

# Spatial Patterns of Parasitism of the Solitary Parasitoid *Pseudapanteles dignus* (Hymenoptera: Braconidae) on *Tuta absoluta* (Lepidoptera: Gelechiidae)

NORMA E. SÁNCHEZ,<sup>1</sup> PATRICIA C. PEREYRA, AND MARÍA G. LUNA

CEPAVE (CCT-La Plata-CONICET-UNLP), Calle 2, No. 584, 1900 La Plata, Argentina

Environ. Entomol. 38(2): 365–374 (2009)

**ABSTRACT** We examined the interaction between the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a key pest of tomato crops in South America, and its main solitary larval parasitoid, *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae). The pattern of parasitism of *T. absoluta* by the parasitoid was studied at three scales on tomato crops: plant, leaf, and leaflet. Host density, spatial distributions of both host and parasitoid, percentages of parasitism, variation in the probability and risk of parasitism in relation to host density, and the spatial density dependence were assessed in a horticultural region in Argentina. The spatial distribution of *T. absoluta* was clumped at all sites and scales, whereas that of *P. dignus* was much more variable, fitting to negative, positive binomial distributions and to Poisson series. Percentages of parasitism were as follows: site 1, 17.06%; site 2, 27.53%; site 3, 26.47%; site 4, 45.95%. Parasitoid aggregation in relation to host density was found at leaf and leaflet scales. However, the proportion of parasitized hosts was independent of host density. The variability of parasitism rates exhibited at the three spatial scales seems to result in partial refuges for the host, which might contribute to the persistence of the interaction between host and parasitoid. We discuss our field observations in relation to ecological theory and its potential application to the biological control of *T. absoluta* on tomato.

**KEY WORDS** host–parasitoid interaction, spatial scales, *Lycopersicon esculentum*, biological control

One of the hypotheses in biological control is that effective natural enemies will contribute to a stable and reduced pest density (Bellows and van Driesche 1999). For parasitoids, theoretical and empirical research carried out in laboratory experiments and natural settings have dealt with evaluating ecological attributes that make them effective natural enemies of pests (Hassell 2000). Among those attributes is the spatial aggregation or nonrandom search by parasitoids in response to patchy distributions of hosts, resulting in the phenomenon that females spend more time and/or concentrate in areas where hosts are more abundant. Based in theoretical models, many authors assumed that an aggregative response would increase efficiency in parasitoid foraging and would lead to a direct density-dependent parasitism, a powerful trait for a successful biological control agent because of its potential to regulate the host population (Hassell and May 1973, 1974; Murdoch and Oaten 1975; Heads and Lawton 1983). However, Reeve and Murdoch (1985), in the California red scale–*Aphytis melinus* system, concluded that spatial aggregation of parasitism in response to local host density is not a necessary condition for stability or for a successful biological control. Subsequent studies indicated that

inverse density-dependent and density-independent patterns of parasitism may also be potential stabilizing mechanisms if the distribution of parasitism is sufficiently aggregated (Walde and Murdoch 1988, Pacala and Hassell 1991, Hassell 2000, van Veen et al. 2002).

The variation in risk of parasitism between individuals in the host population is currently considered an important factor that could contribute to the persistence of the host–parasitoid system (Chesson and Murdoch 1986). This variation can arise from other sources besides host density in the field. Among the sources of variation are lack of synchronization between host and parasitoid life cycles and phenotypic variation in the host population that causes differential susceptibility and/or vulnerability to parasitism (Hassell 2000).

A controversial point in detecting aggregative response in parasitoids is the spatial scale of observation. Indeed, observed natural patterns of parasitism have been scale dependent in some studies (Heads and Lawton 1983, Lill 1998, Jarošik and Lapchin 2001) and scale independent in others (Reeve and Murdoch 1985, Strong 1989, Hails and Crawley 1992). Thus, examining patterns of parasitism at different scales might improve the likelihood of showing the actual interaction mechanism in the field.

<sup>1</sup> Corresponding author, e-mail: plagas@cepave.edu.ar.

In this research, we examined the host–parasitoid system consisting of the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a key pest of tomato crops in South America, and the solitary parasitoid *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae). *T. absoluta* is a neotropical oligophagous species that feeds on wild and cultivated solanaceous plants (Razuri and Vargas 1975, Souza and Reis 1986, Pereyra and Sánchez 2006). On tomato, it feeds on leaves, fruits, and stems, preferably in the apical parts of the plant. It causes yield losses of up to 70% in South America (Vargas 1970, Fernández and Montagne 1990, Ulle and Nakano 1994, Botto et al. 1998, Torres et al. 2002).

Chemical control with neurotoxicant insecticides are used, but because of reduced efficacy (Siqueira et al. 2000, García et al. 2005, Lietti et al. 2005) and significant sublethal effects to natural enemies (Consoli et al. 1998), interest has grown in finding alternative management practices.

In this context, various control methods have been studied, such as (1) resistant plants (Ecole et al. 2001); (2) biological control with the predator *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) (Torres et al. 2002) and (3) the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) (Pratissoli et al. 1998, Faria et al. 2008); and (4) the use of the microbial control agent *Bacillus thuringiensis* variety *kurstaki* (Giustolin et al. 2001). However, effective control of the pest has not been achieved yet.

*Pseudapanteles dignus* is a dominant larval parasitoid of *T. absoluta* in open field and protected crops (Lange and Bronson 1981, De Santis 1983, Uchoa-Fernandes and Campos 1993, Haji et al. 1995, Miranda et al. 1998, Faria et al. 2000, Colomo et al. 2002). This koinobiont endoparasitoid is distributed in the Americas, and its host range is limited to a few gelechiid species (Cardona and Oatman 1971, Oatman and Platner 1989, Bennett 1995). The potential of *P. dignus* as a control agent of *T. absoluta* has recently been considered by studying its life history traits and functional response (Luna et al. 2007).

The objective of this study was to assess the spatial pattern of parasitism of *T. absoluta* by the solitary parasitoid *P. dignus* at different spatial scales on commercial tomato crops. We report the density of the host and the spatial distributions of both host and parasitoid, the percentage of parasitism, the probability and risk of parasitism among different individuals in the host population, and the spatial density dependence. Our findings are discussed in the context of biological control and may be useful for other types of pest–enemy interactions, as well.

