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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1687801> since 2020-04-06T19:43:03Z

Published version:

DOI:10.1007/s10340-019-01079-8

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1 **FUNCTIONAL RESPONSE OF THE MIRID PREDATORS *DICYPHUS BOLIVARI* AND**
2 ***DICYPHUS ERRANS* AND THEIR EFFICACY AS BIOLOGICAL CONTROL AGENTS**
3 **OF *TUTA ABSOLUTA* ON TOMATO**

4

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20

21 **Key words:**

22 Hemiptera Miridae, Lepidoptera Gelechiidae, South American tomato pinworm, Numerical
23 response, Predator voracity

24

25 **Key message:**

- 26 • The potential of *Dicyphus bolivari* and *Dicyphus errans* as biological control agents of *Tuta*
27 *absoluta* was evaluated
- 28 • In laboratory, females and 5th-instar nymphs of both mirids showed a type II functional
29 response to eggs of *T. absoluta*
- 30 • In greenhouse, both predators were able to reduce the population of *T. absoluta*, but less
31 effectively than *Macrolophus pygmaeus*, which showed the strongest numerical response
- 32 • Functional response studies should be combined with population dynamics and multiple-
33 prey studies to evaluate omnivorous predator efficacy

34

35 **Author Contribution Statement**

36 BLI and GM conceived, designed the trials and wrote the manuscript. NB analysed the functional
37 response data. BLI, AI, LD, JBW and AL conducted the experiments. LT supervised the trials and
38 contributed to manuscript writing. All authors read and approved the manuscript.

39

40 **Acknowledgements**

41 The functional response and predation cage trials were partly supported by the Italian national
42 project “Insects and globalisation: sustainable control of exotic species in agro-forestry ecosystems
43 (GEISCA)” of the Italian Ministry of University and Research. The greenhouse trial of Wageningen
44 University & Research was funded by the Dutch top sector Horticulture and Starting Materials. The
45 research was partly carried out during a Short Term Scientific Mission of COST Action FA1105
46 “Towards a sustainable and productive EU organic greenhouse horticulture”.

47

48 **Conflict of Interest**

49 The authors declare that they have no conflict of interest.

50

51 **ABSTRACT**

52 *Dicyphus bolivari* Lindberg and *Dicyphus errans* (Wolff) (Hemiptera: Miridae) are naturally
53 widespread in many crops with low-pesticide pressure, where they prey upon several arthropods,
54 including the tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). However, their
55 efficacy as biological control agents (BCAs) of this pest needs further investigations. Therefore, in
56 this study the predatory efficacy of *D. bolivari* and of *D. errans* on *T. absoluta* was evaluated on
57 tomato in laboratory and greenhouse trials. Their functional response to different numbers of *T.*
58 *absoluta* eggs (up to 350) offered to single females or 5th-instar nymphs for 24 h was assessed in
59 laboratory. Females and nymphs of both predators showed a high voracity and a type II functional
60 response, with an estimated maximum predation rate per day of 189 and 194 eggs for *D. bolivari*
61 females and 5th-instar nymphs, respectively, and 197 and 179 eggs for *D. errans* females and
62 nymphs, respectively. The predators showed similar predation rates of *T. absoluta* eggs on plants in
63 cage trials. However, our greenhouse trial showed that the commonly used *Macrolophus pygmaeus*
64 (Rambur) (Hemiptera: Miridae), which has a lower individual predation capacity than *D. bolivari*
65 and *D. errans*, was more effective in controlling *T. absoluta* than *D. errans* and *D. bolivari*, because
66 of its stronger numerical response to densities of *T. absoluta* and supplemental food than the other
67 two predator species. This shows that long-term greenhouse trials, which include functional and
68 numerical responses to pest densities, are essential to evaluate the efficacy of an omnivorous
69 predator.

70

71

72 INTRODUCTION

73 Tomato crop [*Solanum lycopersicum* L. (Solanaceae)] is affected by several pests (e.g., aphids, leaf
74 miners, spider mites, thrips, whiteflies). Recently, in the Mediterranean area, the exotic tomato
75 pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has become a serious threat for this
76 crop, and chemical control is often ineffective because of its cryptic habits and high pesticide
77 resistance (Desneux et al. 2010; Tropea Garzia et al. 2012; Campos et al. 2017; Biondi et al. 2018;
78 Roditakis et al., 2018). To reduce both pest infestations and pesticide use, sustainable control can be
79 achieved using effective biological control agents (BCAs).

80 Hemipteran predators, such as anthocorids, geocorids, mirids, nabids, and pentatomids, have been
81 identified as potential BCAs against *T. absoluta*. Omnivorous mirids were used soon after the pests'
82 arrival, through augmentative and inoculative releases in fields and plant nurseries, sometimes
83 supported by conservation strategies (e.g., using banker plants) (Biondi et al. 2018). Several species
84 of mirid predators belonging to the tribe Dicyphini (Heteroptera: Miridae) have been reported in
85 many crops with low-pesticide pressure preying upon various pests (Ingegno et al. 2008, Leman et
86 al. 2019). However, only a few dicyphine species [i.e., *Macrolophus pygmaeus* (Rambur) and
87 *Nesidiocoris tenuis* (Reuter)] are commercially produced and released, while many other species
88 belonging to the genus *Dicyphus* have been observed in several crops (Ingegno et al. 2017a;
89 Sanchez and Cassis 2018). Among them, the Mediterranean *Dicyphus bolivari* Lindberg [previously
90 named *Dicyphus maroccanus* Wagner (Sanchez and Cassis 2018)] and the Palearctic *Dicyphus*
91 *errans* (Wolff) live omnivorously on various host plants and pests (Ingegno et al. 2008; Voigt et al.
92 2007; Abbas et al. 2014). Recently, both predators have been reported to prey upon *T. absoluta* in
93 open field and greenhouse crops (Zappalà et al. 2013; Abbas et al. 2014). Moreover, *D. errans*
94 showed potential as a BCA of this exotic pest under laboratory conditions (Ingegno et al. 2013,
95 2017a, b).

