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Some reproduction aspects of *Ephestia kuehniella* Zeller (Lepidoptera, Pyralidae) under mass rearing conditions

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Abstract: The Mediterranean flour moth, *Ephestia kuehniella* Zeller, is a serious cosmopolitan pest for cereal mills and wherever flour is stored, and in the Azores it is used to rear parasites and predators that are used in biological control (Trichogrammatidae, Coccinellidae, Chrysopidae). In order to learn the effect of mass rearing conditions on the reproduction of *E. kuehniella*, four groups of adult moths were studied: (i) isolated couples which were allowed to mate freely during all adult life, and which were not fed; (ii) isolated couples which mated freely and were fed with honey; (iii) couples that mated once and were not fed; (iv) virgins and were not fed. Differences between groups regarding weight, longevity, copulating behaviour, fecundity, fertility and number of spermatophores were evaluated. Females were heavier than males. Males and virgin females lived significantly longer than mated females. The oviposition was mainly concentrated in the first three days of the females lifetime (70-90% laying eggs). A high percentage (88%) of copulations occured within five hours before dawn. No significant differences were found in fecundity and fertility parameters between females coupling once and those mating freely during lifetime, nor between those fed with or without honey. Virgin females laid lower numbers of eggs than mated females.

Aspectos de la reproducción de *Ephestia Kuehniella* Zeller (Lepidoptera, Pyralidae) en crianza masiva

Resumen: La polilla mediterránea de la harina, *Ephestia kuehniella* Zeller, es una grave plaga cosmopolita que afecta cereales allí donde se almacene harina. En las Azores se utiliza para criar parásitos y predadores que luego son empleados en control biológico (Trichogrammatidae, Coccinellidae, Chrysopidae). Para conocer los efectos que las condiciones de cría en masa tienen sobre la reproducción de *E.kuehniella*, se estudiaron cuatro grupos independientes de adultos: (i) parejas aisladas que copularon libremente durante toda la vida y no fueron alimentadas; (ii) parejas aisladas que también copularon libremente pero fueron alimentadas con miel; (iii) parejas que copularon sólo una vez y no fueron alimentadas; (iv) vírgenes aislados y no fueron alimentados. Se midieron el peso, la longevidad, comportamiento reproductivo, fecundidad, fertilidad y número de espermatóforos. Las hembras siempre dieron peso superior al de los machos. En contraste, los machos y las hembras vírgenes vivieron significativamente más que las hembras que copularon. La oviposición está principalmente concentrada en los tres prime-

ros días de vida adulta de las hembras (70-90% del total de los huevos puestos). Un elevado porcentaje (88%) de las cópulas empezaron en las 5 horas previas al amanecer. No se hallaron diferencias significativas en fecundidad y fertilidad entre las hembras que copularon sólo una vez y las que lo hicieron libremente toda su vida adulta, y tampoco entre las que fueron alimentadas con miel y las que no. Las hembras vírgenes pusieron menos huevos que las que habían copulado.

INTRODUCTION

The Mediterranean flour moth, *Ephestia kuehniella* Zeller, 1879 (Lepidoptera, Pyralidae), is a well known pest for stored cereals. It has been largely used as a substitute host for the rearing of predators and parasites aimed for Biological Control (TAVARES *et al.*, 1989; VIEIRA *et al.*, 1992). The characteristics of its reproduction under mass production conditions, i.e. in Biofactories, are of great interest for the management of production units and helps to the understanding of biological cycles of damaging insects (DAUMAL, 1987; TAVARES *et al.*, 1989; TAVARES & VIEIRA, 1992; DAUMAL & BOINEL, 1994).

