



UNIVERSITÀ DEGLI STUDI DI TORINO

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1 **Evaluation of the effectiveness of *Dicyphus errans* (Wolff) as predator of *Tuta absoluta***
2 **(Meyrick)**

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8

9 ABSTRACT

10 Tomato crop in the Mediterranean Basin and in Europe has been recently affected by the exotic pest
11 *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), which is difficult to control due to its high
12 reproduction rate and potential to develop resistance to insecticides. In this paper, the suitability and
13 effectiveness of the predatory bug *Dicyphus errans* (Wolff) (Hemiptera: Miridae), an indigenous
14 species usually found in the IPM tomato crop of northwestern Italy, were evaluated on eggs and
15 larvae of *T. absoluta* under controlled conditions. This generalist predator could be an effective
16 biological control agent against the tomato borer. Both sexes of *D. errans* were proven to prey
17 actively on *T. absoluta* eggs and 1st-instar larvae. In particular, compared to males, females showed
18 a significantly higher egg consumption rate (11.0 ± 0.7 vs 8.6 ± 0.8 day⁻¹), and were also more
19 effective in preying on 1st-instar larvae (2.4 ± 0.5 vs 1.3 ± 0.3 day⁻¹). The mirid was able to develop
20 from egg to adulthood on tomato infested with eggs or with 1st-instar larvae of *T. absoluta*, even if a
21 high mortality of the predator was recorded in the latter case. Moreover, in olfactometer bioassays
22 predator adults proved to be attracted by tomato either infested or previously infested with tomato
23 borer larvae, independently of larval instars. *D. errans* can be considered a promising potential
24 candidate for controlling the pest, and further research is needed to assess its effectiveness under
25 field conditions.

26

27 Keywords: tomato borer, mirid bug, prey consumption, behavioural response, biological control

28

29 **1. Introduction**

30 Several species of Miridae Dicyphini (Hemiptera: Heteroptera) have received much interest for
31 their role in the biological control of many important pests in various vegetable crops (Malausa,
32 1989; Arzone et al., 1990; Tavella et al., 1997; Alomar et al., 2002; Castañé et al., 2004; Perdakis et
33 al., 2008). These generalist predators are characterized by zoophytophagous behaviour; thus, they
34 are strictly associated with the plant in addition to the prey (Alomar and Albajes, 1996; Sanchez et
35 al., 2004; Lykouressis et al., 2008; Ingegno et al., 2011). In the Mediterranean region, dicyphine
36 predatory bugs such as *Dicyphus cerastii* Wagner, *D. errans* (Wolff), *D. tamaninii* Wagner,
37 *Macrolophus costalis* Fieber, *M. pygmaeus* (Rambur), and *Nesidiocoris tenuis* (Reuter) have been
38 successfully established on horticultural crops, especially on tomato, *Solanum lycopersicum* L.
39 (Solanaceae), to control infestation by several pests such as aphids, leafminers, mites, thrips and
40 whiteflies (Cassis, 1984; Malausa and Trottin-Caudal, 1996; Carvalho and Mexia, 2000;
41 Lykouressis et al., 2000; Alomar et al., 2002; Arnó et al., 2003; Agustí and Gabarra, 2009).
42 Currently, the last two mentioned predator species are reared by several private companies and
43 largely used in IPM and biological control programmes throughout Europe, while in North America
44 the most promising agent for pest control on the tomato crop is *D. hesperus* Knight (McGregor et
45 al., 1999; Sanchez et al., 2003; Shipp and Wang, 2006).

46 In the Mediterranean Basin and Europe, the tomato crop has recently been affected by the new
47 exotic pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), which is able to cause serious yield
48 losses with its larval feeding activity (Desneux et al., 2010). Native to South America, it was first
49 reported in Europe in 2006 and in Italy in 2008 (Urbaneja et al., 2009; Viggiani et al., 2009). The
50 tomato borer is a very challenging pest to control by chemicals due to its high reproduction rate and
51 potential to develop resistance to insecticides, as well as the side effects on beneficial organisms
52 used in IPM programmes (Siqueira et al., 2000; Lietti et al., 2005; Cabello et al., 2009a; Roditakis
53 et al., 2013). Research on indigenous predators and parasitoids able to control this exotic borer has
54 been carried out throughout the Mediterranean area. Several species of predators, such as *D.*

55 *tamaninii*, *M. pygmaeus*, *N. tenuis*, and *Nabis pseudoferus* (Remane) (Hemiptera: Nabidae)
56 (Cabello et al., 2009b; Urbaneja et al., 2009; Desneux et al., 2010; Guenaoui et al., 2011; Urbaneja
57 et al., 2012), as well as parasitoids such as *Necremnus* spp. (Hymenoptera: Eulophidae), *Agathis*
58 *fuscipennis* (Zetterstedt) and *Bracon nigricans* Szepilgeti (Hymenoptera: Braconidae) (Cabello et
59 al., 2009a; Loni et al., 2011; Biondi et al 2012 Ferracini et al., 2012a; Zappalà et al., 2012), have
60 been evaluated for their effectiveness as natural enemies of the tomato borer.

