

Location, acceptance and suitability of *Spodoptera littoralis* and *Galleria mellonella* as hosts for the parasitoid *Exorista larvarum*

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Abstract

The location, acceptance and suitability of the phytophagous *Spodoptera littoralis* (Boisduval) by the tachinid larval parasitoid *Exorista larvarum* (L.) was studied in the laboratory. A test was conducted in a cage environment to assess whether *E. larvarum* displays a difference in locating and accepting the laboratory host *Galleria mellonella* (L.) vs. *S. littoralis* and whether the host plant plays a role in host location by the parasitoid. Inexperienced *E. larvarum* females were similarly attracted to, and accepted, *G. mellonella* and *S. littoralis* larvae, but weakly responded to *S. littoralis* larvae feeding on a bean leaf. Since the latter were apparently less mobile compared to the other two targets, the results may support the hypothesis that, at close range, tachinid females use visual cues and, in particular, motion signals in host location. Host acceptance and suitability of *S. littoralis* vs. *G. mellonella* by *E. larvarum* were then further compared. Based on the time needed to obtain the oviposition of 4-6 eggs per larva, acceptance was not significantly different between the two host species. Puparia were however obtained from 1.3% of *S. littoralis* larvae vs. 75% of *G. mellonella* larvae. Despite the low successful parasitization, in parasitized *S. littoralis* larvae mortality was higher compared to control (unparasitized) larvae. This result suggests that *E. larvarum* may be a candidate for biological control of *S. littoralis*.

Key words: parasitoids, host location and acceptance, host plant, host suitability, Tachinidae.

Introduction

Exorista larvarum (L.) (Diptera Tachinidae), a polyphagous gregarious larval parasitoid of Lepidoptera, is well known as an antagonist of *Lymantria dispar* (L.), *Malacosoma neustria* (L.), *Tortrix viridana* L. and other forest defoliators (Herting, 1960; Delrio *et al.*, 1983; 1988). It is also recorded as a natural enemy of noctuid species of agricultural interest, including *Mamestra brassicae* (L.) (Sannino and Espinosa, 1999), *Autographa gamma* (L.) and *Lacanobia oleracea* (L.) (Cerretti and Tschorsnig, in press).

The biology of *E. larvarum* was described by Hafez (1953) and, recently, Michalkova *et al.* (2009). Females lay macrotype eggs on the host body. The newly hatched larvae penetrate the host integument, induce the formation of a primary integumental respiratory funnel and continuously develop until pupation, which generally occurs outside the host remains. The parasitoid development is independent of the hormonal balance of the host larva, which is killed quickly (i.e. 1-2 days after parasitoid egg hatching, at 25 °C). *E. larvarum* can be mass-reared on the factitious host *Galleria mellonella* (L.) (Lepidoptera Pyralidae) or artificial media composed of crude ingredients and devoid of insect material (Bratti *et al.*, 1995; Mellini and Campadelli, 1996; Dindo *et al.*, 1999; 2006). The artificial rearing may be also performed starting from eggs laid away from the host, thus completely excluding the victim from the parasitoid line of production, at least for one generation (Dindo *et al.*, 2007; Marchetti *et al.*, 2008).

To date, *E. larvarum* has been used as a biological control agent only against *L. dispar*, in inoculative releases in the northern United States (Sabrosky and

Reardon, 1976). Yet, the possibility to mass rear this tachinid quite easily, both *in vivo* and *in vitro*, makes it a potential candidate for use in biological control programs also against other lepidopterans of forest and agricultural interest (Grenier, 2009). Research aimed at improving knowledge on its biology, interaction with host and potential for use against selected target pest species is thus justified. In this framework, the experiments described below were aimed at investigating host location, acceptance and suitability of the phytophagous *Spodoptera littoralis* (Boisduval) (Lepidoptera Noctuidae) by *E. larvarum* reared *in vivo* on *G. mellonella*. *S. littoralis* is widespread in the African and Sub-Mediterranean region, it is widely polyphagous and attacks several horticultural plants, strawberry and ornamental plants (EPPO/CABI, 1997). The species was selected as a case-study in the present research, because it is getting more and more harmful to different crops (both in greenhouse and open field) in the central and southern regions of the Italian peninsula and in Sicily (Sannino *et al.*, 2006; Masetti *et al.*, 2008). *S. littoralis* was recorded as a natural host of *E. larvarum* in Egypt (Hafez *et al.*, 1976; Assal and Koilab, 1984).