96 A fundamental aspect in multitrophic interactions and population dynamics in a predator-prey
97 system is the capability of a BCA to find, kill and consume prey (Nachman 2006). Predator's

98 feeding rate related to changes in prey density, the so-called functional response, is one of the key
99 components to assess its effectiveness in controlling pest populations and the stability of prey-
100 predator dynamics (Abrams 1982; Fernández-Arhex and Corley 2003). The numerical response
101 (increase of densities) of the predator to prey densities is another key component for evaluating its
102 potential as BCA, and biological control eventually represents the combined results of functional
103 and numerical responses to pests (Coll and Ridgway 1995; Meiracker and Sabelis 1999).
104 The main aim of this study was to further evaluate, under laboratory and greenhouse conditions, the
105 potential of *D. bolivari* and *D. errans* as BCAs of *T. absoluta* by comparing their predatory traits
106 through assessment of: I) functional responses of females and nymphs to increasing egg densities in
107 arena trials; II) daily predation of eggs on infested tomato plants in cage trials; III) population
108 dynamics of *T. absoluta* and the predators in a greenhouse trial with a time-period long enough to
109 observe the numerical response of the released predators. The commonly used predator *M.*
110 *pygmaeus* was included as a reference in this greenhouse trial.

111

112 **MATERIALS AND METHODS**

113 **Insect and plant rearing**

114 Colonies of *D. bolivari* were started from individuals collected on tomato in the Valencia region,
115 Spain in 2015. They were reared on pods of the flat bean *Phaseolus vulgaris* L. (Fabaceae) in
116 transparent plastic cylinder cages (H 27 cm, Ø 25 cm; JET 107 PM, Jokey plastic GmbH, Sohland,
117 Germany) with the drilled lid covered with a fine net mesh. Colonies of *D. errans* were started from
118 individuals collected on the European black nightshade *Solanum nigrum* L. (Solanaceae) in
119 Piedmont region, NW Italy in 2015. They were reared on this plant species, as well as on tomato
120 and tobacco *Nicotiana tabacum* L. (Solanaceae) in insect cubic cages (47.5 cm edge; BugDorm,
121 MegaView, Taiwan). Both dicyphine species were supplied with eggs of *Ephestia kuehniella* Zeller
122 (Lepidoptera: Pyralidae) mixed with dehydrated and decapsuled cysts of *Artemia* sp. (Anostraca:
123 Artemiidae) (Entofood, Koppert B.V. Berkel en Rodenrijs, The Netherlands). Colonies of *M.*

124 *pygmaeus* were established starting from individuals purchased from Koppert Biological Systems
125 (Berkel en Rodenrijs, The Netherlands) and reared on flat bean pods inside transparent plastic
126 cylinder boxes (H 27 cm, Ø 25 cm, JET 107 PM, Jokey Plastik GmbH, Sohland, Germany).
127 Ventilation was possible through a hole in the lid covered with insect gauze (mesh size 80 µm). All
128 cultures were kept in climatic chambers at 25±1°C, 70±5% RH, 16:8 L:D.
129 Colonies of *T. absoluta* were established from individuals provided by Bioplanet laboratories
130 (Bioplanet s.c.a., Cesena, Italy). A continuous rearing was maintained on tomato plants in net cages
131 (W 150 × L 150 × H 110 cm) consisting of a stainless-steel frame structure supporting an insect-
132 proof net (mesh 0.23×0.23 mm). The rearing was carried out in an experimental heated greenhouse
133 at 27±3°C and 55±23% RH.
134 For plant growing, seeds from seed companies were used, except for seeds of *S. nigrum*, which
135 were collected from wild plants grown in Piedmont region. Seeds were sown in plastic pots (Ø 14
136 cm), watered daily, and fertilized. Plants were grown in an experimental heated greenhouse at
137 27±3°C and 55±23% RH.

138

139 **Functional response trials**

140 In laboratory, 1-week-old females of *D. bolivari* and *D. errans* were used to assess their functional
141 response to *T. absoluta* egg densities. After starving them for 16 h, to standardize individual
142 behaviour, single females were exposed to definite prey densities for 24 h. Specifically, eight
143 treatments consisting of different amounts of *T. absoluta* eggs offered as prey on tomato leaflets
144 (i.e., 5, 10, 25, 50, 90, 150, 250 and 350 eggs) were set up to fit the functional response curve. The
145 experiments were carried out in artificial arenas with excised leaflets, which were shown to be
146 representative for functional response studies on a plant level for the related *M. pygmaeus* (Maselou
147 et al. 2014). *Tuta absoluta* eggs were offered on three tomato leaflets, with stalks placed into 2 ml
148 plastic tubes containing water and sealed with Parafilm[®], inside a Petri dish (Ø 20 cm). After 24 h,

149 the female was removed, and the leaflets were inspected under a stereomicroscope to count
150 predated eggs. Five replicates were performed for each treatment. Experiments were carried out in
151 climatic chambers at $24\pm 1^{\circ}\text{C}$, $65\pm 5\%$ RH and 16:8 L:D.

