterilized knife and removing the sand with a sterilized metal spatula.

P. aequinoctialis Oviposition

Female beetles used in this experiment were between 3 months and 1-year old, obtained from a research colony, and presumably gravid. Previous fecundity observations on this colony confirmed that females which were continuously kept with males in 236.6 mL (8 oz) plastic delicatessen cups were gravid (Weed, 2003). It was also determined that a large variation existed in fecundity between individuals, and on average females began laying eggs within 1 month of adult emergence. Peak mean egg laying for 10 females occurred 16 weeks after oviposition started (Weed, 2003).

For any particular oviposition choice arena, one female was randomly selected from our research colony and after the oviposition period the female was removed from the colony so it was not selected again. Each female was placed into the middle of a vertically oriented Plexiglas® sandwich and the direction each faced was alternated between replications. The top of the sandwich was covered with another Plexiglas® strip (1 cm × 29.5 cm × 0.5 cm) to prevent the beetle from escaping and to hold in moisture. All sandwiches were held vertically and placed so that the broad side of the sandwich was facing the light source in a Florida Reach-In unit (Walker *et al.*, 1993) kept at 27°C, 10:14 (L:D) h, and 38–54% relative humidity. Females were allowed to oviposit for 4 days and were carefully removed after the oviposition period with forceps to not disrupt the arena.

Sampling Oviposition Arenas

After the oviposition period, the sandwiches were opened and the sand was split in half vertically with a sterilized knife to separate the treatments. This left each treatment with a volume of 348 cm^3 of sand. The entire arena of sand was horizontally divided into six, 4 cm intervals giving depth intervals of 0–4, 4–8, 8–12, 12–16, 16–20, and 20–24 cm. Twelve individual samples were obtained in each choice arena. Each depth-interval was scraped into a 236.6 mL (8 oz) plastic delicatessen cup that was labeled with the appropriate depth interval and treatment information.

Egg Extraction

Eggs were extracted from sand by a method developed by Jenkins (1964) to extract nematodes from soil. This method was later modified by Lake (2000) to extract *P. aequinoctialis* eggs from sand, which did so with 98% efficiency. Each depth-interval sample was placed into a 354.9 mL (12 oz) plastic cup. Into each cup was added a 100 mL quantity of a sugar solution, created by combining 300 g of table sugar with 1 L tap water. The contents were stirred continuously until the sand was suspended in the solution and the mixture was left to settle for 5 min. The settled solution with floating eggs was poured off into a 9 cm petri dish and eggs were moved into tap water using a dropper. To make sure all of the eggs were extracted, the sand in the cup was washed a second time with tap water and this mixture was poured into the 9 cm petri dish. Eggs were removed and counted.

Ten arenas were set up for all three oviposition substrates (MCT vs. NT, MCT vs. AT, and AT vs. NT) and one female was allowed to oviposit in each arena. Oviposition in the MCT versus NT arenas was completed during August–October 2002 to obtain tunnel dimensions for the AT and in the MCT versus AT and AT versus NT arenas during November 2002 to February 2003. The mean number of eggs was computed for each depth interval within each treatment. These data were transformed as necessary to fit the normality assumption. Data obtained in the MCT and NT experiment were transformed to a power of 0.15, data from the MCT and AT experiment were transformed to a power of 0.2, and finally data from the AT versus NT experiment were transformed using a logarithmic transformation. A split plot analysis was performed on all three experiments with the SAS PROC MIXED procedure (SAS Institute Inc., 2000) with replication and replication × treatment as the random effects and treatment, depth, and treatment × depth as the fixed effects. In the analysis of the AT and NT experiment, the number of eggs counted from the 0 to 4 cm

interval was compared to all eggs counted in the 4–24 cm depth intervals. This method of comparison was chosen because of the 120 observations in this experiment, 88 were zeroes with no eggs being deposited below 8 cm in the NT treatment. Of the nonzero observations, the majority were in the 0–4 cm depth interval, making it appropriate to compare the eggs counted in this interval to the sum of the remaining intervals. For each experiment, least squares means procedures were performed for mean separation of the main effects. Statistical significance was determined at the $\alpha = 0.05$ level.

