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Epigenetic Variability of *Ephestia kuehniella* Zeller (Lepidoptera, Pyralidae) under mass rearing conditions

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Abstract: *Ephestia kuehniella* Zeller egg production unit (biofactory) is operating at the University of Azores for the last ten years. The Mediterranean flour moth eggs are used to rear entomophagous insects of use in biological control (Trichogrammatidae, Coccinellidae, Chrysopidae). The influence of different temperature regimes, larval density and diet texture (regional maize flour) was studied on ten populations, using the following parameters: pre-imaginal development time, adult longevity and weight, fecundity, fertility, mortality, emergency rate and sex ratio. As a result, optimum conditions for mass production of *E. kuehniella* at the azorean biofactory are: embryonic development and first 15 days of larval development at 25 °C; larval populations with more than 15 days at 12 °C, up to the prepupal stage; the following developmental stages at 25 °C; 4 or 5 eggs per 2 cm^3 of alveolar card volume; medium texture, regional hard-maize semolina; 70 ± 5% relative humidity and 16L:08D photoperiod.

Variabilidad e genética de *Ephestia kuehniella* Zeller (Lepidoptera, Pyralidae) em condições de multiplicação maciça

Resumo: A unidade de produção de ovos de *Ephestia kuehniella* Zeller (biofábrica) da Universidade dos Açores está em funcionamento há uma dezena de anos. Os ovos são usados para a multiplicação maciça de parasitóides (Trichogrammatidae) e predadores (Coccinellidae, Chrysopidae) necessários ao controlo biológico de pragas agrícolas. Foi estudada a influência das variáveis temperaturas alternadas (25 e 12°C), o grau de densidade (4, 5 e 6 ovos por alvéolo de cartão com 2 cm^3) e a textura da dieta (milho branco regional) sobre o desenvolvimento pré-imaginal, peso e longevidade dos adultos, fecundidade, fertilidade, mortalidade, percentagem de emergência e *sex-ratio*, de dez grupos de *E. kuehniella*. Os resultados deste estudo permitem concluir que as melhores condições para a multiplicação maciça de *E. kuehniella* na biofábrica açoreana são: desenvolvimento embrionário e primeiros 15 dias do desenvolvimento larvar a 25 °C; populações com mais de 15 dias submetidas a 12°C até à pré-ninfa; 4 ou 5 ovos por alvéolo de cartão com 2 cm^3 de volume; farinha de milho regional de textura média (meio carolo); 70 ± 5 % de humidade relativa e fotofase de 16 horas.

INTRODUCTION

The eggs of the Mediterranean Flour Moth, *Ephestia kuehniella* Zeller, 1879 (Lepidoptera, Pyralidae), in the Azores Archipelago are used as a factitious host for parasitoids (Trichogrammatidae) and as a food source for predators (Coccinellidae and Chrysopidae) in mass rearing units.

To accomplish such a massive production, a "Biofactory", was built at the University of the Azores (TAVARES, 1983). In the last ten years, this semi-industrial unit was improved in order to allow the automatic collection of the adult moths and the removal of the scales, and a cold storage of the eggs (TAVARES, 1989; TAVARES & VIEIRA, 1992). The unit is now managed in real time by computer.

Meanwhile, several studies were performed to determine the effects of the temperature (TAVARES & DAUMAL, 1983; TAVARES *et al.*, 1989; VIEIRA *et al.*, 1992), and type of diet on the development of the moth (TAVARES, 1989), and to evaluate the variations in temperature and relative humidity in mass rearing conditions (TAVARES, 1989). A new method, that allows for a longer storage of the eggs, under low temperatures was developed by VIEIRA & TAVARES (1992) and VIEIRA & TAVARES (in press).

Due to economic reasons, yellow maize imported flour was replaced by white maize local flour. The alveolated card plates (where the insect pupates) were replaced by card plates with an alveolus of 2 cm^3, a duplication of the previous volume. In the present work, the impact of these new conditions in *E. kuehniella* mass rearing was evaluated. The influence of temperature regime, larval density and diet texture, was studied in ten experimental populations. The following parameters were considered: larval and pupal development time, adult longevity, adult weight, fecundity, fertility, mortality, first 20 days emergency rate, and sex ratio.

