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DISAFA, ULF Entomologia Generale e Applicata, University of Torino, l.go Paolo Braccini 2, Grugliasco (TO), Italy

chiara.ferracini@unito.it; +390116708700

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Abstract

The native parasitoids *Necremnus tutae* Ribes & Bernardo and *N. cosmopterix* Ribes & Bernardo (Hymenoptera: Eulophidae) are potential biocontrol agents of the exotic tomato pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Europe. We investigated the functional response of parasitoid females to different densities of 3rd-instar host larvae (from 3 to 40 hosts offered), as well as some life history traits (longevity, lifetime fecundity, parasitism and host-feeding rate) of *N. tutae* and *N. cosmopterix*. In functional response experiments, the rate of attack of both parasitoids was positively correlated with host density, *N. tutae* and *N. cosmopterix* showing type III and type II functional response, respectively; the latter species exhibited a higher maximum estimated host-killing rate than the former. Both parasitoids showed high fecundity (> 50 eggs) during their lifetime. The potential application of *N. tutae* and *N. cosmopterix* in conservation biological control of *T. absoluta* is discussed.

Keywords: Eulophidae, Gelechiidae, Larval parasitoids, Tomato leafminer, Lifetime fecundity, Biological control

Key message

- Functional responses for different attack behaviours (parasitism, host-feeding) and life history traits of *Necremnus tutae* and *N. cosmopterix*, native larval parasitoids of the exotic tomato moth *Tuta absoluta* in the Mediterranean area were investigated.
- *Necremnus tutae* and *N. cosmopterix* showed type III and type II functional responses, respectively; both parasitoids demonstrated similar high fecundity and non-reproductive host-killing behaviour, even if having different longevity.
- Our study provides useful information to improve exploitation of *Necremnus* spp. biological control against *T. absoluta*.

Author Contribution Statement

NB, CF and LT conceived and designed the experiments. NB performed the experiments and analysed the data. NB and CF wrote the manuscript. All authors read and approved the manuscript.

Introduction

Biological invasions are among the paramount threats of the XXI century for both natural and human-modified ecosystems (Cameron et al. 2016; Guillemaud et al. 2011). Agricultural landscapes are especially sensitive to invasions by exotic pests, with consequent severe economic losses, besides the ecological risks (Pyšek and Richardson 2010; Vilà et al. 2010). Agricultural pests often spread quickly in the invaded regions, usually favoured by the absence of efficient natural control (Colautti et al. 2004; Letourneau et al. 2009). Nevertheless, some native natural enemies may adapt to the exotic pests, therefore exploiting new food and/or reproductive resources (Berthon 2015; Hokkanen and Pimentel 1989; Strauss et al. 2006). The use of indigenous natural enemies as biocontrol agents of agricultural pests (often exotic) is one of the key points of conservation biological control (Kean et al. 2003; Naranjo et al. 2015).

Native natural enemies have often been considered not very effective against exotic pests, compared to exotic natural enemies that share a co-evolutionary adaptation with the target species (Hoddle 2004). Nevertheless, they may present no ecological or conservational problems (Hoddle 2004; Zappalà et al. 2013), and their role as regulators of pests has been increasingly promoted over the last years (Crowder and Jabbour 2014; Symondson et al. 2002). Several biological control programmes have indeed demonstrated the importance in certain agroecosystems of native natural enemies in controlling populations of exotic pests (Balzan et al. 2016; Bianchi et al. 2006; Hogg et al. 2013; Jonsson et al. 2008; Letourneau et al. 2009; Wang et al. 2013; Zappalà et al. 2012). A thorough evaluation of the indigenous natural enemies naturally attacking exotic species is therefore important to identify promising biocontrol agents (Cornell and Hawkins 1993; Gabarra et al. 2014). Functional response to host densities and foraging behaviours performed by biocontrol agents are fundamental aspects to evaluate natural enemies' efficiency for pest control (Chen et al. 2016; Dannon et al. 2010; Fellowes et al. 2005; Garay et al. 2015; Laumann et al. 2008).

The South American tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has rapidly spread in Eurasia and Africa following its accidental introduction into Spain in 2006,

78 becoming a major threat to tomato production in the Old-World (Biondi et al. 2018; Campos et al.
79 2017; Desneux et al. 2010, 2011; Tonnang et al. 2015). Natural enemy communities found attacking
80 *T. absoluta* in the Mediterranean area are quite abundant, and predators, especially predatory mirids
81 [e.g., *Dicyphus errans* (Wolff), *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter)
82 (Heteroptera: Miridae)], are used in biological control programmes (Biondi et al. 2016; Ingegno et
83 al. 2013, 2017; Mollá et al. 2014; Urbaneja et al. 2012; Zappalà et al. 2013).
84 A diverse complex of native hymenopteran parasitoids has been found attacking *T. absoluta* in the
85 Mediterranean area, and some species have been tested for effectiveness on *T. absoluta* (Chailleux
86 et al. 2012; Gabarra et al. 2014; Zappalà et al. 2013). *Necremnus tutae* Ribes & Bernardo
87 [previously referred to as *N. artynes* or *N.* sp. nr *artynes* (Gebiola et al. 2015)] and *N. cosmopterix*
88 Ribes & Bernardo [previously referred to as *N. tidius* or *N.* sp. nr *tidius* (Gebiola et al. 2015)]
89 (Hymenoptera: Eulophidae) are among the most abundant larval parasitoids of *T. absoluta* in the
90 Mediterranean region (Cocco et al. 2015a; Gabarra et al. 2014; Zappalà et al. 2013), and potential
91 candidates for biological control programmes against the tomato pest (Calvo et al. 2013; Ferracini
92 et al. 2012; Urbaneja et al. 2012). Previous studies have assessed some biological and behavioural
93 characteristics of *N. tutae* on both the exotic host and the indigenous host *Cosmopterix pulchrimella*
94 Chambers (Lepidoptera: Cosmopterigidae) (Bodino et al. 2016; Chailleux et al. 2014a), whereas *N.*
95 *cosmopterix* has been seldom studied in relation to its activity on *T. absoluta* (Cocco et al. 2015a;
96 Ferracini et al. 2012). These two *Necremnus* species probably occur in the same environments (i.e.,
97 sympatric species) but in different periods (i.e., no syntopic species) (Cocco et al. 2015a), although
98 very little information is available on their biology and ecology. However, some biological traits of
99 *N. tutae* have been investigated to evaluate its use in an augmentative biological control perspective
100 (Calvo et al. 2016). Despite its promising attack capabilities against *T. absoluta*, this parasitoid
101 seems not to be suitable for augmentative release programmes because of high rates of host-killing,
102 resulting in a non-cost-effective mass rearing, and the intraguild predation exercised by predatory
103 mirids, already widely used in biocontrol programmes against *T. absoluta* (Calvo et al. 2016;

