Susceptibility of the egg parasitoid *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae) to selected insecticides used in tomato greenhouses

Juan R. Gallego, Jesús Guerrero-Manzano, Francisco J. Fernández-Maldonado and Tomás Cabello

*University of Almería, Dept. Biology and Geology, Ctra. Sacramento s/n, 04120 Almería, Spain.*

**Abstract**

The South American tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a pest species of great economic importance in tomatoes, both in greenhouses and in open-air crops. This importance has increased in recent years because it has been introduced in many countries in Europe, Africa, and Asia. Insecticides different active ingredients and biological control agents are being used in the control of this pest species. This implies the need to make both groups compatible within IPM programmes. Therefore, the objective of this work was to study the compatibility between different insecticides and the use of the egg parasitoid *Trichogramma achaeae* Nagaraja and Nagakartti (Hymenoptera: Trichogrammatidae). Three groups of trials were carried out under laboratory and greenhouse conditions. Ten insecticides with the following active ingredient were evaluated: abamectin, azadirachtin, *Bacillus thuringiensis*, chlorantraniliprole, emamectin, flubendiamide, indoxacarb, methomyl, spinosad, and spiromesifen. In the results, three groups of insecticides were established based on their compatibility with the use of biological control: The first group (abamectin, *B. thuringiensis*, flubendiamide, indoxacarb and spiromesifen) showed a high degree of compatibility with egg parasitoid releases. The second group (azadirachtin and chlorantraniliprole, and methomyl) presented compatibility problems. Finally, the last group (emamectin, methomyl, and spinosad) did not appear to be compatible. The results found will allow a better application of IPM programmes in tomato crops for the control of this pest species.

**Additional keywords:** biological control; IPM; ecotoxicology; South American tomato moth; parasitoid; insecticides; side effects.

**Abbreviations used:** a.i. (active ingredient); *Bt* (*Bacillus thuringiensis*); E (percentage reduction of the evaluation parameters with respect to the control); EC (emulsifiable concentrate); GLM (general linear model); GZLM (generalized linear model); HSD (honestly significant difference); IOBC (International Organization for Biological and Integrated Control); IPM (Integrated Pest Management); IRAC (Insecticide Resistance Action Committee); RH (relative humidity); SC (suspension concentrate); SG (soluble granule); UVL (ultraviolet light); WG (water dispersible granules); WP (wettable powder).

**Authors' contributions:** TC designed the study. JGM carried out the laboratory trials. FJFM and JRG carried out the greenhouse trials. TC and JRG analyzed the data, intellectually reviewed the content and collaborated in writing the article. All authors read and approved the final manuscript.

**Citation:** Gallego, J. R.; Guerrero-Manzano, J.; Fernández-Maldonado, F. J.; Cabello, T. (2019). Susceptibility of the egg parasitoid *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae) to selected insecticides used in tomato greenhouses. Spanish Journal of Agricultural Research, Volume 17, Issue 2, e1009. [https://doi.org/10.5424/sjar/2019172-14413](https://doi.org/10.5424/sjar/2019172-14413)

**Received:** 18 Dec 2018. **Accepted:** 25 Jun 2019.

**Copyright © 2019 INIA.** This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC-by 4.0) License.

**Funding:** Ministry of Economy, Innovation and Science of the Andalusian Regional Government [Excellence Project Programme]; FEDER Funds (P09-AGR-5000).

**Competing interests:** The authors have declared that no competing interests exist.

**Correspondence** should be addressed to Tomás Cabello: tcabello@ual.es

**Introduction**

In Europe and the United States, the environmental cost associated with the use of chemical pesticides is considered too high. Thus, there is a general movement towards environmentally safer control and production (Dent, 2000). This has been transcribed into the European Union's legislative policies with the aim of reducing the use of pesticides, removing large quantities of products, and giving renewed importance to Integrated Pest Management (IPM) (Lefebvre et al., 2015). The adoption of IPM in all member states in 2014 is the main pillar of the EU strategy to mitigate the negative impact of rapid removal of chemical pesticides from food production (Clark & Hillocks, 2014).

According to the Food and Agriculture Organization (FAO, 1966), IPM means the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides...
and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agroecosystems and encourages natural pest control mechanisms.

The South American tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the main tomato pests in South American countries (Guedes & Picanço, 2012). In addition, since its accidental introduction in 2006, this species has become a pest of great economic importance in the countries of the Mediterranean area and many others of Europe and Asia (Desneux et al., 2011; Campos et al., 2017; Biondi et al., 2018). In Spain, *Tu. absoluta* rapidly became a serious impediment to biological control programmes in tomato production greenhouses, requiring applications of more than 15 different insecticidal substances directed specifically towards *Tu. absoluta* (Desneux et al., 2011). The damage is caused by larval feeding mainly on leaves and fruits, but the pest can also attack stems, buds and flowers causing severe crop losses that can reach 100% if no control measures are taken. The dynamics of populations of *Tu. absoluta* and their consequent damage differ depending on their presence in greenhouse or outdoor crops, date of transplantation, etc., creating significant challenges for the development and successful application of biocontrol methods (Cabello, 2009), and the results are not always satisfactory due to the overlap of *Tu. absoluta* generations and the continuous re-infestations in the crops, which motivates the need for the application of several treatments per crop cycle to adapt the population levels to the capacity of control by natural enemies.

At present, pest control of *Tu. absoluta* is based on biological control, chemical control or a combination of both, although the most common method of control is based on the intensive use of insecticides and this constitutes the first tool in newly invaded areas (Bielza, 2010; Campos et al., 2017; Biondi et al., 2018).

Parasitic insects of the *Trichogramma* genus have been widely used during the 20th century to control lepidopteran pests in maize and sugarcane crops, and subsequently extended to control many other pests in many different crops; the list of crops continues to increase (Smith, 1996; van Lenteren et al., 2018). *Trichogramma*, with approximately 200 species described, is the best-known genus in the family due to its use in the biological control of pest species in agriculture of which more than 25 species are used in pest control in 34 crops across 30 countries (Pinto & Stouthamer, 1994; Querino et al., 2010; van Lenteren et al., 2018). *Trichogrammatid* can therefore play a vital role in pest control programmes by destroying the first developmental state (egg) of pest, limiting the use of pesticides and contributing to the prevention of environmental contamination (Kumar et al., 2013).

The establishment and subsequent commercialization of the parasitoid *Trichogramma achaeae* Nagaraja and Nagakartti (Hymenoptera: Trichogrammatoidea) has been an important advance in the control of the pest in Spain (Cabello et al., 2009, 2012; Vila & Cabello, 2014). In addition, *T. achaeae* has been or is being used in Europe in the following countries: Germany, Belgium, Spain, France, Greece, the Netherlands, Romania, and Portugal, against several species of Lepidoptera in more than 15 crops both horticultural and ornamental (Leplla et al., 2017; Vila, 2017 pers. com.; van Lenteren et al., 2018).

Studying the side effects of insecticides on natural enemies is necessary to minimize any adverse impacts within the IPM programmes (Goulart et al., 2012). The integration of biological and chemical control tactics requires a thorough understanding of how pesticides affect biological control organisms (Brunner et al., 2001). Prior to the release of *Trichogrammatids* in an IPM system, it is essential to know their compatibility with other pest control methods, including the use of chemical pesticides. Such information will assist in the timing of parasitoid releases regarding the application of chemical pesticides (Jalali et al., 2016).

Currently, the strategy used in IPM programmes in the control of *Tu. absoluta* in Spanish southeast tomato greenhouses consists of the early inoculation of the omnivorous predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) in combination with inundative or inoculative releases of *T. achaeae* (Cabello et al., 2009, 2012; Desneux et al., 2011; Vila & Cabello, 2014).

The objective of this work was to establish the side effects of 10 insecticides commonly used in the chemical control of *Tu. absoluta* on the parasitoid *T. achaeae*. The selected materials are abamectin, azadirachtin, *Bacillus thuringiensis*, emamectin, flubendiamide, indoxacarb, methomyl, chlorantraniliprole, spinosad and spironesifan. All are included in the list of substances authorized in the South zone of Annex I of the EC Regulation (EC, 2009) that covers the substances allowed in the members states of the EU.

