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Biology and Thermal Requirements of *Utetheisa ornatrix* (L.) (Lepidoptera: Arctiidae) Reared on Artificial Diet

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ABSTRACT

A study on the biology of Utetheisa ornatrix *reared on the artificial diet, was conducted to determine the thermal requirements in each development stage. The aim was to find out the thermal regions in the São Paulo state where the pest could develop on* Crotalaria *spp. The insects were reared on an artificial diet based on the white beans and yeast.* U. ornatrix *thermal requirements were tested at 18, 20, 22, 25, 28, 30, and 32ºC 70±20% RH, and 14-h photophase. Lower threshold temperatures (TT) and thermal constants (K) for the egg, larval and pupal stages were 12.7ºC and 51.2 GDD, 13.5ºC and 290.9 GDD, 13.8ºC and 108.4 GDD, respectively; TT and K values for the biological cycle were 13.8ºC and 436.3 GDD.*

Key words: *Crotalaria*, Rattlebox moth, rearing technique

INTRODUCTION

The Rattlebox moth, *Utetheisa ornatrix* (L., 1758) (Lepidoptera: Arctiidae), is considered to be the most important *Crotalaria* spp. (*Crotalaria anagyroides, C. falsajuncea, C. juncea, C. stipularia, C. usaramohensis, C. vittelina*) (Leguminosae) pest in Brazil (Gallo et al. 2002). It occurs on four other plant species (Silva et al. 1968), and has been found from the North America (east of the Rocky Mountains) to South America (Argentina, Brazil and Chile) (Pease 1968; Silva et al. 1968). The larvae feed on the green pods and on the developing seeds, and they may reduce the crop yields when the infestation is high (Ferro 2001).

In the past few years, the area planted to the crotalaria has increased significantly, reaching about 3,000 ha in Brazil (José Aparecido Donizete Cardoso, personal comunication). The crotalaria is used for the green manure and cover crop (Rosa et al. 2004; Castro et al. 2005) as an controlling nematode agent and as a fiber for the tobacco industry (Nogueira et al. 1992).
The definition of the pest thermal requirements has helped the pest management programs to predict the pest occurrence (Nava and Parra 2003) and to mass-rear the natural enemies for the applied biological control programs (Parra et al. 2002). In this study on the biology of *U. ornatrix* reared on the artificial diet in laboratory the thermal requirements for each insect's development stages in the laboratory were determiner. Results aimed

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to predict the insect occurrence based on the number of annual generations and to facilitate the basic laboratory studies to develop alternative pest control methods.

MATERIAL AND METHODS

Biology of *U. ornatrix* in artificial diet

U. ornatrix adults were obtained from *Crotalaria* fields in Piracicaba, SP and were taken to the laboratory. They were maintained in PVC tube cages (10 × 20 cm) until the oviposition and for the preliminary studies, the cages were covered with the multipurpose office paper (as the egg laying substrate). The adults were fed a 10% honey solution and the eggs were collected daily by cutting the containing egg paper.

The first-generation caterpillars (n=150) were inoculated and individually placed into glass vials (2.5 × 8.5 cm) for the biological study on the artificial diet. The glass vials were filled to one third with the artificial diet containing the white bean (130.0 g), carrot (30.0 g), yeast extract (20.0 g), vitamin solution (40.0 ml), Vitagold® (B complex vitamins) (2.0 ml), ascorbic acid (1.6 g), sorbic acid (0.6 g), methyl parahydroxybenzoate (nipagin) (1.1 g), propionic acid (0.4 ml), Tetrex® (tetracycline) (0.3 mg), forlamdehyde 40% (1.0 ml), agar (7.0 g), and destilled water (500.0 ml). The vitamin solution were compose of: niacinamide (1.00 mg), calcium pantothenate (1.00 mg), thiamine (1.00 mg), riboflavin (0.25 mg), pyridoxine (0.25 mg), folic acid (0.25 mg), biotin (0.02 mg), vitamin B12 (0.002 mg) and inositol (20.00 mg).

The diet preparation followed the methodology developed by Parra (2001). During the pupation, the insects were separated and placed in the plastic cups on a tray covered with the filter paper which was daily moistened. The emerging adults (25 pairs) were separated by the sex (based on the abdomen tip) and placed under the air-conditioned room at 25 ± 2ºC, 70 ± 10% RH, and 14-h photophase.