RESULTS

MCT Versus NT

Significantly more eggs were laid in the MCT treatment (836 eggs or 78%) than in the NT treatment (238 eggs or 22%) ($F = 13.83$; $df = 1, 9$; $P = 0.0048$) and depth had a significant effect on the number of eggs laid ($F = 10.12$; $df = 5, 90$; $P < 0.0001$). When oviposition was pooled over both treatments, 31.2% of the eggs were laid within the 0–4 cm depth interval; 50% were laid within the top 8 cm (Fig. 2A). Almost 93% of the eggs counted in the NT treatment were laid within the top 8 cm. Only 38.6% of the eggs in the MCT treatment were counted from the top 8 cm (Fig. 2A). In the two depth intervals ranging from 16 to 24 cm, 314 eggs were counted from the MCT treatment compared to 16 in the NT treatment. Despite these numerical differences, the treatment × depth interaction was not significant ($F = 1.24$; $df = 5, 90$; $P = 0.2992$). Further analysis by the least squares means procedure on the transformed data reported a similar mean number of eggs in the 0–4 and 4–8 cm depth intervals of the MCT treatment, but significantly more eggs were laid in the 0–4 cm depth interval than in four depth intervals ranging from 8 to 24 cm of this treatment (Table I). In the NT treatment, more eggs were laid in 0–4 cm than in all other depth intervals (4–24 cm) and more eggs were laid in the 4–8 cm depth interval than in the remaining four intervals ranging from 8 to 24 cm (Table I).

MCT Versus AT

Significantly higher numbers of eggs were laid in the MCT treatment (663 eggs or 77.5%) than in the AT treatment (193 eggs or 22.5%) ($F = 11.98$; $df = 1, 9$; $P = 0.0071$). In addition, the depth had a significant effect on the number of eggs laid ($F = 9.50$; $df = 5, 90$; $P < 0.0001$). Almost 47% of the eggs were laid within the top 4 cm of both treatments (Fig. 2B).
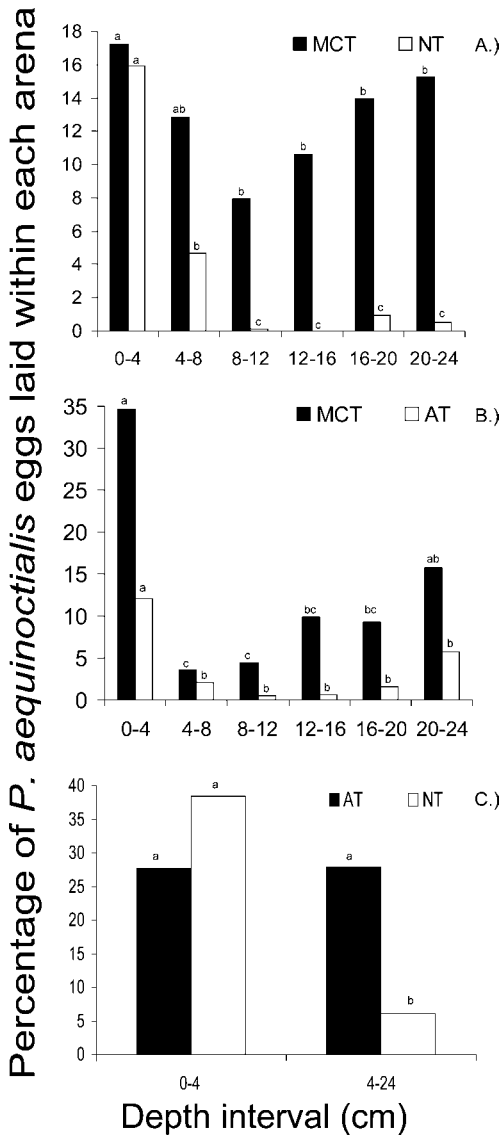
Fig. 2. Percentage of eggs laid within each depth interval and treatment for each arena. A) Mole Cricket Tunnel (MCT) versus No Tunnel (NT), B) MCT versus Artificial Tunnel (AT), and C) AT versus NT. Lower case letters indicate results of least squares means test on transformed data within each treatment. Bars denoted by the same letter within the same treatment are not significantly different at $\alpha = 0.05$.

