Effect of *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) Adult Nutrition on Longevity and Oviposition

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ABSTRACT

The role of *Trichogramma minutum* Riley adult nutrition on longevity of adults and their response to artificial oviposition stimuli in wax artificial eggs was examined. The longevity of *in vitro* or *in vivo* reared parasitoid females fed as adults was significantly longer than unfed females. The number of eggs deposited by raisin- (51.1 eggs/female) or honey-fed (83.0 eggs/female) females reared *in vitro* or females reared *in vivo* (44.9 or 69.5 eggs/female, respectively) was significantly greater than that deposited by unfed females (35.0 eggs/female for *in vitro* reared and 25.6 eggs/female for *in vivo* reared females). Means of the net reproductive rate, the intrinsic rate of increase, and the limiting frequency of reproduction were also significantly higher for fed than unfed females.

RESUMEN

Se examinó el papel de la nutrición del adulto de Trichogramma minutum Riley en la longevidad de adultos y su respuesta a los estímulos de oviposición artificial en huevecillos artificiales de cera. La longevidad de las hembras parasitoides producidas *in vitro* o *in vivo* alimentadas como adultos fue significativamente más larga que la de las hembras no alimentadas. El número de los huevecillos depositados por las hembras producidas *in vitro* y alimentadas con pasa o miel (51.1 y 83 huevecillos/hembra respectivamente) o *in vivo* alimentadas con pasa o con miel (44,9 y 69,5 huevecillos/hembra respectivamente) fue significativamente mayor que el depositado por las hembras no alimentadas (35.0 huevecillos/hembra cuando fueron producidas *in vitro* y 25.6 huevecillos/hembra cuando fueron producidas *in vivo*). Las medias del índice reproductivo neto, del coeficiente de incremento intrínseco, y la frecuencia limitadora de la reproducción fueron también significativamente más altas para las hembras alimentadas que para las no alimentadas.

Additional Index Words: longevity, fecundity, reproductive potential, in vitro, in vivo, rearing

During the last 30 years, *Trichogramma* spp. have been used in biological control on corn, sugarcane, cotton, vegetables, and fruit trees in more than 30 countries against different insects, mostly lepidopterans. Some 32 million ha of agricultural and forest land are treated annually with *Trichogramma* spp. (Li, 1994).

Our limited capacity for mass rearing of entomophagous insects is a primary constraint to successful commercialization for augmentative releases of natural enemies. The objective of a mass rearing program is to produce the maximum quantity of quality-assured individuals by predetermined dates at a minimal cost (King, 1993). Several species of factitious hosts are currently used for rearing *Trichogramma* spp. This requires a large investment in production facility for the host and limits opportunities for automating the *Trichogramma*-rearing process. *In vivo* rearing of *Trichogramma* spp. was recently reviewed by Greenberg et al. (1996). The production costs could be reduced and capacity increased with automated *in vitro* rearing systems for *Trichogramma* spp. *In vitro* rearing of *Trichogramma* spp. was reviewed by Grenier (1994). Our research goal is the development of an effective automated system for *in vitro* mass rearing of *Trichogramma* spp. Our idea of automated in vitro mass rearing for *Trichogramma* spp. involves collection of *Trichogramma* eggs, which would then be mixed with artificial diet. This would ensure efficient diet use. However, our ability to collect large numbers of *Trichogramma* eggs has been limited.

Some authors have demonstrated that feeding honey or sugar syrup to adult *Trichogramma* spp. increased their longevity, vigour, and fecundity in the laboratory (Ashley and Gonzales, 1974; Shchepetilnikova et al., 1979; Zilberg, 1980; Zaslavsky and Kwi, 1982; Greenberg et al. 1986; Hohmann et al., 1988; Bourarach and Hawlitsky, 1989; Greenberg, 1991; Leatemia et al., 1995).

The objectives of this study were to compare the longevity and response to artificial oviposition stimuli in wax artificial

eggs (WAEs) of honey-fed, raisin-fed or unfed *Trichogramma minutum* Riley adults, which had been reared on artificial diet and on a factitious host.

MATERIALS AND METHODS

The Host. *Helicoverpa zea* (Boddie) eggs were used as the host for *in vivo* rearing of *T. minutum*. They were obtained from a laboratory colony maintained in the Subtropical Agricultural Research Center USDA—ARS, Weslaco, Texas. *H. zea* eggs were irradiated with gamma radiation ca. 25 krad, Cs 137 source and 641 sec. The eggs were then used for *Trichogramma* rearing or stored at 3ºC for < 7 days before they were used in our experiments.

