Eur. J. Entomol. **97**: 177–181, 2000
ISSN 1210-5759

Fecundity and survival of *Anagyrus kamali* (Hymenoptera: Encyrtidae) under different feeding and storage temperature conditions

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Key words. *Anagyrus kamali*, Encyrtidae, parasitoid, *Maconellicoccus hirsutus*, hibiscus mealybug, Pseudococcidae, storage, fecundity, survival, egg load

Abstract. The parasitoid, *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae), has been recently introduced into the Caribbean as a biological control agent against the hibiscus mealybug (HMB), *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae). Storage of *A. kamali* that is essential for its use in biological control did not affect the longevity of female and male parasitoids (40.3 ± 14.07 and 31.7 ± 9.57 days, respectively) when kept at 20 ± 2°C in absence of hosts and fed ad libitum with droplets of pure honey. At a storage temperature of 27 ± 2°C the longevity decreased by about 10 days. Fed females did not resorb eggs during the first two weeks of storage at 20 ± 2°. Parasitoid ovogenesis ceased when ovarioles/lateral oviducts were full. The lifetime fecundity was not significantly affected by a storage at 20 ± 2°C of up to 14 days. Foraging activities and oviposition were the main factors influencing the lifespan of female *A. kamali*.

INTRODUCTION

Maconellicoccus hirsutus Green (Homoptera: Pseudococcidae), commonly named the hibiscus or pink mealybug (HMB), was accidentally introduced into the island of Grenada in 1994 and has been inexorably spreading throughout the Caribbean islands where it has become a major pest on several crops. It is now present in 18 Caribbean islands, and also in Guyana (I.I.E., 1997). *M. hirsutus* is a very prolific pest with females laying between 384–540 eggs (Mani, 1989). It injects a toxin at the point of feeding, causing severe distortion of leaves, new shoots and fruit (Williams, 1996), and attacks a wide range of fruit trees, ornamentals, forest trees and weeds (i.e. 125 host species – Mani, 1989).

Anagyrus kamali Moursi (Hymenoptera: Encyrtidae), a solitary endoparasitoid, was imported from China by the CABI International Institute of Biological Control (IIBC), UK, for classical biological control of this pest in the Caribbean. As part of the Technical Co-operation Program funded by the Food and Agriculture Organisation (FAO) of the United Nations for the biological control of *M. hirsutus*, *A. kamali* is currently shipped from Trinidad to several countries in the Caribbean and South America. Parasitoids in transit may be stored for periods of 4 to 10 days between emergence in the rearing facilities and field release. This might result in mortality, and possibly a decrease of lifetime fecundity due to resorption of mature eggs, negatively affecting the efficiency of the released parasitoids.

Little has been published on the biology and behavior of this parasitoid except for Moursi (1948) and Sagarra and Vincent (1999) and Sagarra et al. (2000). Our objectives were to determine (1) the effect of storage conditions on the survival of the parasitoid; and (2) the impact of storage on the egg load of adult female parasitoids, lifetime fecundity and oviposition period.

MATERIALS AND METHODS

Rearing of *M. hirsutus*

Mealybugs were reared on sprouted potatoes in nylon mesh cages (32 sprouted potatoes per cage) supported on steel wire frames (48 × 48 × 68 cm). The cultures were maintained in the dark at 27 ± 2°C. Each week 64 sprouted potatoes were individually infested with 20 adult female mealybugs having well-formed ovisacs. Weekly infestations ensured a continuous supply of different *M. hirsutus* nymphal instars. Three weeks after infestation, the potatoes had *M. hirsutus* populations that mainly consisted of L2 (second instar) and L3 (third instar) mealybugs. Cohorts of adult females with ovisacs were available after 4–6 weeks. Size was used as the primary criterion to distinguish *M. hirsutus* stages (Ghose, 1971).

In each experiment we used L3 female, and the early adult female (preovisac). L3 and adults *M. hirsutus* were sexed using characteristics described in Ghose (1971).