### Materials and Methods

**Study Area.** The study site is located in the surroundings of La Plata, northern Buenos Aires province, Argentina (34°56' S, 57°59' W). This area is representative of an extended and traditional horticultural region, embedded into a landscape com-

prised of cropping and grazing fields, the Rolling Pampas, in the humid-temperate climatic zone (Hall et al. 1992). In this region, tomato is cultivated both in open-field and protected conditions (open-sided plastic greenhouses) (Corvo Dolcet 2005, García et al. 2005), mainly based on fresh-market tomato, and comprises ≈40% of national production. Climatic temperate conditions allow two growing cycles per year: early season (spring, from September to December) and late season (summer, from January to April). Conventionally, producers use insecticides based on calendar applications to control *T. absoluta* and other pests, such as *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) whiteflies. Alternatively, organic farming practices are also used by some producers in this area.

**Insect Sampling.** Tomato leafminer larvae were sampled at four tomato fields (referred to as “sites”), representing different tomato cropping conditions: two conventional greenhouses (sites 1 and 2) in 2004, one organic open field (site 3), and one organic greenhouse (site 4) in 2005. Site sizes ranged from 180 (organic) to 840 m<sup>2</sup> (conventional). Tomato varieties in the locations were indeterminate disease-resistant hybrids and hybrids that prolong product shelf-life (e.g., ‘Superman’). Conventional sites had no pesticide applications for at least 30 d before sampling to allow carrying out this study.

The spatial parasitism pattern was analyzed in the context of discrete host aggregation or “patches” on different scales: plant, leaf, and leaflet. To avoid spatial interdependence, all units from the smaller scale were included in the larger scale (Jarošík and Lapchin 2001). We considered that the upper third of the plant was representative of the distribution of pest and parasitoid at plant scale, because *T. absoluta* concentrates mainly in the apical parts of the tomato plants (Torres et al. 2001). The apical parts of 19–20 tomato plants (containing seven to eight consecutive expanded leaves) were harvested at random at each site, placed individually in plastic bags, and transported to the laboratory. To examine the parasitism at the three scales, each apical part of the plant was sorted in all component leaves and each leaf in all component leaflets. All sampling took place at the harvest season. Samples were taken once at each site at the middle of March (site 1) and at the end of April (site 2) in 2004 and at the end of March (site 3) and at the beginning of April (site 4) in 2005, when host and parasitoid populations were interacting.

**Host Density, Spatial Distribution of Host and Parasitoid, and Percentage of Parasitism.** In the laboratory, the leaves of each plant were examined under a binocular microscope to estimate the densities of immature stages of *T. absoluta* and *P. dignus* at the three spatial scales. Leaflets containing at least one *T. absoluta* larva were reared individually in plastic petri dishes (10 cm diameter). The larvae were fed daily with fresh tomato leaves and maintained in an environmental chamber at 25 ± 2°C, 70 ± 5% RH, and 14:10 (L:D) h, until leafminer pupa formation or parasitoid cocoon emergence. Dead host larvae were dissected

**Table 1.** Mean ( $\pm$ SE) and ranges of densities of *T. absoluta* at three spatial scales in four tomato crops

Location	Plant				Leaf				Leaflet			
	n	Mean (SE)	Min.	Max.	n	Mean (SE)	Min.	Max.	n	Mean (SE)	Min.	Max.
Site 1	20	36.45 b (6.60)	5	96	162	4.39 b (0.50)	0	36	2,201	0.33 b (0.02)	0	12
Site 2	19	69.74 a (12.86)	3	245	154	9.09 a (0.77)	0	54	3,283	0.40 a (0.02)	0	11
Site 3	20	8.55 c (2.13)	0	39	165	1.04 c (0.15)	0	15	1,623	0.11 c (0.01)	0	4
Site 4	19	11.58 c (1.91)	0	26	150	1.43 c (0.17)	0	12	2,144	0.10 c (0.008)	0	6

Site 1, conventional greenhouse, 2004; site 2, conventional greenhouse, 2004; site 3, organic open field, 2005; site 4, organic greenhouse, 2005. All crops were located in the surroundings of La Plata, Buenos Aires province, Argentina. Different letters within a column indicate significant differences ( $P \leq 0.05$ ) according to Tukey test for plants; Tukey type test for nonparametric multiple comparisons for leaves and leaflets.

to search for parasitoid immature stages. Mean densities of *T. absoluta* were calculated considering living and dead larvae. The sampling units were the number of parasitized hosts per plant, per leaf, and per leaflet.

Differences in densities among sites at plant and leaf scales were tested by analysis of variance (ANOVA; square root-transformed data) or with the Kruskal-Wallis tests, when ANOVA assumptions were violated (normality and homoscedasticity). A median test was used for leaflets because of the high number of counts ( $\approx 10,000$ ). To compare mean densities, the Tukey test or Tukey type test for nonparametric analysis was used (Zar 1999).

The spatial distribution of *T. absoluta* was evaluated by the index of dispersion ( $I$ ) calculated as the variance to mean ratio (Southwood 1978), and the significance of the departure from ratio unity was assessed by  $\chi^2$  (Elliot 1979). Additionally, data were fitted to the Poisson, negative, and positive binomial distributions, depending on whether the population distributions were random, aggregated, or uniform, respectively. The same tests were done for the parasitoid, based on numbers of parasitized hosts found in all collected plants, leaves, and leaflets. Goodness-of-fit of the observed data to the theoretical distributions was tested by  $\chi^2$  or  $G$  tests ( $P < 0.05$ ) in cases where the df was  $< 3$ .

Percentage of parasitism by *P. dignus* was calculated as follows: (the number of parasitoid larvae found in dissections + cocoons formed) / [(total number of

hosts (healthy and parasitized)]  $\times 100$ . Differences among sites in mean percentages/leaflet were compared by Kruskal-Wallis and Tukey type test for nonparametric analysis (Zar 1999).

**Probability and Risk of Parasitism.** To examine the aggregation of parasitism in relation to host density, two variables were estimated: (1) probability of parasitism and (2) risk of parasitism. The probability of parasitism among host patches at the different scales was calculated as the proportion of parasitism at each host patch density on which parasitism occurred, independent of the number of parasitized hosts (Bezemer and Mills 2001). This variable represents the proportion of patches at each host patch density and scale that were parasitized. The risk of parasitism per individual host at each host patch density at the different scales was measured according to Lill (1998) as (probability of parasitism)  $\times$  (proportion of larvae parasitized/number of parasitized leaflet, leaf, or plant). Density dependence of these two variables was examined for each spatial scale with linear regression analysis, with previous arcsine transformation of the original data.