61 Among predators, both commercialized dicyphine species, *M. pygmaeus* and *N. tenuis*, were proven
62 to prey on eggs and larval stages of *T. absoluta*. In laboratory, the number of preyed eggs (from 10
63 to 100 day⁻¹) varied in relation to the bug species, the sex, and also the number of daily offered eggs
64 (10, 20, 40, 60, 145), while among the larval instars the 1st-instar was preferred with 1.8 and 2.4
65 larvae daily consumed by *M. pygmaeus* and *N. tenuis*, respectively (Arnò et al., 2009; Mollà et al.,
66 2009; Urbaneja et al., 2009). The predatory activities of these two species have also been reported
67 in Italian protected tomato crops (Viggiani et al., 2009; Fois et al., 2011). In Piedmont, a more
68 continental region in northwestern Italy, the indigenous dicyphine predators usually found in IPM
69 tomato crops are *D. errans* and *M. pygmaeus* (Tavella and Goula, 2001; Ingegno et al., 2009)
70 instead of *N. tenuis*, which has its northernmost distribution limit in Liguria, the coastal region of
71 northwestern Italy (Arzone et al., 1990). While several studies have been carried out on both
72 commercialized species, little is known about the effectiveness of the native *D. errans* as a predator
73 of *T. absoluta*, which has largely been found in infested tomato plantations (Boualem et al., 2011;
74 Ferracini et al., 2012b).

75 This mirid is a Palaearctic species widespread throughout Europe, except in Finland, Latvia,
76 Lithuania, northern Ireland, northern Russia and Slovakia, and western Asia (Kerzhner and Josifov,
77 1999; Aukema, 2005). In Italy its presence has been reported everywhere (Servadei, 1967; Faraci
78 and Rizzotti Vlach, 2003). It lives omnivorously on various host plants (over 150), preying on a
79 wide range of small arthropods (at least 15) (Voigt, 2005). Among plants, it prefers glandular hairy
80 plants such as tomato, eggplant *Solanum melongena* L., potato *S. tuberosum* L., European black

81 nightshade *S. nigrum* L. (Solanaceae), courgette *Cucurbita pepo* L. (Cucurbitaceae), *Calendula*
82 *officinalis* L. (Asteraceae), *Geranium* spp., *Pelargonium* spp. (Geraniaceae), *Stachys sylvatica* L.
83 (Lamiaceae) and *Urtica dioica* L. (Urticaceae) (Arzone et al., 1990; Calabrò and Nucifora, 1993;
84 Tavella and Goula, 2001; Voigt, 2005; Ingegno et al., 2008) as a living substrate because of
85 morphological and behavioural adjustments (Voigt et al., 2007).

86 The present study aimed at investigating the predator capacity of *D. errans* and its potential role as
87 a biological control agent for the exotic tomato borer. In particular, predation rates on eggs and
88 larval instars of *T. absoluta*, survival and developmental time of the predator on this prey, and the
89 attractiveness of infested tomato were assessed under controlled conditions to evaluate if this
90 indigenous dicyphine species can be considered a promising candidate as a biological control agent
91 of the exotic tomato borer.

92

93 **2. Material and methods**

94 **2.1. Plant cultivation and insect rearing**

95 Tomato plants, cv Marmande, approximately 25 cm in height, were used for both mass-rearing and
96 laboratory trials. Seeds were sown in plastic pots (Ø 20 cm), watered daily and fertilized. All
97 tomato plants were cultivated in an open-sided greenhouse at 27 ± 3 °C, and $55 \pm 23\%$ RH, under a
98 photoperiod of 12 hours.

99 *D. errans* was collected on the European black nightshade *S. nigrum* in different localities of
100 Piedmont (NW Italy) and reared on tomato plants. The mirids were fed with eggs of *Ephestia*
101 *kuehniella* Zeller (Lepidoptera: Pyralidae) (Bioplanet Sca, Forlì Cesena, Italy) and reared inside a
102 cubic insect cage, 47.5 cm along each edge (MegaView, Taiwan), at 24 ± 1 °C, 55 ± 5 % RH, with a
103 16:8 L:D photoperiod to maintain the colonies. For the predation trials, newly emerged females and
104 males of *D. errans* were isolated in cages to allow them to mate; they were then used in the
105 experiments at different ages, i.e., five-, 10-, 15-days-old.