The improvement of productivity is highly dependent on the knowledge of the reproductive capacity and related biological traits, such as adult weight, longevity, adult feeding and number of matings. So, the aim of this study was to assess the effect of mass rearing on these life history traits in *E. kuehniella.*

MATERIAL AND METHODS

The reference population was obtained rearing *E. kuehniella* according to the methods described by TAVARES *et al.* (1989) and TAVARES & VIEIRA (1992). Conditions for larval development were those described by VIEIRA *et al.* (1992), but the diet choosen was regional crushed corn grains of a medium texture (instead of imported corn flour), sited in alveolar cardboard. Egg and larval instars were initially maintained at 25 ± 0.5 °C and 70 ± 5 % Relative Humidity. Twenty one days after the beginning, the cultures were changed to 12 ± 0.5 °C and 85 ± 5 % R.H. and thus maintained until the first prenymphal states (36 days after the beginning). The rest of development and adult life continued under the initial conditions. The photoperiod was always 16L:08D.

Adult pairs were divided in four separated groups (M, N, U, and V groups, see below). The two members of each pair had emerged on the same day and were weighed 8 hours after emergence in a METTLER AE 240® balance. They were confined in plastic transparent boxes (53 cm^3).

Pairs of M group were allowed to mate freely all along their adult life but

they were not fed in order to be used as control group, as it corresponds with the normal conditions of mass production. The N pairs were also let to mate freely, but drops of honey (1 ml) were daily subministred as adult nutrition. The U individuals were separated after the observation of the first coupling. Finally, virgin females and males (V group) were isolated before coupling.

Longevity was recorded by means of daily observation, as well as fecundity and fertility. Since the females disperse their eggs, the eggs produced by every female were maintained into the plastic box and counted directly under binocular magnifying glasses. Fertility was estimated 3-5 days later by counting the esterile eggs (white in colour, as opposite to the yellow, fertile eggs). Infertile eggs were defined as those with no development after oviposition (STEELE, 1970).

Copulation behavior was studied using 86 *E. kuehniella* couples. Observations were performed every 15 minutes for a period of 13 hours (including 5 hours before dawn) in order to record the number of mated couples and also the mean copulation time. To the effects of analysis and representation, observations were grouped by hours. Once dead, females were dissected in order to count the spermatophores from the bursa copulatrix.

The data were analised using an analysis of variance (ANOVA) and if significant, the means separated using the Scheffe's test.

RESULTS AND DISCUSSION

Weight of adults: The weights for both sexes and for every one of the four experimental groups are shown in Table 1. Females were always heavier than males, respectively, 18.54 ± 0.26 mg (n = 117) and 13.11 ± 0.20 mg (n = 101). Significant differences between groups were found for males ($F = 19.70$, $p < 0.001$) separating groups M and N from U and V, but not for females ($F = 2.74$, $p \geq 0.05$) (Table 1).

Longevity: Longevity for females (8.97 ± 0.29 days, n = 116) was lower than for males (12.35 ± 0.31 days, n = 98). Moreover, analysing longevity for the four experimental groups of the Table 1, we remark: (i) that, for females ($F = 138.01$, $p < 0.001$), while we found no significant differences among the M, N and U groups (virgins (V) lived significantly longer. (ii) For males ($F = 11.43$, $p < 0.001$): virgins (V) and those that mated only once (U) lived significantly longer than N and M groups, so V and U males seem to save the energy that others spend in reproductive activities. (iii) Finally, feeding adults on honey does not increase longevity (N group front M, Table 1).

Fecundity, fertility and number of spermatophores: Fecundity and fertility resulted similar for M, N and U groups (Table 1). The increase in average fecundity relative to females coupling once to free coupling ones was only of

11.8%. Virgins produced a significantly lower amount of eggs (obviously, infertile eggs).

Table 1

Different biological parameters with indication of the number of analized *E. kuehniella* individuals (n) and mean value with standard error (X ± SE) for the four studied groups: M (multiple copulations and not fed), N (multiple copulations and fed with honey), U (mating once and not fed) and V (virgins and not fed). *For every single parameter, those pairs of values with signifitive differences for the Scheffe's Test ($p < 0.05$) are indicated with the same letter. † Values of fertility were transformated previously to analysis according to the formula $\arcsin\sqrt{x}$.