104 Chailleux et al. 2014b; Naselli et al. 2017). Nonetheless, the positive effect of other herbivorous
105 insects on population dynamics of parasitoid in tomato agroecosystem (Dong et al. 2018), as well as
106 the possible effective coexistence of an idiobiont parasitoid and a mirid predator (Chailleux et al.
107 2017) were already assessed.
108 *Necremnus* species naturally occurring in tomato agroecosystem could be exploited by conservation
109 biological control programmes, contributing to IPM strategies used to control *T. absoluta* in
110 Mediterranean area. Successful programmes of conservation biological control imply a good
111 knowledge of the biology and ecology of both pest and natural enemies, as well as the host-
112 parasitoid dynamics and interactions between biocontrol agents and the ecosystem, with a particular
113 regard to the influence of intraguild predation and other biotic and abiotic factors on biocontrol
114 effectiveness (Dong et al. 2018; Duan et al. 2015; Naselli et al. 2017). Research on functional
115 response and foraging behaviour of natural enemies of *T. absoluta* has focused mainly on predatory
116 mirids and egg parasitoids (Faria et al. 2000; Ingegno et al. 2017; Queiroz et al. 2015; van Lenteren
117 et al. 2016). However, no information is available yet on functional response of larval parasitoids of
118 this exotic pest, with the only exception of *Dineulophus phtorimeae* de Santis (Hymenoptera:
119 Eulophidae), a native parasitoid in Argentina (Savino et al. 2012). Therefore, the present study aims
120 to 1) determine the type of functional response of *N. tutae* and *N. cosmopterix* females to different
121 densities of *T. absoluta* larvae, and 2) evaluate the frequency of attack behaviours along the lifespan
122 of the female of both parasitoid species.
123

124 **Material and methods**

125 **Insect rearing and plant cultivation**

126 Colonies of *T. absoluta* were established starting from individuals provided by Bioplanet
127 laboratories (Bioplanet s.c.a., Cesena, Italy) and regularly enriched with individuals collected in
128 tomato greenhouses in Piedmont (Italy). A continuous mass rearing was maintained on potted
129 tomato plants in cages with an insect-proof net (mesh 680 μm) (BugDorm: 60 × 60 × 60 cm,

130 MegaView Science Co., Taichung, Taiwan). Tomato seedlings were initially sown in plastic pots (∅
131 20 cm), and plants were transplanted individually in pots (∅ 10 cm) after three weeks. Tomato
132 plants of Marmande variety (Green Paradise s.r.l., Milano, Italy) were used for both mass rearing
133 and laboratory trials. Plants used in the experiments had 4-5 true leaves (14-15 BBCH-scale) and
134 were approximately 60 days old.
135 The two parasitoid species *N. tutae* and *N. cosmopterix* were initially provided by Bioplanet and
136 collected on *C. pulchrimella* larvae in Liguria (Italy), respectively. A separate mass rearing for the
137 two parasitoids was set up as follows: about 10 adults (sex ratio ~ 1♀:1♂) were released into a
138 Plexiglas cage (40 × 40 × 50 cm) with 2-3 tomato plants infested by 3rd-instar *T. absoluta* larvae.
139 After four days, all parasitoids were removed and plants were transferred into a screen cage
140 (BugDorm: 47.5 × 47.5 × 47.5 cm) for six days, allowing parasitoid larvae to conclude their
141 development. Plants were then cut and placed in clean Plexiglas cages (35 × 35 × 35 cm) with drops
142 of honey on cardboard, awaiting parasitoid emergence. Mass rearing of parasitoids was maintained
143 in a climate room (25±1°C, 60±5% RH, and L:D 16:8 h). Newly emerged adult parasitoids were
144 transferred daily from the rearing cages to glass vials (24 × 120 mm), supplied with small honey
145 drops, and placed in a climatic chamber (15±1°C, 60±5% RH, and L:D 16:8 h).

146 **_Necremnus_ spp. functional response on _Tuta absoluta_ larvae**

147 Females of the two parasitoids *N. tutae* and *N. cosmopterix* were individually exposed to different
148 densities of host larvae on a tomato plant. Females of each species were collected from mass rearing
149 within 48 h from their emergence, and transferred to plastic cages (24 × 15 × 12 cm) together with
150 males (sex ratio ~ 1♀:1♂). Tomato leaflets infested by two or three 3rd-instar *T. absoluta* larvae per
151 parasitoid female and honey drops on cardboard were supplied. Cages were kept in climatic
152 chambers (25±1°C, 60±5% RH and L:D 16:8 h), allowing parasitoid females to mate and oviposit
153 and/or host-feed on *T. absoluta* larvae for 24 h. The exposition to the host larvae prior to the assays
154 permitted to test parasitoid females with a definite experience on *T. absoluta*, since previous
155 experience can influence foraging behaviour efficiency (Bodino et al. 2016), as well as functional

