Evaluation of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) for the Control of American Cockroaches (Dictyoptera: Blattidae) in Sewers and Crevices Around Buildings

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ABSTRACT The objective of this study was to evaluate the potential of *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae) to control American cockroaches, *Periplaneta americana* (L.) (Dictyoptera: Blattidae), in sewer manholes and in crevices around buildings. Parasitoids were released weekly for 12 wk from laboratory parasitized heat-killed oothecae, and parasitism monitored using sentinel oothecae of American cockroaches. In addition, preference of *A. hagenowii* for 1- to 4-wk-old oothecae was evaluated in the laboratory. *A. hagenowii* females showed no preference for any ootheca age. Twenty of the 30 tested females parasitized one ootheca, whereas the other 10 parasitized two oothecae. The total progeny (males, females, and total) that emerged from a single ootheca parasitized by a female was not significantly different to the total progeny that emerged from two oothecae parasitized by a female. The number of males, females, and total progeny that emerged from the second parasitized ootheca was significantly less than the number that emerged from the first parasitized ootheca. The weekly mean sentinel oothecal parasitism rate in wall crevices was 18.1 ± 3.2% and in sewer manholes was 13.3 ± 2.0%. The mean number of released *A. hagenowii* females per number of parasitized sentinel oothecae recorded in crevices was 189 ± 18, whereas it was 428 ± 50 in sewers. *A. hagenowii* females were more effective at parasitizing sentinel oothecae placed at high and middle levels in manholes than at a low level when releases were made at the midpoint of the manhole shaft.

KEY WORDS *Periplaneta americana*, oothecal parasitoid, cockroach control, host age preference, biological control

The American cockroach, *Periplaneta americana* (L.) (Dictyoptera: Blattidae), is a cosmopolitan insect pest of medical and economic importance. It is a potential mechanical vector of disease organisms, and six allergens have been identified and characterized from *P. americana* (Roth and Willis 1957, Lee 1997, Gore and Schal 2007, Rust 2008). The presence of *P. americana* reduces esthetic value of residential environments (e.g., by causing psychological stress and being linked to unhygienic conditions) and renders business losses that eventually require pest control measures to be taken. In Southeast Asia, it is a dominant cockroach pest in residential premises, and its main reservoir habitat is the sewer (Lee and Lee 2000, Tee et al. 2011).

Currently, chemical methods, such as spraying of insecticides and placement of toxic baits, are widely used to manage American cockroach infestations (Lee and Ng 2009). These methods work against the adult and nymphal stages but they are not effective in killing eggs inside oothecae. Reinfestation by newly eclosed individuals that escape the insecticide treatment is common. Several authors have attempted to evaluate the potential of incorporating oothecal parasitic wasps into chemical control programs to target all three life stages (i.e., adults, nymphs, and eggs) (Hagenbuch et al. 1989, Bell et al. 1998, Reierson et al. 2005). The adverse effects of insecticide use in sensitive environments such as insectariums, zoos, and greenhouses also encourage the development of an environmentally friendly approach to cockroach control (Coler et al. 1984, Reierson et al. 2005, Baldwin et al. 2008, Rust 2008).

Reviews of the natural enemies of *P. americana* suggest that *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae) is one of the most promising candidates for use as a biological control agent (Cameron 1955, Lebeck 1991). This is because of its small size, active searching ability and gregariousness. *A. hagenowii* is a gregarious endoparasitoid wasp of cockroach oothecae. Its immature stages develop entirely inside oothecae, with nutrients obtained from consuming cockroach eggs (Cameron 1955, Narasimham 1984). Parasitism of oothecae collected from the field

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in the United States, India, Saudi Arabia, and Japan ranged from 16 to 48% in *P. americana*, 22–84% in *Periplaneta fuliginosa* (Serville), and 12.5% in *Blatta orientalis* L. (Cameron 1955, Fleet and Frankie 1975, Kanayama et al. 1976, Piper et al. 1978, Narasimham and Sankaran 1979).

Several studies demonstrated that release of *A. hagenowii* can result in high parasitism rates on cockroach oothecae distributed in confined, simulated-room conditions. However, a limited number of field evaluations that had been conducted so far reported mixed results (Pawson and Gold 1993, Suiter et al. 1998, Reierson et al. 2005). Therefore, the possibility of using *A. hagenowii* to control American cockroaches in the field requires further evaluation, particularly its potential usage in sewer—the main reservoir habitat of this pest in warm and humid microclimates—has not been evaluated extensively. In our study, the potential of using *A. hagenowii* as a biological control agent against the American cockroach was evaluated in sewers and crevices around buildings for a 12-wk period. In addition, searching ability of *A. hagenowii* within manhole shaft was also investigated by examining parasitism of oothecae deployed at different height levels. Before the field evaluation, the ootheca age preference of *A. hagenowii* was evaluated in laboratory.

Materials and Methods

Rearing of *A. hagenowii*. Parasitoid strain and colony maintenance were the same as described in Tee et al. (2010). For use in the experiments, 1- to 2-d-old *P. americana* oothecae were individually subjected to parasitization by a female *A. hagenowii* in a 2 ml microcentrifuge tube that was tightened with a piece of chiffon cloth to prevent escape of the wasp and allow ventilation. The purpose of parasitization of one ootheca with one female was to minimize the possibility of variation in wasp size because of superparasitism (ootheca parasitized by more than one female), and clutch size differences that can occur when more than one ootheca is offered simultaneously to a female *A. hagenowii* (Heitmans et al. 1992). Newly emerged males and females from these oothecae were allowed to sibmate for 24 h in the tube. Thus, *A. hagenowii* females used in the experiments were 2 d old, oviposition inexperienced, and assumed to have mated.

