

Development and yields of the tachinid *Exorista larvarum* in three common Noctuidae of Azores Archipelago and in a laboratory host

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Abstract

The development of the tachinid larval parasitoid *Exorista larvarum* (L.) was studied upon *Peridroma saucia* (Hübner), *Pseudaletia unipuncta* (Haworth) and *Xestia c-nigrum* (L.) (Lepidoptera Noctuidae), all of them being common pests of pastures and horticultural crops in the Azores Archipelago, and in the laboratory host *Galleria mellonella* L. The study occurred under laboratory conditions, but relied on noctuids samples collected on Terceira island. The experiments were conducted at 15, 20 and 25 °C to determine the effect of temperatures commonly recorded in the Azores throughout the year on the tachinid development. All host species were accepted by *E. larvarum* females, but more eggs were laid on the noctuids than on *G. mellonella* both at 20 and 25 °C. The effect of temperature on the number of eggs laid was significant only for *G. mellonella* and *X. c-nigrum*, although on all species fewer eggs were laid at 15 °C (the mean winter temperature in the Azores). At 15 °C, no puparia were obtained from any host species. At 20 °C no puparia formed in *G. mellonella* and parasitoid pupation percentages were extremely low also in the noctuids. At 25 °C the puparial yields obtained in the latter were considerably lower than those found in *G. mellonella* (=13%).

In all moth species, including noctuids, host larval mortality was however very high despite the low percentages of puparia obtained. The efficiency of *E. larvarum* as a biocontrol agent could be enhanced by host mortality due to incomplete parasitoid development.

Key words: Tachinidae, Noctuidae, *Exorista larvarum*, *Peridroma saucia*, *Pseudaletia unipuncta*, *Xestia c-nigrum*, *Galleria mellonella*, Biological control, Azores Archipelago.

Introduction

Many tachinid parasitoids are suitable for use in the control of phytophagous insect pests (Grenier, 1988). Despite this, till now they have received relatively little attention both in general parasitoid ecology and biological control (Mellini, 1990; Belshaw, 1994).

Exorista larvarum (L.) is a polyphagous gregarious larval parasitoid of Lepidoptera that is well distributed throughout Europe, northern Africa and several Asian regions (Herting, 1960). It is well-known as an antagonist of *Lymantria dispar* (L.) and other forest lepidopterous defoliators. In cork-oak forests in Sardinia (Italy) Luciano and Prota (1984) reported parasitism by this tachinid of up to 50% of *L. dispar* larvae, thus indicating its potential for use in biological control programmes. *E. larvarum* is also recorded as a parasitoid of noctuid agricultural pests, including *Agrotis segetum* Schiffermüller, *Prodenia litura* F. (Hafez, 1953) and *Mamestra brassicae* (L.) (Sannino and Espinosa, 1999).

A number of studies have shown that *E. larvarum* is particularly suitable for mass production both *in vivo* on a factitious host, the wax moth *Galleria mellonella* L. (Lepidoptera Galleriidae), and *in vitro* on artificial diets composed of crude components (Mellini and Campadelli, 1995; Dindo *et al.*, 1999, 2003). Also this characteristic makes this tachinid a good candidate for use in applied biological control (Grenier *et al.*, 1994). Yet, to date *E. larvarum* has been used as a biological control agent only in inoculative release against *L. dispar* in the

northern United States (Sabrosky and Reardon, 1976).

The noctuids *Peridroma saucia* (Hübner), *Pseudaletia unipuncta* (Haworth) and *Xestia c-nigrum* (L.) are common pests of pastures and horticultural crops in the Azores Archipelago, with *P. unipuncta* causing the major damage, followed by *X. c-nigrum* and *P. saucia*. In the Azores all of them are polyvoltine (Silva, 1992; Araújo, 1994; Simões, 2001). The present study was aimed to investigate in the laboratory the potential of *E. larvarum* as a biological control agent of the three noctuid species. For this purpose, developmental aspects of this tachinid were studied upon *P. saucia*, *P. unipuncta* and *X. c-nigrum* as well as the laboratory host *G. mellonella*, which was maintained as a control. The experiments were conducted at 15, 20 and 25 °C to determine the effect of temperatures commonly recorded in the Azores throughout the year (Simões, 2001) on the tachinid development.