152 The functional response of juvenile predators on *T. absoluta* eggs was assessed by using 5th-instar
153 nymphs of *D. bolivari* and *D. errans* with the same experimental conditions and procedure above
154 reported for females. Ten treatments consisting of different amounts of *T. absoluta* eggs offered as
155 prey on tomato leaflets (i.e., 5, 10, 15, 20, 40, 50, 100, 150, 200, 300 eggs) were set up. Five
156 replicates were performed for each treatment.

157

158 **Predation trials on plants**

159 In laboratory, the predatory efficacy of *D. bolivari* and *D. errans* on tomato plants infested with *T.*
160 *absoluta* was investigated through cage trials. Three treatments were compared: 1) tomato infested
161 with *T. absoluta*; 2) tomato infested with *T. absoluta* plus one female of *D. bolivari*, 3) tomato
162 infested with *T. absoluta* plus one female of *D. errans*. A tomato plant (H 40 cm, with 6-7 leaves)
163 was infested with a constant amount of 20 *T. absoluta* eggs(<24 h), gently transferred with a brush,
164 every 24 h per 3 days (for a total of 60 eggs) and isolated in a net insect tent cage
165 (W 60 × L 60 × H 60 cm, BugDorm, MegaView, Taiwan). After 24 h from first egg insertion, in the
166 treatment 2 and in the treatment 3, one female of *D. bolivari* or of *D. errans* was introduced and
167 removed after 72 h, respectively, while the treatment 1 was kept as control without predators. The
168 number of *T. absoluta* eggs was checked at 24, 48 and 72 h from the predator introduction, and the
169 number of *T. absoluta* larvae was counted at the last egg checking (96 h from the first egg
170 introduction). Ten replicates were performed for each treatment. The experiment was carried out in
171 climatic chambers at $24\pm 1^{\circ}\text{C}$, $65\pm 5\%$ RH and 16:8 L:D.

172

173 **Population dynamics greenhouse trials**

174 A greenhouse trial was set up to evaluate the effects of pre-established populations of *D. bolivari*
175 and *D. errans* on *T. absoluta* establishment and population increase. The commonly used *M.*
176 *pygmaeus* was included in this trial as a reference treatment. The experiment was conducted in a
177 greenhouse compartment of 98 m² at Wageningen University & Research in Bleiswijk, The
178 Netherlands. Three-week-old tomato plants (with each 4 developed leaves), cv Briosso (Rijk Zwaan,
179 The Netherlands), were grown individually in rock wool blocks, and each block was placed on a
180 50 cm rock wool slab. The young plants were vaccinated with a mild isolate of the Pepino mosaic
181 virus (PepMV) (PMV®-01, DCM, Belgium), to offer protection against more aggressive isolates,
182 which is a common practice for Dutch tomato growers. Nutrients for the plants were provided
183 through drip irrigation. Each tomato plant was subsequently enclosed in a mesh cage
184 (60 × 60 × 180 cm) made of fine gauze (mesh size 500 µm, Vermandel, The Netherlands)
185 representing an experimental unit. The experimental units were distributed within the greenhouse
186 using a randomized block design with the following treatments: A) no predators (control), B) *D.*
187 *bolivari*, C) *D. errans*, and D) *M. pygmaeus*. There were five replicates of each treatment, thus 20
188 experimental units were used in total.

189 The predators were introduced 2, 3 and 4 weeks after placing the plants in the cages in densities of
190 respectively 3, 3 and 6 couples of one-week old adults (12 females and 12 males in total). The
191 establishment and population growth of the predators were supported by adding weekly 0.5 gr of
192 sterilized *E. kuehniella* eggs per cage (Koppert B.V. Berkel en Rodenrijs, The Netherlands). The
193 eggs were spread all over the plant with a fine brush and applied for 7 weeks, starting in the week of
194 the first predator introduction. *Tuta absoluta* was introduced as adults, starting in the same week of
195 the last predator introduction. The introduction was spread over 4 weeks to ensure that eggs and
196 first larval stages (vulnerable for predation) were present over a longer time-period. Young mated
197 couples of *T. absoluta* were weekly introduced in densities of 1, 2, 1 and 3 couples per cage (7
198 females and 7 males in total). Densities of predatory mirids and *T. absoluta* were monitored every 2

199 weeks, during a 6-week period, by counting the total number of each species per cage (nymphs and
200 adults of predatory mirids and larvae, pupae and adults of *T. absoluta*).

201 Temperature and relative humidity in the greenhouse compartment were recorded every 5 min using
202 a climate recorder (Hoogendoorn Growth Management, Vlaardingen, The Netherlands) throughout
203 the experiment. The average temperature and relative humidity during the experiment (from the
204 time predators were introduced) was 21.9°C (range 14.9-39.1°C) and 64% (range 32–89%),
205 respectively.