The biological parameters measured were the duration and viability of the egg, larval and pupal stages, and of the biological cycle (egg to adult); number of instars; male and female pupal weight, pre-oviposition and oviposition periods, sex ratio, fecundity, and longevity. The number of the instars was obtained by daily measuring the head capsule width of 20 caterpillars, with a Wild MM 5235 ocular micrometer attached to a stereoscopic microscope and by adopting the multimodal frequency curve for these measurements. The hypotheses were tested using the linear model for Dyar´s rule and the MOBAE software (Parra and Haddad 1989). The sex ratio (sr) was obtained by the formula sr = ♀/♀+♂.

Thermal requirements of *U. ornatrix*

The insects were reared on the artificial diets in incubators maintained at 18, 20, 22, 25, 28, 30, and 32 ± 1ºC. The embryonic development was observed in 120 eggs/temperature. The eggs were distributed on the acrylic dishes (6.0 × 2.0 cm) containing the partially moistened (bottom part) filter paper. The larval development was observed in 150 larvae/temperature; the insects were individualized in the glass vials (2.0 × 7.0 cm) containing the artificial diet. The pupal development was studied using 100 insects and each one was placed in the acrylic dishes (6.0 × 2.0 cm). The dishes were kept moistened by the humid cotton pads placed in the dishes.

The duration and viability of the embryonic period and the larval and pupal stages were assessed. After determining the duration of the development stages and of the biological cycle (egg to adult) at different temperatures from 18 to 30ºC, the threshold temperature (TT) and thermal constant (K) were calculated using the hyperbole method (Haddad et al. 1999). At 32ºC, the duration was not taken into account since it was not statistically different from the duration at 30ºC ($P \geq 0.05$), thus avoiding the linearity of the hyperbole curve that represented the duration and temperature.

Data analysis

The tests were organized in a completely randomized design and the data submitted to ANOVA. The means were compared by the Tukey test at 5% probability level. The viability data were transformed to arc sine $\sqrt{x + 4}$. The probable number of *U. ornatrix* generations per year for the isotherms within the observed range was calculated via the general thermal constant formula $K = D (T – Tb)$, based on the temperatures that enhanced the insect development (Silveira Neto et al. 1976). The number of the annual generations was obtained by dividing 365 days by the duration value obtained for each temperature.

RESULTS AND DISCUSSION

Biology of *U. ornatrix* on artificial diet

The artificial diet containing the bean and yeast extract led to an adequate development of the immature and adult *U. ornatrix* stages. The duration and viability of the egg, larval, and pupal stages were 4.5 and 84.2, 26.5 and 85.3, and 10.3 days and 91.4%, respectively, for a total duration of 42 days and a 65.6% viability of the biological cycle (egg to adult) (Table 1).

The larval head capsules grew in a geometric progression at each ecdysis, at a constant rate of about 1.4, following Dyar's rule (1890). All the larvae had five instars (Fig. 1). The sex ratio was 0.49, or 1M:1F. The pupal weight was 204.1 mg for the males and 184.9 mg for the females (Table 2). The pre-oviposition period lasted 4.1 days and the oviposition period 18.6 days (Table 2). The fecundity mean was 239.5 eggs, with the longevity values of 24.0 and 22.8 days for the males and females, respectively (Table 2). The daily fecundity mean was 12.9 eggs per day per female, which was higher than the nine eggs mentioned by Johnson et al. (1985), when rearing *U. ornatrix* on the natural diet (*Crotalaria*).

Although 75% eggs-adult viability has been considered adequate for the insects reared on artificial diets (Singh 1983), in the present study the viability was below that percentage. However *U. ornatrix* gradually could adapt to the artificial diet in future generations, given that the number of insects reared on the diet for one generation was not different to those on the natural diet, thus showing the adequate nutritional value of the artificial medium. As mentioned earlier, one of the objectives of this work was to study *U. ornatrix* biology when reared on the artificial diet for the mass-rearing production. Apparentlu the insect can be reared on such medium allowing to study the alternative biological control methods, such as with the *Cotesia* sp. parasitoid, frequently found as a parasite on the caterpillar.

This study is the first to record the rearing of *U. ornatrix* on artificial diet, although the data line refers to one generation only. It has been hypothesized in previous studies that continued rearing of *U. ornatrix* requires that the insect feeds on *Crotalaria* sp. because of chemical compound, which are required for the of the plant insect to mate, such as hydroxydanaidal, the precursor of its sex pheromone (Conner et al. 1990). The females select males that sequester the largest amount of the alkaloid as a natural selection strategy because the larger males will probably have more sperm cells to fertilize the eggs. Further studies should be conducted to determine the possibility of rearing the insect on the artificial diet for the successive generations.

