## 1 Title: A new multistage dynamic model for biological control exemplified by the host-parasitoid

## 2 system Spodoptera exigua-Chelonus oculator

- **3 Running title:** Dynamic Model for Biological Control
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16 Abstract. Over the last few decades, important advances have been made in understanding of host-17 parasitoid relations and their applications to biological pest control. Not only has the number of agent species 18 increased, but new manipulation techniques for natural enemies have also been empirically introduced, 19 particularly in greenhouse crops. This makes biocontrol more complex, requiring a new mathematical modelling 20 approach appropriate for the optimization of the release of agents. The present paper is aimed at filling this gap by the development of a temperature- and stage-dependent dynamic mathematical model of the host-parasitoid 21 22 system with an improved functional response. The model is appropriate not only for simulation analysis of the 23 efficiency of biocontrol agents, but also for the application of optimal control methodology for the optimal 24 timing of agent releases, and for the consideration of economic implications.

Based on both laboratory and greenhouse trials, the model was validated and fitted to the data of
 *Chelonus oculator* (F.) (Hym.: Braconidae) as a biological control agent against the beet armyworm, *Spodoptera exigua* Hübner (Lep.: Noctuidae). We emphasize that this model can be easily adapted to other interacting
 species involved in biological or integrated pest control with either parasitoid or predator agents.

Key Words: entomo-ecology, dynamical model, degree-day dependence, functional response, host-parasitoid
 system, greenhouse crop.

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### **37 INTRODUCTION**

38 Recently, a large amount of high-quality data is collected in laboratory and greenhouse conditions. This facilitates the development of mathematical models based on fine-scale details of biological situations. These 39 40 kinds of models have important advantages. First, we can determine whether the parameters (in this study, 41 degree-day dependence, functional response, and population dynamics) measured in laboratory, correspond with 42 the appropriate theoretical population dynamics. If not, different degree-day dependences and functional 43 responses can be considered, respectively, or the theoretical model improved. Second, like in physics, a 44 theoretical model of population dynamics can be used to predict experimental results by simulation, and these 45 predictions can be experimentally tested. Of course, based on general principles, in each concrete biological 46 situation, new models are required for each specific biological situation.

47 Over the last few decades, important advances have been made concerning host-parasitoid relations and 48 their applications to biological pest control. Not only has the number of agent species increased, but new 49 manipulation techniques for natural enemies have also been introduced, particularly for greenhouse crops. This 50 makes biocontrol more complex, requiring a new mathematical modelling approach appropriate for the 51 optimization of the release of agents. The present paper is aimed at filling this gap by the development of a 52 temperature- and stage-dependent dynamic mathematical model of a host-parasitoid system with an improved functional response. This model is appropriate not only for simulation analysis of the efficiency of biocontrol 53 54 agents, but also for the application of control methodology for optimal timing of agent releases, and for the 55 consideration of economic implications.

Based on both laboratory and greenhouse trials, the model was validated and fitted to the data of *Chelonus oculator* (F.) (Hym.: Braconidae), a biological control agent (koinobiont parasitoid) for the beet armyworm, *Spodoptera exigua* Hübner (Lep.: Noctuidae). We emphasize that this model can be easily adapted to other interacting species involved in biological or integrated pest control with either parasitoid or predator agents

### 61 Theoretical preliminaries

62 Mathematical models are widely used in population biology, particularly in entomology. Fecundity, rates of 63 development and mortality are strongly affected by temperature in insects (Chown and Nicolson 2004). These 64 effects are included in most deterministic dynamic stage-dependent models of insect populations (e.g., Birt et al. 65 2009; Bommarco 2001; Prasad et al. 2002; Schmidt et al. 2003; Son and Lewis 2005; Söndgerath and Müller-66 Pietralla 1996; Wagner et al. 1985). For stochastic models, see Castañera et al. (2003) and the references therein. 67 To describe interacting insect populations, functional response has also been built into stage-dependent models. 68 Murdoch et al. (1997) studied the effects of host size and parasitoid state-dependent attacks. Alto et al. (2009) 69 investigated the coexistence of competing mosquitoes when the predation efficiency depends on the size of 70 instars in a marine aquaculture model (Barbeau and Caswell 1999). In our study, we are interested in a concrete 71 mechanism underlying host-parasitoid interaction that can be applied in biological control practice. In our 72 dynamic population model, two factors play an important role: temperature dependence of the developmental 73 rates and density dependence of the functional response.

With the change from chemical to biological control motivated principally by resistances to insecticides,
 pest control in greenhouse crops of northern Europe has experienced an important evolution in the last 30 years

76 (Lenteren 2007). A similar, more recent, shift has occurred in Spanish greenhouse crops (Blom 2010). Again,

excessive use of chemical control (Cabello and Cañero 1994) led to increased resistance to insecticides (Blom

- 78 2010). This change was very rapid; out of approximately 24,000 Ha of crops, the use of biological control has
- 79 increased from 1,400 Ha in the 2006-07 crop season, to 23,500 Ha in 2009-10 (Blom 2010).

## 80 Factors modeled in the literature but not considered in the current study

81 Not only are the developmental rates of insects strongly affected by temperature, but the functional 82 responses also depend on it (Logan et al. 2006; Logan and Wolesensky 2007). For simplicity, we did not account 83 for this dependence. Furthermore, humidity (e.g., Choi and Ryoo 2003) and dispersal and/or spatial population 84 structure (e.g., Ims and Andreassen 2005) also have an effect on insect population dynamics.

Our stage-dependent model is not a spatial model as it ignores the colonization process, since in a greenhouse, we can consider the pest and parasitoid populations uniformly distributed among plants (although we do not consider their distribution on a given plant). This is justified, firstly, by the relatively small crop areas in greenhouses compared to those in open-air crops. Secondly, after the initial infestation of the pest population, crop colonization is very quick because of the temperature and high plant density. Finally, the hypothesis of a uniform distribution of parasitoid populations is justified by their uniform release throughout the greenhouse, and because the released specimens, in our case, are adults with the capacity for flight.

## 92 The host-parasitoid system

93 The beet armyworm, S. exigua, is a lepidopteran species (Family Noctuidae); it is polyphagous and attacks 94 herbaceous plants in greenhouses and open-air crops. It is a serious pest species in pepper and watermelon crops 95 in greenhouses (Cabello 2009). In turn, Ch. oculator is a species in the genus Chelonus (Subfamily Cheloninae) 96 that constitutes a wide group within the Braconidae family (Ichneumonoidea superfamily). All Cheloninae 97 species are egg-larval lepidopteran solitary koinobiont endoparasitoids. Females oviposit inside the host egg and 98 subsequently, at the beginning of the third larval instar, the parasitoid leaves the egg to pupate (Gauld and Bolton 99 1996; Garcia-Martin et al. 2005). Several aspects of its biology, ecology, and functional response have been 100 studied in laboratory conditions, but not on natural hosts (Garcia-Martin et al. 2005, 2008; Ozkan and Tunca 101 2005; Ozkan 2006; Cabello et at., 2011 a). The species has already been used in the biological control of S. 102 exigua in greenhouses of southeast Spain.

