

# Potential of biological control agents against *Tuta absoluta* (Lepidoptera: Gelechiidae): current knowledge in Argentina

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## Abstract

Pest suppression through biological control seeks to maximize the action of the pest's natural enemies with the goal of reducing pesticide use. We present a summary of published studies and original findings on several entomophagous species as biocontrol agents of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), a key pest of tomato crops in Argentina, with the aim to select potential candidates for its management. Spontaneously occurring *T. absoluta* egg parasitism was lower than that inflicted by the larval parasitoids *Dineulophus phthorimaeae* (De Santis, 1983) (Hymenoptera: Eulophidae) and *Pseudapanteles dignus* (Muesebeck, 1938) (Hymenoptera: Braconidae). These parasitoids exhibit important life history traits in laboratory conditions and produce relevant amounts of *T. absoluta* mortality in the field. Surveys carried out in Tucumán and Buenos Aires provinces, Argentina, revealed that *D. phthorimaeae* and *P. dignus* coexist in tomato and eggplant crops; *T. absoluta*-*P. dignus* interaction is also found on other non-cultivated solanaceous species present in horticultural farms. In addition, studies are currently under way to determine the predation ability of *Zelus obscuridorsis* (Stål, 1860) (Hemiptera: Reduviidae) on both larvae and adults of the pest. Finally, we discuss the prospects for implementing experimental augmentative releases of *P. dignus* to control the pest, a candidate selected considering various positive biological traits and because of its simple mass production and manipulation compared with other antagonists of *T. absoluta*.

Key Words: biocontrol, *Lycopersicon esculentum* L., *Dineulophus phthorimaeae*, parasitoid, predator, South American tomato leafminer

## Resumen

El control biológico de plagas tiene como premisa maximizar la acción de sus enemigos naturales para reducir el uso de plaguicidas en la agricultura. En este trabajo se reúne información de estudios sobre las especies de entomófagos de *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) más importantes en el cultivo de tomate de la Argentina, con el objetivo de seleccionar candidatos potenciales para el control de esta plaga. Comparativamente, el parasitismo natural de huevos de *T. absoluta* es más bajo que el provocado por los parasitoides larvales *Dineulophus phthorimaeae* (de Santis, 1983) (Hymenoptera: Eulophidae) y *Pseudapanteles dignus* (Muesebeck, 1938) (Hymenoptera: Braconidae). Estos parasitoides exhiben atributos relevantes como agentes de control en estudios de laboratorio y campo. Muestras realizadas en las provincias de Tucumán y Buenos Aires, Argentina, mostraron que *D. phthorimaeae* y *P. dignus* coexisten en los cultivos de tomate y de berenjena, y también *P. dignus* se encuentra en otras solanáceas no cultivadas presentes en los predios hortícolas. Adicionalmente, se están llevando a cabo estudios para determinar la capacidad de depredación de *Zelus obscuridorsis* (Stål, 1860) (Hemiptera: Reduviidae) sobre larvas y adultos de la plaga. Finalmente, se discute sobre la implementación de liberaciones aumentativas de *P. dignus*, candidato que reúne varios atributos biológicos positivos como potencial agente de control biológico de la plaga, y cuya cría masiva y manipulación son más simples que las de otros antagonistas.

Palabras Clave: biocontrol, *Lycopersicon esculentum* L., parasitoide, *Dineulophus phthorimaeae*, depredador, "polilla del tomate"

The South American tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an endemic neotropical pest that causes significant economical losses in tomato, (*Lycopersicon esculentum* L.; Solanales: Solanaceae) production (Desneux et al. 2011). Injury is caused by the larvae that mine leaves and fruits, mainly on solana-

ceous species, and eventually facilitates plant pathogen invasion (EPPO 2005). Native to South America, *T. absoluta* has recently invaded Europe and it is spreading across Africa and Asia (Desneux et al. 2011), being presently in at least 63 countries. Because of its high invasive potential *T. absoluta* also causes concern to other countries where *T.*

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*absoluta* is considered a quarantine pest, and where it has not yet been recorded (USDA-APHIS 2011; Zlof & Suffert 2012).

