

Effect of food deprivation on survival and development of larvae of the geometrid moth *Idaea inquinata*

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Abstract

Food shortage on the development of larvae of *Idaea inquinata* (Scopoli) was considered. Experiments were carried out at 26 ± 1 °C, $70 \pm 5\%$ RH, and a photoperiod of 16:8 (L:D). Fifty larvae of first, second, third, and fourth instar fasted after the moult, were observed. Larvae from the first to the third instar were deprived of food for two, four, six, eight, and ten days; after the different periods without food, 0.1 g of artificial diet was gradually added. In another experiment, larvae were fed *ad libitum* for three, five, and seven days and then starved. Only fasted fourth instar larvae developed into an adult, the other instars larvae died. When food was reintroduced after a period of fast, 100% mortality was observed after 6 days fast in first instar larvae, after 8 days in second instar larvae and after 10 days in third instar larvae. When food was available after the moult for few days, larvae of first, second, and third instar can afford at most only one moult and died.

Key words: starvation, rusty wave moth, survival, larval instars.

Introduction

Food availability is one of the key factors for insect's development as moult is an energy demanding process and, if food lacks, insects can afford the moult only if they have sufficient nutrient reserves in the fat body (Mirth *et al.*, 2007; Arrese and Soulages, 2010). Food shortage delays the development time and decreases the growth rate, the body size, the production of hormones and influences the reproduction (Berrigan and Charnov, 1994; Blanckenhorn, 1998; Fischer and Fiedler, 2001; Daghli, 2005; Chen and Gu, 2006). Poor food quality or starvation cause an increase of the larval period (Gebhardt and Stearns, 1988; 1993; Tammaru, 1998; Tammaru *et al.*, 2004; Bauerfiend and Fisher, 2005) and when food becomes again available in some cases the development rate increases (Hector and Nakagawa, 2012). Several types of research report last instar larvae survive food stress longer than younger instars. This fact was documented in larvae of the tortrix moth *Epiblema strenuana* (Walker), in larvae of the nymphalid moths *Actinote antea*s (Doubleday) and *Actinote thalia pyrria* (F.) (Ma *et al.*, 2002; Li *et al.*, 2005), and in *Corythucha ciliata* (Say) (Hemiptera Tingidae) (Wu *et al.*, 2016). Also temperature and relative humidity influence survival to food stress or fast. Moth larvae tolerate better food shortage at a temperature lower than the optimal one (Candura, 1928; Morère and Le Berre, 1967; Bell *et al.*, 1979; 1983).

During post embryonic development stored product pests can suffer starvation in warehouse temporary empty where larvae of different instar can be present.

As far moth pests of stored product, more larvae of first, second, and third instar of *Plodia interpunctella* (Hubner), *Cadra cautella* (Walker), *Corcyra cephalonica* (Stainton) and *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) survive longer at 18 °C than at 28 °C. Second instar larvae moulted and third instar larvae pupate in advance (Savoldelli, 2005). Starved first instar larvae of *Idaea inquinata* (Scopoli) (Lepidoptera Geom-

etridae) survive ten days, but environmental conditions are not reported (Candura, 1931). *I. inquinata*, known as rusty wave moth, is a pest of hay, dried medicinal plants and stored food with a high fibre content (Locatelli *et al.*, 2005; Limonta and Locatelli, 2015).

As data on survival to starvation of *I. inquinata* are lacking, it is useful to complete the knowledge on the reaction of larvae to food shortage. These information are important in the integrated pest management of stored products. In the present study, the effect of different patterns of food availability on larvae of *I. inquinata* was investigated at optimal temperature and relative humidity.

Materials and methods

Mass rearing

I. inquinata was collected on medicinal plants in a warehouse in Milano and reared for several years on an artificial diet (Limonta *et al.*, 2010) in a climatic chamber (CFT 600, Piardi Tecnologie per il freddo S.r.l., via Brescia1/A, Castenedolo BS) at 26 ± 1 °C, $70 \pm 5\%$ RH, and a photoperiod of 16:8 (L:D).

