Cabello et al.: coincidental intraguild predation

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Can interactions between an omnivorous hemipteran and an egg parasitoid limit the level of biological control for the tomato pinworm?

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### Abstract

Relationships between the omnivorous predator Nesidiocoris tenuis (Reuter) and the egg parasitoid Trichogramma achaeae Nagaraja and Nagarkatti were studied in the laboratory (no choice and choice assays, and functional responses) and in a greenhouse experiment. Both natural enemies are utilized in the biological control of tomato pinworm on greenhouse-grown tomato crops. Three different food items were offered to the predator: non-parasitized prey, prey parasitized for less than 4 days by T. achaeae, and prey parasitized more than 4 days by the parasitoid. There were significant differences in consumption of food types, with highest consumption for non-parasitized prey followed by parasitized (< 4 days) and then parasitized (> 4 days), both in no-choice and choice trials. At the same time, the predator causes a significant mortality in the prey (over 80%) regardless of previous parasitism; resulting in a very coincidental intraguild predation detrimental to the parasitoid. It has also been observed that there was a change in the functional response by the predator from type II in presence of nonparasitized prey, to type I when there was a combination of parasitized and non-parasitized prey. This represents an increase of instantaneous search rate (a'), and a decrease of handling time  $(T_h)$  which indicates a change in feeding behavior on the two prey types. Under greenhouse conditions, the intraguild predation reduced the percentage of parasitism by T. achaeae in just over 20%. However, when both natural enemies were present, a better control of pest *Tuta absoluta* (Meyrick) was achieved than in the case of application of any of them alone.

Keywords: Omnivore, intraguild predation, prey preference, functional response, biological control.

#### Introduction

In biological control programs in greenhouses, such as those in Spain, several species of natural enemies (predators and parasitoids) are used at the same and/or different times throughout the crop cycle in order to control different pests using different release methods (Vila and Cabello 2014). In the case of Spanish tomato crops in greenhouses, augmentative biological control programs are mainly used in nurseries for the control of the white fly *Bemisia tabaci* (Gennadius) (Hem.: Aleyrodidae), by using inoculative releases of the omnivorous predator *Nesidiocoris tenuis* (Reuter) (Hem.: Miridae) (Gabarra *et al.* 2008, Vila and Cabello 2014), with inundative or inoculative releases of the egg-parasitoid *Trichogramma achaeae* Nagaraja and Nagarkatti (Hym.: Trichogrammatidae) to control the tomato pinworm, *Tuta absoluta* (Cabello et al. 2012, Vila and Cabello 2014).

*N. tenuis* is an omnivorous bug (Sanchez 2008), whose original distribution was palaeotropic, but has subsequently been introduced into Europe (Wheeler and Henry 1992, Rabitsch 2008). The use of this predator in augmentative biological programs remains somewhat controversial because it may behave as a pest of tomato as well as a predator (Wheeler 2000, Sanchez and Lacasa 2008), particularly in greenhouses (Sanchez 2009). Despite the concerns, other omnivorous arthropods, including true bugs, have been used in conservation and augmentative biological control programs in many parts of the world, both in greenhouse and field crop systems (Gillespie and Roitberg 2006). In contrast, *T. achaeae* is a parasitoid that attacks lepidopteran eggs (Cabello et al. 2009, Polaszek et al. 2012). It has a world-wide distribution (Polaszek et al. 2012, Pino et al. 2013).

The use of more than one natural enemy in augmentative biological control programs can lead to direct and indirect interactions such as apparent competition, intraguild predation, and resource competition (see Janssen et al. 1998). These interactions may impact the overall efficiency of these biological control agents (Yano 2005, Messelink et al. 2013). Interest in these interactions has resulted in a remarkable amount of research, both theoretical and experimental. However, studies show the impact of interspecific interactions on biological control are still rather scarce (Janssen et al. 1998). Therefore, more research is needed, especially long-term studies involving intraguild predation (IGP) (Rosenheim and Harmon 2006).

In the past 90 years, the predator-prey mathematical models have been built based on the functional responses as has been reviewed by Holling (1966), Royama (1971), May (1974); Hassell (1978); Jeschke et al. (2002). The functional response is a core component of predatorprey interactions and predator-prey population models, it can be crucial for understanding population dynamics (Harmon, 2003) and it is essentially the interpretation of a bio-assay system in which individual predators have access to fixed numbers of prey for a given period of time (Fenlon and Faddy, 2006).

From an experimental point of view, the functional response has been used to evaluate the effects of various factors, abiotic and biotic ones, such as: Temperature (Wang and Ferro, 1998; Mohaghegh *et al.* 2001; Garcia-Martin *et al.* 2008); sub-lethal insecticide doses (Claver *et al.* 2003); different predator species (Stewart et al, 2002.), different prey species or both (Ables et al, 1978.); intra-specific competition (Garcia-Martin et al. 2006, Martinou et al. 2010), interspecific competition (Cabello *et al.* 2011), etc. However, recently few works have focused on the impact of IGP on the functional response (Sentis et al. 2013).

The present work is aimed to assess whether IGP occurs between *T. achaeae* and *N. tenuis*. This was carried out throughout four sets of trials: the first three in the laboratory conditions. The no-choice test was used to assess the predator feeding on different prey eggs: Non-parasitized or parasitized. Subsequently, a choice test was performed to evaluate the predators' prey preference for the different types of prey eggs and also assess the mortality on both entomofagous. Also in the laboratory, as the mortality is a density-dependent factor, the functional responses of predator were assessed in the presence and absence of parasitized eggs. The results, as well as those from greenhouse trial, should allow to estimate the IGP impact when both entomophagous are used.

### **Materials and Methods**

**Biological material.** The specimens of *Trichogramma achaeae* and *Tuta absoluta* (Meyrick) (Lep.: Gelechiidae) used in the trials were obtained from natural populations collected in Fuencaliente, Isla de la Palma (28° 28' 43" N, 17° 51' 42" W), and Mazarron, Murcia (2 locations: 37° 32' 36" N, 1° 22' 28" W, and 37° 33' 51.64" N, 1° 3' 51" W), Spain, respectively.