In Argentina, pest management to protect tomato, including tactics against *T. absoluta*, involves weekly or bi-weekly pesticide treatments (Desneux et al. 2011). Currently, other complementary techniques, like pheromone trapping and applications of *Bacillus thuringiensis*, are starting to be used by producers as more sustainable forms of pest management (Luna et al. 2012a).

Limited attempts to employ other biological tactics against *T. absoluta* in Argentina have dealt with the use of imported egg parasitoids (Riquelme Virgala & Botto 2010). However, the results are still preliminary and we still cannot conclude that their commercial implementation in tomato crops is a possibility.

Several natural enemies of *T. absoluta* have been reported to occur naturally in tomato crops under various cultural practices in Argentina (Desneux et al. 2010; Luna et al. 2012b). Therefore, there is potential value in selecting those native species that are most effective and promising candidates for developing biological control programs.

In this article, we summarize past work and present recent original findings concerning several natural enemies of *T. absoluta* in tomato crops in Argentina.

## PROSPECTS OF BIOLOGICAL CONTROL AGENTS AGAINST *TUTA ABSOLUTA*

Our initial research on natural enemies of *T. absoluta* took into account their abundance and seasonal synchronization with the pest as the main criterion for selecting species as biocontrol candidates. Subsequently, our studies included additional species of the *T. absoluta* enemy complex so as to have a thorough understanding of how they interact.

### Larval Parasitoids

The parasitoid complex reported for the larval stage of *T. absoluta* consists of approximately 20 hymenopteran species (Colomo et al. 2002; Luna et al. 2007; Luna et al. 2012b). Yet, the koinobiont endoparasitoid *Pseudapanteles dignus* (Muesebeck, 1938) (Hymenoptera: Braconidae) and the idiobiont ectoparasitoid *Dineulophus phthorimaeae* (De Santis, 1983) (Hymenoptera: Eulophidae) accomplish over 50% of natural parasitism and exhibit promising attributes for either augmentative or conservation biological control in the native range of *T. absoluta* (Colomo et al. 2002; Luna et al. 2007; Sánchez et al. 2009; Savino et al. 2012, 2013). They may also be suitable for introduction in the new regions invaded by *T. absoluta* (Savino et al. 2012).

*Pseudapanteles dignus* is a solitary larval endoparasitoid of *T. absoluta* – and also reported for other gelechiids – commonly present in open field and greenhouse tomato crops, whether organic or sprayed with pesticides (Nieves et al. in press). Previous studies indicated that *P. dignus* exhibits some life history and ecological traits that could potentially limit *T. absoluta* populations in this crop (Table 1, Luna et al., 2007, 2010; Sánchez et al. 2009). *Pseudapanteles dignus* parasitizes all larval instars of its hosts. Population parameters estimated by a life table study under laboratory conditions ( $25 \pm 2$  °C,  $65 \pm 5\%$  RH, 14:10 h L:D), yielded a developmental time from egg to adult of 36 days (Nieves 2013; Nieves et al. in press). Female reproductive strategy was moderately synovigenic and time-limited, with a mean egg load of 52 eggs ready to be laid at the emergence of the adult, and a mean fecundity of 192 eggs throughout its adult life (Nieves et al. in press). On average, females laid eggs until the 20th day of adult life, and 50% of the total eggs were laid within the first 6 days (Luna et al. 2007; Nieves et al. in press). Mean parasitism per female (calculated as the total number of parasitized larvae during the female life span divided by the total number of offered larvae) was 47%. Although superparasitism was observed in the laboratory (30%), it rarely occurred in the field (10%) (Nieves 2013). Cohort studies in the laboratory revealed a pre-imago survival rate (egg to pupa) of 38%. Highest mortality occurred at the larval stage, and encapsulation by the host was identified as the main reason (Nieves 2013; Nieves et al. in press). When a single *P. dignus* larva was present in the host, it could suffer up to 28% encapsulation, but superparasitism (~ 5 immatures per host) could lead up to 91% encapsulation (Nieves 2013).

