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The Effect of Mating on The Eggs' Fertility and Fecundity of *Helopeltis antonii* (Heteroptera: Miridae)

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Abstrak: *Helopeltis antonii* merupakan salah satu serangga perosak utama pada pokok gajus (*Anacardium occidentale* L.) yang boleh menyebabkan kerosakan pada pokok tersebut. Perkembangan populasi *H. antonii* dipengaruhi oleh kesuburan dan kadar kesuburan telur-telurnya. Pengaruh persenyawaan keatas kesuburan dan kadar kesuburan telur *H. antonii* telah dikaji. Penyelidikan telah dijalankan di makmal Perkhidmatan ladang daerah Ngadirojo, Wonogiri, Indonesia untuk mengkaji kematangan seksual serangga betina dan jantan, pengaruh nisbah jantina keatas kesuburan telur dan kepanjangan umur betina, dan pengaruh kekerapan persenyawaan keatas kesuburan telur dan penetasan telur. Hasil kajian menunjukkan bahawa betina dan jantan *H. antonii* bersedia untuk melakukan persenyawaan pada umur dua hari. Jumlah telur yang dikeluarkan dan kepanjangan umur betina tidak dipengaruhi oleh nisbah jantina, tetapi nisbah jantina 2:1 dan 1:2 cenderung menghasilkan lebih banyak telur. Kesuburan betina tidak dipengaruhi oleh jumlah persenyawaan. Betina yang tidak disenyawakan menghasilkan kurang bilangan telur berbanding betina yang berpasangan dengan jantan yang matang. Kajian ini menunjukkan betina memerlukan persenyawaan untuk menghasilkan telur yang subur.

Kata kunci: *Helopeltis antonii*, Kesuburan, Kadar Kesuburan, Persenyawaan

Abstract: *Helopeltis antonii* is the major pest affecting cashew plants in Indonesia and causes potential damage to the plant. The development of the population was influenced by the fecundity and fertility of their eggs. The effect of mating on the eggs' fecundity and the fertility of *H. antonii* was studied. Laboratory studies at Wonogiri Estate Service in Ngadirojo District, Wonogiri, Indonesia investigated the sexual maturity, the influence of female to male sex ratio on the females' fecundity and longevity, and the influence of mating frequency to fecundity and the eggs hatchability of *H. antonii*. The study reveals that *H. antonii* females and males are ready to mate when they are two days old. The number of eggs laid and the longevity of the females' lives were not significantly affected by the sex ratio; however, female to male sex ratios of 2:1 and 1:2 tended to produce a greater number of eggs. The fecundity of the female was not significantly influenced by the number of times the female mated. Unmated females laid fewer eggs than females paired with a mature male. The study shows that females need to mate to produce fertile eggs.

Keywords: *Helopeltis antonii*, Fecundity, Fertility, Mating

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INTRODUCTION

The tea mosquito bug *H. antonii* (Heteroptera: Miridae) is one of the major pests affecting the cashew plant in Indonesia. It is also known as a major pest of tea, cocoa (Stonedahl 1991) and neem (Onkarappa & Kumar 1997), and it feeds on allspice, annatto, apples, black peppers, camphire, cinchona, grapevine, guavas, *Moringa oleifera*, jambos, mangoes, *Eucalyptus* sp., cotton, mahogany, redgram and drum stick (Devasahayam & Nair 1986). Both nymphs and adults feed on the young and succulent parts of the plants. Young nymphs that feed on the tender leaves of cashew plants cause necrotic lesions. When they feed on inflorescences, they cause the flowers to dry, and if they feed on the growing tip or bud (primordial), they can kill the bud. Extensive secondary branching that forms a rosette growth usually occurs below the dead tip; however, these branches cannot reproduce. Feeding on young fruit causes the nut to shrivel and die, while feeding on older nuts reduces their size and quality. The adults are poor fliers, and mating usually takes place under the surface of the cashew leaves (Mandal 2000).