MATERIAL AND METHODS

Ten populations of *E. kuehniella* (one thousand eggs each) were taken from the colony of the Azorean biofactory. Embrionic development was completed in five days, at 25 ± 1°C, 70 ± 5% of relative humidity, and a 16 hours photophase. Larvae were reared in 2 liter, transparent plastic boxes, with two 5 cm holes at the top, covered by a fine metallic mesh. Flour was added to each box, and covered with a cardboard plate with 2 cm^3 alveoli. Neonate larvae were placed within the alveoli. Treatments were based on temperature regime, diet texture and larval density. For all treatments, relative humidity and photoperiod were those refered for embryonic development.

Temperature: Larvae were reared in a chamber regulated to 25 °C, during the first 5 days (group B), 10 days (group C), 15 days (group D), 20 days (group E) or 25 days (group F). Afterwards, they were submitted to 12°C until the first prepupa. The following stages were submitted to 25 °C. Group A developed at a constant temperature of 25 °C. Medium texture flour, and 5 larvae per alveolus were used for these groups.

Texture of diet: Three different maize flour textures were used: medium (group D), fine (group G) and coarse (group H). Temperature regime and larval density were those given for group D.

Density: Three different larval densities were used: 5 (group D), 6 (group I) or 4 (group J) larvae per 2 cm^3 alveolus. Temperature regime and diet were those given for group D.

Adult moths were weighed, and couples were formed, until 12 hours after adult emergency. They were not fed. For mating and oviposition, isolated couples were placed in cylindric boxes 3 cm high per 5 cm diameter. Couples were formed for life.

Observations were performed every day. For each parameter (duration of preimaginal development, weight, longevity, fecundity, fertility) differences between treatments were evaluated by ANOVA. When significant differences were found, means were compared using SHEFFE "F" test. Multivariate analysis (AFD) was also performed to better discriminate the groups. A proportion comparison test was used for the following parameters: preimaginal mortality, percentage of emergence for the first 20 days, and sex ratio.

RESULTS AND DISCUSSION

Temperature: Mean duration of preimaginal development (Table 1) ranged from 40.14 (group A) to 101.38 days (group B).

Females always were heavier than males, as found by TAVARES (1989) and VIEIRA *et al.* (1992), but males lived longer (Table 1). The lowest fecundity was found in group B. Group C presented the highest fecundity, followed by group D (Table 1). Mean fertility was always higher than 94 % (Table 1).

Significant differences were found between the six groups, for all the parameters ($p \leq 0.0025$). Meanwhile, differences between two groups were not always significant (Table 1).

Data from Tables 1 and 2 show that, a constant temperature of 25 °C leads to the shortest preimaginal development time, and the lowest mortality, emergency rate and egg production. On the other hand, rearing at 12 °C from the first two larval instars to the pre-pupa, homogenised emergency rate, but gave rise to a higher mortality, and a lower fecundity. Regarding sex ratio, no significant differences ($p > 0.05$) were found between groups.

Table 1

Biological parameters of ten groups of *E. kuehniella* submitted to different temperature regimes, diet textures and larval densities.
* Mean, standard error and statistical analysis. # Values of fertility were transformed previously to the analysis, according to the formula arc sin $\sqrt{x}$.
® A significative difference between *E. kuehniella* groups was found ($P < 0.05$). For example, A≠(C, E) means that A is significantly different from C and E.