Materials and methods

Insects

A colony of *S. littoralis* was started in 2006 from egg masses collected in the field in the province of Latina (Lazio, central Italy) by Alberto Lanzoni and cooperators. The colony was maintained on bean plants (*Phaseolus vulgaris* "Borlotto Firetongue") in a rearing chamber at 25 ± 1 °C, 65 ± 5% RH and 16:8 L:D photoperiod

(El Guindy *et al.*, 1978). The larvae and adults were kept in Plexiglas cages (60 × 35 × 50 cm) and wood and net cages (25 × 30 × 40 cm) respectively. The adults were fed on cotton balls soaked in a honey and water solution (20% honey). As an oviposition substrate, bean plants (about 10 cm high) were placed in the adult cages for 24 h.

A colony of *E. larvarum* was established in 1992 and augmented in 2004 with adults which had emerged from *L. dispar* and *Hyphantria cunea* (Drury) larvae collected in the field in the provinces of Bologna and Modena (Emilia Romagna, northern Italy). Throughout the years, the standard colony consisted of three adult cages at least, each containing 70-80 flies. The colony was maintained in the laboratory using *G. mellonella* as a factitious host. *G. mellonella* larvae were reared on the artificial diet developed by Sehnaal (1966) and modified by Campadelli (1986) at 30 ± 1 °C, 65 ± 5% RH and in complete darkness. *E. larvarum* adults were kept in Plexiglas rearing cages (40 × 30 × 30 cm) at 25 ± 1 °C, 65 ± 5% RH and 16:8 L:D photoperiod. The flies were fed on lump sugar and cotton balls soaked in the above described honey and water solution, as in Dindo *et al.* (1999).

In the experiments, all host larvae were in the last instar, the most suitable for parasitism by *E. larvarum* according to Hafez (1953) and Mellini *et al.* (1993). *S. littoralis* larvae (about 3-3.5 cm long) were newly-moulted, as determined by the presence of a moulted head capsule, whereas *G. mellonella* larvae (about 2.5 cm long) were in advanced last instar so as to minimize the difference in size between the two species. *E. larvarum* females ranged in age from 5-12 days (Dindo *et al.*, 1999).

Location and acceptance of *G. mellonella* vs. *S. littoralis*, alone or in the act of feeding on a bean leaf

In the laboratory, a three-choice test was performed to start assessing whether this parasitoid displays a difference in locating and accepting *G. mellonella* vs. *S. littoralis* and whether the host plant plays a role in host location by *E. larvarum*. The test was conducted at 25 ± 1 °C, 65 ± 5% RH between 12:00 and 18:00 h, when *E. larvarum* females were observed to be more active (Depalo, 2009). Newly-emerged female flies were kept together with an equal number of males for at least four days to ensure that they had the opportunity to mate and develop fertile eggs (pre-oviposition of *E. larvarum*: 2-3 days; Dindo *et al.*, 2007). The parasitoids were fed as in the standard rearing conditions described above. The females used in the experiment were inexperienced (i.e. they had never encountered a host). They were individually presented with three targets in a Plexiglas cage (cm 60 × 35 × 50). The targets consisted of: (1) a *G. mellonella* larva; (2) a *S. littoralis* larva; (3) a *S. littoralis* larva feeding on a leaf of a bean plant. Each target was placed on the bottom of a 5-cm diameter glass Petri dish. A target was considered as chosen when the female laid an egg on the larva. The total duration of time spent by each female in the cage until oviposition (= time to make the choice) was recorded. Forty flies were tested and each was tested only once. For every female, the three targets were renewed and placed in the cage in a different position in order to avoid position

effect on female response. The parameters used to assess location and acceptance of the targets were the number and percentage of females which chose each target and the total duration of time (min) spent by each female in the cage until oviposition.