**Material and methods**

**Insects**

A colony of *T. achaeae* was obtained from wild populations and reared in the entomology laboratory of Almeria University according to the method described...
by Cabello (1985) and maintained in a climatic chamber at 25 ± 1°C, 70 ± 10% RH and a 16:8 h light: dark photoperiod. The wasps were reared on UVL-sterilized eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). A weekly egg supply of *E. kuehniella* was obtained from a commercial supplier (Agrobio S.L, Almeria, Spain). The eggs were glued with distilled water onto paper cards (273 cm²) and exposed to adult *T. achaeae* in 1 L plastic pots sealed with a fine nylon mesh. After 24 h of exposure, the cards were transferred to new plastic pots, where they were held until adult emergence. Adult *T. achaeae* were provided honey as droplets smeared on the inside wall of the pots.

**Insecticides**

Ten commercial formulations, with different insecticide active ingredients (AIs) were used, as listed in Table 1. These compounds were selected because of their current and main use in the chemical management of *Tu. absoluta* in the Mediterranean area, and because they represented a variety of chemical groups used in insecticide resistance management. The doses tested were the maximum authorized or recommended by the manufacturer in greenhouse tomato culture. Application rates of the insecticide formulations used were prepared by diluting the products in distilled water according to the manufacturer’s instructions.

**Insecticide application on pupae and sublethal effects**

The insecticide formulations were applied to the pupa stage inside the host egg. Ten separate trials were carried out with each insecticide a.i. (abamectin, azadirachtin, *B. thuringiensis*, chlorantraniliprole, emamectin, flubendiamide, indoxacarb, methomyl, spinosad, and spiromesifen) plus a control (water). Following Hassan’s (1998) recommendations, each insecticide formulation was tested at its maximum recommended field dose (Table 1). Each trial was carried out in two steps as follows: the first step was to evaluate the effects of each a.i. compared to the control (water) on the pupal survival of *T. achaeae*; the second step, consecutive to the previous one, was to evaluate the effects of the same a.i. on adult longevity and fertility when applied at the pupa stage. In first the steps of each trial, four card disks (17.5 mm diameter each) with over 300 parasitized eggs (containing parasitoid pupae, less than 3 days for adult emergence at 25 °C and a 16:8 h light: dark photoperiod) were treated by spraying with a Potter tower sprayer (Burkard®, Uxbridge, UK) (working pressure: 0.76 atmosphere) for 2-2.5 seconds (time required to apply a product quantity of 0.285 mL/cm² close to the recommended optimum in the field). Additionally, distilled water was applied as described above and to a similar number of card disks with parasite eggs as a control in each trial. After treatments, the cards were kept over filter paper at room temperature until the excess liquid had drained. They were then transferred to emergency tubes and kept in a climatic chamber with controlled conditions. Emergence was evaluated by counting parasitized hosts that presented holes due to the emergence of adult offspring from them. In the second step of each trial, to evaluate the side effects of the insecticides on the longevity and fecundity of adults, subsequent bioassays were performed. Thirty couples ($2\overline{2}$) of adults emerged from the treated pupae, and 30 couples of adults emerged from the control (water) were isolated in glass vials. Each couple was offered 50 UVL-sterilized eggs of *E. kuehniella*, glued with water to a card (5 × 0.9 cm), every 3 days (days: 0, 3, 6, 9, and 12). Isolated couples *T. achaeae* were fed honey, and their survival was assessed daily until death. Host eggs that changed colour to black were tallied as parasitized; all others were counted as non-parasitized (Rodriguez et al., 1994). After female oviposition, the cards were transferred to new glass vials. All trials were conducted in a climatic cabinet (ICP 600, Memmert®, Memmert GmbH+Co. KG, Schwabach, Germany) at chamber conditions.

**Experimental design and data analysis**

The experimental design in each trial was completely randomized with a single factor at two levels (insecticide and control). The data (pupal survival, longevity of females and males, and female fecundity) obtained were analysed using general linear models (GLMs) and mean values for each insecticide were compared using Tukey’s HSD test (at $p = 0.05$) with respect to the respective control (water). Additionally, in each trial for the pupal survival value, the number of replications was 4 (each with 300 parasitized eggs) and for the longevity and fertility values of adults the number of replications was 30 couples ($1\overline{2}+1\overline{5}$). Subsequently, the percentage reduction in emergence from parasitized eggs, adult longevity and percentage of parasitism relative to the control was evaluated by the following equation: $E(\%) = \left[1 - \left(\frac{Q}{q}\right) \times 100\right]$, where $E$ is the percentage of reduction of the capacity of the biological parameter in question, $Q$ is the average value of the parameter to be analysed for the insecticide, and $q$ represents the mean value of the parameter obtained in the control (water). Based on the results obtained in this study, each insecticide was classified according to the IOBC criteria for laboratory tests: class 1 = harmless.
Table 1. General information on tested insecticide formulations: active ingredient/ commercial name, manufacturer/ distributor, mode of action, chemical group, concentration of spray mixtures, formulation type, tested rate, and EU countries in which a.i. is authorised.

| Active Ingredients (AI)/ Trade name | Supplier                | Mode of action | Chemical group                  | Concentration | Formulation | Dose | EU countries in which a.i. is authorised |
|-------------------------------------|-------------------------|----------------|---------------------------------|---------------|-------------|------|----------------------------------------|
| Abamectin /Vertimec®               | Syngenta                | Glutamate-gated (GluCl) allosteric chloride channel modulators | Avermectins, Milbemycins | 1.8%          | EC          | 0.01 mL/L | AT, BE, BG, CY, CZ, DE, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK, UK |
| Azadirachtin /Align®               | Sipcam Inagra           | Compounds of unknown or uncertain MoA | Azadirachtin | 3.2%          | EC          | 1.5 mL/L | AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FR, HR, HU, IT, LT, LU, LV, NL, PT, SE, SI, SK, UK |
| Bacillus thuringiensis /Dipel®     | Valent Biosciences Corporation | Microbial disruptors of insect midgut membranes | Bacillus thuringiensis and the insecticidal proteins they produce | 16%          | WP          | 0.5 g/L | AT, BE, BG, CY, DE, DK, EL, ES, FR, HR, HU, IT, LU, LV, NL, PT, SE, SI, UK |
| Chlorantraniliprole /Altacor®     | Du Pont                 | Ryanodine receptor modulators | Diamides | 35%          | WG          | 0.1 g/L | AT, BE, BG, CY, CZ, DE, EL, ES, FR, HR, HU, IE, IT, LT, LU, MT, NL, PL, PT, RO, SI, SK, UK |
| Emamectin (benzoate)/Affirm®      | Syngenta                | Glutamate-gated (GluCl) allosteric chloride channel modulators | Avermectins, Milbemycins | 0.855%        | SG          | 1.5 g/L | BE, BG, CY, EL, ES, FR, HR, HU, IT, LU, NL, PT, RO, SI, SK, UK |
| Fluubendiamide /Fenos®            | Bayer CropScience       | Ryanodine receptor modulators | Diamides | 24%          | WG          | 0.25 g/L | CY, DK, NL |
| Indoxacarb /Steward®              | Du Pont                 | Voltage-dependent sodium channel blockers | Indoxacarb | 30%          | WG          | 0.126 g/L | AT, BE, BG, CY, CZ, DE, EE, ES, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK, UK |
| Methomyl /Lannate®                | Du Pont                 | Acetylcholinesterase (AChE) inhibitors | Carbamates | 25%          | WP          | 1.25 mL/L | BG, CY, EL, ES, HU, IT, PL, PT, RO, SI, SK, UK |
| Spinosad /Spintor®                | Dow AgroSciences        | Nicotinic acetylcholine receptor (nAChR) allosteric activators | Spinosyns | 48%          | SC          | 0.25 mL/L | AT, BE, BG, CY, CZ, DE, EL, ES, FR, HR, HU, IE, IT, LU, MT, NL, PL, PT, RO, SE, SI, SK, UK |
| Spiromesifen /Oberon®             | Bayer CropSciences      | Inhibitors of acetyl CoA carboxylase derivatives | Tetronic and Tetratomic acid derivatives | 24%          | SC          | 0.6 mL/L | BE, CY, EL, ES, FR, HR, HU, IT, LU, MT, NL, PL, PT |

1Mode of action: IRAC (2016). 2Formulation: EC: emulsifiable concentrate, WP: wettable powder, WG: water dispersible granules, SG: soluble granule, SC: suspension concentrate. The dose used was the highest authorized for application in tomato crops. 4Authorised EU countries at the date of 03.04.2019: AT: Austria, BE: Belgium, BG: Bulgaria, CY: Cyprus, CZ: Czechia, DE: Germany, DK: Denmark, EE: Estonia, EL: Greece, ES: Spain, FI: Finland, FR: France, HR: Croatia, HU: Hungary, IE: Ireland, IT: Italy, LT: Lithuania, LU: Luxembourg, LV: Latvia, MT: Malta, NL: Netherlands, PL: Poland, PT: Portugal, RO: Romania, SE: Sweden, SI: Slovenia, SK: Slovakia, UK: United Kingdom.