T. minutum were also reared on an oligidic diet as described by Nordlund et al. (1997). The diet used in this study consisted of 6 components, including one insect-derived component: 10.0% of a 7% yeast extract (Difco Laboratories, Detroit, MI) solution; 5.0% of Freamine III (Kendall McGaw Laboratories, Inc. Irvine, CA); 15.0% of a 10% of suspension of nonfat dry milk (Parkmanor Instant Nonfat Dry Milk Fortified with Vitamins A and D, H-E-B Food Stores, San Antonio, Texas); 25.0% of chicken egg yolk; 15.0% of chicken embryo extract; and 30.0% of *Manduca sexta* (L.) egg liquid. A 10 mg/ml solution of Gentamicin (Sigma Chemical Company, St. Louis, MO) was mixed into the diet at the rate of 5ml/1 with a vortexer.

The Parasitoid. *T. minutum* used in these studies were originally obtained from Beneficial Insectary, Guelph, Ontario, Canada where they had been reared on *Ephestia küehniella* Zeller eggs.

The *in vivo* rearing was conducted in 3 X 15 cm glass tubes at 26 ± 1ºC, 65 ± 5% RH, and a 14:10 (L:D) h photoregime. The *H. zea* eggs were attached to paper strips (2.5 X 12.5 cm) with chicken egg white. Two cardboard strips, with up to 0.66 g (ca. 7260 eggs) of unparasitized eggs were inserted into a vial and taped end to end to a vial in which *Trichogramma* spp. emergence had begun. The vial containing the adults was covered with black paper. A ratio of ca. 1 parasitoid female: 10 host eggs was maintained and the exposure period was ca. 24 h.

The *in vitro* rearing was conducted in 96 well tissue culture plate Falcon 3070 Microtest III™ (Becton Dickinson & Company, Lincoln Park, NJ). A microscope slide, with 60 WAEs attached, was exposed to 1-d old adult *Trichogramma* (ca. 15 females per WAE) in a 12 X 8 X 2 cm plastic container (two 96 well tissue culture plate covers taped together with masking tape) for 6 h at 24 ± 1ºC and ca. 65 ± 5% RH. WAEs were prepared by placing a mixture of paraffin (75%) (Gulfwax Household Paraffin Wax, Reckitt & Colman Inc., Wayne, NJ) and Vaseline® (25%) (Chesebrough Pound's USA Co., Greenwich, Connecticut) as described by Hagen and Tassen (1965) on top of the artificial diet in a glass vial held at 65ºC. The tip of a capillary pipette was immersed in the liquid, removed, and touched to a paper strip 0.7 X 2.5 cm. The resulting WAEs had a diameter of about 2-3 mm. WAEs that had been exposed to parasitoids were surface sterilized in a 75% alcohol solution for 6 min followed by several rinsings in sterile reverse osmosis water. The diet and *T. minutum* eggs from each WAE were transferred to a well tissue culture plate using a pasteur pipette. The tissue culture plate with eggs was held ca 100% RH, ca. 24 ± 1ºC, and under a 14:10 (L:D) h photoperiod until development to the last instar. The humidity was then reduced to ca. 75% until the adults emerged.

Experimental methods. The female parasitoids used in all experiments were taken from batches of newly emerged *in vivo* and *in vitro* reared adults. Females were divided into 3 groups: unfed females, females fed with a raisin, and females fed with undiluted honey. Thirty five females were tested in each group. Females were held individually in 1 X 3 cm glass vials until death. Fed females were provided one raisin or a drop of honey per vial. Mortality was checked each day and this information was used to determine longevity. The median and mode of longevity for different groups of females were calculated as described by Schefler (1980) and Sokal and Rohlf (1981).

For determination of the response of females to artificial oviposition stimuli, 0.01% sodium bisulfite solution (Baker Chemical Co, Phillipsburg, NJ), each female was provided a paper strip with one WAE in a 1 X 3 cm glass vial, and the paper strip was replaced daily. After exposure to *Trichogramma* females, the WAE was broken open and the *Trichogramma* eggs in each were counted, with the aid of a dissecting microscope. The daily age of females (x), day of oviposition, and number of live females in the interval x (lx) were recorded. The mean percentage of parasitoid eggs hatching (ca. 90.0%) when they were reared *in vitro*, and the mean percentage of *Trichogramma* female progeny reared *in vivo* (ca. 67.9%) and *in vitro* (ca. 72.1%) were based on a previous study (Nordlund et al., 1997). The latter two indices

Table 1. The reproductive rate of fed and unfed adult *T. minutum* females[1].