In most insects, mating stimulates oviposition, and this can be observed in *Helopeltis theivora* on cocoa (Muhamad *et al*. 1990). Females of some insects mate only once during their lifetime, whereas other insects may mate more than once. Male fitness is reflected by the number of females he inseminates, but females often benefit from limiting their number of mates (Campbell 2005). The relationship between the number of copulations and female fecundity can be positive (Pardo *et al*. 1995; Wilson *et al*. 1999; Jimenez-Perez *et al*. 2003), neutral (Van der Kraan & Van der Straten 1988; Kawagoe *et al*. 2001) or negative (Cook 1999; Orsetti & Rutowski 2003). In *H. theivora* on cocoa, no significant different was recorded in the fecundity among females in the various female to male sex ratios. Length of exposure of females to males did not indicate a significant difference in the fecundity of a given female (Muhamad *et al*. 1990).

The mating process of *H. antonii* involves brief courting, mounting and copulation in an end-to-end position (Devasahayam & Nair 1986). However, information on the mating behaviour of this insect was still lacking. This study was conducted to investigate the biology of *H. antonii* in the following aspects: 1) the sexual maturity of male and female *H. antonii*; 2) the effects of sex ratio on the fecundity and longevity of females *H. antonii*; and 3) the influence of mating frequency on fecundity and eggs fertility.

Life tables of the insect feeding on cashew indicated that the population development of the insect is quite low, a finding substantiated by the 0.092 intrinsic rate of increase (r_m) per female per day and the 1.097 daily finite rate of increase (λ) per female per day. The mean generation time (T) was 27.70%, and the net reproductive rate (R_o) of the population was 12.84 (Siswanto *et al*. 2008).

MATERIALS AND METHODS

H. antonii was obtained from a laboratory colony reared at the Wonogiri Estate Service in Ngadirojo District, Wonogiri, Central Java province, Indonesia from May to July 2005. The characteristic appearance of the adult is reddish-brown with a black head, red thorax, and black and white abdomen. A pin-like process rises relatively straight from the dorsal part of the thorax. Body size of the adult is 6–8 mm in length, with the male being smaller in size than the female (Stonedahl 1991; Kilin *et al.* 1998). The adults of *H. antonii* collected from a cashew plantation in Wonogiri, Central Java were used as rearing material. The insects were bred in the laboratory and fed cucumber. The culturing technique of *H. antonii* by Kilin *et al.* (1998) was adopted. The hatching adults were collected every day and separated according to sex.

Sexual Maturity of Male and Female *H. antonii*

The experiment was conducted with twenty age combinations of male (1, 2, 3 and 4 day old) and female (1, 2, 3, 4 and 5 day old) *H. antonii* based on the preliminary studies and sexual maturity of *H. theivora* on cocoa (Muhamad *et al*. 1990). Each treatment consisted of two pairs of insects placed in a rearing box (18 x 18 x 25 cm) containing a cucumber for their food and egg deposition. There were four replicates per treatment. The males were removed after 24 hours, and the cucumber was replaced daily. The number of eggs, which were indicated by a pair of fine, thread-like breathing tubes of different length projecting outside the tissues, was recorded daily for a period of up to seven days. The number of nymphs that hatched from the eggs was also recorded.

Influence of Sex Ratio on The Fecundity and Longevity of Female *H. antonii*

Three-day-old adults were used in six treatments with sex ratios (female:male) as follows: 1. (5:1); 2. (2:1); 3. (1:1); 4. (1:0); 5. (1:2); and 6. (1:5). Each group was placed in plastic rearing containers measuring 18 x 18 x 25 cm that contained a fresh cucumber and were covered with a tiled sheet. Each day the cucumber was collected and replaced with a fresh one. The number of eggs laid was recorded from the collected cucumbers, and the number of dead females was recorded daily. The experiment ended when the last female died. The experimental design was CRD with five replicates. The data were analysed using one-way Anova, and the differences between individual means were tested with Tukey's HSD using PC-SAS.

