Mysore J. Agric. Sci., 58 (3) : 316-324 (2024)

Courtship Behavior and Ecology of Mating in Tea Mosquito Bug, *Helopeltis theivora* Waterhouse (Hemiptera: Miridae)

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Received : May 2024
Accepted : June 2024

Abstract

The tea mosquito bug (TMB), *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) is a major pest of tea in South Indian tea plantations. Since its identification, extensive work has been done on various aspects from bioecology to control measures. The current study explores the sexual maturity, courtship behavior and effects of mating in tea mosquitoes. The sequential courtship behaviors of tea mosquito bugs were observed. For the mating experiments, the treatments namely T1- virgin females, T2- single-mated females, T3- multiple-mated females were studied under laboratory conditions at 19 to 25.4º C with 59 to 89 per cent RH and a photoperiod of 13L: 11D. Biological parameters such as fecundity, incubation period, hatching percentage and adult emerging percentage were recorded during the study period. The experimental results showed that the sexual maturity period for male tea mosquito bugs was one-day, while for females, it was three days. Courtship behaviors including arousal, mounting, copulation and termination were observed. The mean mating duration was 107 ± 4.56 minutes. Fecundity differed significantly (P=0.001) between single-mated and multiple-mated females. Virgin females laid unfertilized eggs during their life span, none of which hatched. A significant difference in female longevity was observed among the virgin, single-mated and multiple-mated females (P=0.001). The study revealed that there is no parthenogenesis in TMB and a single-mating is sufficient to produce maximum and viable offspring.

Keywords : Biology, Fecundity, Fertility, Reproduction, Sexual maturity, Tea mosquito bug

The tea mosquito bug (TMB), *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) is a significant pest of tea in the mid-elevation areas of South Indian tea-growing districts such as Vandiperiyar (Kerala) and Valparai (Tamil Nadu). The infestation caused approximately 17-100 per cent crop loss in South India (Muraleedharan, 1992). Aside from tea, TMB also damages various economic plants such as cocoa, cashew, acacia, pepper and camphor. Researchers have found that TMB can complete its life cycle in a wide range of weeds as well. Several studies have been conducted by various researchers on various aspects of TMB, including biology, life history, host range, feeding behavior, damage potential, crop loss, seasonal abundance, cultural control, biological control, botanical pesticides and sex pheromones (Somnath *et al*., 2015). Research on reproductive biology offers valuable insights that can aid in preventing pest dispersion behavior and developing suitable control strategies (Pallavi *et al.,* 2023 and Sunil & Mohan, 2022). Sudhakaran (2000) conducted some preliminary studies on the reproductive biology of TMB. However, detailed information on the reproductive biology and courtship behavior of TMB remains insufficiently explored. Additionally, understanding the fecundity and longevity of TMB based on the number of matings is a crucial question to address. Knowledge of reproductive behavior is essential for comprehending the life history and population dynamics of any species. To address this gap, this study aims to explore the sexual maturity, courtship behavior and mating behavior of tea mosquitoes.

Material and Methods

Insect Rearing

Adults of *H. theivora* were collected from the experimental farm of the United Planters' Association of Southern India (UPASI) in Valparai and maintained in culture at the Entomology Division, UPASI TRF Tea Research Institute in Valparai, Coimbatore, Tamil Nadu, India. Mass rearing of *H. theivora* was conducted using the method evaluated by Sudhakaran (2000). The insects were fed tea shoots every other day. The tea shoots were carefully examined for the presence of eggs and shoots with eggs were transferred to new plastic rearing containers (30 cm height × 10 cm width). Eggs were monitored daily for hatching, and hatched nymphs were observed until the adult stage. Adults were sexed based on morphological characteristics and virgin males and females were kept separately for the study. All rearing containers were kept at a room temperature of 19 to 23°C with a relative humidity of 75-89 per cent and experiments were conducted under the same temperature and relative humidity conditions.