156 response experiments of parasitoids (Yazdani and Keller 2016). Females were then individually
157 isolated in glass tubes (24 × 120 mm) with honey and humid cotton cap, and maintained in climatic
158 chambers (25±1°C, 60±5% RH, and L:D 16:8 h) for further 24 h before the assays.
159 The test cage ("microcosm") was set up as follows. A single tomato plant (40-50 days from
160 germination, 15-16 BBCH-scale), grown in a plastic pot (∅ 14 cm), was inserted inside a Plexiglas
161 cage (20 × 20 × 31 cm) with a wall and the lid of net (mesh 680 µm). Then, 3rd-instar *T. absoluta*
162 larvae were placed randomly on the tomato plant at different densities (3, 5, 8, 12, 17, 23, 30, 40
163 larvae plant^{-1}), 24 h before starting the functional response experiment. Therefore, larvae had time
164 to infest the plant creating new mines on the tomato leaves. Based on the preliminary results, the
165 maximum host density was established in 30 and 40 larvae for *N. tutae* and *N. cosmopterix*,
166 respectively, in order to approach the attack asymptote and better determine the functional response
167 parameters.
168 A single parasitoid female, previously isolated as described above, was then inserted into the
169 microcosm, together with drops of honey provided on a cardboard. The female was maintained in
170 the cage for 24 h in order to evaluate the attack response to host larvae densities. Afterward, the
171 parasitoid was removed, and all host larvae were immediately checked under a stereomicroscope
172 with transmitted light (12-25×, Wild M5A) to assess total mortality, i.e., parasitism and host-
173 feeding on host larvae as well as number of eggs laid by the parasitoid female. Host larvae were
174 considered parasitised when they were found paralysed, with one or more eggs on or close to the
175 body in the same leaf mine, whereas they were considered killed by host-feeding when they
176 appeared somewhat "empty" and with a wound caused by the ovipositor of parasitoid female,
177 meaning that the parasitoid fed on their haemolymph. Total mortality included all the host larvae
178 found paralysed or dead. Results from cages where the parasitoid female was dead at the end of the
179 assay were discarded. Overall, five successful replicates (i.e., females found alive) were performed
180 per each tested density. Functional response experiments were carried out in a climatic chamber
181 (25±1°C, 60±5% RH, and L:D 16:8 h).

182 **Life history traits of *Necremnus* spp.**

183 Females of the two parasitoids *N. tutae* and *N. cosmopterix* were individually exposed to a tomato
184 leaf infested by 10 host larvae per day during their entire adult lifespan. The number of host larvae
185 was determined through preliminary assays that demonstrated that the females of the two parasitoid
186 species were unable to attack more than 10 host larvae per day (unpublished data). Number of eggs
187 and attack behaviours like parasitism, host-feeding and host-stinging were registered. In particular,
188 host larvae were considered killed by host-stinging when they showed an ovipositor sting mark but
189 no eggs or haemolymph leaking from the wound. Host-killing (i.e., total mortality) was intended as
190 the overall number of host larvae parasitised or killed through host-feeding or host-stinging by the
191 parasitoid. Parasitoid females (emerged < 24 h) were collected and singly inserted into a Petri dish
192 (∅ 20 cm, "arena") containing a filter paper disc on the bottom and one tomato leaf (with 5 leaflets)
193 infested with 10 3rd-instar *T. absoluta* larvae 24 h earlier. One male of the same species was
194 introduced into the Petri dish, allowing the female to mate throughout its life. After 24 h, both
195 female and male were transferred to another arena, containing a filter paper disc and a new tomato
196 leaf infested with *T. absoluta* larvae, as described above. The tomato leaf exposed to the parasitoid
197 was immediately checked under a stereomicroscope with transmitted light (12-25×, Wild M5A) to
198 assess the attack behaviours by the parasitoid female during the previous 24 h. Parasitoid females
199 were provided with a new infested tomato leaf each day of their life, and a total of 10 replicates
200 were performed for each parasitoid species. Ten arenas without any parasitoid were set up as
201 control, and checked after 24 h to assess mortality of *T. absoluta* larvae not caused by the natural
202 enemy. Experiments were carried out in a climatic chamber (25±1°C, 60±5% RH, and L:D 16:8 h).

203 **Statistical analyses**

204 Number of host-killing, parasitism and host-feeding behaviours in relation to parasitoid species and
205 host density were analysed using a two-way ANOVA, whereas the difference between the attack
206 frequencies of the two parasitoid species at diverse densities was tested using Mann-Whitney U test.
207 Functional response type and parameters of attack rate and handling time for the different attack

behaviours were estimated using the general approach proposed by Okuyama (2012a). This approach includes the application of a model selection index (i.e., Akaike information criterion, AIC) directly to candidate models. We used the maximum likelihood approach to obtain parameters estimates of the Holling's type II (Eq. 1), Holling's type III (Eq. 2) and θ-logistic models (Eq. 3) (Bolker 2008; Okuyama 2012b). Maximum likelihood method is less used than the typical estimation method of least squares, but it has been recently accounted of better performances in estimating functional response parameters (Okuyama 2012b). The three used models do not account for prey depletion, since parasitoids do not completely consume their host/prey and hence could re-encounter formerly attacked hosts. The three functional response models were then used to fit the data regarding the different attack behaviours, using the R package bbmle for maximum likelihood estimation (*mle*) (Haddaway et al. 2012). The best model for each attack behaviour was selected using the AIC test (lower AIC means a better model fit), from which the coefficients *a* (attack rate) and *Th* (handling time) were obtained and compared using confidence intervals (95%). Mean values of *Th* were used to calculate the maximum attack rate as *T/Th* (Hassell 2000), representing the maximal number of *T. absoluta* larvae that could be attacked during the considered time interval (i.e., $T = 1$ day).

Differences between lifetime biological traits of the two parasitoid species were analysed using *t*-test, when assumption of normality (Shapiro-Wilk test) and homoscedasticity (Levene's test) were verified, and GLM based on gamma distribution (inverse link function), when assumptions of normality and/or homoscedasticity were rejected. The relationship between parasitoid female age and daily fecundity (no. eggs laid female^{-1} day^{-1}) was fitted by both Bieri (Eq. 4) and Analytis (Eq. 5) models (Kontodimas et al. 2007; Lanzoni et al. 2004). The most appropriate model was selected using the AIC test. Statistical analyses were performed using the statistical software R (R Core Team 2013).