106 A continuous mass-rearing of all development stages of *T. absoluta* was maintained on tomato
107 plants in an open-sided greenhouse, in cages (150 by 150 by 110 cm) with a stainless steel frame
108 structure supporting an insect-proof net (mesh 0.23 × 0.23), starting from an initial culture collected
109 from commercial tomato plantations in Liguria (NW Italy).

110 **2.2. Predation trials on eggs**

111 Healthy tomato plants were periodically exposed to adults of *T. absoluta* to obtain a synchronized
112 cohort of eggs. Fifteen eggs were gently transferred under a stereomicroscope with the aid of a thin
113 brush onto a healthy stem with three leaflets. Each stem was put in a 2 ml plastic tube filled with
114 fertilized water and sealed with Parafilm[®]. Then, they were individually placed inside a Petri dish
115 (Ø 9 cm) on wet coarse paper to maintain leaf turgor during the experiments.

116 Five-, 10- and 15-day-old females and males of *D. errans* were placed individually inside the Petri
117 dish for 24 hours and the number of preyed eggs was then recorded. Before using them in the
118 experiments, the individuals were isolated for 24 hours in a glass tube with a healthy tomato leaf,
119 providing only water to ensure they were starved. Ten replicates were carried out for each sex and
120 age of the mirid, and their feeding activity was evaluated by counting consumed and healthy eggs.
121 Ten replicates without predator introduction were performed as a control. The experiment was
122 carried out in a climatic chamber at 25 ± 1 °C, 60 ± 10% RH, and 16:8 L:D.

123 **2.3. Predation trials on larvae**

124 A preliminary trial was performed to assess whether the predator showed any preference for
125 different larval instars. For this purpose, five larvae of each instar (1st, 2nd, 3rd, 4th) were exposed
126 simultaneously to five-day-old females and males of *D. errans* starved for 24 hours. The larvae
127 were gently transferred under a stereomicroscope with the aid of a thin brush onto a healthy tomato
128 stem with four leaflets, for a total of 20 larvae stem⁻¹. Each stem was put in a 2 ml plastic tube filled
129 with fertilized water and sealed with Parafilm[®]. Then, they were individually placed inside a Petri
130 dish (Ø 20 cm) on wet coarse paper to maintain leaf turgor during the experiments. The

131 consumption rate was evaluated after 24 hours of exposure, counting depleted and healthy larvae.
132 Five replicates were carried out for both sexes of the mirid.
133 To evaluate the effectiveness of *D. errans* as a biocontrol agent, five newly hatched larvae of the
134 tomato borer (< 1-day old) were placed on a stem in a Petri dish as described above in the
135 preliminary trial. After 24 hours of starvation inside a glass tube with a healthy tomato leaf and a
136 water source, five- and 10-day-old females and males were individually placed inside the Petri dish
137 for 24 hours and the number of preyed larvae was recorded. Ten replicates were performed for each
138 sex and age of the mirid, and feeding activity was evaluated by counting consumed and healthy
139 larvae. Ten stems in dishes without predator introduction were also set up as a control. All trials
140 were carried out in a climatic chamber at 25 ± 1 °C, $60 \pm 10\%$ RH, and 16:8 L:D.

141 **2.4. Developmental trials**

142 Developmental time from egg hatching to adulthood and mortality rates of *D. errans* were
143 investigated on tomato leaves in the presence of eggs or 1st-instar larvae of *T. absoluta* in
144 comparison with *E. kuehniella* eggs as a control. For the experiments, 15 fresh eggs or five newly
145 hatched larvae (<1-day old) of the tomato borer, or 15 *E. kuehniella* eggs, were gently transferred
146 onto a tomato stem in a Petri dish as described above, and individually offered to a 1st-instar mirid
147 nymph (<1-day old). Prey consumption, survival and moulting were recorded daily until the adult
148 stage, while leaflets and food were replaced every two days. All emerged adults were examined
149 under a stereomicroscope to determine their sex and measure their length (from vertex to the end of
150 the hemielytra) in order to detect any difference in size between the diets. For each diet (i.e., *T.*
151 *absoluta* eggs and larvae, and *E. kuehniella* eggs) 30 replicates were performed. The trials were
152 carried out in a climatic chamber at 25 ± 1 °C, $60 \pm 10\%$ RH, and 16:8 L:D.

