Suitability of Heat- and Freeze-Killed Oothecae of the American Cockroach (Dictyoptera: Blattidae) as Hosts for an Oothecal Parasitoid, *Aprostocetus hagenowii* (Hymenoptera: Eulophidae)

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ABSTRACT The objective of this study was to evaluate the suitability of heat- and freeze-killed oothecae of *Periplaneta americana* (L.) (Dictyoptera: Blattidae) as hosts for parasitoid *Aprostocetus hagenowii* (Ratzeburg) (Hymenoptera: Eulophidae). The oothecae were subjected to −20, 45, 48, 50, and 55°C at different exposure times (15, 30, 45, and 60 min). The effects of heat- and freeze-killed oothecae on several biological parameters (e.g., parasitism and emergence rates, developmental times, progeny number, and sex ratio) of *A. hagenowii* were determined. Embryonic development of 2-d-old oothecae was terminated by either freezing at −20°C or heating at ≥48°C for ≥30 min. *A. hagenowii* parasitized live oothecae as well as both heat- and freeze-killed oothecae. Percentage parasitism, emergence rates, and developmental times of *A. hagenowii* in both heat- and freeze-killed oothecae were not significantly different from those of the live oothecae. Both heating and freezing did not influence progeny number (male and female) and sex ratio of *A. hagenowii* emerged from killed oothecae.

KEY WORDS *Periplaneta americana*, *Aprostocetus hagenowii*, parasitoid, freeze, heat

The American cockroach, *Periplaneta americana* (L.) (Dictyoptera: Blattidae), is an important species of insect pest in the urban environment. In Southeast Asia, *P. americana* can be found in both indoor and outdoor environments (Lee 2007, Lee and Ng 2009). It is a potential mechanical vector of pathogenic organisms and may produce allergens and inhalants that are responsible for allergies and asthma (Roth and Willis 1957, Rueger and Olson 1969, Lee 1997, Wu et al. 2000). Cockroach management has relied heavily on insecticide application, particularly baits and sprays (Lee and Ng 2009). Several studies reported the potential of using natural enemies for cockroach management, especially oothecal parasitoid wasps (Roth and Willis 1960, Coler et al. 1984, Hagenbuch et al. 1989, Suiter et al. 1998). Conventional cockroach management strategies commonly target on adults and nymphs, such as application of toxic baits and sprays that take effects when cockroaches come into contact and get a lethal dose (Rust et al. 1991, Smith et al. 1997). Integrating oothecal parasitic wasps into a conventional cockroach management program may further reduce cockroach population because of the ability of parasitoids to search for and parasitize oothecae which are either concealed or deposited in areas that require a thorough search for their locations (Rau 1943, Piper et al. 1978, Hagenbuch et al. 1989; Yeh 1995; Gordon et al. 1994a,b; Bell et al. 1998).

Aprostocetus hagenowii (Ratzeburg) (Hymenoptera: Eulophidae) is a cockroach oothecal parasitoid. Its biology and potential as a biological control candidate have been reviewed previously (Cameron 1955, Edmunds 1955, Roth and Willis 1960, Lebeck 1991). Several release programs of *A. hagenowii* have been conducted to evaluate its potential to control *P. americana.* In a simulated room, Roth and Willis (1954) and Hagenbuch et al. (1989) reported a parasitism rate of 83 and >90% by releases of ≈1,000 and 600 wasps, respectively. Suiter et al. (1998) conducted a field release of *A. hagenowii* in treeholes infested by *Periplaneta* spp. and achieved a significantly higher parasitism of the cockroach oothecae compared with the nonrelease sites.

Despite these promising findings, information on the mass rearing of *A. hagenowii* is relatively limited, especially concerning the interaction between *A. hagenowii* and its cockroach hosts (Hagenbuch et al. 1988, Heitmans et al. 1992, Bressan-Nascimento et al. 2008). Rearing for a mass release program requires large production of biological control agents and live hosts. Mass rearing of insects for biological control often involve the use of killed hosts rather than live hosts (Petersen and Cawthra 1995, Geden and Kaufman 2007). Using killed hosts has the advantages of preventing escape of unparasitized hosts, retaining

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hosts at stages favorable for mass production, and for stockpiling for future use.

Several host-killing methods have been employed and studied for this purpose. High and low temperatures, UV radiation, and irradiation were the common techniques used to kill the hosts (Roth et al. 1991, Hwang and Chen 2004, Geden and Kaufman 2007, Moreno et al. 2009). Because an irradiator is often not available or accessible, freezing and heating may provide another economical way to kill the hosts (Geden and Kaufman 2007). Suiter et al. (1998) and Hwang and Chen (2004) found that freeze-killed oothecae were not suitable for the production of *A. hagenowii* or *Evania appendigaster* (L.). However, the optimum duration of heating and freezing was not determined in their studies. Hu et al. (1999) reported that reduced host suitability was incurred when the host was killed under extended durations. In addition, Hwang and Chen (2004) pointed out that there was a relationship between host quality and host treatment duration.

