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Influence of multiple mating and food deprivation on reproduction, longevity and sex ratio of *Amblyseius largoensis* (Acari: Phytoseiidae)

Amira A. Abdel-Khalek and Shimaa F. Fahim*

Pests and Plant Protection Department, National Research Centre (NRC), El-Buhouth St., Dokki, Cairo, **Egypt.**

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***Correspondence:** shfahim@yahoo.com **Accepted:** 10 Feb 2018 **Published online:** 25 Mar. 2018

The influence of single and multiple mating, as well as food deprivation on reproduction, female longevity and sex ratio of the predatory phytoseiid mite *Amblyseius largoensis* (Muma) were studied under laboratory conditions. The results indicated that the total egg production was increased by increasing the number of mating. In addition, the female fecundity was significantly declined as the period of food deprivation increased. However, the oviposition period of *A. largoensis* females was significantly increased by increasing the number of mating and decreased by increasing the food deprivation periods. Generally, both multiple mating and food deprivation apparently influenced the fecundity, female longevity and sex ratio of *A. largoensis*.

Keywords: ***Amblyseius largoensis*, multiple mating, food deprivation, reproduction.**

INTRODUCTION

The phytoseiid mites have attracted the attention of the world due to their significance in the biological control of mite pests and other small insect pests on several crops (Mandape and Shukla, 2017). *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) is a common species that naturally existing in tropical and subtropical zones, frequently in perennial flora (Demite et al., 2016). However, *A. largoensis* has been classified as a generalist predator (type III) which can eat mites of different families in addition to small insect pests (McMurtry and Croft, 1997). *Amblyseius largoensis* has been reported to feed on or found in association with several potential tetranychid mite pests including *Tetranychus urticae* Koch (Galvao et al., 2007), *T. mexicanus* (McGregor), *T. neocaledonicus* Andre, *Oligonychus sp*. (Lawson-Balagbo et al., 2008) and *T. gloveri* Banks (Carrillo et al., 2010).

Many factors influencing reproduction and sex ratio of the predatory phytoseiids have been investigated by various authors (Amano and Chant, 1978; Momen, 1994; Zaher et al., 2007; Gotoh and Tsuchiya, 2009; Rasmy and Abdel-Khalek, 2017). However, several studies were investigated the influence of the multiple mating on the biological characteristics of the phytoseiids (Momen, 1993; Pappas et al., 2007; Zaher et al., 2007. Gotoh and Tsuchiya, 2008). It was reported that some phytoseiid species require multiple mating for full egg production, while a single mating could be sufficient for optimal egg production of other species (Tsunod and Amano, 2001). In the majority of the studied phytoseiids, multiple mating has commonly resulted in an increase in fecundity (Momen, 1993; Pappas et al., 2007; Gotoh and Tsuchiya, 2008). In contrast, *Metaseiulus occidentalis* (Nesbitt) was capable of mate many times, but this was not essential for full reproduction (Laing, 1969).

In addition, the food deprivation is another factor affecting different biological characters of phytoseiids such as longevity, fecundity and sex ratio (El-Sawi and Abou-Awad, 1992; Momen, 1994; Gotoh and Tsuchiya, 2009). However, many phytoseiids feed on spider mites whose numbers in the field are greatly changeable and unexpected (Gotoh and Tsuchiya, 2009). In such habitats, the phytoseiids can expose sequentially to satiation and starvation over a short time (Sabelis and Janssen, 1994). In general, the prey consumption influences the reproduction of phytoseiids because it is known that adult females assign the majority of their food for egg production such as *Phytoseiulus persimilis* Athias-Henriot which transfer 70% of the consumed food to eggs (Sabelis and Janssen, 1994). Thus, food deprivation can cause reduction in phytoseiids fecundity (Zaher et al., 2007). In addition, the food stressed females can adjust the sex ratio of their progeny due to the variation in production costs for daughters and sons (Charnov, 1982).