153 **2.5. Olfactometer bioassays**

154 One-week-old adults of *D. errans* were used to assess their olfactory preference between the odours
155 of tomato plants either uninfested or infested by *T. absoluta*. In particular, six comparisons were
156 performed using healthy leaves, eggs, 1st- and 4th-larval instars, adults, and leaves previously

157 infested by the tomato borer, obtained by removing all larvae with the aid of a thin brush and
158 leaving only larval frass (Table 1). Before using them in olfactometer bioassays, adults were kept
159 without neither prey nor plant in a glass tube (length 12 cm, Ø 2.3 cm) for 18 hours.
160 The bioassays were carried out in a vertical Y-shaped Pyrex tube following the procedure described
161 for another predatory bug, *M. pygmaeus* (Ingegno et al., 2011). Each individual was observed until
162 it had walked at least 6 cm up one of the side arms or until 20 min had elapsed. Adults that did not
163 choose a side arm within 20 min were considered as “no choice” and were not counted in the
164 subsequent data analysis. For each test, an adult was evaluated only once to prevent any behaviour
165 conditioning by experience. The odour sources chosen by the mirid that responded were recorded.
166 Thirty responses were recorded for each pair of odour sources.
167 After testing five adults, the odour sources were switched between the left-hand and right-hand side
168 arms to minimize any spatial effect on choices. The Y-tube and cameras were cleaned with mild
169 soap and alcohol (70%v) and sterilized in an autoclave at 120°C for 20 min. The olfactory bioassays
170 were conducted at 24 ± 2 °C, $50 \pm 10\%$ RH, and 150 ± 10 lux.

171 **2.6. Statistical analyses**

172 After testing for homogeneity of variance (Levene) and normality (Shapiro-Wilk), data on egg and
173 1st-instar larva predation were analysed by one-way analysis of variance (ANOVA) and means were
174 compared by Tukey’s test. Since they were not normally distributed, data on preference for larval
175 instars, developmental time, and adult body length were analysed by Kruskal Wallis and means
176 were separated by the Mann-Whitney U test. In the olfactometer bioassays, the responses of *D.*
177 *errans* females were analysed by a chi-square test. The null hypothesis was that predatory females
178 had a 50:50 distribution across the two odour sources. Females that did not make a choice were
179 excluded from the statistical analysis. All analyses were performed using the software SPSS version
180 17.0 (SPSS, Chicago, IL).

181

182 **3. Results**

183 Both females and males of *D. errans* proved to prey on *T. absoluta* eggs. Predation rates were
184 relatively high, ranging from 6.4 ± 1.5 to 12.4 ± 0.7 eggs day⁻¹, corresponding to 42.7% and 82.7%
185 of total offered eggs, for 15-day-old males and 10-day-old females, respectively (Table 2). Overall,
186 females showed a significantly higher consumption rate: in fact, 11.0 ± 0.7 and 8.6 ± 0.8 eggs day⁻¹,
187 corresponding to $73.6 \pm 4.3\%$ and $57.6 \pm 5.4\%$ of offered eggs, were consumed on average by
188 females and males, respectively (ANOVA: $df = 1, 58, F = 5.30, P = 0.025$). A significantly smaller
189 number of eggs was preyed upon by 15-day-old males compared to 10- or 15-day-old females
190 (ANOVA: $df = 5, 54, F = 3.32, P = 0.011$). In the control without predator after 24 hours, the eggs
191 were still turgid and then hatched.

192 When different instar larvae were provided simultaneously to *D. errans*, both sexes accepted only
193 1st-instar larvae as preys, except for a small percentage (4%) of predation on 2nd-instar larvae by
194 females. By contrast, 3rd- and 4th- larvae showed no evidence of predation (Kruskal Wallis: $df = 3$;
195 $\chi^2 = 35.24, P < 0.001$) (Table 3). When five 1st-instar larvae were offered for 24 hours, females
196 were more effective than males, preying on an average of 2.40 ± 0.54 and 1.30 ± 0.29 larvae,
197 respectively (ANOVA: $df = 1, 38, F = 7.79, P = 0.008$) (Fig. 1). Also on 1st-instar larvae, older
198 males (10-days-old) were the least effective predators (Kruskal Wallis: $df = 3, \chi^2 = 8.33, P = 0.040$)
199 compared to females (Fig. 1). In the control without predator after 24 hours, the larvae were still
200 alive.

201 In the developmental trials, *D. errans* nymphs could develop on all diets; but they showed evident
202 difficulties preying on 1st-instar larvae of *T. absoluta*, attested by their high mortality (89.3%)
203 (Table 4). However, the high mortality rates of 2nd-, 3rd- and 4th-instar nymphs preying on 1st-instar
204 larvae of the tomato borer were also associated with significantly shorter instar duration.

205 Developmental time from egg hatching to adulthood was significantly shorter on tomato leaves with
206 *E. kuehniella* eggs (20.38 ± 0.75 days) than with *T. absoluta* eggs (24.12 ± 1.05) (Kruskal Wallis:
207 $df = 2, \chi^2 = 5.98, P = 0.05$) (Table 4). By contrast, no statistically significant differences were found
208 in body length between diets within sexes, even if the mean size was smaller with *T. absoluta* eggs

209 as diet (males, ANOVA: $df = 2, 23, F = 0.59, P = 0.56$; females, ANOVA: $df = 2, 31, F = 0.89, P =$
210 0.42) (Table 4).