206

207 **Statistical analyses**

208 Functional response type and parameters of attack rate and handling time showed by *D. bolivari* and
209 *D. errans* females and 5th-instar nymphs were estimated using the general approach proposed by
210 Okuyama (2012a). This approach includes the application of a model selection index (i.e., Akaike
211 information criterion, AIC) directly to candidate models. We used the maximum likelihood
212 approach to obtain parameter estimates of the Holling's type II (Eq. 1), Rogers random-predator
213 equation (Eq. 2) and Holling's type III (Eq. 3) (Bolker 2008; Okuyama 2012b). The maximum
214 likelihood method is less used than the typical estimation method of least squares, but it has been
215 recently been accounted for better performances in estimating functional response parameters than
216 the estimation method of least squares (Okuyama 2012b). The three functional response models
217 were then used to fit the data regarding the consumption of *T. absoluta* eggs, using the R package
218 *bbmle* for maximum likelihood estimation (*mle*); since N_a is on both left and right side of the
219 equation, the Eq. 2 has been modified using *LambertW* function (Bolker 2008; Haddaway et al.
220 2012). Rogers random-predator equation describes a type II functional response but accounts for
221 prey depletion, that is the case of our experiments, since mirid predators completely consume
222 preyed eggs. The best model for each mirid species and life stage was selected using the AIC test
223 (lower AIC means a better model fit), from which the coefficients a (attack rate) and Th (handling
224 time) were obtained and compared using confidence intervals (95%). Mean values of Th were used

225 to calculate the maximum attack rate as T/Th (Hassell 2000), representing the maximal number of
226 *T. absoluta* eggs that could be attacked during the considered time interval (in our case $T = 1$ day).
227 Statistical analyses were performed using the statistical software R (R Core Team 2018).
228 Data on cage trials were analysed by performing one-way ANOVA since data satisfied normality
229 and homogeneity criteria (Shapiro Wilk and Levene tests). When significantly different, means
230 were separated by Tukey's test ($P < 0.05$). A Student t test was performed for comparing the two
231 tested species. Statistical analyses were performed using the statistical software SPSS, 25th edition
232 (IBM Corp., NY, USA).
233 Population dynamics of predators and *T. absoluta* in the greenhouse trial were analysed with
234 repeated measures ANOVA. Predator densities were based on the cumulative numbers of nymphs
235 and adults, *T. absoluta* densities were based on the cumulative numbers of larvae, pupae and adults.
236 Data were prior to the analyses $\log(+1)$ transformed to fit a normal distribution. Differences among
237 treatments were tested with Fisher's Least Significance Difference (LSD) test ($P < 0.05$). These
238 analyses were performed using the software package Genstat, 18th edition.

239

240 **RESULTS**

241 **Functional response trials**

242 The two mirids *D. bolivari* and *D. errans* presented very similar functional response curves
243 showing a type II functional response, which is inverse density dependent, both for the adult and
244 nymphal stage (Fig. 1). The model fits were compared and are reported in Table 1. Rogers random-
245 predator function presented lower values for females and 5th-instar nymphs of both predator species,
246 which indicates that accounting for prey depletion results in models of better fit. Parameters
247 estimated by Rogers random-predator equation were all significantly different from zero, showing a
248 marked increase in consumed eggs with an increasing number of offered eggs. Comparing the
249 confidence intervals of functional response parameters, *D. bolivari* and *D. errans* did not differ
250 significantly in attack rate of *T. absoluta* eggs (Table 2), although *D. bolivari* showed a trend

251 towards a greater attack rate compared to *D. errans*, for both females and 5th-nymphs. The two
252 mirids also did not differ in handling time of *T. absoluta* eggs (Table 2), regardless of the
253 considered life stage. These results lead to similar maximum attack rates: 188.52 and 194.18 eggs
254 day⁻¹ for *D. bolivari* females and 5th-instar nymphs, respectively, and 197.24 and 178.58 eggs day⁻¹
255 for *D. errans* females and 5th-instar nymphs, respectively. In our experiment, when exposed to the
256 highest density of 350 *T. absoluta* eggs, predator females consumed on average more than 130 eggs
257 per day.

258

259 **Predation trials on plants**

260 No differences in the number of daily preyed eggs of *T. absoluta* were found between the two
261 predators *D. bolivari* and *D. errans* (Student t test; df=18; after 24 h: F=0.109, P=0.745; after 48 h:
262 F=1.580, P=0.225; after 72 h: F=0.027, P=0.871). Except for the first 24 h, both predators were able
263 to prey around 12-14 eggs per day with a continuous daily offer of 20 eggs (Table 3). The total
264 number of *T. absoluta* eggs consumed over 3 days of exposure by both predators was also similar,
265 namely 29.0±2.1 for *D. bolivari* and 33.5±2.6 for *D. errans* (Student t test; df=18; F=0.732;
266 P=0.404). The total number of viable *T. absoluta* eggs after 3 days of exposure to both predators
267 was significantly different from that one of the control without predators (ANOVA; df=2;
268 F=36.634; P<0.0001) (Table 3). Also the number of emerged larvae at the end of the trial (i.e., 96 h
269 after the first egg insertion) was significant different between treatments with predators and without
270 predators (ANOVA; df=2; F=5.601; P<0.01) (Table 3).

271

272 **Population dynamics greenhouse trials**

273 Densities of *T. absoluta* were significantly different among treatments through time (F_{3,12}=19.12,
274 P<0.001). The predators *D. bolivari* and *D. errans* both equally reduced the population increase in
275 *T. absoluta* compared to the treatment without predators, but *M. pygmaeus* clearly better suppressed
276 *T. absoluta* (Fig. 2A). Densities of predators were also significantly different among species

277 ($F_{2,8}=10.41$, $P=0.006$), with the highest densities observed for *M. pygmaeus* and comparable lower
278 densities for *D. bolivari* and *D. errans* (Fig. 2B).