(E < 30% reduction of emergence, longevity, or fecundity), class 2 = slightly toxic (30% ≤ E ≤ 79% reduction), class 3 = moderately toxic (80% < E ≤ 99% reduction), and class 4 = toxic (E > 99% reduction) (Hassan et al., 1991; Jepson, 1998; Sterk et al., 1999; Amano & Haseeb, 2001). All statistical analyses were carried out using the SPSS software, version 23 (IBM, 2014).

Insecticide application on adults

Evaluation followed the method prescribed by IOBC for selectivity tests with parasitoids of the genus _Trichogramma_ (Hassan, 1998; EPPO, 1999; Hassan et al., 2000). Four trials were carried out. For each bioassay, _T. achaeae_ was exposed to fresh and dried residues of insecticide formulation sprayed on
2 mm thick glass plates measuring 13 cm × 13 cm. The products were sprayed using a Potter tower sprayer under the same conditions indicated in the previous section. After spraying, the plates were kept in the shade for approximately 3 h to dry, forming a dry insecticide film. The surfaces of the two glass plates with the dry insecticide film were used as the internal back and the top of the cage.

Each cage (equal to those described by Hassan et al., 2000) was made of an aluminum frame measuring 13 cm (length) × 13 cm (width) × 1.5 cm (height). Single-coated adhesive foam tape, 1.5 cm wide, was fixed on the aluminum frame to hold the glass plates. Six ventilation holes (~ 1 cm in diameter) were drilled into three sides of the aluminum frame. The holes were covered with thin, black muslin fabric glued onto the frame with adhesive foam tape to promote ventilation. The fourth side of the aluminum frame had two openings. The first opening was 3.5 cm wide × 1 cm high and was used to transfer the eggs to be parasitized and food for the parasitoids into the cage; the second opening was a 1 cm diameter hole to allow for the release of the parasitoids inside the cage. These two openings were closed from the outside with black cardboard and were opened only to place the cards with eggs and the parasitoids into the cages.

To prevent the escape of parasitoids to the margins of the glass plate, the external surfaces (untreated) were covered with black cardboard (7 cm × 7 cm). Because the parasitoids were attracted to light, they were active on the glass surface exposed to light and thus more exposed to the insecticides being tested. Afterwards, the glass plates were fixed to the aluminum frame with four rubber bands.

Approximately 2500 parasitized eggs with a time for the emergence of parasitoid adults of less than 24 h and were placed in a corner in each frame. As food sources, they were given a piece of non-absorbent paper (6 × 1.5 cm) with 6 thin lines of honey. Through the opening of the frame, a strip of paperboard (3 × 10 cm) was supplied with approximately 3000 eggs, which were replaced at 24, 72, and 96 h. The replaced paperboard was placed in a plastic container and evolved in a chamber under chamber conditions. Once all the parasitized eggs (more than 5 days) were evolved and showed black colour they were photographed, and digital measurement of the surface occupied by parasitized eggs was performed by image processing using Photoshop® CS6 software (Adobe System Software Ltd, Ireland) and Fiji software (Schindelin et al., 2012). Previously the average surface (in pixels²) equivalent for an egg was calculated. To avoid and prevent the accumulation of toxic gases, the frames were placed in a closed structure equipped with an air extractor (flow rate = 98 m³/h) which created a continuous air flow during the experiments. All trials were conducted for 24 h in a climatic cabinet (model ICP 600, Memmert GmbH+Co. KG, Schwabach, Germany) (25 °C ± 1, RH: 75-85% and 16:8 h light: dark photoperiod).

**Experimental design and data analysis**

In each trial of insecticide application on adults, the design was completely randomized with a single factor at four levels (trial 1, a.i.: chlorantraniliprole, flubendiamide, indoxacarb, and control; 2, a.i.: abamectine, azadirachtin, spinosad, and control; and 3, a.i.: B. thuringiensis, emamectin, spiromesifen, and control) o at two levels (trial 4, a.i.: methomyl and control) and three replicates per treatment. The data (percentage of parasitism) obtained in the different trials were analyzed by GLMs and their means compared with Tukey’s HSD test (p = 0.05) with SPSS software, version 23 (IBM, 2014). Subsequently, the number of parasitism reductions was evaluated in relation to the control (water). This was calculated with equation (E) and classified according to the IOBC scale as indicated above for laboratory tests.

**Greenhouse evaluation**

To evaluate the effect of the application of the different insecticides under conditions of tomato greenhouse production, in a previous trial, we tried to use the methodology proposed by EPPO (1999) and Hassan et al. (2000) for T. cacoeciae using parasitized sentinel eggs (E. kuehniella eggs stuck on a piece of green cardboard) as a *Trichogramma* activity measurement but it was verified for *T. achaeae* that the number of parasitized sentinel eggs does not well reflect the actual activity of this species. This has also been previously cited by Cabello et al. (2010) and Sanchez et al. (2014). As an alternative, the release-recapture method was used. We used yellow sticky traps because this method has demonstrated efficacy monitoring the activity of others *Trichogramma* species (Romeis et al., 1998; Chapman et al., 2009), and it has been proposed by Yong & Hoffman (2006) and Cabello et al. (2010) for assessing *Trichogramma* adult activity.

Using this methodology, four trials with different insecticides were carried out in four Almeria-type commercial greenhouses with tomato crops located at different locations in the province of Almeria, Spain. In all of them, the crop plant height was greater than 1.40 m, and no prior chemical control had been carried out. In every greenhouse, the insecticide applications were carried out by a backpack sprayer equipped (Maruyama®, model MS073D). Also, in each trial, the equipment was
pre-calibrated, in relation to the application time per plot, for an application rate of 1500 L/ha. The products used, and the tested doses are listed in Table 1. Inside the greenhouses, the different blocks and plots were delimited with a plastic sheet to avoid drift. Where it was not possible to place the plastic sheet two guard lines (4.5 m separation) were left for each treatment.

Experimental design and data analysis

In each of the four trials (greenhouses), the experimental design employed random blocks (four) arranged inside the surface of each greenhouse. Each plot had an area of 120 m². The number of tested insecticides was different according to the greenhouse trial (Trial 1, a.i.: abamectin, azadirachtin, *B. thuringiensis*, spinosad, and spiromesifen; Trial 2, a.i.: chlorantraniliprole, indoxacarb, and methomyl; Trial 3, a.i.: flubendiamide and spiromesifen. Trial 4, a.i.: emamectin); with doses as indicated in Table 1. In addition, in each greenhouse a control (check) was sprayed only with water. In each plot of the four tests, 9 yellow sticky traps (2 × 2 cm) were arranged, according to the arrangement shown in Fig. 1, 24 h prior to the insecticide applications. Later, these sticky traps were visited at 3, 6, 9 and 12 days after treatments, and the number of adults of *T. achaeae* captured per treatment plot was evaluated. These data were analysed by generalized linear models (GZLMs). Then, the mean values were analysed by a pairwise multiple comparison procedure (Wald test) (Aruna & Aruna, 2015). For this, we used the SPSS software, vers. 23 (IBM, 2014). Subsequently, the parasitism reduction was evaluated in relation to the control (*E*). This was calculated with the equation and classified according to the IOBC scale for field trials (Hassan et al., 1991; Jepson, 1998; Amano & Haseeb, 2001): class 1 = harmless (*E* < 25%), class 2 = slightly harmful (25% ≤ *E* ≤ 50%), class 3 = moderately harmful (51% ≤ *E* ≤ 75%), and class 4 = harmful (*E* > 75%).

