Effects of Four Physical Treatments of Oothecae of *Periplaneta americana* on Parasitism and Development of Parasitic Wasp *Evania appendigaster*

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ABSTRACT *Periplaneta americana* (L.) is one of the major hygienic pests distributed worldwide. *Evania appendigaster* (L.) is a parasitic wasp species of oothecae of *P. americana* and could, therefore, be used in the control of *P. americana*. Before releasing the parasitized oothecae, however, it is important to inhibit embryogenesis of *P. americana* to prevent them from hatching. Hence, the objective of this study was to investigate the effect of four physical treatments (freezing, heating, UV irradiation, and gamma irradiation) on the hatchability of the treated oothecae and also to measure the parasitism rate, emergence rate, and developmental time of *E. appendigaster* in the treated oothecae. The results revealed that *P. americana* hatched from UV-treated oothecae, whereas the eggs receiving the other three treatments did not hatch. The results also indicated that, except for the oven-heated oothecae, those receiving the other three treatments had no effect on the parasitism rate of *E. appendigaster*. In addition, the freezing treatment had the highest impact on the emergence rate and developmental times of *E. appendigaster*, with <20% emergence. Overall, our results suggested that gamma irradiation had the lowest impact on the parasitism rate, emergence rate, and the developmental times of *E. appendigaster*. The application of parasitized oothecae to *P. americana* habitats, such as sewer networks, together with bait control, may effectively reduce the population of *P. americana*.

KEY WORDS *Periplaneta americana*, *Evania appendigaster*, freezing, heating, UV irradiation, gamma irradiation

AMERICAN COCKROACH, *Periplaneta americana* (L.), is one of the major hygienic pests distributed worldwide. This cockroach has a wide range of natural enemies (Cameron 1955, 1957; Edmunds 1955, Piper et al. 1978; Hagenbuch et al. 1988, 1989; Lebeck 1991; Rust 1999). Of these, the most important invertebrate enemies of *P. americana* seem to be hymenopterous oothecal parasitoids (Cameron 1955). Because of their generally small size and well-developed searching ability, oothecal parasitoids are usually considered suitable for urban cockroach control (Hagenbuch et al. 1988). The most widely distributed oothecal parasitoids of *P. americana* include *Evania appendigaster* (L.) (Cameron 1955), *Tetrastichus hagenowii* (Ratzeburg) (Roth and Willis 1954; Piper et al. 1978), and *Prosevania punctata* (Brulle) (Edmunds 1954).

Evania appendigaster (L.) is a solitary cockroach oothecae parasitoid, with a global distribution (Cameron 1957; Lebeck 1991). Its biology and behavior have been well studied (Cameron 1955, 1957; Edmunds 1955; Kumarasinghe and Edirisinghe 1987; Lit 1988; Lebeck 1991). *E. appendigaster* is able to parasitize all important cockroach pests except for the brownbanded cockroach, *Supella longipalpa* (F.), and the German cockroach, *Blattella germanica* (L.) (Roth and Willis 1960). Cameron (1957) reported that *E. appendigaster* had a significant impact on cockroach populations, and field parasitization of *P. americana* oothecae ranged from 25 to 29%. This evidence suggests that *E. appendigaster* is, potentially, a desirable biological control agent for *P. americana* oothecae.

The primary aspect related to biological control, through the release of natural enemies, is mass production of the entomophagous insects (Van den Bosch et al. 1973). Mass production of *E. appendigaster*, *P. americana*, and host oothecae is, therefore, the first stage in developing a successful biological control program for cockroaches. To optimally rear and maintain a colony of *E. appendigaster*, the oothecae of *P. americana* were used as the host. Before being parasitized by *E. appendigaster*, the host oothecae had to be treated to both preserve the host eggs and destroy the developing embryo. Little is known, however, about the effects on *E. appendigaster* of various physical and chemical manipulations of the *P. americana* oothecae. Hence, the objective of this study was to investigate the effect of four different treatments (freezing, heating, UV irradiation, and gamma irradiation) on the

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hatchability of the treated oothecae, as well as the parasitism rate, emergence rate, and developmental time of the *E. appendigaster* in the treated oothecae.