**Density Dependence.** Spatial density dependence of proportion of parasitism was analyzed by fitting logistic regressions between the proportion of parasitized hosts and host density. This method is appropriate in cases where the dependent variable is categorical (a discontinuous variable), and it can be used with continuous and/or discrete independent vari-

**Table 2.** Spatial distribution of *T. absoluta* at three spatial scales in four tomato crops

Location	Spatial scale	$I$	df	$\chi^2$ <sup>a</sup>	Dispersion pattern	Frequency distribution	$\chi^2$ <sup>b</sup>	$k$	df	$P$
Site 1	Plant	23.93	19	454.67	Clumped	Negative binomial	73.04	1.59	96	0.93
	Leaf	9.24	161	1487.64	Clumped	Negative binomial	45.45	0.53	36	0.13
	Leaflet	2.39	2201	5260.39	Clumped	Negative binomial	14.38	0.24	13	0.28
Site 2	Plant	44.83	18	806.94	Clumped	Negative binomial	84.44	1.59	245	0.99
	Leaf	10.14	161	1632.54	Clumped	—	—	—	—	—
	Leaflet	2.2	3282	7220.40	Clumped	—	—	—	—	—
Site 3	Plant	10.67	19	202.73	Clumped	Negative binomial	399.93	0.88	39	0.428
	Leaf	3.69	164	605.16	Clumped	Negative binomial	13.44	0.39	15	0.568
	Leaflet	1.37	1622	2222.14	Clumped	Negative binomial	4.31	0.29	4	0.365
Site 4	Plant	5.97	18	107.46	Clumped	—	—	—	—	—
	Leaf	3.19	149	475.31	Clumped	Negative binomial	7.99	0.65	12	0.786
	Leaflet	1.58	2143	3385.94	Clumped	Negative binomial	6.66	0.18	6	0.35

Site 1, conventional greenhouse, 2004; site 2, conventional greenhouse, 2004; site 3, organic open field, 2005; site 4, organic greenhouse, 2005. All crops were located in the surroundings of La Plata, Buenos Aires province, Argentina.

k, negative binomial parameter;  $\chi^2$ <sup>a</sup>, value for the departure from unity of the index of dispersion ( $I = \text{variance}/\text{mean}$ );  $\chi^2$ <sup>b</sup>, test for goodness of fit of negative binomial distribution.  $P \geq 0.05$  indicates agreement with a negative binomial.

**Table 3. Spatial distribution of *P. dignus* at three spatial scales in four tomato crops**

Location	Spatial scale	<i>I</i>	df	$\chi^2$ <sup>a</sup>	Dispersion pattern	Frequency distribution	$\chi^2$ <sup>b</sup>	<i>k</i>	df	<i>P</i>
Site 1	Plant	5.47	19	103.93	Clumped	Negative binomial	27.81	1.35	21	0.15
	Leaf	2.22	117	259.74	Clumped	Negative binomial	7.79	0.84	10	0.65
	Leaflet	1.10	421	463.10	Random	Poisson	2.99	—	3	0.39
Site 2	Plant	22.14	18	398.52	Clumped	Negative binomial	56.85	0.94	94	0.99
	Leaf	3.76	147	552.72	Clumped	Negative binomial	27.26	1.02	17	0.06
	Leaflet	1.14	778	886.92	Clumped	Negative binomial	2.76	2.82	4	0.60
Site 3	Plant	3.30	17	56.10	Clumped	Negative binomial	10.84	1.04	15	0.76
	Leaf	1.14	70	79.80	Random	Poisson	2.75	—	4	0.40
	Leaflet	1.05	149	156.45	Random	Poisson	0.85	—	3	0.84
Site 4	Plant	1.49	16	23.84	Random	Poisson	16.98	—	10	0.07
	Leaf	1.12	75	84.00	Random	Poisson	4.13	—	6	0.66
	Leaflet	0.66	174	114.84	Uniform	Positive binomial	1.13	—	2	0.57

Site 1, conventional greenhouse, 2004; site 2, conventional greenhouse, 2004; site 3, organic open field, 2005; site 4, organic greenhouse, 2005. All crops were located in the surroundings of La Plata, Buenos Aires province, Argentina.

$\chi^2$ <sup>a</sup>, value for the departure from unity of the index of dispersion (*I* = variance/mean);  $\chi^2$ <sup>b</sup>, test for goodness of fit of Poisson, negative or positive binomial distribution, *k*, negative binomial parameter. *P* ≥ 0.05 indicates agreement with a Poisson, negative or positive binomial distribution.

ables. Because the proportion of parasitism is a discrete dependent variable, the errors associated with it may not be normally distributed (Trexler et al. 1988, Trexler and Travis 1993). Other methods, such as linear regression and nonlinear least square, have been usually used to test density dependence in laboratory and field experiments. However, the logistic model that explicitly assumes binomially distributed errors has proved to be more suitable (Juliano 2001). Thus, the statistical model used was:

$$\ln[p/(1 - p)] = \alpha + \beta x$$

where *p* is the proportion of parasitized hosts, and values of  $\beta$  equal to, greater than, or less than zero indicate density independence, direct density dependence, or inverse density-dependence, respectively. This model was fitted to data using the method of maximum likelihood (STATISTICA 6.0; StatSoft 2004). Analyses were carried out for each site, and for all sites combined, at each spatial scale.

**Table 4. Regression analysis of the probability of parasitism of *T. absoluta* larvae parasitized by *P. dignus* on host density at three spatial scales in four tomato crops**

Location	Spatial scale	Slope ± SE	R <sup>2</sup>	<i>F</i>	df	<i>P</i>
Site 1	Plant	0.23 ± 0.24	0.05	0.91	1,16	0.35
	Leaf	0.65 ± 0.16	0.42	15.24	1,21	0.0008
	Leaflet	0.30 ± 0.36	0.09	0.71	1,7	0.43
Site 2	Plant	<sup>a</sup>	—	—	—	—
	Leaf	0.61 ± 0.15	0.37	15.74	1,27	0.0005
	Leaflet	0.17 ± 0.34	0.03	0.25	1,8	0.63
Site 3	Plant	0.37 ± 0.30	0.14	1.45	1,9	0.26
	Leaf	0.83 ± 0.22	0.69	13.27	1,6	0.01
	Leaflet	0.62 ± 0.55	0.39	1.26	1,2	0.38
Site 4	Plant	0.52 ± 0.28	0.27	3.40	1,9	0.10
	Leaf	0.68 ± 0.25	0.47	7.07	1,8	0.03
	Leaflet	0.92 ± 0.19	0.85	21.89	1,4	0.01

Site 1, conventional greenhouse, 2004; site 2, conventional greenhouse, 2004; site 3, organic open field, 2005; site 4, organic greenhouse, 2005. All crops were located in the surroundings of La Plata, Buenos Aires province, Argentina.