Rearing and feeding regimes	R_o	T	r_m	
		In vitro reared females:		
Honey-fed	46.9 ± 2.3a	10.4 ± 0.2	0.370 ± 0.008a	1.448 ± 0.011a
Raisin-fed	27.3 ± 2.1b	10.1 ± 0.1	0.326 ± 0.010b	1.387 ± 0.014b
Unfed	18.5 ± 1.6c	9.5 ± 0.08	0.295 ± 0.009c	1.345 ± 0.012c
		In vivo reared females :		
Honey-fed	37.6 ± 2.5a	10.4 ± 0.11	0.357 ± 0.005a	1.430 ± 0.007a
Raisin-fed	23.4 ± 1.8b	9.8 ± 0.13	0.319 ± 0.009a	1.378 ± 0.013a
Unfed	13.9 ± 2.4c	9.2 ± 0.04	0.266 ± 0.028b	1.312 ± 0.035b

[1]Means (±SEM) in each column from one group of rearing regime followed by different letters are significantly different at 5% level, as determined by Tukey's studentized range test.

were used for calculation of the number of females produced (m_x).

The net reproductive rate (R_o), the mean age of reproduction (T), the intrinsic rate of increase (r_m), and the limiting frequency of reproduction (λ) were calculated as described by Carey (1993). The net reproductive rate was calculated using the formula:

$$R_o = \bullet\, l_x m_x.$$

The intrinsic rate of increase was calculated using the formula:

$$r_m = \ln R_o / T.$$

The mean age of reproduction was calculated using the formula:

$$T = \bullet\, x\, l_x\, m_x / \bullet\, l_x\, m_x.$$

The limiting frequency of reproduction was calculated using the formula:

$$\lambda = e^{rm}.$$

Statistical analyses. Statistical analyses were conducted using analysis of variance (ANOVA) and Tukey's studentized range test of SYSTAT (Wilkinson et al., 1992).

RESULTS AND DISCUSSION

When provided with honey or raisin, adult *T. minutum* females reared *in vitro* or *in vivo* lived significantly longer than unfed females ($F = 13.8$; df = 2, 83; $P < 0.05$ for *in vitro* reared and $F = 18.1$; df = 2, 67; $P < 0.05$ for *in vivo* reared). The mean (±SEM) longevity of *in vivo* reared females was 1.95 ± 0.3 d (range = 1.0 - 4.0 d) for the control (unfed), 3.88 ± 0.4 d (range = 1.0 - 10.0 d) for raisin-fed females, and 5.8 ± 0.5 d (range = 2.0 -14.0 d) for honey-fed females. *T. minutum* females given honey increased their median of longevity 3.3-fold and the mode by 2.4-fold compared with unfed females. The mean longevity of *in vitro* reared females was 3.2 ± 0.2 d (range = 1.0 - 5.0 d) for unfed females compared with 4.5 ± 0.3 d (range = 1.0 - 7.0 d) for raisin-fed and 6.8 ± 0.8 d (range = 2.0 - 19.0 d) for honey-fed females. The median of longevity for honey-fed females increased 2.0-fold and the mode by 2.6-fold compared with control group of unfed females (Fig. 1, 2).

The number of eggs deposited by raisin or honey-fed females of *T. minutum* reared *in vitro* or *in vivo* increased significantly compared with unfed females ($F = 50.0$; df = 2, 83; $P < 0.05$ for *in vitro* reared and $F = 23.2$; df = 2, 67; $P < 0.05$ for *in vivo* reared parasitoids). *In vitro* reared *T. minutum* females given honey increased their egg production 2.5-fold compared with unfed parasitoids (83.0 ± 4.3 [range = 35.0 - 136.0] versus 32.2 ± 2.8 [range = 11.0 - 66.0] eggs per female) and by 1.6-fold compared with raisin-fed females (83.0 versus 51.1 ± 4.0 [range = 0 - 75.0] eggs per female). The situation for *in vivo* reared *T. minutum* females is similar. In this case the mean number of eggs deposited by honey-fed parasitoids was 69.5 ± 5.2 (range = 0 - 108.0) eggs per female. The mean number of eggs oviposited by a honey-fed females was 2.7-fold higher than that oviposited by unfed females (25.6 ± 4.5 [range = 0 - 57.0] eggs per female) and 1.5-fold higher than raisin-fed females (44.9 ± 3.6 [range = 0 - 57.0] eggs per female) (Fig. 3).

