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**Fertility of *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker),
Ephestia kuehniella Zeller (Lepidoptera Pyralidae) after larval starvation**

Abstract - Fertility of *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Ephestia kuehniella* Zeller has been evaluated using ten couples coming from fasting larvae of III instar. Their progeny has been compared to that of 10 referential couples, coming from larvae developed on artificial diet until the complete maturity. The fertility of adults is significantly lower than the relatives couples of reference. About *sex ratio*, the number of females is more than double towards the number of males, in every couple of reference. The number of females increased in descent of couples subjected to alimentary stress, both for *C. cautella* and for *E. kuehniella*, while for *P. interpunctella* the relation among males and females was similar to the descent of referential couples. Larvae coming from adults developed from III instar larvae have been isolated in two groups: a batch of 50 larvae has been submitted to forced fasting, another batch has fed regularly. Couples of adults, coming from III instar larvae subjected to starvation, gave life to a number of larvae that could be compared with their parents progeny. Larvae born from parents subjected to starvation but fed regularly until the end of larval stadium, gave life to adults, whose progeny is possible to compare with the one of referential couples. An adequate feeding can nullify the effects on fertility coming from alimentary stress subjected by parents.

Riassunto - *Fertilità di Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) sottoponendo le larve a digiuno forzato.

È stata valutata la fertilità di *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Ephestia kuehniella* Zeller isolando 10 coppie provenienti da larve di III età sottoposte a digiuno forzato, per paragonarla a quella di altrettante coppie testimone, le cui larve si erano sviluppate su dieta artificiale fino a completa maturità. La fertilità degli adulti è risultata significativamente più bassa di quella delle rispettive coppie testimone. Per quanto riguarda la *sex ratio*, è stato osservato che il numero delle femmine nella discendenza delle coppie testimone è stato più del doppio rispetto a quello dei maschi. Nel caso di *P. interpunctella* il rapporto tra femmine e maschi derivanti da coppie ottenute da larve di III età digiunanti è restato invariato rispetto a quello dei testimoni, mentre è stato riscontrato un aumento del numero di femmine sia per *E. kuehniella*, che per *C. cautella*. Successivamente le larve discendenti da adulti sviluppatasi da larve di III età sono state suddivise in due lotti: uno sottoposto a digiuno forzato, un altro alimentato normalmente. Le coppie

provenienti da larve digiunanti hanno dato vita ad un numero di larve simile a quello dei genitori, mentre larve nate da genitori che avevano subito uno stress alimentare allo stadio giovanile, ma alimentate regolarmente fino alla maturità larvale, hanno dato vita ad adulti la cui progenie è stata simile a quella delle coppie testimone. Un'adeguata alimentazione è quindi in grado di annullare gli effetti dello stress subito dai genitori sulla fecondità della discendenza.

Key words: fecundity, fertility, moths of stored products, *sex ratio*, starvation.

INTRODUCTION

Many factors can influence fertility and fecundity of stored product moths, during embryonic and postembryonic development or during adult stage. In particular, fertility can be affected by larval diet (Almasi *et al.*, 1987; Rodriguez-Menendez *et al.*, 1988; Perez-Mendoza & Aguilera-Pena, 2004; Ryne *et al.*, 2004), by exposition of younger stages to chemical substances (Flaherty *et al.*, 1973; Press & Flaherty, 1973; Bell, 1976; 1977; Al-Hakkak *et al.*, 1985; Al-Hakkak & Hussain, 1990; Locatelli *et al.*, 2006), by exposition of pupae to high temperatures (Lum, 1977; Arbogast, 1981), by exposition of eggs, pupae or adults to radiations (Ahmed *et al.*, 1976; Brower, 1975a; 1976; 1980; Al-Hakkak *et al.*, 1988) and ultrasounds (Huang *et al.*, 2003; Huang & Subramanyam, 2003). Fertility can also be affected by the presence in the larvae of bacteria (Faruki & Khan, 1993) or mycotoxins (Wright & Harein, 1982) and by delay of coupling among males and females (Barrer, 1976).

During some tests about larval survival in fasting situations, III instar larvae of *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Ephestia kuehniella* Zeller have been able to become pupae and prematurely complete postembryonic development (Savoldelli, 2005). In this work it was tested if forced starvation influenced the fertility of adults born prematurely, or on their descent.

