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**Cannibalistic behaviour of the first and second instar larvae of *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Ephestia kuehniella* Zeller, *Corcyra cephalonica* (Stainton) (Lepidoptera Pyralidae) under starvation**

**Abstract** - Tests were carried out at three different environmental conditions: 18°C and 65% RH; 24°C and 63% RH; 28°C and 70% RH. Results can be summarised in the following: cannibalism was confirmed for *P. interpunctella*, *C. cautella*, *E. kuehniella*, *C. cephalonica*; it is more frequent at 28°C than 24°C and 18°C; the probability of cannibalism depends on larval instar; cannibalism is more frequent in presence of different larval stages; the choice of the victim is made towards more vulnerable individuals, that are less resistant to the attack (no first instar larva was able to cannibalise a second instar one); at 28°C the survival time of cannibal first and second instar larvae is, with some exceptions, greater than those of starved larvae of the same stage.

**Riassunto** - *Comportamenti cannibalistici delle larve di prima e seconda età di Plodia interpunctella (Hübner), Cadra cautella (Walker), Ephestia kuehniella Zeller, Corcyra cephalonica (Stainton) (Lepidoptera Pyralidae) in condizioni di digiuno.*

Le prove sono state effettuate in laboratorio a tre diverse condizioni ambientali: 18°C e 65% RH; 24°C e 63% RH; 28°C e 70% RH. I risultati possono essere così sintetizzati: il cannibalismo è confermato per *P. interpunctella*, *C. cautella*, *E. kuehniella*, *C. cephalonica*; è più frequente a 28°C piuttosto che a 24°C e 18°C; la frequenza del cannibalismo dipende dall'età larvale e si verifica maggiormente con la presenza contemporanea di larve con età diversa; le larve più vulnerabili sono le vittime preferite in quanto oppongono meno resistenza all'attacco (nessuna larva di prima età ha divorato quella di seconda età); a 28°C le larve di prima e seconda età sono sopravvissute, con qualche eccezione, più a lungo delle larve della stessa età digiunanti.

**Key words:** cannibalism, larval behaviour, larval survival, moths of stored products.

## INTRODUCTION

Cannibalism, or “intraspecific predation”, is widespread among animals, including insects (Fox, 1975). It is well known for Dictyoptera, Orthoptera, Lepidoptera and Coleoptera. It can be induced by starvation, overcrowding, lack of some nutrients, high level of stress (Mansour & Dimetry, 1972; Abdel-Salam & El-Lakwah, 1973; Tignor & Eaton, 1986). Heinig (1989) observed that cannibalistic behaviour during starvation of *Agrotis segetum* (Denis & Schiffermüller) and *Mamestra brassicae* (L.) (Lepidoptera Noctuidae) larvae was provoked by an extremely low content of trehalose in the haemolymph, and not by gustatory signals, gut emptiness, water and ion deficiency or amino acid shortage. In fact, trehalose feeding reduced cannibalism in *A. segetum*. Tignor and Eaton (1986) noticed that high stress levels, caused by crowding and starvation, increased larval cannibalism of *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) towards all immature stages.

This phenomenon is manifested not only in laboratory conditions but, for some Lepidoptera Noctuidae larvae, it was observed also in field (Gould *et al.*, 1980; Joyner & Gould, 1985).

The benefits of cannibalism in terms of survival, development time, weight of pupa were evaluated by Chapman *et al.* (1999a). They compared cannibalistic and non-cannibalistic larvae of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in presence of low and high food quantity, and reported that the frequency of cannibalism was influenced neither by the sex of larvae, nor by availability of alternative food.

Cannibalism can be considered like a positive behaviour when the individuals that eat conspecifics, can easily acquire essential nourishments, as sterols (Stein, 1986). If they were not synthesized in an enough quantity, they must be acquired with diet (Wigglesworth, 1974). Chapman *et al.* (1999b) referred that cannibalism was observed commonly even when food was not limiting, but occurred more frequently at low food quantities and/or high rearing densities.