The objective of this study was to evaluate the suitability of *P. americana* oothecae, killed after subjected to minimum duration of heating and freezing, as hosts for the production of *A. hagenowii* in comparison with live oothecae. Biological parameters such as parasitism and emergence rates, developmental time, progeny number, and sex ratio of *A. hagenowii* of those produced through live- and killed oothecae were measured and compared.

Materials and Methods

Rearing of Cockroaches. Cultures of *P. americana* were established from stock cultures in the Urban Entomology Laboratory, Universiti Sains Malaysia, Penang, Malaysia, inaugurated in 1997 from wild cockroaches collected from sewers in the Minden campus of Universiti Sains Malaysia and indoor trapping at residential premises in Penang. To supply oothecae for experimental use, 75–90 adult females and 15 adult males were reared in a polyethylene container (45 by 30 by 30 cm), with a screened lid, under environmental conditions of 26.0 ± 0.5°C, 55 ± 3% RH, and a photoperiod of 12:12 (L:D) h. The inner upper wall surface of the container was smeared with a thin layer of petroleum jelly to prevent escape of cockroaches. Harborages consisted of 20 cut polyvinyl chloride pipes (20 by 3 cm). Water and dog chow were provided ad libitum. A piece of Styrofoam (15 by 10 by 3 cm) was placed into the rearing container as oothecal trap for cockroaches to deposit their oothecae (Yeh 1995). Dead cockroaches were replaced weekly. Twenty to 25 containers of this type were set up to provide ≈10–20 oothecae per day each container. Because age of ootheca was known to affect the progeny number, developmental time, and sex ratio of *A. hagenowii*, 2-d-old oothecae were used to compare the effects of heating and freezing on the suitability of killed oothecae as hosts for *A. hagenowii* (Hagenbuch et al. 1988, Suiter et al. 1998).

Rearing of Parasitoids. Initial colonies of *A. hagenowii* were established from the parasitoids emerged from several parasitized sentinel oothecae collected in the Minden campus, Universiti Sains Malaysia, in 2007. The species of the parasitoid was identified and confirmed by Andrew Polaszek (Department of Entomology, Natural History Museum, London, United Kingdom). Parasitoids were reared in a nylon-meshed metal cage (30 by 30 by 30 cm) and provided with 10% sucrose solution under the condition of 26.5 ± 0.5°C, 63 ± 2% RH, and a photoperiod of 12:12 (L:D) h. Thirty-five to 40 oothecae were provided in 100-ml polyethylene cups every 3 to 4 d into the cage and removed after 48 h of exposure (Hagenbuch et al. 1988). The exposed oothecae were either placed back into the cage 2 to 3 d before emergence for parasitoid colony maintenance or allowed to emerge individually in the capped polyethylene cup to provide oviposition-inexperienced females. Two-day-old mated and oviposition-inexperienced female parasitoids used in the experiment were obtained from 2-d-old oothecae exposed individually to a female parasitoid collected from the capped polyethylene cups. This was to minimize the possibility of wasp size differences caused by superparasitism (ootheca oviposited by more than one female), and clutch size differences when more than one host was offered simultaneously to the female *A. hagenowii* (Heitmans et al. 1992).

Determination of Minimum Time of Heating and Freezing to Terminate Embryonic Development of *P. americana*. To determine the minimum temperature and duration of heating and freezing to terminate embryonic development of *P. americana*, four exposure periods of 15, 30, 45, and 60 min were selected. In total, 600 2-d-old oothecae were randomly assigned to one of the 20 combinations of temperature and exposure period. Only oothecae in intact bean-shaped form and with intact keel were selected for this study because damaged keel could lead to desiccation and death (Roth and Willis 1955, Bressan-Nascimento et al. 2008). Six 100-ml polyethylene cups (replicate) of five oothecae each were placed into a digital-controlled oven calibrated at 45, 48, 50, and 55°C with 17 ± 1% RH and into a freezer at −20°C. Another six replicates were not treated and maintained as control. After heating and freezing, the oothecae were retrieved and held under the conditions similar to cockroach rearing. The hatchability of cockroaches from the oothecae was then checked and recorded daily up to 70 d after heating and freezing. Percentage of mortality of oothecae was recorded.

