Selection of *Trichogramma* spp. (Hym.: Trichogrammatidae) for the biological control of *Tuta absoluta* (Lep.: Gelechiidae) in greenhouses by an entomo-ecological simulation model

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Abstract: A greenhouse trial was carried out, between October 2011 and April 2012, on tomato crops with the aim of evaluating the biotic potential of two species of *Trichogramma* (*T. achaeae* and *T. urquijoi*) for the control of the South American tomato moth, *Tuta absoluta*. The trial also studied the relationship of the latter with the predator *Nesidiocoris tenuis*, which is also used in biological control programmes in greenhouses. With this objective, we developed a mathematical model which makes it possible to evaluate and compare the effects of different biological control agents. As a result we established that *T. achaeae* was better at controlling pest populations than any other species. We also noted a significant intra-guild competition effect of the predator on both species of *Trichogramma*.

Key words: *Trichogramma achaeae*, *T. urquijoi*, *Nesidiocoris tenuis*, mathematical model, intra-guild competition

Introduction

Over 500 mathematical models have been developed for crop pest species in an attempt to improve methods for controlling them (Nietschke *et al.*, 2007). However, fewer than 50 such models have been set up for use in more general biological controls (Barlow, 2004). Of them, the predator-prey and parasitoid-host models were the ones that were most developed, but mainly at the theoretical level (e.g. Hawkins & Cornell, 2004); only a few of these models found real applications under field conditions. Moreover, the authors of these models always tried to approach the problem from the mathematical point of view, regarding it as a continuous system. This was particularly true of augmentative biological control by inoculative releases of entomophagous species and pest species with overlapping generations. In contrast, very few mathematical models have been applied to biological controls involving inundative releases and pests with discrete generations; the only the exceptions have been the works of van Hamburg & Hassell (1984), Smith & You (1990), You & Smith (1990) and Rafikov & Limeira (2012). This may be the result of host-parasitoid systems presenting more difficulties and greater complexity. Thus, it has been pointed out that pests and their para-

sitoid species with discrete generations, which show an imperfect phenological synchronization, result in reduced or even no risk of parasitism (Godfray, 1994; Godfray *et al.*, 1994).

Furthermore, the need for mathematical models which can be applied to biological control conditions in greenhouses starts with the combined use of several entomophagous species (parasitoids and predators) in the same crop. It is therefore necessary to study the intra-guild competition. These relationships, as noted in other theoretical and applied studies, can play an important role in the efficient use of natural enemies (Rosenheim *et al.*, 1995, Gillespie & Roitberg, 2006). With this in mind, intra-guild competition must be addressed in the development of such models.

The aim of this paper is to present a mathematical model for phytophagous-parasitoidpredator systems which could be applied in the selection of *Trichogramma* species for use in biological controls of the South American tomato moth, *Tuta absoluta* and also to study the relationships between intra-guild biological control agents in this particular case.

Material and methods

The insects *Trichogramma urquijoi*, *T. achaeae* and *T. Absoluta*, which were used in the different trials that were conducted, came from populations reared at the Entomology Lab of the University of Almeria (Spain); these insects were raised according to the methodology described by Cabello (1985) and Marin *et al.* (2002). At the same time, the predator *Nesidiocoris tenuis* was used in pre-transplantation. The trial was carried out from October 2011 to April 2012 at the Experimental Station of Cajamar Foundation (El Ejido, Almeria, Spain). It took place in a 16×16m greenhouse which was divided into two isolated modules (16×8m). The plants, of the cultivar Iberico[®] Philoseed España, were grown in soil with a gravel-sand mulch, at a density of 2.30 plants/m². Artificial *T. absoluta* infestation was carried out on 4 November 2011(21 days after transplanting, or DAT hereafter) at a rate of 8.4 released adults/m². In one greenhouse module, a *T. achaeae* release was carried out, while *T. urquijoi* was released in the other module; both releases were at a rate of 100 adults/m² and took place on 11 November 2011 (28 DAT). The temperature and relative humidity values for each greenhouse module were monitored by means of thermo-hygrometers (EBI 20-TH1, Ebro Electronic GmbH & Co. KG, Ingolstadt, Germany) placed inside a meteorological box.



Figure 1: Network of interactions considered in the herbivore-parasitoid-predator model (H = *Tuta absoluta*; $E_1 = Trichogramma$ spp. and $E_2 = Nesidiocoris tenuis$); the linking lines show benefits (+ arrow) and losses (- clubs).