Rearing of larvae

Larvae for the different experiments were reared individually in a glass jar (diameter 3.8 cm; height 2.5 cm) closed with a piece of fabric to allow gaseous exchange. Rearing was started from eggs until the larvae moulted in the different instars. Each jar was checked daily and first instar larvae were used in the experiments as eggs hatched. Second, third and fourth instar larvae were selected just after the moult.

Experiments

Experiments were carried out in a thermostatic cell at the same conditions of the rearing chamber (see mass rearing paragraph).

Group of fifty larvae of the first instar just after egg

hatching and fifty larvae each for the second, third and fourth instars just after the moult were individually put in the glass jars for every experiment.

In the preliminary experiment, to observe the effect of food deprivation, the survival of starved larvae of first, second, third, and fourth instar, was observed daily.

Two different experiments were carried out with larvae from the first to the third instar. In one experiment, the effect of different periods of food deprivation was observed. A small amount of artificial diet, 0.1 g, was gradually added to larvae fasted for two, four, six, eight, and ten days.

In another experiment, the effect of fast after different periods with food was observed. Larvae were fed *ad libitum* for three, five, and seven days. At the end of the different periods with food, larvae were starved.

Experiments were daily inspected; mortality of larvae and survival in days were recorded. Data were submitted to one-way ANOVA and Least Significance Difference test ($\alpha = 0.05$) (IBM SPSS Statistics 24).

Results

Effect of food deprivation

Larvae of first, second and third instar of *I. inquinata*, fasted after the moult, survived few days and then died. First and second instar larvae died after three days, 3.4 (± 0.36) and 2.9 (± 0.28) respectively, while third instar larvae survived for a statistically significant longer period (5.7 ± 0.32 days) (ANOVA $F_{2, 147} = 20.483$, $P < 0.001$) (figure 1).

Fourth instar larvae food deprived moulted into fifth instar larvae after 4.0 ± 0.09 days, pupated after 7.5 ± 0.13 days, and adults emerged in 3.4 ± 0.13 days. As fourth instar larvae survived fast, they were excluded from the other experiments.

Effect of different periods of food deprivation

Mortality of larvae, starved in the first days after the moult and then fed, varied according to the considered instar and to the length of the period without food (figure 2). One-hundred percent mortality was observed in first instar larvae when starved for 6 days, in second instar larvae after 8 days. Third instar larvae are less susceptible to food deprivation than second and first instar larvae and total mortality was observed only when they were starved for ten days after the moult.

When first instar larvae were unfed for the first 2 days

and then regularly fed, 26% mortality was observed (figure 2). The surviving larvae presented a mean development period of 43.7 ± 0.62 days (table 1). When unfed for four days, mortality of first instar larvae increased to 74% and the mean developmental period of surviving larvae was 44.7 ± 0.60 days (ANOVA $F_{2, 46} = 0.781$, $P = 0.464$). Ninety-eight percent mortality was observed after 6 days and 100% after 8 days without food (figure 2).

Mortality of second instar larvae increased from 14% after 2 days of fasting to 100% after 8 days unfed (figure 2). The development period of surviving larvae decreased from 45.1 ± 0.63 days after 2 days deprived of food, to 35.7 ± 0.63 after 6 days (ANOVA $F_{2, 66} = 58.799$, $P < 0.001$) (table 1).