Among stored products pests cannibalism was observed for *Plodia interpunctella*, *Catra cautella*, *Oryzaephilus mercator* and *Tribolium* spp. (Stein, 1986; Bartels & Schliesske, 1997; Boots, 2000). In some cases, a self-limitation of eggs or pupae allows a better development of the survivors: an example is in *Tribolium* spp., where eggs are mainly eaten by adult females and pupae are cannibalised by adult males (Stein, 1986). Other studies point out that cannibalism can be considered as a potential way for the diffusion of pathogens among a population. Boots (1998) referred that *P. interpunctella* larvae cannibalise conspecifics infected by virus, although they have the possibility of choosing between healthy individuals and infected ones. For *P. interpunctella* cannibalism occurs without distinction of relationship; in particular, in presence of a sibling and an unrelated individual, they did not avoid to cannibalise siblings (Boots, 2000).

In this research, laboratory trials were carried out to study larval cannibalism of the most common stored products moths, in absence of food. The aim of this work was to contribute to our understanding on the status of the infestation that may occur after production and packaging of some foodstuff.

#### MATERIALS AND METHODS

Larvae of *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Ephestia kuehniella* Zeller and *Corcyra cephalonica* (Stainton) were obtained from the Institute of Agricultural Entomology, University of Milan. They were reared in a climatic room at  $27 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH and light/dark 16:8 hrs, on an artificial diet composed of bran, corn meal, wheat meal, glycerine, wheat germ, honey and yeast (Locatelli & Limonta, 2004).

Fifty pairs of larvae of each species were isolated in Petri dishes ( $\varnothing: 5.5$  cm), without food; the only possibility to survive was to cannibalise each other. Test were carried out on pairs of first and second instar larvae, and pairs made by one larva of the first and of the second instar.

The incidence of cannibalism and the survival time of cannibal larvae were evaluated. Tests were carried out in three different environmental conditions:

- $(18 \pm 1)^\circ\text{C}$  and  $(65 \pm 6)\%$  RH;
- $(24 \pm 1)^\circ\text{C}$  and  $(63 \pm 7)\%$  RH;
- $(28 \pm 1)^\circ\text{C}$  and  $(70 \pm 5)\%$  RH.

Initially the pairs of first instar larvae were composed by newborn larvae, kept without food, but in these cases, for all species, the cannibalistic behaviour was never observed and larvae died of starvation. Then, the newborn larvae were replaced by first instar larvae that ate the artificial diet within the 24 hours following their birth. The composition of diet was the same used for rearing.

Afterwards, other tests were carried out with fifty larvae of first and fifty of second instar, in Petri dishes, without food, to verify if some of them were able to become adult, eating only other individuals.

Results were submitted to ANOVA and Duncan's multiple range test ( $P > 0.05$ ). The tests were performed using the SPSS for Windows version 12.0.

#### RESULTS AND DISCUSSION

Figures 1 - 3 show the number of cannibalistic cases observed between the fifty couples of the first and the second instar larvae and Figures 4 - 6 show the mean time passed from the start of the test until cannibalism was observed.

Cannibalism occurred most at  $28^\circ\text{C}$ , than at  $24^\circ\text{C}$  and  $18^\circ\text{C}$ . It was easier for the

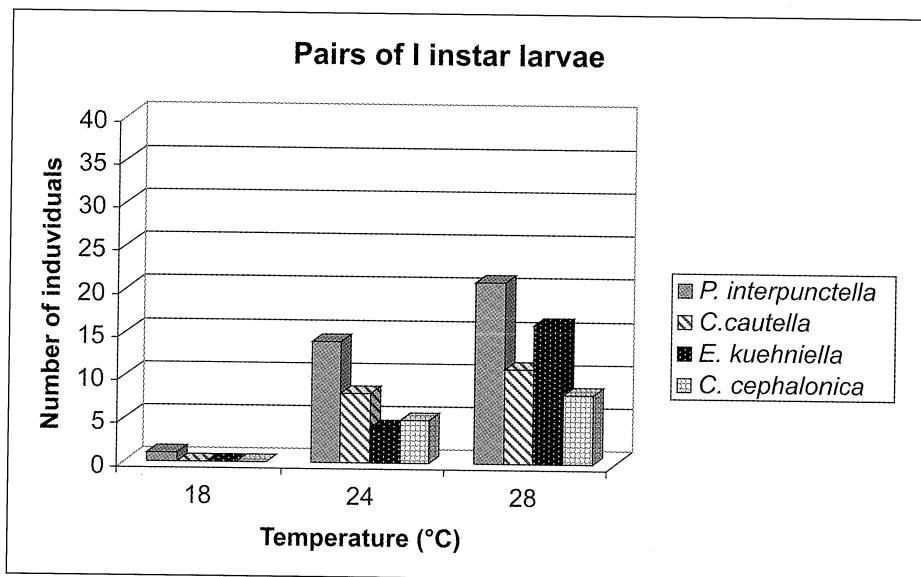


Fig. 1 - Incidence of cannibalism among fifty pairs of first instar larvae exposed to different temperatures.