*T. achaeae* was reared in the Lab of Agricultural Entomology at the University of Almeria (Spain) for 14 generations, while *T. absoluta* was reared for one generation prior to the onset experiments. *T. achaeae* was reared on eggs of the factitious host *Ephestia kuehniella* Zeller (Lep.: Pyralidae) eggs in 1000-ml plastic containers following the methodology of Cabello *et al.* (2012). Then, 12500 *E. kuehniella* eggs were stuck to 13×10.5-cm cardboard pieces, and one piece of the prepared cardboard was then placed in each container. Next, parasitoids were introduced at a ratio of 1:4. *T. absoluta* was grown according to the methodology devised by Marin *et al.* (2002), with the following modifications: 132-ml cylinders with (#1.5-mm) were used as mating and oviposition chambers. A tomato leaf was placed around the cylinder walls as a substrate for oviposition. Larval breeding was completed in 12-1 plastic containers with 6–7 tomato leaves that were replaced on a weekly basis up to

pupation. In both cases, breeding was completed under controlled climate conditions:  $25 \pm 1^{\circ}$ C, 60–80% relative humidity (RH), and 16:8-h light:dark (L:D) photoperiod.

A commercial colony of the predator, *Nesidiocoris tenuis* (Nesicontrol®, Agrobio S.L., Almeria, Spain) was used for the greenhouse trial; it was released within 2 h upon being received. For the remaining trials, mated adult females of *N. tenuis* were obtained from the populations kept at the Agricultural Entomology Laboratory. An alternative host/prey *E. kuehniella* was used in the non choice, choice, and functional response trials due to the complexity of rearing *T. absoluta* under laboratory conditions. *T. absoluta* cannot be reared on an artificial diet, and it is difficult to obtain large quantities of eggs in a timely fashion. However, it has been reported that both the parasitoid and predator have a good acceptation of *E. kuehniella*, and both have been reared on it (Cabello *et al.* 2009, Vila *et al.* 2012, Vila and Cabello, 2014).

#### No-choice trial.

*Experimental design and procedure.* The no-choice test was carried out to assess which type of host prey (non-parasitized or parasitized) would be used as food as well about comparing the level of consumption of *N. tenuis* females. The assay was arranged in a completely randomized design, with only one factor (at three levels or treatments), plus controls, and 12-18 replications per treatment and per control. Types of host eggs (treatments) were: (a) non-parasitized and untreated (non-irradiated, non-frozen) less than 72 h old since oviposition, taken from the populations kept in the Agricultural Entomology Lab at the University of Almeria; (b) *T. achaeae*-parasitized eggs, less than 4 days after their parasitization; and (c) *T. achaeae*-parasitized eggs, over 4 days after their parasitization. Only one type of host eggs listed above was offered to each single female, for a period of 24 hours. Likewise, controls for each prey treatment subjected to the above-mentioned procedure, were not exposed to *N. tenuis* female predation. All treatments and controls were carried out simultaneously during the test time.

Adult mated *N. tenuis* females less than 72 h old taken from the populations kept in the Entomology Lab at the University of Almeria were used. They were individually isolated in Petri dishes and starved for 24 h prior to trial. A distilled water-moistened sponge was provided as a water source.

*N. tenuis* females were introduced individually into a glass test tube ( $\emptyset$  1×7 cm) closed with cotton. The bottom of this tube already contained a distilled water-moistened sponge as well as a 1×5-cm cardboard piece containing 12 eggs corresponding to the above-mentioned types. The eggs were stuck on the cardboard's central part with no contact with the sponge, and they were attached to form two lines separated by 2 mm. The female was allowed to predate for 24 h at 25±1 °C under a 16:8 h L:D photoperiod. After this period, it was removed. Immediately afterwards predation symptoms were evaluated under a binocular microscope. Subsequently, the cardboard was introduced into the test tube and was left to evolve at the same temperature, for

two weeks, until the emergence of *E. kuehniella* or, if applicable, *T. achaeae*. The number of emerged larvae or adults from the above-mentioned species was registered.

*Statistical analyses.* The data obtained from the predated prey eggs, and the number of individuals that emerged from the eggs (prey species or parasitoids), did not meet the requirements for general linear model (GLM) analysis (Rutherford 2001, Ho 2006) owing to their lack of variance normality and homogeneity. According to Kolmogorov-Smirnov's and Leven's tests, these conditions were not met and no data transformation was found to allow the meeting of the requirements. Therefore, data were subjected to generalized linear models (GZLM) with the GENLIN procedure. The following model was used: Factor 1, Factor 2, and Factor 1 × Factor 2 (Factor 1 = prey type; Factor 2 = prey predation), using Poisson distribution and the loglinear link function. For this purpose, software package IBM SPSS 21 (IBM 2012) was used.

#### **Choice trial**

*Experimental design and procedure.* The choice test was used for feeding preferences and level of consumption of host eggs by *N. tenuis* females, when there were simultaneously present non-parasitized and parasitized host eggs. The assay was arranged in a completely randomized design, with only one factor (at four levels or treatments), plus three controls, and 12-18 replications per treatment and per control. Four different combinations (treatments) of host prey were used: (a) 6 non-parasitized eggs + 6 parasitized eggs (< 4 days), (b) 6 non-parasitized eggs + 6 parasitized eggs (< 4 days), (b) 6 non-parasitized eggs + 6 parasitized eggs (< 4 days) + 6 parasitized eggs (> 4 days), and (d) 12 non-parasitized eggs. Only one type of combinations listed above was offered to each single female, for a period of 24 hours. Likewise, controls for each egg type non – parasitized, parasitized (< 4 days), and parasitized (> 4 días)– were not exposed to *N. tenuis* female predation. All treatments and controls were carried out simultaneously during the test time. The procedure followed was the same as that mentioned earlier for the no-choice trial.

Statistical analysis. The procedures were the same as to those used in the no-choice trial. In addition, predator's preference for the prey's parasitized and non-parasitized eggs was studied. Accordingly, Manly's preference index ( $\alpha$ ) (Manly et al. 1972, Chesson 1978) was used, bearing in mind that Cock (1978) pointed out this index as the only method that takes into account lower prey density throughout trial development. This fact was corroborated by Sherratt and Harvey (1993). This index can be expressed as follows:

$$\alpha = \frac{\frac{r_i}{N_i}}{\frac{r_i}{N_i} + \frac{r_j}{N_j}}$$

where  $\alpha$  stands for the preference index,  $r_i$  and  $r_j$  are the ratios of consumed type-*i* and -*j* prey, respectively, and  $N_i$  and  $N_j$  are the ratios of type-*i* and -*j* prey available in each repetition, respectively.

