Abstract

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

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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 (Boisdalv) (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 Hypantria 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 Sehnal (1966) and modified by Campadelli (1986) at 30 ± 1 ºC, 65 ± 5% RH and in complete darkness. E. larvarum adults were kept in Plexiglas rearing cages (40 × 30 × 30 cm) at 25 ± 1 ºC, 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 ºC, 65 ± 5% RH between 12:00 and 18:00 h, when E. larvarum females were observed to be more active (De-palo, 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 ºC, 65 ± 5% RH and 16:8 L:D photoperiod.

The times needed to have 4-6 eggs laid on larvae were analysed by Kruskall–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 $\chi^2$ found in the 3 by 2 contingency table was 15.51 while the critical $\chi^2$ (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 (± 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

![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.](image1)

![Figure 2. The means (± 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.](image2)

![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.](image3)
(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 parasitoid 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 (Hafiez 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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References


Cerretti P., Tschorsnig H.-P.- Annotated host catalogue for the Tachinidae (Diptera) of Italy.- Stuttgartter Beiträge zur Naturkunde, Neue Serie 3 (In press).


MELLINI E., CAMPADELLI G., 1996.- Formulas for “inexpensive” artificial diets for the parasitoid Exorista larvarum (L.).- Bollettino dell’Istituto di Entomologia “Guido Grandi” della Università degli Studi di Bologna, 50: 95-106.

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