Laboratory Evaluation of Ootheca Age Preference of *A. hagenowii*. Ootheca age preference of *A. hagenowii* was evaluated using a laboratory multiple-choice test. This experiment was designed to evaluate the effect of ootheca age on choice by *A. hagenowii*. This was an important preliminary test, as preference for any ootheca age could affect interpretation of biological control activity in the field, which is usually monitored using sentinel oothecae. It is preferable to use young oothecae as sentinels because they can remain in the field for a longer period of time than older oothecae; older oothecae that are near to hatching have a short window during which parasitism can occur, and they may result in accidental releases of cockroaches if they are left too long in the field.

In this experiment, oothecae were collected weekly from styrofoam oothecal traps (15 × 10 × 3 cm) placed in rearing containers of *P. americana* (polyethylene container, 45 × 30 × 30 cm) (Yeh 1995). Thus, age of collected oothecae could be determined to within 7 d. Four ootheca age groups were used in this experiment: 1- (0–7 d), 2- (8–14 d), 3- (15–21 d), and 4-wk-old (22–28 d) oothecae. A total of 120 oothecae (30 from each of the four age groups) were used in this experiment ($n = 30$). The testing arena of the ootheca age preference experiment consisted of a petri dish (9 cm diameter, 1.5 cm height) fitted inside with a piece of filter paper (9 cm diameter). A circle (5 cm diameter) was drawn at the center of the filter paper and four oothecae (one from each of the four age groups) were distributed and glued (solvent-free glue, UHU, Gmbh & CO, Baden, Germany) at four points at equal distance from one to another along the circumference of the circle. A female *A. hagenowii* was introduced and allowed to acclimate for 30 min at the center of the circle by being held in place inside an inverted 2 ml microcentrifuge tube, the inner side of which was smeared with a layer of Fluon (Asahi Glass Company, Tokyo, Japan) to prevent it from crawling up into the tube. After 30 min of acclimatization, the microcentrifuge tube was removed and the petri dish was covered. Oviposition of *A. hagenowii* was then observed for 3–4 h. Oothecae that were parasitized by *A. hagenowii* during this observation period were recorded. If a female did not parasitize an ootheca within this observation period, the replicate was discarded. This experiment was repeated until 30 females had been recorded to parasitize an ootheca within this observation period. After the observation period, *A. hagenowii* were kept in the petri dish for 48 h. After 48 h, each of the four oothecae in the test arena were individually transferred to a 2 ml microcentrifuge tube and tightened with a piece of chiffon cloth to prevent escape of wasps and allow ventilation. Sixty days after emergence of parasitoids, oothecae were dissected to investigate their contents. Oothecae from which parasitoids had not emerged but that contained dead parasitoids were considered parasitized. The proportion of parasitism for each ootheca age group (total number of parasitized oothecae in each age group divided by total number of parasitized oothecae) was determined, and the number of progeny (males, females, and total progeny) that emerged from each ootheca was recorded.

Evaluation of Biological Control Activity of *A. hagenowii* in Crevices Around Buildings and in Sewer Manholes. *Study Sites.* The potential of *A. hagenowii* to control *P. americana* was evaluated for 12 wk from January to April 2010 by monitoring parasitism of sentinel oothecae placed inside crevices at the base of outer walls of buildings and in sewer manholes at the Minden Campus of Universiti Sains Malaysia, Penang. In total, 13 crevices and 10 sewer manholes (1-m-diameter manhole shaft) from buildings that included student dormitories, administrative offices, and cafeterias were selected for this experiment. Outdoor glass-jar trapping around the buildings (>25 cockroaches per building) (Ooi 2009) and visual inspection of the sewer manholes (>50 cockroaches per manhole) indicated that these areas were infested by *P. americana.* Eight of the 13 crevices and six of the 10 manholes were used as sites for release of *A. hagenowii;* the remaining sites were used as control sites without parasitoid release.

Release of A. hagenowii. *A. hagenowii* parasitoids were released weekly in the form of parasitized oothecae. The 1- to 2-wk-old laboratory-reared *P. americana* oothecae used for release of *A. hagenowii* were heat-killed at 48°C for 45 min before being subjected to parasitism by *A. hagenowii.* This step is performed to prevent accidental release of *P. americana* from unparasitized oothecae into the field (Suiter et al. 1998). This combination of minimum temperature and exposure duration to kill oothecae was assumed to have the least effects on the integrity of oothecae as hosts for *A. hagenowii* (Tee et al. 2010). Heat-killed oothecae were individually introduced into a 2 ml microcentrifuge tube that contained a female *A. hagenowii.* Two days later the wasp was removed, the tube was covered with a lid perforated with two holes (1–2 mm diameter, to allow ventilation and emergence of wasps during field release), and the ootheca in the tube was allowed to incubate in the laboratory for 30–33 d. To estimate the number of female *A. hagenowii* being released in the field, a sample of 5 to 10 parasitized oothecae was taken from each batch of parasitized oothecae prepared for weekly releases. They were allowed to emerge in a microcentrifuge tube sealed with a piece of chiffon cloth to prevent wasp escape. Upon emergence the number of female *A. hagenowii* produced per ootheca was determined.