**Functional response.** The functional response trials had been carried out using only adult females of *N. tenuis*; since both sexes have the same type of functional response (Wei et al. 1998); this has also been indicated for another hemipteran predator species (e.g.: Isenhour et al. 1990, Emami et al. 2014).

*Experimental design and procedure*. The trial was arranged in a completely randomized design, with only one factor: prey density (at seven levels or treatments). The number of replications for each treatment was 10. The age and handling procedures of adult females were the same as described for previous trials except that females were isolated in Petri dishes (0.97 cm), which contained a  $3\times3$ -cm white cardboard piece containing *E. kuehniella* eggs, separated by 2-mm and stuck only by a single stroke of a distilled water-moistened brush. The prey density levels used were 10, 30, 50, 80, 110, 140 and 170 eggs per cardboard piece. Each dish also contained a distilled water-moistened sponge. Predator females were removed after 24 h, and data were collected using the same procedure described in the no-choice trial.

*Statistical analysis*. Two types of statistical analyses were applied. First, logic regression was completed between the ratio of predator-killed preys and available prey density according to the polynomial function used by Juliano (2001) by means of the following equation:

$$\frac{N_e}{N_0} = \frac{EXP(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{1 + EXP(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}$$

where  $N_e$  is the number of killed preys,  $N_0$  is the initial value of available preys, and  $P_0$ ,  $P_1$ ,  $P_2$ and  $P_3$  stand for cut-off, linear, square, and cubic coefficients respectively, estimated according to the method of maximum likelihood.  $P_0$ — $P_3$  parameters were obtained by logic regression. The logic regression procedure and the method of maximum likelihood estimation were carried out using statistical software package Statgraphic Centurion XVI version 16.1.18 (Statgraphics 2010). Regarding the results, if coefficient  $P_1$  was significantly non-different from zero, it represented a type-I functional response (it was considered different from zero when the latter was not included in its confidence interval); a significantly negative value of  $P_1$  indicated type-II; and a significantly positive value of  $P_1$  indicated type-III.

Secondly, the data were adjusted to the three types of functional response, according to the expressions provided by Hassell (1978) and Cabello et al. (2007), as follows:

Type-I functional response:  $N_a = N[1 - EXP(-a'TP)]$ where  $N_a$  is the number of dead preys, N is the number of available preys, a' is the instantaneous search rate (days<sup>-1</sup>), T is the time available for search (days), and P is the number of predators. In this case P = 1 (predator) and T = 1 (day).

Type-II functional response:  $N_a = N \left\{ 1 - EXP \left[ a' P \left( T - T_h \frac{N_a}{P} \right) \right] \right\}$ where  $T_h$  is the handling time (days). Also, P = 1 (predator) and T = 1 (day). Type-III functional response:  $N_a = N \left\{ 1 - EXP \left[ -\frac{\delta \cdot N \cdot P}{1 + T_h (EXP(-\alpha) - 1)N} (T - T_h \frac{N_a}{P}) \right] \right\}$ where  $\delta$  measures the predation potential (values ranging from 0 to 1), and the remaining variables are similar to those in previous responses. Also P = 1 (predator) and T = 1 (day).

The adjustments to the previous equations were completed by non-linear regression using statistical software package Tablecurve 2D version 5.0 (Jandel Scientific 1994). To choose the best adjustment, the corrected Akaike criterion ( $AIC_c$ ) was used because it is a more precise statistic for comparisons among models than the regression coefficient ( $r^2$ ) (Motulsky and Christopoulus 2003). However, this coefficient was also calculated to determine the nonlinear regression adjustment's goodness.

## Functional response with parasitization.

*Experimental design and procedure*. The same foregoing procedure was used, but including the following differences: prey egg density was 10, 30, 50, 80, 110, and 170 eggs per cardboard piece. In addition, each of these density values corresponded to 50% of *T. achaeae*-parasitized (< 4 days) and non-parasitized eggs.

*Statistical analysis.* The same foregoing procedure was followed for testing the functional response, except that it was applied to the whole number of dead preys as well as to the types of preys used, both parasitized and non-parasitized eggs, respectively.

#### Greenhouse trial.

*Experimental design and procedure*. The trial design was completely randomized with one factor: treatment and two repetitions per treatment and with subsamples. The used treatments were as follows: parasitoid releases (*T. achaeae*), predator releases (*N. tenuis*), joint releases of both natural enemies and control.

The trial was developed between March 16 and April 23, 2012 in an Almeria-style greenhouse located in the municipality of La Mojonera, Spain ( $36^{\circ} 47^{\circ} 23.5^{\circ}$  N,  $2^{\circ} 42^{\circ} 6.2^{\circ}$  W). This greenhouse contained eight-m<sup>2</sup> mesh-closed ( $10 \times 20$  threads/cm) cages. In every cage, 20 tomato potted plants were used (Cultivar Josefina®, Philoseed España S.L., El Ejido, Almeria, Spain). At the onset trial, the plants had 13–16 leaves. Temperature and RH values were monitored by means of thermohydrographs (EBI 20-TH1, Ebro Electronic GmbH & Co. KG, Ingolstadt, Germany) placed inside the cages. Watering and fertilization were managed according to local commercial practices. All cages were inoculated on Day 1, which included releases of *T. tenuis* adults at a ratio of 4 individuals per plant (around 5 adults/m<sup>2</sup>).

*N. tenuis* release into the cages was completed by means of one release of adult specimens on March 19, 2012 (Day 4) at a ratio of 1 adult/plant (around 2.5 adults/m<sup>2</sup>). *T. achaeae* releases into cages were completed five times at a ratio of 100 adults/m<sup>2</sup> on March 22, 27, and 30, and on April 2 and 5 (Days 7, 12, 15, 18, and 21, respectively).

At present, *N. tenuis* is used in nurseries, at a dose of 0.4-0.5 adults/plant, resulting in a presence of nymphs on 90% of the plants 10 days after transplantation; or in greenhouses, at a dose up to 1.5 adults/m<sup>2</sup> (Vila et al., 2012; Vila and Cabello, 2014). *T. achaeae* is used with inundative releases at a dose up to 50 adults/m<sup>2</sup>. Thus, the dosages used in this trial are almost twice the commercially recommended for both natural enemies.