279

280 **DISCUSSION**

281 The functional responses of *D. bolivari* and *D. errans* to *T. absoluta* eggs, which were compared
282 here for the first time, show that these mirids can consume large quantities of *T. absoluta* eggs. The
283 high estimated maximum attack rate (i.e., 180-200 eggs per day) for both predators and the short
284 handling time highlight the voracity of these predator species. The predatory response of females
285 and 5th-instar nymphs of *D. bolivari* and *D. errans* to increasing densities of *T. absoluta* eggs was
286 described by a type II functional response, consistent with the results reported in most studies
287 involving dicyphine species (Alvarado et al. 1997; Foglar et al. 1990; Montserrat et al. 2000;
288 Fantinou et al. 2008; Maselou et al. 2014, 2015). The type II functional response is often
289 characteristic of invertebrate predators that provide efficient pest control, even though it is often
290 associated with unstable predator-prey population dynamics (Juliano 2001; Briggs and Hoopes
291 2004; Nachman 2006). However, these fluctuating dynamics are less crucial for omnivores that
292 stabilize their densities by feeding on plants and/or on provided alternative food sources (Messelink
293 et al. 2014).

294 Several studies have also reported that dicyphine predators show a type III functional responses
295 (Enkegaard et al. 2001; Hamdan 2006). A recent study on three Neotropical dicyphine species
296 showed a type II functional response for one species and type III for the other two species to egg
297 densities of *T. absoluta* (van Lenteren et al. 2016). The different outcomes achieved by these studies
298 may be due to difficulties in discriminating readily between type II and III functional response
299 curves or to biological differences in both prey and predator species investigated. At relatively low
300 prey densities, it may be difficult to distinguish between different types of functional responses
301 (Fantinou et al. 2008). In the present study, we provided a large range of densities of eggs to
302 females (from 5 to 350 eggs) and to 5th-instar nymphs (from 5 to 300 eggs) of both predator species.

303 This enabled us to clearly define the response type as type II, with the average proportion of
304 consumed eggs steadily declining as the number of offered eggs increased.

305 Previous studies showed a preference by the mirid predators *D. errans*, *M. pygmaeus* and *N. tenuis*
306 for *T. absoluta* eggs rather than larvae (Ingegno et al. 2013; Jaworski et al. 2013; Urbaneja et al.
307 2009), probably because of the concealing behaviour of the larvae. Van Lenteren et al. (2016)
308 reported *Macrolophus basicornis* (Stål) as the best candidate for control of the tomato pinworm in
309 Brazil, compared to other two tested species. At the highest provided egg density (256), this species
310 consumed 100 eggs per day, whereas the other two predator species *Campyloneuropsis infumatus*
311 (Carvalho) and *Engytatus varians* (Distant) consumed 51 and 91 eggs per day, respectively. In our
312 study, *D. bolivari* and *D. errans* were able to prey around 115-125 eggs at similar prey density (250
313 offered eggs), respectively, showing even better performances compared to their Neotropical
314 relatives. These two predator species show also higher maximum predation rates than that one
315 reported for the commonly used predator *M. pygmaeus* (ca. 90 eggs day⁻¹, Michaelides et al. 2018).

316 Mirid predator preference towards eggs was especially evident for nymphal stages of *M. pygmaeus*
317 and *N. tenuis*, probably due to their small size (Jaworski et al. 2013; Urbaneja et al. 2009). In our
318 study, 5th-instar nymphs of *D. bolivari* and *D. errans* were able to predate more than 100 eggs per
319 day when exposed to the highest density of *T. absoluta* eggs (i.e., 300). Comparing the maximum
320 attack rates, females and nymphs showed to prey almost the same amount of *T. absoluta* eggs, even
321 though females are larger than 5th-instar nymphs and have different feeding requirements, also
322 related to the biomass needed for egg development (Fellowes et al. 2007). Nevertheless, 5th-instar
323 nymphs of both predator species consistently preyed *T. absoluta* eggs at each offered density, thus
324 highlighting the strong predatory capabilities of the late nymphal instar of these mirids on the pest.

325 *Dicyphus bolivari* and *D. errans* showed their predation capability on *T. absoluta* also in cage and
326 in greenhouse trials, being able to reduce the population of *T. absoluta*. On plants in cage, after a
327 period of adaptation of 24 h, both predators preyed on average 12-14 eggs of the tomato pinworm
328 over the 20 fresh eggs daily offered, as estimated by the trend of the functional response curves

329 (~15) (Fig. 1). *Tuta absoluta* usually has an oviposition peak in the first 3 days after adult
330 emergence, during which each female can lay up to 12-14 eggs per day (Lee et al. 2014), although
331 higher peaks were also recorded (Pereyra and Sánchez 2006). Therefore, at the beginning of
332 infestation, a 1:1 predator:prey ratio could lead to a successful control of the pest. However, since
333 the viable eggs were not daily removed, the number of available eggs per day per female raised the
334 quote of 20 with a consequent higher hypothetic expected number of consumed eggs. This
335 discrepancy between real and predicted values of preyed eggs can be attributed to the different
336 experimental scales. The presence of an entire plant could have influenced several aspects, such as
337 phytophagy and searching time, compared to arena scale with excised leaflets, in contrast with what
338 observed in Maselou et al. (2014).

