Evaluation of parasitism and predation of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) by *Diglyphus isaea* (Walker, 1838) (Hymenoptera: Eulophidae)

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Abstract

To evaluate an eventual parasitism and/or predation behaviour of the eulophid parasitoid *Diglyphus isaea* (Walker, 1838) on *Tuta absoluta* (Meyrick, 1917) larvae two assays were performed releasing adult parasitoids. The first assay was conducted in Petri dishes using tomato leaflets havig mines with *T. absoluta* larvae inside. The second, was performed on previously infested potted tomato plants. In both assays no parasitism but only predation was found. Predation was observed, mainly on L_2 and L_3 larvae which had moulted. Proportion of clearly preyed larvae reached 17% in Petri dishes and 38% in potted plants. Predation was attested by the presence of punctures done by the oviscapt of the female parasitoid. A significant higher proportion of dead larvae where it was not possible to see punctures occurred in the presence of the parasitoid females. Some of them probably were also preyed on by *D. isaea* females.

KEY WORDS: Lepidoptera, Gelechiidae, Hymenoptera, Eulophidae, South American tomato moth, predation, host feeding or exploitation punctures, biological control.

Evaluación del parasitismo y depredación de *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) por *Diglyphus isaea* (Walker, 1838) (Hymenoptera: Eulophidae)

Resumen

Se realizaron dos ensayos para evaluar un eventual comportamiento de parasitismo y/o depredación del parásito eulófido *Diglyphus isaea* (Walker, 1838) en orugas de *Tuta absoluta* (Meyrick, 1917). El primero se llevó a cabo en placas de Petri con hojas de tomate conteniendo minas con orugas de *T. absoluta* y el segundo utilizando plántulas de tomate previamente infestado en maceta. No se ha observado parasitismo pero se verificó depredación en ambos ensayos, especialmente en larvas L_2 y L_3 que han sufrido muda. La proporción de orugas que mostraron claramente señales de depredación (presencia de picaduras hechas con el oviscapto de las hembras) alcanzó el 17% en placas de Petri y el 38% en plántulas. Una proporción significativamente mayor de larvas muertas sin que haya sido posible encontrar picaduras, se observó en presencia de hembras del parásito. Probablemente, algunas de estas larvas fueran también depredadas por hembras del parásito.

PALABRAS CLAVE: Lepidoptera, Gelechiidae, Hymenoptera, Eulophidae, polilla del tomate, depredación, picaduras de alimentación o exploración, lucha biológica.

Introduction

Tuta absoluta (Meyrick, 1917) is a Neotropical microlepidopteran. In Europe, it was detected for the first time at the end of 2006, in Castellón de la Plana, Spain (EPPO, 2008) and since then it spread very rapidly along the Mediterranean Basin and in Central Europe and the Middle East

(DESNEUX *et al.*, 2010, 2011). In Portugal, it was reported only in May 2009, in the South (Algarve), and in July 2009, in the Oeste region (ANONYMOUS, 2009; FIGUEIREDO *et al.*, 2010) but there are records of this species damaging greenhouse tomatoes in the South of Lisbon, in April 2009 (MATOS, 2011). It is a key-pest in protected and processing tomato crops, causing leaf mines, galleries in the fruits and stems, and damages that can reach 100% of production (ANONYMOUS, 2009; DESNEUX *et al.*, 2010). Besides tomato, this species attacks all cultivated solanacean species (potato, eggplant, tobacco, groundcherries), and weeds (*Solanum nigrum* L., *Solanum elaeagnifolium* Cav., *Solanum americanum* Mill., *Datura stramonium* L., *Datura ferox* L. and *Nicotiana glauca* Graham (EPPO, 2005; ROBLEDO-CAMACHO *et al.*, 2009; PAYER & FIGUEIREDO, data not published). Green beans (Fabaceae) (ANONYMOUS, 2009; MONSERRAT, 2009) and *Chenopodium murale* L. (Amaranthaceae) (ROBLEDO-CAMACHO *et al.*, 2009) can also be attacked.