Life history traits and population parameters of *P. dignus* showed that it had the potential ability to suppress *T. absoluta* densities (Nieves et al. in press). The intrinsic rate of natural increase ( $r_m$ ) of *P. dignus* was equal to that of *T. absoluta* ( $r_m = 0.14$ ), reared under the same experimental conditions (Pereyra & Sánchez 2006), and the instantaneous attack rate ( $a'$ ) of 0.22 (Luna et al. 2007) was larger than the  $r_m$  of the pest.

In the field, *P. dignus* showed a considerable ability to attack *T. absoluta* either at low or high densities (Sánchez et al. 2009). Parasitism by *P. dignus* was density-independent, and thus the probability of being parasitized and the risk of parasitism increased with increasing population densities of *T. absoluta* (Sánchez et al. 2009). In greenhouse tomato, parasitism exhibited a high pest-parasitoid synchrony throughout the crop cycle. By calculating the impact of parasitism on the pest population by means of comparing the areas under the density curves of both populations throughout the season (as in Carey 1993), we showed that *P. dignus* was able to reduce *T. absoluta* populations by 33–49% in early tomatoes (Sep to Dec) and up to 64% in late tomatoes (Jan to Jun) (Nieves et al. in press).

**Table 1.** Life history traits comparison between *Pseudapanteles dignus* and *Dineulophus phthorimaeae*, parasitoids of *Tuta absoluta* (summarized from Luna et al. 2007, 2010, 2012a; Sánchez et al. 2009, Nieves 2013, Nieves et al. in press, Savino et al. 2012, Savino 2014).

	<i>Pseudapanteles dignus</i>	<i>Dineulophus phthorimaeae</i>
Life history strategy	Endoparasitoid, koinobiont	Ectoparasitoid, idiobiont
Mean life-time fecundity	192.36 ( $\pm 17.17$ ) eggs per female	4.15 ( $\pm 0.4$ ) eggs per female
Sex ratio (females per offspring)	1: 1.25	1:1
Mean ( $\pm$ SE) developmental time (from egg to adult emergence) (days)	22.69 $\pm$ 0.22	11.17 $\pm$ 0.60
Life cycle (days)	$\approx$ 36	$\approx$ 23
Host larval instar attacked	From 1 <sup>st</sup> to 4 <sup>th</sup>	Only 3 <sup>rd</sup>
Host-feeding	No	Yes
Aggregative response to host density	Yes	Yes
Foraging behavior	Time limited	Egg limited
Mass rearing	Straight forward	Not available yet

The mode of parasitism of *D. phthorimaeae* contrasted with that of *P. dignus* (Table 1). *Tuta absoluta* and another gelechiid, *Phthorimaea operculella* (Zeller), were reported as hosts (De Santis 1983). With an ovigeny index equal to 0 (Savino et al. 2012), its female reproductive strategy was extremely synovigenic. Adult females were anautogenous, i.e., they needed to acquire additional nutrients to mature eggs by destructive host feeding. Host feeding by the wasp paralyzed *T. absoluta* larvae, causing arrest of further development (Luna et al. 2010; Savino et al. 2012). This requirement to host feed on larvae to develop eggs led to *T. absoluta* mortality by both host-feeding and larval parasitism. The mean lifetime fecundity of *D. phthorimaeae* was of  $4.15 \pm 0.4$  eggs per female and showed a type I functional response; hence this species was found to be an egg-limited parasitoid (Luna et al. 2010; Savino et al. 2012) (Table 1). The potential of *D. phthorimaeae* as natural enemy of *T. absoluta* was based not only on the quantity of hosts it could parasitize but also on its host feeding behavior; the latter can cause 30% of extra mortality (Savino et al. 2012).