Table I. Mean Number of Eggs (Transformed Least Squares Means Estimate) Laid at Each Depth Interval in Three 2-Choice Oviposition Arenas

	Oviposition arena					
	MCT and NT		MCT and AT		AT and NT[a]	
	MCT	NT	MCT	AT	AT	NT
Depth (cm)						
0–4	18.5 (1.43) a	17.1 (1.32) a	29.6 (1.60) a	10.3 (1.46) a	12.3 (0.83) a	17.1 (1.08) a
4–8	13.8 (1.16) ab	5.0 (0.68) b	3.0 (0.91) c	1.8 (0.39) b	4.5 (0.60) a	2.7 (0.26) b
8–12	8.5 (0.86) b	0.1 (0.10) c	3.8 (0.80) c	0.4 (0.22) b	4.1 (0.60) a	0 (0.26) b
12–16	11.4 (0.86) b	0 c	8.5 (1.04) bc	0.5 (0.23) b	2.9 (0.60) a	0 (0.26) b
16–20	15 (0.82) b	1.0 (0.14) c	7.9 (1.12) bc	1.4 (0.30) b	0.2 (0.60) a	0 (0.26) b
20–24	16.4 (0.81) b	0.6 (0.13) c	13.5 (1.40) ab	4.9 (0.38) b	0.7 (0.60) a	0 (0.26) b

Note. Lower case letters indicate results of least squares means test on transformed data within each treatment. Means within a column followed by the same letter are not significantly different at $\alpha = 0.05$.
[a]The least squares means analysis on the AT vs. NT data compared the collective mean number of eggs counted from the depth intervals 4–24 cm to the depth interval 0–4 cm instead of each interval individually (see Methods).

About 50% of the eggs laid in the MCT treatment were in the top 8 cm. In contrast, 53.4% of the eggs in the AT treatment were laid in the top 4 cm.

The treatment by depth interaction was not significant ($F = 1.69$; $df = 5, 90$; $P = 0.1461$). The results from the least squares means procedure indicated that in the MCT treatment, more eggs were laid in 0–4 cm than in the depth intervals ranging from 4 to 20 cm, but a similar number was laid in the 0–4- and 20–24 cm depth intervals (Table I). More eggs were laid in the 20–24 cm interval than in the two depth intervals ranging from 4 to 12 cm, but were similar to the 12–20 cm depth intervals of the MCT treatment (Table I). Similar numbers of eggs were laid in the five depth intervals ranging from 4 to 20 cm of the MCT treatment (Table I). In the AT treatment, beetles laid more eggs in the 0–4 cm depth interval than in the five deeper depth intervals (4–24 cm) (Table I). A similar mean number of eggs was laid within the 4–24 cm depth intervals.

AT Versus NT

In this arena there was no significant difference between treatments, with 247 eggs being laid in the AT and 198 in the NT ($F = 0.05$; $df = 1, 9$; $P = 0.8289$). There was a significant effect due to depth ($F = 9.88$; $df = 1, 18$; $P = 0.0056$). Sixty-six percent of the eggs were found in the 0–4 cm interval of both treatments (Fig. 2C.). Beetles laid about 56% of the eggs in the AT treatment, and within this treatment, almost 50% were laid in the 0–4 cm interval of sand (Fig. 2C). In the NT treatment, eggs were only extracted from the top 8 cm of sand, with 86% being counted from the 0 to 4 cm interval (Fig. 2C).

The treatment × depth interaction was not significant ($F = 3.12$; $df = 1, 18$; $P = 0.0944$). The least squares means analysis determined that in the AT treatment similar numbers of eggs were laid within both depth intervals (Table I). In the NT treatment, beetles laid more eggs in the 0–4 cm interval than in the 4–24 cm depth interval (Table I).