Results

Insecticide application on pupae and sublethal effects

The side effects of the different insecticides on pupal survival, longevity, and fecundity of *T. achaeae* after application of insecticide formulations to the pupal stage are shown in Table 2. The pupal survival decreased significantly, with respect to controls, after treatment with six AIs (azadirachtin, chlorantraniliprole, emamectin, indoxacarb, methomyl and spinosad) (Table 2). These values less than 30% (IOBC class 1-harmless) for the AIs: abamectin (*E* = 1.3%), azadirachtin (*E* = 20.77%), *B. thuringiensis* (*E* = 5.58%), emamectin (*E* = 19.78%), flubendiamide (*E* = 12.63%), indoxacarb (*E* = 16.38%) and spiromesifen (*E* = 0). In turn, the decrease in pupal survival was between 30 and 50% (IOBC class 2-slightly toxic) for the AIs: chlorantraniliprole (*E* = 33.72%) and methomyl (*E* = 41.92%). Only one, AI spinosad (*E* = 79.16%) showed a greater decrease in the pupal survival (IOBC class 3-harmless) (Fig. 2).

The longevity of female that emerged from pupae treated with insecticides decreased significantly with respect to controls, after treatment with six AIs (azadirachtin, chlorantraniliprole, emamectin, indoxacarb, methomyl and spinosad) (Table 2). All of the AIs, except for the first one, show decreases in female longevity under 30% (IOBC class 1-harmless); emamectin showed a greater reduction (*E* = 34.20%) (IOBC class 2-slightly toxic) (Fig. 2). In turn, the deleterious effects of treatments on male longevity were not significant, with the exception of the AI emamectin (*E* = 37.50%) (IOBC class 2-slightly harmful (Fig. 2).

Finally, when the applications were carried out in the pupal stage, significant reductions were found in the fecundity of females for six AIs (abamectin, azadirachtin, chlorantraniliprole, emamectin, flubendiamide and indoxacarb) (Table 2). For these, only the AI azadirachtin (*E* = 31.55%) presented a decrease in fertility greater than 30% (IOBC class 2-slightly harmful). For the rest, the value of *E* is located within IOBC class 1 (Fig. 2).

Analysis of the effects of the AI spinosad treatment on the *F₁* generation was not performed, as the number

Figure 1. Chromatic trap distribution in tomato plants for *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae) greenhouse dispersal study after insecticide applications.
of offspring females was very low, and they died in less than 24-48 h. It should be noted that the pupal survival trial for this AI was repeated up to 3 times.

**Insecticide application on adults**

The effect on parasitism by *T. achaeae* females, when they were exposed to the residue of freshly sprayed insecticides is shown in Table 3. A statistically significant decrease was found in relation to the control (water) for four AIs: azadirachtin (*E* = 33.89%), emamectin (*E* = 75.19%), methomyl (*E* = 80.29%), and spinosad (*E* = 69.38%). This allows the classification of the AI methomyl into the moderately harmful IOBC class 3, and the other three AIs into the slightly harmful IOBC class 2.

**Greenhouse evaluation**

The results obtained in the four commercial greenhouse trials are shown in Table 4. Three AIs showed statistically significant decreases with respect to the water control (check): emamectin, methomyl, and spinosad. The AI emamectin showed an *E* = 40.00% (IOBC class 2-slightly harmful), and the others IA methomyl (*E* = 51.97%) and IA spinosad (*E* = 52.30%) were grouped into the IOBC class 3-moderately harmful.

**Discussion**

In Europe, *T. achaeae* has been shown to be a suitable biological control agent against *Tu. absoluta*, as mentioned above. However, the control of this pest is difficult to manage alone, with the simultaneous use of natural enemies or insecticides being necessary for a satisfactory pest control (Campos et al., 2017). This is more pronounced at present; thus, the incidence of the pest has increased in tomato crops in Spain and Europe in recent years, especially in greenhouse crops (Vila, 2018, pers. com.), possibly motivated by problems of resistance to the AIs currently used in these crops. Thus, Roditakis et al. (2018) cited several cases of resistance to emamectin, spinosad, indoxacarb,
Juan R. Gallego, Jesús Guerrero-Manzano, Francisco J. Fernández-Maldonado and Tomás Cabello

Spanish Journal of Agricultural Research
June 2019 • Volume 17 • Issue 2 • e1009

Figure 2. Reduction of adult emergence of $F_0$ generation, longevity and fecundity of adults of the $F_1$ generation of Trichogramma achaeae (Hymenoptera: Trichogrammatidae) after application of insecticide formulations to parasitized eggs of Ephestia kuehniella (Lepidoptera: Pyralidae) when the immature parasitoid was in the pupal stage and under laboratory conditions. Class of toxicity according to the IOBC, where: 1-harmless, $E < 30\%$; 2-slightly harmful, $30 \leq E \leq 79\%$; 3-moderately harmful, $80 \leq E \leq 99\%$; and 4-harmful, $E > 99\%$.

and chlorantraniliprole in that geographic area. In this sense, it has been mentioned that multiple sublethal effects, sometimes counterintuitive ones, on natural enemies have been reported for modern slower-acting insecticides and/or biopesticides. This highlights the need to revise the labelling of these products to indicate their compatibility with sustainable IPM programmes (Biondi et al., 2018).

Table 3. Parasitism (mean±SE) of Ephestia kuehniella eggs (Lepidoptera: Pyralidae) by Trichogramma achaeae (Hymenoptera: Trichogrammatidae) when parasitoid females were exposed to residues of insecticide formulations, under laboratory conditions, for four trials.

| Trial | Treatment | Parasitism (%)$^1$ | $E^2$ | C$^3$    |
|-------|-----------|-----------------|-------|---------|
| 1     | Chlorantraniliprole | 87.83±8.78 b $^{F_{1,8}} = 10.8$ | 0.00  | 1       |
|       | Flubendiamide    | 59.81±9.56 a $^{p = 0.004}$ | 20.69 | 1       |
|       | Indoxacarb      | 63.53±2.69 a $^{p = 0.001}$ | 15.75 | 1       |
|       | Control         | 75.41±2.34 ab |       |         |
| 2     | Abamectine      | 63.79±8.65 ab $^{F_{1,8}} = 48.2$ | 16.79 | 1       |
|       | Azadirachtin    | 50.68±3.23 b $^{p < 0.001}$ | 33.89 | 2       |
|       | Spinosad        | 23.47±6.04 c   | 69.38 | 2       |
|       | Control         | 76.66±2.67 a   |       |         |
| 3     | B. thuringiensis| 72.57±11.95 a $^{F_{1,8}} = 26.5$ | 11.04 | 1       |
|       | Emamectin       | 20.24±5.74 b $^{p < 0.001}$ | 75.19 | 2       |
|       | Spiromesifen    | 68.04±9.40 a   | 16.60 | 1       |
|       | Control         | 81.58±12.85 a  |       |         |
| 4     | Methomyl        | 15.38±5.18 a $^{F_{1,4}} = 39.3$ | 80.29 | 3       |
|       | Control         | 78.04±11.76 b $^{p < 0.001}$ |       |         |

$^1$In each trial, different letters indicate significant differences verified by GLM and Tukey’s HSD at $p < 0.05$; $^2$Reduction in parasitism (%); $^3$IOBC Classes: 1, harmless ($E < 30\%$); 2, slightly harmful ($30 \leq E \leq 79\%$); 3, moderately harmful ($80 \leq E \leq 99\%$); 4, harmful ($E > 99\%$).
**Table 4.** Total number (mean±SE) of *Trichogramma achaeae* adult parasitoid (Hymenoptera: Trichogrammatidae) caught on yellow sticky traps (release-recapture method), per experimental plot (9 traps/plot), after application of insecticide formulations in four trials carried out under commercial greenhouse conditions.