Results

234 ***Necremnus* spp. functional response on *Tuta absoluta* larvae**

235 The number of host larvae killed, parasitised or host-fed by both *N. tutae* and *N. cosmopterix*
236 increased with the host density offered (Tab. 1 and Fig. 1), whereas the percentage of host larvae
237 killed, parasitised or host-fed decreased, although differently between the two parasitoid species
238 (Fig. 2). The interaction effect between parasitoid species and host density on host mortality and
239 parasitism highlighted relevant differences between *N. tutae* and *N. cosmopterix* (Host-killing: F =
240 3.68, df = 6,60, P = 0.003; Parasitism: F = 5.70, df = 6,60, P < 0.001), with the latter parasitoid
241 attacking more host larvae, especially when provided at the highest densities (23-30 host larvae)
242 (Tab. 1). Indeed, the highest host density supplied to *N. cosmopterix* was increased to 40 larvae in
243 order to approach the upper asymptote of the functional response curve. Number of host-feeding
244 attacks also increased with host density (F = 48.40, df = 7,60, P = 0.009), but without significant
245 difference between the two parasitoid species (F = 0.61, df = 1,60, P = 0.638) (Tab. 1).
246 The calculated AIC values for *N. tutae* by the Holling's type III equation (Eq. 2) were lower than
247 the ones calculated by Holling's type II equation (Eq. 1) and theta-logistic (Eq. 3) for total killed
248 larvae, parasitism and host-feeding (Tab. 2). On the contrary, AIC values for *N. cosmopterix* fitted
249 by Holling's type II equation (Eq. 1) were lower than the ones calculated by the Holling's type III
250 equation (Eq. 2) and theta-logistic (Eq. 3) for the total killed, parasitised and host-fed larvae (Tab.
251 2). Thus, *N. tutae* functional responses were better modelled by type III model, whereas *N.*
252 *cosmopterix* functional responses were better modelled by type II model (Fig. 1).
253 Host-killing rate of *N. cosmopterix* was higher than the one estimated for *N. tutae*, comparing 95%
254 confidence intervals, whereas both parasitism and host-feeding rates were similar in the two
255 parasitoid species (Tab. 3). Host-killing and parasitism handling times of *N. cosmopterix* were
256 lower than those ones of *N. tutae*. Hence, the maximum estimated host-killing rate per day ($1/T_h$)
257 was higher for *N. cosmopterix* (23.66 larvae day^{-1}) than for *N. tutae* (8.23 larvae day^{-1}). In the same
258 way, the maximum number of parasitised larvae (*N. cosmopterix*: 12.5 larvae day^{-1}; *N. tutae*: 3.12
259 larvae day^{-1}) was higher for the former species than for the latter one. Handling time of host-feeding

260 was instead similar between the two *Necremnus* species, leading to not significantly different
261 maximum number of host-fed larvae (*N. cosmopterix*: 6.25 larvae day^{-1}; *N. tutae*: 3.03 larvae day^{-1}).

262 **Life history traits of *Necremnus* spp.**

263 Lifetime fecundity experiment showed some differences in biological traits between *N. tutae* and *N.*
264 *cosmopterix* (Tab. 4). Specifically, *N. cosmopterix* females lived significantly longer than *N. tutae*
265 ones, but with a lower number of daily ovipositions. Therefore, the total lifetime fecundity was not
266 different between the two parasitoid species. The shorter lifespan of *N. tutae* females caused both a
267 shorter pre-oviposition period and a shorter oviposition period compared to the ones observed for *N.*
268 *cosmopterix*. Parasitism and host-killing (i.e., parasitism + host-feeding + host-stinging) followed
269 the same pattern of ovipositions, with *N. tutae* parasitizing and killing a higher number of larvae per
270 day than *N. cosmopterix*, leading to not significant differences between the total lifetime number of
271 parasitised or killed larvae between the two parasitoid species. Conversely, both lifetime and daily
272 host-feeding activities were not different between the two parasitoid species. Likewise, both
273 lifetime and daily host-stinging activities were not different between *N. tutae* and *N. cosmopterix*
274 (lifetime *t*-test: t = 0.98, df = 18, P = 0.340; daily *t*-test: t = 1.50, df = 18, P = 0.151). No mortality
275 of *T. absoluta* larvae was observed in control arenas, thus all dead larvae recorded in experimental
276 arenas were assumed to be caused by the foraging activity of the parasitoid female.
277 Comparing the age-specific fecundity of the two parasitoid species, a different pattern could be
278 recognized; the ovipositions of *N. tutae* were slightly better described by the Analytis model (AIC:
279 Analytis = 1009.93; Bieri = 1011.39), whereas the ovipositions of *N. cosmopterix* were slightly
280 better fitted by Bieri model (AIC: Analytis = 672.72; Bieri = 670.91) (Fig. 3). The highest daily
281 mean oviposition value was at the 6th day of life for *N. tutae*, whereas was between the 15th and the
282 18th day of life for *N. cosmopterix*. Hence, *N. tutae* had a higher age-specific fecundity during the
283 first days of adult life, until 10-12 days old, then the number of daily ovipositions steadily declined
284 to zero. Conversely, *N. cosmopterix* exhibited a lower daily fecundity, especially during the first

285 days of adult life, reaching the maximum number of eggs later, reflecting the higher longevity of the
286 females of this species.
287
288 **Discussion**
289 The results of the present study provide novel information on biological traits and foraging
290 behaviour of the two parasitoid species *N. tutae* and *N. cosmopterix* on the exotic host *T. absoluta*.
291 This is also the second study, after Ferracini et al. (2012), investigating on several biological traits
292 of *N. cosmopterix* and comparing the efficiency of the two *Necremnus* species on *T. absoluta*. Both
293 these natural enemies demonstrated a good capability in killing and parasitising 3rd-instar *T.*
294 *absoluta* larvae in laboratory conditions. However, the two parasitoid species showed marked
295 differences regarding their functional response and lifetime foraging behaviours.
296 In functional response assays, *N. tutae* exhibited density-dependent (type III) parasitism and host-
297 killing of *T. absoluta*, whereas *N. cosmopterix* exhibited inversely density-dependent (type II)
298 attack behaviours. Type III functional response is often considered to have a stabilizing effect on
299 host-parasitoid interactions, potentially leading to a successful regulation of the pest population
300 (Fernández-Arhex and Corley 2003; Hassell 2000; Yazdani and Keller 2016). However, the low
301 maximum attack rate exhibited by *N. tutae* in this study highlights a moderate capacity of this
302 parasitoid to efficiently respond to a strong increase of pest density. Nonetheless, parasitoids
303 showing a type III functional response should be more efficient at low host densities, and thus *N.*
304 *tutae* could usefully control *T. absoluta* populations at the beginning of the growing season, when
305 their infestation level is still low, even though augmentative releases of *N. tutae* alone are probably
306 not sufficient to keep pest density lower than the economic threshold (1-3% damaged fruits) (Calvo
307 et al. 2016; Cocco et al. 2015b; Desneux et al. 2010).
308 Conversely, *N. cosmopterix* exhibited an inversely density-dependence functional response of
309 parasitism and host-killing (type II), characterized by a constant increase of number of larvae
310 attacked with an augmentation of larvae offered (Juliano 2001). Type II functional response is