VARIABLE *	GROUP	N	Preimaginal development time (days)	Adult weight (mg)		Longevity (days)		Fecundity	Fertility (%) #
				Female	Male	Female	Male		
Temperature	A	37	40.14±0.12	17.94±0.40	12.52±0.27	7.51±0.22	12.22±0.36	248.84±08.70	98.6±0.003
	B	32	101.38±0.09	18.60±0.52	12.30±0.26	9.07±0.18	12.40±0.34	240.53±11.43	94.1±0.014
	C	39	72.31±0.16	20.36±0.44	14.70±0.31	7.90±0.17	13.88±0.33	343.03±11.04	98.6±0.004
	D	39	51.80±0.14	19.37±0.36	13.19±0.31	7.39±0.12	11.71±0.48	340.92±08.36	97.6±0.004
	E	39	50.46±0.14	20.36±0.43	13.36±0.30	7.51±0.20	12.59±0.33	320.33±07.31	98.8±0.003
	F	37	42.60±0.21	18.51±0.44	12.40±0.24	7.65±0.20	12.73±0.40	241.27±08.92	98.7±0.002
	Analysis of variance								
	F - Value		21889.88	5.16	10.19	10.08	3.82	29.29	8.66
	P - Value		< 0.0001	0.0002	< 0.0001	< 0.0001	0.0025	< 0.0001	< 0.0001
	Scheffe test®		A≠B≠C≠D≠E≠F	A≠[C,E]	C≠[A,B,D,E,F]	B≠[A,C,D, E, F]	C≠D	A≠[C,E]; C≠[B,F]; D≠[A,B,F]; E≠[B,F]	B≠[A,C,E,F]
Texture of diet	D	39	51.80±0.14	19.37±0.36	13.19±0.31	7.39±0.12	11.71±0.48	340.92±08.36	97.6±0.004
	G	26	104.39±0.24	21.81±0.71	14.77±0.37	8.15±0.25	11.19±0.55	212.81±17.68	92.2±0.018
	H	30	54.90±0.27	18.30±0.60	12.38±0.27	6.82±0.16	12.35±0.53	218.70±09.75	99.1±0.003
	Analysis of variance								
	F - Value		17708,99	10.01	13.56	13.00	1.18	42.47	13.52
	P - Value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.3135	< 0.0001	< 0.0001
	Scheffe test®		D≠G≠H	G≠[D,H]	G≠[D,H]	G≠[D,H]	.	D≠[G,H]	D≠G≠H
Larval density	D	39	51.80±0.14	19.37±0.36	13.19±0.31	7.39±0.12	11.71±0.48	340.92±08.36	97.6±0.004
	I	27	54.15±0.24	20.07±0.52	13.56±0.47	7.50±0.28	12.35±0.46	286.89±19.08	91.5±0.029
	J	25	53.20±0.14	21.90±0.83	13.67±0.44	7.61±0.23	12.85±0.49	345.08±13.90	95.1±0.017
	Analysis of variance								
	F - Value		50.70	5.52	0.42	0.31	1.41	5.49	3.34
	P - Value		< 0.0001	0.0055	0.6587	0.7320	0.2496	0.0057	0.0402
	Scheffe test®		D≠I≠J	D≠J	.	.	.	I≠[D,J]	D≠I

Table 2

Preimaginal mortality rate, 20 first days emergency rate, and sex ratio in ten groups of *E. kuehniella*, submitted to different temperature regimes, diet textures and larval densities.
* For every variable, those pairs of values followed by the same letter (only between bold letter and this one in plain text) are significantly different at the 5% level according to a proportions comparison test.

Variable*	*E. kuehniella* group	N	Preimaginal mortality rate	Emergency rate during first 20 days	Sex ratio (% of female)
Temperature	A	641	08.3 **a**	72.8 **a**	48.8
	B	668	36.5 ab	100.0 ab	53.3
	C	683	37.3 ab	99.8 ab	48.4
	D	660	36.8 ab	97.8 ab	50.8
	E	710	35.4 ab	96.1 ab	52.3
	F	737	23.1 **ab**	81.7 **ab**	51.1
Texture of diet	D	660	36.8 c	97.8 c	50.8 c
	G	699	67.1 cd	100.0 cd	40.9 cd
	H	695	37.6 d	97.9 **d**	50.5 **d**
Larval density	D	660	36.8 e	97.8	50.8
	I	886	34.9	99.1	47.8
	J	546	30.8 **e**	97.9	47.6

A discriminant analysis based on 5 parameters of the females shows four different groups (Figure 1). Preimaginal development and fecundity are the parameters with a higher discriminating effect. These results agree with the accepted idea that an alternating temperature increases the rentability of the mass production of adults and eggs (DAUMAL *et al*, 1981; VIEIRA *et al.*, 1992), allowing to choose a faster or slower development, depending on the need to replace the egg stock.

Texture of diet: Significant differences were found between group D and groups G (fine flour) and H (coarse flour) for all the parameters, except male longevity (Table 1).

Fine flour caused higher mortality, higher proportion of males, and an higher emergency rate after 20 days (Table 2). Fine and coarse flour caused a lower fecundity (Table 1).

A discriminant analysis based on five parameters of the females shows that group G is separated from the intersection of groups D and H (Figure 2). Correlation circle analysis shows that preimaginal development, weight and fecundity, are the parameters with an higher discriminating effect.

Density: A comparison of groups D, I and J shows that higher population density resulted in a decrease in fecundity and fertility (Table 1), but also in lower mortality (4 larvae per alveolus). No significant differences were found for emergency rate and sex ratio (Table 2).