First, we summarize the methods of data collection and evaluation of the biological parameters of the host and parasitoid species, for laboratory and greenhouse trials. A dynamic mathematical model of the hostparasitoid system was developed for the analysis of the biological control mechanism and its validation with the greenhouse data is presented. It describes the results of laboratory and greenhouse trials, as well as simulation results of the dynamic biological-control model. Applications for predicting the effect of release of *Chelonus* during different stages of *Spodoptera* development are also considered. Finally, the results are summarized and discussed.

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## 111 MATERIAL AND METHODS

## 112 Rearing conditions

113 The species used in the different trials come from populations maintained in laboratory conditions  $(25 \pm 114 \ 1 \ ^{\circ}C, \ 60-80\% \ R.H.$  and 16 h: 8 h light: dark cycle) at the University of Almeria. *S. exigua* was reared on an artificial diet, according to the methodology described by Cabello et al. (1984 a,b). The parasitoid species, *Ch.* 

116 oculator, was reared according to the methodology used by Cabello et al. (2011 a), on alternative host, Ephestia

117 *kuehniella* Zeller (Lep.: Pyralidae), which was reared according to Daumal et al. (1975).

### 118 Evaluation of biological parameters of the host species

a) <u>Laboratory trial</u>

120 The experimental design was randomized with one factor (temperature) at four levels (15, 20, 25, and 121  $30 \pm 1$  °C). The number of replications was variable for each temperature tested, with a minimum of 515 eggs, 122 477 larvae, 240 pupae, and 99 couples of adults. Rearing conditions were 60-80% R.H. and 16 h: 8 h of light: dark cycle. Egg masses were obtained from the adult couples reared at one of the four temperatures for at least 123 124 one generation. Small egg masses (presenting less than 50 eggs arranged in a single layer) were cleaned of scales 125 and isolated in a container (25 ml) with a lid that was provided with a hole (0.75 cm in diameter) and closed 126 with a metallic mesh. Then, neonate larvae were isolated from the hatched eggs in the same type of container and 127 an artificial diet was used and replaced every 24 hours. The containers were placed in climatic cabinets at each 128 of the four temperatures. After pupation, the sexes were separated by their morphological characters and 129 maintained at each temperature until they reached the adult stage. Later, pairs of adults  $(1 \oplus +1 )$  were placed in 130 cylindrical containers (Ø 85 mm x 70 mm) of filter paper used as the ovipositional container. As food, honey in 131 water (10 %) was provided on cotton, and was replaced every 24 hours.

The times spent in the egg, pupa, and larval stages were recorded. Longevity and fecundity of adults were also recorded for each temperature. The data were statistically analyzed using a generalized linear model (GLM) and Tukey's test with the SPSS software package (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, version 21.0, Armonk, NY, USA). To calculate the thermal units required for growth and development, we used the improved linear model of Ikemoto and Takai (2000):

137

$$(D \cdot T) = k + t \cdot D$$

where D = length of stage in days, T = temperature in °C, k = thermal units required for growth and development in accumulated degree-days (ADD) and t = minimum threshold temperature in °C. The linear regressions were realized using SPSS.

b) <u>Greenhouse trial</u>

The greenhouse trial was carried out between June and August in an 'Almeria' type greenhouse with a soil mulch system and pepper crop (variety INIA) located at La Mojonera, Almeria, Spain, on a 750 m<sup>2</sup> surface. The crop management was the same as traditional practice in the area except that no phytosanitary treatment was applied. Four cages were made with nonwoven fabric (2.25 m above and 0.15 m below the level of the soil; 2.0 m wide and 5.0 m long) located at random inside the crop surface. Each cage contained five pepper plants (1.2 m height). A mature adult couple (1  $\wp$  + 1  $\stackrel{\circ}{\supset}$ ) of *S. exigua* from the laboratory population was also released into every cage.

The plants inside every cage were numbered and the phenological states were recorded (height, number of side branches, and number of leaves, flowers, buds, and fruits in the different branches). In the first five days, samples were collected every day to count the eggs oviposited by the females. Later, even in the pupal stage, sampling was conducted twice per week to count the larvae and record their instars. Then, the entire soil in every cage of the greenhouse up to 15 cm depth was sifted to obtain the pupae. Immediately after, the pupae of each cage were introduced in a wooden box with walls of a metallic mesh (# 0.5 mm) that was buried in the same soil of every cage in the greenhouse; these were observed, every two days, until the emergence of the adults. Duringthe trial period, a thermo-hygrometer was placed in each cage.

To analyze the stage-frequency data of the host species, we have considered a simplified version of the Kiritani, Nakasuji and Manly's method (Manly, 1990; Southwood and Henderson, 2000) as it was described for zooplankton instar development (Rigler and Cooley 1974; Hairston 1985). However, we consider single cohort data (i.e., for populations where all individuals enter stage 1 at about the same time) (Manly 1990).

161 We consider  $\overline{b}_{j+1,j}$ , the number of individuals entering stage j + 1:

162 
$$\overline{b}_{j+1,j} \coloneqq \frac{A_j}{T_j},$$

where  $A_j$  is the area under a stage-frequency curve for stage j, and  $T_j$  is the developmental time for stage j + 1, expressed in ADD.

165 We also consider  $\overline{b}_{i,j}$ , the number of individuals that remain in state *j*:

166 
$$\overline{b}_{j,j} \coloneqq \frac{A_j}{T_j} - \frac{A_{j+1}}{T_{j+1}}$$

167 For each stage, values were obtained from the experiments. They were interpolated in order to have168 equidistant times for function fitting to calculate the transition coefficients between the stages.

# 169 Evaluation of biological parameters of the parasitoid species

# a) <u>Laboratory trial</u>

The experimental design was randomized with one factor (temperature) and two levels (20 and  $30 \pm 1$ °C). The number of replications for each temperature treatment was variable, with a minimum of 350 larvae inside the host, 172 larvae in the third-instar outside the host, 125 pupae, and 21 parasitoid adult couples. Rearing conditions were, 60–80 % R.H., and 16 h: 8 h of light: dark cycle. Egg masses of the host, *S. exigua*, were obtained from laboratory rearing.

The eggs were parasitized by isolated *Ch. oculator* females that were mated previously with two males. Fifty eggs were offered for 4–5 hours to every parasitoid female for both temperature treatments. Once the eggs hatched, the neonate host larvae were placed individually in a plastic container (20 ml). Host larvae were reared in similar conditions, up to the emergence of the parasitoid larvae, the third-instar. At this time, they were isolated in a clean container and preserved, at each test temperature until the emergence of the adult.