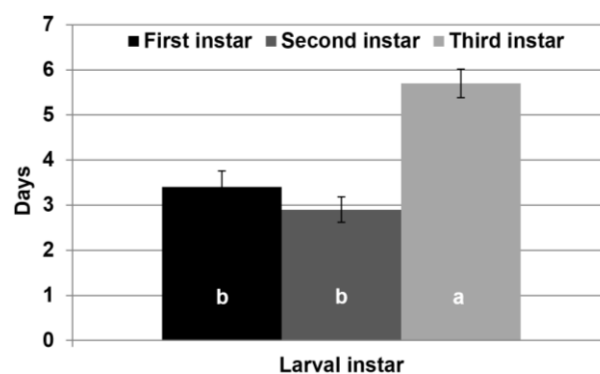


Figure 1. Mean survival (\pm SE) (days) of first, second and third instars larvae of *I. inquinata* food-deprived after egg hatching (first instar) or after the moult (second and third instars).

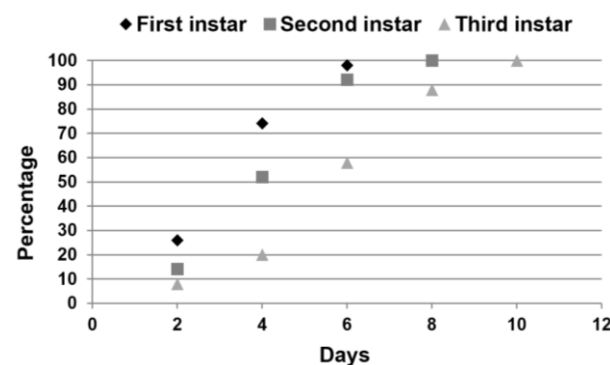


Figure 2. Mortality (%) of first, second, and third instar larvae of *I. inquinata* deprived of food for 2, 4, 6, 8, and 10 days after the moult.

Table 1. Mean developmental period (\pm SE) (days) of surviving first instar larvae of *I. inquinata* after first 2, 4, 6 and 8 days of starvation after the moult and then fed.

Fasting period (days)	First instar		Second instar		Third instar	
	N	Mean \pm SE	N	Mean \pm S.E.	N	Mean \pm S.E.
2	35	43.7 \pm 0.62	41	45.1 \pm 0.63a	47	36.2 \pm 0.45c
4	13	44.7 \pm 0.60	24	35.2 \pm 0.66b	40	30.9 \pm 0.34d
6	1	41.0	4	35.7 \pm 0.63b	21	42.0 \pm 2.08b
8	-	-	-	-	6	66.8 \pm 1.60a

ANOVA: first instar $F_{2, 46} = 0.781$, $P = 0.464$; second instar $F_{2, 66} = 58.799$, $P < 0.001$; third instar $F_{3, 110} = 107.123$, $P < 0.001$. Means followed by a different letter are significantly different for LSD test.

Table 2. Mean survival (days) (\pm SE) of first, second, and third instar larvae of *I. inquinata* fed after the moult for 3, 5, and 7 days and then deprived of food.

Days with food	First instar	Second instar	Third instar
3	4.3 \pm 0.25b	4.8 \pm 0.31	5.8 \pm 0.74c
5	4.3 \pm 0.22b	4.9 \pm 0.28	7.8 \pm 0.49b
7	6.1 \pm 0.42a	5.0 \pm 0.23	11.0 \pm 0.63a

ANOVA: first instar $F_{2, 147} = 11.348$, $P < 0.001$; second instar $F_{2, 147} = 0.088$, $P = 0.916$; third instar $F_{2, 147} = 17.075$, $P < 0.001$. Means followed by a different letter are significantly different for LSD test.

Third instar larvae tolerated food deprivation better and one-hundred percent mortality was observed after 10 days (figure 2). The development period of surviving larvae increased from 36.2 ± 0.45 days after 2 days without food to 66.8 ± 1.60 after 8 days (ANOVA $F_{3, 110} = 107.123$, $P < 0.001$) (table 1).

Effect of fast after different periods with food

First, second, and third instar larvae of *I. inquinata* fed for 3, 5, and 7 days and then starved were not able to complete the development into an adult (table 2).