339 The high predation rates of *T. absoluta* eggs we observed for *D. bolivari* and *D. errans* suggests
340 that these predators can potentially be more effective in controlling *T. absoluta* than the commonly
341 used *M. pygmaeus*. However, the greenhouse trial showed the opposite effect: *M. pygmaeus* was
342 clearly more effective in controlling the tomato pinworm than *D. bolivari* and *D. errans*. At the
343 same time, we observed a much faster population increase for this predator compared to *D. bolivari*
344 and *D. errans*, which is a likely explanation for the difference in suppression of *T. absoluta*. This
345 shows that lower predation capacity per individual can soon be compensated by a stronger
346 population increase, which increases the predation capacity of the total predator population. The
347 predation capacity per individual probably had a stronger influence at the start of the experiment
348 when equal numbers of predators were released, but in a longer time-period the numerical response
349 of the predators was eventually a more defining factor. Yet, it needs to be considered that these
350 results were achieved in the summer period with high temperature peaks. The numerical response of
351 the predators may have been different under other climatic conditions, for example at lower
352 temperatures, which would have changed the outcome of the experiment. Also a longer time period
353 to build up predator densities prior to the introduction of *T. absoluta* could have resulted in a more
354 effective control of *T. absoluta* by *D. bolivari* and *D. errans* than achieved in the trial of this study.

355 The results of the greenhouse study show that functional response studies for omnivorous predators
356 are relevant to assess their behaviour at different prey densities and their potential predation
357 capacity, but they are not completely defining their predation efficacy to evaluate them as BCAs.
358 Since they are omnivores, their functional response to a specific pest will also be strongly affected
359 by the presence of other prey. For example, increases in the numbers of one prey often decrease the
360 predator's functional response to other prey, either due to satiation or switching (Murdoch 1969).
361 Additionally, increases in the density of one prey increase predator numbers (numerical response),
362 which can reduce the numbers of a second prey species (Holt 1977). For these reasons, the
363 functional response to a single pest can be considered as a starting point to evaluate omnivorous
364 predators but it should go together with studies on population dynamics, which allow assessing
365 numerical response during the experimental time-period, and multiple-prey studies. Moreover, the
366 nutritional quality of their host plant will also affect predation rates by changing their extent of plant
367 feeding and flexible feeding behaviour (Castañé et al. 2011; Biondi et al. 2016). Plant quality may
368 also indirectly affect the functional response to pests through the accumulation of secondary
369 metabolites in prey (Koller et al. 2007).

370 In conclusion, our study shows the potential of *D. bolivari* and *D. errans* as BCAs of *T. absoluta*,
371 but further investigations are still needed to evaluate their ability of establishment, reproduction
372 rate, developmental time and survival in different environmental and agronomic conditions, and
373 behaviour in multiple-prey communities that are common in tomato crops. Moreover, also their
374 degree of phytophagy and potential crop damage should be studied as a part of their evaluation for
375 use in biological control.

