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IMPORTANCE OF EXORISTA LARVARUM (LINNAEUS, 1758) (DIPTERA: TACHINIDAE) SPECIES IN THE BIOLOGICAL REGULATION OF LEPIDOPTERA SPECIES

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Abstract. Exorista larvarum is a dipteran polyphagous larval endoparasitoid particularly known as antagonist of Lepidoptera. Exorista larvarum is a good biocontrol candidate against forest lepidopterous defoliators. This parasitoid has positive features, among which, it can be efficiently reared in vivo and in vitro. In the laboratory, box tree moth larvae were accepted by E. larvarum females. A lower number of eggs were laid on C. perspectalis than on G. mellonella, but the difference between the two moth species was not significant, although a quite long 3 hours exposure time was necessary for oviposition. We can speculate that, although not the preferred host, C. perspectalis may be accepted by E. larvarum also in nature. The overall results suggest that the mortality of C. perspectalis and G. mellonella larvae due to the partial development of E. larvarum may be useful to regulate the populations of this invasive pest in a context of conservative biological control.

Introduction

Exorista larvarum (Linnaeus, 1758) is a dipteran endoparasite known especially as an antagonist of Lepidoptera species. This species is part of the Tachinidae family, which includes about 8,500 species of parasitoids present worldwide [17]. This Palaearctic tachinid fly is distributed from Europe, to northern Africa, and certain Asian regions. It has also become established in North America during the 20th century, when it was introduced there to control *Lymantria dispar* (Linnaeus, 1758), which is a lepidopterous defoliator species causing serious damages in the forests [12].

The species develops in the caterpillars of about 45 species of host butterflies [13, 14]. It is well known as an enemy of *Lymantria dispar* and other defoliating butterflies: *Malacosoma neustria* (Linnaeus 1758), *Tortrix viridana* (Linnaeus, 1758), *Hyphantria cunea* (Drury, 1773) *Dendrolimus pini* (Linnaeus, 1758), *Peridroma saucia* (Hübner, l808), *Spodoptera littoralis* (Boisduval, 1833), *Pseudaletia unipuncta* (Haworth 1809) [5, 9, 18, 28]. This tachinid is also an antagonist of some agricultural noctuid pests as *Xestia c-nigrum* (Linnaeus, 1758), *Agrotis segetum* (Denis & Schiffermüller, 1775), *Prodenia litura* (Fabricius, 1775), *Mamestra brassicae* (Linnaeus,1758), *Pieris brassicae (Linnaeus*, 1758), *Autographa gamma* (Linnaeus, 1758), *Lacanobia oleracea* (Linnaeus, 1758) [6, 7, 12 ,15, 19].

Moreover, other lepidopterous species, not attacked in nature, may be suitable for the development of *E. larvarum* in vitro, one of these is the wax moth *Galleria mellonella* (Linnaeus, 1758). Due to the potential to reduce and regulate the number of phytophagous insects, Tachinidae species can be used in classical biological control programs [10].

The purpose of this study is to investigate the potential of E. larvarum as a biological control agent of lepidopteran pests by testing the effectiveness of fly on the larvae of artificial hosts *G. mellonella* and *Cydalima perspectalis*.

Materials and methods

Rearing of *Exorista larvarum:* A laboratory colony of *E. larvarum* was maintained on the factitious host *Galleria mellonella* [14]. The artificial medium used during the study consisted of skim milk (30 ml), egg yolk (5.5 ml), yeast extract (2.7 g), sucrose (0.8 g), gentamicin (10-1mg solution), 0.01 ml/ml [2, 15].

Plastic plates with 24 holes were used as growth containers. Each hole had a diameter of 17 mm, a height of 18 mm and a capacity of 3.3 ml. *E. larvarum* eggs were collected from infected larvae of *G. mellonella* and were placed by the method an egg on an orifice [2].

After laying the parasitoid eggs, the plates were sealed with paraffin paper and kept in the dark at 26 ± 1°C and 70% humidity throughout the development period, except for daily inspections. The food was prepared according to the method of Farneti et al. [6]. The diet of adult flies consisted of sugar cubes mixed with pollen, yeast extract and cotton balls soaked with a prepared solution of 20% honey and 80% water. The change of these cotton balls was done three times a week to avoid drying and contamination with mold [8].

For the standard colony maintenance, up to 2-5 parasitization procedures were performed weekly, by exposing last instar larvae of *G. mellonella* to flies however younger larvae can also be suitable for them [1]. According to the number of flies 40-70 host larvae were inserted into the adults cage. Exposure occurred after about 5 days from the beginning of fly emergence: the females were, therefore, mated, since in *E. larvarum* mating 18 occurs soon after emergence [1].

Prior to exposure, cotton balls, pollen, yeast and sugar, as well as the remaining pupae were removed from the cage. Alternative hosts were removed after infestation with 3-4 parasite eggs clearly visible on their cuticle, this being the optimal number for each host, in order to avoid excessive over parasitization [14, 15].

The dolls collected from the previous procedures were placed in a new box. Sometimes, the hatched flies earlier were gathered together with the hatched ones later to keep about 50-70 individuals in the box [16].

Rearing of *Galleria mellonella:* The colony of *Galleria mellonella* species was grown in the laboratory under controlled conditions [3].

In a sideboard at a fixed temperature of 30-32 ± 1°C, humidity of 65 ± 5% in complete darkness were kept a number of 10 boxes (24 x 13 x 8 cm) of different *Galleria mellonella* larvae continuous ages. Three times a week, the larvae were fed the ground diet. The requirements for the amount of food were different depending on the stages of development, the young larvae consumed more food. Adult moths lay eggs on filter papers, which were collected weekly. Unused eggs were stored in a refrigerator at 4°C.