Three samples were completed on March 28 (Day 13), March 10 (Day 26), and March 23 (Day 39). The number of eggs, larvae, *T. absoluta* mines, and *T. achaeae*-parasitized eggs were determined. Eggs, larvae, and mines were counted in situ on a leaf in each of the high, medium, and lower sections of randomly selected plants (6 plants per cage). To assess parasitism, a total of 30–60 egg-containing leaves were randomly taken in each sample from the remaining 14 plants in each cage. The leaves were collected into labelled plastic bags, which were cold-stored. The total number of hatched and predated eggs was determined under a binocular microscope at the Entomology Lab at the University of Almeria. The remaining eggs were individually isolated and evolved at  $25\pm1^{\circ}$ C and 60-80% RH, until hatching, and *T. achaeae*-parasitization was determined. The number of *N. tenuis* nymphs and adults were evaluated in situ when the pest species populations were assessed.

*Statistical analyses.* To avoid overestimation of parasitism ratios, the following equation was used for parasitism calculation (Cabello *et al.* 2012):

$$\%P = \left(\frac{b_p}{b_e}\right) \cdot 100; b_i = \frac{A_i}{T_i}$$

where %*P* is the actual parasitism ratio,  $b_p$  is the total number of parasitized eggs,  $b_e$  is the total number of eggs entering this stage on each day,  $A_i$  is the area under the state frequency curve (total collection number), and  $T_i$  is days of development time.

The percentage of parasitism by *T. achaeae* was subjected to univariate general linear model (GLM) analysis with repeated measurements and arc-sine square-root transformed. The evolution of the *T. absoluta* population (eggs, larvae, and mines) was analyzed by comparing the effects of treatment factors and sampling by means of GZLM following the abovementioned procedure for the choice and no-choice trials. All analyses were done with the statistical software package IBM SPSS 21 (IBM 2012).

#### Results

**No-choice trial.** Prey consumption in the no-choice trial is presented in Fig. 1. For nonparasitized eggs and parasitized eggs (< 4 days), prey were acknowledged as "consumed" if only the chorion or egg external structure was left and when the chorion appeared creased. In the case of parasitized eggs (> 4 days), they were considered as consumed if they had a normal structure, but there was no content inside. The designation "collapsed" corresponds to creased chorion, yet also with partial contents inside; collapsed eggs may correspond to partial prey consumption by the predator, and they can also be due to accidental chorion breakage in experimental manipulation, which can lead to partial egg emptying with no predation. The eggs labelled as "normal" were those presenting fully turgid chorion and whole content inside, as well as showing no apparent symptoms of predation under the binocular microscope.

GZLM adjustment of consumed prey showed that residual deviance (46.217) was lower than twice the degrees of freedom (df = 42), which means that — according to Anderson et al. (1994) — the use of the Poisson distribution led to no over-dispersion problems. In the Omnibus test, when the adjusted model was compared with the model including only the intersection, it was observed that the model-explained variance exceeded the one that remained unexplained (likelihood ratio  $\chi^2 = 195.520$ , df = 2, P < 0.0001). Likewise, in the model-effect testing a highly significant effect was found in the type of eggs available for predatory females (Wald  $\chi^2 = 99.975$ , df = 2, P < 0.0001).

These results point out that the predator showed significantly greater consumption of non-parasitized prey eggs, followed by parasitized ones (< 4 days first, then > 4 days) (Fig. 1). In addition, the number of normal eggs (apparently not consumed) increased inversely to that of consumed eggs.

Fig. 2 shows the number of emerged specimens (*E. kuehniella* first-instar larvae or *T. achaeae* adults) from the different prey eggs used for the no-choice trial and their relation to controls.

GZLM analysis of the emerged specimens was found to present no over-dispersion problems (deviance/df = 0.987). The Omnibus test showed the high significance of the modelexplained variance (likelihood ratio  $\chi^2 = 413.298$ , df = 5, P < 0.0001). Both the analyzed factors (egg type, and predation) had equally significant effects on the emergence of individuals from these eggs (Wald  $\chi^2 = 25.525$ , df = 2, P < 0.0001, and Wald  $\chi^2 = 174.610$ , df = 1, P < 0.0001, respectively), as well as their interaction (Wald  $\chi^2 = 22.045$ , df = 2, P < 0.0001).

Controls presented no significant differences among them regarding phytophagous larval and parasitoid emergence (Fig. 2). No differences were observed among individuals which emerged from non-parasitized or parasitized eggs (< 4 days), but there were differences between these two types and parasitized eggs (> 4 days). The values for the mortality were 95.0, 96.7, and 80.0%, respectively for non-parasitized eggs, parasitized (< 4 days), and parasitized (> 4 days).

**Choice trial.** The prey consumption in the choice trial, according to available prey typology, is shown in Fig. 3. GZLM analysis of consumed prey showed no over-dispersion problems (deviance/df = 0.727). The Omnibus test also showed high significance in the model-explained variance (likelihood ratio  $\chi^2 = 100.738$ , df = 342, P < 0.0001). The model-analyzed factor (prey

typology) had a highly significant impact on the number of consumed prey eggs (Wald  $\chi^2$  = 84.395, df = 3, P < 0.0001). The mean values of the consumed eggs per treatment differed significantly.

The prey preference index ( $\alpha$ ) also reflected these results (Table 1). The values of this index indicate indifference, if equal to 0.5, rejection if below 0.5, and attraction when over 0.5. As shown in Table 1, the predator showed clear rejection to the consumption of parasitized eggs (> 4 days) in the presence of both non-parasitized and parasitized eggs (< 4 days): 0.03 and 0.08, respectively. Conversely, it showed high preference for non-parasitized eggs and intermediate preference for parasitized eggs (< 4 days).

No treatment effect was found on the number of *E. kuehniella* or *T. achaeae* individuals which emerged in the choice trial (Fig. 4) in the GZLM analysis (Wald  $\chi^2 = 2.623$ , df = 3, P = 0.453). The mortality was 97.9 % in *E. kuehniella* when non-parasitized eggs were exposed to the predator; 96.0 % in *E. kuehniella* and 96.0 % in *T. achaeae* for non-parasitized eggs and parasitized (< 4 days); 91.2 in *E. kuehniella* and 94.2 % in *T. achaeae* for non-parasitized eggs and parasitized (> 4 days), and 96.8 % in *T. achaeae* for parasitized eggs (< 4 days) and (> 4 days).