The Nearctic *Liriomyza trifolli* (Burgess, 1880) and the Neotropical *L. huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae) were reported in Portugal on protected crops since the middle 80's (SEYMOUR *et al.,* 1986) and 1990-91 (MEXIA & GODINHO, 1993), respectively. Native parasitoid species that naturally parasitized already existing agromyzids, such as *L. strigata* (Meigen, 1830), *L. bryoniae* (Kaltenbach, 1858), *Chromatomyia horticola* (Goureau, 1851) and *Ch. syngenesiae* Hardy, 1849, rapidly adopted these recently available pests as new hosts. Within these species there were four species of *Diglyphus* Walker, 1844 wasps (Hymenoptera: Eulophidae): *D. crassinervis* Erdos, 1958, *D. isaea* (Walker, 1838), *D. mineus* (Walker, 1838) and *D. poppoea* Walker, 1848 (GODINHO & MEXIA, 2000). They are all solitary ectoparasitoids and highly polyphagous. *Diglyphus isaea* is utilized worldwide as a biological control agent against pests of the genera *Liriomyza* Mik, 1894, *Phytomyza* Fallen, 1810 and *Chromatomyia* Hardy, 1849 (BENE, 1990; LaSALLE & PARRELLA, 1991; van LENTEREN, 2012). Nevertheless, *D. isaea* was reported parasitizing also Tephritidae species and microlepidoptera species of the Lepidoptera families Gracillariidae, Lyonetiidae and Nepticulidae (LaSALLE & PARRELLA, 1991; FISHER *et al.*, 2005). AGUIAR (1999) and MASSA *et al.* (2001) observed *D. isaea* parasitizing *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae), in citrus, in Madeira, Portugal and in Sicily, Italy, respectively.

Besides the mortality induced by larval parasitic activity, *D. isaea* can also cause host mortality by the adult activity of host feeding. Females of the parasitoid sting host larvae (normally, 1st and 2nd instar larvae) with their ovipositor, feed on the body fluids that come out and kill them (MINKENBERG, 1989; BENUZZI & RABONI, 1992; NICOLI & PITRELLI, 1993; ONILLON, 1999). This behaviour, known as "host-feeding", is necessary to egg production since this species is synovigenic (MALAIS & RAVENSBERG, 1996). In this case, the host feeding behaviour is dependent on the quantity of eggs present inside the female: a female with a high quantity of mature eggs can gain more from ovipositing while a female with immature eggs or which has laid many of her eggs can gain more from host-feeding (JERVIS & KIDD, 1996). The developmental stage of the host larvae influences also which of these two behaviours is chosen by the female parasitoid (BENUZZI & RABONI, 1992).

New associations between exotic pests and native natural enemies often occur in a spontaneous way (NICOLI, 1997) when pesticide pressure is low, making the importation of new species for their control unnecessary and avoiding possible side-effects from the importation and release of exotic species. In the case of *T. absoluta*, the native predators *Macrolophus pygmaeus* (Rambur, 1839) and *Nesidiocoris tenuis* (Reuter, 1895) became rapidly adapted to this new prey (e.g. URBANEJA *et al.*, 2009; DESNEUX *et al.*, 2010; MOLLÁ *et al.*, 2010). In the Oeste region, Portugal, the mirids *N. tenuis* were also seen feeding on *T. absoluta* (Sofia Rodrigues, pers. comm.) and probably the same is done by *Dicyphus umbertae* Sánchez & Cassis, 2006 often observed on the tomato plants (FIGUEIREDO *et al.*, 2012). More slowly, native parasitoids, such as the idiobiont *Necremnus artynes* (Walker, 1839) (Hymenoptera: Eulophidae) and other eulophid, ichneumonid and braconid species have been observed parasitizing *T. absoluta* in regions where this pest has been established for longer periods (DESNEUX *et al.*, 2010; MOLLÁ *et al.*, 2010; ZAPALLÀ *et al.,* 2012)*.* In this study, *T. absoluta* was evaluated as a

potential host for *D. isaea* since this parasitoid is able to parasitize leafminer larvae of Gracillariidae, and both Gelechiidae and Gracillariidae are related families.