A 3 by 2 contingency table was used for testing the independence of target type and number of females which chose each target. Separate 2 by 2 contingency tables were then created to test any possible combination of targets; the partition of chi-square was calculated by using Kimball's formula (Kimball, 1954). The times spent by females in the cage until oviposition on each target were analysed by one-way ANOVA and then compared by Tukey HSD multiple range test.

Acceptance and suitability of *S. littoralis* vs. *G. mellonella*

This experiment was carried out to further test the acceptance and start testing the suitability of *S. littoralis* vs. *G. mellonella* as hosts for *E. larvarum*. The experiment consisted of four treatments each comprising 80 larvae: *S. littoralis* larvae (a) exposed or (b) not exposed to *E. larvarum* and *G. mellonella* larvae (c) exposed or (d) not exposed to *E. larvarum*. In treatments (a) and (c) the larvae were individually exposed to 70-80 parasitoids in a rearing cage (one per treatment) and removed when 4-6 eggs/larva had been laid (the optimal egg number per host according to Mellini and Campadelli, 1997). The duration of time (min) needed to have these eggs laid on each larva was recorded and used as a parameter to assess acceptance. *S. littoralis* larvae (with or without eggs) were placed singly into plastic cylindrical containers (10-cm diameter × 10-cm height), supplied with bean leaves and daily observed. *G. mellonella* larvae (with or without eggs) were placed together into plastic boxes (22 × 15 × 10 cm) without food, as in the standard rearing condition (1 box per treatment). To assess suitability, for treatments (a) and (c) the number of successfully parasitized larvae (= larvae from which puparia were obtained) and the percentage of successful parasitization were calculated. The latter percentage was based on the original number of larvae infested with parasitoid eggs (= 80). Moreover, for all treatments the total number of dead larvae and pupae and the percentage of total mortality were also calculated. The experiment was conducted at 25 ± 1 °C, 65 ± 5% RH and 16:8 L:D photoperiod.

The times needed to have 4-6 eggs laid on larvae were analysed by Kruskal-Wallis test. The nonparametric test was necessary because of heteroscedasticity in the data. The independence of parasitization by *E. larvarum* and total number of dead *S. littoralis* or *G. mellonella* was tested using 2 by 2 contingency tables. Two separate 2 by 2 contingency tables were created to test the independence of host species (*S. littoralis* vs. *G. mellonella*) and total number of dead lepidopterans (a) exposed or (b) not exposed to *E. larvarum*.

The data concerning successful parasitization were not subjected to statistical analysis, because puparia were obtained from only one *S. littoralis* larva.

All statistical tests were done with STATISTICA 6.0 (StatSoft, 2001).

Results

Location and acceptance of *G. mellonella* vs. *S. littoralis*, alone or in the act of feeding on a bean leaf

The results concerning female choice are shown in figure 1. This parameter was significantly influenced ($P < 0.01$) by the target type, as the calculated χ^2 found in the 3 by 2 contingency table was 13.51 while the critical χ^2 (0.01, 2) was 9.21. In detail, *S. littoralis* larvae on a bean leaf were significantly less frequently chosen compared to *G. mellonella* larvae ($\chi^2 = 25.21$, $P < 0.01$) and *S. littoralis* larvae alone ($\chi^2 = 11.25$, $P < 0.01$), but female choice was not significantly affected by the target type when *G. mellonella* was compared to *S. littoralis* alone ($\chi^2 = 2.81$, $P > 0.05$). Females spent a significantly longer time to choose *S. littoralis* larvae on the bean leaf compared to *S. littoralis* alone and *G. mellonella* ($F_{2,37} = 4.63$, $P < 0.05$). No significant difference was found for this parameter between *S. littoralis* alone and *G. mellonella* (figure 2). *S. littoralis* larvae on the bean leaf were apparently less mobile compared to the other two targets.