311 usually associated with the incapability of a natural enemy to modulate its response to different
312 prey/host densities, and has often been considered less stable in regulating the host-parasitoid
313 dynamics (Hassel 2000). Nonetheless, several natural enemies showing this type of functional
314 response have become successful biocontrol agents, and the supposed host-parasitoid population
315 instability arising from this type of functional response is still debated (Fernández-Arhex and
316 Corley 2003; Kidd and Amarasekare 2012). Furthermore, both higher values of attack rate and
317 smaller values of handling time estimated for *N. cosmopterix*, compared to those ones estimated for
318 *N. tutae*, reveal the capability of the former to quickly attack high numbers of host larvae even at
319 high densities, thus showing an important requisite for controlling the pest also in case of high
320 infestation levels.
321 In lifetime fecundity assays, the two parasitoid species attacked similar amounts of host larvae, but
322 *N. tutae* showed a significantly shorter adult life and consequently higher daily rates of parasitism,
323 host-feeding and host-killing compared to *N. cosmopterix*. Thus, despite some biological
324 differences, both *Necremnus* species killed high number of hosts during their lifetime (~ 100
325 larvae), that is an important characteristic for biological control agents. Host-feeding and host-
326 stinging were important components of attack activities of the two parasitoids during both
327 functional response and lifetime assays. These types of attacks are possibly advantageous for pest
328 control (Jervis et al. 1996, 2008) but can lead to non-cost-effective massive production of these
329 natural enemies to be used in augmentative or inundative biological control programmes (Calvo et
330 al. 2016).
331 Our results about *N. tutae* biology are consistent with those ones previously achieved for *N. artynes*
332 or *N.* near *artynes* (Calvo et al. 2013; Chailleux et al. 2014a; Ferracini et al. 2012), which are now
333 considered to belong to the same species (Gebiola et al. 2015). Longevity, fecundity and host-
334 killing of *N. tutae* observed in our study are also very similar to those ones observed at the same
335 temperature (25°C) by Calvo et al. (2013), who however recorded a higher frequency of host-
336 feeding behaviour compared to our results. Such a difference could be due to the fact that in those

337 experiments host-stinging behaviour was not accounted and/or that larger arenas were used.
338 Conversely, Chailleux et al. (2014a) and Ferracini et al. (2012) reported a lower overall efficiency,
339 i.e., both lower number of eggs laid and of host larvae killed. These differences are likely due to the
340 experimental setup, but it should not be overlooked also that those studies were conducted before
341 the systematic revision by Gebiola et al. (2015), and therefore, some other cryptic species of
342 *Necremnus* may had been used. Despite the promising host attack capabilities of *N. tutae*, in a study
343 on augmentative release of *N. tutae* in semi-field conditions, the parasitoid alone was not able to
344 reduce the damage caused by *T. absoluta* below the economic threshold (Calvo et al. 2016).
345 Furthermore, larval ectoparasitoids, such as *Necremnus* species, are prone to intra-guild predation
346 or kleptoparasitism by predatory mirids, which are already widely used in biological control
347 programmes on tomato, and can significantly reduce parasitoids' efficiency (Chailleux et al. 2014b;
348 Naselli et al. 2017). However, in a recent study the ectoparasitoid *Stenomesius japonicus*
349 (Ashmead) proved to coexist successfully with the mirid *M. pygmaeus* and contribute with an
350 additive effect to lower *T. absoluta* population (Chailleux et al. 2017).
351 Given the few works dealing with the biology of larval parasitoids of *T. absoluta* in Mediterranean
352 region, it is useful to compare our results also to those ones achieved in studies on larval parasitoids
353 in South America. *Pseudoapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae), a larval
354 endoparasitoid attacking *T. absoluta* in South America, is a promising candidate for augmentative
355 and conservation biological control of this tomato pest in Argentina (Luna et al. 2007; Sánchez et
356 al. 2009). In our study, *Necremnus* species showed higher functional response and lifetime
357 fecundity-attack parameters, but lower fecundity and parasitism levels than those reported for *P.*
358 *dignus* in both laboratory and field experiments (Nieves et al. 2015; Savino et al. 2012). Despite
359 some methodological differences between the studies, biological parameters observed for *P. dignus*
360 and *Necremnus* spp. are somehow comparable, suggesting *N. tutae* and *N. cosmopterix* as possibly
361 successful biological control agents.

362 *Necremnus tutae* and *N. cosmopterix* should be considered as potential effective natural enemies in
363 conservation biological control programmes against *T. absoluta*, rather than in augmentative release
364 strategies. An early establishment of these species in tomato agroecosystem, with a consequent
365 increase of their resident populations, could help keeping *T. absoluta* populations under economic
366 threshold, preventing the pest populations to increase uncontrolled. However, to achieve a
367 successful conservation biological control programme, more information about biology, ecology
368 and multitrophic interactions at agroecosystem and landscape level should be acquired (Jonsson et
369 al. 2008; Tscharntke et al. 2007). For example, understanding possible effects of native plants as
370 bankers of alternative hosts or providers of food supplies (nectars), as suggested for *N. tutae* by
371 Balzan and Wäckers (2013), can lead to attraction and establishment of native parasitoids in tomato
372 agroecosystem. Studying similar ecological dynamics, as well as multitrophic relationships in the
373 field with other native enemies like predatory mirids (e.g., *N. tenuis* and *D. errans*) (Ingegno et al.
374 2013; Mollá et al. 2014; Naselli et al. 2017), would help to achieve a more efficient natural control
375 of *T. absoluta* in Mediterranean areas. Furthermore, also the coexistence and possible competition
376 of *N. tutae* and *N. cosmopterix* in tomato crop should be investigated, as this aspect could have
377 important consequences on conservation biocontrol efficiency. Until now, experiments have in fact
378 focused on microcosm or semi-field assays, but further research is needed to evaluate these natural
379 enemies in field conditions, where the intra-guild competition/predation with predatory mirids could
380 be less severe than the one registered in artificial assays, and the coexistence of different natural
381 enemies could lead to a more efficient pest control.
382

383 **Ethical approval**

384 All applicable international, national, and/or institutional guidelines for the care and use of animals
385 were followed. All procedures performed in studies involving animals were in accordance with the
386 ethical standards of the institution or practice at which the studies were conducted.