Materials and Methods

Cockroach Rearing. To mass generate oothecal parasitoids, methods for rearing *P. americana* were developed to ensure a sufficient supply of oothecae while maintaining stock colonies of cockroaches (Hagenbuch et al. 1988). *P. americana* adults were captured from a local farmer's market in Taichung, Taiwan, during fall 2001. Ten male and 40 female cockroaches were placed into a plastic rearing container (60 by 30 by 35 cm^3) with a screen cover and placed in a walk-in growth chamber at 27 ± 1°C, 80 ± 10% RH with a photoperiod of 12:12 (L:D) h. The cockroaches were provided with vials (250 ml) of water and cat chow, as well as toilet paper core tubes for harborage. Because previous studies have indicated that the female *P. americana* prefer to deposit her oothecae into styrofoam board (Yeh 1995), a piece of Styrofoam board (≈2 cm in thickness) was placed on the rearing container for female *P. americana* to deposit oothecae; fresh oothecae were obtained daily from the Styrofoam board.

Parasitoid Rearing. *E. appendigaster* parasitoids were reared in the laboratory in chambers similar to those used for the cockroaches. Adult *E. appendigaster* were collected at the campus of the National Chung Hsing University, Taiwan. These parasitoid wasps were put into a glass container (12 cm in diameter by 20 cm in height) for mating and were provided with American cockroach oothecae for ovipositing. Newly emerged adults were reared individually in 250-ml plastic cups and provided with 10% sugar water. Every day, one to three cockroach oothecae were provided to each individual mated female *E. appendigaster*, to deposit her eggs. The newly parasitized oothecae were collected to be used for bioassays.

Effects on Parasitism of Freezing Host Oothecae. To determine the optimal duration for the freezing treatment to inhibit hatching, and also to measure the parasitism rate, emergence rate, and developmental time of the *E. appendigaster* in the treated oothecae, vials containing fresh American cockroach oothecae (within 24 h after deposition) were placed in a freezer at −16°C for 6, 12, 18, 24, 30, and 36 h. Oothecae, which had not been frozen, served as the control. Four vials (replicates) of oothecae (five per vial for a total of 140 oothecae) were each randomly assigned to one of the seven freezing regimes at −16°C for 0, 6, 12, 18, 24, 30, and 36 h. After the freezing treatment, each individual ootheca was placed into an empty rearing cup and a mated female *E. appendigaster* was released into the cup to oviposit into the ootheca. Oviposition behavior of *E. appendigaster* was recorded with videocamera to confirm completion of oviposition. One hour later, each individual ootheca was removed and placed in a test tube (1 by 9 cm), which was capped with cotton and placed in a Percival growth chamber (27°C). The rates of parasitism, emergence, and developmental time of the *E. appendigaster* were determined. In addition, to determine the effect of the length of freezing on oothecal development, another set of oothecae were treated, as described previously (without parasitism), placed in a Percival growth chamber (27°C), and the emergence of *P. americana* recorded. All the performance parameters of *E. appendigaster* (rates of parasitism, emergence, and developmental time) were arcsine transformed and analysis of variance (ANOVA) (PROC GLM, SAS Institute 1988) was performed, followed by comparisons of means using Tukey's studentized range (honestly significant difference [HSD]) test.

Effects on Parasitism of Heating Host Oothecae. To assess the optimal duration for heating on hatchability and also to measure the parasitism rate, emergence rate and developmental time of the *E. appendigaster* in the treated oothecae, the American cockroach oothecae were heated in an oven at 50°C for 6, 12, 18, 24, 30, and 36 h. As was done in the freezing experiment, oothecae with no heating treatment served as the control. Four vials (replicates) of oothecae (five per vial for a total of 140 oothecae) were each randomly assigned to one of the seven heating treatments at 50°C for 0, 6, 12, 18, 24, 30, and 36 h. After the heating treatment, the subsequent procedure for each individual ootheca was the same as was done in the freezing treatment. After the treatment, the rates of parasitism, emergence, and developmental time of the *E. appendigaster* also were measured. Again, as with the freezing treatment, another set of oothecae also were heated (without parasitism) to evaluate the length of heating time on oothecal development. All the performance parameters of *E. appendigaster* (rates of parasitism, emergence, and developmental time) were arcsine transformed and ANOVA (PROC GLM, SAS Institute 1988) was performed, followed by comparisons of means using Tukey's studentized range (HSD) test.