Weekly samplings (total no = 7) began on 20 December 2011 (35 DAT) for both the pest (number of eggs) and predator (nymphs and adults) on each leaf. For sampling, whole upper-, mid- and low-thirds of selected leaves were examined *in situ*. This sampling system has been

reported to be the most suitable for analysing predator behaviour (Sanchez, 2009). A total of 15 plants were sampled in each greenhouse module and every week (total number of leaves sampled = 630). The sampled eggs were collected and taken to the laboratory where they were examined under a binocular microscope to determine whether they had hatched or had been killed by predators. The remaining eggs were individually isolated and left to evolve (at $25 \pm 1^{\circ}$ C and 60-80% RH) until they hatched in order to determine *Trichogramma* parasitism (total eggs sampled = 1,539). Figure 1 shows the network interactions considered according to the nomenclature employed by Mills (2006) and used in the model. Its mathematical expression is shown below:

Phytophagous species:	$x_1' = x_1 (m_1 - \alpha \cdot x_2 - \gamma_1 \cdot x_3)$
Parasitoid species:	$x_2' = x_2(-m_2 + \beta \cdot \alpha \cdot x_1 - \gamma_2 \cdot x_3)$
Predator species:	$x_{3}' = x_{3}(-m_{3} + \gamma_{1} \cdot x_{1} + \gamma_{2} \cdot x_{2})$

Where x_1 , x_2 and x_3 are the densities (number / m2) of the phytophagous, parasitoid and predator species, respectively; m_1 is the phytophagous growth rate; m_2 and m_3 are the parasitoid and predator mortality rates, respectively; α is the parasitism rate; β is the parasitoid emergence rate; γ_1 and γ_2 are the predation rates on phytophagous and parasitoid, respectively.

The previous model was fitted to the data using the software Simfit version 6.6.2 (Bardsley, 2010).

Results and discussion

The model adjusted very well to the data for both *Trichogramma* species (Figure 2). The pest populations were slightly higher on the *T. achaea* than on the *T. urquijoi* plots, but the former produced a better and faster control of the pest population. This would seem to suggest that *T. achaeae* offered a better potential to control *T. absoluta*. The observed differences may have had several explanations, including abiotic factors, such as temperature. During the trial, the average, maximum and minimum temperature values were 19.9, 38.9 and 6.7°C, respectively.

From the temperatures observed, it appears that T. achaeae adapts well to unheated greenhouses with extreme (minimum and maximum) temperature values. This confirms previous work conducted on this species (Kabiri et al., 2010; Vila et al., 2010). Contrary to some previous reports (Urbaneja et al., 2012), temperature does not, therefore, hinder the parasitic activity of T. achaeae. It should also be noted that T. achaeae has been used in Central Asia in cotton crops under high temperature and low relative humidity conditions (Puerta, 1979). In contrast, such extreme temperatures appear to have a detrimental effect on T. urquijoi. These effects have also been cited in the biology (Cabello & Vargas, 1989) and parasitization rate (Oliveira et al., 2003) of other species of the same genus. Other results obtained from the data and from the model that was developed include the importance of intra-guild competition between N. tenuis - species of palaeotropical origin that were introduced into Europe (Goula, 1985; Malausa & Ehanno, 1988; Wheeler & Henry, 1992; Rabitsch, 2008) - and the two species of Trichogramma (Figure 2). This was recently demonstrated by Calvo et al. (2012). These authors found that when a high N. tenuis population was used under semi-field conditions (with average values of 1.3 individuals/leaf and extreme values of 0.4 and 2.0 individuals/leaf) in the presence of only T. absoluta eggs

(with the absence of any other prey like *B. tabaci*), no effects of *T. achaeae* parasitization were observed. This phenomenon had also previously been observed in cotton crops in which the appearance of *Orius insidiosus* (Say) predated up to 90% of *T. pretiosum*-parasitized eggs, as these eggs were exposed for longer than the non-parasitized ones (Lingren & Wolfenbarger, 1976; King *et al.*, 1985; Knutson, 1998).



Figure 2: Simulation model applied to data showing the evolution of the pest *Tuta absoluta*, *Trichogramma* parasitoids (A = T. *urquijoi*, B = T. *achaeae*) (both values on the left axis) and the predator *Nesidicoris tenuis* (values on the right axis), in greenhouse tomato crops.

Intra-guild predation has been widely discussed (e.g.: Brodeur & Boivin, 2006) and also in greenhouse crops (Lucas & Alomar, 2002; Castañé *et al.*, 2004). However, this is the first

time that intra-guild competition has been demonstrated for an herbivore- predator-parasitoid system in greenhouse crops. It is also the first time that a mathematical model has been developed and applied to these intra-guild relationships.

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