DISCUSSION

The results of this study suggested that the presence of mole cricket tunnels influenced the number and depth at which eggs were laid by *P. aequinoctialis* when placed in two-choice oviposition arenas. The presence of a tunnel, even when made artificially, influenced oviposition depth by allowing beetles access to the lower depth intervals. However, we could not conclude whether the presence of a tunnel in sand was the single contributing

factor to the observed directed oviposition behavior. Because mole cricket tunneled sand was the preferred ovipositional substrate compared to all others offered, perhaps a semiochemical associated with these tunnels may have influenced beetle oviposition.

The treatment $\times$ depth interaction was not significant for any oviposition arena. This was particularly surprising when considering the MCT versus NT arena because we would have expected a dissimilar oviposition response to the treatments with increasing depth. For instance, we would have expected beetles to consistently lay more eggs in the deeper depths of mole cricket tunnels compared to the same depths of the sand without tunnels. However, on closer inspection of the arenas after beetle oviposition, some beetles actually used the MCT treatment to reach the deeper depths and then burrowed horizontally into the NT treatment. Therefore, perhaps a clearer understanding of the influence that mole cricket tunnels have on ovipositional depth would have been obtained by placing the barrier a few cm under the sand surface to allow beetles to select the substrate but to prevent beetles from crossing into the other treatment.

Although we could not determine how any particular treatment influenced the number of eggs laid at any particular depth, some general conclusions could be made. In the AT versus NT and the MCT versus NT arenas, the number of eggs laid within each interval of both treatments consistently decreased with each succeeding depth interval. In the MCT versus AT arena, the number of eggs laid in the MCT treatment initially decreased from 0 to 12 cm, but steadily increased in the depth intervals ranging 12–24 cm deep, where the 20–24 cm interval had a similar number of eggs to the 0–4 cm interval. For the AT treatment, a similar pattern to that of the other arenas was observed, where lower numbers of eggs were consistently laid the deeper the depth interval. Although a general pattern existed for each arena, the interaction was not strong enough to conclude how each treatment influenced the distribution and number of eggs laid.

In each choice arena, females deposited the highest number of eggs within the 0–4 cm sand interval for all treatments. However, the presence of tunnels largely influenced the depth at which the eggs were laid. For instance, in the NT treatment a much higher majority of eggs was laid closer to the sand surface than in the MCT and AT treatments for all arenas. In fact, when tunnels were present, eggs were consistently laid at greater depths. For example, in the MCT and NT choice arena, close to 40% of the eggs laid in the MCT treatment were laid in the sand intervals deeper than 12 cm compared to about 1.5% in the NT treatment. In addition, almost 15% of the eggs laid in the AT treatment of the AT versus NT arena were below 8 cm compared to 0% in the NT treatment. Therefore, our results suggest that the presence of tunnels provided easier access to the lower

depth intervals and may indicate that beetles in the field either locate tunnels for daytime refuge or for oviposition purposes. However, because such a high percentage of eggs was consistently laid in the 0–4 cm depth interval over all arenas, perhaps females simply oviposit near the soil surface and allow larvae to find the host's eggs.

Beetles preferred to lay eggs in the MCT treatment compared to the artificially tunneled and no tunneled substrates. In fact, females laid over 75% of the total number of eggs in the MCT treatment when it was offered. There was no apparent oviposition site preference between the AT and NT treatments when offered in an arena. Lastly, when the MCT treatment was not available, substantially lower numbers of eggs were laid (AT vs. NT = 447), compared to the number laid when the MCT treatment was available (MCT vs. AT = 856; MCT vs. NT = 1074).

The physical presence of a tunnel was not the sole factor influencing beetle oviposition. Because *P. aequinoctialis* laid significantly more eggs in the MCT treatment when given the choice between the MCT and AT treatments, a semiochemical associated with mole cricket-excavated tunnels may have influenced oviposition behavior. If the presence of tunnels was the single most important factor to females, similar numbers of eggs would have been expected within each of the AT and MCT treatments. Similarly, in the AT and NT oviposition arena, similar numbers of eggs were laid in the AT and NT treatments despite the presence of a tunnel in the AT treatment. Therefore, if physical presence of a tunnel was influential, we would have expected beetles to lay higher numbers of eggs in the AT treatment.