Rearing of the parasitoid

Each week, 100 *A. kamali* adult females were released into two cages each containing 32 infested potatoes supporting three-week old populations of *M. hirsutus*. Insects were maintained at 27 ± 2°C, 60 ± 10% RH, under a photoperiod of 12L : 12D. Light was provided by two fluorescent lamps (70 W) suspended 30 cm above the cages. Two weeks after the release of the parasitoids, mummies were collected from the potatoes and individually placed in gelatin capsules. Mummies were observed daily and newly (<24 h) emerged parasitoids were used for

experiments. All parasitoids used for experiments were the same size (1.5–1.8 mm in length).

Longevity

Newly (<24 h) emerged female and male parasitoids were individually placed in transparent plastic vials (7 cm long, 2 cm in diameter) with a 1 cm hole covered with fine mesh cloth in the cap. The effect of temperature and food supply was assessed by storing half of the insects (i.e. 60 males and 60 females) at 27 ± 2°C and the other half (i.e. 60 males and 60 females) at 20 ± 2°C. Within each temperature treatment, half of the parasitoids (i.e. 30 males and 30 females) were fed with droplets of pure honey and the other half (i.e. 30 males and 30 females) were not fed. Observations were made at 24 h intervals to determine the longevity of the adults. For each of the eight combinations sex-temperature-food treatment, 30 parasitoids were tested. t-tests ($p \leq 0.05$) were used to compare longevities. Interaction between temperature and food supply was determined using two-way ANOVA (Systat 7.0 for Windows). Cohort survival curves (fraction of each cohort surviving at a particular moment in time) were plotted and compared using the Weibull frequency distribution model. The proportion of individuals surviving at time t is given by:

$$F(t) = 1 - \exp(-[(t/b)^c])$$

Where b and c are the scale and shape parameters of the distribution (Pinder et al., 1978). These parameters were estimated and compared using Systat 7.0 for Windows.

Egg load

Groups of ten newly (<24 h) emerged female and five male parasitoids were stored in transparent plastic vials (as described above). The parasitoids were stored at 20 ± 2°C and fed daily with droplets of pure honey. Every second day, ten of the stored female parasitoids were dissected and the number of mature oocytes (Moursi, 1948) was recorded. Insects were not dissected beyond 14 days, as this represented the maximum storage period. Egg loads were compared using a t-test (Systat 7.0 for Windows).

Fecundity after storage

Groups of ten newly (<24 h) emerged female parasitoids were stored in transparent plastic vials (as described above). Ten male parasitoids were also introduced into each vial in order to ensure mating. The parasitoids were kept at 20 ± 2°C and fed daily with droplets of pure honey. Batches of parasitoid females were stored for 1, 4, 7, 10, and 14 days. Fecundity was assessed for each of these periods.

Third-instar mealybugs were collected from potato sprouts and placed in groups of 20 on a hibiscus (*Hibiscus rosa-sinensis* L.) leaf. The hibiscus leaf was then placed inside a cylindrical transparent plastic vial (10 cm in diameter, 6 cm in height) with a 1 cm hole covered with mesh on the screw cap. One female parasitoid was introduced into each vial and fed with a drop of honey deposited on the mesh window of the cap. The parasitoids were left to forage and oviposit for 24 h. The adult parasitoids were then removed and transferred to another vial with 20 new mealybugs for another 24 h. This was repeated until the death of the parasitoids. Parasitized mealybugs were dissected in a drop of ethanol (70%) and the number of parasitoid eggs in each host was recorded. Oviposition tests were conducted at 27 ± 2°C, 60 ± 10% RH. Fifteen parasitoids were tested for each storage period (total of 75 parasitoids tested). The data were analyzed with ANOVA and means were separated with Tukey tests ($p \leq 0.05$) (Systat 7.0 for Windows).