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$$N_a = \frac{aN}{1 + aN}$$

Eq. 1 Holling's type II functional response model

$$N_p = \frac{aN^2}{1 + aN^2}$$

Eq. 2 Holling's type III functional response model.

$$N_p = \frac{aN^\theta}{1 + aN^\theta}$$

Eq. 3 θ-logistic functional response model.

$$y = [P_1(x - P_2)]/\{\exp[\ln(P_3)\,(x - P_2)]\}$$

Eq. 4 Bieri model, where x = age of female (days), and P_1, P_2 and P_3 are coefficients.

$$y = P_1((x - x_{min})^{P_2}.(x_{max} - x)^{P_3}$$

Eq. 5 Analytis model, where x = age of female (days), x_{min} and x_{max} are the first and final age of oviposition, and P_1, P_2 and P_3 are coefficients.

590 **Tab. 1** Mean number (±SE) of *Tuta absoluta* larvae killed, parasitised or host-fed by *Necremnus tutae*
591 (*N.t.*) and *N. cosmopterix* (*N.c.*) females at different host densities in functional response assays. At
592 each host density, means followed by asterisk are significantly different between the two parasitoid
593 species (Mann-Whitney U test, $P < 0.05$).
594

Host density (larvae plant^{-1})	**No. killed larvae**			**No. parasitised larvae**			**No. host-fed larvae**		
	N.t.	*N.c.*		*N.t.*	*N.c.*		*N.t.*	*N.c.*	
3	2.4 ± 0.75	2.2 ± 0.37		1.4 ± 0.4	1.6 ± 0.51		1.2 ± 0.2	0.8 ± 0.2	
5	4.0 ± 1.98	4.0 ± 0.4		2.8 ± 0.6	2.6 ± 0.51		2.0 ± 0.3	1.2 ± 0.49	
8	6.4 ± 2.50	5.2 ± 0.63		3.6 ± 0.6	4.0 ± 0.55		2.6 ± 0.4	0.4 ± 0.24	*
12	8.0 ± 2.00	7.2 ± 0.45		4.4 ± 1	4.2 ± 1.11		2.8 ± 0.7	3.0 ± 1.05	
17	7.8 ± 2.74	11.6 ± 0.86		2.8 ± 1.6	6.8 ± 1.46		3.2 ± 0.8	2.6 ± 0.81	
23	7.0 ± 2.43	11.8 ± 0.51		2.4 ± 1.1	7.4 ± 1.54	*	2.4 ± 0.5	3.4 ± 1.17	
30	8.0 ± 1.44	18.0 ± 1.07	*	2.2 ± 1	12.6 ± 2.64	*	2.2 ± 0.6	3.8 ± 0.86	
40	−	11.4 ± 1.05		−	5.8 ± 0.73		−	3.2 ± 0.86	

595

596 **Tab. 2** Akaike information criterion (AIC) of the functional response models tested for *Necremnus*
597 *tutae* (*N.t.*) and *N. cosmopterix* (*N.c.*) females. Functional response models that fitted better the data
598 (lower AIC) are underlined.

Functional response	**No. killed larvae**		**No. parasitised larvae**		**No. host-fed larvae**	
	N.t.	*N.c.*	*N.t.*	*N.c.*	*N.t.*	*N.c.*
Type II	171.59	204.45	155.06	195.47	114.18	142.70
Type III	169.80	205.69	153.39	196.76	113.23	143.99
Θ-sigmoid	171.69	204.70	153.76	196.67	114.97	144.58

599

600

601 **Tab. 3** Attack coefficient (*a*), handling time (*Th*) estimates (± SE) and confidence intervals from
602 functional responses of attack behaviours displayed by *Necremnus tutae* and *N. cosmopterix* to
603 increasing densities of *Tuta absoluta* larvae.

Species	**Attack type**	***a*** (larvae day^{-1})		***Th*** (day)	
		Estimate (± SE)	95% C.I.	Estimate (± SE)	95% C.I.
N. tutae					
	Host-killing	0.37 ± 0.11	(0.216-0.745)	0.12 ± 0.01	(0.101-0.145)
	Parasitism	0.46 ± 0.31	(0.172-1.436)	0.31 ± 0.04	(0.248-0.402)
	Host-feeding	0.27 ± 0.18	(0.103-7.932)	0.36 ± 0.05	(0.275-0.472)
N. cosmopterix					
	Host-killing	0.95 ± 0.15	(0.709-1.309)	0.04 ± 0.01	(0.023-0.054)
	Parasitism	0.67 ± 0.14	(0.461-1.046)	0.07 ± 0.02	(0.039-0.102)
	Host-feeding	0.26 ± 0.08	(0.151-0.558)	0.16 ± 0.06	(0.046-0.292)

604

605

606 **Tab. 4** Mean lifetime biological traits (±SE) of *Necremnus tutae* and *N. cosmopterix* on the host *Tuta*
607 *absoluta*. Differences between biological traits of the two parasitoids were tested by *t* test or GLM
608 with Gamma distribution.