**Predator functional response.** In the logit regression analysis, according to the polynomial function of Juliano (2001), a value for  $P_1$  (estimate = -0.0206, SE = 0.0034 and CI = -0.0292 to -0.0119) was found which was significantly negative throughout the whole confidence interval, which would indicate that the functional response is type-II. This was confirmed by fitting the three types of functional response to the data, according to the equations of Hassell (1978) and Cabello et al. (2007), and their subsequent comparison by means of the corrected Akaike index (*AIC<sub>c</sub>*). The type-II functional response (Fig. 5) showed the lowest value in this index (*AIC<sub>c</sub>* = 11.22) and it presented the following parameters: a' = 2.5945 (±0.3813) days<sup>-1</sup> and  $T_h = 0.0043$  (±0.0007) days ( $R^2 = 0.9948$ , df = 6, P < 0.001).

Effect of parasitization on the predator's functional response. Prey consumption — according to the available prey typology — is shown in Fig. 6. GZLM analysis showed no overdispersion problems (deviance/df = 1.297). The Omnibus test also found high significance in the model-explained variance (likelihood ratio  $\chi^2 = 1659.751$ , df = 11, P < 0.001). Prey-density (Wald  $\chi^2 = 37.668$ , df = 5, P < 0.001), previous parasitization (Wald  $\chi^2 = 172.150$ , df = 1, P < 0.001), and interaction (Wald  $\chi^2 = 11.586$ , df = 5, P = 0.041) had significant effects on prey consumption by predatory females.

Estimation according to Juliano's (2001) methodology indicated that when half of the available eggs have been previously parasitized by *T. achaeae* (< 4 days), the functional response for both parasitized and non-parasitized eggs, and also for the whole sample, was type-I ( $P_I = -0.0697$ , SE = 0.0703, and CI = -0.3720 to 0.2326;  $P_I = -0.0304$ , SE = 0.0418, and CI = -0.2104 to 0.1495; and  $P_I = -0.0119$ , SE = 0.0172, and CI = -0.0861 to 0.0623; respectively).

This was confirmed by fitting to the three types of functional response to the data, according to the equations of Hassell (1978) and Cabello *et al.* (2007) which is confirmed to be of Type-I, both for total prey, with a value of a' = 1.0895 days<sup>-1</sup> ( $AIC_c = 6.57$ ) (Fig. 7), as when they were analyzed separately, a' = 1.6958 (±0.0593) (F = 1548.61; df = 5; P < 0.001), and 1.7860 (±0.1894) (F = 164.3571; df = 5; P < 0.001) for parasitized and non-parasitized eggs, respectively (Fig. 8).

**Influence of IGP on biological control of** *T. absoluta.* The evolution of egg and larval populations, as well as their damages, for the pest species *T. absoluta* in tomato plants is presented in Figs. 9, 10, and 11 for all treatments: biological control with parasitoid only, biological control with predator only, joint releases of both natural enemies, and control.

In the GZLM analysis of the number of *T. absoluta* eggs by leaf, although the deviance coefficient/*df* exceeded 2 (4.06), over-dispersion was discarded because it did not exceed 5 (Anderson et al., 1994). In the Omnibus test, high significance was found for the model-explained variance (likelihood ratio  $\chi^2 = 864.923$ , df = 11, P < 0.0001). The two factors: treatment and sampling date, had highly significant effects on the production of the number of eggs (Wald  $\chi^2 = 17.948$ , df = 3, P < 0.0001, and Wald  $\chi^2 = 414.571$ , df = 2, P < 0.0001, respectively), and there was also a significant as well as on their interaction between the two factors (Wald  $\chi^2 = 90.865$ , df = 6, P < 0.0001). Fig. 9 shows that the number of pest eggs per leaf was very similar between the control and the *T. achaeae* (parasitoid) treatment. On the contrary, the inclusion of the predator, *N. tenuis*, significantly reduced egg numbers.

In the GZLM analysis of the number of pest larvae per leaf (Fig. 10) (deviance/df = 1.983 < 2), high significance was found for the model-explained variance (likelihood ratio  $\chi^2$  = 439.456, df = 11, P < 0.0001). The factors analyzed, treatment, and sampling date had highly significant effects on the production of the number of *T. absoluta* larvae (Wald  $\chi^2$  = 93.724, df = 3, P < 0.0001, and Wald  $\chi^2$  = 10.239, df = 2, P = 0.006; respectively), as well as on their interaction (Wald  $\chi^2$  = 56.975, df = 6, P < 0.0001). Similarly, GZLM analysis for *T. absoluta* damages (Fig. 11) (deviance/df = 3.030 < 5) revealed high significance for the model-explained variance (likelihood ratio  $\chi^2$  = 1568.353, df = 11, P < 0.0001). In addition, significant effects were observed for both treatment and sampling date (Wald  $\chi^2$  = 18.685, df = 3, P < 0.0001, and Wald  $\chi^2$  = 12, P < 0.0001, respectively), as well as for their interaction (Wald  $\chi^2$  = 117,696, df = 6, P < 0.0001, respectively).

Unlike the number of eggs (Fig. 9), the values of larvae and pest damage, particularly towards the end of the trial, were significantly higher in the control group, followed by plots in which only the predator was released. Below these values were plots with parasitoid-only releases, followed by plots with joint releases of both natural enemies (Fig. 10 and 11).

Fig. 12 shows the production of *T. achaeae* parasitism in plots where they were released, either alone or jointly with the predator. GLM analysis showed that the presence of the

predator had a significant effect on the percentage of parasitism (F = 22.893, df = 1, P = 0.041). In all three samplings, the average parasitism values were significantly lower in the presence of *N. tenuis*, than when it was absent.

The production of *N. tenuis* populations (nymphs and adults) in plots where *N. tenuis* was released was very similar to plots with parasitoid only (Fig. 13). No effects were related to parasitoid presence. The values obtained in the present study correspond to regular *N. tenuis* settling and colonization values in greenhouse tomatoes (Vila et al. 2012).

#### Discussion

The data obtained from lab trials showed the existence of IGP by *N. tenuis* adult females, which attack eggs that had previously been parasitized by *T. achaeae*, which Rosenheim and Harmon (2006) referred to as the "intermediate predator". It partially confirms the results found for the same parasitoid specie in relation to another predator *Macrolophus pygmaeus* (Rambur) (Het: Miridae) (Chailleux *et al.* 2013).