| Trial | Treatment       | Adults caught$^a$ | Omnibus test ($\chi^2$ likelihood ratio) | $E^2$ | C$^0$         |
|-------|-----------------|-------------------|------------------------------------------|------|---------------|
| 1     | Abamectin       | 87.25±4.67        | $\chi^2 = 132.611$                       | 5.42 | 1 Harmless    |
|       | Azadirachtin    | 109.75±5.24 *     | $p < 0.001$                              | 0    | 1 Harmless    |
|       | *B. thuringiensis* | 97.25±4.93       | $p < 0.001$                              | 0    | 1 Harmless    |
|       | Spinosad        | 44.00±3.32 *      |                                          | 52.30| 3 Moderately harmful |
|       | Spiromesifen    | 91.00±4.77        |                                          | 1.36 | 1 Harmless    |
|       | Control         | 92.25±4.80        |                                          |      |               |
| 2     | Chlorantraniliprole | 44.50±3.35     | $\chi^2 = 48.840$                       | 0    | 1 Harmless    |
|       | Indoxacarb      | 34.75±2.95        |                                          | 8.55 | 1 Harmless    |
|       | Methomyl        | 18.25±2.14 *      |                                          | 51.97| 3 Moderately harmful |
|       | Control         | 38.00±3.08        |                                          |      |               |
| 3     | Flubendiamide   | 57.50±3.79        |                                          | 6.50 | 1 Harmless    |
|       | Spiromesifen    | 58.50±3.82        |                                          | 4.88 | 1 Harmless    |
|       | Control         | 61.50±3.92        |                                          |      |               |
| 4     | Emamectin       | 66.00±5.75 *      |                                          | 40.00| 2 Slightly harmful |
|       | Control         | 110.00±7.42       |                                          |      |               |

$^a$For each trial, treatments differing significantly (GZLM analyses and pairwise multiple comparison procedure and Wald test) at $p < 0.01$ from the water control are indicated by an asterisk (*); $\epsilon$: reduction in parasitism (%); $^0$IOBC class 1 = harmless ($E < 25\%$), class 2 = slightly harmful ($25\% \leq E \leq 50\%$), class 3 = moderately harmful ($51\% \leq E \leq 75\%$), and class 4 = harmful ($E > 75\%$).

In our work we have shown a broad picture of the side effects of the AIs used in the chemical control of *Tu. absoluta* on the parasitoid *T. achaeae*.

First, the group of AIs, abamectin, *B. thuringiensis*, flubendiamide, indoxacarb, and spiromesifen did not present side effects, or these were negligible, on *T. achaeae* for all the trials carried out under laboratory or greenhouse conditions (IOBC class 1).

It should be noted that in other studies with the AI abamectin, discrepant results have been found for other *Trichogramma* species. Thus, Brunner et al. (2001) found a high mortality of adults (56-60\%) for *T. platneri* Nagakartti, when they were exposed to leaf residues less than 3 days old. Additionally, Carvalho et al. (2003) found side effects on adult emergence and the longevity and fecundity of adults when this AI was applied on the pupal stage. This was corroborated by Moura et al. (2006) for the same species and AI. On the other hand, Consoli et al. (1998) and Nornberg et al. (2009) reported that this AI had no side effects for *T. pretiosum* Riley when it was applied in the protected life stage (pupa). Our results seem to be intermediate those previously mentioned; thus, side effects were observed in female fecundity ($E = 16.27\%$, IOBC class 1) (Fig. 2), parasitism ($E = 16.79\%$, IOBC class 1) (Table 3) (both IOBC class 1); and adult activity in the greenhouse trial ($E = 5.42\%$, IOBC class 1) (Table 4). Perhaps the differences noted above may be due to different degrees of susceptibility; this has been reported for different populations of *T. pretiosum* by Vianna et al. (2009). For this AI, we can highlight what was indicated by Gentz et al. (2010) that despite significant toxicity to several non-target species, abamectin was once considered suitable for use with many beneficial insects due to its short environmental persistence.

In relation to the AI *B. thuringiensis*, several authors agree that side effects have not been found in *T. achaeae* (Saélices et al., 2012; Fontes et al., 2018), and in other species of the same genus: *T. dendrolimi* (Matsumura), *T. pretiosum*, *T. bourarachae* Pintureau and Babault, *T. cacoeciae* Marchal, and *T. evanescens* Westwood (Takada et al., 2001; Vianna et al., 2009; Ksentini et al., 2010). Our results (Fig. 2; Tables 3 and 4) corroborate the above.

Our results for the AI flubendiamide indicate that there were significant side effects on female longevity ($E = 23.97\%$) and female fecundity ($E = 24.59\%$), when the application was made on the pupal stage (Fig. 2), as well as in the exposed phase of the parasitoid (adult) ($E = 20.69\%$); all of them were within IOBC class 1. However, in the greenhouse trial, these side effects were lower ($E = 6.5\%$, IOBC class 1) (Table 4). In addition,
the compatibility between the AI flubendiamide and
*T. achaeae* found in this work corroborates the results
found with other species of the same genus, such as *T. chilonis* Ishi and *T. pretiosum*. Thus, side effects were
not found on adults (Sattar et al., 2011; Martins et al.,
2011) or on the developmental stages of *T. pretiosum*
(Carvalho et al., 2005). The same results have been
reported for *T. atropurpurea* Oatman and Platner
(Rezende et al., 2005), that is, in the latter case with a
different methodology from the one used in the present
work.

Similar effects, to those previously indicated for the
AI flubendiamide, were found in our work for the AI
indoxacarb, both in laboratory and greenhouse trials
(Fig. 2, Table 4). The values found were less than a
30% reduction in pupal survival (*E* = 16.38%), female
longevity (*E* = 9.15%), female fecundity (*E* = 23.53%)
(Fig. 2), and parasitism (*E* = 15.75%) with respect
to the control for laboratory trials (Table 3). In the
greenhouse trial, the AI indoxacarb presented (*E*
= 8.55%) harmless side effects, grouping into IOBC
class 1. Similar side effects have been found by Scholz
& Zalucki (2000) for *T. pretiosum* and Hewa-Kapuge
et al. (2003) for *T. sp. nr brassicae* under laboratory
and field conditions. Only, Sattar et al. (2011) found
a slightly harmful effect (IOBC class 2) on the adult
emergence and female fecundity of *T. chilonis*.

Finally, in relation to the first five AIs indicated at
the beginning of this discussion, the AI spiromesifen
did not present harmful effects on the biological
parameters analysed in the laboratory trial (Tables 2
and 3). The same result was obtained in the greenhouse
trial (Table 4). This AI is highly compatible with *T.
achaeae*. The same results have been cited by Kavitha
et al. (2006) for *T. chilonis*.

Second, there is another group of two AIs that
presented slightly harmful side effects (IOBC class
2): azadirachtin and chlorantraniliprole, in laboratory
trials, but that, under greenhouse conditions, showed
no side effects (IOBC class 1-harmless).

Additionally, the AI azadirachtin had side effects
on pupal survival (*E* =20.77%) (IOBC class 1) (Fig. 2).
Similar side effects on adult emergence have been
cited for *T. cacocoeae* for this AI (Saber et al., 2004). It
should also be noted that this AI presented side effects
on female fecundity (*E* = 31.55%) (IOBC class 2) (Fig.
2). However, in the greenhouse trial no side effects were
found on parasitoid activity (IOBC class 1) (Table 4).

The AI chlorantraniliprole, in tests carried out with
predatory species, presents different degrees of toxicity;
from very high in some species of Coccinellids and
Chrysopids, to no side effects in other species (Stanley
& Preetha, 2016). In relation to the species of the genus
*Trichogramma*, the first studies have indicated that
this AI is safe for *T. chilonis*, *T. galloi* (Zucchi), and *T.
pretiosum* (Preetha et al., 2009; Brugger et al., 2010;
Oliveira et al., 2013). Additionally, it does not affect
the emergence of *T. chilonis* and *T. pretiosum* adults
(Brugger et al., 2010). However, our laboratory data
differ from those found in these species; thus, pupal
survival (*E* = 33.7%) (IOBC Class 2) and, to a lesser
extent, female fertility (*E* = 12.4%) (IOBC class 1)
were affected by this AI (Fig. 2). Our results in relation
to *T. achaeae* female fertility agree with those cited
by Fontes et al. (2018). Despite these effects in the
laboratory, no side effects were found under greenhouse
conditions (IOBC class 1) (Table 4).

For the two insecticide groups discussed above,
abamectin, azadirachtin, *B. thuringiensis*, chlorantraniliprole,
flubendiamide, indoxacarb, and spiromesifen, can be considered very compatible with the use of the parasitoid
*T. achaeae*.

On the other hand, there is a third group of AIs:
emamectin, methomyl, and spinosad, which presented
side effects both in the laboratory and in greenhouse
trials.