*E. kuehniella**	EXPERIMENTAL GROUP							
	M		N		U		V	
	n	X ± SE	n	X ± SE	n	X ± SE	n	X ± SE
Weight (mg)								
Female	36	19.21 ± 0.38	29	18.13 ± 0.50	22	19.32 ± 0.55	30	17.56 ± 0.63
Male	39	12.66 ± 0.29a	29	12.07 ± 0.28b	22	14.16 ± 0.32ab	11	15.36 ± 0.61ab
Longevity (days)								
Female	36	7.33 ± 0.13a	28	7.36 ± 0.18b	22	7.36 ± 0.15c	30	13.62 ± 0.45abc
Male	38	11.26 ± 0.54a	27	11.22 ± 0.42b	22	14.16 ± 0.45ab	11	15.27 ± 0.63ab
Fecundity	36	337.39 ± 8.65a	29	295.72 ± 13.58b	22	301.86 ± 14.12c	30	123.77 ± .58abc
Fertility (%)†	36	97.58 ± 0.44	29	96.78 ± 1.48	22	96.49 ± 2.13		
Spermatophores	36	2.64 ± 0.18a	29	2.48 ± 0.28b	22	1 ab		

Females copulated from one to seven times, as determined by the number of spermatophores present in the bursa copulatrix at death. The number of spermatophores found in females of the M and N groups was similar (Table 1). The relationship between fecundity and number of spermatophores is shown in Table 2. No correlation existed between these parameters ($r = 0.20$, $p = 0.0628$, $n = 87$). Thus, reproductive efforts seem similar for the three groups, independently on the number of couplings or on the nutrition with honey at the adult stage.

Table 2

The effect of the number of copulations on the fecundity (mean ± standard error) of *E. kuehniella*.

COPULATION FREQUENCY	n	FECUNDITY (X ± SE)
4 spermatophores	9	338.67 ± 14.81
3 spermatophores	19	333.32 ± 15.03
2 spermatophores	21	321.33 ± 11.82
1 spermatophore	35	295.00 ± 12.40
Virgin	30	123.77 ± 10.60*

*There are significative differences ($p < 0.05$) between virgin and the other ones, according Sheffe's test.

The study of fertility permitted to detect some cases of sterility. During our experiences only for the U group there appeared females (14.8 %) whose eggs did not develop at all, or only did in a small number. These females were not considered for statistical analysis. The fact that these females had a single spermatophore means that we are directly testing the number of those spermatozoids. Moreover, the lack of sterility in the groups of females containing the majority of them more than one spermatophore can point to the possibility that the successive copulations hide the possible presence of some unable or infertile spermatozoids.

Accumulated mean fecundity was studied for the first eight days of adult life (Figure 1). Little difference exists among M, N and U (Figure 1). Day-to-day comparison gives significant differences ($p < 0.05$) between days 1-2 and 2-3 for the first two groups. After that, number of eggs per day diminishes and accumulated fecundity seems to reach a maximum. So, oviposition is mainly concentrated in the first three days, where 77.27 %, 82.14 % and 90.69 % (M, N and U, respectively) of the total egg mass is concentrated.