According to Polis et al. (1989), these types of interactions can be considered as "omnivorous" intraguild predation (OIGP), or "coincidental intraguild predation" (CIGP). OIGP occurs without a joint attack on the herbivore when one predator encounters and consumes another predator. In contrast, CIGP, occurs most often when a predator (the IG predator) attacks an herbivore that has previously been attacked by a parasitoid (or a pathogen), and which therefore harbors a developing offspring of the parasitoid (the IG prey). Both types can be asymmetrical if one of the two species (the IG predator) prey on the other (the IG prey), or symmetrical when both species prey on each other to a greater or lesser extent (Pell et al. 2008). By the results found for *N. tenuis-T. achaeae*, it is evident to see it not as an asymmetric CIGP (Perhaps this CIGP should be called non-reciprocal, rather than asymmetric).

For both CIGP and OIGP, Rosenheim and Harmon (2006) take into account that a key determinant of the overall implications for biological control is the IG predator's preference for consuming the IG prey (or intermediate predator) versus the herbivore, and they suggest that OIG predators may be more likely to exhibit a preference for consuming the intermediate predator than CIG predators. Thus, our case seems to confirm the above mentioned hypothesis. The prey consumption of *N. tenuis* is higher in non-parasitized than in parasitized eggs. The latter was those with lower parasitoid development in both the non choice (Fig. 1) and choice (Fig. 3) trials. This was also pointed out by the values of the prey preference index in this trial (Table 1).

This lower consumption may be primarily due to the presence of melanin in prey eggs with parasitoids in the prepupal stage. For example, several authors have noted that hosts parasitized by *Trichogramma* species turn black due to the deposition of melanin-containing granules on the internal surface of the chorion at the beginning of the third-instar (Clausen

1940, Metcalfe and Breniere 1969, Alrouechdi and Voegele 1981). As reviewed by Pintureau *et al.* (1999), these substances serve a number of functions, including protection against natural enemies. One mechanism shown by Alrouechdi and Voegele (1981) was mechanical protection of the parasitoid inside the host egg from first-instar larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). However, protection did not extend to later lacewing instars. In addition, Pintureau *et al.* (1999) hypothesized that substances inside host eggs allow faster parasitoid development which reduces the time of exposure to predators and, therefore, the risk of IGP.

Other hypotheses for predator avoidance of *Trichogramma*-parasitized egg may be relate to substances other than those mentioned above, such as the venom injected by adult female parasitoids or induced changes they cause in the host, including tissue necrosis (Takada *et al.* 2000, Jarjees and Merritt 2003, 2004). On the other hand, parasitoid larvae may themselves produce substances other than melanin (Jarjees *et al.* 1998, Wu *et al.* 2000).

The direct effects of one or several of the above-mentioned substances — which vary throughout the development of immature parasitoids — may explain the significant differences observed in the consumption of both types of parasitized eggs (< and > 4 days) relative to non-parasitized eggs.

The literature contains several examples where predator species show no preferences between non-parasitized and parasitized eggs by *Trichogramma*. These include *Orius insidiosus* (Say) (Hem.: Anthocoridae) (Lingren and Wolfenbarger 1976), *Chrysoperla carnea* Stephens (Neur.: Chrysopidae) (Alrouechdi and Voegele 1981), and *Coleomegilla maculata* De Geer (Col.: Coccinellidae) (Roger et al. 2001). However, other studies have reported predator preference for non-parasitized eggs, e.g., *Xylocoris flavipes* (Reuter) (Hem.: Anthochoridae) (Brower and Press 1988) or *Podisus maculiventris* Say (Hem.: Pentatomidae) (Oliveira et al. 2004). Rosenheim and Harmon (2006) indicated that the most common result is that predators do not distinguish between parasitized and non-parasitized individuals early in the parasitoid's development, but later develop an increasing preference for non-parasitized hosts. This pattern is consistent with the consumption behavior we observed in *N. tenuis*.

Rosenheim and Harmon (2006) considered multiple published experimental examples and concluded that CIGP may have less potential for disrupting biological control than OIGP. Therefore, the negative impact of *N. tenuis* on *T. achaea* may not reduce the level of biological control of *T. absoluta* in programs where both natural enemies are released together. However, if *N. tenuis* consumption (Fig. 1 and 3) is compared with prey survival (Fig. 2 and 4) in no-choice and choice trials, it shows that the prey/pest mortality caused by the predator was high in parasitized eggs (96.7 and 80.0 %) and similar to that of non-parasitized (95.0 %). Those values are as much as 10 times larger than the ones found by Chailleux et al. (2013) for *M. pygmaeus* also in eggs parasitized by *T. achaeae*, which showed different predation behavior between the

two species *N. tenuis* and *M. pygmaeus*. Moreover, as shown in Fig 1 and 3, the consumption of parasitized eggs, especially for > 4 days, as well as the mortality in them (Fig. 2 and 4) were lower in the no-choice than in the choice trial.

Pest mortality due to *N. tenuis*, mainly parasitized eggs (> 4 days), when few or no eggs were consumed (Fig. 1 and 3), seems to be due to probing behavior of the predator. On one hand, it is known that phytophagous cimicomorphs (tingids and phytophagous myrids) are all plant-feeders and utilize the lacerate-and-flush strategy (Bacus, 1988), producing mechanical damage with their serrate mandibular stylets (Raman and Sanjayan 1984, Raman et al. 1984, Wheeler 2001). On the other hand, *N. tenuis* is known to inject several substances, including oral pre-digestive enzymes, which are also found in other heteropteran families, into their prey causing tissue damage (Cohen 1990, 1995). If, after the feeding probes, the prey are not accepted by the predator, the prey may die. This could explain the mortality found either in the immature parasitoids stage, or in the non-consumed prey eggs. Thus, there is additional mortality to parasitioids that may occur other than from true IGP.

Our results from the no-choice and choice trials involving the interaction between *N*. *tenuis* and *T. achaeae*, at first glance, do not support the assertion of Rosenheim and Harmon (2006), that in cases of CIGP, the IG predator will impose mortality on the IG prey or "intermediate" predator population that is often similar in magnitude to the mortality it imposes on the herbivore, as discussed below.

The functional response presented by adult *N. tenuis* females — according to Fig. 5 — is type-II for the use of *E. kuehniella* eggs as prey. This type of response seems the commonest in this species with different prey: Aphids, whiteflies, eggs and larvae of Lepidoptera (Wei et al., 1998, Boabin et al. 1999, Ling-Rui et al. 2008), and also common in other Heteroptera predatory species (Foglar et al. 1990, Isenhour et al. 1990, Montserrat et al. 2000, Emami et al. 2014).