211 In olfactory bioassays, *D. errans* proved to be more attracted by tomato infested by the tomato borer
212 compared to uninfested tomato. In particular, significant differences in the responses of adults were
213 found when healthy tomato was compared with a previously infested one ($\chi^2 = 4.83, P = 0.03$), and
214 with tomato infested by 1st- or 4th-instar larvae of *T. absoluta* ($\chi^2 = 4.83, P = 0.03$; $\chi^2 = 6.53, P =$
215 0.01) (Fig. 2). On the contrary, no statistically significant differences were found between
216 uninfested tomato *versus* *T. absoluta* adults ($\chi^2 = 1.20, P = 0.27$), or *versus* tomato with *T. absoluta*
217 eggs ($\chi^2 = 3.33, P = 0.07$), and between plants infested by 1st-instar larvae and 4th-instar larvae ($\chi^2 =$
218 $3.33, P = 0.07$) (Fig. 2).

219

220 **4. Discussion and conclusions**

221 To control a cosmopolitan pest, as *T. absoluta* has become, exploration of the efficiencies of several
222 biological control agents suitable to different habitats is almost compulsory. Generalist predator
223 species belonging to Miridae Dicyphini live in different habitats and have a wide host plant range
224 that makes them suitable candidates for use as biological control agents in different environments.
225 In particular, *D. errans* has a distribution area similar to *M. pygmaeus*, whereas it is more
226 continental than *N. tenuis*, being reported mainly in localities with a mild climate along the
227 Mediterranean coast (Kerzhner and Josifov, 1999; Aukema, 2005). Regarding the host range, some
228 plants are known as natural hosts for all three species [tomato, pepper *Capsicum annuum* L.,
229 *Dittrichia viscosa* (L.) Greuter, *C. officinalis*, *S. nigrum*] (Tavella and Goula, 2001; Cano et al.,
230 2009) even if *D. errans* seems to have a wider host plant range, as so far it has been reported on
231 more than 150 plant species (Voigt, 2005).

232 In Italy, all three dicyphine species, *D. errans*, *M. pygmaeus* and *N. tenuis*, are naturally present and
233 able to establish on IPM tomato crop if not disturbed by chemical treatments; however, they have
234 been found to colonize the crop in different areas in relation to environmental conditions and wild

235 flora (Tavella and Goula, 2001; Ingegno et al., 2009). In Piedmont, the predominance of *D. errans*
236 may be due to the natural abundance of *S. nigrum*, one of the most favourite hosts of this species as
237 well as of *M. pygmaeus* (Tavella et al., 1997; Lykouressis et al., 2000; Perdakis et al., 2008,
238 Ferracini et al., 2012b). In our climate, this cosmopolitan annual herbaceous plant blooms from
239 March to November and is very common in pioneer nitrophilic communities that often surround
240 tomato crop (Pignatti, 1982; Ingegno et al., 2009). *D. errans* is able to colonize tomato early in the
241 season when it is present in the surroundings (Ingegno et al., 2009). Moreover, since it shares some
242 other host plants with *T. absoluta* (i.e., *S. melongena*, *S. nigrum*, *S. tuberosum*, *Datura* spp.,
243 *Nicotiana* spp.) (EPPO, 2005; Voigt, 2005), this predator could carry on a strategic control role
244 throughout the whole pest cycle.

245 In this study, the ability of the indigenous *D. errans* to prey on *T. absoluta* is reported for the first
246 time. Previous studies showed that both commercialised dicyphine species, *M. pygmaeus* and *N.*
247 *tenuis*, prey actively on *T. absoluta* (Arnó et al., 2009; Urbaneja et al., 2009, 2012; Mollá et al.,
248 2009). In our experiments, *D. errans* showed to accept the tomato borer as prey. On *T. absoluta*
249 eggs, adult predators were able to consume more than 10 eggs daily on 15 eggs total offered. This
250 rate was certainly lower in comparison with numbers of eggs consumed by *M. pygmaeus* and *N.*
251 *tenuis*, when higher amounts of eggs were provided (Arnó et al., 2009; Urbaneja et al., 2009; Mollá
252 et al., 2009). However, since the consumption rate could vary in relation to the amount of offered
253 eggs, the actual effectiveness of *D. errans* should be further assessed by offering bug adults
254 different amounts of eggs. Overall, females of *D. errans* consumed eggs at a significantly higher
255 rate compared to males ($73.6 \pm 4.3\%$ against $57.6 \pm 5.4\%$) as it has also been observed for two other
256 species (Urbaneja et al., 2009). Older females (10- and 15-days-old) were hungrier than older males
257 (15-days-old), probably due to the effort of mature egg production. In fact, the pre-oviposition
258 period of closely related species lasts between 3 and 6 days (Agustí and Gabarra, 2009), and it is
259 known that predatory abilities can also change with age (Abrams, 2000).