The population of honey-fed *in vitro* reared *T. minutum* females grew at a mean constant exponential rate of 0.37 ± 0.08 (r_m) individuals per day. In these same conditions the population was multiplying 46.9 ± 2.3 times each generation (R_o) for a length of generation (in this study was 10.4 ± 0.2 d), and finite rate of increase (λ) for an interval of time equal to 1

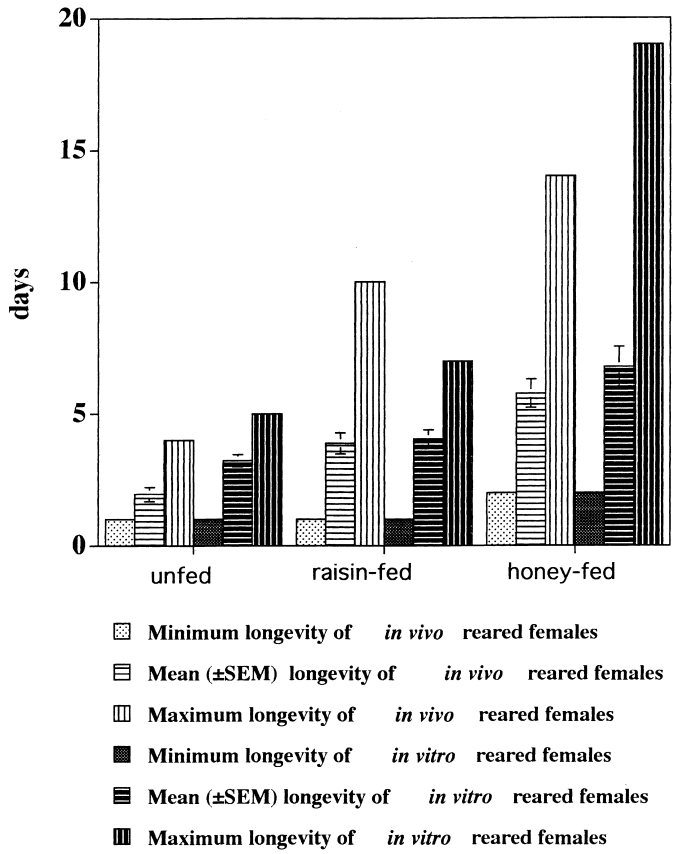

Fig. 1. The longevity of *in vitro* and *in vivo* reared *T. minutim* females, which were unfed or fed as adults.

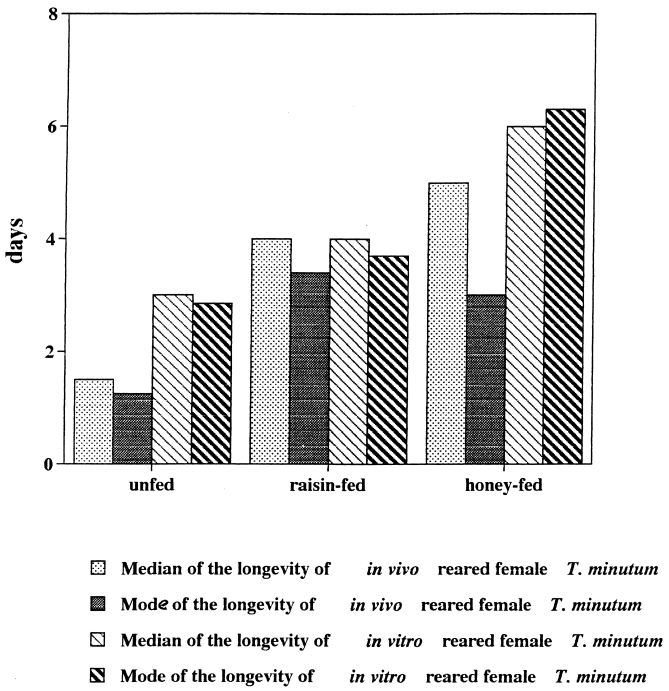

Fig. 2. The median and mode of longevity of *in vitro* and *in vivo* reared *T. minutim* females.

was 1.448 ± 0.011. The means for the indices for honey-fed females were significantly higher than those for raisin-fed and unfed females. This was also true for *in vivo* reared females (Table 1).