376

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511

512 **EQUATIONS**

513

514
$$N_a = \frac{aN_0}{1 + aThN_0}$$

515

516 **Eq. 1** Holling's type II functional response model

517

518
$$N_a = N_0(1 - e^{\alpha(NT_h)})$$

519

520 **Eq. 2** Rogers random-predator equation

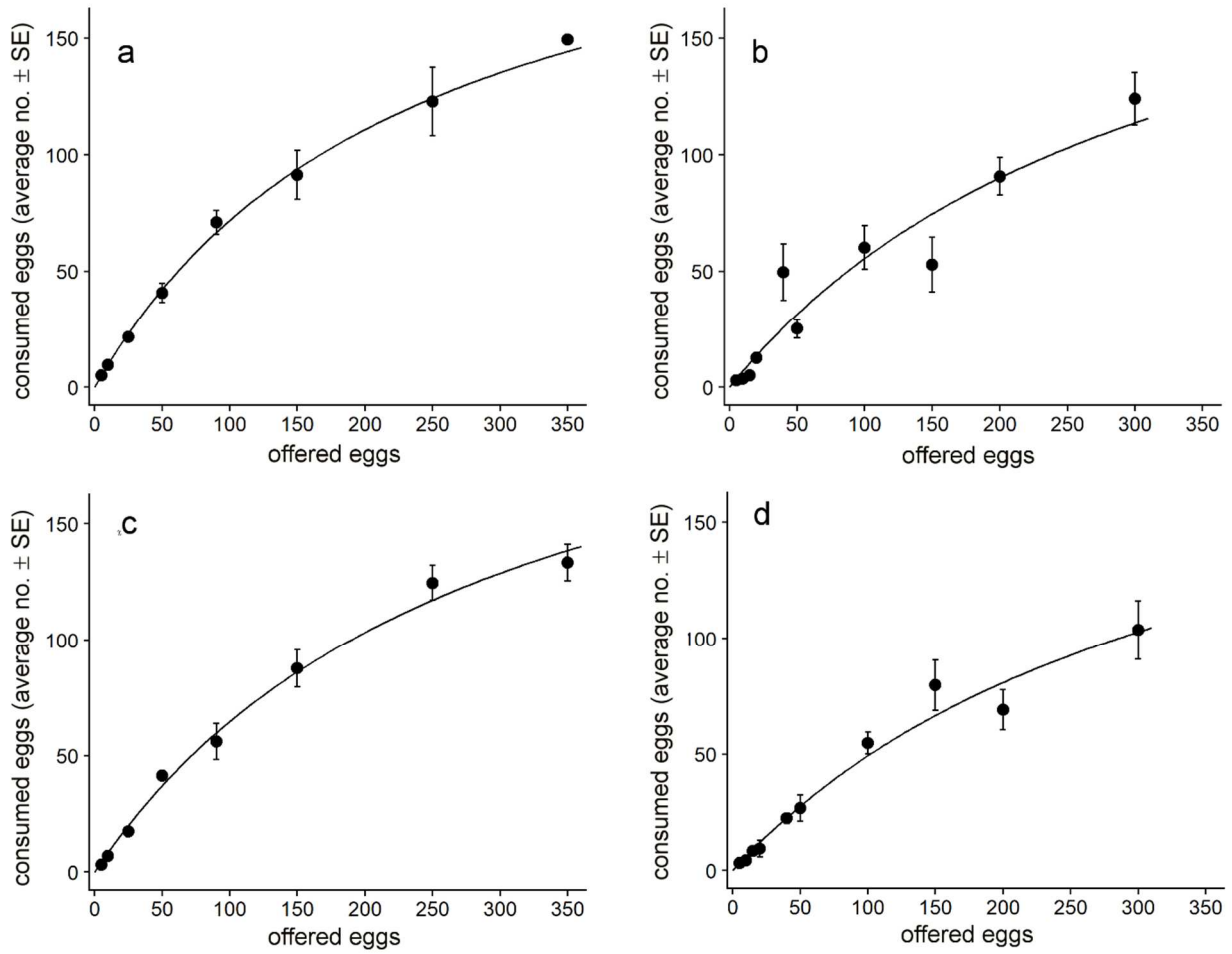
521

522
$$N_a = \frac{aN_0^2}{1 + aThN_0^2}$$

523 **Eq. 3** Holling's type III functional response model

524

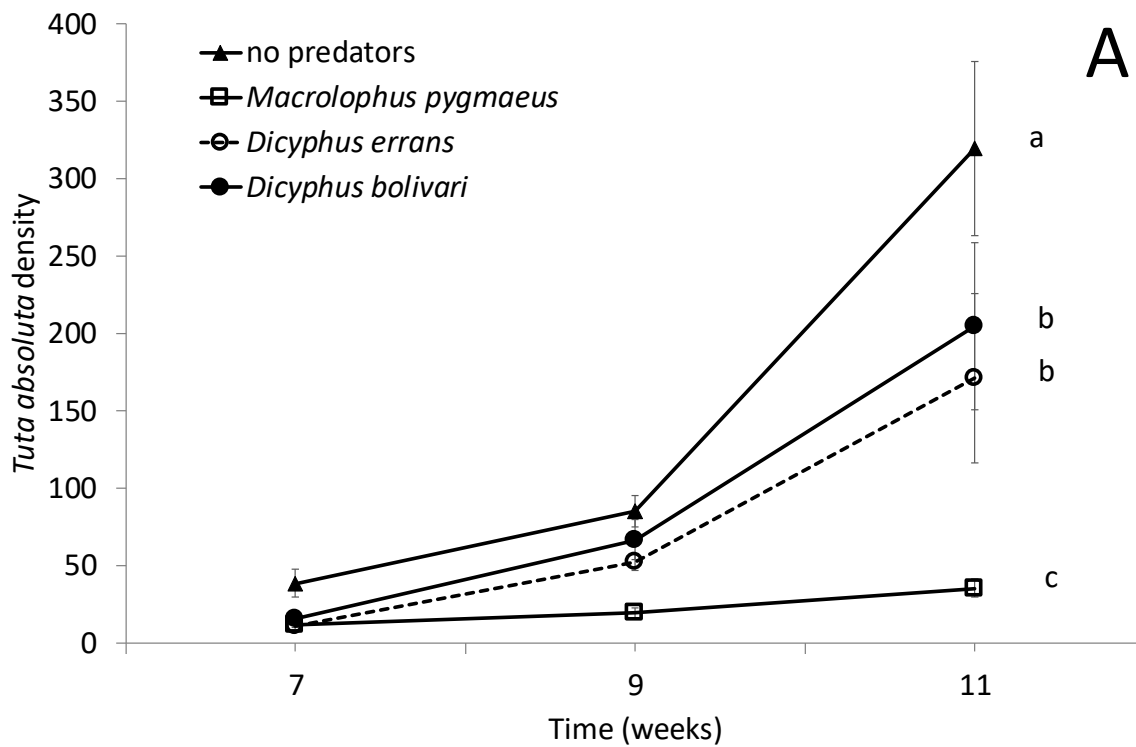
525 **FIGURES**



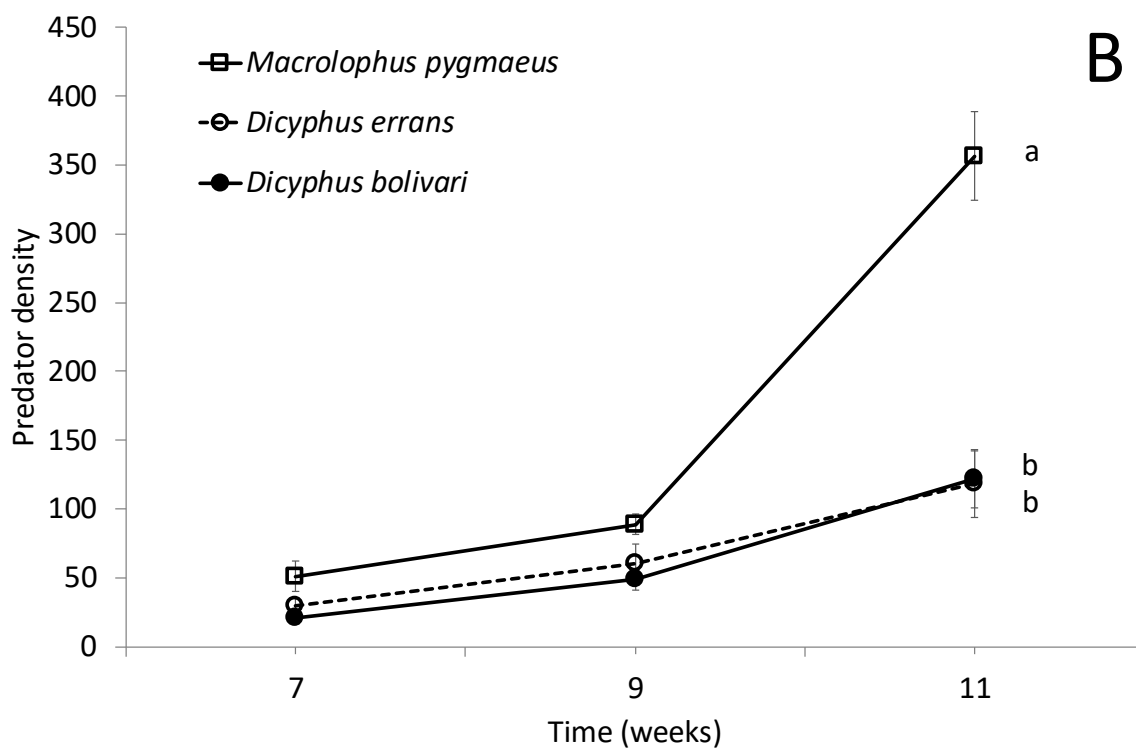
526

527 **Fig. 1** Type II functional response of (a) *Dicyphus bolivari* female adults, (b) *D. bolivari* 5th-instar
528 nymphs, (c) *Dicyphus errans* female adults, and (d) *D. errans* 5th-instar nymphs when exposed to
529 increasing densities of *Tuta absoluta* eggs during a 24 h period

530



531



532

533 **Fig. 2** Population growth in greenhouse trials of (A) *Tuta absoluta* in treatments with no predators

534 (untreated) and three species of mirid predators, and (B) the three species of the mirid predators.

535 Data shown are the mean (\pm SE) densities of (A) larvae, pupae and adults of *T. absoluta* and (B)

536 nymphs and adults of mirid predators per experimental unit. Predators were released in week 1, 2

537 and 3 and *T. absoluta* in week 3, 4, 5 and 6. Different letters indicate significant differences among
538 treatments through time (Fisher's LSD test, $P < 0.05$)
539

540 **TABLES**

541 **Table 1** Functional response trials: Akaike Information Criterion (AIC) of three functional response
 542 models fitted for females and 5th-instar nymphs of *Dicyphus bolivari* and *Dicyphus errans*. A lower
 543 AIC value indicates a better fit