The purpose of this study was to determine the effect of the single and multiple mating of the female, as well as food deprivation on some biological characteristics of *A. largoensis* which might have considerable effects on the predator population. In addition, such information may be valuable for mass-rearing of *A. largoensis* and the successful using in biological control.

MATERIALS AND METHODS

Mite culture

The culture of *A. largoensis* used in the present work has been kept in the laboratory for more than ten generations before the experiments. *Amblyseius largoensis* was reared on mulberry, *Morus alba* L., leaves. The mulberry leaves were placed on wet cotton wool in Petri-dishes. *Tetranychus urticae* was provided as food for *A. largoensis*.

Experimental units

The mites were kept individually on the experimental units which consist of mulberry leaf discs (3-cm in diameter) placed on wet cotton wool in Petri-dishes. A thin layer of wet cotton was surrounded every disc to prevent the mites from escaping. The experiments were carried out at 25±1°C, 65-70 % RH and under photoperiod of 14 L: 10 D. Once the leaf discs started to decay, the tested mites were transferred to new discs by a fine hair brush. In all experiments, *T. urticae* nymphs were provided as food.

Two series of experiments were conducted

The first series was conducted to study the influence of single and multiple mating on reproduction, female longevity and sex ratio of *A. largoensis*. In these series, three groups of predatory females were used. Fifteen newly emerged unmated females for every group were placed singly on the discs of mulberry as previously described. The leaf discs were provided with ample of food. In the first group (single-mated females), the female was allowed to mate once with one newly emerged unmated male in the first day of adult emergence. After copulation was ended, the male was removed to prevent the multiple mating. In the second group (females mated twice), the female was allowed to mate twice. The female was allowed to mate in the first day of adult emergence with one newly emerged unmated male, and then the male was removed. Five days later, the female was allowed to mate for the second time with another one newly emerged unmated male, and then the male was removed. For the third group (females mated three times), the same procedure was followed as in case of females of the second group except that the female of the third group was allowed to mate for the third time (five days after the second mating) with another one newly emerged unmated male, and then the male was removed.

The second series was conducted to study the influence of food deprivation on reproduction, female longevity and sex ratio of *A. largoensis*. In these series, three groups of predatory females were used. Fifteen newly emerged unmated females for every group were placed singly on the discs of mulberry as previously described. Each female was inseminated with one newly emerged unmated male within the first day after adult emergence (first mating). In each group, females were allowed to copulate with males for three times as described before in first series of experiments.

In the first group of females (control), the females were supplied with ample of food until the end of their lives. However, the predator starvation has defined as full satiety immediately after food deprivation periods (Fransz, 1974). Therefore, the other two tested female groups were exposed to two types of food regimes until the end of their lives. In the second group of females (moderate food deprivation), the females were exposed to the first food regime consists of repetitive series of two days of fasting then followed by two days of ample food. In the third group of females (severe food deprivation), the females were exposed to the second food regime consists of repetitive series of four days of fasting then followed by two days of ample food.

In the two series of experiments, the leaf discs were daily examined to determine the female longevity and fecundity. The sex ratio of the progeny was also recorded.

Statistical analysis

To compare fecundity and longevity between various groups, one-way analysis of variance (ANOVA) were used and the means were separated by Tukey´s test using SPSS program.

RESULTS AND DISCUSSION

Influence of single and multiple mating on reproduction, female longevity and sex ratio of *A. largoensis*.