Parameter	***N. tutae***	***N. cosmopterix***	**t test$_{(18)}$**	**F$_{(1, 18)}$**	***P***
Longevity (days)	14.00 ± 1.96	24.50 ± 3.11	2.86		0.011
Pre-oviposition (days)	0.30 ± 0.15	1.30 ± 0.39		6.54	0.019
Oviposition (days)	12.80 ± 1.99	22.00 ± 2.52	2.86		0.011
Fecundity (eggs female^{-1})	53.50 ± 8.09	59.50 ± 11.98	0.41		0.683
Oviposition rate (eggs female^{-1} day^{-1})	3.89 ± 0.32	2.32 ± 0.37	3.21		0.005
Host-killing (larvae)	95.40 ± 13.25	125.60 ± 23.59		1.38	0.255
Host-killing rate (larvae day^{-1})	6.82 ± 0.35	4.90 ± 0.68		5.03	0.038
Host-feeding (larvae)	21.70 ± 2.83	34.60 ± 7.30		3.51	0.077
Feeding rate (larvae day^{-1})	1.61 ± 0.10	1.32 ± 0.21		1.35	0.261
Parasitism (larvae)	47.50 ± 7.12	55.70 ± 10.97	0.63		0.539
Parasitism rate (larvae day^{-1})	3.44 ± 0.25	2.17 ± 0.33	3.07		0.007

609

610

611 **Fig. 1** Functional response of *Necremnus tutae* and *N. cosmopterix* for total host-killing (a, b),
612 parasitism (c, d) and host-feeding (e, f). Points represent mean number of *Tuta absoluta* larvae
613 attacked (±SE) for the different larval densities offered to the parasitoid female. *Solid lines*
614 represent type II functional response and *dashed lines* represent type III functional response.
615
616 **Fig. 2** Functional response of *Necremnus tutae* and *N. cosmopterix* for host-killing (a, b), parasitism
617 (c, d) and host-feeding (e, f). Points represent mean percentage of *Tuta absoluta* larvae attacked
618 (±SE) for the different larval densities offered to the parasitoid female. *Solid lines* represent
619 quadratic polynomial function and *dashed lines* represent cubic polynomial function.
620
621 **Fig. 3** Age-specific fecundity as a function of age of *Necremnus tutae* (a) and *N. cosmopterix* (b)
622 females fitted using Bieri (*solid line*) and Analytis (*dashed line*) models.
623

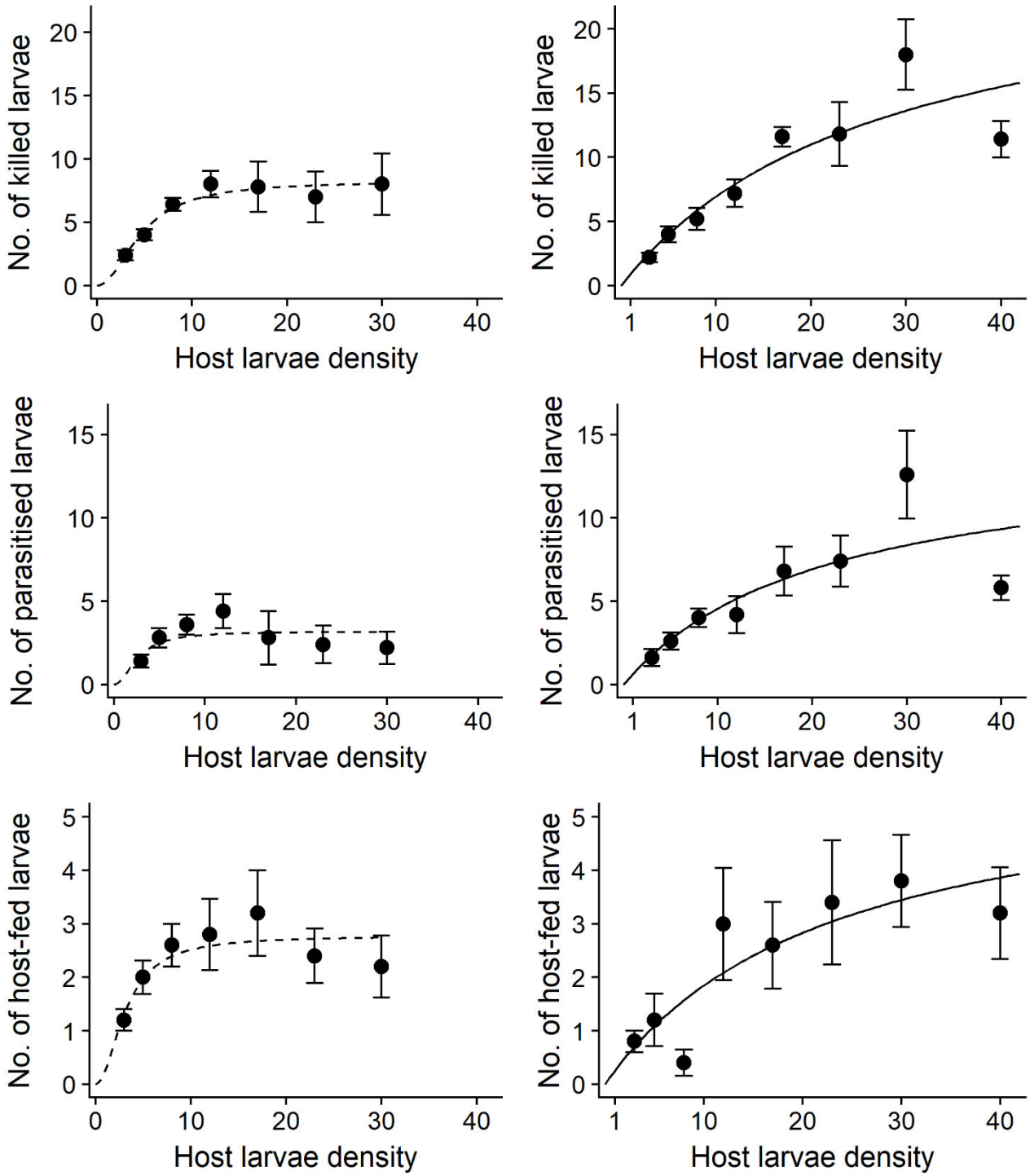

No. of killed larvae
Host larvae density
No. of parasitised larvae
Host larvae density
No. of host-fed larvae
Host larvae density