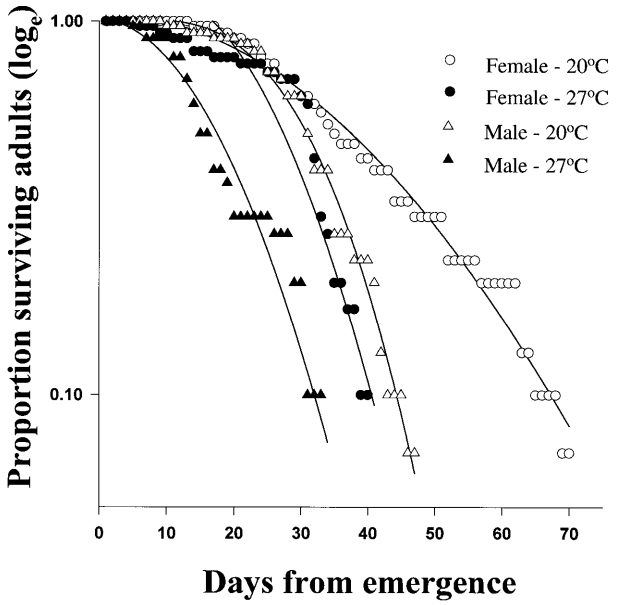

Fig. 1. Adult survival (days) of female and male *A. kamali* at 20 ± 2 and 27 ± 2°C (n = 30 individuals per temperature-sex combination).

RESULTS

Longevity

Female parasitoids lived longer than males in all treatments ($p \leq 0.05$). *Anagyrus kamali* individuals that were fed on honey lived longer than individuals stored without food at both temperature treatments ($p \leq 0.05$) (Table 1, Fig. 1). Unfed parasitoids did not survive beyond 48 h at both 20 ± 2 and 27 ± 2°C. However, *A. kamali* fed on honey survived significantly longer ($p \leq 0.05$) when stored at 20 ± 2 than at 27 ± 2°C. Female longevity dropped from 40.3 ± 14.07 to 29.0 ± 9.94 days, and male longevity from 31.7 ± 9.57 to 18.9 ± 8.92 days (Table 1, Fig. 1). At 20 ± 2°C with food supply a maximum longevity of 71 days was recorded for female parasitoids and 48 days for male parasitoids. Median lethal time 50 (LT_{50}) occurred at 36 and 22 days at respectively 20 ± 2 and 27 ± 2°C for the female parasitoids, and after 21 days at 20 ± 2°C and 18 days at 27 ± 2°C for male parasitoids, respectively (Table 2). If stored at 20 ± 2°C with regular food supply, female parasitoids could survive up to 28 days, and males up to 23 days before reaching 10% mortality.

Cohort survival curves (Fig. 1) were plotted only for parasitoids which were provided with food, as unfed indi-

TABLE 1. Average (n = 30 individuals) longevity (days) male and female adult *A. kamali*, according to food type and temperature, in absence of hosts (i.e. no oviposition).

Food supply	Temperature (°C) ± 2	Mean (± SD) longevity (days)	
		Female	Male
Control (no food)	20	1.7 ± 0.47 b*	1.3 ± 0.47 a
Control (no food)	27	1.8 ± 0.41 b	1.1 ± 0.31 a
Pure honey	20	40.3 ± 14.07 e	31.7 ± 9.57 d
Pure honey	27	29.0 ± 9.94 d	18.9 ± 8.92 c

* Pairs of means within rows and columns followed by the same letters are not significantly different (t-test, $P < 0.05$).

TABLE 2. Survival analysis data of *A. kamali* fed with honey, according to the sex and temperature (n = 30 individuals – means ± SD).

	Male		Female	
	20 ± 2°C	27 ± 2°C	20 ± 2°C	27 ± 2°C
Weibull parameter b	23.9 ± 2.30 a*	20.3 ± 2.13 a	40.2 ± 3.00 b	27.7 ± 2.57 a
Weibull parameter c	1.73 ± 0.225 a	1.69 ± 0.245 a	1.66 ± 0.168 a	1.64 ± 0.205 a
LT_{50}	21.5	18.0	36.0	24.6

* Within rows, pairs of means followed by the same letters are not significantly different (t-test, $p < 0.05$).

viduals did not survive long enough to perform this analysis. At both temperatures, the shape of the cohort survival curves of the fed parasitoids was of type I (Weibull c > 1 – Table 2) (Pinder et al., 1978), the age specific mortality rate increasing with age. A t-test performed on the value of the b parameter (scale parameter of the distribution) showed that survival of females at 20 ± 2°C was significantly (t-test, $p \leq 0.05$) greater than females and males stored at 27 ± 2°C.

Egg load

The number of mature oocytes in *A. kamali* females increased during the first four days, rising from 29.1 ± 6.25 mature oocytes to 41.2 ± 7.18. There was no significant difference between egg load of female parasitoids stored between 4 to 14 days ($p > 0.05$), with an average of 43.0 ± 12.2 mature eggs (Fig. 2).