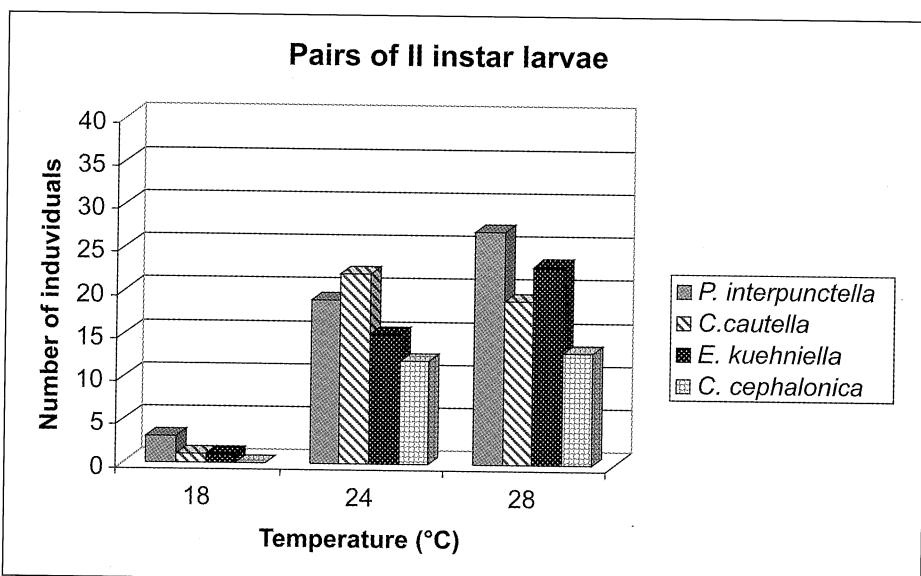


Fig. 2 - Incidence of cannibalism among fifty pairs of second instar larvae exposed to different temperatures.

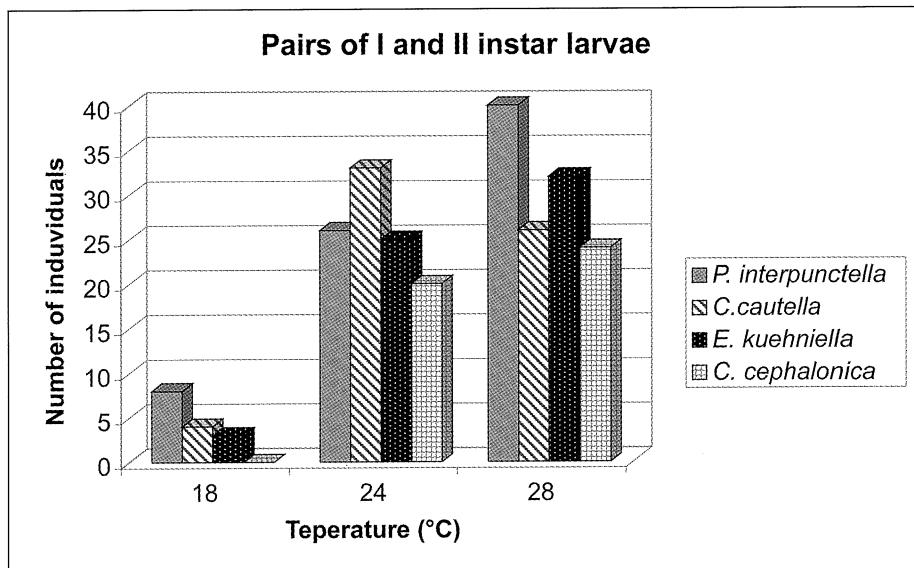


Fig. 3 - Incidence of cannibalism among fifty pairs of first and second instar larvae exposed to different temperatures. In all cases second instar larva cannibalised that of first instar.