Acceptance and suitability of *S. littoralis* vs. *G. mellonella*

The mean time (\pm s.d.) to have 4-6 tachinid eggs laid per larva was 5.2 ± 3.8 min for *S. littoralis* and 4.1 ± 2.7 min for *G. mellonella*. The difference was not significant ($H = 3.5$; $N = 160$; $P > 0.05$). Only one *S. littoralis* larva (1.3% of the total) produced a puparium, from which a parasitoid adult emerged. In contrast, 60 *G. mellonella* larvae (75% of the total) were successfully parasitized.

Independently of parasitization success, the effect of *E. larvarum* on *S. littoralis* and *G. mellonella* mortality was significant (*S. littoralis*: $\chi^2 = 14.1$, $P < 0.01$; *G. mellonella*: $\chi^2 = 92.2$, $P < 0.01$) (figure 3). Percent mortality of the larvae accepted by the parasitoid females was significantly lower for *S. littoralis* compared to *G. mellonella* ($\chi^2 = 15$, $P < 0.01$). It has to be noted that, contrary to *S. littoralis*, all *G. mellonella* larvae which died following oviposition by *E. larvarum* were successfully parasitized. In the absence of parasitoidism, mortality was significantly higher for *S. littoralis* compared to *G. mellonella* ($\chi^2 = 12.5$, $P < 0.01$) (figure 3). Nearly all the non-exposed *G. mellonella* larvae survived, pupated and emerged as adults.

Discussion

The results obtained in the first experiment demonstrated that inexperienced *E. larvarum* females were attracted to, and accepted, *G. mellonella* and *S. littoralis* larvae with no significant difference between the two lepidopterous species. Females showed a dramatically lower response to *S. littoralis* larvae in the act of feeding on a bean leaf, compared to the other two targets. Thus, in the cage environment where the test was conducted, the phytophagous-infested plant decreased the attractiveness of the noctuid larvae to *E. larvarum*. It is likely that, compared to the other two targets, *S. littoralis* larvae feeding on the bean leaf were less perceived, and therefore less frequently chosen, by parasitoid females, because of factors linked to the presence of the plant. Most studies concerning host selection behaviour have involved hymenopterous parasitoids, for which chemical cues have been shown to play a major role (Godfray, 1994). In particular, a number of authors

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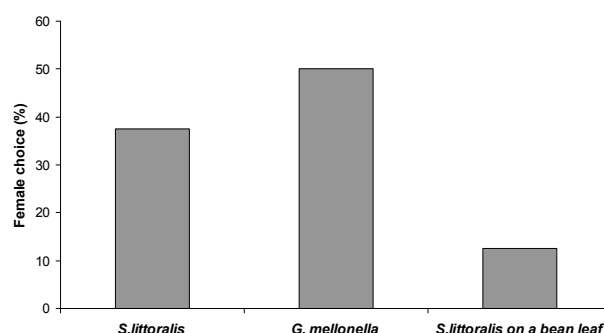


Figure 1. Choice (%) by *E. larvarum* females among the three target types: 1) a *S. littoralis* larva; 2) a *G. mellonella* larva; 3) a *S. littoralis* larva in the act of feeding on a bean leaf. A target was considered as chosen when the female laid an egg on the larva. Number of flies tested = 40. See text for statistics.

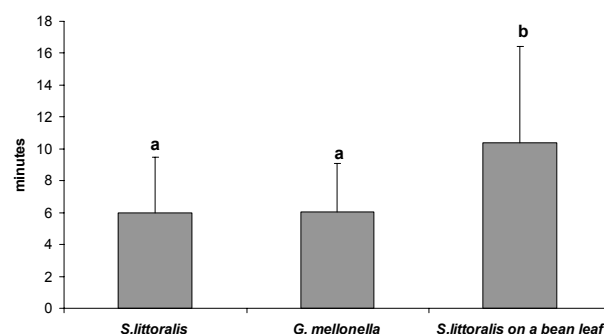


Figure 2. The means (\pm s.d.) of the total time spent by *E. larvarum* females to choose *S. littoralis* larvae on a bean leaf compared to *S. littoralis* alone and *G. mellonella*. Number of flies tested = 40. Letters above columns indicate significantly different means. See text for statistics.

