Bol. San. Veg. Plagas, 31: 21-25, 2005

Productivity and quality aspects concerning the laboratory rearing of *Trichogramma* spp. (Hym.: Trichogrammatidae) and its factitious host, *Ephestia kuehniella* Zeller (Lep.: Pyralidae)

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The mass rearing of natural enemies for use in integrated pest management requires the availability of factitious hosts capable of maintaining the potentialities of the biological control agents, for many successive generations, and that can be reared at low costs. *Ephestia kuehniella* is a widely used factitious host for the mass rearing of oophagous parasitoids of the genus *Trichogrammma*.

In this study, a laboratory rearing technique for *Trichogramma* spp. capable of providing parasitoids for use in bioassays is described. This method was developed based on the techniques used in the Biology Department of Azores University and in the Stored Products Phytosanity Study Centre, as well as literature and personal experience.

Some aspects reflecting the quality of the parasitoids rearing technique were evaluated, such as emergence rate and sex ratio of adults emerging from parasitized eggs.

To obtain host eggs for parasitism by *Trichogramma*, it is necessary to rear *E. kuenhiella*. Therefore, some aspects of the hosts rearing technique were evaluated, such as female fecundity and sex ratio.

The results indicate that the rearing techniques used for both *Trichogramma* sp. and *E. kuehniella* are acceptably productive and adequate to obtain the necessary amount of parasitoids for bioassays.

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Key words: *Trichogramma*, *Ephestia kuehniellla*, laboratory rearing, factitious host, productivity, quality.

INTRODUCTION

The genus *Trichogramma* constitutes a hymenopteran group of the Trichogrammatidae family which includes only oophagous parasitoid species, with hundreds of host species, mostly among the Lepidoptera (NAGARKATTI & NAGARAJA, 1977; PINTO, 1997). HASSAN (1997) refers to the use of *Trichogramma* spp. as biological control agent in 33 crops, against 52 genuses of lepidopteran pests.

The mass rearing of natural enemies for use in integrated pest management requires the availability of factitious hosts capable of maintaining the potentialities of biological control agents for many successive generations, and that can be reared at low costs (VOEGELÉ, 1986a; TAKADA *et al.*, 2001).

Flanders opens way to the mass rearing of *Trichogramma*, in 1929, by using the factitious host *Sitotroga cerealella* (Olivier) (Lep.: Gelechiidae), concept adopted by Meier who, in 1931, establishes the first mass rearing unit

for an oophagous insect, in Leningrad; this technique rapidly spread to several countries (review by VOEGELÉ, 1986b). *S. cerealella* is a small host, therefore being replaced, in many countries, by *Ephestia kuehniella* Zeller (Lep.: Pyralidae), a bigger and easier to rear host. A third small species, *Corcyra cephalonica* Stainton (Lep.: Pyralidae) is used in several Asian countries given its local availability (BRENIÈRE, 1965; SMITH, 1996). RÍOS & TERÁN (1995) further refer to the use of *Trichoplusia ni* (Hübner) (Lep.: Noctuidae) and *Colias eurytheme* Boisduval (Lep.: Pyralidae), both easy to rear under laboratory conditions. The most widespread species used to rear *Trichogramma* parasitoids are *S. cerealella* and *E. kuehniella*.

E. kuehniella, known as the mediterranean flour moth, is an important pest of stored products, (HAINES, 1991; VICENTE, 1998). The development of this species is possible between 12 and 30ºC; each female can lay 200 to 300 eggs, 95% of which during the first five days after emergence (TAVARES *et al.*, 1989).

The quality of the parasitoids reared from factitious hosts can be evaluated using biological parameters such as emergence rate, fecundity, life span, sex ratio, activity and parasitism rate (BIGLER *et al.*, 1987).

It is intended with this study to develop and apply simple, not very time consuming and reasonably productive rearing methods for *Trichogramma* and its host, *E. kuehniella*, with the purpose of using the parasitoids in laboratory bioassays.

MATERIALS AND METHODS

Laboratory conditions

The first part of this study took place in the Secção de Protecção Integrada (Integrated Pest Management Section), in Instituto Superior de Agronomia (Agronomy Superior Institute) of Lisbon Technical University. *E. kuehniella* was maintained at 22-25ºC, 60-70% R.H. and a 16L:8D photoperiod. In this phase the quality of the *E. kuehniella* rearing technique was evaluated.