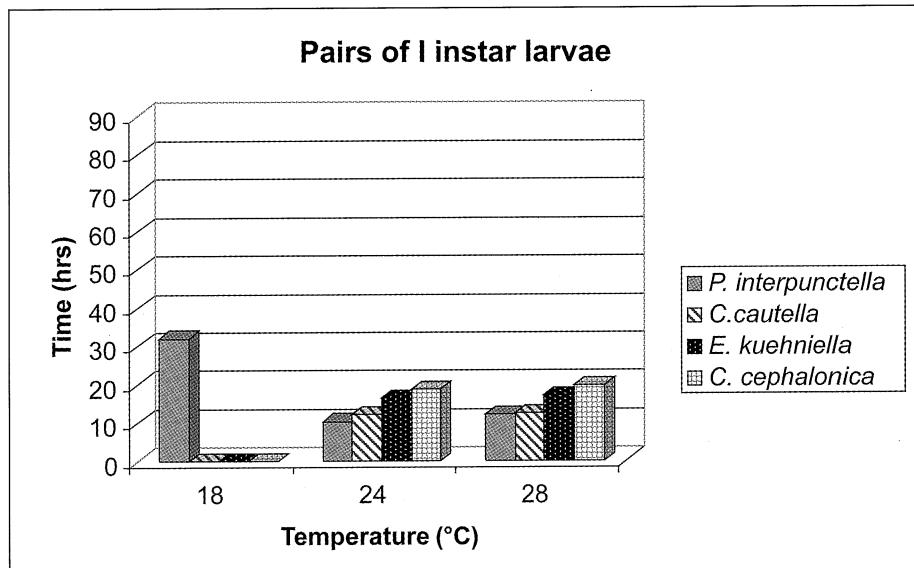


Fig. 4 - Mean time to cannibalise the larva exposed together to different temperatures.

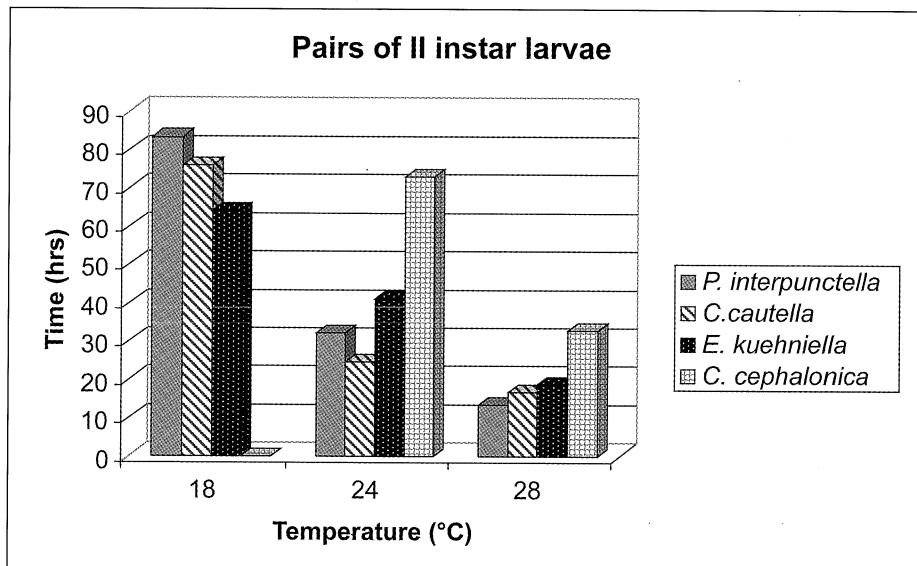


Fig. 5 - Mean time to cannibalise the larva exposed together to different temperatures.