Effects of Killed Oothecae on Biological Parameters of *A. hagenowii*. We assume that minimum duration of heating and freezing to terminate embryonic development of *P. americana* would be the least disruptive to the integrity of the oothecae and would have the least effect on their suitability as hosts to *A. hagenowii*. Therefore, to evaluate parasitism rate, emergence rate, developmental time, number of progeny (male and female), and sex ratio of *A. hagenowii* in killed oothecae, five 2-d-old oothecae were subjected to one of the following conditions: 1) −20°C for 30 min, 2) 48°C for 30 min, and 3) no treatment (live oothecae). There were five replicates in each condition. Thirty

Table 1. Mean percentage mortality of *P. americana* oothecae after exposure to heating and freezing[a]

Temp (°C)	% ootheca mortality (mean ± SE) at exposure time (min)			
	15	30	45	60
−20	83.3 ± 9.6	100	100	100
45	6.7 ± 4.2	3.3 ± 3.3	6.7 ± 4.2	10.0 ± 4.5
48	6.7 ± 4.2	100	100	100
50	100	100	100	100
55	100	100	100	100

[a] Mortality (%) of control sets at room temp (26.4 ± 0.2°C) = 3.3 ± 3.3.

minutes after heating or freezing, each ootheca was placed into a 2-ml microcentrifuge tube with a 2-d-old unfed, mated and oviposition-inexperienced female parasitoid. At 48 h, the female parasitoid was removed and the tube was capped with cotton. Sixty days after the parasitoid emergence, the oothecae without any parasitoid emergence were dissected to investigate their internal contents. Oothecae from which parasitoids had not emerge but contained dead parasitoids were considered as parasitized oothecae.

We determined the following parameters: 1) parasitism rate, 2) emergence rate, 3) developmental time, 4) number of progeny, and sex ratio (number of males divided by number of females). Percentage parasitism and percentage emergence of *A. hagenowii* in different treated oothecae were subjected to arsine transformation, followed by analysis of variance (ANOVA). Developmental time, number of males, females, and sex ratios were subjected to ANOVA. All analyses were performed using Statistix 7.0 software (Analytical Software, Tallahassee, FL) at a significance level of $P = 0.05$.

Results

For oothecae subjected to heating, there was <11% mortality at 45°C for up to 60 min and 48°C for 15 min (Table 1). However, 100% mortality of oothecae was achieved when heating duration was increased to 30 min at 48°C. At ≥50°C, 100% mortality was recorded at all durations. For oothecae subjected to freezing at −20°C, ≥30 min was sufficient to terminate embryonic development of *P. americana* (Table 1).

A. hagenowii parasitized both freeze-killed and heat-killed oothecae. Parasitism rates were not significantly different among the different treatments with percentage parasitism of ≥84% ($F = 1.10$; df = 2, 12; $P = 0.3638$) (Table 2). The emergence rates as well as developmental times of *A. hagenowii* were not significantly different among treatments ($F = 0.25$; df = 2, 12; $P = 0.7830$, and $F = 2.01$; df = 2, 12; $P = 0.1769$, respectively) (Table 2). The number of male and female progeny and their sex ratio produced from live oothecae parasitized by *A. hagenowii* did not differ significantly from those of the killed oothecae ($F = 2.23$; df = 2, 57; $P = 0.1169$, $F = 0.37$; df = 2, 57; $P = 0.6928$ and $F = 2.76$; df = 2, 57; $P = 0.0716$, respectively) (Table 2). *A. hagenowii* produced a mean of 6.6 males, 88.6 females, and female-biased sex ratio of 0.076.

Discussion

In augmentative biological control programs, using hosts killed at a level not reduce host quality has several advantages over the use of live hosts. For field control, the risk of accidentally releases of pests from unparasitized live hosts can be prevented by using killed hosts (Suiter et al. 1998). In addition, host killing also eliminates the need to remove hatching or emerging individuals that escape from unparasitized hosts in parasitoid rearing cages (Geden and Kaufman 2007). In our study, exposure of oothecae to minimum duration of heating and freezing was able to terminate embryonic development of *P. americana.* Other studies also have shown that freezing and heating could terminate embryonic development of *P. americana* (Suiter et al. 1998, Hwang and Chen 2004). However, minimum time required to terminate embryonic development of *P. americana* were not determined in their studies. Our study also provided a quicker option of host-killing treatment for *P. americana* oothecae than that reported by Bressan-Nascimento et al. (2008), who showed that embryonic development of *P. americana* can be terminated by chilling oothecae at 0–5°C for 5 d.