Materials and Methods

All tests were conducted in 1998 and 1999 in the laboratory of Entomology, Department of Agricultural Sciences, University of Azores, Terceira, Portugal. *E. larvarum* was maintained on larvae of *P. saucia*, *P. unipuncta* and *X. c-nigrum*. When the noctuids were not available, the parasitoid rearing was performed with *G. mellonella* larvae. These larvae were reared on an artificial diet under conditions described by Campadelli

(1973). The parasitoid colony was established from puparia obtained from the laboratory of Entomology, University of Bologna, Italy in 1998. This colony was started from adults which had emerged from *L. dispar* and *Hyphantria cunea* Drury larvae field-collected in the province of Bologna in 1992.

Adults of *P. saucia*, *P. unipuncta* and *X. c-nigrum* were field-collected overnight, throughout the year, on Terceira island pastures using light traps. The moths of each species were transferred into the laboratory, placed inside separate plastic containers (21x10x15 cm) covered with a net. Adults were fed on cotton balls soaked in a sucrose and water solution (10% sucrose). To obtain noctuid eggs, pieces of filter paper (for *P. saucia* and *X. c-nigrum*) or pleated wax paper (for *P. unipuncta*) were placed on the bottom of the containers as oviposition substrate. For each species, the paper sheets with eggs were daily transferred into similar containers. Upon hatching the larvae were fed on a semi-synthetic diet developed by Poitout and Bues (1974). All noctuid stages were kept at room environmental conditions (namely at 26 ±1 °C, 60±10% RH and 16:8 L:D photoperiod).

The development of *E. larvarum* in each host species (*G. mellonella*, *P. saucia*, *P. unipuncta* and *X. c-nigrum*) at three temperatures (15 °C, 20 °C and 25 °C) was studied. Newly-emerged *E. larvarum* pairs were placed inside 21x15x10 cm glass cages (1 pair per cage) and supplied with lump sugar and a honey and water solution (20% honey) as in the standard rearing procedure. The cages with the pairs were placed in environmental chambers at 15 °C (31 pairs) or 20 °C (31 pairs) or 25 °C (36 pairs). All rearing chambers were set at the same humidity and photoperiod conditions, namely 70±5% RH and 16:8 (L:D) photoperiod. At each temperature mature larvae of *G. mellonella*, *P. saucia*, *P. unipuncta* or *X. c-nigrum* were daily exposed to parasitoids from emergence until death. For each species, 5 larvae per cage were placed and removed after 24 h. The total number of larvae used was different among species and temperatures, depending on availability.

At each temperature, upon removal from the parasitoid cages the eggs which had been laid on the body of each larva were counted. The larvae were then placed singly inside a 30 ml capacity plastic cup until puparium formation or moth emergence. Each host larva was considered as a replicate.

For each host species and temperature, the results were evaluated in terms of the following parameters:

1. Number of *E. larvarum* eggs laid on larvae and mean number of eggs/larva.
2. Number and % puparia obtained (= no. puparia obtained/no. eggs x 100).
3. Number and % parasitoid adults emerged (=no. parasitoid adults emerged/no. puparia x 100).

The data were analysed by Kruskal-Wallis non parametric test (for the number of eggs) or 4x2 or 2x2 contingency tables (for the number of puparia and adults). For each host species, the relationship between temperature and number of eggs laid was analysed by Spearman rank correlation test (Zar, 1984).

For each lepidopterous species we have also calculated the percentages of larvae carrying 1-3, 4-7, 8-12, 13-20 or >20 eggs/larva at 15, 20 and 25 °C, the percentages of larvae producing 1, 2, 3, 4 or 5 puparia/larva at 20 and 25 °C and the percentages of larvae with *E. larvarum* eggs which produced puparia, which died without producing puparia and which developed until moth at 15, 20 and 25 °C.