It is generally accepted that *E. kuehniella* concentrates much of its egg pro-

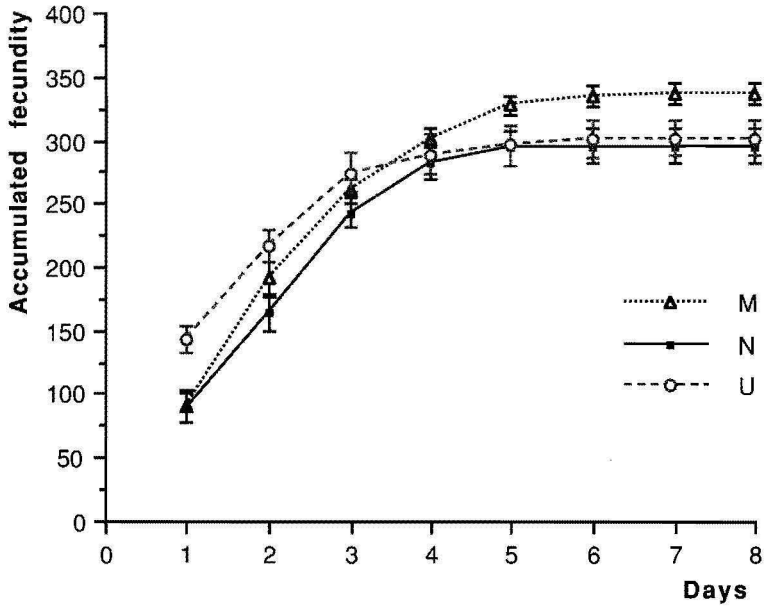

Figure 1. Accumulated mean fecundity (mean ± standard error) in function of time (days) for the three coupling groups of *Ephestia kuehniella*, "M" (multiple copulations and not fed), "N" (multiple copulations and fed) and "U" (mating once and not fed).

duction in the first five days of adult life. This fact permits to maximize the use of a unity of production (Biofactory) to obtain a high amount of eggs, which can be used to rear parasites and predators (see also TAVARES & VIEIRA, 1992).

Females weight and fecundity were positively correlated ($r = 0.475$, $p < 0.001$), according to the regression line $y = 81.97 + 12.32\ x$ ($F = 24.77$, $p < 0.001$, $df = 85$), where y is fecundity and x is weight in mg. However, this relation can be disturbed by many factors which act before the mating, namely temperature, photoperiod and nutrition (DAUMAL, 1987; DAUMAL & BOINEL, 1994).

Copulating behaviour: A high percentage (88%) of copulations occured within five hours before dawn (Figure 2). Proportion of moths calling for *E. kuehniella* increases during scothophase with a maximum at about dawn (TRAYNIER, 1970; TAKAHASHI, 1973). During day time it slowly decreases towards a minimum coinciding with the start of scotophase (TRAYNIER, 1970; TAKAHASHI, 1973). The average duration of copula was longest for females that started mating two hours before, and one hour after the light was on (Figure 2). We reported a maximum copulation duration of 220 minutes.

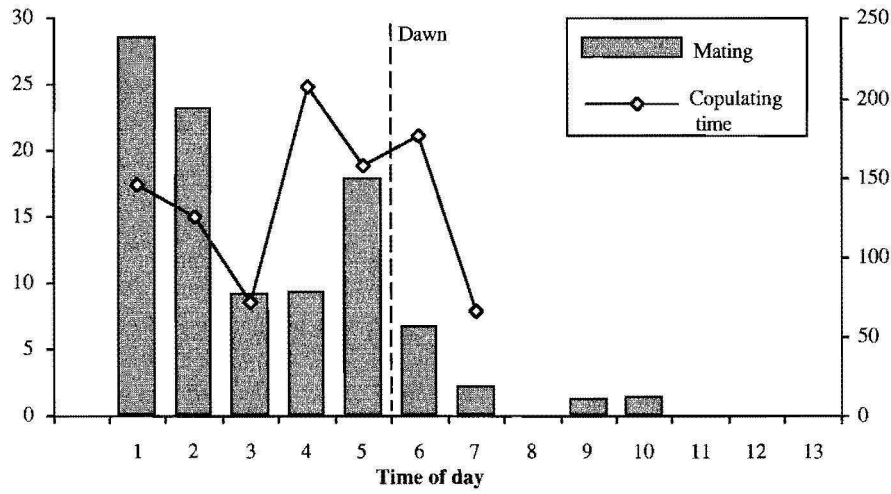

Figure 2. Percentage of couples of *E. kuehniella* (total number = 86) which began to mate at every hour for the period of 13 hours observed, which includes 5 hours before dawn. Also the mean copulation time (in minutes) for each hour is represented.

CONCLUSIONS

The main conclusions of the present work are the following:

— Females of *E. kuehniella* always weigh more than males;
— Males live longer than females;
— Among females, virgin ones live longer;
— Feeding adults with honey does not increase longevity;
— Fecundity and fertility do not result modified when adults only mate once or when they are fed with honey;
— 77-90% of eggs are laid in the first three days;
— 88% of matings begin in the five hours previous to dawn;
— The mean copulation time is of about 120 minutes;
— Matings beginning from two hours before to one hour after dawn are longer.

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