MATERIALS AND METHODS

Lepidoptera used in this tests have been reared in laboratories of Institute of Agrarian Entomology of University of Milan, in climatic rooms, at the temperature of $27\pm1^{\circ}\text{C}$, $70\pm5\%$ RH and photoperiod 16:8 (light:dark). Diet used in feeding of larvae is made of bran, corn flour, maize flour, germ of corn, brewer's yeast, honey, glycerine (Locatelli & Limonta, 2004). Third instar larvae of males and females have been isolated singularly in Petri's dishes. Sex determination was possible thanks to the presence of a dark spot on male's abdomen of every species. After emerging of adults, ten couples of every species have been singularly transferred in a box of polyethylene (\varnothing : 10 cm; h: 12 cm) with the cover closed by a metallic net (120 mesh). Inside every box have been placed 400g of diet used for rearing with the purpose of stimulate oviposition and to allow descent development. At the same time 10 couples of adults of every species, coming from larvae developed on same artificial diet until the complete maturity, have been isolated. The fertility was evaluated for these too, and was compared to fertility of

the previous couples. After 20-25 days larvae have been counted, distinguishing males and females and *sex ratio* was calculated.

In a following test, larvae coming from adults developed from III instar larvae have been isolated. A batch of 50 larvae has been submitted to forced fasting, another batch has fed regularly. Test had a double goal: on the one hand to verify if alimentary stress prolonged for a second generation could affect a new adult's fertility; on the other hand to verify if the chance of regular feeding could allow, to larvae coming from parents grown in lack of food of III instar larvae, to have adults whose progeny was possible to compare with that of referential couple. All tests have been done at $27\pm1^{\circ}\text{C}$, $70\pm5\%$ RH. Results have been elaborated with ANOVA and Duncan's test (SPSS 12.0 for Windows).

RESULTS AND DISCUSSION

Many researches pointed out that the number of progeny is affected by larval diet. For example, the fecundity of *P. interpunctella* on fresh garlic seed ranged from 117 to 303 eggs per female (Perez-Mendoza & Aguilera-Pena, 2004), while on dried food was 170 (Simmons & Nelson, 1975). The average number of adults progeny produced by a female of *P. interpunctella* reared on walnuts, almonds and wheat bran diet was 258, 274, and 280 respectively (Johnson *et al.*, 1992). The average number of eggs per female of *C. cautella* on semi-dry dates was 90.1-104.5 (Kamel *et al.*, 1976); under optimum conditions (26°C and available water) was 383.3, while the maximum number laid was 738 (Tsvetkov & Latif, 1987). On the artificial diet used in this tests (Locatelli & Limonta, 2004), couples of *Plodia interpunctella* were the most prolific, while the lowest number of larvae has been get from couples of *Ephestia kuehniella* (Table 1). In general the fertility of adults born from III instar larvae is significantly lower than the relatives couples of reference. *Cadra cautella* is the species that has most suffered starvation; its fertility in stress conditions was 5.2 time lower than in normal condition. Fertility of *P. interpunctella* and *E. kuehniella* was a little more than the half (respectively 2.1 time lower for *P. interpunctella* and 2.6 time lower for *E. kuehniella*). In stress condition the average number of *P. interpunctella* progeny was reduced from 401 to 202 larvae per couple, similar to that one of the couples of reference of *C. cautella* (214 larvae per couple) and *E. kuehniella* (229 larvae per couple) (Table 1).

Table 1 - Average number of larvae (F2) descending from a couple of adults (F1) coming from III instar larvae subject to starvation, compared to progeny of referential couple. Means followed by the same letter are not significantly different (Duncan's test; $P<0.05$).

| | Number of larvae \pm S.D. | | |
|---|------------------------------|-----------------------|----------------------------|
| | <i>Plodia interpunctella</i> | <i>Cadra cautella</i> | <i>Ephestia kuehniella</i> |
| Progeny of referential couple | 401.1 \pm 55.3 c | 214.5 \pm 39.7 b | 229.0 \pm 40.0 b |
| Progeny (F2) of adults (F1) coming from III instar larvae | 202.5 \pm 29.5 b | 59.2 \pm 28.1 a | 82.1 \pm 20.9 a |

About *sex ratio* the number of females is more than double towards the number of males, in every couple of reference (Table 2). Some experiences on *C. cautella* showed that when the larvae develop themselves on foods with high susceptibility index (barley, mais, sorghum, corn) will born more females than males (Teotia & Pandey, 1968). These results differ from observations on *E. kuehniella* by Hassanein and Kamel (1965). The Authors refer that the relation among males and females is 1:1, also if it can change a little bit according to larvae feeding. Kamel *et al.* (1976) refer that *sex ratio* of *C. cautella* developed on semi-dry dates is 1:1. The same relation is for individuals reared on soya's seeds (Fenilli, 1983). Podoler (1974) remark that the relation among males and females in *P. interpunctella* is nearly 1:1, but emergence of adults of both sex can be affected by the food's availability. This highlights the need of managing further test to verify if the predominance of females observed during this study, comes from a lack of balance in rearing diet or if the preponderance of females comes from high index of susceptibility of diet, as observed by Teotia and Pandey (1968). Many works published about possible link between feeding and *sex ratio* in animals, insects included, suggest that in non optimal conditions of feeding, it is possible to have a predominance of males, however Holdaway and Smith (1932) observed a preponderance of females if new born larvae of *Tribolium confusum* Jacquelin du Val stay without food for 2-3 days. In these tests it has observed an increase of females number in descent of couples subjected to alimentary stress, both for *C. cautella* and for *E. kuehniella*, while for *P. interpunctella* the relation among males and females it was similar to the descent of referential couples (Table 2). With a low number of eggs deposited for the lack of food, the survival seems guaranteed by an higher number of females, because every male can get more females. Brower (1975b) verified the effects of *sex ratio* on the numbers of coupling of males and females of *P. interpunctella*, varying the relation from 6:1 to 1:6. He has observed that increasing the number of females, every male coupled until 10 times; for females, instead, there weren't differences in number of coupling, in spite of they could have more males. The total number of progeny from a male is strictly linked to the number of his couplings with virgin females; in this test, the higher number of progeny coming from a single male was 2200 individuals. The progeny of females is not linked to the