- Later, the parasitoid adult couples  $(1 \bigcirc + 1 \circlearrowleft)$  were placed in isolation in Petri dishes (Ø 85 mm). Food, honey diluted in water (50%) was provided on cardboard and replaced every 24 hours. In addition, every 24 hours, a cardboard with 300 *E. kuehniella* eggs was added to each container. Later, the cardboard pieces with the parasitized eggs were moved to a plastic container (100 ml) with flour, beer yeast, and wheat germ, and they developed at  $30 \pm 1$  °C until the emergence of the adult.
- 186 The times spent inside or outside the host larvae and in the pupa stage were recorded. Longevity and187 fecundity of adults were also recorded. These data were collected for both temperature treatments.
- 188 The data for each stage of host development as well the longevity and fecundity of adults were analyzed 189 by GLM and the Tukey's test by using the SPSS software package. From the values obtained, the transition 190 coefficients of the species for each developmental stage were determined as described above.

b) <u>Greenhouse trial</u>

- Trials were carried out with three different parasitoid release rates (0.5, 1.0, and  $1.5 \text{ }^{2}/\text{m}^{2}$ ). For each trial, the experimental design was randomized with one factor (host density) at five levels (50, 100, 150, 200, or 250 host eggs). The number of replications was 12 for each host density. A multi-tunnel greenhouse (969 m<sup>2</sup>) located in La Cañada, Almeria, Spain, was used. There were two lines for each of the following crops: tomato, pepper, bean, cucumber, melon, and watermelon to minimize the effect of the plant in the behavior of parasitoid females. The three trials were realized between May and July and were separated by 15 days each. During the trial period, a thermo-hygrometer was placed in each cage.
- The parasitism rate in each trial was evaluated by the sentinel method (Mills 1997). *E. kuehniella* eggs (factitious host) were used: they were glued on a white cardboard ( $70 \times 30$  mm) with water at the different host densities. The cardboard pieces with the host eggs were distributed uniformly at random in the greenhouse and placed over the leaves using a paper clip. The eggs were left for 72–90 hours. Then, the cardboard was collected, and transferred into plastic containers with flour, beer yeast, and wheat germ. They developed at  $30 \pm 1$  °C until adult emergence. During the test period, temperature and relative humidity were measured using a thermohygrometer.

For each trial, the numbers of adult *Ch. oculator* and *E. kuehniella* that emerged from the cardboard wererecorded.

Based on the collected data, we determined the type of functional response to adapt in our dynamic model.
 The functional response was fitted to the average number of parasitized host eggs according to the following
 equations:

**211** Type II (Hassell 1978):

212 
$$x_a = x \cdot \left[ 1 - \exp\left( -\frac{a' \cdot T \cdot y}{1 + a' \cdot T_h \cdot x} \right) \right]$$

Type III (Cabello et al. 2007):

214 
$$x_{a} = x \cdot \left[ 1 - \exp\left( -\frac{\hat{\alpha} \cdot T \cdot x \cdot y}{1 + \hat{\alpha} \cdot T_{h} \cdot x + \hat{\alpha} \cdot T_{h} \cdot x^{2}} \right) \right],$$

where:  $x_a$  = number of parasitized hosts, x = host density, a' = instantaneous search rate (1/days),  $\hat{\alpha}$  = potential of mortality, T = total time available for search (days), and  $T_h$  = handling time (days), y = number of parasitoids.

The least-squares method in TableCurve 2D and 3D software packages (TableCurve 2D version 5.01
and TableCurve 3D version 4.0, Systat Software, San Jose, CA, USA) was used for parameter estimation.

219 Dynamic mathematical model

220 Our model is based on the mechanism displayed in the flow diagram in Figure 1.

For the *host species*, we consider five model stages (simply called stages) of the life cycle: egg, small larvae (1st to 3<sup>rd</sup> -instar), large larvae (4th and 5<sup>th</sup> -instar), pupa, and adults. Entomophagous parasites (parasitoid and predator species) are generally identified as small or large larvae (Cabello 1988).

For the lifecycle of the *parasitoid species*, three model stages, egg, larva-pupa, and adult were considered.

Since some coefficients in our model are functions of biological time (in ADD), we recall their general definitions in the discrete time form. Let T(t) (t = 0, 1, 2, 3,...) be the temperature scenario, then biological time,  $\tau_t$ , corresponding to physical time *t* is defined as

229 
$$\tau_t := \sum_{i=0}^t (T(i) - T_{\min}),$$

assuming that the temperature only changes within the physiological range of  $T_{\min}$  and  $T_{\max}$  valid for both species.

232 Let  $b_{j+l,i}(\tau)$  be the proportion of individuals passing from stage j to stage j + 1 during the biological 233 time  $\tau$  (*j* = 1,...,4), and  $b_{jj}$  ( $\tau$ ) be the proportion of individuals in stage *j* that remain in this stage; during the 234 same biological time (j = 1, ..., 4),  $\alpha_5(\tau)$  is the number of eggs laid per host female during this period, and  $\sigma$  is 235 the proportion of females among the host insects. Certainly,  $b_{j,j}(\tau) + b_{j+1,j}(\tau) \le 1$  must hold (j = 1, 2, 3, 4, 5) and 236  $1 - (b_{j,j}(\tau) + b_{j+1,j}(\tau))$  is interpreted as the death rate for stage j during the period considered. For the dependence of the transition rates on biological time, we apply the Weibull cumulative distribution function that 237 238 is widely used for the description of the development of ectothermic organisms (Wagner et al. 1984; Manly 1990; Söndgerath and Müller-Pietralla 1996; Choi and Ryoo 2003; Schmidt et al., 2003). Suppose that, after a 239 long period of biological time, each individual passes to the next stage with probability  $\lambda \in ]0,1[$ . Then, the 240 241 long-term death rate is  $1 - \lambda$ . With constants  $\alpha, \beta > 0$ , we define

242 
$$b_{j+1j}(\tau) := \lambda \left( 1 - e^{-(\frac{\tau}{\alpha})^{\beta}} \right) \text{ and } b_{jj}(\tau) = \lambda \left( e^{-(\frac{\tau}{\alpha})^{\beta}} \right).$$

Evidently, these constants may depend on the stage *j*. In the above expressions, for the *i*-th cohort in a given stage, it is necessary to substitute

245

$$au := au_t - au_{t-i}$$

which is the accumulated degree-day during the last *i* time units and can be easily calculated using online
software (Zalom et al. 1983). The number of eggs can also be expressed as

248 
$$\alpha_5(\tau) := \mu \left( 1 - e^{-(\frac{\tau}{\varepsilon})^{\circ}} \right)$$

249 where  $\mu$  is the maximal number of eggs per host female (after a long biological time), and  $\varepsilon$  and  $\varphi$  are 250 constants. For the Weibull cumulative distribution function, the TableCurve 2D software package was used for 251 parameter estimation by the Marquardt algorithm (Conway et al. 1970) and the least-squares method.