According to Weseloh (1981), many parasitoid wasps use visual and chemical cues in coordination to arrive at their intended host. For ground beetles, orientation behavior typically proceeds similarly through visual and chemical cues acting together (Thiele, 1977; Lövei and Sunderland, 1996), but these determinations were elucidated from studies on prey or habitat searching behavior and not oviposition. For example, in the studies conducted by Evans (1988), de Ruiter *et al.* (1989), Chiverton (1988), and Wheater (1989), each verified that carabids use semiochemical cues to locate either a food source or habitat. Only a few studies have directly addressed oviposition behavior of ground beetles, and none has specifically addressed the influence of semiochemicals on their oviposition behavior. Huk and Kühne (1999) experimentally demonstrated the influence of soil moisture and substrate type on oviposition behavior of *Carabus clatratus* and determined that females detected ideal moisture conditions rather than substrate type because moisture was the major influential factor of egg and larval mortality. Weseloh (1993) and Spieles and Horn (1998) demonstrated the importance of prey to initiate oviposition behavior of *Calosoma*

sycophanta L., a predator with a particular attraction to *Lymantria dispar* L. caterpillars.

Studies dealing with ectoparasitoid carabids have mainly described their basic biology, host biology, and larval behavior; and have only provided guesses on adult oviposition behavior rather than having been demonstrated in studies. Erwin and Erwin (1976) described the intricate relationship between *Eurycoleus macularis* Chevrolat (Lebiini) and its only known host, polypore-fungus-inhabiting *Amphix* spp. (Coleoptera: Endomychidae) in South America. The authors observed female *E. macularis* depositing an egg clutch a few cm away from aggregations of *Amphix* pupal colonies. Although it was not determined experimentally, the authors assumed that *E. macularis* was ovipositing in response to chemical cues either associated with the fungal growth or pupal colonies. They suggested that over time the searching behavior of *E. macularis* may have been fine-tuned to the semiochemicals associated with *Amphix* pupae or the fungal growths. The same may occur in *P. aequinoctialis.* Investigations by Erwin (1967) and Juliano (1985) on the behavior of female *Brachinus* spp., a genus in the same tribe as *Pheropsophus* and with a well-known ectoparasitoid relationship with water beetle pupae in the families Gyrinidae, Hydrophilidae, and Dytiscidae, determined that females preferred to lay eggs near the water's edge, an area where the water beetle pupal hosts are located in underground chambers. Erwin (1967) observed females inserting eggs into previously created mud balls that were deposited on or under rocks. Emerging larvae were observed to immediately begin searching for water beetle pupal chambers. This observation does not suggest that *Brachinus* females search for their larval host like *E. macularis*, but rather that the first instar is the host-finding stage. However, it does imply that *Brachinus* females oviposit in habitats likely to contain water beetle pupae and thus potentially increase the chances for larval survival.

Observations on the fecundity of laboratory-reared *P. aequinoctialis* determined that some females can lay a relatively large number of eggs (average of 80 eggs per week for a 20-week period), but a large variation existed among females, with one female only laying about 6 eggs per week (Weed, 2003). Fertility was very low, never exceeding on average 30% of the total eggs laid for one female (Weed, 2003). Lastly, no difference in the ability of first instars to find egg chambers located at depths ranging from 5 to 30 cm was observed (unpublished data). These unpublished observations and those found in this study suggest that *P. aequinoctialis* has developed oviposition behavior similar to that of *Brachinus*. *P. aequinoctialis* might emerge from mole cricket egg chambers, mate, and then lay eggs in an area likely to contain mole cricket eggs and allow the triungulin larvae to search for the egg chambers. Unlike parasitoid wasps which typically need to find the habitat and then the host, it is likely that *P. aequinoctialis* is already

in close proximity to mole cricket tunneled areas and simply oviposits in areas close to where it has emerged. Although there have been no observations on the flight behavior of *P. aequinoctialis*, adults have fully functional wings and may disperse to new areas when the competition for mole cricket eggs is high or when *Scapteriscus* mole crickets disperse.