Table 2 - Sex ratio of descent coming from a couple of adults (F1) descending from III instar larvae subject to starvation, compared to sex ratio of referential couple. Means followed by the same letter are not significantly different (Duncan's test; $P<0.05$). F=number of females; M=number of males.

| | Sex ratio (F/M) \pm S.D. | | |
|---|------------------------------|-----------------------|---------------------------|
| | <i>Plodia interpunctella</i> | <i>Cadra cautella</i> | <i>Ephesia kuehniella</i> |
| Progeny of referential couples | 2.35 \pm 0.84 a | 2.56 \pm 0.74 a | 2.42 \pm 0.52 a |
| Progeny (F2) of adults (F1) coming from III instar larvae | 2.34 \pm 0.59 a | 5.00 \pm 3.30 b | 4.32 \pm 1.15 b |

Table 3 - Average number of larvae (F3) coming from a couple of adults (F2) whose parents (F1) were get from III instar larvae subject to starvation, compared to progeny of referential couple. Means followed by the same letter are not significantly different (Duncan's test; $P < 0.05$).

| | Number of larvae \pm S.D. | | |
|---|------------------------------|-----------------------|----------------------------|
| | <i>Plodia interpunctella</i> | <i>Cadra cautella</i> | <i>Ephestia kuehniella</i> |
| Progeny (F3) of adults (F2) coming from mature larvae | 360.8 \pm 57.7 d | 201.0 \pm 27.6 c | 186.5 \pm 42.2 bc |
| Progeny (F3) of adults (F2) coming from III instar larvae | 166.6 \pm 33.5 b | 38.4 \pm 15.6 a | 70.3 \pm 20.4 a |
| Progeny of referential couples | 401.1 \pm 55.3 d | 214.5 \pm 39.7 c | 229.0 \pm 40.0 c |

Table 4 - Sex ratio of descent (F3) coming from a couple of adults (F2) whose parents (F1) were get from III instar larvae subject to starvation. Means followed by the same letter are not significantly different (Duncan's test; $P < 0.05$). F=number of females; M=number of males.

| | Sex ratio (F/M) \pm S.D. | | |
|---|------------------------------|-----------------------|----------------------------|
| | <i>Plodia interpunctella</i> | <i>Cadra cautella</i> | <i>Ephestia kuehniella</i> |
| Progeny (F3) of adults (F2) coming from mature larvae | 2.05 \pm 0.43 a | 2.50 \pm 0.57 ab | 2.44 \pm 0.34 ab |
| Progeny (F3) of adults (F2) coming from III instar larvae | 3.04 \pm 0.63 b | 4.27 \pm 1.56 c | 4.46 \pm 0.74 c |

number of coupling, and the higher number of individuals coming from a female was 450 larvae.

Larvae (F2) born from parents (F1) subjected to starvation but fed regularly until the end of larval stadium, gave life to adults (F2), whose progeny (F3) is possible to compare with the one of referential couples (Table 3). It is so possible to say that an adequate feeding can cancel the effects on fertility coming from alimentary stress subjected by parents.

Couples of adults (F2), coming from III instar larvae subjected to starvation, gave life to a number of larvae (F3) (Table 3) that could be compared with their parents progeny (F2) (Table 1), witch larvae were subjected to starvation too. Sex ratio of descent (F3) coming from a couple of adults (F2) whose parents (F1) were get from III instar larvae subjected to starvation was higher than sex ratio of progeny (F3) of adults (F2) coming from mature larvae without alimentary stress (Table 4). The results of this test showed that, although the starvation is repeated again on III instar larvae, the number of progeny F3 could be compared with those of parents F1 (Tables 1, 3). Starvation of III instar larvae significantly reduces fertility of adults, but the number of progeny doesn't decrease if larval starvation is protracted in F1 and F2 generations.

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