Results

The mean number of *E. larvarum* eggs/larva was not significantly influenced by host species at 15 °C. For the same parameter, both at 20 °C and 25 °C significant differences were found among the host species. At both temperatures (and also at 15 °C) the lowest value was found for *G. mellonella* (table 1). The highest number of eggs laid on one larva was 59 (on *X. c-nigrum*) at 15 °C, 72 (on *X. c-nigrum*) at 20 °C and 50 (on *P. saucia*) at 25 °C. For all lepidopterous species and at all temperatures the number of eggs laid on one larva ranged from 1 to 3 or from 4 to 7 for most larvae (>60%) (figure 1).

Table 1. Mean numbers of eggs/larva laid by *Exorista larvarum* on different lepidopterous host species at 15, 20 or 25 °C. Means (± SE) in a column followed by the same letter are not significantly different (P<0.05, non parametric multiple range test).

Host species	Mean number of <i>E. larvarum</i> eggs/larva (± SE)		
	15 °C	20 °C	25 °C
<i>Galleria mellonella</i>	(n=13) 2.0±0.4a	(n=56) 2.4±0.3a	(n=335) 3.2±0.2a
<i>Peridroma saucia</i>	(n=97) 5.3±0.8a	(n=261) 7±0.5b	(n=519) 5.4±0.3b
<i>Pseudaletia unipuncta</i>	(n=146) 3.3±0.3a	(n=202) 7.6±0.6c	(n=355) 5.1±0.3c
<i>Xestia c-nigrum</i>	(n=234) 3.8±0.4a	(n=219) 5.4±0.5d	(n=233) 5.3±0.4d
H (N)	6.4 (490)	49.6 (738)	41.5 (1442)
P	0.09	0.00001	0.00001

n= number of host larvae (= number of replicates)

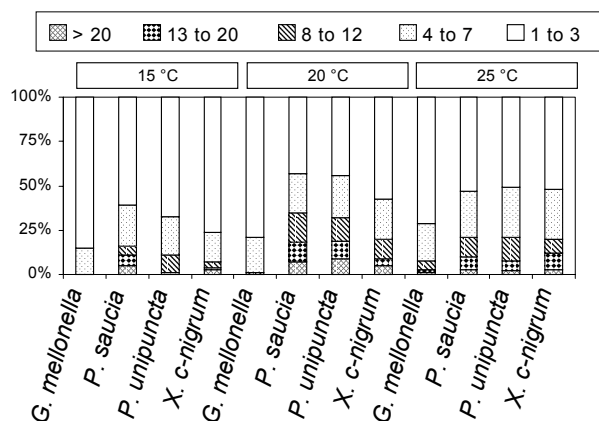


Figure 1. Percentages of *G. mellonella*, *P. saucia*, *P. unipuncta* and *X. c-nigrum* larvae carrying 1-3, 4-7, 8-12, 13-20 or >20 *E. larvarum* eggs/larva at 15 °C, 20 °C and 25 °C.

According to the Spearman rank correlation analysis, the number of eggs laid on larvae was positively correlated with temperature in a significant way for *G. mellonella* (n= 404, R= 0.120, P= 0.012) and *X. c-nigrum* (n= 686, R= 0.198, P<0.001) but not for *P. saucia* (n= 877, R= -0.045, P= 0.174) and *P. unipuncta* (n= 703, R= 0.072, P= 0.055). The data showed high variability for all species. The relationship between temperature and eggs laid on *G. mellonella* and *X. c-nigrum* larvae is illustrated in figure 2.