Material and methods

In the first assay (Assay 1) the predation/parasitism behaviour was evaluated in Petri dishes. Tomato leaflets infested with 1st and 2nd instar larvae of *Tuta absoluta* were collected in the greenhouses (Silveira, Torres Vedras, Portugal). No phytosanitary treatments had been made in the fortnight previous to picking up the leaflets. These leaflets presented larvae in different instars. The leaflets were inspected: other insects were removed, larvae were observed under backlight to verify their developmental stage and whether they were healthy. A filter paper, one to three of these leaflets with healthy *T. absoluta* larvae, two adults of *D. isaea* from Biobest Biological Systems and a small card (about 2 cm) with droplets of water-diluted honey (1:1) to feed the parasitoids, were put together in each of 40 Petri dishes (9 cm diameter). The Petri dishes were sealed with Parafilm® and maintained at 25.0±1.0º C, 80±10% relative humidity (RH) and photoperiod 16h L: 8h D, in an acclimatized rearing chamber (Aralab Fitoclima S600PLH). Two days later, the parasitoids were removed, killed and observed for sex determination (males present one yellow fringe in the centre of the tibia whereas females have tibias entirely dark green (BEITIA *et al.*, 1999) and females are, in general, larger than males). Fresh and non-infested leaflets were put in the dishes when necessary to allow the larvae to have enough mesophyll. A final checkup of healthy, parasitized and preyed larvae was done on the 7th day under stereoscopic microscope. Predation was assumed when feeding punctures were observed on the larvae. Petri dishes with two males were used as control.

Afterwards, in Assay 2, evaluation of predation was made on small potted tomato plants using a methodology adapted from HAGHANI *et al*. (2007). Three plants were put in each transparent acrylic cage of about 4 L (30 x 30 x 40 cm³), with mesh covering one side and holes in the top. *Tuta absoluta* was reared in laboratory on tomato plants in acrylic and mesh cages. Previously to the experiments, moth adults were allowed to lay eggs on tomato plants and those plants infested with 1st and 2nd instar larvae were selected for the assay. In each assay cage four adults of *D. isaea* from Biobest Biological Systems were released. A small card with water diluted honey (1:1) was put in each cage to feed the parasitoids. For control we used two similar cages where no parasitoids were released. Cages were maintained at 24.0 ± 1.0º C, 55 ± 5% RH and a 16h L: 8h D photoperiod, in an adapted storage shelf rearing facility, for seven days. Then, the larvae were inspected for signs of parasitism or predation under a stereoscopic microscope.

In both cases (Petri dish and plant assays) the parasitoids were kept at 10º C until use. Parasitoid age was not controlled since they were acquired in the same way the growers do. In both cases, they were used in the assays before the date indicated on the tube label as their "expiration date".

Both assays were performed at the laboratory *Insectário,* at the Instituto Superior de Agronomia (Universidade de Lisboa).

Statistical analysis for the Petri dishes assay (Assay 1) was performed by the non-parametric Kruskal-Wallis test because normality of residuals and homoscedasticity were not achieved even after angular transformation (or arcsine square root) of proportions; for the plant assay (Assay 2) an ANOVA was performed after angular transformation of data. These analyses were performed using IBM SPSS Statistics (version 20.0).

Results and discussion

In both assays no parasitism was observed. However, a behaviour designated in this study as predation was observed. That can consist of either host feeding, or probing and rejection for oviposition, or both. This predation behaviour was verified by the presence of punctures. Some larvae presented more than one puncture. Melanised tissue bordered the hole in most of the cases, revealing an attempt to heal the wound. It is important to note that the adults of the parasitoids had honey to feed on

inside the dishes but even so the females punctured some *T. absoluta* larvae probably due to protein need to produce their eggs (MALAIS & RAVENSBERG, 1996) or in an attempt to lay eggs.

Besides the high number of larvae on which these punctures were seen no reason was associated with the death of some dead larvae. The majority of these too dark, mushy and squeezed out to find eventual holes. Consequently, the predation rate could be under evaluated. This is highlighted by the higher proportion of deaths for unknown reasons in the dishes where one or two female parasitoids were released compared with the control dishes (Table 1).

Predation was detected mainly on 2nd and 3rd instar larvae. The majority of the larvae with visible holes moulted to 3rd instar during the assay. Probably, lower sclerotization and lower mobility presented by the *T. absoluta* larvae during pre-moult and immediately after moulting facilitate the attack. Larvae that pupate were probably 3rd or 4th instar at the beginning of the assay. Using this information, the proportion of larvae that were preyed on that died during the assay in relation to those that would be more able to be attacked (L_2 and L_3) in the assay was calculated for each dish [number of preyed or death / (number of larvae present in the dish - number of pupae at the end of the assay)] (Table 1).