A discriminant analysis based on five parameters of the females shows con-

siderable overlaping of the populations (Figure 3). Correlation circle analysis demonstrates that preimaginal development and fecundity are the parameters with an higher discriminating effect, although the biological parameters studied are unable to separate the groups.

Fecundity of *E. kuehniella* is affected both by larval density and by the number and size of the alveoli used to collect the pupae in the cardboard plate (DAUMAL & BOINEL, 1994). For this reason, alveolated cardboard plate should be adopted to the oviposition behaviour of the moth.

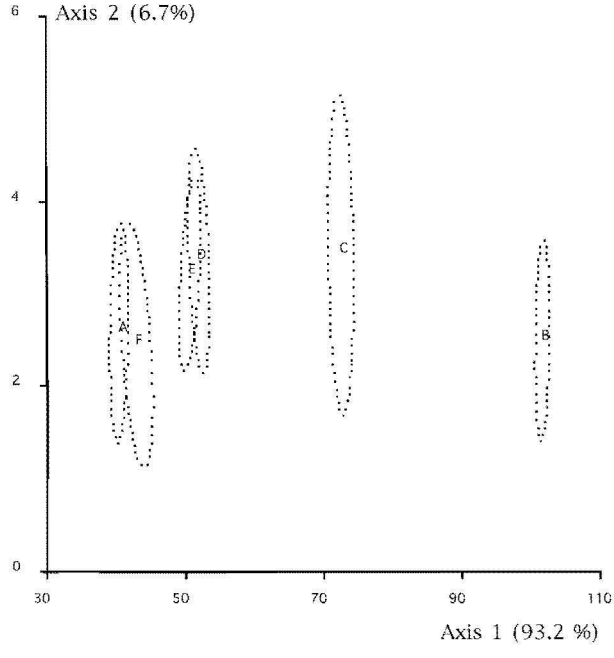

Figure 1. Factorial discriminant analysis (FDA) based on five parameters (preimaginal development time, weight an longevity of the adults, fecundity and fertility) measured on six groups of *E. kuehniella* submitted to different temperature regimes. Elipses containing 95% of the observations.

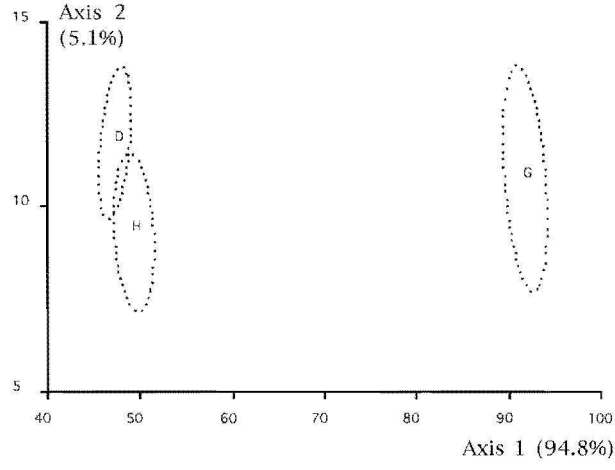

Figure 2. Factorial discriminant analysis (FDA) based on five parameters (preimaginal development time, weight an longevity of the adults, fecundity and fertility) measured on three groups of *E. kuehniella* submitted to different diet textures. Elipses containing 95% of the observations.

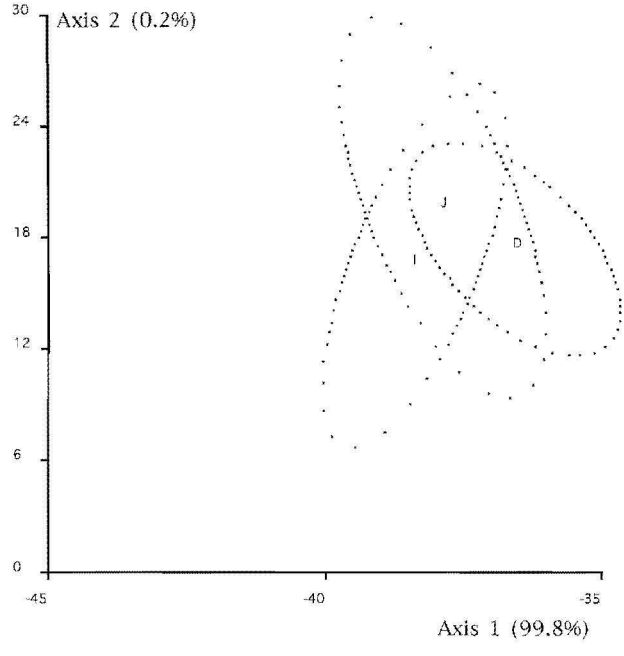

Figure 3. Factorial discriminant analysis (FDA) based on five parameters (preimaginal development time, weight an longevity of the adults, fecundity and fertility) measured on three groups of *E. kuehniella* submitted to different larval densities in 2 cm^2 alveolated card. Elipses containing 95% of the observations.