260 In a simultaneous exposure to different larval instars of *T. absoluta*, both sexes of *D. errans*
261 preferred 1st-instar larvae, which are less mobile and smaller in size; only one female accepted 2nd-
262 instar larvae and none chose the latter two instars. On the contrary, *M. pygmaeus* and *N. tenuis* were
263 able to accept all larval instars even if they showed a preference for the 1st-instar (Urbaneja et al.,
264 2009). Overall, males and females of the indigenous predator preyed on a significantly different
265 number of 1st-instar larvae (2.4 ± 0.5 and 1.3 ± 0.3 larvae day⁻¹, respectively, on average), while no
266 differences between the sexes were reported for *M. pygmaeus* and *N. tenuis*, which preyed on about
267 2 larvae day⁻¹ (Urbaneja et al., 2009).

268 Furthermore, in order to assess the potential of a species as a biological control agent it is important
269 to take into account the time from egg to adulthood of both the predator and its prey. When the
270 developmental time of a generalist predator is long compared to its prey, the predators are unlikely
271 to be effective classical biological control agents because their abundance is strongly regulated by
272 cannibalism (Kindlmann and Dixon, 1999, 2001, 2002; Hamdi et al., 2013). The duration of the life
273 cycle of *T. absoluta* depends greatly on environmental conditions, varying from 23.8 days at 27.1°C
274 and 28.0 days at 25°C to 39.8 days at 19.7°C and 76.3 days at 14°C (Barrientos et al., 1998; Pereyra
275 and Sanchez, 2006). In the Mediterranean climate, adult *T. absoluta* can be detected all through the
276 year because there is no clear distinction between the generations (about 12 year⁻¹) that overlap
277 throughout the entire crop cycle (Vercher et al., 2010). In our experimental conditions, *D. errans*
278 proved to develop successfully on tomato infested with *T. absoluta* eggs: at 25°C more than 80% of
279 nymphs reached adulthood in 24.1 ± 1.1 days, a longer time than on tomato infested with *E.*
280 *kuehniella* eggs (20.4 ± 0.8 days) but still shorter compared to the life span of the pest under the
281 same conditions. This difference could be attributed to the smaller size of *T. absoluta* eggs, 360 µm
282 long and 220 µm in diameter (EPPO, 2005), in comparison to *E. kuehniella* eggs, 500–550 µm long
283 and 290–325 µm wide (Moreno et al., 1994), with a consequently reduced protein intake.

284 Differences in developmental time have also been observed in other dicyphine species reared on
285 different diets, such as artificial food, bee pollen, various preys and plants (Gillespie and McGregor,

286 2000; Perdakis and Lykouressis, 2000, Castañé et al., 2002). In previous studies on *M. pygmaeus*,
287 90% of nymphs reached adulthood preying on *E. kuehniella* eggs at 24–25°C in 17–18 days
288 (Grenier et al., 1989; Castañé and Zapata, 2005; Vanderkekhove et al., 2006). Development on *E.*
289 *kuehniella* eggs was generally more rapid than on natural preys such as *Myzus persicae* Sulzer,
290 *Trialeurodes vaporariorum* Westwood, and *Tetranychus urticae* Koch; on these preys
291 developmental times were 27.0 days at 22°C, 29.4 days at 22°C and 35.7 days at 25°C, respectively
292 (Fauvel et al., 1987; Tedeschi et al., 1999). The high mortality of 2nd-, 3rd-, and 4th-instar nymphs of
293 *D. errans* feeding on 1st-instar larva of *T. absoluta* might be explained by the small size of the
294 nymphs compared with the 5th-instar nymph and adult. By contrast, the complete survival of 1st-
295 instar nymphs could be due to its only taking in nourishment from the plant; in fact, it is known that
296 a basal level of plant feeding is necessary to provide water to sustain vital functions in dicyphine
297 species (De Puyseleir et al., 2013). However, *T. absoluta* larvae would seem to be more nutritive
298 for the predator, as the development time was shorter.

299 Recently, both commercialised dicyphine species, *M. pygmaeus* and *N. tenuis*, have been used
300 successfully in IPM and biological control programmes against the tomato borer (Belda et al., 2011;
301 Harpaz et al., 2011; Jacobson, 2011; Mollá et al., 2011; Seguret et al., 2011, Urbaneja et al., 2012),
302 advancing the opportunity to also use *D. errans* in more continental areas. These predators, if
303 present in the surroundings, are usually attracted by volatile organic compounds emitted by healthy
304 tomato plants as assessed in laboratory experiments for *M. pygmaeus* (Ingegno et al., 2011). In
305 olfactometric assays used here for the first time on this dicyphine species, *D. errans* was more
306 attracted by tomato plants infested with *T. absoluta*, when larvae of different instars were present
307 and even when the larvae were removed. Probably residues of larval activity, together with
308 chemical signals transmitted by injured plants, play an active role in tritrophic interactions. A
309 greater attraction due to volatile organic compounds emitted by tomato plants after whitefly
310 infestation was also observed in *M. pygmaeus* (Ingegno et al., 2011).

