

Performance of *Pseudapanteles dignus* (Hymenoptera: Braconidae), a Natural Enemy of *Tuta absoluta* (Lepidoptera: Gelechiidae) in Eggplant

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Abstract

Pseudapanteles dignus (Muesebeck; Hymenoptera: Braconidae) is an American endoparasitoid that attacks the South American tomato leafminer *Tuta absoluta* (Meyrick; Lepidoptera: Gelechiidae). The interaction between *P. dignus* and *T. absoluta* in tomato (*Solanum lycopersicum* L.; Solanales: Solanaceae) crops has demonstrated that this enemy exhibits some desirable ecological traits as an effective biological control agent of this pest. With the aim of extending the use of *P. dignus* to other solanaceous crops, laboratory experiments were carried out to assess some life history traits and the parasitism efficiency when parasitizing *T. absoluta* larvae fed on eggplant (*Solanum melongena* L.; Solanales: Solanaceae). Rearings and experiments were conducted at $25 \pm 2^\circ\text{C}$, $\approx 70\%$ RH, and 14:10 (L:D) h photoperiod, and *T. absoluta* was fed with eggplant. *P. dignus* developmental times of immature stages were lower (~ 5 d) on *S. melongena* than on *S. lycopersicum*. The female did not exhibit a pre-reproductive period, and its oviposition period lasted longer (~ 4 d) than that determined in tomato plants. Adult longevity was ca. 24 d for both sexes. Females produced ca. 61 cocoons during their lives and the maximum daily percentage of parasitism was 50% at the first day of adult emergence. Functional response of *P. dignus* on eggplant was density-independent of the host density offered, as in tomato plant, and the instantaneous attack rate (a') was 0.24 attacked larvae/available larvae, in 24 h. Our results indicate that although there are differences, *P. dignus* would have a similar performance in eggplant and tomato in terms of its efficacy in the control of *T. absoluta*.

Key words: South American tomato leafminer, Solanaceae, parasitoid, biocontrol agent, *Solanum melongena*

The tomato leafminer *Tuta absoluta* (Meyrick; Lepidoptera: Gelechiidae) native to the South America has been reported as causing significant economic losses to tomato (*Solanum lycopersicum* L.; Solanales: Solanaceae) production in many countries in Europe, Africa and Asia (Parra and Zuchi 2004, Desneux et al. 2011, Luna et al. 2015, Ponti et al. 2015). Its spread is also threatening North American crops (USDA 2011). Besides tomato, *T. absoluta* feeds on other host plants from the Solanaceae family, including crop and non-crop species (Galarza 1984, Estay 2000, Desneux et al. 2010, USDA 2011, Bawin et al. 2015, Salas Gervassio et al. 2016). Particularly, after its initial detection in tomato crops in Spain in 2006 (Urbaneja et al. 2007), it has been found causing economic damage in eggplant (*Solanum melongena* L.; Solanales: Solanaceae) crops in southern Italy (Speranza et al. 2009, Tropea Garzia et al. 2009). Some years later, in Africa, Mohamed et al. (2012) reported severe losses in *S. melongena* crops of Sudan, and El Arnaouty and Kortam (2012) recorded its presence in plantations in Egypt.

Studies carried out in Argentina demonstrated that *T. absoluta* is attacked by a diverse complex of natural enemies in tomato crops (Colomo et al. 2002; Sánchez et al. 2009; Luna et al. 2015; Savino et al. 2012, 2016), being *Pseudapanteles dignus* (Muesebeck; Hymenoptera: Braconidae) the main larval parasitoid (Luna et al. 2007). More recently, Salas Gervassio et al. (2016) found that *T. absoluta* populations can also reach up to 33% of natural parasitism in protected eggplant crops in northeastern Buenos Aires province, Argentina.