Sexual Maturity of Male and Female *H. Theivora*

An experiment was conducted to study sexual maturity in *H. theivora*, involving twenty different age combinations of females (1, 2, 3, 4 and 5 days old) and males (1, 2, 3 and 4 days old). Each treatment consisted of a pair of insects and was replicated three times. The paired insects were placed in a plastic rearing container and fed daily as described. After 24 hours, the males were removed and the number of eggs laid was recorded daily for up to ten days, along with the number of nymphs hatched. Each treatment combination was replicated three times.

Courtship Behavior

To study the courtship behavior and copulation frequency of *H. theivora*, ten pairs of newly emerged males and females (<24h after eclosion) were randomly selected from the mass culture unit. They were then allowed to pair in individual plastic container measuring 30cm in height and 10cm in diameter. The paired insects were observed and sequential courtship behaviors such as arousal (sexual excitement or readiness for copulation), mounting (the act of one insect climbing onto the back of another in preparation for mating), copulation (sexual intercourse between male and female), termination (end of copulation) and post-copulatory behavior (behavior after successful mating) were recorded. Additionally, the pre-copulatory period, time taken for the formation of a stable pair, mating duration and copulation frequencies were all recorded. Observations were recorded daily from 8:00 to 20:00 hours, excluding the scotophase.

Mating Experiments

Virgin Females Alone

Virgin females (<24h after the final molt; *n*=20) were randomly collected from a mass-rearing unit and kept individually in a plastic container (30cm height × 10cm diameter). They were not allowed to mate throughout their lifespan. Parameters such as longevity, fecundity and hatchability were recorded daily until their death.

Single-Mated Females

Twenty virgin females and males (<24h after the final molt) were randomly collected and placed in pairs in individual plastic containers (30cm height × 10cm diameter). To prevent a second mating, the paired insects were observed every 30 minutes from 8:00 to 20:00 hours. Once successful mating occurred, each male was removed and the females were then observed individually to record parameters such as longevity, fecundity and fertility. Fifteen females that successfully mated were included in the data analysis.

Multiple-Mated Females

Virgin females and males (<24h after the final molt; *n*=20) were randomly collected and placed together in individual plastic containers (30cm height × 10cm diameter). The paired insects were kept together until death to increase mating frequencies. The pairs were monitored every hour from 8:00 to 20:00 for the first 10 days. Fifteen pairs that mated at least twice during the observation period were included in the data analysis.

Statistical Analysis

The data from the sexual maturity study and mating experiments were pooled and subjected to analysis of variance (*ANOVA*). The means were compared at a significance level of 5 per cent using Duncan's multiple range test (DMRT). Biological parameters including incubation period, nymphal durations, total developmental period and nymphal survival percentage were analyzed using an independent *t*-test. All statistical analyses were performed using SPSS v.23 software.

Results and Discussion

Sexual Maturity of Male and Female *H. Theivora*

Reproductive regulation in insects is generally controlled by translating various external and internal stimuli into appropriate physiological and behavioral responses by the central nervous and neuroendocrine systems (Gillot, 2003 and Raikhel *et al.*, 2005). The results of the sexual maturity study are presented in Table 1. The study indicates that females paired with one-day-old males cannot produce nymphs. However, males that are two days old can produce nymphs with mature females (>2 days old). Similarly, one-day-old and two-day-old females are unable to produce nymphs when mating with males of any age (Table 1). However, three-day-old females can produce nymphs when mated with mature males (>2 days old). From the study, it was found that the sexual maturity period of male and female tea mosquitos was two days and three days, respectively after eclosion and the sexual maturity period was significantly different (df=19, F=347.76, P<0.0001). A similar sexual maturation phenomenon was observed in other hemipterans such as *Trioza erytreae* (Hemiptera: Triozidae) and *Diaphorina citri* (Hemiptera: Psyllidae) (Van Den Berg *et al.*, 1990 and Wenninger & Hall, 2007). The results of this study demonstrate that the sexual maturation of tea mosquitoes is strongly associated with changes in the reproductive physiology of both sexes. The sexual maturity period may be necessary for insects to promote gonadal activity as well as reproductive behaviors to enhance individual fitness. Until this sexual maturity period, neither sex exhibited mating behaviors. In the sorghum plant bug, *Stenotus rubrovittatus* males require sexual maturation for mating, while females are receptive to male courtship when their ovaries are partially developed (Oku *et al.*, 2010).