<sup>a</sup> Could not be calculated because all plants with hosts had at least one parasitized.

**Results**

**Density of Host, Spatial Distributions of Host and Parasitoid, and Percentage of Parasitism.** A total of 9,251 leaflets from 631 leaves of 78 tomato plants were examined. Overall mean densities (±SE) for *T. absoluta* at the three spatial scales were 31.35 (±4.51) leafminer larvae/plant; 3.96 (±0.27) leafminer larvae/leaf; and 0.26 (±0.008) leafminer larvae/leaflet. Densities of *T. absoluta* were much lower in sites 3 and 4 sampled in 2004 than in sites 1 and 2 sampled in 2005 at the three scales (Table 1): plants (ANOVA: *F* = 22.14; df = 3,74; *P* = 0.001); leaves (Kruskal-Wallis test:  $H_{(3, n = 631)} = 196.51$ ; *P* = 0.001); and leaflets (Median test:  $\chi^2 = 262.62$ ; df = 3; *P* = 0.001).

Leafminer spatial distribution was clumped at all scales and sites, with the index of dispersion (*I*) significantly greater than one (Table 2). In most cases (9 of 12), observed frequencies did not deviate significantly from the expected frequencies based on the negative binomial distribution. Three cases did not adjust either to Poisson or to negative or positive

**Table 5. Regression analysis of risk of parasitism of *T. absoluta* larvae parasitized by *P. dignus* on host density at three spatial scales in four tomato crops**

Location	Spatial scale	Slope ± SE	R <sup>2</sup>	<i>F</i>	df	<i>P</i>
Site 1	Plant	0.22 ± 0.24	0.05	0.81	1,16	0.38
	Leaf	0.30 ± 0.20	0.09	2.11	1,21	0.16
	Leaflet	0.55 ± 0.31	0.30	2.99	1,7	0.13
Site 2	Plant	-0.24 ± 0.24	0.06	0.99	1,16	0.33
	Leaf	0.51 ± 0.16	0.26	9.35	1,27	0.005
	Leaflet	0.66 ± 0.26	0.44	6.32	1,8	0.04
Site 3	Plant	-0.16 ± 0.32	0.02	0.23	1,9	0.65
	Leaf	0.94 ± 0.13	0.89	48.92	1,6	0.0004
	Leaflet	0.98 ± 0.15	0.95	39.99	1,2	0.02
Site 4	Plant	0.02 ± 0.33	0.0004	0.004	1,9	0.95
	Leaf	0.86 ± 0.18	0.74	23.00	1,8	0.001
	Leaflet	0.80 ± 0.29	0.66	7.61	1,4	0.05

Site 1, conventional greenhouse, 2004; site 2, conventional greenhouse, 2004; site 3, organic open field, 2005; site 4, organic greenhouse, 2005. All crops were located in the surroundings of La Plata, Buenos Aires province, Argentina.

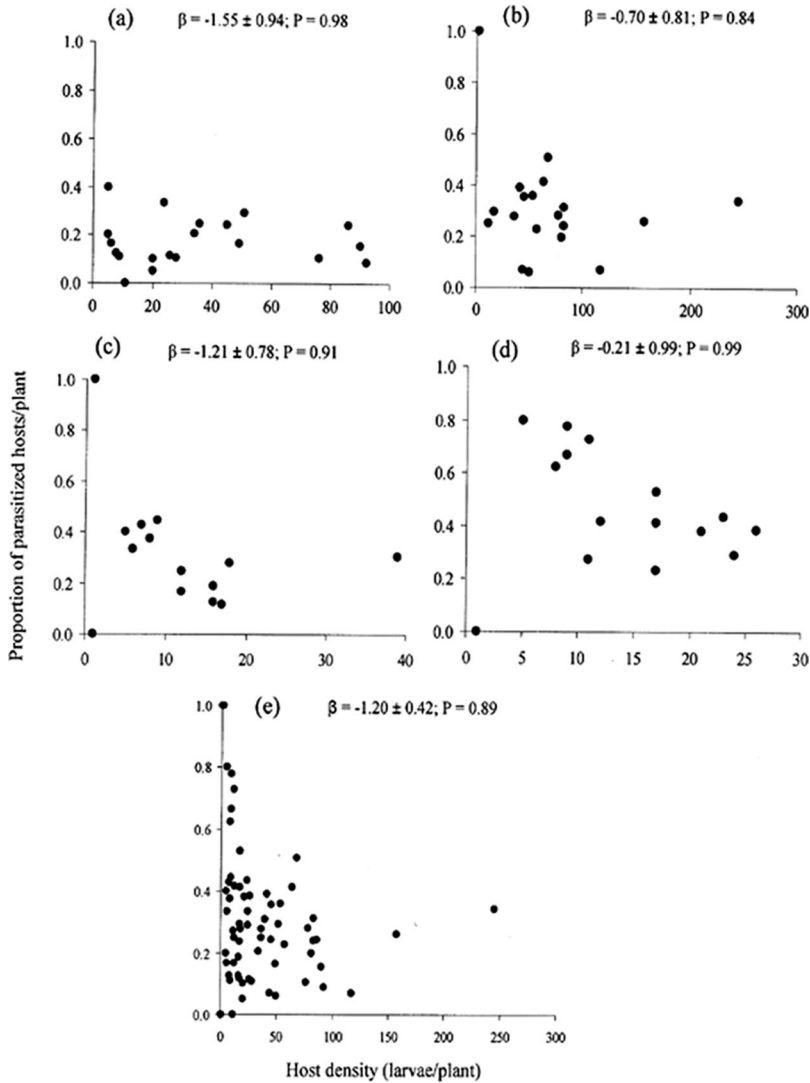


Fig. 1. Relationship between the proportion of parasitized *T. absoluta* per plant by the parasitoid *P. dignus* against *T. absoluta* density in four tomato crops representing different cropping conditions, located in the surroundings of La Plata, Buenos Aires province, Argentina. (a and b) Sites 1 and 2, respectively; both are conventional greenhouses, 2004. (c) Site 3, organic open field, 2005. (d) Site 4, organic greenhouse, 2005. (e) All sites combined.  $\beta \pm SE$ : slope of logistic regression curve. \*Significant level:  $P \leq 0.05$ .

binomial distributions. Values of  $k$ , the binomial distribution parameter, decreased from plant to leaflet, indicating a more aggregate distribution as scale diminishes.