Influence of Mating Frequency on The Fecundity and Eggs Hatchability

Three-day-old adult females and males of *H. antonii* were used in the experiment. The following treatments were prepared: 1) a female with more than once mating; 2) a female with a single mating; 3) a female with continuous exposure to one male; and 4) a virgin female. Each treatment was placed in a plastic container measuring 18 x 18 x 25 cm with a tiled sheet cover containing a cucumber. In treatments where females were allowed to mate more than once, one female was held with two males that were marked with ink and left until mating occurred. The observation was carried out throughout the days to record the mating. Then the

males were removed, and the female was transferred to a new container with a fresh cucumber on which she could lay eggs. After 48 hours in the egg-laying container, the female was exposed to the males again for the next mating period. After the females had mated, they were transferred to a new container for a subsequent 48-hour egg laying period. In treatments where females were exposed to a single mating period, a female was confined to two males. After mating, the males were removed, and the female was transferred individually to a new container with a fresh cucumber for egg laying. In another treatment, females were subjected to continuous exposure to one male in a plastic container with a fresh cucumber for food and egg laying. The last treatment consisted of virgin females in a plastic container with a cucumber as food. Six replicates were set up. Observations of the number of eggs laid and the number of eggs hatched were carried out daily. Data was statistically analysed by one-way Anova, and differences between individual means were tested with Tukey's HSD using PC-SAS.

RESULTS

Sexual Maturity of Male and Female *H. antonii*

Table 1 shows that, in all treatments, except treatment (4:1), females could lay a varying number of eggs. However, not all of the eggs hatched. Any female paired with one-day-old males could not produce nymphs, but males that were two days old or older were capable of fertilising mature females, a finding demonstrated by the number of eggs that hatched into nymphs. A similar pattern was seen with the females. No nymph was produced when one-day old females were paired with mature males (two days old or older), while two day old or older females could produce nymphs. This indicates that the sexual maturity of the male and female (the premating period) is one day. The unmated females in the control also laid eggs, but all of them were sterile.

Table 1: Fecundity of female *H. antonii* in a sexual maturity study of the male and female.

No.	Age (days) of treatment combination		Mean no. of egg laid per female up to 7 days	Mean no. of eggs hatched
	Female	Male	(fecundity)	(fertility)
1	1	1	21.8	0.0
2	1	2	13.6	0.0
3	1	3	8.1	0.0
4	1	4	10.5	0.0
5	2	1	10.9	0.0
6	2	2	51.5	40.3

(continued on next page)

Table 1: *(continued)*

No.	Age (days) of treatment combination		Mean no. of egg laid Per female up to 7 days (fecundity)	Mean no. of eggs hatched (fertility)
	Female	Male		
7	2	3	46.6	25.8
8	2	4	23.5	15.3
9	3	1	18.2	0.0
10	3	2	87.3	65.5
11	3	3	69.1	68.8
12	3	4	108.0	78.5
13	4	1	0.0	0.0
14	4	2	8.0	0.0
15	4	3	24.9	17.5
16	4	4	12.4	4.3
17	5	1	1.6	0.0
18	5	2	55.4	32.8
19	5	3	42.6	18.8
20	5	4	7.8	0.0
21	Control (1 female)		6.4	0.0

Influence of The Sex Ratio on The Fecundity and Longevity of Female *H. antonii*

The sex ratio had no significant effect on the average number of eggs laid per female, except between the sex ratios of 1:0 and 2:1 and between the sex ratios of 1:0 and 1:2 (Table 2). The sex ratio treatments of 2:1 and 1:2 produced more eggs than the other sex ratio treatments, but both were significantly different only compared with the sex ratio 1:0, that of the unmated females. The unmated females laid eggs, but none hatched, i.e., they were sterile. It appears that the sex ratio of females to males did not have any influence on the number of eggs laid. However, it was obvious from the data obtained that females need to mate in order to fertilise their eggs.

Table 2: Influence of sex ratio on fecundity and longevity of *H. antonii* females.

Sex ratio (F:M)	Fecundity per female	Longevity of females (days)	Egg hatching (%)
5:1	104.1 (±12.2) ab	23.6 (±2.2) a	17.30
2:1	149.2 (±15.1) a	21.6 (±1.2) a	18.39
1:1	128.0 (±33.7) ab	21.4 (±0.7) a	10.54
1:0	80.0 (±4.6) b	22.4 (±2.4) a	0.00
1:2	153.0 (±28.7) a	23.4 (±2.1) a	53.84
1:5	95.4 (±10.1) ab	15.8 (±1.7) b	17.93

*Means within column followed by the same letter are not significantly different at 5% level (Tukey) of probability.
Note: Most oviposited foods decayed about 10–14 days after exposure to *H. antonii*.