In our model,  $x_1, ..., x_5$  will denote the numbers (densities) of individuals in the above specified stages of the *host* population. Similarly, for the parasitoid,  $y_1$ ,  $y_2$ ,  $y_3$  indicate the densities for each of the three considered stages.

Furthermore, we distinguish *N* and *M* cohorts in each stage of the host and the parasitoid, respectively, with respective densities

257 
$$x_{ji}$$
  $(j = 1, 2, 3, 4, 5; i = 1, ..., N)$ , and  $y_{ji}$   $(j = 1, 2, 3; i = 1, ..., M)$ , and set  $x_1 := \sum_{i=1}^{N} x_{1i}$ ,  $y_3 := \sum_{i=1}^{M} y_{3i}$ 

For the model, it is also supposed that the parasitoid female only parasitizes the host eggs (Garcia-Martin *et al.*, 2008). Then, we applied the following functional response suggested by Cabello et al. (2007):

260 
$$f_1(x_1, y_3) := 1 - \exp_{10} \left( -\frac{\hat{\alpha} T x_1 y_3}{1 + \hat{\alpha} T_h x_1 + \hat{\alpha} T_h x_1^2} \right), \tag{1}$$

where T = 1 (day),  $T_h$ , the estimated handling time of the host (days) and  $\hat{\alpha}$  are fitting parameters. A further model parameter is  $\alpha_s(\tau)$ , the number of eggs laid per host female during this period.

### a) <u>Dynamics of the host population</u>

To describe the development of the host population, we propose the following multi-stage dynamic model:

Suppose that, for a unit of time, individuals in each stage j = 1, 2, 3, 4, in each cohort i = 1, 2, ..., N-1, either pass into the first cohort of the next stage, or pass into the next cohort of the same stage. Individuals of the Nth cohort in the stage j = 1, 2, 3, 4 either die or pass into the first cohort of the next stage. Individuals in the fifth stage (adults) either die or pass into the next cohort.

270 *Egg*:

$$x_{11}(t+1) = \sigma \sum_{i=1}^{N} \alpha_{5}(\tau_{t} - \tau_{t-i}) x_{5i}(t)$$

271

$$x_{1i}(t+1) = b_{11}(\tau_t - \tau_{t-(i-1)}) [1 - f_1(x_{1(i-1)}(t), y_3(t)] x_{1(i-1)}(t) \quad i = 2, ..., N$$

ı.

273 Small larva:

274 
$$x_{21}(t+1) = \sum_{i=1}^{N} b_{21}(\tau_t - \tau_{t-i}) [1 - f_1(x_{1i}(t), y_3(t)] x_{1i}(t).$$

3.7

275 
$$x_{2i}(t+1) = b_{22}(\tau_t - \tau_{t-i+1})x_{2(i-1)}(t) \quad i = 2,...N.$$

276 *Large larva, pupa, adult:* the densities of the cohorts in these three stages are expressed similarly:

277 
$$x_{31}(t+1) = \sum_{i=1}^{N} b_{32}(\tau_t - \tau_{t-i}) x_{2i}(t) ; \ x_{3i}(t+1) = b_{33}(\tau_t - \tau_{t-i+1}) x_{3i-1}(t) \quad (i=2,...,N);$$

278 
$$x_{41}(t+1) = \sum_{i=1}^{N} b_{43}(\tau_t - \tau_{t-i}) x_{3i}(t) \; ; \; x_{4i}(t+1) = b_{44}(\tau_t - \tau_{t-i+1}) x_{4;i-1}(t) \quad (i=2,...,N)$$

279 
$$x_{51}(t+1) = \sum_{i=1}^{N} b_{54}(\tau_t - \tau_{t-i}) x_{4i}(t) ; \quad x_{5i}(t+1) = b_{55}(\tau_t - \tau_{t-i+1}) x_{5i-1}(t) \quad (i=2,...,N).$$

b) <u>Dynamics of the parasitoid population</u>

The corresponding multi-stage dynamic model for the parasitoid is as follows. Assuming that every host egg is parasitized at most once, consider rates  $d_{jj}(\tau)$  (j = 1, 2, 3) and  $d_{j+1j}(\tau)$  (j = 1, 2) defined analogously as  $b_{j,j}(\tau)$  and  $b_{j+1,j}(\tau)$ , respectively. Then, for the parasitoid dynamics we have the following:

284 Egg:

285 
$$y_{11}(t+1) = f_1(x_1(t), y_3(t))x_1(t) ; y_{1i}(t+1) = d_{11}(\tau_t - \tau_{t-i+1})y_{1;i-1}(t) \quad (i=2,...,M)$$

286 *Larva-pupa:* 

287 
$$y_{21}(t+1) = \sum_{i=1}^{M} d_{21}(\tau_t - \tau_{t-i}) y_{1i}(t) \; ; \; y_{2i}(t+1) = d_{22}(\tau_t - \tau_{t-i+1}) y_{2;i-1}(t) \; (i=2,...,M).$$

288

Adult:

289 
$$y_{31}(t+1) = \sum_{i=1}^{M} d_{32}(\tau_t - \tau_{t-i}) y_{2i}(t) ; y_{3i}(t+1) = d_{33}(\tau_t - \tau_{t-i+1}) y_{3i-1}(t) \quad (i = 2, ..., M).$$

290

The coefficients  $b_{i,i}$  and  $d_{i,i}$  correspond to transitions between the different stages of the life cycle of the host and parasitoid, respectively, and are estimated according to the Weibull cumulative distribution functions. 291

292 The thermal parameter values used in the model are indicated in Table 2. For the minimum temperature 293 of activity of adult females of S. exigua, we used a minimum threshold of 10 °C according to estimates of Aarvik 294 (1981) and Belda (1994).

#### 295 Data and method for validation of the model in the greenhouse

296 Trials were carried out, between August and September, in an 'Almeria' type greenhouse with soil with 297 gravel-sand mulch and pepper crop (Lamuyo variety) located at El Ejido, Almeria, Spain, with a surface of 5,000 298  $m^2$ . The traditional crop management practiced in the area was adopted for the trial except that no phytosanitary 299 treatment was applied. Four cages were made with nonwoven fabric (3.0 m high, 18.0 m wide and 18.0 m long) 300 located at random on the crop surface; every cage contained 1,300 pepper plants (1.5 m height). During the trial 301 period, a thermo-hygrometer was placed in each cage.