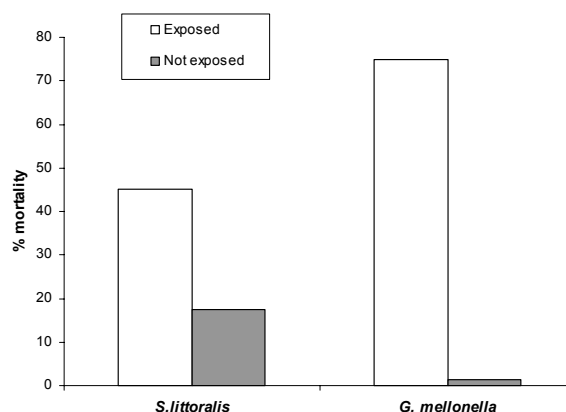


Figure 3. Percent mortality of *S. littoralis* and *G. mellonella* larvae exposed or not exposed to *E. larvarum*. Number of larvae tested = 80 per treatment. See text for statistics.

(e.g., Turlings *et al.*, 1990; De Moraes *et al.*, 1998; Fukushima *et al.*, 2002) have demonstrated that the volatiles produced by plants infested with phytophagous insects are important cues for host location by these parasitoids. As emphasized by Mellini (1991), and later Stireman *et al.* (2006), the mechanisms of host selection in Tachinidae, including the role of host plants, are far less known. Chemical stimuli released by phytophagous-infested plants have been shown to attract some tachinid species, however, including the polyphagous larval parasitoids *Exorista mella* Walker (Stireman, 2002) and *Exorista japonica* Townsend (Kainoh *et al.*, 1999) which lay macrotype eggs on the host cuticle, similarly to *E. larvarum*. Recently, in tests performed in a wind tunnel, *E. japonica* was found to be more attracted to plants infested with larvae of the noctuid moth *Mythimna separata* (Walker), compared to artificially damaged or undamaged plants (Ichiki *et al.*, 2008). The results achieved in our research are not consistent with those obtained in the above mentioned studies. Considering that *S. littoralis* larvae on the bean leaves were apparently less mobile compared to the other two targets, our results may support the hypothesis that, at close range (e.g., in the cage environment), tachinid females primarily use visual cues and, in particular, motion signals in host location. Olfactory cues such as volatile chemicals associated with host plants may attract tachinid females to particular habitats (and therefore be active at longer range) (Stireman, 2002; Stireman *et al.*, 2006). This aspect certainly deserves further research.

The results of the second experiment further suggested that *S. littoralis* and *G. mellonella* larvae are equally accepted by *E. larvarum*, but *S. littoralis* proved less suitable for parasitoid development. One hypothesis for this result is that *E. larvarum*, maintained in continuous culture on a laboratory host for many generations, has considerably decreased its capability to successfully parasitize a different host. Similar issues have to be addressed when entomophagous insects are mass reared on laboratory hosts/preys (van Lenteren, 2003). A wild strain of *E. larvarum* will have thus to be tested. Another hypothesis is that *S. littoralis* itself is only marginally suitable for the development of *E. larvarum*. Actually, records of successful parasitization of this noctuid species by *E. larvarum* in nature are few and not very recent (Hafez *et al.*, 1976; Assal and Koilab, 1984).

Grenier and De Clercq (2003) have stated that the efficiency of parasitoids as biological control agents is usually evaluated by the number of hosts successfully parasitized, but it is also necessary to take into account other parasitoid-related mortality factors, including incomplete parasitoid development. Probably due to the latter factor, *S. littoralis* larvae accepted by *E. larvarum* showed higher mortality than control (unparasitized) larvae, despite the low successful parasitization. This result suggests that *E. larvarum* may be a candidate for biological control of *S. littoralis*. More research is however needed to better evaluate this issue. In particular, host mortality following parasitization may be higher in younger larvae. Therefore, it will be crucial to study the effects of host age, a key aspect for all parasitoids including Tachinidae (Mellini, 1986), on *S. littoralis* mortality.

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