Fecundity

A. kamali oviposited successfully after all the storage periods tested. Lifetime fecundity (total number of eggs oviposited through the parasitoid lifespan) was not significantly ($p > 0.05$) affected by the increase of the storage period, the total number of eggs oviposited going from 99.9 ± 24.9 after one day of storage to 74.2 ± 32.5 after two weeks (Table 3). The oviposition period was also not significantly different ($p > 0.05$) according to the storage period, which went from 9.4 ± 3.17 days after one day storage to 7.3 ± 2.87 days after two weeks. The oviposition rate increased during the first three days of oviposition, and then gradually decreased until it reached a plateau at the end of oviposition period (Fig. 3). The slope and the value of the plateau of the curves corresponding to 10 and 14 days of storage were less than (average of 20%) for 7 days of storage curves, showing a decrease in oviposition rate. The longevity of the parasitoid significantly increased with an increase of the storage period, the parasitoid lifespan going from 9.4 ± 3.2 to 21.3 ± 2.9 days after one and 14 days, respectively.

TABLE 3. Average lifetime fecundity and oviposition period of *A. kamali* according to the storage period prior to first oviposition (n = 15 individuals, 27 ± 2°C).

Storage period (days)	Mean (± SD)		
	Lifetime fecundity (eggs #)	Oviposition period (days)	Total longevity (days)
1	99.9 ± 24.94 a*	9.4 ± 3.17 a	9.4 ± 3.17 a
4	100.1 ± 21.29 a	9.0 ± 3.27 a	13.0 ± 3.27 b
7	99.4 ± 23.37 a	9.8 ± 2.90 a	16.8 ± 2.90 bc
10	84.3 ± 29.72 a	8.5 ± 3.90 a	18.5 ± 3.90 cd
14	74.2 ± 32.48 a	7.3 ± 2.87 a	21.3 ± 2.87 d

* Within columns, pairs of means followed by the same letter are not significantly different (Tukey, $P < 0.05$).

DISCUSSION

Longevity test

Large insects tend to live longer than small insects (Godfray, 1994). This positive correlation between body

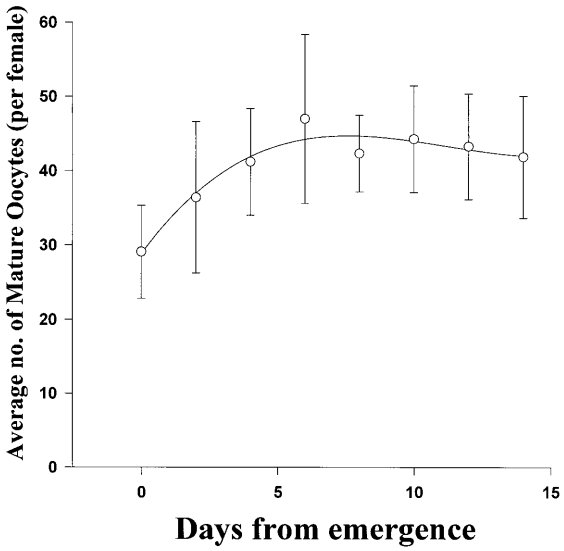

Fig. 2. Average number of mature oocytes of female *A. kamali* from 0 to 14 days after emergence at 20 ± 2°C (n = 10 females per treatment).

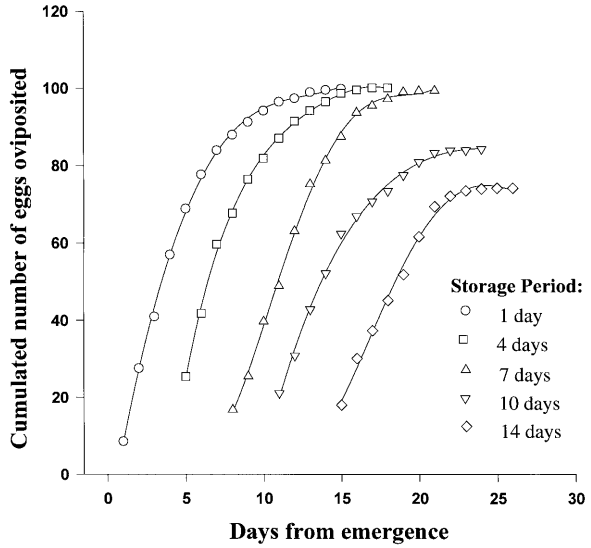

Fig. 3. Cumulative fecundity of female *A. kamali* following storage period from 1 to 14 days (n = 15 individuals per storage period).

size of adults and their longevity has been shown for several parasitoid species (Harvey et al., 1994; Waage & Ng, 1984). In *A. kamali*, females are generally larger and they have a greater longevity than males.