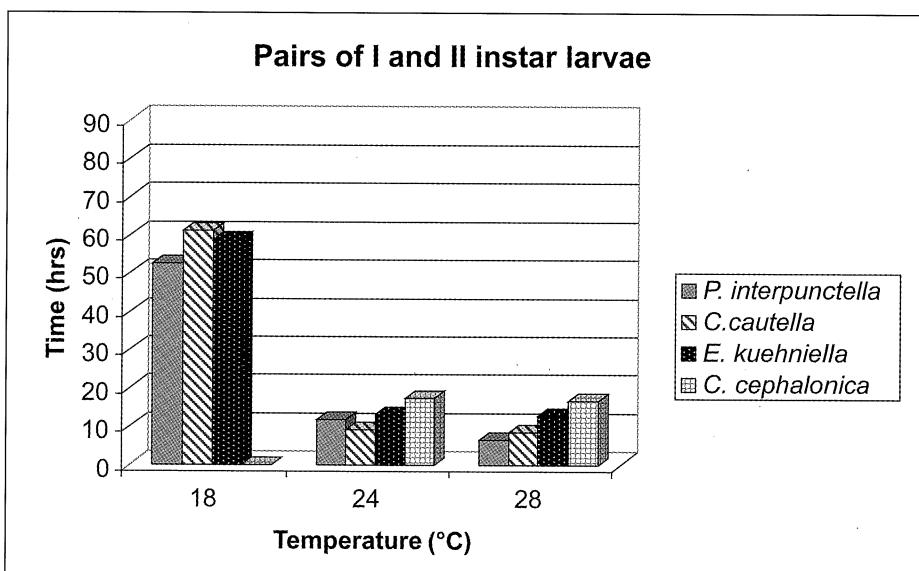


Fig. 6 - Mean time to cannibalise the larva exposed together to different temperatures.

second instar larvae to cannibalise younger individuals, rather than larvae of the same stage; in fact a greater incidence of cannibalism was observed in pairs with one larva of first and one of second instar (Fig. 3). In all such pairs the older individual that attacked and eaten the first instar larva, could have been facilitated due to their bigger size. The easiness in cannibalising the younger larvae is pointed out by the short time required from the beginning of the test: second instar larvae are faster in cannibalising those of first instar, rather than second instar larvae in eating individuals of the same stage (Figures 5-6).

Boots (1998) referred that old instar larvae of *P. interpunctella* cannibalised preferentially young early larvae, while in presence of infected and healthy larvae of the same stage, the infected individuals are more likely to be cannibalised. Reed *et al.* (1996) noticed that *P. interpunctella* larvae parasitized by *Venturia canescens* (Gravenhorst) were easier cannibalised by healthy individuals. Chapman *et al.* (1999b) observed the same phenomenon on *Spodoptera frugiperda* among fifth and sixth instar larvae that were more likely to consume younger conspecifics than larvae of the same stage.

The cases of cannibalism between first instar larvae have been sporadic; in particular at 18°C only one case of cannibalism has been observed in *P. interpunctella* (Fig. 1). In general *P. interpunctella* showed the highest incidence of cannibalism, while in *C. cephalonica* cannibalism was the lowest.

Figures 7 and 8 show the calculated additional time cannibalistic larvae were able to survive without food, after they consumed their prey. This additional time was calculated based on survival time without food experimented by Savoldelli (2005) and for comparison was included in Tables 1 and 2. Therefore, figures 7 and 8 are the graphical presentation of the calculated survival time differences obtained from Tables 1 and 2.

At 28°C the cannibalistic larvae of first instar of all species and *P. interpunctella* larvae at 18°C are able to survive significantly more time of the conspecifics, isolated without food. At 24°C there were no significant differences in survival times of cannibalistic larvae with others of the same species, having nothing to eat (Fig. 7 and Table 1).

Second instar larvae, which cannibalised an individual of the same stage, survived significantly more time than larvae without food, with the exception of *P. interpunctella* at 18°C and *E. kuehniella* at 18°C and 28°C. The survival times of larvae, which cannibalised a younger individual, were comparable with those of the larvae confined without food (Tables 2-3).

In some cases larvae were able to survive for more than 24 hours, if compared to those dead without food: *C. cautella* at 18°C (A); *E. kuehniella* and *C. cephalonica* at 24°C (A) (Fig. 8).

Additional tests were carried out to verify that eating only conspecifics, if some moths are able to become adult and to establish how many individuals are needed, but there were not valuable results. Consequently, ten groups for each species, each one composed by fifty larvae of first and fifty of second instar, have been isolated at 28°C; only in two cases, two adult males of *P. interpunctella* emerged.