Two to 3 d before wasp emergence (mean developmental time of *A. hagenowii* determined in a preliminary study was 35 d), the 2 ml microcentrifuge tubes containing parasitized oothecae were taken to the field sites for release. For release of *A. hagenowii* into crevices around buildings, four to five tubes were affixed with cloth tape to the wall ≈15 cm above the selected crevice. For release into sewers, 10–15 tubes were held together by rubber bands and lowered to the midpoint inside the manhole shaft using a length of nylon rope. Parasitized oothecae were left in the field for 2 wk to ensure parasitoid emergence, and then the oothecae were brought back to the laboratory to evaluate the emergence success. Oothecae with holes on the oothecal shell and that were empty and had a smooth inner wall without ridges were defined as oothecae from which wasps had successfully emerged whereas oothecae with holes but that contained dead parasitoids were considered oothecae from which wasps had not emerged. The number of oothecae from which wasps emerged and the average number of female *A. hagenowii* produced in the laboratory samples were used to estimate the number of released female *A. hagenowii* per site on each release date.

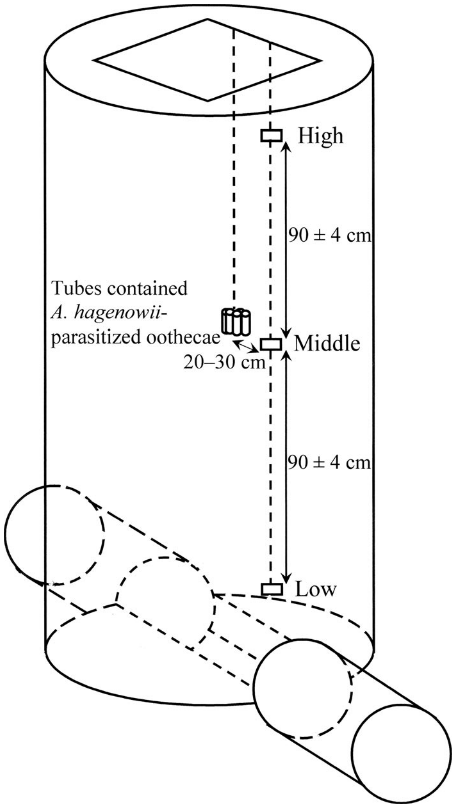

Fig. 1. Diagram of the release point of *A. hagenowii*-parasitized oothecae and locations (high, middle, and low) of the three sentinel oothecal bags (□) in the sewer manhole. Solid lines with arrows indicate distances (mean ± SE cm) between sentinel oothecal bags and release point of *A. hagenowii*-parasitized oothecae.

Monitoring of Parasitism of Sentinel Oothecae of P. americana. To monitor biological control activity of *A. hagenowii,* 10-mesh polyethylene net bags (5 × 3 cm), each containing three 1-wk-old, laboratory-reared *P. americana* oothecae, were placed at the crevice and sewer manhole sites. These bags were made by folding a piece of 10-mesh polyethylene net (6 × 5 cm) in half and stapling the edges closed to secure the three oothecae inside (Reierson et al. 2005). One bag of sentinel oothecae was placed into each crevice, and three bags were used in each manhole. The mean depth of the sewer manholes used in this study was 180 ± 8 cm. Therefore, the effect of height on sentinel ootheca parasitism of *A. hagenowii* in sewer manholes was also evaluated. One of the three sentinel oothecal bags within a manhole was suspended at a level close to the top, another was suspended at the midpoint 20–30 cm away from the *A. hagenowii* release point, and the third was placed at a level close to the bottom of the manhole shaft (Fig. 1). These locations were defined as high (mean distance above the wasp release point was 90 ± 4 cm), middle (20–30 cm away from the wasp release point), and low (mean distance below the wasp release point was 90 ± 4 cm). Sentinel

Table 1. The no. of males, females, and total progeny produced by individual *A. hagenowii* females that oviposited into one ootheca or two oothecae

Sex	Mean ± SE no. wasps/ootheca					
	n	Oviposition in only one ootheca	*n*	Two ovipositions in two oothecae		
				First ootheca	Second ootheca	Total
Female	20	77.7 ± 2.3a	10	57.8 ± 1.9b	16.8 ± 2.8c	74.6 ± 2.4a
Male	20	5.2 ± 0.5a	10	4.4 ± 0.5a	1.5 ± 0.4b	5.9 ± 0.5a
Total progeny	20	82.9 ± 2.4a	10	62.2 ± 1.9b	18.3 ± 2.8c	80.5 ± 2.2a

Means within the same row followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey's HSD).

oothecal bags were in place for 1 wk, after which they were brought back to the laboratory. Oothecae from the same bag were placed inside a 2 ml microcentrifuge tube to assess emergence of *A. hagenowii.* After 2–3 mo, sentinel oothecae from which wasps had not emerged were dissected to investigate their contents. Sentinel oothecae from which wasps had not emerged but that contained dead parasitoids were considered to have been parasitized.

The parameters used to evaluate the performance of *A. hagenowii* as a biological control agent were: 1) percentage parasitism of sentinel oothecae per site each week; 2) number of released females per parasitized sentinel oothecae per site; and 3) percentage parasitism of sentinel oothecae placed at the three height levels in the manholes.