Spatial distributions of *P. dignus* were much more variable than those of the host (Table 3). Seven cases were clumped and fitted to a binomial distribution. The other five fitted to Poisson series: two at leaf level (sites 3 and 4), two at leaflet level (sites 1 and 3), and one at plant level (site 4). Only one case showed a regular dispersion and adjusted to positive binomial distribution at the leaflet scale (site 4). At site 2 where the spatial distribution was aggregated at three scales,  $k$  values increased while scale decreased, indicating a

trend toward a less clumped distribution at leaflet scale.

Percentages of parasitism were as follows: site 1, 17.06%; site 2, 27.53%; site 3, 26.47%; site 4, 45.95%. Mean percent parasitism/leaflet at site 4 was significantly higher than at the other sites (Kruskal-Wallis test:  $H_{(3, n = 1508)} = 57.33; P = 0.001$ ).

**Probability and Risk of Parasitism.** The probability of parasitism was aggregated at leaf scale, indicating that it increased with host density. At larger (plant) or smaller (leaflet) scales, no relationship between proportion of parasitism and host density was found, exception for site 4 at the leaflet scale, where it was positively density dependent (Table 4). The risk of

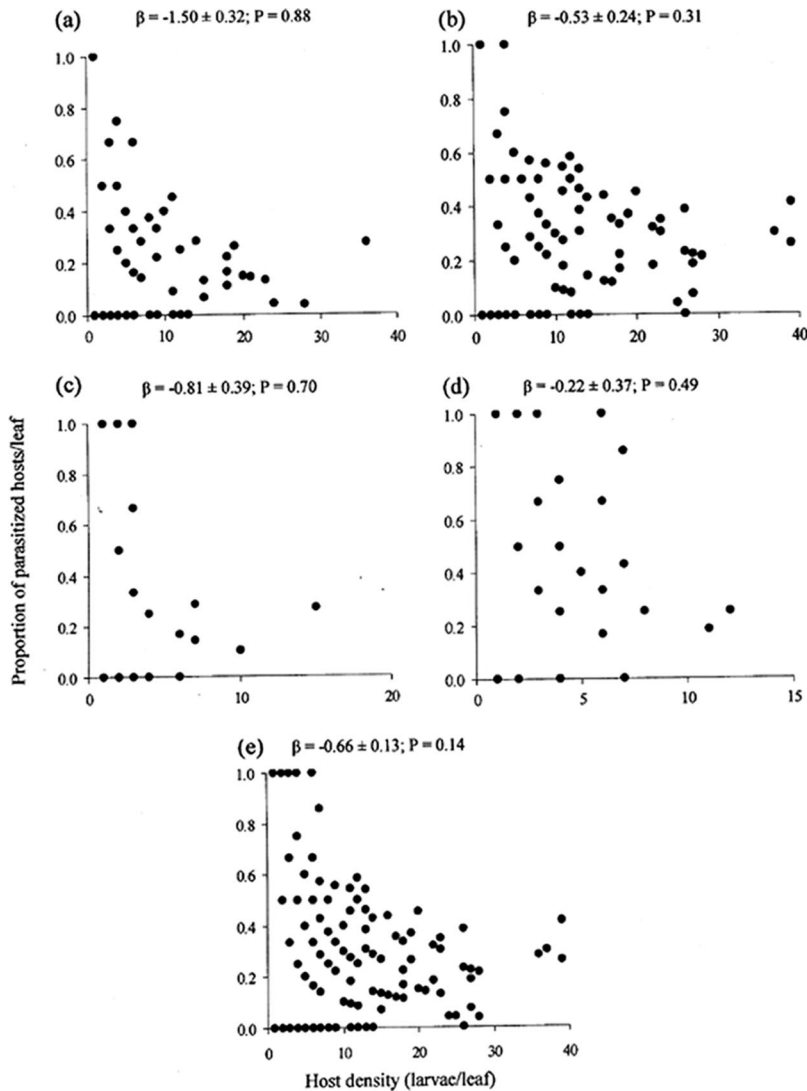


Fig. 2. Relationship between the proportion of parasitized *T. absoluta* per leaf by the parasitoid *P. dignus* against *T. absoluta* density in four tomato crops representing different cropping conditions, located in the surroundings of La Plata, Buenos Aires province, Argentina. (a and b) Sites 1 and 2, respectively; both are conventional greenhouses, 2004. (c) Site 3, organic open field, 2005. (d) Site 4, organic greenhouse, 2005. (e) All sites combined.  $\beta \pm SE$ : slope of logistic regression curve. \*Significant level:  $P \leq 0.05$ .

parasitism increased with host density both at leaf and leaflet scales, with the exception of site 1, where there was density independence at all scales (Table 5).

**Density Dependence.** Linear coefficients ( $\beta$ ) were not significantly different from zero at all scales and sites, indicating that the proportion of parasitized hosts was independent of host density (Figs. 1–3). Despite lack of significance, slopes exhibited a negative trend in most cases, and when data of all sites were combined, these resulted in a significant inversely density dependence of the proportion of parasitized hosts at the leaflet scale (Fig. 3). The relationship between the proportion of parasitized hosts and host abundance showed a high variability at the three spatial scales and at all sites.

## Discussion

As most phytophagous hosts (Wajnberg 2006), *T. absoluta* populations occurred in discrete patches in the environment. In contrast, parasitoid population distribution showed an opposite trend by underdispersing when scale decreased, and spatial distribution became random and even uniform at the leaflet scale (Table 3).

Although parasitoid foraging behavior and/or host recognition mechanisms of females in the field were not directly tested in this study, the outcome of these can be inferred from the observed *P. dignus* parasitism. Results suggest that females are initially attracted to and spend more time foraging on leaves with high host