The result of additional adult feeding is not the same in different species of parasitoids but depends on the status of oogenesis of females at the moment of emergence. In this point Flanders (1950) divides parasitic Hymenoptera into two main groups: proovigenic and synovigenic. In proovigenic species oogenesis is completed before the females start to deposit eggs. They were characterized by having a short lifespan and ovipositional period. The maturing of additional eggs occurred due to resources accumulated in the larval stage. In synovigenic species, the oogenesis is prolonged and occurs throughout the life of the adult. Chumakova (1971) modified the classification of Flanders, to classify Hymenoptera into three groups: pro-, syn-, and epiovigenic species. The maturing of eggs in proovigenic species takes place in the pupal stage, in synovigenic species - partly in pupal stage and partly in the adult stage, and in the epiovigenic species - only in the adult stage. In the majority of *Trichogramma* spp. the maturing of eggs occurrs at the end of the pupal stage. Parasitoid females emerged with most of their eggs mature and ready to be oviposited, and part of them as oocytes in ovarioles, which continue to develop throughout the life of the adult. The initial quantity of eggs is determined by the conditions of preimaginal development. However, total fecundity is determined by conditions of preimaginal development and during the life of the adult. Through adult feeding, the longevity and fecundity of *Trichogramma* females are increased. Our data are similar to those recorded for *Trichogramma* spp. by other authors. Shchepetilnikova et al. (1979) observed that the lifespan of unfed females of *T. euproctidis* Girault and *T. evanescens* Westwood was 3 - 4 days while sugar syrup-fed females was 8 - 9 days. They also observed that under natural conditions the lifespan of females *Trichogramma* spp. fed on nectar of blooming plants and dew increased to 14 - 15 days. Zilberg (1980), Zaslavsky and May Fu Kwi (1982), Greenberg et al. (1986), and Bourarach and Hawlitzky (1989) demonstrated that fecundity of fed females *T. euproctidis*, *T. pintoi* Voegele, *T. evanescens*, *T. embryophagum* Harting, and *T. lutea* Girault increased by 1.5-2.0 times and longevity by 4.0 - 5.0 times compared with unfed females. Leatemia et al. (1995) found that honey-fed *T. minutum* females lived 26.4 days and produced 260 offspring while unfed females lived 3.5 days and produced 80 offspring.

In summary, we want to emphasize that the fed female *T. minutum*, especially when fed on undiluted honey, showed an increase in longevity and number of eggs deposited compared with unfed parasitoid females. The introduction of adult feeding into an *in vitro* rearing system would allow parasitoid females to produce more eggs and thus, significantly improve the efficiency of the system.

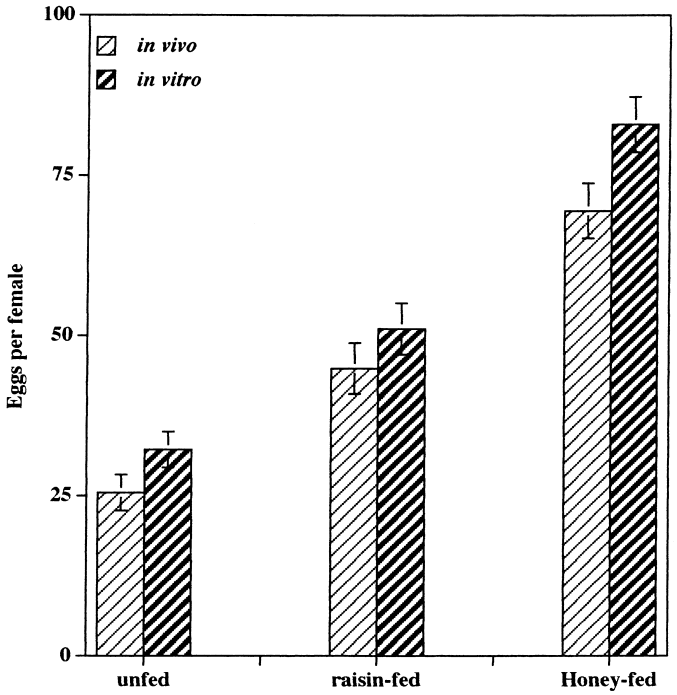

Fig. 3. The fecundity of *in vitro* and *in vivo* reared *T. minutum* females, which were unfed or fed as adults.

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