P. dignus is a solitary koinobiont endoparasitoid. The parasitoid female is able to attack all *T. absoluta* larval instars but exhibits better performance when parasitizing mid-instar larvae. After completing its development, the larva leaves the dead host and the pupa is covered by a white cocoon. This parasitoid species has been reported parasitizing few gelechiid species in the Americas (Cardona and Oatman 1971, Fernández-Triana et al. 2014). Research on the *P. dignus*-*T. absoluta* interaction in tomato crops has demonstrated that the parasitoid has some desirable ecological traits to be

considered an effective biocontrol agent of *T. absoluta* in open-field and protected tomato crops (Luna et al. 2007, Sánchez et al. 2009, Nieves et al. 2015). Among these, it shows seasonal synchronization with host populations, aggregative response to host density, and an instantaneous attack rate greater than the intrinsic growth rate of the host.

Although it is known that *P. dignus* establishes a persistent interaction with *T. absoluta* in eggplant (Salas Gervassio et al. 2016), similar to that registered in tomato by Nieves et al. (2015), more information is necessary to confirm the potential of this parasitoid for the biological control of *T. absoluta* on eggplant crops.

The objective of this study was to determine some life history traits of *P. dignus* and its parasitism efficiency against *T. absoluta* on eggplant. We assessed developmental times of immature stages, adult longevity, daily parasitism rate, and functional response under laboratory conditions.

Materials and Methods

Source of Plants and Insects

T. absoluta and *P. dignus* were originally collected from commercial eggplant organic crops (var. Barcelona F₁) in northern Buenos Aires province, Argentina. Colonies of both species were maintained in a walk-in growth chamber at 25 ± 2°C, ≈70% RH, and 14:10 (L:D) h photoperiod. Individuals used in this study were F₅ progeny of founder colonies. Seedlings (var. Barcelona F₁), grown in 3-liter plastic pots with peat and perlite, and fertilized every 15 d (Nitrofoska foliar PS COMPO: www.compo-expert.com, Santiago, Chile) (1.5 mg), were used to feed *T. absoluta* in our colony and in the trials conducted in this study. Plants were held in a pesticide-free greenhouse, and to avoid their infestation with any possible insects present in the environment, they were placed inside BIOQUIP (Rancho Dominguez, CA) meshed cages (35 cm in width, 35 cm in length, 60 cm in height) until seedlings reached approximately 20 cm of height. *T. absoluta* adults were kept in voile- meshed cages (40 × 40 × 40 cm) with potted eggplant seedlings as substrate for oviposition, and fed honey solution (*ad libitum*). Seedlings were replaced every 2 d, their leaves were removed and placed in plastic boxes (13 cm in width, 18 cm in length, 5 cm in height) covered at the top with voile, to rear the larvae until pupae formation. The obtained pupae were incorporated into the colony. *P. dignus* adults were placed in plastic containers (22 cm in diameter, 25 cm in height) and provided with eggplant seedlings infested with *T. absoluta* larvae for parasitization. After 48 h of exposure, seedlings were removed and their leaves were placed in plastic boxes as described above, to rear the larvae until host pupae or parasitoid cocoon were formed. *T. absoluta* pupae were incorporated into the colony and *P. dignus* cocoons were either stocked individually in Petri dishes until adult emergence and then added into the colony or used in the trials.

Developmental Time of Immature Stages and Adult Longevity

P. dignus life-history attributes when parasitizing *T. absoluta* in tomato plants were determined elsewhere (Luna et al. 2007, Nieves et al. 2015). To assess differences in life parameters when the pest attacks eggplant, an experiment was set up. Newly emerged adult wasps were selected from the above-mentioned colony (one female × ≈ five males) and observed until mating in plastic mating chambers (8 cm diameter, 10 cm height), which occurred within the first 5 h of observation. After this, each female ($n = 12$) was individually transferred into a plastic container (22 cm in diameter, 25 cm in height).

