Effect of nutritional stress and larval crowding on survival, development and reproductive output of Mediterranean flour moth, *Ephestia kuehniella* Zeller

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Abstract Eggs of the Mediterranean flour moth (MFM) are widely used for rearing biocontrol agents. Understanding how nutritional stress and larval crowding affect MFM fitness is essential to the development of an optimal production programme. Such knowledge could also help lead to novel management strategies because it is an important storage pest. The development, survival and reproductive output of MFM under five densities (50, 100, 200, 400 and 800 larvae reared on 50 g of diet) were examined. As larval density increased the larval developmental period significantly increased from 27.8 to 33.7 days, and the larval survival rate and resulting females' fecundity significantly decreased from 77.6 to 47.6% and 326.8 to 132.6 eggs, respectively ($P < 0.0001$). There was no significant difference in fertility, survival, pupation and emergence rates between densities of 50, 100 and 200 larvae. Therefore, the optimal rearing density could be up to 200 larvae on 50 g of diet.

Keywords *Ephestia kuehniella*, larval density, survival, developmental period, fecundity.

INTRODUCTION

The Mediterranean flour moth (*Ephestia kuehniella* Zeller) (MFM) is an economically important pest of a wide range of stored commodities throughout the world. Despite its destructive nature, this species is widely used as food for mass rearing of biological control agents (e.g. Brower 1983; Samara et al. 2008), which requires a well-established laboratory colony.

Previous studies revealed that nutritional stress and crowding during juvenile stages affect various fitness components in many insect species. For example, larvae reared at higher densities have decreased survival rate (Hooper et al. 2003), prolonged larval period (Bauerfeind & Fischer

2005; Fantinou et al. 2008), reduced growth rates and body size (Cerutti et al. 1992), and lower fecundity (Peters & Barbosa 1977; Xu et al. 2007). Larval crowding in MFM may also lead to cannibalism of early instars (Savoldelli 2006). Xu et al. (2007) reported that adequate adults with satisfactory quality can be obtained from 100 larvae reared on 50 g of diet with minimal labour. However, the wide gap between densities (1, 100, 500 and 1000) has made the recommendations of the optimal rearing density difficult.

In the present study, the effect of five larval densities on six fitness parameters was examined in MFM, aiming to determine the best

rearing practice for this species, and to provide fundamental information for further studies on its reproductive behaviour.

MATERIALS AND METHODS

Insects

Initial populations of *E. kuehniella* were collected from a poultry farm near Foxton, New Zealand. Colonies were established in plastic containers (8 cm in diameter $\times$ 10 cm in height) according to Xu et al. (2007) by rearing 100 larvae on 50 g of diet comprising wholemeal wheat flour (43.5%), maize meal (43.5%), brewer's yeast (3%) and glycerine (10%) (Lima et al. 2001) at $25 \pm 1^\circ\text{C}$ and $60 \pm 10\%$ RH with 14:10 h (light:dark) in the Entomology and IPM Laboratory of Massey University, Palmerston North, New Zealand. All experiments were carried out in the above mentioned plastic containers under these environmental conditions. The containers were provided with crumpled paper towel (25 cm $\times$ 25 cm) for pupation and covered with double-layered nylon mesh for ventilation and to exclude parasitoids.

Larval densities

To establish five rearing larval densities, eggs were collected (<24 h) from a number of females kept in above mentioned plastic containers lined with a plastic porous sheet as an oviposition substrate. A preliminary incubation test showed that hatch rate of this species was $96 \pm 0.3\%$. Therefore, to obtain densities of 50, 100, 200, 400 and 800 neonate larvae, 52, 104, 208, 416 and 832 randomly selected eggs from the breeding colony were assigned to different treatment groups. For each replicate, the eggs were placed in an above mentioned plastic container containing 50 g of diet. Unlike larvae of many other insect species including moths that live in the open air, MFM larvae live and feed inside their food (stored products such as flour). Therefore, the amount of food more or less reflects living space. In this experiment, 50 g of food occupied about 1/4 of the container space and the top of the container was covered with mesh for ventilation. As a result, the containers are not limiting the space and not having an impact on the stress of the larvae.

A crumpled paper towel was provided in each container for pupation. To minimise the variation in egg period, eggs collected on the same day were used for each replicate. There were ten replicates for densities of 50, 100, 200 and 400 larvae and eight replicates for the density of 800 larvae.