Table 1

Sexual maturity of the tea mosquito bug, *Helopeltis theivora*

Trt. no.	Treatment combinations (adult age in days)		Mean no. of eggs laid/ female up to 10 days (fecundity)	Mean no. of eggs hatched (fertility)	Mean fertility %
	Male	Female			
1	1	1	27.0	0	0.0
2	1	2	31.7	0	0.0
3	1	3	25.0	0	0.0
4	1	4	42.0	0	0.0
5	1	5	50.7	0	0.0
6	2	1	23.3	0	0.0
7	2	2	30.3	0	0.0
8	2	3	38.3	15.3	40.2
9	2	4	40.3	28.3	70.7
10	2	5	46.0	36.7	80.3
11	3	1	23.3	0	0.0
12	3	2	23.3	0	0.0
13	3	3	39.3	15.7	40.1
14	3	4	47.3	22.0	47.2
15	3	5	52.7	38.0	73.4
16	4	1	25.7	0	0.0
17	4	2	35.3	0	0.0
18	4	3	45.3	12.3	27.6
19	4	4	49.3	26.3	54.0
20	4	5	50.3	34.7	69.6
SEM			1.472	1.544	3.136
CD @ 5%			5.771	6.051	12.292

Courtship Behavior

The tea mosquitoes exhibited courtship behavior mainly in the morning (8:00-11:30) and evening (14:00-18:00), but never displayed courtship behavior between 11:30 and 14:00.

Arousal

Most of the mating behavior is initiated by male TMBs, with females rarely taking the lead. When males initiate the sequences, they begin by approaching females either by walking or flying. Once a male is near a female, it will use its antennae to probe the female's body. If the female responds positively, the mating sequence will continue. However, most of the time, females signal negatively and attempts to move away from the male. On the other hand, if a female initiates the mating sequences by releasing its sex pheromones, multiple males are attracted to the single female. In this scenario, all the males will attempt to mate with the female TMB at a time. At the end of this mating competition, a single mating will occur or mating may not occur at all. The duration of this stage was found to be 1.43 ± 0.24 minutes with a range of 1 - 1.8 minutes.

Mounting

After receiving a positive signal during arousal, the male attempts to mount the female from the dorsal region of the female. The duration of this phase was 57.47 ± 7.56 seconds (range 42 - 67 sec.). During this phase, the male's aedeagus become erect and bends the tip of its abdomen towards the female's genital aperture to facilitate insertion. In some cases, the female may bend her abdomen towards the ventral side to prevent the male from inserting. In response, the male will move aside slightly and continue to attempt mounting multiple times. However, most of the time, female reject mating and do not allow the male to successfully mount.

Copulation and Termination

After successful mounting, the male's genitalia rotates from side to side and ends up in an 'end to end' position. The coupled pairs remain static and do not feed till the completion of copulation. Any disturbance during copulation does not lead to termination and occasionally, couples in copula move away with the female leading the way and the male following. Termination of copulation begins after the successful completion of copulation. During the termination process, both insects mutually try to release their pairing and walk away from each other. Sometimes, a male's aedeagus is unable to withdraw from the female's genital aperture during the termination process, leading to the death of the male. After a successful termination, both sexes clean their appendages and antennae and then begin feeding vigorously. Females do not attempt to mate again for a while.

In the present study, courtship behavior in TMB was initiated by males. Additionally, females naturally produce sex pheromones to attract males (Sachin *et al*., 2008 and Bharathi *et al*., 2022). The aggressive courtship behavior of males might be due to the release of sex pheromones by females. However, there are no detailed studies on the production and timing of sex pheromone release in females. Similarly, courtship behavior was exhibited by various males soon after the release of sex pheromones. Thube *et al*. (2019) reported that the courtship behavior of tea mosquito bugs predominantly occurs during the morning hours, which may be due to the courtship pheromone activity of the females. Courtship behavior significantly influences mating status in many insects (Gillot, 2003 and Gemeno *et al*., 2007). Strong *et al*. (1970) reported that the interest of male *Lygus hesperus* in mating was completely associated with the development of accessory glands.