	<i>Dicyphus bolivari</i>		<i>Dicyphus errans</i>	
	Females	5th-instar nymphs	Females	5th-instar nymphs
Holling's type II	336.38	603.75	302.83	452.91
Rogers random-predator	334.57	601.07	300.15	451.20
Holling's type III	488.50	667.53	403.46	519.20

544

545

546 **Table 2** Functional response trials: functional response estimates of a = attack rate, Th = handling
 547 time during the considered time interval ($T = 1$ day) of females and 5th-instar nymphs of *Dicyphus*
 548 *bolivari* and *Dicyphus errans*; CI = confidence interval

		a	CI	Th	CI
<i>Dicyphus bolivari</i>	Females	2.187	1.724 - 2.662	5.31×10^{-3}	$(4.49 - 5.91) \times 10^{-3}$
	5th-instar nymphs	1.155	0.972 - 1.351	5.15×10^{-3}	$(3.94 - 6.18) \times 10^{-3}$
<i>Dicyphus errans</i>	Females	1.603	1.314 - 1.916	5.07×10^{-3}	$(4.18 - 5.74) \times 10^{-3}$
	5th-instar nymphs	0.956	0.814 - 1.116	5.60×10^{-3}	$(4.30 - 6.75) \times 10^{-3}$

549

550

551 **Table 3** Predation trials on plants: number of daily preyed eggs of *Tuta absoluta* exposed to a
 552 female of *Dicyphus bolivari* or *Dicyphus errans* checked starting from 24 h after the predator
 553 introduction (20 eggs day⁻¹ per 3 days); number of viable eggs and of larvae of *Tuta absoluta* at the
 554 end of the trials (i.e., 72 h and 96 h after inserting predator and first eggs, respectively). In column,
 555 values followed by different letters are significantly different (ANOVA, Tukey test, P<0.05)
 556

Treatment	Daily no. of preyed eggs			No of viable eggs	No. of larvae
	After 24 h	after 48 h	after 72 h		
+ <i>Dicyphus bolivari</i>	5.0±1.3	11.2±2.3	12.8±1.9	25.9±1.7 a	5.1±1.0 a
+ <i>Dicyphus errans</i>	5.7±1.2	13.7±1.7	14.1±2.2	20.8±2.3 a	5.7±1.2 a
Without predator (control)	-	-	-	47.4±2.5 b	12.6±2.5 b

557
 558