*Pseudapanteles dignus* and *D. phthorimaeae* commonly were observed to occur together in tomato crops in Argentina (Luna et al. 2010; Savino 2014). Therefore, we examined the interspecific relationships between both larval parasitoid species, to determine possible multiparasitism, i.e., the use of a single host individual by 2 or more different species of parasitoids. *Pseudapanteles dignus* and *D. phthorimaeae* were found to have an overlapping niche, sharing *T. absoluta* as their host mostly at the third larval instar (Luna et al. 2010; Savino 2014). Under these conditions, it was expected that the idiobiont ectoparasitoid, *D. phthorimaeae*, should outcompete the koinobiont endoparasitoid, *P. dignus*, by at least 2 mechanisms (Hawkins 1994). Firstly, by killing heterospecific immatures due to host-feeding of already parasitized *T. absoluta* larvae. Secondly, by paralyzing *T. absoluta* larvae upon host feeding and by parasitization, thus removing hosts of *P. dignus*, while the opposite it is not true, i.e., *T. absoluta* larvae parasitized by *P. dignus* may remain suitable for *D. phthorimaeae* (Savino 2014).

Host discrimination ability of *D. phthorimaeae* including its ability to distinguish *P. dignus* parasitized hosts from unparasitized hosts was experimentally examined as follows: *Tuta absoluta* larvae were offered to individual endoparasitoid females (*P. dignus*) in a cylindrical plastic box (600 mL) arena for 48 h. After that, these previously exposed host larvae were exposed to individual *D. phthorimaeae* females of 3 ages (1, 5, and 7 days old). Results indicated that young (3-day old) *D. phthorimaeae* females tended to attack healthy *T. absoluta* larvae; however, as each female's age progressed (5 days old and older) multiparasitism frequently occurred (Savino 2014). The observed coexistence of both parasitoid species in the field could be promoted by the behavior of *D. phthorimaeae* to partially reject heterospecifically parasitized larvae. The combined effects of the age of the *D. phthorimaeae* females and the availability of hosts in a patch where both *P. dignus* parasitized and non-parasitized *T. absoluta* larvae were already present could have played an important role in the dynamics of the interactions between the 2 parasitoid species.

Field surveys carried out in extensive areas of Argentina during 2009 through 2012 (Figure 1 and Table 2) showed a general spatial host density-independent pattern of parasitism for both parasitoid species occurring together on the same plant, regardless the region. Evidence of low-level and host density-independent multiparasitized *T. absoluta* were also found, indicating a weakly negative interspecific interaction between both parasitoid species (Savino et al. 2013).

Knowledge of host species and host plants that can provide refugia and alternative food resources for the population growth and maintenance of natural enemies is important in the design of biological control strategies for a pest species. Because of the lack of information related to the host ranges of *D. phthorimaeae* and *P. dignus*, we initiated

a survey on horticultural farms in northeastern Buenos Aires province by means of a centrifugal phylogenetic approach (Wapshere 1974). Solanaceous plants commonly cultivated in the region [tobacco *Nicotiana tabacum* L., eggplant *Solanum melongena* L., and sweet pepper *Capiscum annuum* L.], and spontaneous non-cultivated [such as the fierce thorn-apple *Datura ferox* L., longflower tobacco *Nicotiana longiflora* Cav., tree tobacco *Nicotiana glauca* Graham, lily of the valley vine *Salpichroa origanifolia* (Lam.) Baill., American black nightshade *Solanum americanum* Mill., and the sticky nightshade *Solanum syssimbrifolium* Lam.] were examined during 2013-2014, to detect signs of *T. absoluta* or damage of other gelechiid species. Leafminer hosts and parasitoids collected were identified and numbers of each per plant species were registered. So far, we recorded *P. dignus* parasitizing *T. absoluta* on eggplant around the year and sporadically on *S. americanum*, while *D. phthorimaeae* was detected on *T. absoluta* only occasionally on eggplant (Salas Gervassio et al. 2014). This information was indicative that host plant species that are alternatives to *L. lycopersicum* could be playing a role in the maintenance of natural parasitoids of *T. absoluta* when this crop is absent.

## OTHER ANTAGONISTS

By means of the sentinel egg technique (Moya-Raygoza et al. 2012), we obtained 2 species of egg parasitoids naturally occurring in tomato crops in Argentina: *Trichogramma pretiosum* (Riley, 1879) (Trichogrammatidae) and *Encarsia porteri* (Mercet, 1928) (Aphelinidae) (Luft et al. ms submitted). *Trichogramma pretiosum* had a broader distribution than the aphelinid wasp, which was collected only in Tucumán (northwestern Argentina). Percentages of parasitism were low (< 5%). When these 2 egg parasitoids co-occurred, 50% of the parasitized *T. absoluta* eggs were attacked by either one of the 2 species, and superparasitism was not observed.