Food is also an important factor for parasitoid survival, and it is essential to ensure mating and subsequent foraging and oviposition of the parasitoid (van Lenteren et al., 1987; Finch & Coaker, 1969). Adult *A. kamali* that were provided with food lived approximately 20 times longer than unfed individuals. Moursi (1948) recorded a maximum longevity of 131 days for *A. kamali* during the Egyptian winter at temperatures under 20°C. In our study, out of the 240 tested parasitoids a maximum of 71 days was recorded for two individual female parasitoids at 20 ± 2°C. Host feeding did not affect positively the adult parasitoid longevity since in the fecundity tests, where *A. kamali* females were exposed to their host and could host feed on them, their average longevity was 9.4 ± 3.2 days.

The impact of temperature on longevity of *A. kamali* was significant. Storage at 20 ± 2°C increased the average survival period by approximately 30% for both female and male fed parasitoids, as a result of a reduction in their activity.

For other Anagyrini species, like *Anagyrus mangicola* Noyes, the average survival of male and female parasitoids at 25 ± 2°C was shorter than *A. kamali*, with 16.7 ± 0.46 and 20.2 ± 0.30 days, respectively (Cross & Moore, 1992). Similar trends were observed for longevity of *Anagyrus pseudococci* (Girault) and *Leptomastix abnormis* (Girault) (Tingle & Copland, 1989), and also *Anagyrus dactylopii* (Howard) (Mani & Thontadarya, 1988).

Egg load

The egg load of the parasitoid determines the number of eggs the female can lay at any given time. As a synovogenic species, only a fraction of *A. kamali* oocytes are mature at emergence, and four days are required to fill the ovarioles with mature oocytes (41.2 ± 7.18 oocytes). Similar observations were also made for the synovogenic species *Coccophagus atratus* Compère (Hymenoptera: Aphelinidae) (Donaldson & Walter, 1988).

Lifetime fecundity

The impact of storage period on the lifetime fecundity of parasitoids has been reported for only one other Encyrtid species, *Anabrolepis mayurai* Subba Rao, a parasitoid of the sugarcane scale, *Melanaspis glomerata* (Green) (Dutta & Devaiah, 1988). In this species, the storage period negatively affected the lifetime fecundity of the parasitoid. In *A. kamali*, neither lifetime fecundity nor oviposition period were significantly affected during the storage periods tested in this study. The females generally oviposited more eggs during the first three to four days of their oviposition period. Oviposition and foraging activities had a profound effect on *A. kamali* longevity since the oviposition period was the same for parasitoids stored for 1–14 days. These activities appeared to exhaust the resources of the parasitoid and shorten its survival time. Hohmann et al. (1989) observed the same phenomenon with *Trichogramma platneri* Nagarkatti, where oviposition was the main factor influencing the lifespan of female parasitoids.

Implications for mass-production and biological control shipments

Emerging parasitoids should be supplied with food to increase their longevity. Storage periods of four to ten days between emergence and release of the parasitoids should cause minimal losses as 97% of the female parasitoids and 93% of the male parasitoids stored at 20 ± 2°C with a food supply were alive after 14 days. The fecundity of *A. kamali* can be sustained for up to 14 days if they are stored at 20 ± 2°C. Thus, the parasitoid can be collected, stored for a period of several days and that should allow for shipments of high quality parasitoids.

ACKNOWLEDGEMENTS. We thank N. Peters for technical assistance. We thank J. Etienne, INRA/URPV Petit Bourg – Guadeloupe, French West-Indies, and D.E. Meyerdirk, USDA/APHIS, Maryland, USA, for commenting an early version of the manuscript. This is contribution no. 335/2000.03.02R of Horticultural Research and Development Research Center, Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, QC, Canada.

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Received June 28, 1999; accepted February 3, 2000