Effects on Parasitism of UV-Irradiated Host Oothecae. To determine the effects of different lengths of UV exposure on hatchability and also to measure the parasitism rate, emergence rate, and developmental time of *E. appendigaster* in the treated oothecae, the cockroach oothecae were irradiated with UV light for durations of 0, 10, 30, 60, 90,120, and 150 min. Oothecae that had not been irradiated served as the controls. Four vials (replicates) of oothecae (five per vial for a total of 140 oothecae) were each randomly assigned to one of the seven irradiation treatments (0, 10, 30, 60, 90, 120, and 150 min). Oothecae were irradiated with UV in a UVE Cabinet (model CC10, UVP, Upland, CA), with a mineralight UV lamp, which emitted radiant energy at 254 nm. After the irradiation treatment, the subsequent procedure for each individual ootheca was the same as was done in the freezing treatment. After the treatment, the rates of parasitism, emergence, and developmental time of the *E. appendigaster* also were measured. In addition, as with the freezing treatment, another set of oothecae also were irradiated (without parasitism) to evaluate the length of irradiation on oothecal development. All the per-

formance parameters of *E. appendigaster* (rates of parasitism, emergence, and developmental time) were arcsine transformed and ANOVA (PROC GLM, SAS Institute 1988) was performed, followed by comparisons of means by Tukey's studentized range (HSD) test.

Effects on Parasitism of Gamma-Irradiated Host Oothecae. To assess the effects of radiation treatment on hatchability and also to measure the parasitism rate, emergence rate, and developmental time of *E. appendigaster* in the treated oothecae, the cockroach oothecae were irradiated with various doses of gamma irradiation: 20, 40, 160, and 320 Gy. Oothecae, that had not been irradiated, served as the control. Four vials (replicates) of oothecae (five per vial for a total of 100 oothecae) were each randomly assigned to one of five irradiation treatments of 0, 20, 40, 160, and 320 Gy. Irradiation was applied via a Gammacell irradiator equipped with cobalt 60 containing 26,060 curies, at the Laboratory of Nuclear Science and Technology Development Center, National Tsing Hua University, Taiwan. As with the previous treatments, after irradiation the subsequent procedure for each individual ootheca was same as was done in the freezing treatment. After the treatment, the rates of parasitism, emergence, and developmental time of the *E. appendigaster* also were measured. Likewise, as with the freezing treatment, another set of oothecae also were irradiated (without parasitism) to evaluate the dose of irradiation on oothecal development. All the performance parameters of *E. appendigaster* (rates of parasitism, emergence, and developmental time) were arcsine transformed and ANOVA (PROC GLM, SAS Institute 1988) was performed, followed by comparisons of means using Tukey's studentized range (HSD) test.

Results

Freezing Effects on Parasitism.

Freezing at −16°C terminated the embryogenesis of *P. americana* eggs; after an exposure of 6 h to a temperature of −16°C, no *P. americana* nymphs hatched.

E. appendigaster laid their eggs in host oothecae that had been frozen as well as in those not exposed to freezing. The parasitism rates of the oothecae were not significantly different between the different exposure periods (Table 1). Longer periods of exposure to a temperature of −16°C, however, reduced host suitability. The rate of adult emergence of *E. appendigaster* was significantly ($P < 0.001$) higher (93.75%) when oothecae, not exposed to freezing, were used as the host (Table 1). Emergence rates decreased with increased exposure to a temperature of −16°C (Table 1). The results also indicated that the developmental period of *E. appendigaster*, in differently treated oothecae, was not significantly different ($P = 0.102$) (Table 1).