In one dish with two males of *D. isaea* one larva of *T. absoluta* was found to be preyed on. This

Table 1.– Mortality of *Tuta absoluta* larvae by *Diglyphus isaea* predation and total mortality in the Petri dishes assay (Assay 1).

D. isaea females (number)[1]	N[2]	*Tuta absoluta* larvae (mean per dish[3])	*Tuta absoluta* L_2+L_3 larvae (mean per dish[3])	Predation rate (%) (mean[3,4])	Predation rate on L_2+L_3 (%) (mean[3,4])	Death by unknown causes (%) (mean[3,4])
0	10	6.8±1.6	2.6±0.6	3.3 ± 3.3 a	6.2 ± 6.2 a	2.2±1.7 a
1	24	9.2±0.9	5.5±0.8	17.1 ± 2.3 b	30.1 ± 4.4 b	16.4±2.9 b
2	6	7.7±1.5	4.5±1.2	15.9 ± 5.4 ab	26.1 ± 8.6 ab	12.0±4.3 ab

[1,] Number of females in the pair of parasitoids released in the Petri dish; [2,] Number of replications (number of Petri dishes); [3,] Mean and standard error of the mean; [4,] Means followed by different letters in the same column correspond to medians which differ significantly at p= 0.05.

larva had probably been attached in the field, possibly by mirids that were very abundant in the greenhouses, despite having verified that the larvae were healthy prior to performing the assay. However, all the modalities were performed in similar conditions.

The proportions of larvae preyed upon (predation rate) and of L_2+L_3 death by predation and by unknown causes in the presence of one female of *D. isaea* were significantly different from the control (respectively: H=8.578, p=0.014; H=6.829, p=0.033; H=9.276, p=0.010; for all the Kruskal-Wallis tests: N=40, d.f.=2). No differences were found when releasing one and two *D. isaea* females for both mortalities (Table 1). The lower number of replications with two female parasitoids released and the associated higher variance can be the reason for not detecting significant differences between this modality and the control.

In the plant assay (Assay 2) no parasitism was found either, but in the cages where *D. isaea* was released a high proportion of larvae were preyed on (mean ± SE: 38 ±15%) or death by unknown cause (mean ± SE: 55±12%); total mortality was higher than 70% in all these cages (mean ± SE: 93±5%). Highly significant differences were found between mortality in *D. isaea* cages and the control (t= 58.748; d.f.= 1, 5; p=0.001).

All the larvae that showed punctures died. Some of them presented more than one hole. In the control, all the *T. absoluta* larvae present on the plants were alive and healthy; none presented holes. Since, no other insects were present in the cages besides *T. absoluta* and *D. isaea* and no larvae died in

the controls, the majority of the larvae that died from an unknown cause can be attributed to predation by *D. isaea* females.

Mines where larvae with punctures were found were more sinusoidal and narrower than the mines with healthy larvae from the same instar.

It is known that some parasitoids may kill more host individuals by host-feeding than by parasitism (JERVIS & KIDD, 1996). This feeding behaviour of *D. isaea* has been known for a long time and its implication for leafminer biological control is being studied at least since the 90's (BENUZZI & RABONI, 1992; FRANCO & BAUDRY, 1993; NICOLI & PITRELLI, 1993, MARQUES, 1996; ONILLON, 1999). The punctures on *T. absoluta* here described occurred even in the presence of honey droplets and they could have been caused by host feeding or by probing and rejection to oviposit. Since all punctured larvae died and appeared to be squeezed and no live larvae showed punctures it is possible that extensive depletion of fluids took place and so feeding could be the reason for the punctures. In any case, this behaviour may represent a first adaptation to the availability of this pest.

It is necessary to note that the parasitoids used were mass produced and had no previous contact with *T. absoluta*. Subsequently, evaluation should be made with field parasitoids, collected in the tomato greenhouses where *T. absoluta* is an important pest and consequently, an easily available host. LARA *et al.* (2010) found some *D. isaea* adults emerging from tomato leaves infested with *Tuta absoluta* collected in greenhouses with low leafminer populations leading to the hypothesis that this adaptation can actually be occurring in agroecosystems where this parasitoid is naturally present.

Conclusion

Females of *D. isaea* are able to predate larvae of *T. absoluta* but apparently they do not use this species as a host to parasitize. Nevertheless, studies with naturally occurring populations should be performed in order to evaluate if any adaption in this behaviour is going on in nature.

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