Artificial diet preparation was made monthly. The diet contained corn flour, white flour, and wholemeal flour, milk powder, lyophilised brewer's yeast; the ingredients were mixed together in a big pot; solid wax and liquid glycerin were put in two other pots, and two bottles of honey were prepared in advance. All of the ingredients were placed in a special oven set at 100°C for 3-4 hours. This is a method to disinfest the materials and to melt the solid ingredients. In

a special mixing machine all components were mixed until they got mash texture; before the whole dollop became rigid it was mounted to a plastic tray and cut into smaller pieces. When the diet cubes were cold they became solid and some of the cubes were grinded in a shredding machine. The grinded diet was placed for 24 h into a freezer (-24°C), and the following day it was unfrozen and was used for larval feeding. The remaining diet was stored in a fridge set at 4-5°C until further use [3].

Infection of *G. mellonella* larvae with eggs of *E. larvarum*. For the experiment, a total of 34 instare larvae of *G. mellonella* and *C. perspectalis* were exposed separately to *E. larvarum*. The exhibition took place in plastic cages of 20 x 20 x 20 cm. After exposure, the parasitoid females were removed and placed in another cage, then the eggs of the parasitoids on the larvae were counted (fig.1).The infected larvae were placed separately in plastic boxes with a diameter of 9 cm and were kept in the climate chamber and monitored daily until the visible effects of parasitization or non-parasitism by the fly. The larvae were considered infected when at least one parasitoid egg was found on their integument [2].

Figure 1. Infection of G. mellonella larvae with E. larvarum eggs in the growth box

Statistical analysis. For statistical analysis, the software STATISTICA 10.0 (StatSoft, 2010) was used. The data were analyzed by one-way ANOVA or, in case of variance heterogeneity, by Kruskal-Wallis nonparametric test. The percentages of moth adults were analyzed separately for the larvae exposed to *E. larvarum* females and for those which were not. Prior to analysis, the percentage values were transformed using an arcsine transformation [21].

Results and discussions

In the laboratory, box tree moth larvae were accepted by *E. larvarum* females. A lower number of eggs were laid on *C. perspectalis* than on *G. mellonella*, but the difference between the two moth species was not significant, although a quite long 3 hours exposure time was necessary for oviposition. We can speculate that, although not the preferred host, *C. perspectalis* may be accepted by *E. larvarum* also in nature.

As a result of the study, as expected, no significant difference was obtained between *G. mellonella* larvae that were fully accepted and between those of *C. perspectali*s accepted on av-

erage by 86%. No significant difference was also found for the parasitoid eggs/accepted larva, but higher oviposition was obtained on *G. mellonella*. No puparia formed in any accepted C. perspectalis larva. Conversely, puparia were obtained from *G. mellonella* larvae and all puparia emerged as adults (Table 1). The percentage of moths obtained from the larvae exposed to *E. larvarum* was low for both *C. perspectalis* (4.2 ± 2.6) and G. mellonella (7.3 ± 1.8) [2].

Importance of *E. larvarum* as an applied biological control agent. Effective production of biological control agents requires efficient mass-rearing techniques at competitive costs [2]. The role of tachinid parasitoids in applied biological control programs has been sometimes underestimated; they receive less attention than hymenopteran parasitoids, on which more research has been carried out [9].

Table 1. Acceptance and suitability of C. perspectalis and G. mellonella for the parasitoid E. larvarum: accepted larvae (%) based on moth larvae exposed to parasitoids, E. larvarum eggs/accepted larva, E. larvarum puparia (%) based on parasitoid eggs laid on larvae, adults (%) based on parasitoid puparia

Host species	Accepted larvae (%)	*E. larvarum* eggs/ accepted larva (n.)	*E. larvarum* puparia (%)	*E.larvarum* adults (%)
C. perspectalis	86.1 ± 11.1	4.2 ± 2.6	0	0
G. mellonella	100	7.3 ± 1.8	15.5 ± 5.4	100

The members of Tachinidae can be applied in classical and augmentative biological control programs. Yet, the potential of these beneficial enemies as antagonist of target insect pests is still underestimated and misunderstood. Difficulties may arise from the scarcity of knowledge about the biology, behavior and ecology of both the tachinids to be used as biological control agents and the target insect pests. Also, the rearing techniques and shipping methods of tachinids flies are relatively poorly known in comparison with hymenopteran parasitoids [9].

Exorista larvarum is a good candidate for biocontrol against forest Lepidoptera defoliators. One of the most important target pest is L. dispar (native in Eurasia), impairing deciduous and evergreen species. This defoliator damages more than 300 species of trees. The pest was introduced from Europe into the USA in the end of the 19th century [17]. To date, however, E. larvarum has been used as a biological control agent against L. dispar only in inoculative releases in the northern United States, where it has become established [18]. The potential of *E. larvarum* as an antagonist of forest and agricultural insect pests deserves to be better exploited. This parasitoid has positive features, among which, it can be efficiently reared in vivo and in vitro (on artificial media, without the host) too [9].The results of laboratory studies are encouraging regarding the possible augmentative releases of *E. larvarum* against populations of agricultural interest, such as *Spodoptera littoralis or Mithymna unipuncta*, and also such species as *Agrotis segetum, Prodenia litura, Mamestra brassicae, G. mellolnella, C. perspectalis* [6, 7, 11, 19].

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