Table 1. Effects of freezing (−16°C) on parasitism by *E. appendigaster* on oothecae of American cockroach

Treatment (h)	Means ± SE		
	Parasitism (%)	Emergence (%)	Development (d)
0	95.00 ± 5.00	93.75 ± 6.25a	42.78 ± 0.54
6	95.00 ± 5.00	33.75 ± 15.99b	46.17 ± 1.64
12	100.0 ± 0.00	20.00 ± 14.14b	47.50 ± 3.43
18	95.00 ± 5.00	22.50 ± 13.15b	45.00 ± 1.78
24	100.0 ± 0.00	10.00 ± 10.00b	48.00 ± 0.00
30	100.0 ± 0.00	10.00 ± 5.77b	45.50 ± 1.50
36	95.00 ± 5.00	10.00 ± 5.77b	44.00 ± 0.00
F value	0.50	6.12	0.20
df	27	27	27
P value	0.801	< 0.001	0.102

Within a column, means bearing the same letter are not significantly different ($P > 0.05$).

Heating Effects on Parasitism.

Heating at 50°C can terminate the embryogenesis of *P. americana* eggs. After a 6-h exposure to 50°C, no *P. americana* nymphs hatched.

It also was revealed that long periods of exposure to 50°C did not change the acceptability of the host oothecae to *E. appendigaster*. Parasitism rates were not significantly different ($P = 0.163$) among the different treatment periods (Table 2). The emergence rate of adult *E. appendigaster*, however, varied significantly between the different heating periods ($P < 0.001$) (Table 2). Emergence rates decreased with increasing length of exposure to 50°C temperatures (Table 2). However, the developmental period of *E. appendigaster* in differently treated oothecae was not significantly different ($P = 0.333$) (Table 2).

UV Irradiation Effects on Parasitism. In contrast to the results of the previous two treatments, UV irradiation had no effect on the embryogenesis of *P. americana* eggs. After a 150-min exposure to UV irradiation, all of the *P. americana* nymphs hatched from the treated oothecae.

The effects of UV irradiation of *P. americana* oothecae on parasitization and development of *E. appendigaster* are provided in Table 3. For all durations tested, irradiated and nonirradiated host oothecae

Table 2. Effects of heating (50°C) on parasitism by *E. appendigaster* on oothecae of American cockroach

Treatment (h)	Mean ± SE		
	Parasitism (%)	Emergence (%)	Development (d)
0	95.00 ± 5.00	83.75 ± 9.87a	42.24 ± 0.30
6	85.00 ± 9.57	81.67 ± 6.87ab	42.00 ± 0.51
12	90.00 ± 10.00	66.67 ± 9.43abc	42.17 ± 0.32
18	65.00 ± 15.00	40.00 ± 13.54bc	42.00 ± 0.45
24	85.00 ± 5.00	16.25 ± 9.87c	43.00 ± 1.00
30	65.00 ± 5.00	29.17 ± 10.49bc	40.33 ± 0.33
36	90.00 ± 5.77	17.50 ± 5.95c	41.33 ± 0.33
F value	1.73	6.01	1.18
df	27	27	27
P value	0.163	< 0.001	0.333

Within a column, means bearing the same letter(s) are not significantly different ($P > 0.05$).

Table 3. Effects of UV irradiation (254 nm) on parasitism by *E. appendigaster* on oothecae of American cockroach

Treatment (min)	Means ± SE		
	Parasitism (%)	Emergence (%)	Development (d)
0	90.00 ± 10.0	90.00 ± 5.77	41.06 ± 0.85a
10	100.0 ± 0.00	100.0 ± 0.00	42.30 ± 0.52a
30	90.00 ± 10.0	90.00 ± 5.77	41.88 ± 1.02a
60	95.00 ± 5.00	85.00 ± 9.57	38.00 ± 0.37b
90	95.00 ± 5.00	100.0 ± 0.00	42.21 ± 0.73a
120	100.0 ± 0.00	100.0 ± 0.00	41.45 ± 0.43a
150	100.0 ± 0.00	95.00 ± 5.00	42.79 ± 0.69a
F value	0.52	1.42	5.19
df	27	27	27
P value	0.784	0.253	<0.001

Within a column, means bearing the same letter are not significantly different ($P > 0.05$).

were equally well accepted by ovipositing females ($P = 0.784$) (Table 3). In addition, the suitability of the hosts, based on percentage of adult emergence of *E. appendigaster*, was not significantly different between irradiated and nonirradiated host oothecae ($P = 0.253$) (Table 3). The developmental period of *E. appendigaster* also was found to be similar among the differently treated oothecae (Table 3).