Table 2 also shows that the longevity of females in the sex ratio of 1:5 is significantly shorter (15.8 days) than all the other sex ratio treatments (5:1, 2:1, 1:1, 1:0 and 1:2). Meanwhile, treatments other than 1:5 show no significant difference. Thus, the overcrowded males in the sex ratio of 1:5 could be exhausting the females due to mating with so many males, which resulted in the lower longevity and fecundity of the female.

Influence of Mating Frequency on The Fecundity and Egg Hatchability

Table 3 shows the influence of mating frequency on the fecundity and egg hatchability of *H. antonii*. The frequency of mating did not significantly influence the fecundity of female *H. antonii*. However, unmated females laid fewer eggs than females paired with matured males. The virgin females laid eggs that were sterile. Fertilisation occurred when the ovum was inseminated with male sperm during mating. Among the females that were paired with mature males, it appeared that when females mated more than once, they laid more eggs than females that had mated only once. Table 3 also indicates that mating frequency did not significantly influence the percentage of egg hatchability. However, this experiment indicated that females tend to mate more than once during their lifespan.

Table 3: Influence of mating frequencies on fecundity and egg hatchability of *H. antonii*.

Mating * treatment	Mean no. of laid eggs	Mean no. of hatched eggs	Mean no. of hatched percentage
1	91.7 (±11.4) a	64.0 (±5.5)	72.3 (±5.1)
2	85.8 (±5.5) a	61.5 (±5.1)	69.0 (±3.8)
3	98.3 (±12.6) a	60.8 (±7.9)	63.7 (±3.9)
4	61.2 (±20.5) a	0	0

Means within column followed by the same letter are not significantly different at P = 0.05 (Tukey) of probability.
*Note**: 1. Females that mated more than once; 2. Females with a single mating; 3. Females with continuous exposure to one male; 4. Virgin females

DISCUSSION

The results of these experiments showed that male and female *H. antonii* become receptive to mating at two days old or older. One-day-old females did not produce any nymphs when paired with mature males that were two days old or older, and similarly mature females that were two days old or older did not produce any nymphs when paired with one-day-old males. A field study of insects on cocoa by Muhamad *et al*. (1990) revealed that the pre-mating period (before sexual maturity) for female and male *Helopeltis theobromae* was two days. The premating period seems to influence the insect's inclination to mate and produce fertile eggs. Studies on the influence of sex ratio showed that the sex ratio did not influence the fecundities of *H. antonii*. However, virgin females that were not paired with males laid fewer eggs. This indicates that females needed to mate in order to elicit oviposition. The need for mating in order to elicit oviposition also occurred in female *H. theobromae* on cocoa (Muhamad *et al*. 1990). However, there were no significant differences among sex ratio treatments on the females' longevity except for the sex ratio (1:5), which resulted in a significantly shorter longevity. Male and female *H. antonii* usually mate more than once. Studies of *Sitophilus oryzae* L. by Campbell (2005) showed that at a higher male density, progeny production was greatly reduced compared to females that mated with lower male density. In addition, the negative effect on female survival was even greater at a higher male density. With increasing male density, females spent more time and energy in copulation but laid fewer eggs. Injury and interference from male-male competition for copulation might also be a factor, because males interrupted ongoing copulations and could expel a copulating male. Increased exposure to males also resulted in a shorter lifespan (Fowler & Partridge 1989). This study also indicated that mating frequency did not significantly influence fecundity and egg hatchability, even though females that mated more than once showed a tendency to lay more eggs.

Laboratory observations on females during this study revealed that *H. antonii* mated more than once with one male (repeated mating) or different males (multiple mating) with an average mating duration (copulation) of more than 3 hours (n: 34), and the interval of mating periods was about 48 hours (n: 21).

CONCLUSION

The premating period of female and male *H. antonii* on cashew plants was one day. The sex ratio did not influence the fecundity of *H. antonii*; however the females need to mate in order to fertilise their eggs. Overcrowded males disturb the females during mating and oviposition and reduce the females' longevity. Females that mated more than once tended to lay more eggs than females that mated only once. However, mating frequency did not influence the females' overall fecundity and egg hatchability.

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