On the contrary to this, the functional response of *N. tenuis* in the presence of *T. achaeae*-parasitized eggs (< 4 days) — as pointed out in Fig. 6-7 — changes from type-II to type-I. This is caused by changes of the values of *a*' (instantaneous search rate) from  $1.09\pm0.19$  to  $2.59\pm0.38$  days<sup>-1</sup>, and  $T_h$  (handling times) from  $0.004254\pm0.00074$  days to 0. As *a*' is the average of prey encounters per prey and per unit of searching time, there are more encounters between predator and prey in the presence of parasitized prey; probably because the predator does not accept parasitized eggs. At the same time, the non-acceptance causes that  $T_h$  tends to zero. A short handling time increases the time available for search and hence the likelihood of finding further prey (Hassell1978). This seems to support the aforementioned, in non-choice and choice trials, that there is an additional mortality in parasitioids that may occur for a reason other than true IGP. But at a high prey density (> 100) (Figs 5 and 7), the effects of parasitism are not so marked, or are even favorable for non-parasitized prey. This may be important from a

theoretical point of view; but at the practical level of biological control in greenhouses, it seems uncommon in normal situations.

According to the reviewed literature, natural enemies can change their type of functional response (of type I to II or II to III), which implies a reduction in the natural enemy's efficacy because they face different prey or host types or environmental conditions. Thus, the generalist predator C. maculata increased handling time  $(T_h)$  in the presence of T. evanescens Westwood-parasitized eggs (Roger et al. 2001). For the case of N. tenuis, change of type-II to type-I is reported in the literature for the first time. This may be associated with two possible hypotheses: A change in the predatory behavior of N. tenuis because parasitized eggs are less nutritious (or worse structured) than non-parasitized eggs, or due to the above-mentioned substances which could produce prey rejection. This has been reported in the case of Trichogramma-parasitized eggs and the larvae of predator C. maculata, which frequently leaves aside parts of the eggs and often removes the parasitoid pupae without attempting to consume them (Roger et al. 2001), even though the food quality does not always seem to be a determining factor. The predator Geocoris punctipes Fallen (Hem: Geocoridae) faces two prey: eggs of corn earworm *Helicoverpa zea* (Boddie) (Lep.: Noctuidae) that are nutritionally superior to pea aphids *Acyrthosiphum pisum* Harris (Hem: Aphididae), but attack the nutritionally inferior prey, which is pea aphids (Eubanks and Denno 2000).

The results found in functional response assays, in which an increase of the predator attack rate was observed in the presence of parasitized eggs, seem to corroborate the results of no-choice and choice assays; there were a higher consumption and mortality of parasitized eggs when offered in various combinations than when offered separately.

Another hypothesis, different from the mentioned above, could explain our results: the elimination of a competitor. Thus, Pell et al. (2008) have stated that the IG predator not only benefits from the nutritive value of prey, but also from the removal of a competitor. But, this hypothesis must be assessed in subsequent studies in our case.

In summary, according to the results, we could advance two alternative assumptions: (1) the parasitized prey eggs are not accepted by the predator, which induces a higher attack rate (for an increase of the value of a' the instantaneous search rate) or a more speculative one: (2) Another possible assumption might be the elimination of a competitor.

In support of the first hypothesis, and based on data found in greenhouses, a better pest control results when both natural enemies are used (Fig. 9, 10 and 11) without reducing the number of predators (Fig. 13) with the presence of parasite species; especially in the last sampling. This supports an increase of the attack rate also expressed in the last sampling, in the presence of a smaller number of eggs and larvae in parasitoid+predator plots than in the other plots (Figs. 9 and 10). Probably there was a higher mortality in *T. absoluta* egg (as prey) — this

supported by the results found in laboratory assays concerning the change of functional response type — as well as in larvae (as extraguild prey).

Thus, on one hand, it has been cited that after 70 minutes since egg hatching, *T. absoluta* first-instar larvae are totally encased inside the tomato leaves (Cuthbertson et al., 2013); later, second-instar larvae frequently leave the mines and walk on the leaves especially during the morning hours (Coelho et al. 1984, Haji et al. 1988, Torres et al. 2001); finally, Torres et al (2001) and Cely et al. (2010) stated that third- and fourth-instar larvae move to other parts of the plant diminishing their mobility in the prepupal stage. On the other hand, according to Urbaneja et al. (2009) *N. tenuis* could kill *T. absoluta* larvae with percentages up to 31.25, 12.5 and 6.25 for first-, second-, third- and fourth-instar, respectively. It is probably an increased *T. absoluta* larval mortality on the plots with the presence of eggs parasitized by *T. achaeae*. Furthermore, it must be pointed out that among heteropteran predators, the increase in extraguild prey leads to increased IG prey survival (Lucas and Rosenheim 2011, Jaworski et al. 2013).

The latter synergy effect of the parasitoid (IG prey) and the predator (IG predator) on phytophagous (pest) biological control has been proven in numerous empirical studies (Snyder and Ives 2008). We note that a generalization of our previous dynamic optimal foraging model (Garay *et al.* 2012) to the present tree-species system (host/prey-parasitoid-omnivore predator) might also give a theoretical insight into the coexistence problem of this system, thereby giving a theoretical answer to the question formulated in the title of the present study.

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Finally, from the point of view of the application of both natural enemies in greenhouse crops of the Mediterranean area, a quick comparative cost analysis can be done, at Spanish prices of 2014: (a) Only one release of *N. tenuis*, at commercial dose of 0.5 adult/plant (+ releases of *E. kuehniella* eggs as prey), at nurseries costs  $0.05 \notin / m^2$ . (b) Each *T. achaeae* release, also at commercial dose of 50 adults/m<sup>2</sup> costs  $0.00029 \notin /m^2$  (as free parasitized pupae) or  $0.00045 \notin /m^2$  (as card with 2000 parasitized pupae). A simple equivalence is that 127-197 releases of the parasitoid have the same cost as one release of the predator. However, the problem is not simply a cost analysis. In fact, *N. tenuis* is always used in greenhouse tomatoes for whitefly control (Vila and Cabello, 2014), and this predator species is also a promising biocontrol agent of *T. absoluta* (Sanchez et al. 2014).

When do *T. absoluta* control problems arise? In two situations: (1) when predator populations are not established at the start of the crop cycle, as reported by Cabello et al. (2012). This problem has been partly solved by changing the timing and method of predator's releases. Instead of inoculative releases in the first weeks of the crop cycle, these are usually carried out earlier at nurseries; although there are farmers who still use the first method (Vila et al., 2012; Vila and Cabello, 2014). (2) When there are unexpected increases in pest population during the crop cycle (population outbreaks in the crops or high immigration into the greenhouse). In this situation, the predator population does not respond quickly enough to control the pest population, as recently it has been reported by Sanchez (2014).