A survey of tomato crops in northwestern Argentina revealed the presence of a native true bug preying a variety of mobile insects. The species was identified as *Zelus obscuridorsis* (Stål) (Hemiptera: Heteroptera: Reduviidae) (Speranza et al. 2014). Assays were conducted to assess its capability to prey on various developmental stages of *T. absoluta*. We found that *Z. obscuridorsis* preyed on the mobile stages, i.e., free larvae and adults, but did not eat larvae in their mines, pupae or eggs.

## Discussion and Future Prospects

Any biological control program should select the best strategy for the use of natural enemies, and this includes a thorough knowledge of suitable biocontrol agents that naturally occur in a given region. Among the natural enemy complex of *T. absoluta* under study, the biological and ecological characteristics of *P. dignus* presented here and elsewhere (Luna et al. 2012b) meet various criteria as a potentially good biocontrol agent likely to induce sufficient pest suppression when applied in seasonal augmentative releases in commercial crops. For this reason, we have chosen *P. dignus* to conduct experimental releases in tomato greenhouses, a technique to be used alone or in combination in IPM programs. Like many other Braconidae species, *P. dignus* is quite easy to rear and manipulate under laboratory conditions. Results achieved in our studies allow improved *P. dignus* methods for mass rearing, by reducing the frequency of encapsulation and increasing the survival of the offspring. In addition to developing additional knowledge of *P. dignus* rearing, it would be essential to develop quality control guidelines for mass production.

In relation to the other natural control agents of the tomato moth in Argentina, we have continued to study the impact of their trophic