Table 1 - Mean (± SD) duration and viability of *Utetheisa ornatrix* eggs, larvae, and pupae reared on artificial diet. Temperature 25 ± 2ºC, relative humidity 70 ± 10%, and 14-hour photophase.

Stage/Period	Duration (days)	Viability (%)
Egg	4.5 ± 0.1	84.2 ± 12.34
Larva	26.5 ± 2.52	85.3 ± 12.89
Pupa	10.3 ± 0.96	91.4 ± 9.15
Life cycle (egg-adult)	42.0	65.6

Table 2 - Number of instar, duration of pre-oviposition and oviposition periods, sex ratio, fecundity, longevity of male e female, and pupal weight of male e female of *Utetheisa ornatrix* reared on artificial diet. Temperature 25 ± 2ºC temperature, relative humidity 70 ± 10%, and 14-hour photophase.

Biological parameters		Mean ± SD
Number of instars		5.0
Pre-oviposition (days)		4.1 ± 1.74
Oviposition (days)		18.6 ± 5.18
Sex ratio		0.49
Mean fecundity (number of eggs)		239.5 ± 128.4
Longevity (days)	Male	24.0 ± 4.35
	Female	22.8 ± 6.74
Pupal weight (mg)	Male	204.1 ± 031.1
	Female	184.9 ± 024.5

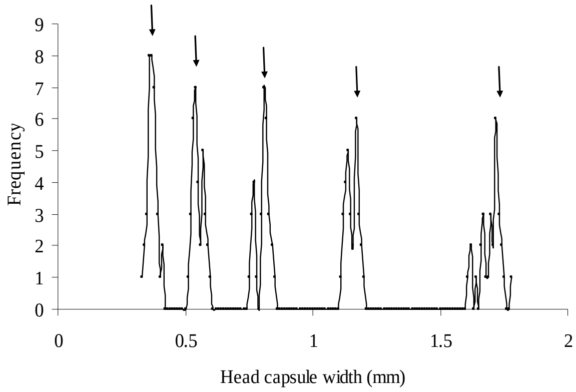

Figure 1 - Frequency distribution of head capsule widths of *Utetheisa ornatrix* reared on artificial diet. Arrows indicate the number of instars (n=5).

Thermal requirements

All *U. ornatrix* stages developed within the temperature range chosen for the study (18 to 32ºC), although at the higlest temperature (32ºC), the viability was significantly lower than the smaller (Table 3). The duration of *U. ornatrix* development stages decreased as the temperature increased (Table 3). The egg stage lasted eight days at 18ºC and three days at 32ºC. The larval stage duration was 56.5 days at 18ºC and 17 days at 32ºC. At the pupal stage, the duration decreased from 24.1 days at 18ºC to 6.3 days at 32ºC. The duration of the biological cycle (egg to adult) varied from 90.6 days at 18ºC to 26.9 days at 32ºC. At 28, 30 and 32ºC, all stage durations were similar, which indicated that the development might be hindered at the temperatures higher than 32ºC.

The temperature did not affect the *U. ornatrix* egg viability, which was above 89.3% (Table 3). For the larval and pupal stages, the viability was significantly lower at 32ºC. The viability was 78.1 at 70.0% and 98.8 at 77.9% within the thermal range of 18 to 30ºC (Table 3). Likewise, 32ºC was harmful for the development of the biological cycle of *U. ornatrix;* at the other temperatures, the viability varied from 56.8 to 75.3% (Table 3). Therefore, it could be attributed that within the temperature range under the study, the best temperatures for *U. ornatrix* development were from 18 to 30ºC. Also, apparently, the insect prefer lower temperatures because all the stages were similar at 28ºC and higher temperatures. This adverse situation was demonstrated from the decrease in the viability for the larval and pupal stages at 32ºC (Table 3).