Each female was offered one eggplant seedling 20-cm height, previously infested with 20 *T. absoluta* third-instar larvae, and supplied with honey droplets and water *ad libitum*. After 24 h of exposure, *T. absoluta* infested leaves from each experimental unit were placed into plastic boxes as described above, and larvae were fed every 72 h by supplying new eggplant leaves. Boxes were put inside Ziploc bags to prevent larval escape. Daily, the units were checked to register host larvae mortality, and formation of *T. absoluta* pupae or *P. dignus* cocoons, using a stereoscopic microscope (SMZ 1270, Nikon Corporation, Tokyo, Japan). Parasitoid cocoons were individually stored in Petri dishes, and monitored daily until adult emergence for sex determination.

The developmental times from egg to pupa and from pupa to adult of *P. dignus* were determined by counting the total number of days from the time of parasitization until cocoon formation, and then from cocoon formation until adult emergence.

Newly emerged adults of both sexes were used to assess their longevity. To determine female longevity the following aspects were taken into account: 1) the better performance when *P. dignus* parasitizes mid-instar host larvae; and 2) parasitoid female life span is influenced -among other factors- by the availability of hosts throughout its life (Quicke 1997). Then, in this experiment, 2nd and 3rd instars of *T. absoluta* larvae were provided to wasps *ad libitum*. For estimating male longevity, individuals were only fed with honey and water. Individuals of both sexes were monitored until death.

Differences in immature development times and adult longevity data (mean ± SEM), between sexes, were compared using Student's *t*-tests with a significance value of $P < 0.05$, by using the Bipartite package in the R environment for statistical computing (R Development Core Team 2014).

Daily Parasitism Rate

Newly emerged *P. dignus* adults were selected from the colony and allowed to mate by grouping one female × ≈ five males in individual mating chambers. Then, mated females ($n = 10$) were individually transferred into plastic containers (22 cm in diameter, 25 cm in height). As females do not show pre-oviposition period (Nieves et al. 2015), a potted eggplant seedling containing approximately 15 *T. absoluta* larvae—2nd and 3rd instars, already installed in mines—was offered daily for parasitization, from the first day of adult emergence until its death. Adult females were daily provided with honey droplets and water *ad libitum*. Each day, infested seedling leaves were removed, placed into plastic boxes, and checked every 48 h to record the formation of moth pupae or parasitoid cocoons. Leafminers were fed with fresh eggplant leaves from the greenhouse.

The following biological parameters were determined: 1) The reproductive period: the mean (±SEM) number of days a *P. dignus* female parasitized hosts; 2) The daily parasitism rate per female, calculated as the mean (±SEM) number of cocoons obtained in relation to the number of hosts offered; and, 3) The sex ratio of the offspring: the proportion of females emerged (number of females/number of females + males).

Functional Response

Newly emerged (less than 3-d-old) adults of both sexes from the colony were grouped as described above, to allow mating. Next, females were immediately transferred to plastic containers (22 cm in diameter, 25 cm in height) and offered hosts at five densities: 3, 7, 10, 15, 20, or 25 second and third instar *T. absoluta* larvae, installed in potted eggplant seedlings. The range of densities tested in this trial was selected according to a previous study on *P. dignus* functional

response in tomato (Luna et al. 2007), and also considering the population parameters registered for this parasitoid (Nieves et al. 2015). Ten replicates were carried out for each host density. The experiment lasted 24 h, and then, infested leaves with exposed larvae were removed and disposed in labeled plastic boxes with fresh eggplant leaves until *T. absoluta* pupae or *P. dignus* cocoon formation.

Number (N_p) and proportion (N_p/N_0) of parasitized hosts at each host density offered (N_0) were calculated to determine type I, II and III functional response curves, described by Holling (1959). For doing so, the logistic model was fit to the data through stepwise nonlinear regressions between the proportion of parasitized hosts and host density (Trexler et al. 1988, Juliano 2001, Fernández Arhex and Corley 2004, Luna et al. 2007).

The significance of coefficients was calculated using the maximum likelihood method Statistical program R (R Development Core Team 2014).

Since both the quadratic and cubic expressions were not significant, the instantaneous parasitism rate (a') (*sensu* the 'attack rate' by Holling 1959) of the parasitoid, i.e., the proportion of host parasitized by time unit, was calculated by linear regression using the following equation:

$$N_p = a'TN_0$$

where: T is the time that hosts were exposed to the parasitoid female (24 h).