During the study, a total of 76 mating attempts were observed, with 68.42 per cent (52 instances) resulting in successful mating and 31.58 per cent in mating rejections. All mating rejections were exhibited by females. Out of the 52 successful matings, 25 matings were randomly selected for data analysis. It was found that the mean time taken for the formation of a stable pair was 1.45 ± 1.10 minutes and the mean mating duration (after successful genital connection) was 107 ± 4.56 minutes. Various researchers have reported

TABLE 2

The biological parameters of single-mated, multiple-mated and virgin females of the tea mosquito bug

Treatments	Female longevity	Mean fecundity	Fertility %
Single-matted females	41.9 ± 3.87 [b]	297.8 ± 10.13 [ab]	50.95 ± 9.49 [b]
Multiple-matted females	43.3 ± 3.47 [b]	322.3 ± 17.96 [b]	80.84 ± 1.47 [c]
Virgin females	37.1 ± 2.77 [a]	286.5 ± 16.45 [a]	0 [a]

Mean ±SD (n=15) followed by the same letter in the vertical column is not significant at the 5 per cent level according to Duncan's Multiple Range Test (DMRT)

different mating durations in Miridae such as two minutes in *Lygocoris pabulinus* (Groot *et al*., 1998), 15 minutes in *Cyrtorhinus lividipennis* (Liquido and Nishida, 1985), 37 minutes in *Trigonotylus caelestialium* (Kakizaki and Sugie, 1997) and up to three hours in *Nesiodiocoris caesar* (Chatterjee, 1984). An average of 31.58 per cent of mating rejections were observed in the present study, mostly exhibited by females. This rejection rate may be normal in all insects. A very high rejection rate was observed in *Murgantia histrionica* (Zahn *et al*., 2008) and *Edessa meditabunda* (Silva *et al*., 2012).

Mating Experiments

The pre-oviposition period for virgin females, single-mated females and multiple-mated females was one day and not significantly different ($p > 0.05$). The mean fecundity of virgin, single-mated and multiple-mated females was 286.5, 297.8 and 322.3 eggs, respectively with a significantly different fecundity rate ($df = 44$, $F = 9.248$, $P = 0.001$) among the females (Table 2). The hatchability percentage significantly differed ($P = 0.001$) between single-mated and multiple-mated females with the mean hatchability percentage being higher in multiple-mated females (80.84%) compared to single-mated females (50.95%). Virgin females also laid unfertilized eggs throughout their lifespan, but none of the eggs hatched. The average fecundity and fertility percentage of single-mated females and multiple-mated females throughout their life span are represented in Fig. 1 and 2, respectively.