Effect of larval density on survival, development and fecundity

Pupae were collected daily from all replicates of each treatment and placed individually in glass vials (2 cm in diameter $\times$ 7.5 cm in height). Larval period (hatching to pupation), pupation rate (number of pupae/number of neonate larvae), emergence rate (number of emerged adults/number of pupae) and survival rate (number of emerged adults/number of neonate larvae) were recorded.

To compare the effect of rearing larval density on female fecundity and fertility, 20 newly emerged virgin males and females from each treatment were randomly collected and paired, placing each pair in a plastic container for their lifetime. Eggs were collected daily from each pair until the female died. Collected eggs were placed in Petri dishes (8.5 in diameter $\times$ 1.5 cm in height) for incubation. After 3 days of incubation, eggs were observed for fertility under a dissecting microscope and eggs with black dots (larval heads) were counted as fertile.

Statistics

All statistical analyses were conducted using SAS 9.3. A goodness-of-fit test was carried out to test the distribution of data. Data on pupation rate were not normally distributed even after transformation and thus analysed using non-parametric Kruskal-Wallis test followed by Dunns' procedure for multiple comparisons. Other data were normally distributed and analysed using ANOVA followed by Tukey's HSD test for multiple comparisons. Percentage data were arcsine square root transformed before analysis. The hypothesis rejection level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Larval survival rate was not significantly different between densities from 50 to 400 larvae ($P > 0.05$),

but was significantly higher than that in the density of 800 larvae ($P<0.0001$) (Table 1). For all rearing densities adult emergence rate was not significantly different ($P>0.05$) (Table 1). These results indicate that the species survives very well until the larval density reaches 800. The decreased survival rate in the extremely crowded environment may be due to food shortage (Hooper et al. 2003) or cannibalism (Savoldelli 2006).

Larval developmental period in the density of 50 larvae was significantly shorter than that in the density of 800 larvae ($P<0.0001$) (Table 1). Larval developmental period for densities of 100 to 400 larvae was between the two extremes and was not significantly different from either ($P>0.05$) (Table 1). The increased larval development time in higher densities is probably attributed to the limitation of food resources, where larvae delay their development to gain minimum weight required for successful emergence (Hooper et al. 2003; Bauerfeind & Fischer 2005). Due to increasingly delayed development with the increase of larval density, significantly fewer larvae pupated, with the lowest pupation rate occurring in the density of 800 larvae ($P<0.0001$) (Table 1).

Both fecundity and fertility of resultant females decreased with the increase in larval rearing density ($P<0.0001$) (Figure 1). Similar results have been reported in the beetle *Dacne picta* Crotch (Sato & Amano 2004) and pink stalk borer *Sesamia nonagrioides* (Lefebvre) (Fantinou et al. 2008). It is suggested that nutritional stress and larval crowding also negatively affect reproductive fitness. Although fecundity in the density of 50

larvae was the highest in MFM, there was no significant difference in fertility (the realised reproductive output) between rearing densities of 50 to 200 larvae ($P>0.05$). When the larval density reached 400, both fecundity and fertility significantly decreased ($P<0.0001$) (Figure 1).

The results of the present study suggest that the optimal rearing density for this species should be 200 larvae per 50 g of diet. In Xu et al. (2007) 100 larvae per 50 g of diet was recommended but the density treatments in that study were too wide and the density of 200 larvae was not tested.

Figure 1 Mean number of eggs and fertile eggs laid under five different larval densities. Columns with the same letters are not significantly different at $P=0.05$.

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Table 1 Mean ($\pm$ SE) larval developmental period (days), and pupation, emergence and survival rates (%) of Mediterranean flour moth reared at five densities. Means in each columns followed by the same letter are not significantly different at $P=0.05$.

Density	Larval period	Pupation rate	Emergence rate	Survival rate
50	27.80 $\pm$ 0.16a	89.60 $\pm$ 1.65a	86.67 $\pm$ 2.10a	77.60 $\pm$ 2.15a
100	28.36 $\pm$ 0.15ab	86.90 $\pm$ 5.37a	84.00 $\pm$ 4.02a	72.30 $\pm$ 5.06a
200	28.97 $\pm$ 0.33b	86.40 $\pm$ 2.68ab	83.04 $\pm$ 1.46a	71.71 $\pm$ 2.50a
400	28.89 $\pm$ 0.13b	80.28 $\pm$ 2.19b	86.58 $\pm$ 1.80a	69.47 $\pm$ 2.27a
800	33.69 $\pm$ 0.48c	56.89 $\pm$ 1.83b	83.60 $\pm$ 1.36a	47.59 $\pm$ 1.66b

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