Results

Developmental Times of Immature Stages and Adult Longevity

P. dignus developmental times for females and males assessed on the *T. absoluta*-eggplant system are shown in Table 1. There were not significant differences between sexes (egg-pupa: $t = 0.09$; $df = 148$, $P = 0.92$; pupa-adult: $t = 0.02$; $df = 148$, $P = 0.98$; egg-adult: $t = 0.08$; $df = 148$, $P = 0.93$). Adult longevity did not differ between sexes; on average it was of ca. 24 d ($t = 1.18$; $df = 48$, $P = 0.24$).

Daily Parasitism Rate

Females did not show a pre-oviposition period and usually did not exhibit a post-oviposition period, laying eggs until their death. The reproductive period lasted 18.5 ± 0.5 d. The maximum daily percentage of parasitism was 50% and it was reached at the first day of female adult emergence, then decreased and remained fairly constant until the 20th day (Fig. 1). *P. dignus* females produced 61.3 ± 5.23 cocoons. The sex ratio was biased towards females (0.7).

Table 1. Life history attributes of *P. dignus* when parasitizing *T. absoluta* larvae, fed with eggplant (*Solanum melongena* L.) leaves, assessed under laboratory conditions ($25 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH, 14:10 [L:D] h)

Attributes	Females	n	Males	n	t -test
Developmental time					
Mean \pm SEM (d)		64		86	
Egg-pupa	15.56 ± 0.30		15.38 ± 0.25		ns
Pupa-adult	12.33 ± 0.23		12.32 ± 0.19		ns
Egg-adult	27.83 ± 0.37		27.51 ± 0.35		ns
Adult longevity					
Mean \pm SEM (d)	24.81 ± 1.51	32	23.33 ± 2.58	18	ns

ns: not significant differences at $P < 0.05$.

Functional Response

The number of *T. absoluta* larvae parasitized by *P. dignus* increased linearly with host density and it was highly variable at all densities (Fig. 2). The maximum value of *T. absoluta* larvae attacked by a single *P. dignus* female in 24 h was 10, at a density level of 25 hosts. The proportion of parasitized *T. absoluta* larvae was also highly variable and independent of the host density tested (Fig. 3). Logistic regression parameters showed a Type I functional response, since cubic and quadratic coefficients (Type II and III, respectively) were not significant (Table 2). Linear regression parameters were: $y = 0.22 + 0.24x$; $r^2 = 0.4$; $F = 46.35$; $df = 1, 58$; $P < 0.001$, indicating an instantaneous attack rate (a') of 0.24 attacked larvae/available larvae, in 24 h.

Discussion

In this paper we report for the first time some biological attributes of the solitary larval endoparasitoid *P. dignus*, when parasitizing the South American tomato moth in eggplant. We emphasize those traits having implications for the biological control of this pest in horticultural greenhouse crops. Considering that this parasitoid is being evaluated as a biocontrol agent of *T. absoluta* in tomato, to be used by conservation or augmentative methods (Luna et al. 2007,

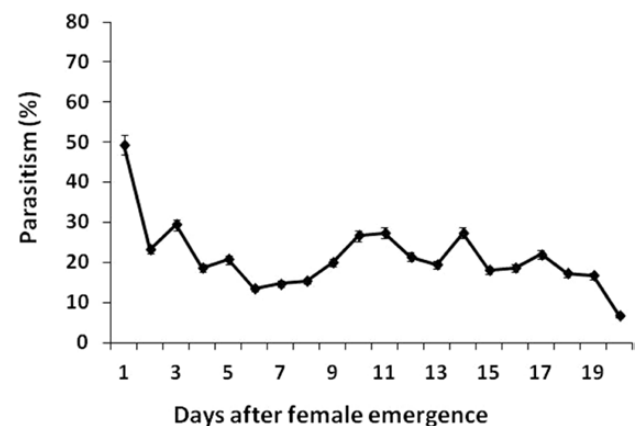


Fig. 1. Daily parasitism rate (mean \pm SE) over the lifetime of *P. dignus* females attacking *T. absoluta* larvae in eggplant.