Data Analyses. Data from the ootheca age preference experiment were analyzed using the chi-squared goodness-of-fit test. The number of progeny (males, females, and total progeny) produced from each ootheca in the ootheca age preference experiment were analyzed by one-way analysis of variance (ANOVA) followed by mean separation using Tukey's honestly significant difference (HSD). The data on the effect of height within a manhole on percentage parasitism were subjected to arcsine square-root transformation followed by one-way ANOVA and mean separation using Tukey's HSD (Zar 1999). Data on percentage parasitism of sentinel oothecae obtained from both crevices and sewer manholes were subjected to arcsine square-root transformation and were compared between these two study sites ($n = 12$) using Student's *t*-test. The number of released females per parasitized sentinel ootheca in crevices was compared with that obtained from sewer manholes using Student's *t*-test. All analyses were performed using SPSS version 11.0 at $\alpha = 0.05$ (SPSS 2002).

Results

In the ootheca age preference experiment, the proportion of *A. hagenowii* parasitism of oothecae aged 1, 2, 3, and 4 wk were 0.33, 0.23, 0.25, and 0.20, respectively. There was no significant difference in the proportion of parasitism among oothecae of different ages ($\chi^2 = 1.400$; df = 3; $P > 0.05$). *A. hagenowii* females oviposited into either one ootheca or two oothecae during the 48 h oviposition period. Twenty of the 30 *A. hagenowii* females oviposited in one ootheca, whereas the other 10 females oviposited in two oothecae. The mean number of males, females, and total progeny from oothecae parasitized by a female wasp that stung one ootheca did not differ significantly from the sum of the two oothecae parasitized by a female that stung two oothecae (Table 1). However, the mean number of females and total progeny that emerged from the two oothecae parasitized by a female *A. hagenowii* was significantly less than the mean number of females and total progeny that emerged from oothecae parasitized by a female *A. hagenowii* that had only stung one ootheca (females, $F = 106.5$; df = 3, 46; $P < 0.05$; total progeny, $F = 114.3$; df = 3, 46; $P < 0.05$; Table 1). The fewest number of males, females, and total progeny occurred in oothecae that were parasitized by *A. hagenowii* females during their second oviposition (Table 1).

Over the 12-wk evaluation period, 459 and 995 release-oothecae were deployed in crevices and sewer manholes, respectively. This resulted in a weekly average of 4.8 ± 0.1 release-oothecae deployed in each crevice and 13.8 ± 0.4 in each sewer manhole. Of these deployed oothecae, weekly average emergence rates of 56.2 ± 4.2% and 57.5 ± 3.8% were recorded for the crevices and sewer manholes, respectively. The mean number of *A. hagenowii* females that were released per site per week was 207 ± 15 for the releases at crevices around buildings and 616 ± 43 in sewer manholes.

The weekly mean percentage sentinel ootheca parasitism in crevices was 18.1 ± 3.2% (range, 4.2–37.5) and in sewer manholes was 13.3 ± 2.0% (range, 5.6–24.1%; Fig. 2A) over the 12-wk evaluation period. Sentinel oothecae retrieved from control sites in crevices and sewer manholes were not parasitized by *A. hagenowii.* Three *Evania appendigaster* (L.) (Hymenoptera: Evaniidae) (a solitary oothecal parasitoid) emerged from the sentinel oothecae collected from control sites (one ootheca from a crevice and two oothecae from sewer manholes). The weekly mean percentage parasitism of sentinel oothecae placed in crevices did not differ significantly from that recorded in sewer manholes ($t = 1.168$; df = 22; $P > 0.05$). However, the weekly mean number of released females per number of parasitized sentinel oothecae in crevices (189 ± 18) was significantly different from the mean recorded in sewer manholes (428 ± 50) ($t = -4.458$; df = 14; $P < 0.05$; Fig. 2).

The mean weekly percentage parasitism of sentinel oothecae located among high, middle, and low levels in sewer manholes was 20.8 ± 2.9%, 13.9 ± 2.3%, and 2.8 ± 1.5%, respectively. This parameter did not differ