311 These results, besides underlining the great value of native fauna and the importance of preserving
312 its natural presence on crops, serve as the basis for further investigations on the efficacy of *D.*
313 *errans* as a pest predator. Therefore, further research is needed to evaluate its candidacy for mass
314 production as a biological control agent against the tomato borer. However, the predator, because of
315 its high performance on eggs and 1st-instar larvae, should be released on banker plants at the
316 beginning of the crop season to enhance early establishment.

317

318 **Acknowledgments**

319 We wish to thank Dr Marco Mosti of Bioplanet s.c.a. (Italy) for providing insects and Evaggelia

320 Kalaitzoglou for technical assistance.

321

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514

515 **Figure 1.** Average number (\pm SE) of 1st-instar *T. absoluta* larvae preyed on by *D. errans*. Ten
516 replicates were performed for each sex and age (5 and 10 days). Bars topped by the same lower-
517 case letter represent means that are not statistically different (ANOVA, $P < 0.05$). Bold-case letters
518 are referred to the comparison between total females and males (ANOVA, $P < 0.05$).

519

520 **Figure 2.** Responses of *D. errans* in a Y-tube olfactometer to the odours of healthy tomato (α),
521 adults of *T. absoluta* (β), tomato infested with eggs (π), 1st-instar larvae (γ) and 4th-instar larvae (δ)
522 of *T. absoluta*, or tomato previously infested with *T. absoluta* (ϵ), for each compared pair. Numbers
523 in bars represent individuals that moved toward the volatiles. χ^2 statistics (* $P < 0.05$; DF:1) tested
524 the hypothesis that the distribution of side-arm choices deviated from a null model where odour
525 sources were chosen with equal frequency.

1 **Table 1.** Comparison of treatments in olfactometric bioassays.

odour source 1	odour source 2
	<i>T. absoluta</i> adults
	tomato infested with eggs
healthy tomato	vs tomato infested with 1 st -instar larvae
	tomato infested with 4 th -instar larvae
	tomato previously infested with larvae
tomato infested with 1 st -instar larvae	vs tomato infested with 4 th -instar larvae

2

Table 2. Number (mean \pm SE) of *T. absoluta* eggs preyed on by 5-, 10- and 15-day-old females and males of *D. errans* from 15 eggs offered for 24 hours. Means followed by different letters are significantly different (Tukey's test, $P < 0.05$).

Adult age (d)	% of eggs preyed on by	
	females	males
5	9.1 \pm 1.4 ab	10.8 \pm 1.3 ab
10	12.4 \pm 0.7 a	8.7 \pm 1.2 ab
15	11.6 \pm 1.0 a	6.4 \pm 1.5 b
tot	11.0 \pm 0.7 A	8.6 \pm 0.8 B

Table 3. Number (mean \pm SE) of *T. absoluta* larvae per instar preyed on by females and males of *D. errans* from 20 larvae (five per instar) for 24 hours. Means followed by different letters are significantly different (Kruskal-Wallis test, $P < 0.05$).

Larval instar	% of larvae preyed on by	
	females	males
1 st	3.4 \pm 0.5 a	2.2 \pm 0.6 a
2 nd	0.2 \pm 0.2 b	0.0 \pm 0.0 b
3 rd	0.0 \pm 0.0 b	0.0 \pm 0.0 b
4 th	0.0 \pm 0.0 b	0.0 \pm 0.0 b

1 **Table 4.** Nymphal development time in days per instar and adult length of *Dicyphus errans* (mean \pm SE) with different diets (*Ephestia kuehniella*
 2 eggs, *Tuta absoluta* eggs, *Tuta absoluta* 1st instar larvae). Means followed by different letters are significantly different (Kruskal-Wallis test P <
 3 0.05).