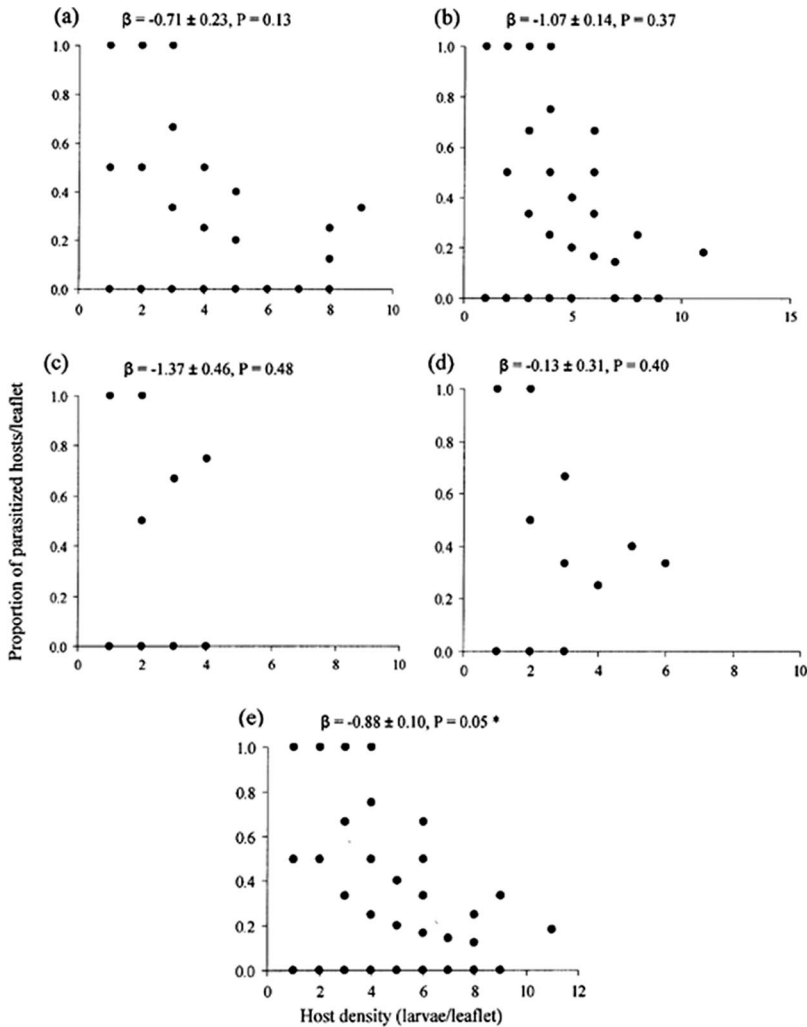


Fig. 3. Relationship between the proportion of parasitized *T. absoluta* per leaflet by the parasitoid *P. dignus* against *T. absoluta* density in four tomato crops representing different cropping conditions, located in the surroundings of La Plata, Buenos Aires province, Argentina. (a and b) Sites 1 and 2, respectively; both are conventional greenhouses, 2004. (c) Site 3, organic open field, 2005. (d) Site 4, organic greenhouse, 2005. (e) All sites combined.  $\beta \pm SE$ : slope of logistic regression curve. \*Significant level:  $P \leq 0.05$ .

densities. Once on the leaf, females may increase the duration of patch visit time on those leaflets where higher host densities occur. As a consequence, individual hosts in leaflet patches of higher densities are at a greater risk of being parasitized.

Interestingly, there was a density-independent pattern of parasitism in all sites and scales, with a tendency toward inverse density dependence, because it was statistically detected at the leaflet scale when all sites were pooled. The density-independent pattern of field parasitism is consistent with the type I functional response of *P. dignus* observed in the laboratory, where the curve reached an asymptote at a mean density of six hosts per day (Luna et al. 2007). Moreover, direct observations from ovipositing females showed that 75% of them did not attack any hosts, exhibiting slow movements and very low activity dur-

ing at least the first 2 h of experiments. The variation in the number of parasitized hosts by *P. dignus* over all ranges of densities observed both in the laboratory and in the field would suggest that females have a higher egg load than the number of hosts they are able to attack, being more time limited (handling time) than egg supply limited when interacting with *T. absoluta*. These results are consistent with those reported by Waage (1983) on *Diadegma* spp., where parasitoids showed aggregation in Brussels sprout plants with higher densities, although the rate of parasitism was density independent. Based on field and laboratory studies, Waage (1983) concluded that handling time could have resulted in limited oviposition rates at the highest densities. Hassell (1982, 2000) suggested that long handling times might prevent rising the parasitism as host density increases and would lead to inverse

density dependence. However, Heimpel and Casas (2008) argued that the combination of handling time limitation and aggregation at higher densities might lead to density-independent parasitism.

We found a great variability of the proportion of parasitism of *P. dignus* at a wide range of host densities in the field, corresponding with our results from laboratory studies (Luna et al. 2007). However, natural conditions are much more complex than those in simplified laboratory assays, and unevenness of field foraging efficiency of *P. dignus* may depend not only on female behavior but also on differences in host distribution at different spatial scales, variability in the susceptibility of different hosts, lack of synchrony between host and parasitoid life cycles, and interference, among other factors (Hassell 2000). The variation in risk of parasitism between individuals in the host population is currently considered an important factor that could contribute to the persistence of host-parasitoid interactions.

*Pseudapanteles dignus* seems to be a promising candidate for conservation biological control and/or seasonal augmentative releases (van Lenteren and Manzaroli 1999) and shows several positive biological traits for a natural enemy of a pest: (1) it is present in open field and protected tomato (under either conventional or organic management practices), indicating its capacity to survive under different cropping conditions; (2) parasitism levels can reach relatively high levels ( $\approx 46\%$ ) in late tomato crops, which is the most susceptible period to tomato leafminer attack; (3) females attack hosts daily; (4) they do not exhibit a preference among larval instars; (5) they have a relatively narrow host range (Oatman and Platner 1989); and (6) they are able to synchronize its larval developmental time with that of the host (Luna et al. 2007). Moreover, the aggregative response at leaf and leaflet scales, and the relatively high variation of density-independent parasitism rates, observed over all ranges of densities at all scales, could create a partial refuge effect for the host, contributing to the persistence of the interaction. Nevertheless, to determine how crucial the density relationships and the aggregation of parasitism are to promote stability in a host-parasitoid natural interaction, more long-term laboratory and field experiments are needed.

Because of crop, pest, and natural enemy removal at the end of the growing season in annual crops, and because of severe pesticide applications, reliable and persistent biological control seems to be difficult to obtain in such crops. According to Landis and Menalled (1998), the difficulty in using parasitoids for biological control in a classical or conservation approach in annual crops stems primarily from the disturbance imposed by humans on agroecosystems. However, the reappraisal of conservation of natural enemies as biological control agents has started to increase, changing the current dogma and creating new management perspectives for annual and perennial agroecosystems (Nyrop et al. 1998).

## Acknowledgments

We thank tomato growers M. Maita, M. del Pino, and R. López, and the authorities of Gorina Experimental Station (Ministry of Agriculture, Buenos Aires province) for permission to collect material at their farms. Field and laboratory help was provided by V. Wada, E. Nieves, D. Oliveira, M. Guzmán, L. Saldúa, L. Haudemand, and V. Fernández (UNLP). We thank J. van Lenteren for substantial comments concerning the manuscript and improvement of English grammar and the Editor and two anonymous reviewers for comments on the manuscript. This research was supported by grants from CONICET (PIP 2000), UNLP (Projects 396, 01/01/2002, and 452, 01/01/2005), and ANPCyT (IM 40-2000).