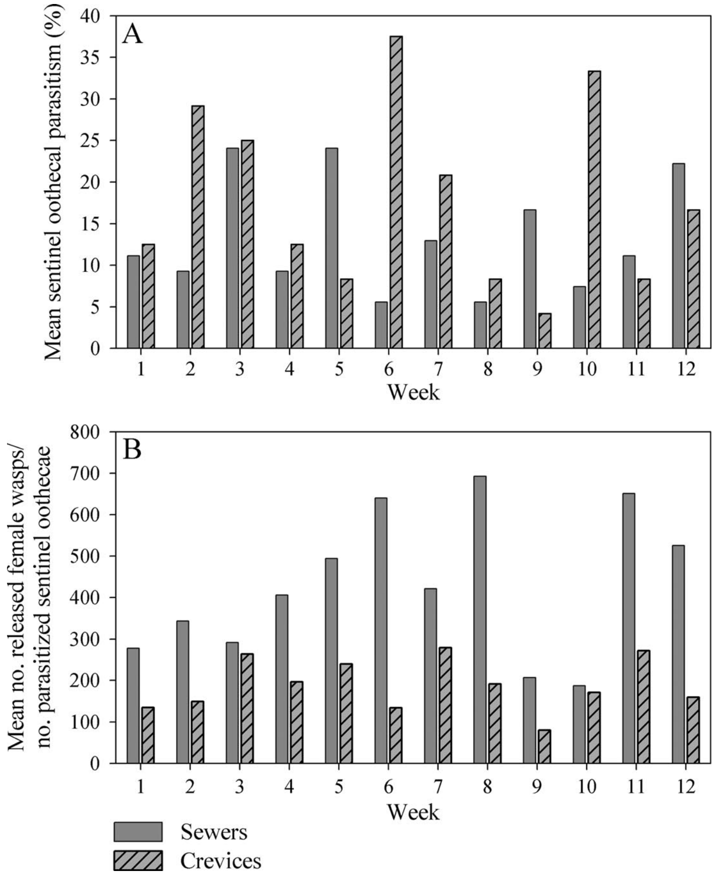

Fig. 2. Weekly mean percentage parasitism of sentinel oothecae (A) and mean number of released *A. hagenowii* females per number of parasitized sentinel oothecae (B) recorded per crevice around buildings and per sewer manhole in the weekly releases of *A. hagenowii* for 12 wk from January to April 2010 at Minden Campus of Universiti Sains Malaysia, Penang.

significantly between oothecae located at high and middle levels, but the values recorded for high and middle levels were significantly greater than that recorded at the low level ($F = 25.4$; df = 2, 33; $P < 0.05$).

Discussion

Understanding host age preference of parasitoids is important in efficient production of biological control agents, as parasitism rates could enhanced by offering preferred hosts. Knowledge about host age preference may also help in interpreting the results of field trials in which sentinel hosts are used as a monitoring tool. Bias in host age preference may indicate a possible means of escape from parasitism by hosts in less preferred stages, and lack of consideration of host preference may lead to misinterpretation of biological control activity results. Hagenbuch et al. (1988) described differences in developmental time and sex ratio of *A. hagenowii* progeny that emerged from oothecae of different ages. Of oothecae of five different ages (1–5 wk) that were subjected to parasitization by five pairs of male and female wasps, the shortest developmental time and a more female biased sex ratio occurred in the 2-wk-old oothecae. Suiter et al. (1998) demonstrated that ootheca age affected the number of *A. hagenowii* progeny that emerged: younger oothecae (aged 3–18 d) produced more progeny than older oothecae (aged 19–25 d). The effect of ootheca age on biological parameters such as developmental time, emergence rate, and progeny number were not determined in our study because 33% of the *A. hagenowii* females that oviposited twice deposited a smaller proportion of their eggs into the second ootheca. Heitmans et al. (1992) reported that *A. hagenowii* females may invest a smaller proportion of their eggs to a second ootheca when two oothecae are presented simultaneously. Thus, comparison of these biological parameters between oothecae of different ages that were offered simultaneously to individual *A. hagenowii* females is inappropriate. However, ootheca age preference by *A. hagenowii* was tested in this study, and oothecae of different ages were found to be equally preferred by *A. hagenowii* females. This agrees with the results reported by Hagenbuch et al. (1988), who found that parasitism by *A. hagenowii* did not differ significantly among 1- to 5-wk-old oothecae. The results from the ootheca age preference experiment conducted here suggest that oothecae aged 1–4 wk are equally preferred by *A. hagenowii* in the field; thus, the use of 1-wk-old sentinel oothecae to monitor *A. hagenowii* in the field probably was not affected by ootheca age.

The egg allocation strategy used by *A. hagenowii* females is poorly understood. In the current study, *A. hagenowii* females parasitized either one or two oothecae. However, the number of progeny produced per female was not significantly different between females that parasitized only one ootheca and those that parasitized two oothecae. Roth and Willis (1954) reported that the number of progeny (94.1 wasps) produced by *A. hagenowii* females that had parasitized an average of 2.1 oothecae was very similar to the number of progeny (103.3 wasps) produced by females that had parasitized an average of 4.8 oothecae. In contrast, Heitmans et al. (1992) reported that females that parasitized two oothecae produced a larger number of progeny than those that parasitized only one ootheca. In both of these studies, the sequence of the ootheca encounter and the number of oothecae presented were involved in how the eggs were invested. Roth and Willis (1954) showed that an ootheca offered to individual *A. hagenowii* females every 24 h throughout their entire lifespan resulted in a greater number of oothecae being parasitized (mean 4.8 oothecae), whereas five oothecae simultaneously offered once to individual females throughout their entire lifespan resulted in a smaller number of parasitized oothecae (mean 2.1 oothecae). Heitmans et al. (1992) found that females tended to allocate a smaller proportion of their eggs to the second ootheca when two oothecae were presented simultaneously, whereas females tended to allocate a similar proportion of their eggs for a second oviposition when the two oothecae were offered in sequence. The effects of oothecal density and their sequence of encounter on oviposition by *A. hagenowii* require further study to better understand the egg allocation strategies used by *A. hagenowii.*