Gamma Irradiation Effects on Parasitism. Iirradiation can terminate the embryogenesis of *P. americana* eggs. No *P. americana* nymphs hatched after treatment with ≥20 Gy.

E. appendigaster laid their eggs in host oothecae that had been irradiated as well as in those receiving no irradiation. The parasitism rates were not significantly different between the different treatment doses (Table 4). The rate of adult emergence and developmental period of the *E. appendigaster*, in differently treated oothecae, also were not markedly different among the different treatment doses (Table 4).

In comparing the four treatments (freezing, heating, UV irradiation, and gamma irradiation), a significantly higher effect on emergence rate was found than on the parasitism rate or the developmental period of *E. appendigaster* (Table 5). Parasitism rates were especially high (>96%) for three of the treatments, freezing, UV irradiation, and gamma irradiation, whereas the parasitism rate for the heated oothecae was lower (80%). The emergence rate, however, varied significantly, depending on the treatment. The emergence rate of *E. appendigaster* was very high (>87%) from UV- and gamma-irradiated oothecae, but very low from frozen oothecae. The developmental period of *E. appendigaster* was similar for the heated, UV-, and gamma-irradiated (42 d) host oothecae, but longer for the ones exposed to freezing (46 d).

Discussion

One of the primary prerequisites for the field release of parasitoids from laboratory-parasitized hosts is to prevent further cockroach infestation from failed laboratory parasitism. Our results indicate that treatment by freezing, heating, and gamma irradiation can

Table 4. Effects of gamma irradiation on parasitism by *E. appendigaster* on oothecae of American cockroach

Treatment dosage (Gy)	Means ± SE		
	Parasitism (%)	Emergence (%)	Development (d)
0	100.0 ± 0.00	90.00 ± 5.77	41.50 ± 0.27
20	95.00 ± 5.00	88.75 ± 6.57	42.24 ± 0.30
40	100.0 ± 0.00	75.00 ± 9.57	42.07 ± 0.50
160	100.0 ± 0.00	95.00 ± 5.00	42.58 ± 0.31
320	100.0 ± 0.00	90.00 ± 5.77	41.72 ± 0.25
F value	1.00	0.81	1.77
df	19	19	19
P value	0.438	0.540	0.142

effectively terminate host embryogenesis. The results also reveal that there is no difference in the parasitism rate and developmental period of *E. appendigaster* between different treatments, whereas the emergence rates vary significantly.

Freezing is a common method of killing host embryos in biological control programs. In our study, 6 h of exposure to −16°C terminated embryogenesis of *P. americana* eggs. The minimum time required to terminate embryonic development of *P. americana* eggs, however, was not identified in this study. Results from other studies also have indicated that exposing eggs to freezing is a viable alternative for killing host embryos (Maini and Nicoli 1990; Nagarkatti et al. 1991; Suiter et al. 1998). Although it is not clear why freezing stops embryogenesis, the freezing process typically denatures large proteins and kills embryos (Hu et al. 1999). In addition, because freezing can change the physical, biochemical, and physiological characteristics of the host, it was anticipated that host acceptability and suitability would be reduced after such treatment (Hu et al. 1999). Hu et al. (1999) reported that the parasitism rates of *Edovum puttleri* Grissell, in Colorado potato beetle, *Leptinotarsa decemlineata* (Say), decreased after freezing for 5 min at −20°C. In our experiments, the parasitism rates of *E. appendigaster*, however, were similar among the controls and the oothecae that had been frozen. Although >90% of the *P. americana* eggs were still successfully parasitized after 36 h of freezing, the hatching rates of the *E. appendigaster* decreased significantly with the

Table 5. Mean parasitism rate, emergence rate, and developmental period of *E. appendigaster* on differently treated oothecae of American cockroach

Treatment	Mean ± SE		
	Parasitism (%)	Emergence (%)	Development (d)
Freezing	97.50 ± 1.38a	17.70 ± 4.54c	46.10 ± 0.88a
Heating	80.00 ± 3.99b	41.88 ± 6.20b	41.95 ± 0.23b
UV irradiation	96.67 ± 1.97a	95.00 ± 2.17a	41.53 ± 0.30b
Gamma irradiation	98.75 ± 1.25a	87.19 ± 3.65a	42.16 ± 0.17b
F value	3.01	15.76	7.01
df	87	87	239
P value	<0.001	<0.001	<0.001