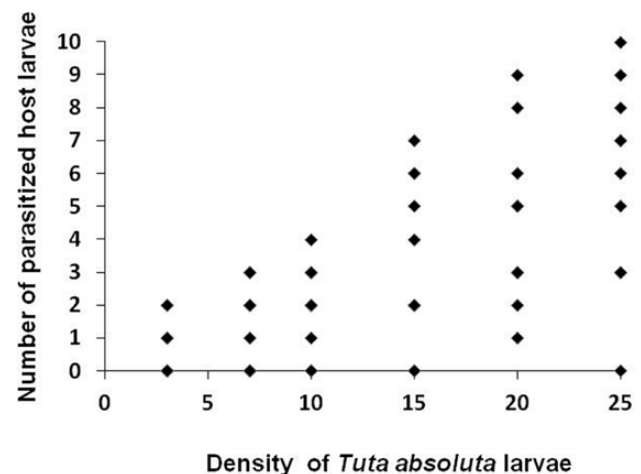


Fig. 2. Relationship between the number of *T. absoluta* larvae parasitized by *P. dignus*, at different host densities offered.

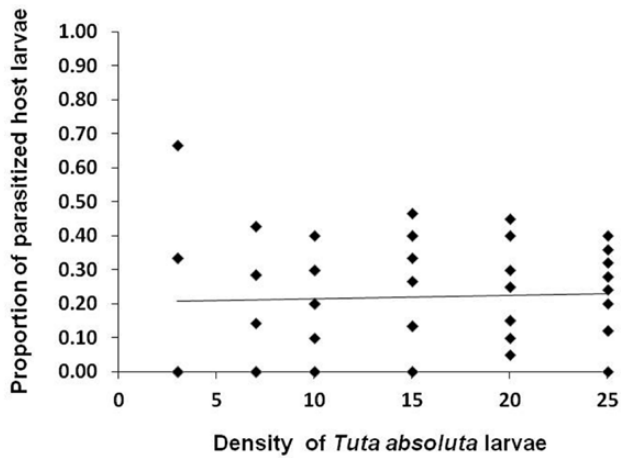


Fig. 3. Type I functional response of *P. dignus* at different densities of *T. absoluta* larvae. The equation is shown in the text.

Sánchez et al. 2009, Nieves et al. 2015, Salas Gervassio et al. 2016), results of the present study contributes new knowledge to promote its manipulation in other solanaceous crops.

When comparing developmental times of *P. dignus* with those recorded in tomato (Nieves et al. 2015), it can be observed that from egg to adult emergence it took ≈ 5 more days, while adult longevity was slightly shorter for females (≈ 2 d), but similar to males when parasitizing *T. absoluta* on eggplant.

In relation to the reproductive period, it was longer than that observed in tomato (≈ 4 d) (Luna et al. 2007, Nieves et al. 2015), with a higher cocoon production than that reported by Luna et al. (2007) (32 cocoons per female) in tomato. However, it should be noted that the lower number of hosts offered to the parasite in the tomato experiment could have limited, at least in part, the rate of female oviposition.

The parasitism rate of *P. dignus* found in this study appears to be quite important when compared to that of other *T. absoluta* larval parasitoids. Biondi et al. (2013) reported for the female of the larval ectoparasitoid *Bracon nigricans* (Szépligeti; Hymenoptera: Braconidae), had a much lower performance than that of *P. dignus*. This common natural enemy of *T. absoluta* in European crops produced an average of 29.8 offspring, and reached a maximum weekly parasitism rate of 1.8 (± 0.09). However, *B. nigricans* could be considered a suitable biological control agent since it causes high mortality in the host population (70% of available larvae) due to high rates of nonreproductive killing behavior (host-feeding).