The AI emamectin showed side effects on the lon-
gevity of females and males (IOBC class 2) and, to a
lesser extent, in the survival of pupae and fecundity of
females (IOBC class 1) in the laboratory tests (Fig. 2). The
decrease in fecundity of females is lower than that
cited for the same species and AI by Fontes et al. (2018)
(IOBC class 2). Additionally, the reduction of the values
of parasitism when the females were exposed to the fresh
residue of the AI (IOBC class 2) (Table 3) was lower
than that cited, also for the same species and AI, by Saelices et al. (2012). Similar results have been cited
for this AI in relation to the *Trichogramma* species
*T. chilonis* and *T. sp. nr brassicae* (Hewa-Kapuge
et al., 2003; Sattar et al., 2011). The detrimental effects
of the AI emamectin in *T. achaeae* are also shown in
greenhouse trials (IOBC class 2) (Table 4).

The AI methomyl has shown an important side effect
on *T. achaeae* pupae. It represented a reduction in
adult emergence (*E* = 41.92%) (IOBC class 2-slightly
harmful) (Fig. 2). In contrast, there were no such effects
on adult longevity and female fecundity (IOBC class
1) (Fig. 2). In turn, this insecticide shows an important
side effect on parasitism (*E* = 80.29%) (IOBC class
3-moderately harmful) (Table 3). This value is very
similar to that found by Fontes et al. (2018) for the
same species. Similar results have been reported for
other species of *Trichogramma* (Bull & House, 1983;
Hassan et al., 1987; Scholz & Zalucki, 2000; Takada
et al., 2001; Bueno et al., 2008). In the greenhouse trial,
it was also shown to be moderately harmful (IOBC class
3) (Table 4). This corroborates the results found by
Tipping & Burbutis (1983) and Campbell et al. (1991)
for *T. nubilale* Ertle and Davis, *T. exiguum*, *T. minutum* and *T. pretiosum*.

Finally, the AI spinosad had significant side effects for *T. achaeae*. This AI decreased pupal survival (*E* = 79.16%) (IOBC class 3-moderately harmful) (Fig. 2) and parasitism (*E* = 80.29%) (IOBC class 3-moderately harmful) (Fig. 2, Table 3). These results corroborate those found by Fontes et al. (2018) for this AI in the same species. At the same time, the toxicity of the AI spinosad in other species of *Trichogramma* has been studied by several authors (Suh et al., 2000; Consoli et al., 2001; Maia et al., 2010; Liu & Zhang, 2012; Saljoqi et al., 2012; Costa et al., 2014), who have cited side effects in some of the biological parameters of *Trichogramma* spp. In our work, the AI spinosad also had side effects for *T. achaeae* in the greenhouse trial (*E* = 51.97) (IOBC class 3-moderately harmful) (Table 4). This insecticide has traditionally been included in IPM programmes because of its low toxicity to mammals and birds, its slight or moderate toxicity to aquatic organisms, and its relative harmless effect for a wide range of natural enemies (Gentz et al., 2010). However, William et al. (2003) have cited that this AI shows a significant side effect on the Hymenopteran parasitoid complex, both in the field and laboratory trials.

According to the values indicated above for this third group of AIs: emamectin, methomyl, and spinosad, we must mention that their use is not compatible with the release of *T. achaeae* in tomato crops.

Recently it has been mentioned that pesticide risk assessments for entomophagous species are being performed by categorizing pesticides based on mortality in laboratory and semi-field trials and reduction in field studies. Testing the pesticides under field recommended concentrations at laboratory conditions does not exactly reveal how the pesticides behave in complex field conditions (Stanley & Preetha, 2016). This last point has also been previously indicated by other authors (e.g., Stark et al., 1995).

Therefore, in the present work the tests were carried out in laboratory and field conditions indicated by the IOBC WPRS methodology, without ruling out any AI in the different stages of the sequential testing scheme methodology.

In this sense, we must indicate, on the one hand, that a very good correlation has been found between the results of laboratory tests and those carried out under greenhouse conditions. Thus, the AIs emamectin, methomyl, and spinosad presented the highest side effects, and in more tests, under laboratory conditions, they also had the highest side effects under greenhouse conditions.

On the other hand, based on the results found, we consider that the new methodology used in the evaluation of adult activity of *T. achaeae* could be more feasible under field conditions and provide reliable results to evaluate the secondary effects these conditions. This is compared to the methodology recommended by the IOBC WPRS (Hassan, 1985; EPPO, 1999) for field tests for the evaluation of parasitism in sentinel eggs and those that could be extended to other species of *Trichogramma*.

Based on the results found in this work, we can conclude that there is an important group of insecticide formulations, especially those of the new generation, which present a high degree of compatibility with the use of the egg parasitoid *T. achaeae*. This allows a better adaptation of the use of both control systems of *Tu. absoluta* in tomato, both in greenhouses and open-air crops. This aligns with the recommendations indicated by Campos et al. (2017) for better control of this pest species.

**References**

Amano H, Haseeb M, 2001. Recently-proposed methods and concepts of testing the effects of pesticides on the beneficial mite and insect species: study limitations and implications in IPM. Appl Entomol Zool 36: 1-11. https://doi.org/10.1303/aez.2001.1

Aruna KT, Aruna RK, 2015. Pairwise comparison of coefficients of variation for correlated samples. Int J Stat Appl 5: 231-236.

Bielza P, 2010. La resistencia a insecticidas en *Tuta absoluta*. Phytoma España 217: 103-106.

Biondi A, Guedes RNC, Wan FH, Desneux N, 2018. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: past, present, and future. Annu Rev Entomol 63: 239-258. https://doi.org/10.1146/annurev-ento-031616-034933

Brugger KE, Cole PG, Newman IC, Parker N, Scholz B, Suvagia P, Walker G, Hammond TG, 2010. Selectivity of chlorantraniliprole to parasitoid wasps. Pest Manag Sci 66: 1075-1081. https://doi.org/10.1002/ps.1977

Brunner JF, Dunley JE, Doerr MD, Beers EH., 2001. Effect of pesticides on *Colpoclypeus florus* (Hym.: Eulophidae) and *Trichogramma plateni* (Hym.: Trichogrammatidae), parasitoids of leafrollers in Washington. J Econ Entomol 94: 1075-1084. https://doi.org/10.1603/0022-0493-94.5.1075

Bueno AF, Bueno COF, Parra JRP, Vieira SS, 2008. Effects of pesticides used in soybean crops to the egg parasitoid *Trichogramma pretiosum*. Cienc Rural 38: 1495-1503. https://doi.org/10.1590/S0103-84782008000600001

Bull DL., House VS, 1983. Effects of different insecticides on parasitism of host eggs by *Trichogramma pretiosum* Riley. Southwestern Entomol 8:46-53.
Cabello T, 1985. Biología de dos especies de *Trichogramma* (Hym.: Trichogrammatidae) parasitas de *Helicoverpa* spp. (Lep.: Noctuidae) en algodonero. Posibilidades de su empleo como agentes de control biológico. Dissertation, Universidad de Cordoba, Spain.

Cabello T, 2009. Cultiivos hortícolas bajo abrigo: control biológico de *Tuta absoluta* en tomate. In: Uso sostenible de fitosanitarios; pp: 199-217. Junta de Andalucía, Sevilla, Spain.

Cabello T, Gallego JR, Vila E, Soler A, Pino M del, Carnero A, Hernández-Suárez E, Polaszek A, 2009. Biological control of the South American tomato pinworm *Tuta absoluta* (Lep.: Gelechiidae), with releases of *Trichogramma cacoeciae* (Hym.: Trichogrammatidae) in tomato greenhouses of Spain. IOBC/WPRS Bull 49: 225-230.

Cabello T, Gallego JR, Fernandez, de Scals D, Rubio A, Salvatierra S, Parra A, 2010. New simple methodology to evaluate the insecticide side-effects on *Trichogramma* species (Hym.: Trichogrammatidae) in greenhouse crops. IX Eur Congr Entomol, Budapest, Aug 22-27. p: 222.