An understanding of the effect of spatial distance on the host searching ability of *A. hagenowii* is critical when releasing *A. hagenowii* in the field for biological control of cockroaches. Field surveys of parasitism rates of *A. hagenowii* on naturally deposited oothecae of *P. fuliginosa* and *P. americana* revealed no correlation between parasitism rates and height of oothecae. Oothecae deposited on substrates as high as 3 m were parasitized by *A. hagenowii* (Fleet and Frankie 1975, Piper et al. 1978). In studies conducted in laboratory rooms (4.9 × 5.2 × 3.9 m) and inside houses, released *A. hagenowii* females parasitized *P. americana* oothecae distributed from the floor to areas near the ceiling (Roth and Willis 1954, Piper and Frankie 1978). However, a few researchers have reported limited host searching ability of *A. hagenowii* in enclosed spaces. In releases of *A. hagenowii* in plumbing chases, Pawson and Gold (1993) reported a parasitism rate of 21–35% for sentinel oothecae placed 0.5–5 m away from the release point of *A. hagenowii;* the rate was only 9% in oothecae placed 16 m away. In another release of *A. hagenowii* in simulated rooms (2.4 × 2.4 × 2.8 m), parasitism rates of oothecae deposited near the ceiling were significantly lower than those of oothecae deposited on the floor and in cabinets (Hagenbuch et al. 1989). In our study, sentinel oothecae that were placed at the low level in sewer manholes had a significantly lower parasitism rate compared with the oothecae placed at the high and middle levels. The sentinel oothecae placed at high and low levels were equidistant (mean 90 cm) from the *A. hagenowii* release point located at the midpoint of the manhole shaft, thus our results suggest that *A. hagenowii* females tended to search for hosts upward rather than downward in sewer manholes. Therefore, the release points of *A. hagenowii* in sewer manholes should include areas near the bottom of the manhole shaft. In a previous study conducted at Santa Monica, CA, Reierson et al. (2005) reported that there was no parasitism activities on sentinel oothecae after *A. hagenowii* were released into sewers. In our study, occurrence of parasitism activities on sentinel oothecae deployed into sewers suggested that parasitoid strain and environmental factors may have caused the differences in the performance of *A. hagenowii.* Similarly, environmental factors may have also contributed to the discrepancy found between the emergence rates of oothecae placed in the laboratory and field. Study on the effects of temperature and humidity on biology of *A. hagenowii* is needed to resolve these questions.

The parasitism rates in crevices around buildings and in sewer manholes documented in our study were lower than those reported by Roth and Willis (1954) and Hagenbuch et al. (1989) for releases of *A. hagenowii* in simulated rooms. However, the parasitism rates reported herein were close to those reported by Pawson and Gold (1993) for plumbing chases in which weekly releases of *A. hagenowii* were conducted. Roth and Willis (1954), Hagenbuch et al. (1989), and Pawson and Gold (1993) obtained a parasitism rate of 74–83, 96, and 23–39% by releasing 800-1000, weekly 300 and weekly 275–415 females, respectively. Therefore, the ratio of the number of released females: sentinel ootheca in Roth and Willis (1954), Hagenbuch et al. (1989), and Pawson and Gold (1993) was 10.6–12.5: 1, 8.3: one and 27.5–41.5: 1, respectively. Although the ratio was higher in Pawson and Gold (1993) and the current study compared with that in Roth and Willis (1954) and Hagenbuch et al. (1989), lower parasitism rates were recorded. This result may indicate the possible presence of competition from naturally occurring oothecae that are deposited by natural populations of cockroaches or a less efficient parasitism of sentinel oothecae by *A. hagenowii* in field conditions. A hydrocarbon (6, 9-heptacosadiene) identified from oothecae, frass and adult females of *P. americana* has been demonstrated as a host-location kairomone that attract *A. hagenowii* (Suiter et al. 1996). Therefore, locations of where naturally occurring oothecae are deposited may have a greater source of this hydrocarbon compared with where only sentinel oothecae are placed and this may lead to a lower parasitism rate of sentinel oothecae in field conditions.

In an evaluation fixed with a known number of oothecae and released wasps such as simulated room condition, the percentage parasitism of oothecae is predictive of the efficacy of *A. hagenowii* parasitization. This information is useful as a reference for planning an optimal release rate in conditions in which estimates of the number of oothecae can be generated. However, the number of oothecae presented in the field is difficult to estimate. Comparison of the biological control efficacy of *A. hagenowii* between different evaluations based on percentage parasitism of sentinel oothecae may not accurately reveal their differences. In an evaluation that documented low parasitism rates, the number of sentinel oothecae used for monitoring may have an influence on the value of percentage parasitism of sentinel oothecae. For example, a lower efficacy of *A. hagenowii* parasitization is expected in an evaluation in which 10 sentinel oothecae are used compared with another evaluation that used only five sentinel oothecae. Therefore, we also use the mean number of released females per number of parasitized sentinel oothecae (this was used to measure the number of females needed to locate and parasitize a sentinel ootheca in each condition) to measure the biological control efficacy of *A. hagenowii* in various conditions, and this allowed the efficacy of *A. hagenowii* in different conditions to be compared (Fig. 2). In this study, the sentinel oothecal parasitism rates and the number of released females: sentinel ootheca ratio in both the crevices and manholes were very similar. However, the mean number of released females/number of parasitized sentinel oothecae in sewer manholes was greater than that generated in crevices. Roth and Willis (1954) demonstrated that 10.6–12.5 *A. hagenowii* females were needed to parasitize an ootheca distributed in their simulated rooms. Pawson and Gold (1993) showed that 70.5–180.4 *A. hagenowii* females were needed to parasitize a sentinel ootheca placed in plumbing chases infested with American cockroaches. In our study, a mean of 189 and 428 females were needed to parasitize a sentinel ootheca under natural conditions (i.e., crevices) and in an enclosed condition (i.e., sewer manholes), respectively. Two releases of 30 parasitized oothecae in sewer manholes did not result in any parasitism of sentinel oothecae in Reierson et al.'s (2005) evaluation of the possibility of using *A. hagenowii* to control *P. americana* in sewers. This suggests that a greater number of females need to be released to compensate for the less satisfactory host searching ability in open-air conditions and to increase parasitism in areas with large infestations of cockroaches, such as sewers. Suiter et al. (1998) showed that a maximum of 18,780–20,322 *A. hagenowii* females needed to be released per treehole over a 30-wk evaluation period to result in significantly higher sentinel oothecal parasitism rates (50–100%) compared with that recorded at control treeholes (10–46%) without releases.