## References Cited

- Bellows, T. S., and R. G. van Driesche. 1999. Life table construction and analysis for evaluating biological control agents, pp. 103–124. In T. S. Bellows and T. W. Fisher (eds.), *Handbook of biological control. Principles and applications of biological control*. Academic, San Diego, CA.
- Bennett, F. D. 1995. Parasites of the pepper flower-bud moth (Lepidoptera: Gelechiidae). *Fla. Entomol.* 78: 546–549.
- Bezemer, T. M., and N. J. Mills. 2001. Host density responses of *Mastrus ridibundus*, a parasitoid of the codling moth, *Cydia pomonella*. *Biol. Control* 22: 169–175.
- Botto, E., S. Ceriani, S. López, E. Saini, C. Cédola, G. Segade, and M. Viscarret. 1998. Control biológico de plagas hortícolas en ambientes protegidos. La experiencia hasta el presente. *Revista de Investigaciones Agropecuarias* 29: 83–98.
- Cardona, C., and R. Oatman. 1971. Biology of *Apanteles dignus* (Hymenoptera: Braconidae), a primary parasite of the tomato pinworm. *Ann. Entomol. Soc. Am.* 64: 996–1007.
- Chesson, P. L., and W. W. Murdoch. 1986. Aggregation of risk: relationships among host-parasitoid models. *Am. Nat.* 127: 696–715.
- Colomo, M. V., D. C. Berta, and M. J. Chocobar. 2002. El complejo de himenópteros parasitoides que atacan a la "polilla del tomate" *Tuta absoluta* (Lepidoptera: Gelechiidae) en la Argentina. *Acta Zool. Lilloana* 46: 81–92.
- Consoli, F. L., J.R.P. Parra, and S. A. Hassan. 1998. Side-effects of insecticides used in tomato fields on the egg parasitoid *Trichogramma pretiosum* Riley (Hym. Trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). *J. Appl. Entomol.* 122: 43–47.
- Corvo Dolcet, S. 2005. Zonas de producción del cultivo del tomate en la Argentina. Secretaría de Agricultura, Ganadería, Pesca y Alimentos. (<http://www.sagpya.mecon.gov.ar/>).
- De Santis, L. 1983. Un nuevo género y dos nuevas especies de eulófidios neotropicales (Insecta, Hymenoptera). *Rev. Per. Entomol.* 26: 1–4.
- Ecole, C. C., M. C. Picanço, R. N. Guedes, and S. H. Brommonschenkel. 2001. Effect of cropping season and possible compounds involved in the resistance of *Lycopersicon hirsutum* t. *typicum* to *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). *J. Appl. Entomol.* 125: 193–200.
- Elliot, J. M. 1979. Statistical analysis of samples of benthic invertebrates. *Freshwater Biology Association Scientific Publication* 25, The Ferry House, Ambleside, UK.