instar	DIET								
	<i>E. kuehniella</i> eggs			<i>T. absoluta</i> eggs			<i>T. absoluta</i> 1 st instar larvae		
	N	no. days	mortality	N	no. days	mortality	N	no. days	mortality
I-II	31	4.03 \pm 0.23 _a	0.0%	30	3.63 \pm 0.19 _a	0.0%	56	3.64 \pm 0.22 _a	0.0%
II-III	31	3.42 \pm 0.26 _b	0.0%	30	5.80 \pm 0.48 _a	0.0%	37	3.84 \pm 0.31 _b	33.9%
III-IV	31	6.03 \pm 0.57 _{ab}	0.0%	29	7.72 \pm 0.81 _a	3.3%	17	4.47 \pm 0.45 _b	54.1%
IV-V	29	3.76 \pm 0.23 _a	6.5%	26	4.15 \pm 0.44 _a	10.3%	6	4.17 \pm 1.19 _a	64.7%
V-A	29	3.24 \pm 0.25 _a	0.0%	25	3.52 \pm 0.34 _a	3.8%	6	2.67 \pm 0.21 _a	0.0%
tot	29	20.38 \pm 0.75 _b	6.5%	25	24.12 \pm 1.05 _a	16.7%	6	23.33 \pm 2.06 _{ab}	89.3%
female length	(mm)	4.91 \pm 0.06 _a			4.78 \pm 0.08 _a			4.85 \pm 0.05 _a	
male length	(mm)	4.72 \pm 0.08 _a			4.60 \pm 0.08 _a			4.75 \pm 0.38 _a	

Figure 1

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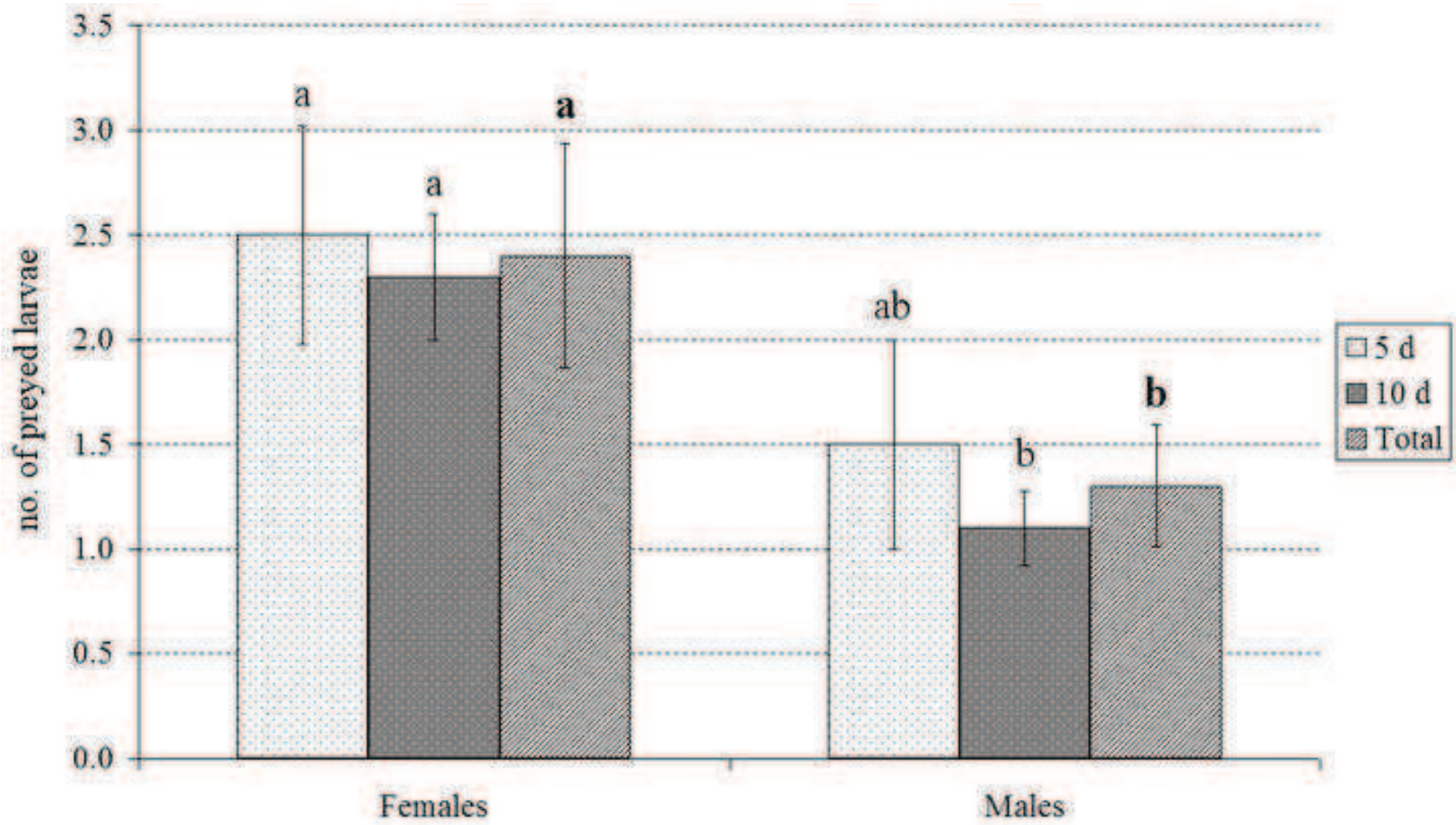


Figure 2
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