The threshold temperature (TT) for the embryonic period was 12.7ºC and for the larval and pupal stage, they were 13.5 and 13.8ºC, respectively. The thermal constants (K) for the egg, larval, and pupal stages were 51.2, 290.9, and 108.4 GDD, respectively. The TT and K values for the biological cycle (egg to adult) were 13.8ºC and 436.3 GDD. As reported by Honek (1996), in general, the species adapted to the tropical conditions had a TT of 13.7ºC, which indicated that the 13.8ºC TT value for the biological cycle of *U. ornatrix* was close to this value (Table 4). The insect populations of different regions can have different thermal requirements and the factors other than the latitude (such as food) can affect the insect development (Zeiss et al. 1996; Mihsfeldt 1998). The regions with the isotherms within the 18 to 30ºC range, which were appropriate temperatures for the insect development (Table 3) could have 3.5 to 13.5 generations annually (Fig. 2). In the situations where crotalaria has been the host specially *C. juncea*, cultivated predominantly in the northern part of the São Paulo state (São José do Rio Preto), monthly temperatures varied from 25.6 to 20.1 and the insect might go up to 10 generations/year (Fig. 2).

Table 3 - Mean duration and viability (± SD) and variation interval (values in parentheses) for egg, larval, and pupal stages, and for the biological cycle (egg-adult) of *Utetheisa ornatrix* reared on artificial diet at different temperatures, relative humidity 70 ± 10%, and 14-h photophase.

Temperatura (ºC)	Egg stage	Larval stage	Pupal stage	Life cycle
		Duration (days)		
18	8.0 ± 0.00 a	56.5 ± 1.96 a	24.1 ± 1.23 a	90.6 ± 0.89 a
	(8.0-8.0)	(46.0-74.0)	(20.0-27.0)	(84.0-93.0)
20	7.0 ± 0.00 ab	45.9 ± 2.25 b	18.2 ± 0.98 b	71.9 ± 2.54 b
	(7.0-7.0)	(40.0-60.0)	(15.0-21.0)	(65.0-77.0)
22	6.3 ± 0.33 b	40.7 ± 1.52 b	14.9 ± 1.05 c	63.2 ± 1.89 c
	(6.0-7.0)	(36.0-60.0)	(14.0-17.0)	(56.0-70.0)
25	4.7 ± 0.67 c	24.0 ± 0.91c	9.1 ± 0.75 d	38.3 ± 1.64 d
	(4.0-6.0)	(24.0-34.0)	(8.0-10.0)	(29.0-42.0)
28	3.0 ± 0.00 d	18.6 ± 0.59 d	7.3 ± 1.00 e	28.8 ± 0.81 e
	(3.0-3.0)	(14.0-24.0)	(6.0-9.0)	(21.0-34.0)
30	3.0 ± 0.00 d	18.5 ± 0.36 d	7.0 ± 0.65 e	27.9 ± 0.63 e
	(3.0-3.0)	(15.0-22.0)	(6.0-9.0)	(20.0-32.0)
32	3.0 ± 0.00 d	17.0 ± 0.59 d	6.3 ± 0.49 e	26.9 ± 0.91 e
	(3.0-3.0)	(14.0-22.0)	(5.0-7.0)	(18.0-32.0)
		Viability (%)		
18	98.1 ± 1.93 a	70.0 ± 7.69 a	93.6 ± 4.72 a	64.3 ± 6.32 a
	(94.2-100.0)	(54.5-84.9)	(80.0-100.0)	(51.2-75.0)
20	98.2 ± 1.19 a	70.4 ± 11.16 a	82.2 ± 2.95 a	56.8 ± 4.08 a
	(95.9-100.0)	(56.7-85.6)	(77.8-90.9)	(50.5-71.3)
22	98.6 ± 1.35 a	77.3 ± 9.64 a	98.8 ± 1.25 a	75.3 ± 3.57 a
	(95.9-100.0)	(62.2-90.9)	(95.0-100.0)	(63.9-82.0)
25	100.0 ± 0,00 a	77.7 ± 11.9 a	82.4 ± 6.83 a	64.0 ± 6.44 a
	(100.0-100.0)	(63.6-95.5)	(72.9-95.3)	(56.8-73.2)
28	100.0 ± 0,00 a	78.1± 6.88 a	77.9 ± 6.08 a	60.8 ± 3.91 a
	(100.0-100.0)	(63.6-95.5)	(61.1-80.0)	(49.1-72.4)
30	89.3 ± 9.99 a	78.1 ± 1.25 a	90.9 ± 4.01 a	63.4 ± 6.49 a
	(69.4-100.0)	(76.2-81.8)	(81.3-100.0)	(56.4-75.3)
32	95.8 ± 2.72 a	60.7 ± 6.64 b	62.2 ± 8.32 b	30.2 ± 2.50 b
	(90.3-98.7)	(54.6-80.9)	(40.0-75.0)	(17.2-43.1)

Mean values followed by the same letter in the columns are not statistically different according to Tukey test ($P \geq 0.05$).