Cabello T, Gallego JR, Fernandez FJ, Gamez M, Vila E, Pino M del, Hernandez-Suarez E, 2012. Biological control strategies for the South American tomato moth (Lep.: Gelechiidae) in greenhouse tomato fields. J Econ Entomol 105: 2085-2096. https://doi.org/10.1603/EC12221

Campbell CD, Walgenbach JF, Kennedy GG, 1991. Effect of parasitoids on lepidopterous pest in insecticide treated and untreated tomatoes in western North Carolina. J Econ Entomol 84: 1662-1667. https://doi.org/10.1093/jee/84.6.1662

Campos MR, Biondi A, Adiga A, Guedes RNC, Desneux N, 2017. From the Western Palearctic region to beyond: *Tuta absoluta* 10 years after invading Europe. J Pest Sci 90: 787-796. https://doi.org/10.1007/s10340-017-0867-7

Carvalho GA, Reis PR, Rocha LCD, Moraes JC, Fuiii LC, Ecole CC, 2003. Side-effects of insecticides used in tomato fields on *Trichogramma pretiosum* (Hym.: Trichogrammatidae). Acta Sci-Agron 25: 275-279. https://doi.org/10.4025/actasciagron.v25i2.1771

Carvalho GA, Rezende DT, Moura AP, Moscardini VF, Lasmar O, Souza JR, 2005. Selectivity of flubendiamide, a new insecticide used to control tomato pests in Brazil to *Trichogramma pretiosum* Riley (Hym.: Trichogrammatidae). J Econ Entomol 111:1219-1226. https://doi.org/10.1093/jee/toy064

Chapman AV, Kuhar TP, Schultz PB, Brewster CC, 2009. Dispersal of *Trichogramma ostriniae* (Hym.: Trichogrammatidae) in potato fields. Environ Entomol 38: 677-685. https://doi.org/10.1603/022.038.0319

Clark B, Hillocks R, 2014. Integrated pest management for European agriculture. In: Integrated pest management; Pimentel D, Peshin, R (eds.). pp: 73-97. Springer, NL. https://doi.org/10.1007/978-94-007-7796-5_3

Consoli FL, Parra JRP, 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. https://doi.org/10.1111/j.1439-0418.1998.tb01459.x

Consoli FL, Botelho PM, Parra JRP, 2001. Selectivity of insecticides to the egg parasitoid *Trichogramma galloi* (Hym., Trichogrammatidae). J Appl Entomol 125: 37-43. https://doi.org/10.1046/j.1439-0418.2001.00513.x

Costa MA, Moscardini VF, da Costa-Gontijo P Carvalho GA, Oliveira RL, Oliveira BH, 2014. Sublethal and transgenerational effects of insecticides in developing *Trichogramma galloi* (Hym., Trichogrammatidae). Ecotoxicology 23: 1399-1408. https://doi.org/10.1007/s10646-014-1282-y

Dent D, 2000. Insect pest management. CAB Int, Wallingford, UK. 410 pp. https://doi.org/10.1079/9780851993409.0000

Desneux N, Luna MG, Guillemaud T, Urbaniea J, 2011. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. J Pest Sci 84: 403-408. https://doi.org/10.1007/s10340-011-0398-6

EC, 2009. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32009R1107

EPPO, 1999. Standards PP1/180(2) - Side-effects on *Trichogramma cacoeciae*. In: EPPO standards: guidelines for the efficacy evaluation of plant protection products. Efficacy evaluation of plant protection products; EPPO. Vol 1, pp: 165-172. European and Mediterranean Plant Protection Organization, Paris.

FAO, 1966. Proceeding of the FAO symposium on integrated pest control. Rome. 129 pp.

Fontes J, Sanchez-Roja I, Tavares J, Oliveira L, 2018. Lethal and sublethal effects of various pesticides on *Trichogramma cacoeciae* (Hym.: Trichogrammatidae). J Econ Entomol 111:1219-1226. https://doi.org/10.1093/jee/toy064

Gentz MC, Murdoch G, King GF, 2010. Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. Biol Control 52: 208-215. https://doi.org/10.1016/j.biocontrol.2009.07.012

Goulart RM, Volpe HXL, Vacari AM, Thuler RT, de Bortoli GA, Oliveira RL, Oliveira HN, 2014. Sublethal and transgenerational effects of insecticides in developing *Trichogramma galloi* (Hym., Trichogrammatidae). Ecotoxicology 23: 1399-1408. https://doi.org/10.1007/s10646-014-1282-y

Gentz MC, Murdoch G, King GF, 2010. Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. Biol Control 52: 208-215. https://doi.org/10.1016/j.biocontrol.2009.07.012

Goulart RM, Volpe HXL, Vacari AM, Thuler RT, de Bortoli SA, 2012. Insecticide selectivity to two species of *Trichogramma* in three different hosts, as determined by IOBC/WPRS methodology. Pest Manag Sci 68: 240-244. https://doi.org/10.1002/ps.2251
Guedes RNC, Picanço MC, 2012. The tomato borer *Tuta absoluta* in South America: pest status, management and insecticide resistance. EPPO Bull 42: 211-216. https://doi.org/10.1111/epp.2557

Hassan SA, 1985. Standard methods to test the side effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group 'Pesticides and beneficial organisms'. EPPO Bull 15: 214-255. https://doi.org/10.1111/j.1365-2338.1985.tb00224.x

Hassan SA, 1998. Defining the problem. Introduction. In: Ecotoxicology. Pesticides and beneficial organisms; Haskell PT, McEwen P (eds.). pp: 55-68. Springer, Boston, USA.

Hassan SA, Albert R, Bigler F, Blaisinger P.; Bogenschütz H, Boller E, Brun J, Calis WD, Huang P, et al., 1987. Results of third joint pesticide testing programme by the IOBC/WPRS-working group pesticides and beneficial organisms. J Appl Entomol 103: 92-107. https://doi.org/10.1111/j.1439-0418.1987.tb00963.x

Hassan SA, Bligler F, Bogenschutz H, Boller E, Brun J, Calis JNM, Chiverton P, Coremans-Pelseneer J, Duso C, Lewis GB, et al., 1991. Results of the fifth joint pesticide testing programme carried out by the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms". Entomophaga 36: 55-67. https://doi.org/10.1007/BF02374636

Hassan SA, Halsall N, Gray AP, Abdelgader H, 2000. Laboratory method to evaluate the side effects of plant protection products on *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae). In: Guidelines to evaluate side effects of plant protection products to non-target arthropod; Candolfi MP, Blumel S, Forster R (eds.). pp: 107-119. Dreier Druck, Reinheim, Germany.

Hewa-Kapuge S, McDougall S, Hoffmann AA, 2003. Effects of methoxyfenozide, indoxacarb, and other insecticides on the beneficial egg parasitoid *Trichogramma nr. brassicae* (Hym.: Trichogrammatidae) under laboratory and field conditions. J Econ Entomol 96: 1083-1090. https://doi.org/10.1111/j.1365-2338.1985.tb00224.x

IRAC, 2016. Mode of action classification: insecticide resistance management. Insecticide Resistance Action Committee. http://www.irac-online.org/documents/moa-classification/?ext-pdf. [19 Feb 2018].

Jalali SK, Mohanraj P, Lakshmi BL, 2016. Trichogrammatids. In: Ecofriendly pest management for food security; Omkar (ed.). pp: 139-181. Acad Press, San Diego, USA. https://doi.org/10.1016/B978-0-12-803265-7.00005-1

Jepson PC, 1998. Insects, spiders and mites. In: Handbook of ecotoxicology; Calow P (ed.). pp: 299-325. Blackwell, Oxford, UK. https://doi.org/10.1002/9781444313512.ch15

Kavitha J, Kuttalam S, Chandrasekaran S, Ramaraju, K, 2006. Effect of spironemifen 240 SC on beneficial insects. Ann Plant Protect Sci 14: 343-345.

Ksentini I, Jardak T, Zeghal N, 2010. *Bacillus thuringiensis*, deltamethrin and spinosad side-effects on three *Trichogramma* species. Bull Insectol 63: 31-37.