CONCLUSIONS

Successful rearing of parasitoids and predators for biological control requires constant and reliable production of *E. kuehniella* eggs by mass rearing. Larvae that were submitted to 12°C in the first two instars had a significantly longer development time. Females were always heavier, but their life-span was significantly shorter than that of males. Fecundity is affected by temperature, texture of diet, and densities higher than 5 initial eggs.

In summary, optimum conditions for mass production of *E. kuehniella* at the azorean biofactory can be described as:

— embryonic development and larval populations during the first 15 days at 25 °C; larval populations with more than 15 days, at 12 °C up to the prepupal stage; the following development stages at 25 °C;

— density between 4 and 5 eggs per 2 cm^3 of alveolar card volume;

— larvae fed with medium texture, regional hard-maize semolina;

— 70 ± 5 % relative humidity;

— photoperiod of 16L:8D.

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REFERENCES

DAUMAL, J., J. VOEGELE & B. PINTUREAU, 1981. Durée de développement d'*Ephestia kuehniella* ZELLER (Lep., Pyralidae) en fonction de la température à laquelle est soumise la chenille. *IX Reunion Nacional de Control Biologico, Oaxaca* (Mexique): 96-106.

DAUMAL, J. & H. BOINEL, 1994. Variability in fecundity and plasticity of oviposition behavior in *Anagasta kuehniella* (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. Am.* 87(2): 250-256.

TAVARES, J. & J. DAUMAL, 1983. Durée de developpement et fécondité d'*Ephestia kuehniella* ZELLER (Lep., Pyralidae) après le ralentissement de quelques jours à l'état prénymphal et nymphal à basse température. *Arquipélago (Série Ciencias da Natureza)* 4: 46-63.

TAVARES, J., 1983. Unidade de processamento automático para adultos das traças da farinha, usadas como hospedeiros intermediários nas biofábricas de insectos oófagos. *Boll. Prop. Ind. Patente* 76184, 2: 258.

TAVARES, J., L. AUNCIADA, L. OLIVEIRA & V. VIEIRA, 1989. Produção em massa de *Ephestia kuehniella* ZELLER (Lep. Pyralidae). III - Metodologia para a avaliação da produção de adultos e ovos. *Bolm. Soc. port. Ent.*. 104 (IV-2): 13-24.

TAVARES, J., 1989. *Mythimna unipuncta* (Haworth) *(Lep., Noctuidae) aux Açores. Bioécologie et lutte biologique.* Thèse d'État Ès-Sciences — Université d'Aix-Marseille, France.

TAVARES, J., & V. VIEIRA, 1992. Produção em massa de *Ephestia kuehniella* Zeller (Lep. Pyralidae). IV - Técnicas de recolha dos adultos e ovos. *Açoreana* 7(3): 461-469.

VIEIRA, V., J. TAVARES & J. DAUMAL, 1992. Influence des temperatures alternées sur le developpement larvaire d'*Ephestia kuehniella* Zeller (Lep., Pyralidae). *Açoreana* 7(3): 471-477.

VIEIRA, V. & J. TAVARES, 1992. Multiplicação de *Trichogramma cordubensis* Vargas & Cabello (Hym., Trichogrammatidae), usando ovos de *Ephestia kuehniella* Zeller (Lep., Pyralidae) conservados ao frio. *Bolm. Soc. port. Ent.*. 1 (Supl. 3): 445-452.

VIEIRA, V. & J. TAVARES, in press. Rearing of *Trichogramma cordubensis* Vargas & Cabello (Hymenoptera: Trichogrammatidae) on Mediterranean flour moth cold-stored eggs. *Proceedings of 4th International Symposium on* Trichogramma *and other egg parasitoids, Cairo, Egypt, from 4 to 7 October 1994.*