The second part was conducted in the Plant Protection Department of Estação Agronómica Nacional (Agronomical National Station), and the species were kept in an artificially acclimatized chamber. Both temperature and relative humidity fluctuated in a regular way, temperature oscillated between 17-23ºC and relative humidity between 55-75%, with mean values of around 20ºC and 65%, respectively. Photoperiod was 16L:8D. Considering the regularity of the temperature variation in this study, a rearing temperature of 20ºC was assumed, based on the findings by CALVIN *et al.* (1984) who observe that fluctuating temperatures between 28-32ºC and 26-34ºC do not cause changes in the development time of *Trichogramma pretiosum* Riley reared on *Diatraea grandiosella* Dyar eggs, when compared to a constant temperature of 30ºC.

Under these conditions, the quality of the *Trichogramma* rearing technique was evaluated based on one laboratory strain.

Trichogramma sp.

To establish a laboratory population of *Trichogramma* sp., a noctuid egg from which two females and one male emerged was selected. The egg was collected in a processing tomato field in Vila Franca de Xira. Assuming that all wasps emerging from one egg belong to the same species and strain, the parasitoids were placed in a glass vial (10x1cm) along with a U.V. sterilized *E. kuehniella* egg card and a drop of honey solution (50% in water) as food source. An egg card is obtained using double sided sello tape, where one of the sides sticks to a 0.8x7cm yellow card and the other to *E. kuehniella* eggs. Around ten days after parasitism, at 25ºC, adults emerge and a new egg card with a drop of honey solution is added. At the fourth/fifth day of parasitism, the newly parasitized egg card is moved to a clean glass vial. After the emergence of a few adults, a fresh egg card is added. This procedure is repeated until a well parasitized egg card is obtained and can be cut and split into several glass vials. The multiplication of the parasitoids proceeds until the necessary

amount of wasps needed for the bioassay is obtained.

To evaluate the quality of the rearing technique for the *Trichogramma* sp. strain, the following analysis' were performed: (1) determination of the emergence rate of adults from parasitized (darkened) *E. kuehniella* eggs, by counting the number of parasitized eggs with emergence hole(s) (viable) and the number of parasitized eggs without emerge hole (unviable) in a total of 4070 darkened eggs; and (2) determination of the sex ratio of a sample of 668 adults.

Ephestia kuehniella

The rearing method for *E. kuehniella* was adapted from that used in the Centro de Estudos de Fitossanidade do Armazenamento (Stored Products Phytosanity Study Centre), which is described in PEREIRA (1996).

The *E. kuehniella* laboratory population was established from eggs obtained in this hosts mass rearing unit in the Biology Department of Azores University. Glass jars with a volume of 0.7-1L were filled up to one third of their volume with a diet of wheat feed, yeast and glycerol, with a weight ratio of 10:1:2, as indicated by HAINES (1991). The diet was infested with *E. kuehniella* eggs, previously disinfected in a 15 minute wash with 6% formaldehyde solution, to minimize the risk of bacterial contamination. For each 100g of diet, around 1000 eggs were used. After a period of around 2 months, the adults emerged, were removed from the diet jars and placed in empty glass jars. These were covered with plastic nets, held in place by plastic rings and inverted over a Petri dish, where the eggs fell. The eggs were collected daily, sterilized with U. V. radiation and stored at 8ºC until they were used for *Trichogramma* rearing.

A U.V. sterilization wooden box was built, following the characteristics of the one used in Azores University. In this box, two 15Watt U.V. lamps were positioned 23cm away from a metal tray where the eggs were placed for 20 minutes, therefore preventing the development of the embryos.

To infest the diet it was necessary to estimate the number of *E. kuehniella* eggs to use. This was achieved by weighing 50, 100, 150, 200, 250, 300 400 and 500 eggs and calculating the regression equation for the values obtained using Excel® software.

The evaluation of the rearing technique's quality was done by: (1) estimating the fecundity of a group of 45 females, kept together with 46 males, throughout their first five days of life. The eggs were collected daily, weighed and, using the regression equation, the number of eggs produced was estimated; and (2) determining the sex ratio of the laboratory population from a sample of 889 individuals, collected in 10 consecutive days.

RESULTS AND DISCUSSION

***Trichogramma* spp.**

Determination of the emergence rate of the adults

In six egg cards, 4070 parasitized eggs were observed, from which 3813 were viable and 257 unviable, resulting in an emergence rate of 93.69±2.8%. Several authors have reported, for different *Trichogramma* species, under similar physical conditions, emergence rates ranging from 95.2% to 100% on *E. kuehniella* eggs (GARCIA & TAVARES, 1994; GRENIER *et al.*, 1995; GARCIA & TAVARES, 1997). These rates were obtained on bioassays where parasitoids were kept at low densities so, in order to increase emergence rates in the rearing, a higher availability of host eggs is advisable in order to avoid superparasitism.