Kumar P, Sekhar JC, Kaur J, 2013. Trichogrammatids: integration with other methods of pest control. In: Biological control of insect pests using egg parasitoids; Sithanantham S et al. (eds.). pp: 191-208. Springer, New Delhi, India. https://doi.org/10.1007/978-81-322-1181-5_9

Lefebvre M, Langrell SRH, Gomez-y-Paloma S, 2015. Incentives and policies for integrated pest management in Europe: A review. Agron Sustain Dev 35: 27-45. https://doi.org/10.1007/s13593-014-0237-2

Leppla NC, Johnson MW, Merritt JL, Zalom FG, 2017. Applications and trends in commercial biological control for arthropod pest of tomato. In: Sustainable managements of arthropod pest of tomato; Wakil W, Brust GE, Perring TM (eds.). pp: 283-303. Acad Press, London. https://doi.org/10.1016/B978-0-12-802441-6.00013-9

Liu TX, Zhang Y, 2012. Side effects of two reduced-risk insecticides, indoxacarb and spinosad, on two species of *Trichogramma* (Hym.: Trichogrammatidae) on cabbage. Ecotoxicology 21: 2254-2263. https://doi.org/10.1007/s1007-012-0981-5

Maia JB, Carvalho GA, Leite MIS, Lopes-de-Oliveira R, Makyama L, 2010. Selectivity of insecticides used in corn crops to adult *Trichogramma at opovirilia* (Hym.: Trichogrammatidae). Rev Colomb Entomol 36: 202-206.

Martins TB; Pereira SM, Carneiro AV, Betetto MJ, Bueno AF, 2011. Selectivity of products fitossanitários a pupas de *Trichogramma pretiosum* em ovos de *Anagasta kuehniella*. In: VI Jornada Acadêmica da Embrapa Soja; Saraiva OF, Melo PGS (eds.). pp: 38-41. EmbrapaSoja, Londrina, Brazil.

Moura AP, Carvalho GA, Pereira AE, Ricca KCD, 2006. Selectivity evaluation of insecticides used to control tomato pests to *Trichogramma pretiosum*. BioControl 51: 769-778. https://doi.org/10.1007/s10526-006-0001-x

Nornberg SD, Grützmacher AD, Kovaleski A, Camargo ES, Pasini RA, 2009. Toxicidade de agrotóxicos utilizados na produção integrada de maçã a *Trichogramma pretiosum* Riley, 1879 (Hym.: Trichogrammatidae) em condições de laboratório. Curr Agric Sci Technol 15: 1-4.

Oliveira HN de, Antigo MR, de Carvalho GA, Glaeser DF, Pereira FF, 2013. Seletividade de produtos fitossanitários usados na cana-de-açúcar a adultos de *Trichogramma galloi* (Hym.: Trichogrammatidae). Rev Colomb Entomol 36: 202-206.

Pinto JH, Stouthamer R, 2013. Seletividade de inseticidas utilizados no controle de *Trichogramma pretiosum* em ovos de *Anagasta kuehniella*. In: VI Jornada Acadêmica da Embrapa Soja; Saraiva OF, Melo PGS (eds.). pp: 38-41. EmbrapaSoja, Londrina, Brazil.
Rezende DT, Carvalho GA, Moura AP, Moscardini VF, Souza JR, Lasmar O, 2005. Side effects of some pesticides used in maize crops in Brazil to the egg parasitoid Trichogramma atrovirilia (Hym.: Trichogrammatidae). Egg Parasitoid News, IOBC 17: 16.

Roditakis E, Vasakis E, García-Vidal L, Martínez-Aguirre MR, Rison JL, Haixare-Lutun MO, Nauen R, Tsagkarakou A, Bielza P, 2018. A four-year survey on insecticide resistance and likelihood of chemical control failure for tomato leaf miner Tuta absoluta in the European/Asian region. J Pest Sci 91: 421-435. https://doi.org/10.1007/s10340-017-0900-x

Saber M, Hejazi MJ, Hassan SA, 2004. Effects of azadirachtin/neemazal on different stages and adult life table parameters of Trichogramma cacoeciae (Hym.: Trichogrammatidae). J Econ Entomol 97: 905-910. https://doi.org/10.1093/jee/97.3.905

Saelices RM, López A, Amor F, Bencochëa P, Fernández MM, Garzon A, Morales I, Velazquez E, Medina P, Adaán A, et al., 2012. Ecotoxicidad de insecticidas de uso frecuente en el cultivo del tomate, en el enemigo natural Trichogramma achaeae (Hym.: Trichogrammatidae). Bol San Veg Plagas 38: 95-108.

Saljooqi AUR, Nawaz M, Farid A, Khan IA, 2012. Compatibility of spinosad with Trichogramma chilonis (Hym.: Trichogrammatidae) in integrated pest management of Sitotroga cerealella. Pak J Zool 44: 133-139.

Sanchez C, Gallego JR, Gamez M, Cabello T, 2014. Intensive biological control in Spanish greenhouses: problems of the success. Int J Biol Biomol Agric Food Biotechnol Eng 8: 1128-1132.

Sattar S, Farmanullah, Saljooqi AR, Arif M, Sattar H, Qazi JI, 2011. Toxicity of some new insecticides against Trichogramma chilonis (Hym.: Trichogrammatidae) under laboratory and extended laboratory conditions. Pak J Zool 43: 1117-1125.

Schindelin J, Arganda-Carreras I, Frise E, Aymig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, et al., 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9: 676-682. https://doi.org/10.1038/nmeth.2019

Scholz BCG, Zalucki MP, 2000. The effects of two new insecticides on the survival of adult Trichogramma pretiosum Riley in sweet corn. In: Hymenoptera: evolution, biodiversity and biological control; Austin A, Dowton M (eds.). pp: 381-388. CSIRO Publ, Clayton, AU.

Smith SM, 1996. Biological control with Trichogramma: advances, successes, and potential of their use. Annu Rev Entomol 41: 375-406. https://doi.org/10.1146/annurev.en.41.011996.021111

Stanley J, Preetha G, 2016. Pesticide toxicity to non-target organisms: exposure, toxicity and risk assessment methodologies. Springer Science+Business Media, Dordrecht: 502 pp. https://doi.org/10.1007/978-94-017-7752-0

Stark J, Jepson PC, Mayer DF, 1995. Limitations to use of topical toxicity data for predictions of pesticide side effects in the field. J Econ Entomol 88: 1081-1088. https://doi.org/10.1093/jee/88.5.1081

Sterk G, Hassan SA, Baillod M, Bakker F, Bigler F, Blümel S, Bogenschütz H, Boller E, Bromand B, Brun J, et al., 1999. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". BioControl 44: 99-117.

Suh CPC, Orr DB, Van Duyn JW, 2000. Effect of insecticides on Trichogramma exiguum (Hym.: Trichogrammatidae) preimaginal development and adult survival. J Econ Entomol 93: 577-583. https://doi.org/10.1603/0022-0493-93.3.577

Takada Y, Kawamura S, Tanaka T, 2001. Effects of various insecticides on the development of the egg parasitoid Trichogramma dendrolimi (Hym.: Trichogrammatidae). J Econ Entomol 94: 1340-1343. https://doi.org/10.1093/jee/94.6.1340

Tipping PW, Burbulis PP, 1983. Some effects of pesticide residues on Trichogramma nobilae (Hym.: Trichogrammatidae). J Econ Entomol 76: 892-896. https://doi.org/10.1093/jee/76.4.892

van Lenteren JC, Bolckmans K, Kohl J, Ravensberg WJ, Urbanja A, 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl 63: 63-59. https://doi.org/10.1007/s10526-017-9801-4

Vianna UR, Pratissoli JD, Zanuncio C, Serrao JE, 2009. Insecticide toxicity to Trichogramma pretiosum (Hym.:
Trichogramma achaeae) females and effect on descendant generation. Ecotoxicology 18: 180-186. https://doi.org/10.1007/s10646-008-0270-5

Vila E, Cabello T, 2014. Biosystems engineering applied to greenhouse pest control. In: Biosystems engineering: biofactories for food production in the Century XXI; Guevara-Gonzalez R, Torres-Pacheco I (eds.), pp: 99-128. Springer, Cham, CH. https://doi.org/10.1007/978-3-319-03880-3_4

Williams T, Valle J, Viñuela E, 2003. Is the naturally derived insecticide spinosad compatible with insect natural enemies? Biocontrol Sci Technol 13: 459-475. https://doi.org/10.1080/0958315031000140956

Yong TH, Hoffmann MP, 2006. Habitat selection by the introduced biological control agent Trichogramma ostriniae (Hym.: Trichogrammatidae) and implications for nontarget effects. Environ Entomol 35: 725-732. https://doi.org/10.1603/0046-225X-35.3.725