Based on this, at present, biological control *T. absoluta* on greenhouse must be a flexible system; relying primarily on the predator species, that is necessary for whitefly control, as noted; but in the possible lack of control of *T. absoluta*, *T. achaeae* releases are absolutely necessary.

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**Table 1**. Mean ( $\pm$ SE) values for the prey consumption preference (Manly's index  $\alpha$ ) of adult females of predator *N. tenuis* in a choice trial on non-parasitized and *T. achaeae*-parasitized (< 4 and > 4 days) prey eggs under lab conditions (25 $\pm$ 1°C, 60–80% RH, and 16:8 h L:D cycles) (*i* and *j* types of prey in columns).

Treatment		Prey preference index (α)		
<i>i</i> + <i>j</i>		i	j	Sum
Non-parasitized	Parasitized (< 4 days)	$0.66 (\pm 0.03)$	$0.34 (\pm 0.03)$	1.00
Non-parasitized	Parasitized (> 4 days)	$0.98 (\pm 0.01)$	$0.03 (\pm 0.01)$	1.00
Parasitized (< 4 days)	Parasitized (> 4 days)	0.67 (±0.11)	0.08 (±0.04)	0.75

**Fig. 1**. Mean (±SE) number of prey consumption in a no-choice trial with *N. tenuis* adult females on non-parasitized and *T.achaeae*-parasitized (< and > 4 days) under lab conditions ( $25\pm1^{\circ}$ C, 60–80% RH, and 16:8 h L:D cycles) (Bars followed by different letters indicate significant differences at *P* = 0.05 by means of pair comparisons of the estimated marginal means based on the dependent variable's original scale in the GZLM analysis).

**Fig. 2**. Mean ( $\pm$ SE) number of emergence of *E. kuehniella* larvae or *T. achaeae* adults from non-parasitized and parasitized (< and > 4 days) eggs when exposed to *N. tenuis* adult females in a no-choice trial under lab conditions (25 $\pm$ 1°C, 60–80% RH, and 16:8 h L:D cycles) (Bars followed by different letters indicate significant differences at *P* = 0.05 by means of pair comparisons of the estimated marginal means based on the original scale of the dependent variables in the GZLM analysis).

**Fig. 3.** Mean ( $\pm$ SE) number of prey consumption of *E. kuehniella* non-parasitized and parasitized (< and > 4 days) eggs by *N. tenuis* female adults in a choice trial under lab conditions (25±1°C, 60–80% RH, and 16:8 h L:D cycles) (Bars followed by different letters indicate significant differences at *P* = 0.05 by means of pair comparisons of the estimated marginal means based on the original scale of dependent variables in the GZLM analysis).

**Fig. 4**. Mean ( $\pm$ SE) number of emergence of *E. kuehniella* larvae or *T. achaeae* adults from non-parasitized and parasitized (< and > 4 days) eggs when exposed to *N. tenuis* adult females in a choice trial under lab conditions (25 $\pm$ 1°C, 60–80% RH, and 16:8 h L:D cycles) (Bars followed by different letters indicate significant differences at *P* = 0.05 by means of pair comparisons of the estimated marginal means based on the original scale of the dependent variables in the GZLM analysis).

**Fig. 5**. Mean ( $\pm$ SE) number of dead *E. kuehniella* eggs by the feeding activity of *N. tenuis* and predicted values according to a type-II functional response model at different density levels under lab conditions (25 $\pm$ 1°C, 60–80% RH, and 16:8 h L:D cycles).

**Fig. 6**. Mean (±SE) number of *E. kuehniella* eggs consumed by *N. tenuis* adult females at different density levels under lab conditions, when 50% had previously been parasitized eggs by *T. achaeae* (25±1°C, 60–80% RH, and 16:8 h L:D cycles) (Bars followed by different letters indicate significant differences at P = 0.05 by means of pair comparisons of the estimated marginal means based on the original scale of the dependent variables in the GZLM analysis).

**Fig. 7**. Mean ( $\pm$ SE) number of dead *E. kuehniella* eggs by the feeding activity of *N. tenuis* and predicted values according to a type-I functional response model at different density levels (50% of these had previously been parasitized by *T. achaeae*) under lab conditions (25 $\pm$ 1°C, 60–80% RH, and 16:8 h L:D cycles).

**Fig. 8**. Mean ( $\pm$ SE) number of dead *E. kuehniella* eggs by the feeding activity of *N. tenuis* and predicted values according to a type-I functional response model at different density levels (50% of these had previously been parasitized by *T. achaeae*) under lab conditions (25 $\pm$ 1°C, 60–80% RH, and 16:8 h L:D cycles) when non-parasitized and *T. achaeae*-parasitized eggs were analyzed separately.

**Fig. 9**. Mean ( $\pm$ SE) number of *T. absoluta* eggs per leaf in a greenhouse tomato crop, according to the strategy for pest control: parasitoid releases (*T. achaeae*), predator releases (*N. tenuis*), and joint releases of both, as well as control (no natural enemies).

**Fig. 10**. Mean ( $\pm$ SE) number of *T. absoluta* larvae by leaf in a greenhouse tomato crop, according to the strategy developed for pest control: parasitoid releases (*T. achaeae*), predator releases (*N. tenuis*), and joint releases of both, as well as control (no natural enemies).

**Fig. 11**. Mean ( $\pm$ SE) number of *T. absoluta* mines per leaf caused by *T. absoluta* larvae in a greenhouse tomato crop, according to the pest control strategy: parasitoid releases (*T. achaeae*), predator releases (*N. tenuis*), and joint releases of both, as well as control (no natural enemies).

**Fig. 12**. Mean ( $\pm$ SE) number of *T. achaeae* parasitism in *T. absoluta* eggs in a greenhouse tomato crop, in plots where parasitoid *T. achaeae* releases and joint parasitoid and predator *T. tenuis* releases were completed.

**Fig. 13**. Mean ( $\pm$ SE) number of *N. tenuis* total population (nymphs and adults) in a greenhouse tomato crop, in plots where *N. tenuis* and/or parasitoid *T. achaeae* had been released.

Figure 1.



Figure 2.



# Figure 3.



# Figure 4.



Figure 5.







Figure 7.



Figure 8.







Figure 10.







Figure 12.



Figure 13.