Table 4 - Lower threshold temperature (TT), thermal constant (K), regression analysis (1/D), and determination coefficient (R^2) for the egg, larval, and pupal stages and for the life cycle (egg-adult) of *Utetheisa ornatrix* in the laboratory.

Stages/Period	TT (ºC)	K (GDD[1])	Regression equation	R^2	*P*
Egg	12.7	51.2	$1/D = 0.019524t_i - 0.247656$	0.9336	≤ 0.05
Larva	13.5	290.9	$1/D = 0.003438t_i - 0.046346$	0.9598	≤ 0.05
Pupa	13.8	108.4	$1/D = 0.009227t_i - 0.127699$	0.9781	≤ 0.05
Egg-adult	13.8	436.3	$1/D = 0.002292t_i - 0.031721$	0.9704	≤ 0.05

(1) GDD: Growing Degree-days.

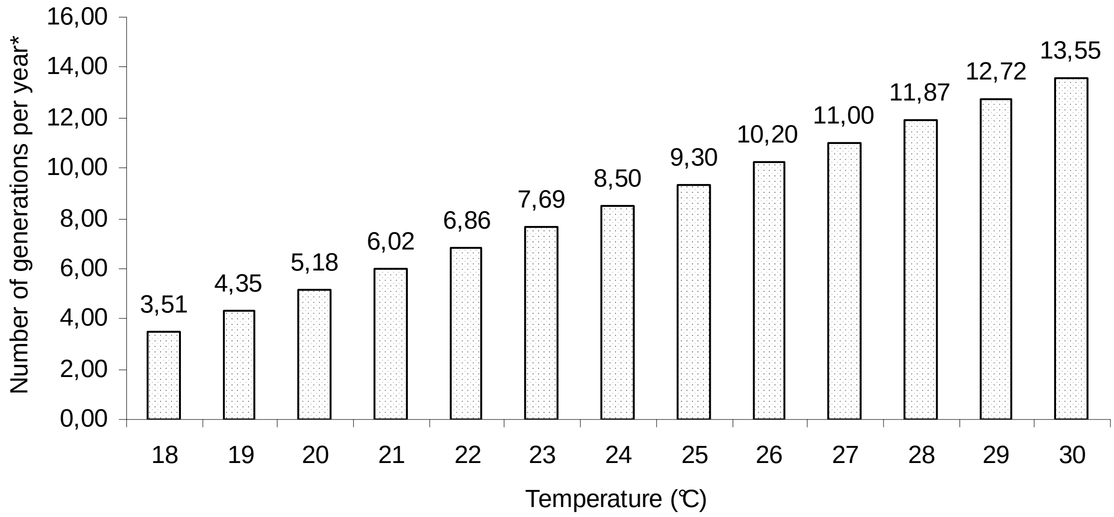

Figure 2 - Number of *Utetheisa ornatrix* generations in regions with same isotherms for the appropriate temperature ranges for insect development. Obtained with the formula K= D(T–TT), where K = 436.28GDD; T = variable, 18 to 30ºC; Tb =13.8ºC and D = variable to be determined. The number of generations per year was obtained by dividing 365 days by D.

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RESUMO

Estudou-se a biologia de *Utetheisa ornatrix* em dieta artificial, visando a determinação das exigências térmicas dos diferentes estágios de desenvolvimento, numa tentativa de avaliar as regiões do estado de São Paulo em que a praga tem maiores condições de se tornar problema para *Crotalaria* spp. Foram criados insetos em dieta artificial à base de feijão e levedura de cerveja. Para a determinação das exigências térmicas de *U. ornatrix* foram utilizadas as temperaturas de 18, 20, 22, 25, 28, 30 e 32ºC, umidade relativa de 70 ± 20% e fotofase de 14 horas. Os limiares térmicos inferiores de desenvolvimento (Tb) e as constantes térmicas (K) para os estágios de ovo, lagarta e pupa foram de 12,7ºC e 51,2 GD, 13,5ºC e 290,9 GD, 13,8ºC e 108,4 GD, respectivamente, resultando em valores de Tb e K para o ciclo biológico (ovo-adulto) de 13,8ºC e 436,3 GD. O inseto pode dar até 10 gerações anuais nas regiões em que *Crotalaria* spp. é mais importante para o estado de SP.

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