Within a column, means bearing the same letter are not significantly different ($P > 0.05$).

length of freezing time. After 6 h of freezing, adult *E. appendigaster* emerged from only 30% of the parasitized oothecae. Thus, freezing may reduce the nutrient quality of the host oothecae and make it more difficult for the developing parasitoid to complete its development (Hu et al. 1999). Developmental time of *E. appendigaster* was similar between the controls and the host oothecae that had been frozen. Studies with beetles and other insects also have indicated a similar developmental time for the parasitoid *Aprostocetus hagenowii* Ratzeburg and *E. puttleir* in eggs that had been frozen (Maini and Nicoli 1990; Suiter et al. 1998).

The exposure of eggs to heating may be another approach to kill host embryos (Ashley et al. 1974). In our experiments, only 6 h of exposure to temperatures of 50°C was required to terminate embryonic development. As with the freezing treatment, heating also may change the physical, biochemical, and physiological characteristics of the hosts, and so it was felt that host acceptability and suitability would be reduced after such treatment. Our results were consistent with the prediction that the parasitism rates of *E. appendigaster* in *P. americana* oothecae decreased slightly as heating time increased. In addition, emergence rates of *E. appendigaster* decreased with increased heating periods. After 18 h of heating, <40% of the *E. appendigaster* adults emerged from the parasitized oothecae. Developmental time of *E. appendigaster* also was similar between the controls and the oothecae that had been heated. Thus, heating at 50°C for ≈6 h can be an effective method of both killing *P. americana* oothecae and maintaining its quality as a parasitic host.

Short wavelength (254 nm) UV irradiation has been frequently used in mass rearing insectaries to prevent host eggs from developing beyond a stage that is appropriate for parasitization (Palmer 1996). In general, the effects of UV irradiation differ, depending on the length of time irradiated and the developmental stage of the treated eggs (Hu et al. 1999). Unlike the results of the other three treatments studied, UV irradiation did not influence the embryogenesis of *P. americana* eggs, even for a duration of up to 150 min. Thus, the acceptability of the host egg (i.e., for parasitization), and the suitability of the host for parasitoid development, were similar between the controls and the eggs receiving UV irradiation treatment. This finding differs from other studies that have reported that UV irradiation can terminate host egg embryogenesis and affect host quality for parasitization (Calderon and Navarro 1971; Goldstein et al. 1983; Hu et al. 1999). Hu et al. (1999) have suggested that the variation in insect eggs to UV sensitivity may be due to egg structures or rates of embryonic development. Because UV is a nonpenetrating radiation (Calderon and Navarro 1971), it may not be able to penetrate the oothecae and affect *P. americana* eggs.

Use of gamma irradiation to prevent host emergence has been reported in many previous studies (Brower 1982; Morgan et al. 1986; Burditt and Hungate 1988; Goodwin and Wellham 1990; Johnson et al. 1990; Toba and Burditt 1992; Suiter et al. 1998). In our study, the lowest treatment dose (20 Gy) caused severe reduction in the embryogenesis of *P. americana* eggs. This result agrees with findings from previous studies done on the eggs of many other insect species (Brower 1982; Tilton and Brower 1983; Morgan et al. 1986; Burditt and Hungate 1988; Goodwin and Wellham 1990; Johnson et al. 1990; Toba and Burditt 1992; Suiter et al. 1998). Differing from the results of the other three treatments (freezing, drying, and UV irradiation), however, our study indicated that gamma irradiation did not influence the acceptability and suitability of the host for the parasitoid. The rates of parasitism and adult emergence of *E. appendigaster* were similar among the controls and all the treated eggs, regardless of the dose.

In summary, these results clearly indicate that gamma irradiation is the most effective treatment for terminating *P. americana* embryogenesis and retaining egg quality for parasitization. Our results also suggest that short term heating (6 h) are viable alternatives for killing host embryos; the suitability of the host for parasitoid development may be reduced, however. UV irradiation does not seem to be a good candidate for this purpose.

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