At 15 °C no puparia were obtained from any host species. At 20 °C no puparia formed in *G. mellonella* and parasitoid pupation percentages were extremely low also in the noctuids (table 2). A 2x4 contingency table was used for testing the independence of host species and number of parasitoid puparia obtained. The calculated chi-square was 7.38 and the critical chi-square (0.05, 3) is 7.82. The effect of the host species on the parasitoid pupation was therefore not significant (P>0.05). At 25 °C parasitoid pupation percentages remained low. The effect of host species on parasitoid pupation was significant (P<0.05). A 2x4 contingency ta-

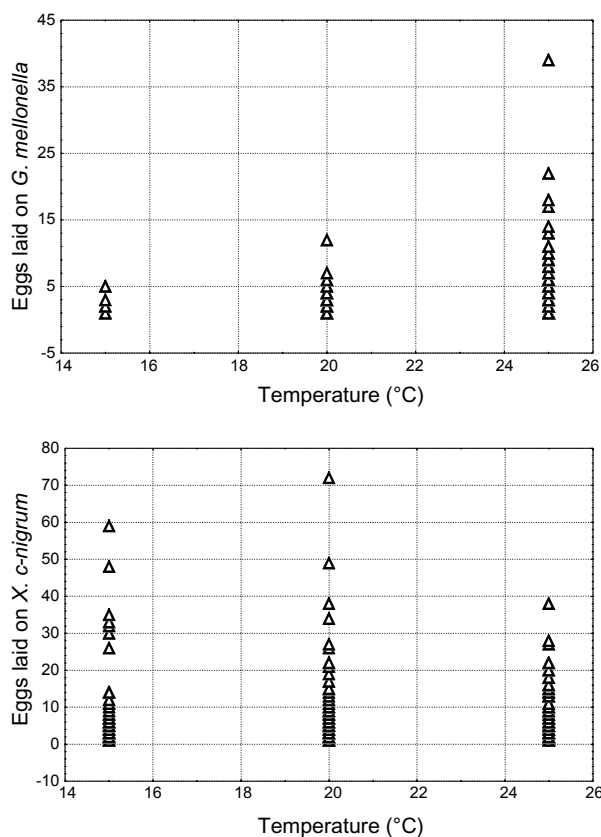


Figure 2. Relationship between temperature and *E. larvarum* eggs laid on *G. mellonella* and *X. c-nigrum* larvae.

ble was used for testing the independence of host species and number of parasitoid puparia obtained. The calculated chi-square was 233.39. Separate 2x2 contingency tables were used to test the independence of 2 host species and number of parasitoid puparia obtained. The results are shown in table 3.

Adult emergence percentages were not affected by the host species both at 20 °C and at 25 °C (table 2). A 2x3 (20 °C) and a 2x4 (25 °C) contingency table were used to test the independence of host species and number of

Table 2. Total number of eggs, puparia and adults, percentages of puparia and adults of *Exorista larvarum* reared in 4 host species at 20 and 25 °C.

Host species	No. eggs	No. puparia	% puparia ^a	Temperature 20 °C	
				No. parasitoid adults	% adult ^b emergence
<i>Galleria mellonella</i>	132	0	0	--	--
<i>Peridroma saucia</i>	1830	44	2.4%	25	56.8%
<i>Pseudaletia unipuncta</i>	1545	26	1.7%	20	76.9%
<i>Xestia c-nigrum</i>	1188	13	1.1%	11	84.6%
Temperature 25 °C					
<i>Galleria mellonella</i>	1075	140	13%	113	80.7%
<i>Peridroma saucia</i>	2777	120	4.3%	89	74.2%
<i>Pseudaletia unipuncta</i>	1796	17	0.94%	12	70.6%
<i>Xestia c-nigrum</i>	1226	40	3.3%	27	67.5%

^a Percentages based on eggs laid on hosts

^b Percentages based on puparia obtained

Table 3. The 2x2 contingency table for testing the independence of lepidopterous host species and the number of *Exorista larvarum* puparia obtained at 25 °C.

Host species	No. puparia obtained	No. dead host larvae	χ^2	P
<i>Galleria mellonella</i>	140	935	93.24	0.00001
<i>Peridroma saucia</i>	120	2657		
<i>Galleria mellonella</i>	140	935	189.73	0.00001
<i>Pseudaletia unipuncta</i>	17	1779		
<i>Galleria mellonella</i>	140	935	75.68	0.00001
<i>Xestia c-nigrum</i>	40	1186		
<i>Peridroma saucia</i>	120	2657	42.74	0.00001
<i>Pseudaletia unipuncta</i>	17	1779		
<i>Peridroma saucia</i>	120	2657	2.48	>0.05
<i>Xestia c-nigrum</i>	40	1186		
<i>Pseudaletia unipuncta</i>	17	1779	21.12	0.00001
<i>Xestia c-nigrum</i>	40	1186		

adults obtained. The calculated chi-squares were 4.42 (20 °C) and 3.45 (25 °C). The critical chi-square (0.05, 2) is 5.99. For both 20 °C and 25 °C we therefore had non-significant chi-square tests.