302 The pest infestation was artificial and was done by placing eggs of S. exigua that were less than 24 -303 hours -old obtained from laboratory cultures, and they were uniformly distributed at a density of 12.5 eggs/m<sup>2</sup>. 304 At 24 hours, adult parasitoids were released uniformly and at a dose of 6 females/m<sup>2</sup>. Later, seven samplings 305 were performed, twice a week, for 20 plants per plot, in which all larvae found on the sampled plants were 306 collected. The larvae were isolated and reared in the laboratory with an artificial diet, as described above, until 307 the emergence of adults. Due to the death of some larvae, they were dissected under a binocular microscope to 308 check for the presence of the parasitoid larva.

309 Data for the parasitized and non-parasitized larvae were compared with the data obtained in the mathematical model using the  $R^2$  statistic, which was close to one indicating the validity of our model 310 311 (Montgomery 2010).

312

313 RESULTS

#### 314 **Biological parameters of the host species**

315 a) In laboratory

316 The average values of the development as well as the longevity and fecundity of the adults are shown in Table 1. In the statistical analyses, significant effects of temperature on the duration were found for all stages of 317 the host species ( $F_{3,511} = 349.74$ , P < 0.0001;  $F_{3,473} = 145.76$ , P < 0.0001;  $F_{3,454} = 179.10$ , P < 0.0001;  $F_{3,450} = 179.10$ , P < 0.0001;  $F_{3,450} = 100000$ 318 304.10, P < 0.0001;  $F_{3,444} = 205.07$ , P < 0.0001;  $F_{3,327} = 171.30$ , P < 0.0001;  $F_{2,237} = 200.34$ , P < 0.0001 for the 319 duration of egg, first-, second-, third-, fourth-, and fifth-instar, larva and pupa, respectively). Similarly, there 320 321 were significant effects of temperature on longevity ( $F_{3,95} = 16.83$ , P < 0.0001;  $F_{3,95} = 9.98$ , P < 0.0001) for 322 female and male, respectively. However, there was no significant effect of temperature on the fecundity of 323 females. Moreover, at 15 °C, the pupal stage is not completed, in any case, and adult emergence did not occur;

- 324 therefore, longevity and fecundity of adults was calculated from the individuals that developed at 20 °C (Table
- 1). From the above data, the thermal relationships were calculated and are shown in Table 2.
- b) <u>In greenhouse</u>

327 The transition coefficients of the host species, *S. exigua*, calculated for each developmental stage in the 328 greenhouse pepper crop are shown in Table 3. The cumulative numbers of individuals entering each 329 developmental stage are shown in Figure 2.

330 For the parameters of  $\alpha_5(\tau)$  the following values were obtained:  $\varepsilon = 49.87$ ,  $\varphi = 1.21$ ,  $\mu = 1292.8$  (see

- the model description in 2.4).
- **332** Biological parameters of the parasitoid species
- a) <u>Laboratory</u>

The average values for the rate of development as well as the longevity and fecundity of adults are shown in Table 4. In the statistical analyses, significant effects of temperature were found on the duration of the stages of the parasitoid species ( $F_{1,348} = 5345.25$ , P < 0.0001;  $F_{1,170} = 64.82$ , P < 0.0001;  $F_{1,123} = 3202.74$ , P <0.0001), for the duration of immaturity inside the host: egg to third -instar; outside: until pupa, and pupa, respectively. There were significant effects of temperature on the longevity of the female and male and apparent parasitism ( $F_{1,19} = 38.45$ ,  $d_{\cdot}f_{\cdot} = 1$ , P = 0.01;  $F_{1,19} = 94.33$ ,  $d_{\cdot}f_{\cdot} = 1$ , P < 0.0001;  $F_{1,19} = 20.99$ , P = 0.0002, respectively).

341

The transition coefficients were calculated and are shown in Table 5.

b) <u>Greenhouse</u>

The functional response found for each field trial, corresponds to type III (Table 6) that showed lower corrected Akaike information criterion (AICc) values (Motulsky and Christopoulos 2003) than did type II. This was performed in laboratory conditions (Garcia-Martin et al. 2008). The joint functional response was estimated and is shown in Figure 3.

From the almost perfect fitting shown in Figure 2, one may suspect that the model (or its components)was overfitted. In fact this is not the case:

1) Figure 2 is related to Table 3 and comes from the fitting of field data for five host stages (Section M&M, Evaluation of biological parameters of the host species, b) Greenhouse trial). The collected data correspond to 4 replications, 13 samplings and 5 stages (total data points = 260), and the total fitted parameters is 15 (5 stages and 3 parameters per stage), which is not considered overfitted. The appearance of overfitting may be due to the fact that Figure 2 corresponds to mean values for the 4 replicates. For the fitting of parameters in Table 3, all

- data were used, not only mean values.
- 2) Similarly, in Table 4 (there is no corresponding figure), there are nine total parameters for the parasitoid

356 species (3 stages and 3 parameters per stage) corresponding to data obtained in the laboratory (Section M&M,

- 357 Evaluation of biological parameters of the host species, b) Laboratory trial). The minimum number of data points
- is 350 larvae inside the host, 172 larvae in the third-instar outside the host, 125 pupae, and 21 parasitoid adult
- 359 couples for 2 temperature treatments (at least 1336 data points). Therefore, there is no overfitting.
- 360 3) Finally, Table 6 and Figure 3 correspond to field data (Section M&M, Evaluation of biological parameters of
- the parasitoid species, b) Greenhouse trial). Three trials with 5 parasitoid densities and 12 replications per
- density were realized (total number of data points = 180) and there were two (Table 6) or three parameters
- 363 (Figure 3). Hence no overfitting occurred in this case either.

364 **Biological control in greenhouse** 

a) Validation of the model

In order to validate our model, we compared the percentage of parasitized larvae with the value 366 367 calculated in the model using the same densities of host adults and released parasitoid adults for the sum of the 368 stages, egg + larva + pupa of the model. Larval parasitism was 92.16%, which is a high value. We carried out a 369 partial validation of our model (Figure 4). The validation is partial in the sense that we could use only a part of 370 the life cycle data of the parasitoid without using those corresponding to the host species. Comparing the real data to those of the model, a rather good matching was found since the coefficient of determination,  $r^2$ , is 0.8139 371 372 (d.f. = 8, P = 0.05). We emphasize that Montgomery (2010) proposed this type of validation pointing out that the 373 closer this value is to one, the more valid is the model.