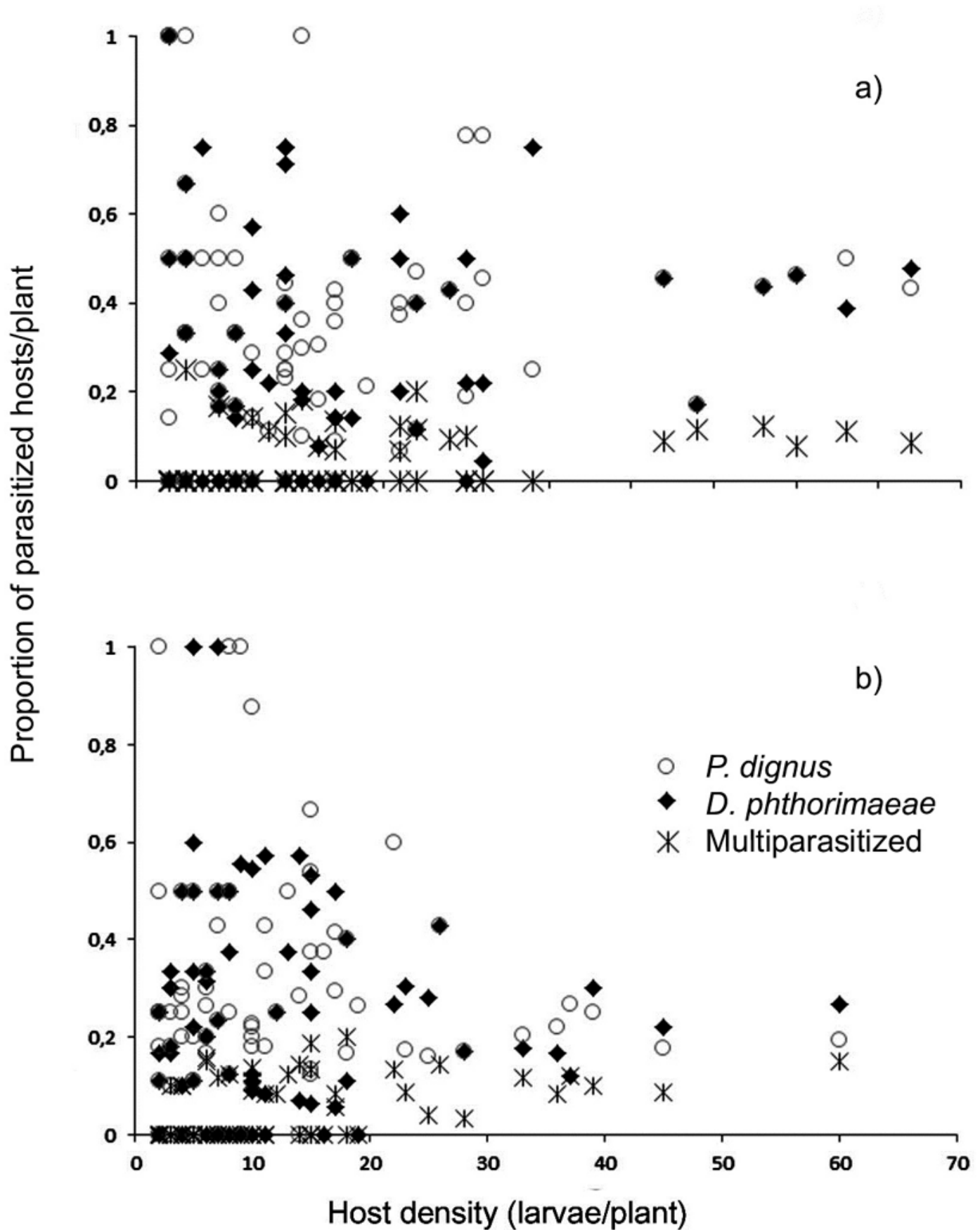


Fig. 1. Relationships among the proportions of *Tuta absoluta* per tomato plant parasitized by *Pseudapanteles dingus*, or by *Dineulophus phthorimaeae phthorimaeae* or by both species (multiparasitism) at various densities of *T. absoluta* in 2 regions of Argentina, i.e., (a) La Plata, northern Buenos Aires province and (b) Tucumán. Relevant logistic regression parameters are shown in Table 2.



**Table 2.** Parameters of the logistic regression between the proportion of *Tuta absoluta* larvae per plant parasitized by *Dineulophus phthorimaeae*, *Pseudapanteles dignus* or multiparasitized by both parasitoids against *T. absoluta* density in Argentina.

Years	Tucumán (26° 54' 34" S, 65° 20' 37" W)*														
	<i>Dineulophus phthorimaeae</i>					<i>Pseudapanteles dignus</i>					Multiparasitized				
	B0	$\beta$	P	$\chi^2$	N	B0	$\beta$	P	$\chi^2$	N	B0	$\beta$	P	$\chi^2$	N
2009, 2011, 2012	-1.35	0.0006	NS	0.0035	58	-0.9	-0.009	NS	0.08	58	-3.55	0.03	NS	0.33	58
Years	La Plata (34° 56' 08" S, 58° 06' 03" W)†														
	<i>D. phthorimaeae</i>					<i>P. dignus</i>					Multiparasitized				
	B0	$\beta$	P	$\chi^2$	N	B0	$\beta$	P	$\chi^2$	N	B0	$\beta$	P	$\chi^2$	N
2010, 2011, 2012	-1.33	0.02	NS	0.54	50	-0.99	0.02	NS	0.27	50	-3.8	0.04	NS	0.31	50

B0: coefficient for the intercept, regression parameter.

Linear coefficients ( $\beta$ ) not significantly different from zero indicate that the proportions of parasitized hosts were independent of host densities.

\* sampling size: 7 sites, 20 plants per site.

† sampling size: 5 sites, 20 plants per site.

N = number of valid cases for logistic regression (logit) model

NS: not significant at  $P \leq 0.05$

interactions (parasitism, predation, competition, intra-guild predation, etc.) in pest suppression. Although less studied to date, these natural enemies could be important in taking advantage of little exploited host niches, i.e., eggs, mobile larvae, etc., by other species antagonistic to *T. absoluta*.

A thorough research program towards developing an IPM program for a given species should take into account all relevant natural enemies, as well as consider other co-occurring complexes of pests and beneficial species, under an agro-ecosystem approach. Such an approach would not only allow for the development of the best IPM strategy, but also result in a better understanding of the pest's trophic relationships in a given region.

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