Fig. 1

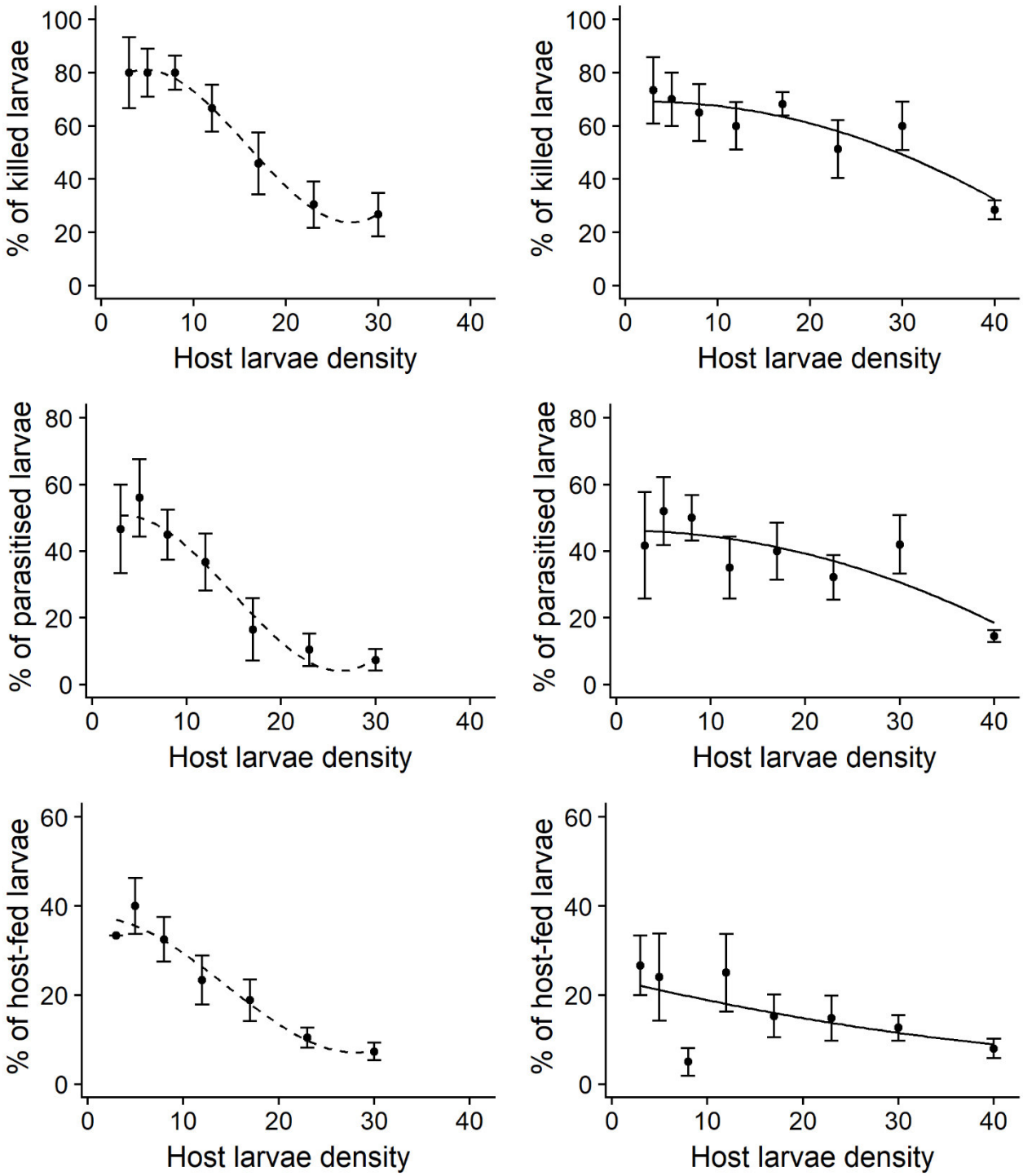
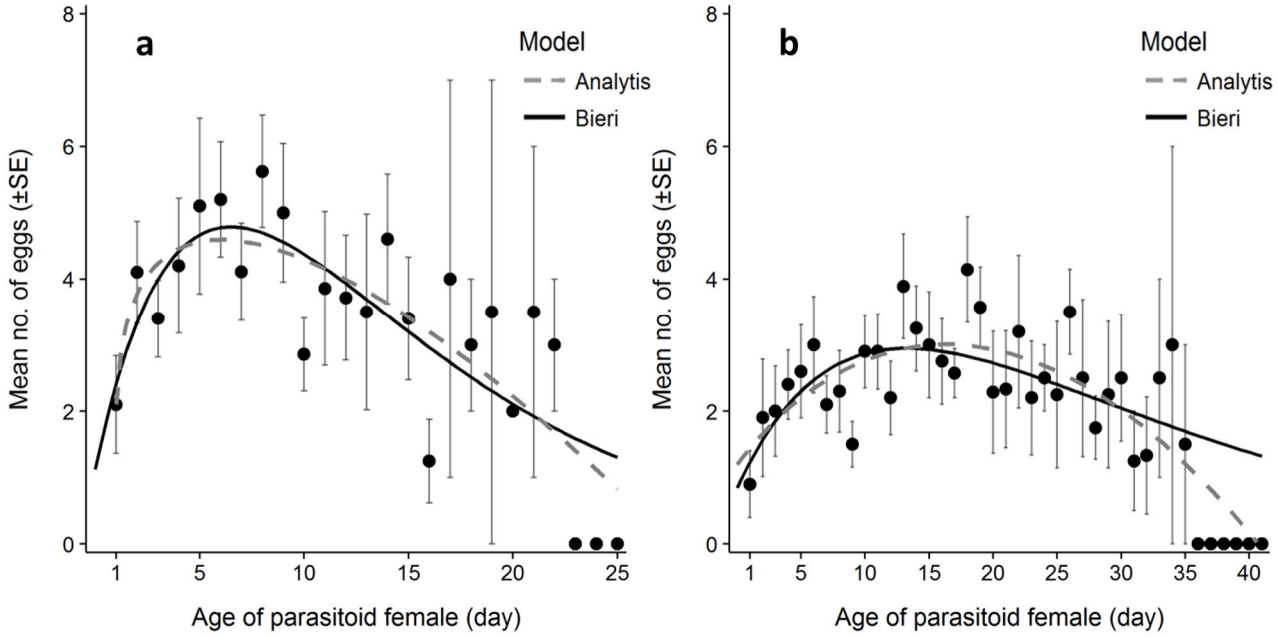

% of killed larvae
Host larvae density
% of parasitised larvae
Host larvae density
% of host-fed larvae
Host larvae density

Fig. 2

629



630

631 **Fig. 3**

632