374

365

b) Model runs showing the dynamics of different stages of both species

375 For the proportion of host females we have  $\sigma = 0.5$ . Tables 3 and 5 show the estimated parameters of 376 the Weibull function. The parameters of  $\alpha_5(\tau)$  are:  $\varepsilon = 49.87$ ,  $\varphi = 1.21$ ,  $\mu = 1292.8$ . Based on the maximal possible time an individual can remain in a particular stage, we set the number of cohorts for both species to N =377 378 M = 40. For the parameters of functional response  $f_1$  of *Chelonus* we have  $T_h = 0.0076$  and  $\hat{\alpha} = 0.00828$ . In 379 Figure 5, we show the rate of development of different stages of host (Figure 5 A) and parasitoid (Figure 5 B) 380 obtained by simulation. Furthermore, for the pest species it is supposed that, from day 30 until day 60, entrance 381 of adults to the greenhouse takes place at a rate of 50 adults per day. As for the parasitoid species, releases of 382 adults (600 adults/100 m<sup>2</sup>, with sex ratio 1:1) on days 32 and 38 were considered.

On may think that 2 -3 cohorts is sufficient. Nevertheless, we wanted to construct a more realistic model that can be applied to the *S. exigua-Ch. oculator* system in commercial greenhouse cultivars. Under these conditions, the cultivation cycle is summer through the following spring, and the infestation in the greenhouse occurs in the first 2 -3 months of the cultivation cycle; afterwards, the populations outside the greenhouse are very rare or absent, because the temperatures are higher inside than outside. The interior populations are important in terms of the crop damage.

According to Wagner et al. (1985) (op. cit.): "The number of cohorts in a simulation is determined by the number of classes in the oviposition distribution. Unfortunately, no method has been developed relating class interval length to the accuracy of the final prediction for a general population. One way to ensure precision is to set the sampling interval of eggs, and thus the class interval of the oviposition distribution, equal to or less than the development time of eggs (e.g., oviposition to hatch) in the field."

Taking into account the temperatures inside the greenhouse, and considering the data of Table 1, the duration of the egg stage is 2 days. For the indicated period, 40 cohorts (equivalent to 200 host population groups) have been considered. Furthermore, considering again the high inside temperatures, the durations of different host stages are reduced considerably in comparison to the outside conditions, generating great divergences between cohorts, justifying why, for a more precise model, it was necessary to consider such a high number of cohorts.

In case of the parasitoid, it was "only" necessary to consider 120 population groups to obtain a more realistic model under the conditions of greenhouse cultivars where usually two releases of parasitoid adults are realized, with a 7 -10 day interval between releases. (We note that the question of improving the efficiency of natural enemies by optimal timing of releases will be the subject of a forthcoming paper.) 404

### All data used in the model are available at Dryad Digital Repository: <u>http://doi.org/10.5061/dryad.t3b5s</u>.

## 405 DISCUSSION

## 406 Biological parameters of the host species

407 The results obtained for the developmental stages of the host species, S. exigua (in days) are similar to 408 those found by other authors (Butler 1966; Cayrol 1972; Fye and McAda 1972; Hogg and Gutierrez 1980; 409 Sannino et al. 1986; Tisdale and Sappington 2001; Elvira et al. 2010). The slight differences might be 410 attributable to the different food provided to larvae (Awmack and Leather 2002; Azidah and Sofian-Azirun 411 2006) or different origins of populations (Pashley 1986). Some differences were also found in the calculated 412 developmental threshold temperatures with respect to those reported by other authors for the same species. The 413 values threshold temperatures found for eggs (13.9 °C) are similar to those cited by El-Refai and Degheele 414 (1988), but they are somewhat higher than those indicated by Cayrol (1972) and McNally (1983). In turn, for 415 small larvae temperatures (12.5 °C) are similar to those quoted by other authors (Cayrol 1972; Hogg and 416 Gutierrez 1980; Ali and Gaylor 1992). The threshold temperature for the pupal stage (8.3 °C) is quite different 417 from those mentioned by El-Refai and Degheele (1988) and Ali and Gaylor (1992) for this species. The 418 differences may be owing to the different geographic locations of populations as demonstrated for this and other 419 insect species (Honek 1996).

The duration of the adult stage of *S. exigua* in our assay is similar to that of Hogg and Gutierrez (1980) and both are somewhat higher than those cited for the species by Sannino et al. (1987). In addition, female fecundity is very similar to those reported by Hogg and Gutierrez (1980) and in both cases are higher than those quoted by other authors for this species (Sannino et al. 1987; Wakamura 1990).

Based on the above, the thermal requirements used in the mathematical model for the eggs, small and large larvae, and pupae are the average of the measured values (10 °C), corresponding to the characteristics for the species in our geographic area.

## 427 Biological parameters of the parasitoid species

428 The development time for the parasitoid species in the natural host (S. exigua) (Table 1) is much shorter 429 than that cited in the factitious hosts: E. kuehniella, Plodia interpunctella (Hübner) (Lep.: Pyralidae) or Cadra 430 cautella (Walker) (Lep.: Pyralidae), which are the only data published until now for the species (Garcia-Martin 431 et al. 2005; Ozkan 2006; Tunca et al. 2011). Two life history strategies exist among parasitoid wasps: idiobionts, 432 where the host does not grow during the development of the parasitoid larvae and koinobionts, where the host 433 continues to grow during the parasitoid development (Askew and Shaw 1986). In idiobionts, the female wasp 434 uses its ovipositor to sting and kill or immobilize the host. The female's progeny thus have to develop on a fixed 435 amount of food. For this group, the influence of the host (size, species, age, etc.) on the biology of the parasitoid 436 has been demonstrated (Thompson and Hagen 1999). In koinobionts, the host is not killed or immobilized and 437 continues to grow after the female wasp oviposits. The progeny are thus not restricted to the original amount of 438 food (Cabello et al. 2011 a). Although the reason for this parasitoid group is less clear, the host may have an 439 influence on the koinobiontic parasitoids that develop on the hosts and continue to feed and grow (Thompson 440 and Hagen 1999).