In conclusion, the ootheca age preference test showed that oothecae aged 1–4-wk had an equal chance of being parasitized by *A. hagenowii.* The oviposition sequence of *A. hagenowii* affected the number of progeny that emerged from parasitized oothecae. *A. hagenowii* females were less effective at parasitizing sentinel oothecae placed near the bottom of a manhole shaft when *A. hagenowii* females were released at the midpoint of the manhole. Nevertheless, occurrence of parasitism on sentinel oothecae deployed at different heights inside sewer shaft indicates this reservoir habitat of *P. americana* may be a suitable site to release *A. hagenowii* for cockroach control. The parasitism rate of sentinel oothecae placed in crevices around buildings did not differ significantly from the rate found in sewer manholes. However, a larger numbers of *A. hagenowii* females were required to effectively parasitize a sentinel ootheca placed in a sewer manhole compared with one in crevices around buildings. Based on the parasitism rate and number of released females per number of parasitized sentinel oothecae reported in our study, it is suggested that large number of *A. hagenowii* is needed to be released to effectively reduce cockroach populations. Because of limitation on mass-production of *P. americana* oothecae [*P. americana* female produces an average of one ootheca every 6 d in its life time and two oothecae during its peak reproductive period (Gould and Deay 1938, Roth and Willis 1956)] and unavailability of artificial diets for mass-rearing *A. hagenowii*, periodic releases of *A. hagenowii* through a limited number of laboratory-parasitized *P. americana* oothecae alone may not sufficient to reduce cockroach populations. Several studies demonstrated that target-specific insecticide baits applied outdoors and in sewers can effectively control cockroaches (Smith et al. 1998, Reierson et al. 2005) while a few studies reported that combined usage of insecticide baits and *A. hagenowii* did not have a detrimental effect on this wasp (Hagenbuch et al. 1989, Bell et al. 1998). Therefore, by treating adults and nymphal stages with insecticide baits, periodic releases of *A. hagenowii* to kill oothecae may negate the rebound of cockroach populations. In addition, the use of insecticide baits instead of sprays also can conserve naturally occurring parasitoid populations for natural control and has less impact on other beneficial organisms (Suiter 1997).

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References Cited

Baldwin, R. W., P. G. Koehler, and R. M. Pereira. 2008. Toxicity of fatty acid salts to German and American cockroaches. J. Econ. Entomol. 101: 1384–1388.

Bell, H. A., G. C. Marris, and J. P. Edwards. 1998. The influence of the juvenile hormone analogue (S)-hydroprene on *Aprostocetus hagenowii* (Hymenoptera: Eulophidae), an oothecal parasitoid of the oriental cockroach *Blatta orientalis* (Dictyoptera: Blattidae). Bull. Entomol. Res. 88: 231–238.

Cameron, E. 1955. On the parasites and predators of the cockroach. I. *Tetrastichus hagenowii* (Ratz.). Bull. Entomol. Res. 46: 137–147.

Coler, R. R., R. G. Van Driesche, and J. S. Elkinton. 1984. Effect of an oothecal parasitoid, *Comperia merceti* (Compere) (Hymenoptera: Encyrtidae), on a population of the brownbanded cockroach (Orthoptera: Blattellidae). Environ. Entomol. 13: 603–606.

Fleet, R. R., and G. W. Frankie. 1975. Behavioral and ecological characteristic of a eulophid egg parasite of two species of domiciliary cockroaches. Environ. Entomol. 4: 282–284.

Gore, J. C., and C. Schal. 2007. Cockroach allergen biology and mitigation in the indoor environment. Annu. Rev. Entomol. 52: 439–463.

Gould, G. E., and H. O. Deay. 1938. The biology of the American cockroach. Ann. Entomol. Soc. Am. 31: 489–498.

Hagenbuch, B. E., R. S. Patterson, P. G. Koehler, and R. J. Brenner. 1988. Mass production of the cockroach oothecal parasitoid, *Tetrastichus hagenowii* (Hymenoptera: Eulophidae), and its host, the Am. cockroach (Orthoptera: Blattidae). J. Econ. Entomol. 81: 531–535.

Hagenbuch, B. E., R. S. Patterson, and P. G. Koehler. 1989. Biological control of the American cockroach (Orthoptera: Blattidae) with inundative releases of *Tetrastichus hagenowii* (Hymenoptera: Eulophidae). J. Econ. Entomol. 82: 90–94.

Heitmans, W.R.B., P. Haccou, and J.J.M. van Alphen. 1992. Egg supply, clutch size and survival probability in *Aprostocetus hagenowii* (Ratz) (Hymenoptera: Eulophidae), a gregarious parasitoid of cockroach oothecae. Proc. Exp. Appl. Entomol. 3: 62–69.

Kanayama, A., E. Yoshida, and T. Honma. 1976. The parasitism by *Tetrastichus hagenowii* (Ratzeburg) on oothecae of the smoky brown cockroach, *Periplaneta fuliginosa* (Serv.), collected in Shizuoka city. Jpn. J. Sanit. Zool. 27: 157–62.