The results represented in Table (1) indicated that the number of eggs laid / female was significantly increased by increasing the number of mating and ranged from 23.67 to 36.67 eggs/ female in case of females mated once and those mated three times, respectively. Also, there was a positive correlation between the total egg production and mating times in *Neoseiulus californicus* (McGregor) (Gotoh and Tsuchiya, 2008). However, a higher fecundity as a result of multiple mating has been recorded for many phytoseiids such as *Amblyseius hibisci* (Chant) (Muma, 1964), *A. andersoni* (Chant) (Amano and Chant, 1978), *A. deleoni* (Muma and Denmark) (Zaher et al., 2007) and *Kampimodromus aberrans* (Oudemans) (Pappas et al., 2007). This increase in the total egg production can be attributed to the noteworthy prolongation in oviposition period of females as a result of multiple mating (Rasmy and Hussein, 1996; Gotoh and Tsuchiya, 2008). Alternatively, *M. occidentalis* was capable of copulating several times, but this was not needed for full egg production (Laing, 1969). However, other predatory phytoseiid mites, such as *P. persimilis* need to copulate only once to give its maximum fecundity (Rasmy and Hussein, 1996).

The oviposition period was expanded from 15.00 days in females that mated once to 24.93 days in females mated three times (Table 1). This finding is similar to that observed in *Neoseiulus barkeri* (Hughes) (Momen, 1993) and *N. cucumeris* (Oudemans) (Zhang et al., 2007). The female longevity was significantly shorter in case of multiple-mated females than that of the single-mated females (Table 1). Likewise, the longevity of the single-mated females found to be longer than that of the multiple-mated females (Amano and Chant, 1978; Pappas et al., 2007; Gotoh and Tsuchiya, 2008). This decline in the longevity of multiple-mated females may be caused by the mating cost (for example, physical harm caused by repeated copulation) as well as the cost of egg production was increased (Arnqvist and Nilsson, 2000). The obtained results revealed that post-oviposition period of females were significantly reduced by increasing the number of mating. The shortest post-oviposition period was reported in case of females mated three times (2.33 days) while the longest was reported in case of those mated once (13.07 days) (Table 1). Similarly in *N. californicus*, Gotoh and Tsuchiya (2008) observed that the post-oviposition period of single-mated females was longer than that of multiple-mated females. The present data displayed that multiple mating seemingly influenced the sex-ratio of *A. largoensis* progeny. In the three female groups, the sex-ratio of the progeny was in favor of females whereas the proportion of the females in progeny was increased as the mating times increased (Table 1). This observation comes in agreement with Momen (1993). Therefore, the current study revealed that *A. Largoensis* females need to multiple mating in order to increase their fecundity.

Influence of food deprivation on reproduction, female longevity and sex ratio of *A. largoensis*.

In Table (2), the calculation of the female oviposition period was begun on the day where the first egg was deposited to the day where the final egg was deposited. Thus, the oviposition period contained the non-oviposition periods due to the periods of food deprivation.

The obtained results exhibited that the fecundity was significantly declined as the period of food deprivation increased. The lowest fecundity (1.60 eggs / female) was reported in case of females subjected to severe deprivation of food as compared to those supplied with ample food in the control (37.00 eggs / female) (Table 2). These findings were in agreement with those observed on other predatory mites such as *A. deleoni* (Zaher et al., 2007) and *N. californicus* (Gotoh and Tsuchiya, 2009). In several organisms such as insects, females exposed food-stress was generally change energy from the reproduction to their survival and reducing the number of their progeny (Agarwala et al., 2008).

Table (1): Influence of single and multiple mating on fecundity, female longevity and sex ratio of *A. largoensis* at 25 ± 1°C.

Item	Single-mated females	multiple mating		F	*P*
		Females mated twice	Females mated three times		
No. of tested females	15	15	15		
No. of eggs laid/female	23.67±0.30**c**	30.00±0.22**b**	36.67±0.26**a**	623.98**	0.000
No. of eggs laid/female/day	1.58±0.03**a**	1.34±0.02**c**	1.47±0.01**b**	28.18**	0.000
Pre-oviposition period (days)	2.00±0.00**a**	1.67±0.13**ab**	1.60±0.13**b**	4.17*	0.022
Oviposition period (days)	15.00±0.22**c**	22.53±0.31**b**	24.93±0.21**a**	437.69**	0.000
Post-oviposition period (days)	13.07±0.21**a**	3.47±0.13**b**	2.33±0.13**c**	1369.26**	0.000
Total adult longevity (days)	30.07±0.36**a**	27.67±0.29**c**	28.87±0.22**b**	16.80**	0.000
Sex ratio (♀♀: ♂♂)	1.7:1	1.95:1	2.33:1		

Mean values within a row followed by different letters were different significantly at the 5% level (Tukey´s test). ** Highly significant * Significant

Table (2): Influence of food deprivation on fecundity, female longevity and sex ratio of *A. largoensis* at 25 ± 1°C.