One important requirement of host-killing treatment is that killed hosts have to be remained acceptable for parasitization by parasitoid and suitable for development by parasitoid larvae. In our study, *A. hagenowii* parasitized live oothecae as well as freeze- and heat-killed oothecae. Reduced host suitability was not observed based on parasitism rates, emergence rates, and developmental times recorded from live and killed oothecae. Bressan-Nascimento et al. (2008) showed similar findings where oothecae subjected to minimum chilling (0–5°C) of 5 d resulted in no differences in parasitism and emergence rates, and developmental times of *A. hagenowii* and *E. appendigaster* compared with that in live oothecae. Previous

Table 2. Biological parameters (mean ± SE) of *A. hagenowii* on live and killed oothecae of *P. americana*

Treatment	Biological parameter (mean ± SE)							
	n	Parasitism (%)	Emergence (%)	Development (d)	*n*	No. male/ootheca	No. female/ootheca	Sex ratio (male/female)
Live	5	84.0 ± 7.5	80.0 ± 8.9	38.9 ± 0.7	19	6.4 ± 0.4	89.2 ± 2.1	0.071 ± 0.004
Heat killed (48°C)	5	88.0 ± 4.9	84.0 ± 7.5	37.5 ± 0.2	20	6.1 ± 0.5	89.5 ± 2.1	0.069 ± 0.006
Freeze killed (−20°C)	5	96.0 ± 4.0	88.0 ± 4.9	37.0 ± 0.5	21	7.4 ± 0.5	87.1 ± 2.4	0.088 ± 0.008

studies have shown that overexposure of hosts to host-killing treatment resulted in reduced host suitability (Hu et al. 1999, Hwang and Chen 2004). Hwang and Chen (2004) reported that *E. appendigaster* parasitized both heat- and freeze-killed oothecae (50 and −16°C, respectively). However, emergence rates of this evaniid wasp decreased as exposure time (ranging from 6 to 36 h) to heating and freezing increased. Hu et al. (1999) also showed that acceptability and suitability of freeze-killed eggs of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), as hosts for a eulophid egg parasitoid, *Edovum puttleri* Grissell, reduced when exposure time to −20°C increased from 5 to ≥10 min. It is likely that the nutrients required by the parasitoid larvae to complete development may deteriorate under prolonged host-killing process.

In our study, in addition to freezing, minimum exposure of hosts to heating also proved to provide another effective way to produce suitable killed hosts. The results of Hwang and Chen (2004) showed similarly that heat-killed oothecae (50°C for 6 h) can be as suitable as live oothecae for *E. appendigaster* development. Application of heating to kill hosts also was reported by Geden and Kaufman (2007) on pupae of the house fly, *Musca domestica* L. They produced pupal parasitoids *Spalangia cameroni* Perkins and *Muscidifurax raptor* Girault & Sanders by using fly pupae heat killed at the minimum combination of temperature and duration (55°C for 15 min). They even maintained the quality of these pupae for a period up to 4 mo for *M. raptor* and up to 2 mo for *S. cameroni* by storage in a refrigerator (4°C).

The time required for *A. hagenowii* to develop in both freeze- and heat-killed oothecae was similar to that in live oothecae. Suiter et al. (1998) and Bressan-Nascimento et al. (2008) also found that developmental times of *A. hagenowii* in frozen oothecae was the same as in live oothecae. However, the mean developmental time for all types of oothecae was 38.1 d in this study, which was longer than the 28.8 d reported in Suiter et al. (1998) and shorter compared with 43.6 d in Bressan-Nascimento et al. (2008). Varying developmental times of *A. hagenowii*, ranging from 22 to 90 d, have been reported (Roth and Willis 1960). Variation in biological strains and environmental factors (especially temperature) may contribute to the differences in biology and behaviors of *A. hagenowii* (Cameron 1955, Narasimham 1984). Therefore, determination of the developmental time is critical for a proper schedule of parasitoid colony maintenance and field release program.

Knowledge of the effects of host quality on parasitoid wasp production is essential for the use of parasitoids in biological control (King 2002). When rearing parasitoid wasps for insect control programs, hosts that produce larger proportion of offspring and a female-biased sex ratio are preferable (King 2002). Results from this study showed that the number of progeny and the female-biased sex ratio in freeze- and heat-killed oothecae remained similar to that in live oothecae. With fewer viable oothecae, Suiter et al. (1998) also found that the number of progeny and the female-biased sex ratio of parasitoids emerged from frozen oothecae did not differ significantly from those of live oothecae. In their study, one frozen ootheca exposed to a mated oviposition-inexperienced female produced an average of seven male and 67 female wasps (1:9.6). Nevertheless, in the mass production process described by Hagenbuch et al. (1988), oothecae which were subjected to superparasitism produced fewer female-biased clutches of offspring, with an average 27 male and 30 female wasps (1:1.1). Thus, knowledge on the effects of different combination of parasitoid and host densities on the production of parasitoid is needed to investigate for an effective mass rearing of *A. hagenowii* to produce larger number of clutches that are female biased.

In conclusion, we demonstrated that a minimum exposure of oothecae to heating at 48°C and freezing at −20°C for 30 min were sufficient to terminate embryonic development of *P. americana*, while maintaining their suitability as hosts for *A. hagenowii*.

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