- Faria, C., J. B. Torres, and A. M. Farias. 2000. Resposta funcional de *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) parasitando ovos de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): efeito da idade do hospedeiro. An. Soc. Entomol. Brasil 29: 85–93.
- Faria, C., J. B. Torres, A. M. Fernandes, and A. M. Farias. 2008. Parasitismo de *Tuta absoluta* in tomato plants by *Trichogramma pretiosum* Riley in response to host density and plant structures. Ciênc. Rural 6: 1504–1509.
- Fernández, S., and A. Montagne. 1990. Biología del minador del tomate *Scrobipalpa absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Bol. Entomol. Venez. 5: 89–99.
- García, M., M. E. Strassera, M. G. Luna, and N. Mezquiriz. 2005. Análisis técnico-económico en una producción de tomate bajo invernáculo del Cinturón Hortícola Platense. Bol. Hortícola 10: 31–37.
- Giustolin, T. A., J. D. Vendramin, S. B. Alves, S. A. Vieira, and R. M. Pereira. 2001. Susceptibility of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae) reared on two species of *Lycopersicon* to *Bacillus thuringiensis* var. *kurstaki*. J. Appl. Entomol. 125: 551–556.
- Hails, R. S., and M. J. Crawley. 1992. Spatial density dependence in populations of a cynipid gall-former *Andricus quercuscalicis*. J. Anim. Ecol. 61: 567–583.
- Haji, F. N., L. C. Freire, F. G. Roa, C. N. da Silva, M. M. Souza, Jr., and M. I. da Silva. 1995. Manejo integrado de *Scrobipalpaloides absoluta* (Povolny) (Lepidoptera: Gelechiidae) no submédio São Francisco River. An. Soc. Entomol. Brasil 24: 587–591.
- Hall, A. J., C. M. Ghersa, and J. P. Culot. 1992. Field-crop systems of the Pampas, pp. 413–450. In C. J. Pearson (ed.), Ecosystems of the world. 1. Field crop ecosystems. Elsevier Publishers, Amsterdam, The Netherlands.
- Hassell, M. P. 1982. Patterns of parasitism by insect parasitoids in patchy environments. Ecol. Entomol. 7: 365–377.
- Hassell, M. P. 2000. Host-parasitoid population dynamics. J. Anim. Ecol. 69: 543–566.
- Hassell, M. P., and R. M. May. 1973. Stability in insect host-parasite models. J. Anim. Ecol. 42: 693–726.
- Hassell, M. P., and R. M. May. 1974. Aggregation of predators and insect parasites and its effect on stability. J. Anim. Ecol. 43: 567–594.
- Heads, P. A., and J. H. Lawton. 1983. Studies on the natural enemy complex of the holly leaf-miner: the effects of scale on the detection of aggregative responses and the implication for biological control. Oikos 40: 267–276.
- Heimpel, G., and J. Casas. 2008. Parasitoid foraging and oviposition behavior in the field, pp. 51–70. In E. Wajnberg, C. Bernstein, and J. van Alphen (eds.), Behavioral ecology of insect parasitoids. Blackwell, Malden, MA.
- Jarošík, V., and L. Lapchin. 2001. An experimental investigation of patterns of parasitism at three spatial scales in an aphid-parasitoid system (Hymenoptera: Aphidiidae). Eur. J. Entomol. 98: 295–299.
- Juliano, S. A. 2001. Non-linear curve fitting: predation and functional response curves, pp. 178–196. In S. M. Scheiner and R. B. Gurevitch (eds.), Design and analysis of ecological experiments. Oxford University Press, New York.
- Landis, D. A., and F. D. Menalled. 1998. Ecological considerations in the conservation of effective parasitoid communities in agricultural systems, pp. 101–121. In P. Barbosa (ed.), Conservation biological control. Academic, San Diego, CA.
- Lange, W. H., and L. Bronson. 1981. Insect pests of tomato. Annu. Rev. Entomol. 26: 345–371.
- Lietti, M. M., E. Botto, and R. A. Alzogaray. 2005. Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotrop. Entomol. 34: 113–119.
- Lill, J. T. 1998. Density-dependent parasitism of the huckleberry nipplegall maker (Homoptera: Psyllidae): a multiple-scale analysis. Environ. Entomol. 27: 657–661.
- Luna, M. G., N. E. Sánchez, and P. C. Pereyra. 2007. Parasitism of *Tuta absoluta* (Lepidoptera: Gelechiidae) by *Pseudapanteles dignus* (Hymenoptera: Braconidae) under laboratory conditions. Environ. Entomol. 36: 887–893.
- Miranda, M. M., M. Picanço, G. L. Leite, J. C. Zanuncio, and P. de Clercq. 1998. Sampling and non-action levels for predators and parasitoids of virus vectors and leaf miners of tomato in Brazil. Med. Fac. Landbouw. Univ. Gent. 63: 519–526.
- Murdoch, W. W., and A. Oaten. 1975. Predation and population stability. Adv. Ecol. Res. 9: 1–131.
- Nyrop, J., G. English-Loeb, and A. Roda. 1998. Conservation biological control of spiders mites in perennial cropping systems, pp. 307–333. In P. Barbosa (ed.), Conservation biological control. Academic, San Diego, CA.
- Oatman, E. R., and G. R. Platner. 1989. Parasites of the potato tuberworm, tomato pinworm, and other closely related gelechiids. Proc. Hawaii. Entomol. Soc. XXIX: 23–30.
- Pacala, S. W., and M. P. Hassell. 1991. The persistence of host-parasitoid associations in patchy environments. II. Evaluation of field data. Am. Nat. 138: 584–605.
- Pereyra, P. C., and N. M. Sánchez. 2006. Effect of two solanaceous host plants on development and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae). Neotrop. Entomol. 35: 671–676.
- Pratissoli, D. J., E. Pezzone, M. D. Esposti, L. C. Bertazo, and J. M. Fornazier. 1998. Estimativa do número de gerações de *Trichogramma pretiosum* Riley na traça do tomateiro *Tuta absoluta* (Meyrick), com base nas exigências térmicas. An. Soc. Entomol. Brasil 27: 109–115.
- Razuri, V., and E. Vargas. 1975. Biología y comportamiento de *Scrobipalpa absoluta* (Meyrick) (Lep.: Gelechiidae) en tomatera. Rev. Per. Entomol. 18: 84–89.
- Reeve, J. D., and W. W. Murdoch. 1985. Aggregation by parasitoids in the successful control of the California red scale: a test of theory. J. Anim. Ecol. 54: 797–816.
- Siqueira, H. A., R. N. Guedes, and M. C. Picanço. 2000. Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). Agr. For. Entomol. 2: 147–153.
- Southwood, T.R.E. 1978. Ecological methods. Chapman & Hall, London, United Kingdom.
- Souza, J. C., and P. R. Reis. 1986. Controle de traça-do-tomateiro em Minas Gerais. Pesq. Agropec. Bras. 21: 343–354.
- StatSoft. 2004. Statistica user's manual, 6th ed. Statsoft, Tulsa, OK.
- Strong, D. R. 1989. Density independence in space and inconsistent temporal relationships for host mortality caused by a fairyfly parasitoid. J. Anim. Ecol. 58: 1065–1076.
- Torres, J. B., C. A. Faria, W. S. Evangelista, and D. Pratissoli. 2001. Within-plant distribution of the leafminer *Tuta absoluta* (Meyrick) immatures in processing tomatoes, with notes on plant phenology. Int. J. Pest Manag. 47: 173–178.
- Torres, J. B., W. S. Evangelista, R. Barras, and R. N. Guedes. 2002. Dispersion of *Podisus nigrispinus* (Het., Pentatomidae) nymphs preying on tomato leafminer: effect of predator release time, density and satiation level. J. Appl. Entomol. 126: 326–332.

- Trexler, J. C., and J. Travis. 1993. Nontraditional regression analysis. *Ecology* 74: 1629–1637.
- Trexler, J. C., C. E. McCulloch, and J. Travis. 1988. How can the functional response best be determined? *Oecologia* (Berl.) 76: 206–214.
- Uchoa-Fernandes, M. A., and W. G. Campos. 1993. Parasitoides de larvas e pupas da traça-do-tomateiro, *Scrobipalpuloides absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae). *Rev. Bras. Entomol.* 37: 399–402.
- Ulle, J. A., and O. Nakano. 1994. Damage assessment of *Scrobipalpuloides absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato plants with different levels of infestation. *An. Soc. Entomol. Brasil* 23: 155–162.
- van Lenteren, J. C., and G. Manzaroli. 1999. Evaluation and use of predators and parasitoids for biological control of pests in greenhouses, pp. 1–13. *In* R. Albajes, M. Lodovica Gullino, J. C. van Lenteren, and Y. Elad (eds.), *Integrated pest and disease management in greenhouse crops*. Kluwer Academic, Dordrecht, The Netherlands.
- van Veen, F. J., C. B. Müller, I. C. Adriaanse, and H. C. Godfray. 2002. Spatial heterogeneity in risk of secondary parasitism in a natural population of an aphid parasitoid. *J. Anim. Ecol.* 71: 463–469.
- Vargas, H. C. 1970. Observaciones sobre la biología y enemigos naturales de la polilla del tomate, *Gnorimoschema absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Idesia* 1: 75–110.
- Waage, J. K. 1983. Aggregation in field parasitoid populations foraging time allocation by a population of *Dia-degma* (Hymenoptera: Ichneumonidae). *Ecol. Entomol.* 8: 447–453.
- Wajnberg, E. 2006. Time-allocation strategies in insect parasitoids: from ultimate predictions to proximate behavioural mechanisms. *Behav. Ecol. Sociobiol.* 60: 589–611.
- Walde, S. J., and W. W. Murdoch. 1988. Spatial density dependence in parasitoids. *Annu. Rev. Entomol.* 33: 441–466.
- Zar, J. H. 1999. *Biostatistical analysis*. Prentice Hall, Upper Saddle River, NJ.

Received 11 July 2008; accepted 7 December 2008.

---