As this study only demonstrated an ovipositional preference and we suggest that a kairomone may be responsible, further study is still needed to determine whether oviposition behavior of *P. aequinoctialis* is chemically orientated and to determine the influence, if any, of the oviposition site on larval survival. Other subsequent studies may need to develop bioassays based on the attraction of adult females or first instar beetles to mole cricket cuticular hydrocarbons, anal gland secretions, or excretory products, all compounds likely found in mole cricket tunnels.

Understanding the reproductive strategy of *P. aequinoctialis* not only provides fascinating information for those interested in the evolution of ground beetles and their behavior, but also provides essential information when considering the release of an insect into a foreign country for control purposes. Determining the reproductive behavior of *P. aequinoctialis* through laboratory experiments provides pertinent information concerning what measures need to be adopted for successful establishment and will also help to clarify the potential of *P. aequinoctialis* to reduce *Scapteriscus* mole cricket populations by understanding host finding behavior. The observations from this study suggest that either releasing gravid females or first instars onto turf or pasture grass displaying mole cricket damage will probably increase the likelihood that *P. aequinoctialis* larvae will find mole cricket egg chambers and limit the number of newly emerging *Scapteriscus* nymphs, and would also increase the chances for establishment of *P. aequinoctialis*.

ACKNOWLEDGMENTS

REFERENCES

Chaboussou, F. (1939). Contribution à l' étude biologique de *Lebia grandis* Hentz., prédateur américain du doryphore. *Ann. Epiphyt. Phytogen.* **5:** 387–433.

Chiverton, P. A. (1988). Searching behaviour and cereal aphid consumption by *Bembidion lampros* and *Pterostichus cupreus*, in relation to temperature and prey density. *Entomol. Exp. Appl.* **47:** 143–182.

de Ruiter, P. C., van Stralen, M. R., van Euwijk, F. A., Slob, W., Bedaux, J. J. M., and Ernsting, G. (1989). Effects of hunger and prey traces on the search activity of the predatory beetle *Notiophilus biguttatus*. *Entomol. Exp. Appl.* **51:** 87–95.

Dimmock, G., and Knab, F. (1904). Early stages of Carabidae. *Springfield (Mass.) Mus. Nat. Hist. Bull.* **1:** 1–56.

Erwin, T. L. (1967). Bombardier beetles (Coleoptera: Carabidae) of North America: Part II. Biology and behavior of *Brachinus pallidus* Erwin in California. *Coleop. Bull.* **21:** 41–55.

Erwin, T. L. (1970). A reclassification of bombardier beetles and a taxonomic revision of the North and Middle American species (Carabidae: Brachinida). *Quaest. Entomol.* **6:** 4–215.

Erwin, T. L. (1979). A review of the natural history and evolution of ectoparasitoid relationships in carabid beetles. In Erwin, T. L., Ball, G. E., and Whitehead, D. R. (eds.), *Carabid beetles: Their evolution, natural history, and classification*, Junk, Boston, MA, pp. 479–484.

Erwin, T. L., and Erwin, L. J. M. (1976). Relationships of predacious beetles to tropical forest wood decay. Part II. The natural history of *Eurycoleus macularis* Chevrolat (Carabidae: Lebiini) and its implications in the evolution of ectoparasitoidism. *Biotropica* **8:** 215–224.

Evans, W. G. (1988). Chemically mediated habitat recognition in shore insects (Coleoptera: Carabidae; Hemiptera: Saldidae). *J. Chem. Ecol.* **14:** 1441–1454.

Forrest, T. G. (1985). Reproductive behavior. In Walker, T. J. (ed.), *Mole crickets in Florida*, Florida Agricultural Experiment Stations Bulletin No. 846, pp. 10–15.

Frank, J. H., and Parkman, J. P. (1999). Integrated pest management of pest mole crickets with emphasis on the southeastern USA. *Integr. Pest Manage. Rev.* **4:** 39–52.

Habu, A., and Sadanaga, K. (1965). Illustrations for identification of larvae of the Carabidae found in cultivated fields and paddyfields. *Bull. Nat. Inst. Agric. Sci. (Jpn.) Ser. C* **3:** 169–177.