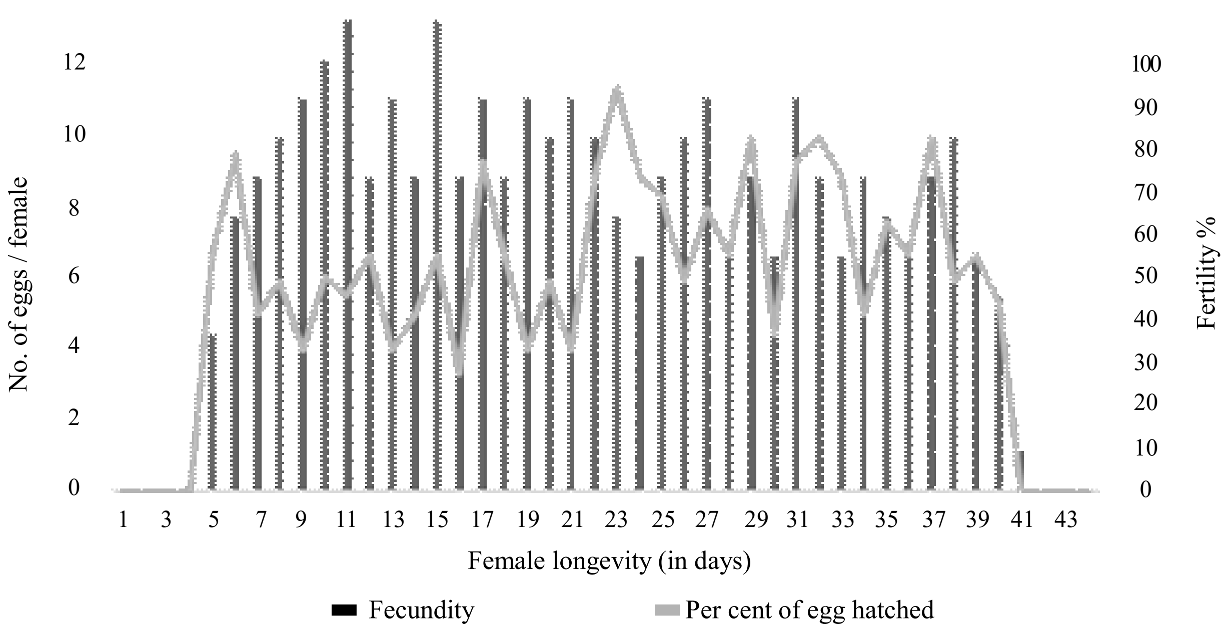

Fig. 1: Mean fecundity and fertility percentage of single-mated females throughout their life span

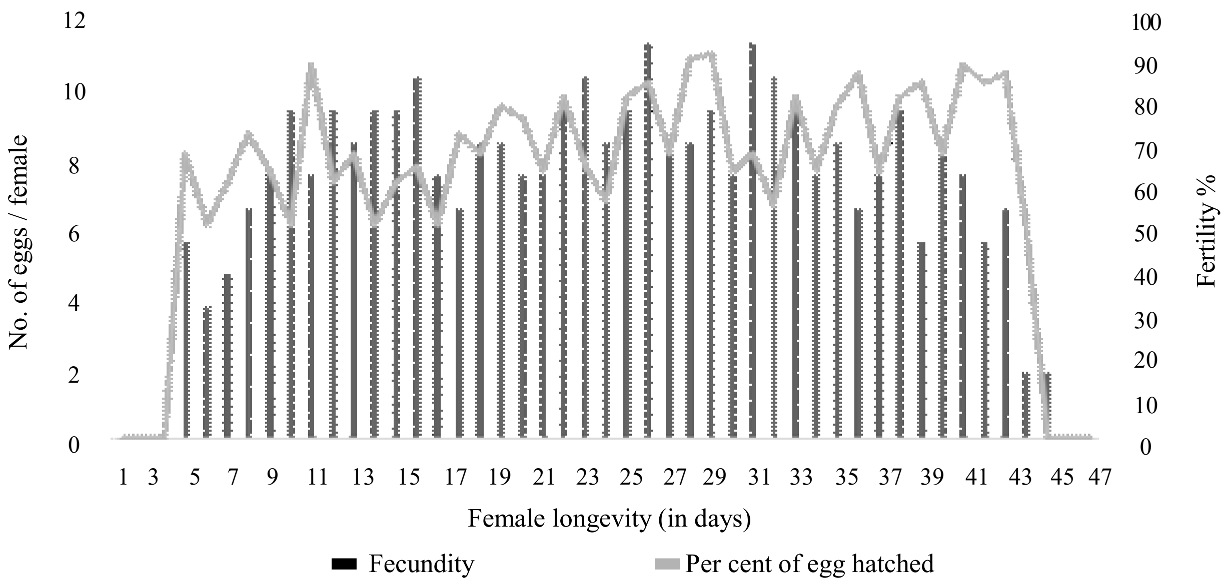

Fig. 2 : Mean fecundity and fertility percentage of multiple-mated females throughout their life span

The biological parameters such as incubation period, nymphal duration and total developmental period of single-mated and multiple-mated females were not significantly different ($P > 0.05$) according to the independent *t*-test (Table 3). Similarly, the nymphal survival percentage and adult emergence were also not significantly different ($P > 0.05$) (Table 4). The study results showed that the number of matings does not affect the growth duration and survival percentage of the early stages of tea mosquitoes.