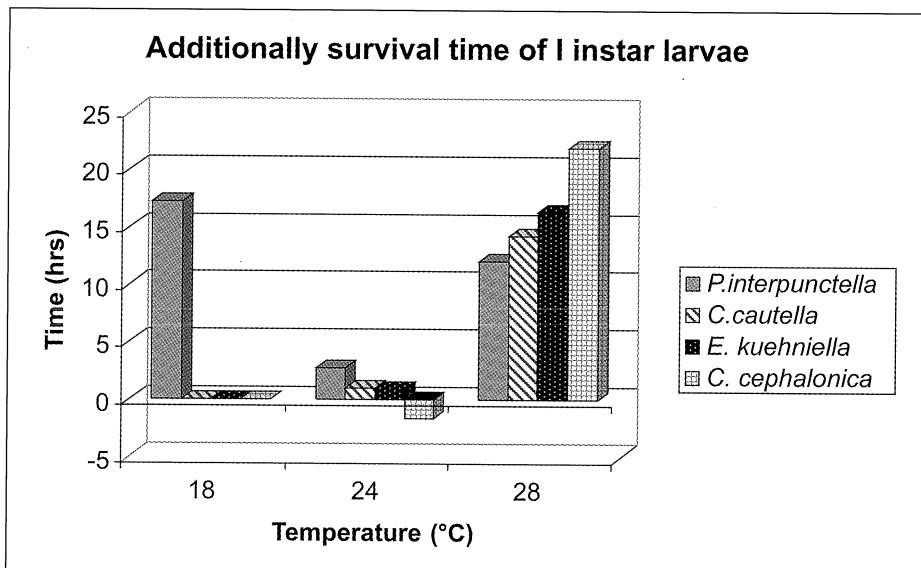


Fig. 7 - Additional survival time of cannibalistic I instar larvae compared to conspecifics of the same age, confined without food.

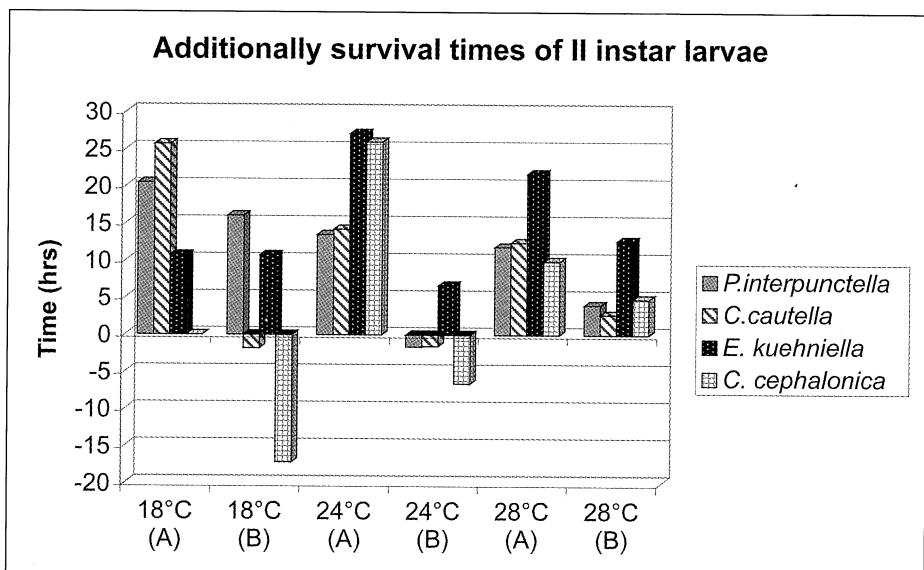


Fig. 8 - Additional survival time of cannibalistic II instar compared to conspecifics of the same age, confined without food.

Table 1 - Survival times (hours) of I instar larvae: confined without food (A); which have cannibalised conspecific of the same stage (B). Means followed by the same letter, on the same line, are not significantly different (Duncan's test;  $P>0.05$ ).