Huk, T., and Kühne, B. (1999). Substrate selection by *Carabus clatratus* (Coleoptera, Carabidae) and its consequences for offspring development. *Oecologia* **121:** 348–354.

Jenkins, W. R. (1964). A rapid centrifugal-flotation technique for extracting nematodes from soil. *Plant Dis. Rep.* **48:** 692.

Juliano, S. A. (1984). Multiple feeding and aggression among larvae of *Brachinus lateralis* Dejean (Coleoptera: Carabidae). *Coleop. Bull.* **38:** 358–360.

Juliano, S. A. (1985). The effects of body size on mating and reproduction in *Brachinus lateralis* (Coleoptera: Carabidae). *Ecol. Entomol.* **10:** 271–280.

Lake, P. C. (2000). Behaviors of *Pheropsophus aequinoctialis* (Coleoptera: Carabidae) affecting its ability to locate its larval food, eggs of *Scapteriscus* spp. (Orthoptera: Gryllotalpidae); and the effect of moisture on oviposition depth in *Scapteriscus abbreviatus*, MS Thesis, University of Florida, Gainesville.

Liebherr, J. K., and Ball, G. E. (1990). The first instar larva of *Eripus oaxacanus* Straneo & Ball (Coleoptera: Carabidae: Peleciini): Indicator of affinity or convergence? *Syst. Entomol.* **15:** 69–79.

Lindroth, C. H. (1954). Die Larve von *Lebia chlorocephala* Hoffm. (Coleoptera, Carabidae). *Opusc. Entomol.* **19:** 29–33.

Lövei, G. L., and Sunderland, K. D. (1996). Ecology and behavior of ground beetles (Coleoptera: Carabidae). *Annu. Rev. Entomol.* **41:** 231–256.

Salt, G. (1928). Notes on the life history of *Pelecium sulcatum* Guérin. *Psyche* **35:** 131–134.

SAS Institute (2000). *SAS Software Version 8.01*, SAS Institute, Cary, NC.

Silvestri, F. (1905). Contribuzione alla conoscenza della metamorfosi e dei costumi della *Lebia scapularis* Fourc. *Redia* **2:** 68–82.

Spieles, D. J., and Horn, D. J. (1998). The importance of prey for fecundity and behavior in the gypsy moth (Lepidoptera: Lymantriidae) predator *Calosoma sycophanta* (Coleoptera: Carabidae). *Environ. Entomol.* **27:** 458–462.

Thiele, H. U. (1977). *Carabid Beetles in Their Environments. A Study on Habitat Selection by Adaptations in Physiology and Behaviour*, Springer, Berlin, Germany.

Vinson, S. B. (1984). Parasitoid–host relationship. In Bell, W. J., and Cardé, R. T. (eds.), *Chemical Ecology of Insects*, Chapman and Hall, New York, pp. 205–233.

Walker, T. J., Gafney, J. J., Kidder, A. W., and Ziffer, A. B. (1993). Florida Reach-Ins: Environmental chambers for entomological research. *Am. Entomol.* **39:** 187–192.

Weed, A. S. (2003). Reproductive strategy of *Pheropsophus aequinoctialis* L.: Fecundity, fertility, oviposition behavior; and influence of mole cricket egg chamber depth on larval survival, MS Thesis, University of Florida, Gainesville.

Weseloh, R. M. (1981). Host location by parasitoids. In Nordlund, D. A., Jones, R. L., and Lewis, W. J. (eds.), *Semiochemicals: Their Role in Pest Control*, Wiley, New York, pp. 79–95.

Weseloh, R. M. (1993). Behavior of the gypsy moth predator, *Calosoma sycophanta* L. (Carabidae: Coleoptera), as influenced by time of day and reproductive status. *Can. Entomol.* **125:** 887–894.

Wheater, C. P. (1989). Prey detection by some predatory Coleoptera (Carabidae and Staphylinidae). *J. Zool.* **218:** 171–185.

Wickham, H. F. (1894). On some aquatic larvae with notice of their parasites. *Can. Entomol.* **26:** 39–41.