Determination of the sex ratio of the emerging parasitoids

Of the 668 individuals observed, 461 were females and 207 were males, resulting in a sex ratio of 69.0%, similar to the one indicated by MEIERROSE *et al.* (1990) for parasitoids collected in their natural hosts in processing tomato fields.

Ephestia kuehniella

Determination of the number of *E. kuehniella* eggs as a function of their weight

A regression equation correlating egg number and weight was calculated, and a mean weight of 0.0266mg per *E. kuehniella* egg was obtained, close to 0.0280mg, indicated by TAVARES *et al.* (1989). There is a good adjustment between the number and weight of the eggs ($R^2 \approx 1$), so it was assumed that the equation obtained remains valid for values higher than those observed.

Estimation of the fecundity of *E. kuehniella* females

A mean fecundity of 271 eggs per female in the first five days of life was observed. Considering, as TAVARES *et al.* (1989) suggest, that 95% of all eggs produced by an *E. kuehniella* female are laid on the first five days after adult emergence, the rearing method produced females with an approximate mean fecundity of 285 eggs. This is a high value when compared to the 200-300 eggs indicated by the same authors.

Determination of the sex ratio of the laboratory population

Of the 889 observed individuals, 429 were females whereas 460 were males, resulting in a sex ratio of 48.3%. Therefore, a sex ratio of approximately 50% is obtained, which is typical for most insects, indicating an appropriate rearing system.

CONCLUSIONS

Among the parameters studied to determine the productivity and quality of the rearing techniques described, only *Trichogramma* adult emergence seemed slightly lower than desirable, although it was still over 90%. This can be easily avoided by ajusting the number of host eggs per parasitoid female and/or the number of females per glass vial. As for the other parameters, it appears that the biological features studied for both *Trichogramma* sp. and *E. kuehniella* remain overall unafected by possible negative side effects of the rearing methods.

The rearing techniques revealed simple, practical and acceptably productive, supplying an adequate number of *Trichogramma* wasps for laboratory bioassays.

ACKNOWLEDGEMENTS

This study was supported by the national research project Agro 189 "Integrated Pest Management in Processing Tomato". We wish to thank Sílvia Albano, Eva Salvado and António Câmara for their support in the field prospecting for noctuid eggs.

ABSTRACT

GONÇALVES, C.I., F. AMARO, E. FIGUEIREDO, M.C. GODINHO, A. MEXIA. 2005. Productivity and quality aspects concerning the laboratory rearing of *Trichogramma* spp. (Hym.: Trichogrammatidae) and its factitious host, *Ephestia kueniella* Zeller (Lep.: Pyralidae). *Bol. San. Veg. Plagas*, **31**: 21-25.

Aspectos de la productividad y calidad respecto de la cría en laboratorio de *Trichogramma* spp. (Hym.: Trichogrammatidae) y su huésped alternativo, *Ephestia kuehniella* Zeller (Lep.: Pyralidae).

La cría masiva de enemigos naturales a utilizar en manejo integrado de plagas obliga al uso de huéspedes alternativos capaces de mantener las potencialidades de los agentes de control biológico por generaciones sucesivas y que puedan ser criados a bajo costo. *Ephestia kuehniella* Zeller es un huésped alternativo ampliamente utilizado en la cría masiva de parasitoides de huevos del género *Trichogramma*.

En esto estudio se describe una técnica de cría en laboratorio de *Trichogramma* sp. que pueda suministrar parasitoides para uso en ensayos de laboratorio. El desarrollo de este método se basa en las técnicas utilizadas en el Departamento de Biologia de la Universidade dos Açores y en el Centro de Estudos de Fitossanidade do Armazenamento, en datos bibliográficos e experiencia personal.

Se evaluaron algunos aspectos que reflejan la calidad de la técnica de cría en laboratorio del parasitoide, como el porcentaje de emergencia y la proporción hembra:macho de los adultos emergentes de huevos parasitados.

Para obtener huevos para parasitismo por *Trichogramma*, es necesario criar *E. kuehniella*. Asimismo, se evaluaron algunos aspectos de la técnica de cría del huésped, como fecundidad de las hembras y la proporción hembra:macho.

Los resultados indican que las técnicas de cría utilizadas para *Trichogramma* sp. y *E. kuehniella* son productivas y adecuadas para obtener la cantidad necesaria de parasitoides para ensayos en el laboratorio.

Palabras clave: *Trichogramma*, *Ephestia kuehniellla*, cría en laboratorio, huésped artificial, productividad, calidad.

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(Recepción: 16 marzo 2004)
(Aceptación: 7 marzo 2005)