Lebeck, L. M. 1991. A review of the hymenopterous natural enemies of cockroaches with emphasis on biological control. Entomophaga 36: 335–352.

Lee, C.-Y. 1997. Medical importance of domiciliary cockroaches. Sing. Microbiol. 11: 14–17.

Lee. C.-Y., and L.-C. Lee. 2000. Diversity of cockroach species and effect of sanitation on level of cockroach infestation in residential premises. Trop. Biomed. 17: 39–43.

Lee, C.-Y., and L.-C. Ng. 2009. Pest cockroaches of Singapore: a scientific guide for pest management professionals. Singapore Pest Management Association, Singapore.

Narasimham, A. U. 1984. Comparative studies on *Tetrastichus hagenowii* (Ratzeburg) and *T. asthenogmus* (Waterston), two primary parasites of cockroach oothecae, and on their hyperparasite *Tetrastichus* sp. (*T. miser* (Nees) group) (Hymnoptera: Eulophidae). Bull. Entomol. Res. 74: 175–189.

Narasimham, A. U., and T. Sankaran. 1979. Domociliary cockroaches and their oothecal parasites in India. Entomophaga 24: 273–279.

Ooi, Y. S. 2009. Outdoor distribution of the American cockroach *Periplaneta americana* (L.) (Dictyoptera: Blattidae) in Minden Campus of Universiti Sains Malaysia. B.S. thesis, Universiti Sains Malaysia, Penang, Malaysia.

Pawson, B. M., and R. E. Gold. 1993. Evaluating different release strategies for the control of American cockroaches using the egg parasitoid, *Aprostocetus hagenowii*, pp. 407–413. *In* K. B. Wildey and W. H. Robinson (eds.), Proceedings, First International Conference on Insect Pests in the Urban Environment, 30 June–3 July 1993, Cambridge, United Kingdom. BPCC Wheatons, Exeter, United Kingdom.

Piper, G. L., and G. W. Frankie. 1978. Integrated management of urban cockroach populations, pp. 249–266. *In* G. W. Frankie and C. S. Koehler (eds.), Perspectives in urban entomology. Academic, New York.

Piper, G. L., G. W. Frankie, and J. Loehr. 1978. Incidence of cockroach egg parasites in urban environments in Texas and Louisiana. Environ. Entomol. 7: 289–293.

Reierson, D. A., M. K. Rust, and E. Paine. 2005. Control of American cockroaches (Dictyoptera: Blattidae) in sewer systems, pp. 141–148. *In* C. Y. Lee and W. H. Robinson (eds.), Proceedings, Fifth International Conference on Urban Pests, 10–13 July 2005, Singapore. Perniagaan Ph'ng, Penang, Malaysia.

Roth, L. M., and E. R. Willis. 1954. The biology of the cockroach egg parasite, *Tetrastichus hagenowii* (Hymenoptera: Eulophidae). Trans. Am. Entomol. Soc. 80: 53–72.

Roth, L. M., and E. R. Willis. 1956. Parthernogenesis in cockroaches. Ann. Entomol. Soc. Am. 49: 195–204.

Roth, L. M., and E. R. Willis. 1957. The medical and veterinary importance of cockroaches. Smithsonian Misc. Coll. 134: 1–147.

Rust, M. K. 2008. Cockroaches, pp. 53–84. *In* X. Bonnefoy, H. Kampen, and K. Sweeney (eds.), Public health significance of urban pests. World Health Organization, Geneva, Switzerland. (http://www.euro.who.int/_data/assets/pdf_file/0011/98426/E91435.pdf).

Smith, L. M., A. G. Appel, T. P. Mack, and G. J. Keever. 1998. Comparison of conventional and targeted insecticide application for control of smokybrown cockroaches (Dictyoptera: Blattidae) in three urban areas of Alabama. J. Econ. Entomol. 91: 473–479.

SPSS. 2002. SPSS, version 11.0. SPSS, Chicago, IL.

Suiter, D. R. 1997. Biological suppression of synanthropic cockroaches. J. Agric. Entomol. 14: 259–270.

Suiter, D. R., D. A. Carlson, R. S. Patterson, and P. G. Koehler. 1996. Host-location kairomone from *Periplaneta americana* (L.) for parasitoid *Aprostocetus hagenowii* (Ratzeburg). J. Chem. Ecol. 22: 637–651.

Suiter, D. R., R. S. Patterson, and P. G. Koehler. 1998. Seasonal incidence and biological control potential of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) in treehole microhabitats. Environ. Entomol. 27: 434–442.

Tee, H.-S., A. R. Saad, and C.-Y. Lee. 2010. Suitability of heat- and freeze-killed oothecae of the American cockroach (Dictyoptera: Blattidae) as hosts for an oothecal parasitoid, *Aprostocetus hagenowii* (Hymenoptera: Eulophidae). J. Econ. Entomol. 103: 1770–1774.

Tee, H.-S., A. R. Saad, and C.-Y. Lee. 2011. Population ecology and movement of the American cockroach (Dictyoptera: Blattidae) in sewers. J. Med. Entomol. 48: 797–805.

Yeh, C. C. 1995. Oviposition concealment behavior of *Periplaneta americana* L. and its application on the oothecal trap in the laboratory. Chinese J. Entomol. 15: 153–161.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ.

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