Other braconid species have been pointed out as potential agents for biological control but with a lower performance than *P. dignus*. For example, *Spathius galinae* (Belokobylskij and Strazhenac; Hymenoptera: Braconidae), is a larval parasitoid of the emerald ash borer *Agrilus plannipennis* (Fairmaire; Coleoptera: Buprestidae), and it is currently being evaluated for field releases in the United States to control this buprestid (Gould and Duan 2013, Duan et al. 2014). The female of *S. galinae* produced an average of 47 (± 5.3) eggs throughout its lifespan and weekly parasitism rates ranged from 39% to less than 25%.

The female biased sex ratio was similar to that obtained by Luna et al. (2007) in tomato, while Cardona and Oatman (1971) found a sex ratio deviated to males when *P. dignus* is reared on other gelechiid, *Keiferia lycopersicella* (Walsingham; Lepidoptera: Gelechiidae), under laboratory conditions. A female-biased sex ratio is considered a beneficial trait for hymenopteran biocontrol agents, since females are

Table 2. Results of the logistic regression analyses of the proportion of *T. absoluta* larvae parasitized by *P. dignus* in relation to different host densities (3, 7, 10, 15, 20, or 25 second- to third-instar larvae) offered in potted eggplant seedlings, during a 24-h experiment

Parameter	Estimator	Standard error	Wald	P
Cubic expression				
Intercept	-1.07	0.89	-1.2	0.23
N_0	-0.08	0.22	-0.39	0.69
N_0^2	0.007	0.015	0.45	0.65
N_0^3	-0.0001	0.0003	-0.45	0.65
Quadratic expression				
Intercept	-1.41	0.5	-2.8	0.0005
N_0	0.007	0.07	0.10	0.92
N_0^2	0.00007	0.002	0.03	0.97
Linear expression				
Intercept	-1.42	0.24	-5.82	5.7 e-09
N_0	0.009	0.01	0.72	0.47

responsible of attacking hosts (Wajnberg et al. 2008). Besides, an offspring deviated to females can be another advantageous condition for mass rearing and the use of *P. dignus* through augmentative releases, as reported for many arrhenotokous braconid species (Green et al. 1982, Luck et al. 1999, Dannon et al. 2010). It could therefore be suggested that the highest proportion of females in *P. dignus* offspring exhibited in eggplant—as in tomato—contributes to the performance of this parasitoid as a biocontrol agent of *T. absoluta*.

The type-I functional response of *P. dignus* displayed when attacking *T. absoluta* in eggplant is coincident with that found in tomato, and the attack rates were similar in both solanaceous plants (Luna et al. 2007). Coincidentally, Savino et al. (2012) reported that *Dineulophus phthorimaeae* (de Santis; Hymenoptera: Eulophidae), a larval ectoparasitoid coexisting with *P. dignus* in tomato and eggplant crops in the main horticultural regions of Argentina (Salas Gervassio et al. 2016), exhibited a type-I functional response and a similar attack rate ($\alpha' = 0.22$) to *P. dignus*.

P. dignus showed density independent parasitism in the solanaceous species studied, and this attribute could be explained by its foraging behavior. As it was discussed in Luna et al. (2007), we suggested that *P. dignus* behaves as a time-limited species when interacting with *T. absoluta* in a fixed-time experiment.

The attack rate of a parasitoid provides a valuable measure of its parasitism efficiency and its potential for successful biological control. van Lenteren and Manzaroli (1999) pointed out that a parasitoid can be an effective biological control agent if, in addition to other factors, its r_m and kill rate are equal or greater than the r_m of the targeted pest. In this sense, *P. dignus* exhibited an $r_m = 0.14$ (Nieves et al. 2015) equal to that of *T. absoluta*'s ($r_m = 0.14$; Pereyra and Sánchez 2006) and instantaneous attack rates higher than r_m of the pest in both solanaceous plants.

Results of this study suggest that *P. dignus*, in spite of differences in some life history traits, would have a similar performance in tomato and eggplant in terms of its efficacy in the control of *T. absoluta*. Further field studies aimed to design strategies for manipulating this parasitoid could provide growers with an alternative tool for *T. absoluta* control in both crops.

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