Item	Control (ample food)	Food deprivation regimes of females		F	*P*
		Moderate	Severe		
No. of tested females	15	15	15		
No. of eggs laid/female	37.00±0.38**a**	19.53±0.27**b**	1.60±0.13**c**	4001.03**	0.000
No. of eggs laid/female/day	1.34±0.01**a**	0.89±0.02**b**	0.18±0.01**c**	1741.86**	0.000
Pre-oviposition period (days)	1.50±1.33**b**	1.73±0.12**b**	2.33±0.49**a**	10.92**	0.000
Oviposition period (days)	27.67±0.29**a**	21.93±0.23**b**	9.07±0.21**c**	1536.56**	0.000
Post-oviposition period (days)	2.47±0.13**b**	4.47±0.13**a**	0.00±0.00**c**	422.38**	0.000
Total adult longevity (days)	31.67±0.35**a**	28.13±0.29**b**	11.40±0.21**c**	1402.00**	0.000
Sex ratio (♀♀: ♂♂)	2.46:1	2.16:1	1:1.5		

Mean values within a row followed by different letters were different significantly at the 5% level (Tukey´s test) ** highly significant

Table (2) indicated that the oviposition period of *A. largoensis* females was significantly affected by food deprivation periods. The shortest oviposition period had been recorded in case of females undergo severe food deprivation (9.07 days) as compared to those undergoing moderate food deprivation (21.93 days) or those in the control (27.67 days) (Table 2). In the same context, the food deprivation was found to cause decrease in the oviposition period of *N. barkeri* (Momen, 1994). Additionally, the current data revealed that the food deprivation significantly influenced the longevity of *A. largoensis* females. The shortest female longevity was observed in females subjected to severe deprivation (11.40 days) as compared to those in the control (31.67 days) (Table 2). Comparably, the longevity of phytoseiid adults becomes shorter under food shortage conditions than under abundant food conditions (Momen, 1994; Gotoh and Tsuchiya, 2009).

The sex ratio of the offspring produced by *A. largoensis* females which subjected to moderate deprivation of food was 2.16 females to 1 male. This proportion becomes 1 female to 1.5 male in case of females subjected to severe deprivation of food (Table 2). Under ideal environmental conditions, maternally controlled progeny sex ratio is female-biased in phytoseiid mites (Sabelis et al., 2002). However, the production costs of female eggs are higher than those of male eggs (Sabelis et al., 2002). Therefore, when the maternal food deprivation increased, the proportion of female eggs in their offspring was decreased (Amano and Chant, 1978; Walzer and Schausberger, 2015) in order to provide more energy for the mother's existence (Walzer and Schausberger, 2015). Generally, the present data obviously indicated that the severe food deprivation had adverse effects on the predatory mite *A. largoensis*

CONCLUSION

Information on the requirement of *A. largoensis* females to repeated mating and their capability to tolerate food deprivation can be significant for the successful mass-rearing of *A. largoensis* and its use in the biological control. The present study revealed that *A. largoensis* females mated more than once had a higher fecundity than those mated only once. In addition, the severe food deprivation had negative effects on fecundity, female longevity and the sex ratio of *A. largoensis*.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

The two authors (Amira A. Abdel-Khalik and Shimaa F. Fahim), shared in putting the idea, designed the research and wrote the manuscript. The two authors, also, shared in performed the experiments, data analysis as well as reviewed the manuscript and approved the final version.

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