The aforementioned effect of shorter development time in *S. exigua* can be explained by the amount of food available; therefore, the maximum weight of the last-instar larvae is 55 mg (unpublished data) unlike weights presented by *E. kuehniella* (31 mg) (Kallenborn and Mosbacher 1983) or *P. interpunctella* (16 mg)

- (Silhacek and Miller 1972). An increase in developmental time due to amount of food was shown in koinobiont solitary parasitoids (Harvey et al. 1995). This is motivated by host quality, and the koinobiont parasitoids are crucially dependent on the host growth rate after parasitism and on the final size of the host when it is destroyed by the parasitoid. When attacking nutritionally suboptimal hosts, such as early instars, the host may grow too slowly for the parasitoid to maximize size and minimize development time (Harvey 2005).
- 449 Moreover, in the adult, longevities found for males and females (Table 1) are unaffected by the rearing
  450 host; the values found are similar to previous studies that consider the same species, but in the host *E. kuehniella*451 (Garcia-Martin et al. 2005).
- The longevities of females and males (Table 4) are not affected by the rearing host at their immature stages since their longevities are very similar to those found in a different host by Garcia-Martin et al (2005). In contrast, the fecundity of the females is influenced by the rearing host at their immature stages; therefore, the fecundity values (Table 4) are 5–6 times higher than those reported by Garcia-Martin et al. (2005) for the host *E. kuehniella*. Similar effects for fecundity were published for other *Chelonus* species (Legner and Thompson 1977) and also in other hymenopteran parasitoids (Harvey and Thompson 1995; Stoepler et al. 2011).
- 458 The minimum development threshold -temperatures could not be determined from the measured data. The only value quoted for this species is 12.5 °C, but in a different host species (Tunca et al. 2011). As noted 459 above, the development rate is affected by the host species, and this in turn influences, according to the method 460 461 of calculation, the threshold temperatures; this issue has not been considered in the literature. Therefore, for the 462 simplicity of the model, we have considered the same development threshold temperature of the host species. 463 We did not consider this as causing serious error in the model, since for other species of the genus, for example, 464 Ch. texanus, Cresson (Hym.: Braconidae) in the same natural host, S. exigua, Butler (1966) found that the rate of 465 development of the parasitoid was no different than that of the host.
- 16

466 Using this temperature (10 °C), the average development time (expressed in ADD) was estimated
467 (Table 4) for the parasitoid species *Ch. oculator* reared in the host *S. exigua*.

## 468 Dynamic model

469 Over 500 mathematical models have been applied to establish the phenology of pest species in relation 470 to the biological time expressed in ADD (Nietschke et al. 2007), many of which considered the age-structure 471 (e.g.: Curry et al. 1978; Osawa et al. 1983; Hudes and Shoemaker 1988; Munholland and Dennis 1992) for their 472 applications to pest control (Nietschke et al. 2007). However, far fewer (50) are applicable in biological control 473 (Barlow 2004). Some refer to host-parasitoid models that are already applied. Concerning host-parasitoid 474 systems, this may be due, in part, to the fact that the host and parasitoid populations with discrete generations 475 frequently show imperfect phenological synchronization resulting in some hosts experiencing reduced or even no 476 risk of parasitism (Godfray et al. 1994).

- Although our model building approach is general, a novelty of our model is the use of an improved
  Holling type III functional response found in an earlier paper by some of the authors (Cabello et al. 2007). In
  fact, a statistical analysis demonstrates that (at least under the considered temperature conditions) this functional
  response fits the data better than the Holling type II functional response. To our knowledge, no multistage
  dynamic model has been built with this improved functional response.
- 482 In general terms, similar well-fitted dynamic models can be used in biological pest control for several
  483 purposes: 1. They may facilitate the selection of an adequate control agent. 2. The model simulations help

determine the most efficient release strategy, and in particular, the optimal timing of the release(s) of the agent
for biological control in greenhouse crops. 3. Such models can be extended to include economic aspects of
biological control for the anticipated analysis of cost-efficiency of biological control. Such developments
concerning the *S. exigua–Ch. oculator* system may be the subject of further research.

### 488 Efficiency of biological control

The genus *Chelonus* has received little attention as a natural or biological control agent of pest species, especially, compared to other species of Lepidopteran parasitoids (e.g., *Trichogramma*, *Cotesia*). Nine *Chelonus* species have been used in the control of Lepidopteran pests, worldwide, through classical biological control (introduction into a new geographic area). In most cases, the parasitoid was not established, or information on it does not exist, but in three cases, when they became established, they colonized new geographical areas and achieved satisfactory control of the pest (Greathead 1976; Clausen 1978; Nechols et al. 1995; Neuenschwander et al. 2003).

496 Furthermore, the use of the Chelonus species through augmentation has been minor (Elzen and King 497 1999; Etzel and Legner 1999). In this regard, four species were tested: Ch. eleaphilus, Silvestri (Hym.: 498 Braconidae) for the control of Prays oleae Hübner (Lep.: Praydidae) had a very high level of parasitism (92.0%) 499 (Stavraki 1970; Stinner 1977) and Chelonus sp. p. curvimaculatus, at a release rate of 0.3 females/m<sup>2</sup>, for control 500 of the Pectinophora gossypiella (Saunders) (Lep.: Gelechiidae) with a good rate of parasitism (69.9%) (Legner 501 and Medved 1979). In contrast, low levels of parasitism were detected in two cases. In Ch. inanitus (L.) (Hym.: Braconidae), the rate of 0.1 females/m<sup>2</sup> achieved a parasitism of 23.6% for *S. littoralis* in cotton crops (Rechav 502 1976) and for Ch. heliopae, a high release rate (10 adults/m<sup>2</sup>) presented a very low level of parasitism of 503 504 Spodoptera litura (F.) (Lep.: Noctuidae) (8.8%) on a cauliflower crop (Patel et al. 1979).

The parasitism found in our study (92.16%), indicates that *Ch. oculator* is a good biological control agent of *S. exigua* in the greenhouse pepper crop. In fact, the species is used for the control of *S. exigua* in southern Spain.

508 The total area that can be covered by biological pest control using the presented application in Spanish 509 greenhouses will be over 25,000 Ha in the next growing season of 2013–14, representing 90 % of the total area. 510 This implies that the utilization of a large amount of natural enemies through the application of different 511 techniques is beneficial. Therefore, to understand the efficiency and cost -effectiveness of this method, it is 512 important to understand how, when and in what doses natural enemies of pests should be released? Despite the great experience of technicians and farmers, this work should not be left to them (Cabello et al. 2011b) alone. 513 514 Therefore, we consider that similar research works must be carried out to improve and optimize biological 515 control of pests in greenhouses.

516 We emphasize that, although our multistage dynamic model is fitted only to the data of S. exigua and 517 Ch. oculator, the model can be easily adapted to other pairs of interacting species involved in biological or 518 integrated pest control with either parasitoid or predator agents. Indeed, the possibility of such extensions of our 519 model rests on the following pillars necessary for the adequate description of stage-specific interactions between 520 the two insect populations: (a) It accounts for the specific developmental temperature threshold. (b) The 521 population densities are structured by developmental stages and by cohorts within each stage. (c) Transition rates 522 depend on biological time according to the Weibull cumulative distribution function generally accepted in 523 modeling of insect development (Wagner et al. 1984). (d) The stage-dependent interspecific interaction is