The adult emergence rate was 73.74 per cent in single-mated females and 79.41 per cent in multiple-mated females. The female-to-male ratio was 1:0.72 and 1:0.78 in single-mated and multiple-mated females, respectively. The hatchability percentage of *H. theivora* was 79.74 per cent in cashew and 66 per cent in cocoa (Srikumar & Bhat, 2012 and Thube *et al.*, 2019). The variation in fertility rate may be due to differences in food and turgor pressure at the oviposition site. The reasonable fecundity and fertility

TABLE 3

Incubation period, nymphal period and developmental periods were observed in single-mated and multiple-mated females of the tea mosquito bug (*n*=15; Mean ± SD)

Particulars	Single-mated female	Multiple-mated female	*t-value*	*P*
Incubation period	8.14 ± 0.49	8.36 ± 0.53	-2.088	>0.05
Nymphal duration				
First	3.67 ± 0.24	3.7 ± 0.30	-0.436	>0.05
Second	3.38 ± 0.41	3.4 ± 0.40	-0.346	>0.05
Third	3.16 ± 0.45	3.11 ± 0.43	0.657	>0.05
Forth	3.3 ± 0.35	3.27 ± 0.35	0.426	>0.05
Fifth	3.35 ± 0.47	3.33 ± 0.46	0.259	>0.05
Total nymphal duration	16.92 ± 0.89	16.81 ± 0.87	0.568	>0.05
Total developmental duration	25.06 ± 1.12	25.18 ± 1.20	-0.517	>0.05

TABLE 4

Percentage of nymphal survival, adult emergence and female-to-male ratio in single-mated and multiple-mated females (*n*=15; Mean ± SD)

Particulars	% Survival		*t-value*	*P*
	Single-mated female	Multiple-mated female		
First instar	95.18 ± 3.28	97.33 ± 1.29	1.362	>0.05
Second instar	90.96 ± 6.48	93.47 ± 2.30	0.779	>0.05
Third instar	86.03 ± 7.29	89.30 ± 4.02	0.879	>0.05
Forth instar	78.99 ± 8.82	84.72 ± 4.16	1.311	>0.05
Fifth instar	73.28 ± 11.04	80.89 ± 4.64	1.416	>0.05
Adult emergence (%)	73.75 ± 10.03	79.41 ± 3.36	1.196	>0.05
Female: Male ratio	1:0.72	1:0.78	-	-

in single-mated females may be attributed to the presence of a spermatheca. Most female insects possess a spermatheca, which plays a crucial role in insect reproduction by allowing for the temporary storage of sperm for an extended period (Parker, 1970). Studies on *Murgantia histrionica* (Hemiptera: Pentatomidae) have shown that the secretory cells of the spermatheca are involved in the production and release of various chemical substances into the lumen of the spermatheca to maintain the viability and mobility of sperm during insemination (Stacconi and Romani, 2010). In addition to the female spermatheca, the male spermatophore also plays a vital role in regulating female fecundity. Increased egg production rates in other species may be due to the secretion of different factors in male accessory glands.

In the mirid bug *L. hesperus*, the size of the spermatophore that males transfer during copulation varies with male age. An average-sized spermatophore contains a significant number of sperm, which may be enough to fertilize eggs while the sperm is viable (Brent, 2010). Single-mated females showed comparable fecundity to multiple-mated females, likely due to the presence of a spermatheca capable of storing sperm for an extended period. The total developmental period was similar between single-mated and multiple-mated females. A significant difference ($df = 29$, $F = 9.153$, $P = 0.001$) in longevity was observed among the evaluated females with the mean longevity of virgin, single-mated and multiple-mated females being 37.1, 41.9 and 43.3 days, respectively. In some cases, repeated mating increases female fecundity but reduces female longevity (Wang & Millar, 2000 and Silva *et al*., 2012).

In conclusion, the sexual maturity period of male and female tea mosquitoes was one day and three days, respectively, after eclosion. The courtship behavior of the tea mosquito bug was well explained. The mating experiments concluded that even a single mating is sufficient to produce viable offspring in the tea mosquito bug. Further detailed studies on the physiology and morphology of male and female reproductive organs would be helpful for a better understanding of the life table and ecology of tea mosquitoes.

Acknowledgement: The authors are thankful to the Director, UPASI Tea Research Foundation, Tea Research Institute, Valparai for his continuous support and encouragement during the work.

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