When first instar larvae were fed 3 and 5 days they survived 4.3 days, while the feeding period of 7 days significantly increased the survival to 6.1 days (ANOVA $F_{2, 147} = 11.348$, $P < 0.001$). Ninety-eight percent and 90% first instar larvae, fed for 5 and 7 days respectively, moulted.

Second instar larvae survived 5-6 days after the different feeding period (ANOVA $F_{2, 147} = 0.088$, $P = 0.916$). Moult was observed in 62% of second instar larvae fed 5 days and 86% fed 7 days.

Third instar larvae survived 5.8 ± 0.74 after being fed for 3 days, 7.8 ± 0.49 when fed for 5 days, and 11.0 ± 0.63 when fed for 7 days (ANOVA $F_{2, 147} = 17.075$, $P < 0.001$) (table 2). Moult was observed in 30% third instar larvae fed 3 days, 46% fed 5 days and 60% fed 7 days.

Discussion

The effect of different patterns of fasting on larvae of *I. inquinata* was observed at a constant temperature and relative humidity, favourable to the development of this species (Limonta *et al.*, 2013). Larvae of first, second and third instar, food-deprived after the moult, survived few days. First instar larvae of *I. inquinata* survived longer than larvae of the same instar of the stored pests *P. interpunctella*, *C. cautella*, *E. kuehniella* and *C. cephalonica* (Savoldelli, 2005), second instar survival was analogous to the first three species and shorter than *C. cephalonica*. In three cases, third instar larvae of pyralid moths pupated and emerged as adults, while third instar larvae of *I. inquinata* die when fasted. Fasted fourth instar larvae of *I. inquinata* moulted into the fifth instar and adults emerged in 14.9 days. Regularly fed, at temperature from 24 to 30 °C, this species has five larval

instars, and the development from the fourth instar to adult lasts longer than when fasted, namely 23.5 days (Limonta and Locatelli, 2010; 2013). As observed by different authors, stressed animals present a reduced lifespan (Criscuolo *et al.*, 2008; Monaghan *et al.*, 2011) and larvae prematurely pupate when food lacks (Shafiei *et al.*, 2001; Sato and Suzuki, 2001; Munyiri *et al.*, 2003; Shintani *et al.*, 2003; Terao *et al.*, 2015). It is evident that only the fourth instar larvae of *I. inquinata* have accumulated reserves sufficient to complete development, as fasted insects can survive on reserves stored when food is available (Arrese and Soulages, 2010).

Larvae, starved in the first days after the moult and then fed, responded differently according to the considered instar. Survival increases with the instar and decreases depending on the number of days of fast. Seventy percent first instar larvae overcomes 2 days of fast, the percentage lowers to 26% when starved for 4 days, and the days necessary to complete the development are equal for both the groups of the surviving larvae. Eight percent of second instar larvae survives 6 days of fast, while 12% of third instar larvae complete the development after the first 8 days without food. The development of second instar larvae is accelerated after the fast as observed in *Onthophagus fasciatus* Boucomont (Coleoptera Scarabaeidae) (Shafiei *et al.*, 2001). On the contrary, the development of third instar larvae, that survived after 8 days without food, lengthened as observed in fruit flies and moths (Gebhardt and Stearns, 1988; 1993; Tammaru, 1998; Tammaru *et al.*, 2004; Bauerfeind and Fischer, 2005).

First, second, and third instar larvae of *I. inquinata* fed 3 days and then starved survived few days but they cannot afford the moult, that means that they do not accumulate enough nutrient to face moult (Mirth *et al.*, 2007). Five and 7 days of feeding were sufficient to allow first, second and third instar larvae to moult one time but the moult consumes all their nutrient supply as they are not able to develop into an adult.

On the whole *I. inquinata* first and second instar larvae survival to fast is similar to the one of other moth pests of stored product, third instar larvae are more susceptible to starvation. Moreover its life cycle is longer than the one of pyralid moths, therefore this species has less chances to survive in an empty warehouse.

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