For all species, most larvae were successfully parasitized (i.e. gave puparia) when 1-3 eggs had been laid on their body. In most cases only one puparium was obtained per larva both at 20 °C and 25 °C (figure 3).

The percentages of successfully parasitized larvae were low for all species. Despite this, host larval mortality was very high. Some of the larvae which were not successfully parasitized developed up to the adult stage and moth emergence occurred (figure 4).

Discussion

All host species were accepted by parasitoid females, but more eggs were laid on the three noctuids than on *G. mellonella* both at 20 and 25 °C. At 20 °C the highest values for the mean number of parasitoid eggs/larva was found for *P. unipuncta* and *P. saucia*. At 25 °C, though the differences were significant among the four different hosts, the values were very close for the noctuid species. The effect of temperature on the number of eggs laid was significant only for *G. mellonella* and *X. c-nigrum*. Yet on all species fewer eggs were laid at 15 °C. Moreover at this temperature (the mean winter temperature at the Azores as reported by Simões, 2001), no puparia were obtained from any host species. This result was expected in the case of *G. mellonella* (which did not produce puparia also at 20 °C). The wax moth is normally found in hives at 35 °C, although it can also live in stored honeycombs at lower temperatures (Mellini *et al.*, 1978). *G. mellonella* has been successfully employed at 26-27 °C as a laboratory host for many tachinid parasitoids (Campadelli and Baronio, 1978; Baronio and Campadelli, 1978; Grenier and Delobel, 1984; Bonnot *et al.*, 1991; Bratti and Costantini, 1991) including *E. larvarum*. At this temperature, the puparial yields of *E. larvarum* obtained from mature *G. mellonella* larvae usually range from 25 to 50% (Mellini and Campadelli, 1996). In the present study, at 25 °C the yield obtained from *G. mellonella* did not exceed 13%.

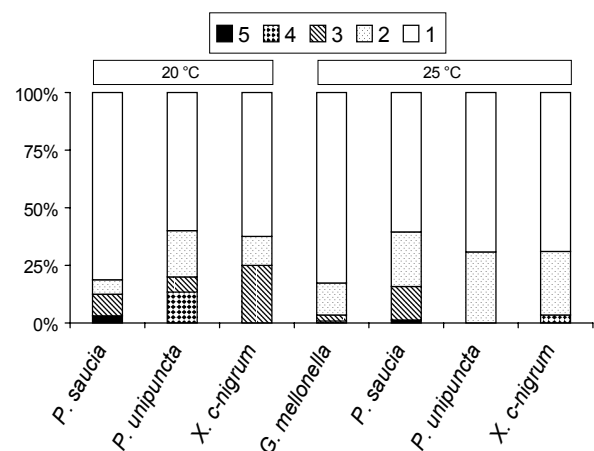


Figure 3. Percentages of *G. mellonella*, *P. saucia*, *P. unipuncta* and *X. c-nigrum* larvae producing 1, 2, 3, 4 or 5 puparia/larva at 20 °C and 25 °C.