- by a functional response fitted to the data on the concrete host-parasitoid or prey-predator interaction.
- 525 Of course, for the model fitting (estimation of the model parameters), trials analogous to ours should be carried
- 526 out, combined with data available in the literature. Thus, an immediate adaptation of our model to similar
- 527 important koinobiont parasitoids-host pairs can be obtained, such as *Aphidius* spp.-aphids, *Dacnusa* sibirica
- 528 Telenga-leaf miners, Eretmocerus mundus Mercet-withefly and Encarsia formosa Gahan-withefly; and to
- 529 idiobiont parasitoids such as: Diglyphus isaea (Walker)-leaf miners and Trichogramma achaeae Nagaraja &
- 530 Nagarkatti-*Tuta absoluta* (Meyrick), see Vila and Cabello (2014). Analogously to our model, corresponding
- 531 multistage dynamic models can be built for predator-prey interactions, as well.
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538

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	Immature							Adult			
Temp.	Εσσ	Larval instar					Longevity			Fecundity	
(°C)	- 55	I	П	Ш	IV	V	VI		(days)		(eggs/♀)
		(days)							9	8	(1881)
15	7.0±0.0a	5.7±0.6a	4.4±0.9a	7.2±1.9a	6.0±1.1a	9.4±2.4a	2.8±5.1	_	25.8±9.0a	21.8±8.5a	932.2±656.5
20	5.3±1.8b	5.2±1.9a	4.0±1.5b	3.7±1.2b	3.9±1.2b	6.2±1.1b	_	8.9±1.7a	15.4±4.0b	36.6±8.0a	1081.0±489.4
25	3.0±0.0c	3.4±0.7b	2.4±0.6c	2.2±0.7c	2.4±0.6c	5.2±1.3c	_	7.8±0.9b	16.4±7.7b	26.2±8.4ab	1296.6±565.9
30	2.0±0.0d	2.6±0.6c	1.5±0.5d	1.4±0.6d	2.0±0.6d	3.2±0.9d	_	5.1±0.9c	10.2±3.9c	12.0±5.6b	911.5±554.1

Table 1. Average values (±SE) of development, longevity, and fecundity of Spodoptera exigua according to temperature under laboratory conditions.

Values with the same letter in the columns do not show significant differences (P = 0.05).

	Danalan mandal tina	Minimum		
Period	(ADD)	temperature	$r^2$	Р
	(ADD)	threshold (°C)		
Egg	32.8±1.3	13.9±0.4	0.9987	0.05
Small larva	98.0±5.0	12.5±0.5	0.9963	0.05
Large larva	116.4±8.0	8.7±0.7	0.9805	0.01
Pupa	115.2±51.7	8.3±7.0	0.9173	0.05

**Table 2.** Thermal relations (± SE) found for Spodoptera exigua in laboratory conditions.

Transition coefficients	Parameters of the Weibull function						
	α	β	λ				
<i>b</i> <sub>21</sub>	$219.3207 \pm 3.3445$	3.7774±0.2948	0.9983±0.0120				
<i>b</i> <sub>32</sub>	264.6238±1.5076	3.7869±0.2325	0.9977±0.0062				
$b_{43}$	390.3434±1.1066	5.6226±0.2566	0.9951±0.0053				
$b_{54}$	415.2787±1.7123	7.8542±0.3246	0.9998±0.0099				
$b_{55}$	227.0177±1.9429	2.7281±0.1128	_				

**Table 3.** Transition coefficients ( $\pm$  SE) fitted to the data found at the corresponding stages of Spodoptera exigua in greenhousepepper crop.

 Table 4. Average values of developmental time (± S.E.), longevity, and fecundity of adults of *Chelonus oculator* reared in

 Spodoptera exigua for two temperature treatments under laboratory conditions.

		Immature		Adult			
Temp.	Inside	Outside	D	Long	evity	F 14	
(°C)	the host	the host	rupa	(da	recundity (parasitized eggs/♀)		
		(days)		Ŷ	2	_ (Fri	
20	29.8±2.9a	3.0±1.7a	21.6±2.1a	13,26±2.6a	12.7±2.8a	481.2±125.6a	
30	12.2±1.6b	1.9±1.6b	6.4±0.3b	6.7±2.8b	5.3±2.6b	234.3±106.1b	

Values with the same letter in the columns do not show significant differences (p < 0.05).

Transition coefficients	Parameters of the Weibull function					
	α	β	λ			
<i>d</i> <sub>21</sub>	131.2823± 2.5023	$8.7543 \pm 0.2286$	$0.9996 \pm 0.0095$			
$d_{32}$	$210.1514 \pm 1.5861$	$5.6363 \pm 0.1852$	$0.9998 \pm 0.0098$			
$d_{33}$	335.2885± 4.1281	$5.2801 \pm 0.5981$	_			

 Table 5: Transition coefficients (± SE) fitted to stage-frequency data of *Chelonus oculator* for the considered stages in laboratory conditions.

		Model parameters (*)		Statistical parameters				
Parasitoid release rate (no. ♀/m²)	Function type	T <sub>h</sub>	a'/Â	AICc	r <sup>2</sup>	d.f.	Р	
0.5	Π	0.00153 (0.00149)	0.92544 (0.31135)	37.6264	0.97619	4	< 0.05	
	III	0.00322 (0.00055)	0.00842 (0.00299)	34,5651	0.98462	4	< 0.05	
1.0	Π	0.00007 (0.00274)	1.82227 (2.2702)	42.4425	0.97310	6	< 0.01	
	III	0.00418 (0.00315)	0.00669 (0.00726)	40.8764	0.97849	6	< 0.01	
1.5	Π	0.00896 (0.00541)	0.48090 (0.93924)	38.2238	0.97435	4	< 0.05	
	III	0.01512 (0.00199)	0.00674 (0.00909)	37.1488	0.97800	4	< 0.05	
All	III	0.00487 (0.00140)	0.00705 (0.00279)	-	0.89976	4	< 0.01	

**Table 6.** Fitting parameters ( $\pm$  SE) for the number of host eggs, *E. kuehniella*, parasitized by *Ch. Oculator* according to the function used and its statistical significance in greenhouse conditions.

**Figure 1**. Flow diagram of the host-parasitoid model (HE = host eggs, HSL = host small larvae, HLL = host large larvae, HP = host pupae, HA = host adults, PE = parasitoid egg, PLP = parasitoid larvae+pupae, PA = parasitoid adults; N = cohort; for *bij* and *dij* see the text).

**Figure 2**. Cumulative number of individuals of *Spodoptera exigua* that entered the small larvae (first to third instar), large larvae (fourth to fifth-instar), pupa, and adult stages in a greenhouse pepper crop.

Figure 3. Surface corresponding to the functional response of *Chelonus oculator* in greenhouse crop.

**Figure 4**. Partial validation of the model *Spodoptera exigua–Chelonus oculator* in pepper crop in a greenhouse.

**Figure 5**. Simulation results for the development of different stages of host and parasitoid: (A) Dynamics of the host eggs, small and large host larvae, host pupae, and adults. (B) Dynamics of the parasitoid eggs, larvae + pupae, and adults.