	<i>Plodia interpunctella</i>		<i>Cadra cautella</i>		<i>Ephestia kuehniella</i>		<i>Corcyra cephalonica</i>	
T (°C)	I A (hrs ± S.D.)	I B (hrs ± S.D.)	I A (hrs ± S.D.)	I B (hrs ± S.D.)	I A (hrs ± S.D.)	I B (hrs ± S.D.)	I A (hrs ± S.D.)	I B (hrs ± S.D.)
18	78.72±18.72 a	96.00±0.00 b	79.68±16.32 ab	-	81.12±16.8 ab	-	86.40±13.68 b	-
24	39.84±7.44 a	42.57±5.17 ab	37.20±9.12 a	38.25±2.25 a	39.60±8.16 a	40.50±3.41 ab	44.40±7.68 b	42.80±2.28 b
28	21.84±3.84 a	33.90±3.77 c	25.68±6.72 b	36.91±4.50 cd	27.36±9.36 bc	43.75±4.95 d	28.80±3.84 c	50.75±3.99 d

Table 2 - Survival times (hours) of II instar larvae of *P. interpunctella* and *C. cautella*: confined without food (A); which have cannibalised conspecific of the same stage (B); which have cannibalised conspecific of I instar larvae. Means followed by the same letter, on the same line, are not significantly different (Duncan's test;  $P>0.05$ ).

	<i>Plodia interpunctella</i>			<i>Cadra cautella</i>		
T (°C)	II A (hrs ± S.D.)	II B (hrs ± S.D.)	II C (hrs ± S.D.)	II A (hrs ± S.D.)	II B (hrs ± S.D.)	II C (hrs ± S.D.)
18	139.20±31.44 a	159.67±16.80 ab	155.25±15.93 ab	160.32±17.76 b	186.00±0.00 c	158.50±16.11 ab
24	90.00±20.16 ab	103.47±10.85 c	88.38±5.44 ab	84.96±22.80 a	99.18±11.29 bc	83.39±6.35 ab
28	60.48±27.60 a	72.30±10.01 c	64.45±5.86 ab	63.84±20.40 ab	76.24±8.30 c	66.54±5.44 b

## CONCLUSIONS

The general conclusion is that the incidence of cannibalistic behaviour in *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Ephestia kuehniella* Zeller, *Corcyra cephalonica* (Stainton) increases with the increase in temperature. Second instar larvae of these moths attack easier and more quickly the younger larvae, rather than larvae of the same stage. No first instar larva is able to cannibalise an indi-

*Table 3 - Survival times (hours) of II instar larvae of *E. kuehniella* and *C. cephalonica*: confined without food (A); which have cannibalised conspecific of the same stage (B); which have cannibalised conspecific of I instar larvae. Means followed by the same letter, on the same line, are not significantly different (Duncan's test;  $P>0.05$ ).*

T (°C)	<i>Ephestia kuehniella</i>			<i>Corcyra cephalonica</i>		
	II A (hrs $\pm$ S.D.)	II B (hrs $\pm$ S.D.)	II C (hrs $\pm$ S.D.)	II A (hrs $\pm$ S.D.)	II B (hrs $\pm$ S.D.)	II C (hrs $\pm$ S.D.)
18	161.28 $\pm$ 19.44 a	172.00 $\pm$ 0.00 a	172.00 $\pm$ 8.00 a	176.64 $\pm$ 22.56 b	-	159.47 $\pm$ 15.35 a
24	95.04 $\pm$ 18.00 a	122.13 $\pm$ 13.82 bc	101.68 $\pm$ 5.00 ab	115.20 $\pm$ 16.32 b	141.17 $\pm$ 13.09 c	108.60 $\pm$ 4.41 ab
28	71.52 $\pm$ 14.88 a	93.22 $\pm$ 6.92 ab	84.19 $\pm$ 7.14 a	95.04 $\pm$ 18.24 ab	104.92 $\pm$ 5.75 c	99.83 $\pm$ 4.79 b

vidual of second instar and no cases of cannibalism have been observed among new-born starved larvae, while some cannibalism was noticed among first instar larvae, that have eaten the artificial diet for 24 hours after their birth. Probably the new-born starved larvae were too weak for attacking conspecific individuals.

The possibility of feeding on conspecific individuals has allowed in some cases to cannibalistic larvae to survive significantly more time if compared with survival time of starved larvae, of the same stage. The survival times have been up to 24 hours greater for cannibalistic larvae of second instar, thanks to the fact that individuals of the same species represent a great food resource.

It was impossible to define how many larvae were necessary to cannibalise so that the post-embrial development of first or second instar larva could be complete. The only result that can be referred is that *P. interpunctella* larvae were able to reach adult stage feeding only with individuals of the same species.

These results can help to explain how an oviposition near packaged products could cause successive infestations: the larvae can survive different hours without food or can cannibalise conspecifics for recovering strength to penetrate packaging.

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