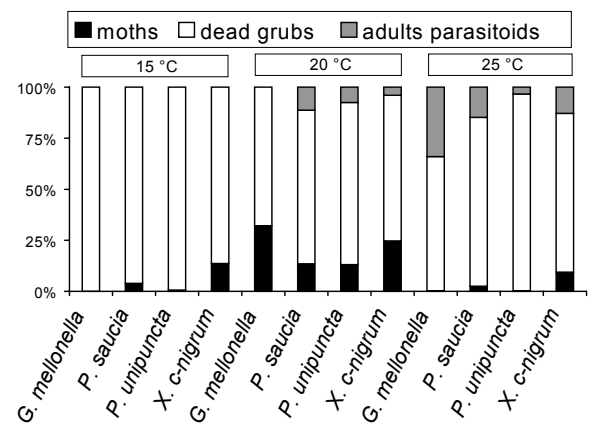


Figure 4. Percentages of *G. mellonella*, *P. saucia*, *P. unipuncta* and *X. c-nigrum* larvae contaminated with *E. larvarum* eggs which produced adult flies, which died without producing puparia and which developed up to moth at 15 °C, 20 °C and 25 °C.

As to the noctuids, in a field study conducted in Egypt, Hafez (1953) showed that *E. larvarum* could complete its life-cycle in the natural host *P. litura* also in winter at 13 °C, although at a slower rhythm than at 24, 27 and 30 °C. Otherwise, in the present study, also at 20 and 25 °C a few puparia were obtained from *P. saucia*, *P. unipuncta* and *X. c-nigrum* (which can be found in the larval stage in the Azores all around the year, although they are far more abundant in spring and summer as reported by Simões, 2001). Mellini and Campadelli (1996) showed that, in *G. mellonella*, considerable parasitoid losses result from the high number of eggs which detach from the host integument before hatching. In the present study, the low puparial yields obtained in all species were probably partially ascribable to the same phenomenon. But since the yields obtained in *G. mellonella* at 25 °C were considerably higher than those found in the noctuids, the latter proved to be less suitable than the wax moth as hosts for *E. larvarum*. In the literature, no record of the three noctuids as natural hosts of *E. larvarum* was found. Arnaud (1978) however reported *P. unipuncta* as a natural host of *Exorista mella* Walker. According to Sabrosky and Reardon (1976) and Morewood and Wood (2002) *E. mella* and *E. larvarum* are exceedingly similar, and there appear to be intermediate (hybrids?) or specimens that are difficult to identify with certainty. Yet the puparial yields obtained from *P. unipuncta* were very low compared to the other noctuids and *G. mellonella*, especially at 25 °C.

Parasitoid adult emergence was not affected either by temperature or host species. However, this phenomenon is generally not affected by the rearing conditions as shown by Mellini and Campadelli (1995, 1996, 1997) and Dindo *et al.* (1999, 2002).

The fact that, for all species, most larvae gave puparia when 1-3 eggs had been laid on their body was consistent with the findings of Mellini and Campadelli (1997).

It is worth pointing that in all hosts, including noctuids, larval mortality was very high despite the low yields of puparia obtained. Similarly to the findings of previous laboratory and field studies performed with the natural host *L. dispar* (Dindo *et al.*, 2002) this mortality was probably related to partial *E. larvarum* activity inside the host. Further research (including non-parasitized larvae maintained as controls) is needed to ascertain if this relationship between host mortality and parasitoid action exists. Inundative field releases of *E. larvarum* against noctuids might be effective despite the low parasitization percentages. As emphasised by Grenier and De Clercq (2003), field efficiency is usually evaluated by the number of hosts successfully parasitized, but it is also necessary to take into account other parasitoid-related mortality factors, such as host stinging and host feeding for parasitic wasps and host mortality due to incomplete parasitoid development for both wasps and tachinids.

Several authors, including Howarth (1997), have addressed the issues of risks of biological control. Negative impacts of introduced exotic entomophagous insects may include suppression of indigenous natural enemies as well as non-target host/prey species (Lynch

et al., 2001). Since *E. larvarum* has never been recorded in the Azores Archipelago, the potential risks of the use of this tachinid under field conditions should be taken into account. Field and laboratory post-release studies, combined with predictive methods, should permit to outline a framework for evaluating environmental effects of agents introduced for arthropod biological control (van Lenteren *et al.*, 2003). More extensive testing are recommended for projects of introduction of parasitoids and predators onto islands (Van Driesche and Hodde, 1997). In the case of *E. larvarum*, this topics will be investigated in future studies.

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