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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)
- 3 B.L. Ingegno, C. Ferracini, D. Gallinotti, A. Alma, L. Tavella*
- 4 Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino, Via L. da
- 5 Vinci 44, 10095 Grugliasco (TO), Italy
- 6
- 7 *Corresponding author. E-mail: <u>luciana.tavella@unito.it</u>, phone number +39-011/6708533

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 prev actively on *T. absoluta* eggs and 1st-instar larvae. In particular, compared to males, females showed 17 a significantly higher egg consumption rate $(11.0 \pm 0.7 \text{ vs } 8.6 \pm 0.8 \text{ day}^{-1})$, and were also more 18 effective in preying on 1st-instar larvae $(2.4 \pm 0.5 \text{ vs } 1.3 \pm 0.3 \text{ day}^{-1})$. The mirid was able to develop 19 from egg to adulthood on tomato infested with eggs or with 1st-instar larvae of *T. absoluta*, even if a 20 high mortality of the predator was recorded in the latter case. Moreover, in olfactometer bioassays 21 predator adults proved to be attracted by tomato either infested or previously infested with tomato 22 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.

- 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; Perdikis et
- 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
- 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 Szepligeti (Hymenoptera: Braconidae) (Cabello et

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 prev on eggs and larval stages of *T. absoluta*. In laboratory, the number of preved 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

(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

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,

1999; Aukema, 2005). In Italy its presence has been reported everywhere (Servadei, 1967; Faraci

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

tomato plants were cultivated in an open-sided greenhouse at 27 ± 3 °C, and $55 \pm 23\%$ RH, under a 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**

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

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

123 **2.3. Predation trials on larvae**

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

133To evaluate the effectiveness of *D. errans* as a biocontrol agent, five newly hatched larvae of the134tomato borer (< 1-day old) were placed on a stem in a Petri dish as described above in the</td>135preliminary trial. After 24 hours of starvation inside a glass tube with a healthy tomato leaf and a136water source, five- and 10-day-old females and males were individually placed inside the Petri dish137for 24 hours and the number of preyed larvae was recorded. Ten replicates were performed for each138sex and age of the mirid, and feeding activity was evaluated by counting consumed and healthy139larvae. 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, \emptyset 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.

After testing five adults, the odour sources were switched between the left-hand and right-hand side arms to minimize any spatial effect on choices. The Y-tube and cameras were cleaned with mild soap and alcohol (70%v) and sterilized in an autoclave at 120°C for 20 min. The olfactory bioassays 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**

Both females and males of *D. errans* proved to prey on *T. absoluta* eggs. Predation rates were 183 relatively high, ranging from 6.4 ± 1.5 to 12.4 ± 0.7 eggs day⁻¹, corresponding to 42.7% and 82.7% 184 of total offered eggs, for 15-day-old males and 10-day-old females, respectively (Table 2). Overall, 185 females showed a significantly higher consumption rate: in fact, 11.0 ± 0.7 and 8.6 ± 0.8 eggs day⁻¹, 186 corresponding to $73.6 \pm 4.3\%$ and $57.6 \pm 5.4\%$ of offered eggs, were consumed on average by 187 188 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 (ANOVA: df = 5, 54, F = 3.32, P = 0.011). In the control without predator after 24 hours, the eggs 190 191 were still turgid and then hatched. 192 When different instar larvae were provided simultaneously to *D. errans*, both sexes accepted only 1st-instar larvae as preys, except for a small percentage (4%) of predation on 2nd-instar larvae by 193 females. By contrast, 3^{rd} - and 4^{th} - larvae showed no evidence of predation (Kruskal Wallis: df = 3; 194 $\chi^2 = 35.24$, P < 0.001) (Table 3). When five 1st-instar larvae were offered for 24 hours, females 195 were more effective than males, preying on an average of 2.40 ± 0.54 and 1.30 ± 0.29 larvae, 196 respectively (ANOVA: df = 1, 38, F = 7.79, P = 0.008) (Fig. 1). Also on 1st-instar larvae, older 197 males (10-days-old) were the least effective predators (Kruskal Wallis: df = 3, χ^2 = 8.33, P = 0.040) 198 199 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 difficulties preying on 1st-instar larvae of *T. absoluta*, attested by their high mortality (89.3%) 202

203 (Table 4). However, the high mortality rates of 2^{nd} -, 3^{rd} - and 4^{th} -instar nymphs preying on 1^{st} -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

E. kuehniella eggs (20.38 ± 0.75 days) than with *T. absoluta* eggs (24.12 ± 1.05) (Kruskal Wallis:

207 df = 2, χ^2 = 5.98, P = 0.05) (Table 4). By contrast, no statistically significant differences were found

in body length between diets within sexes, even if the mean size was smaller with *T. absoluta* eggs

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

In olfactory bioassays, D. errans proved to be more attracted by tomato infested by the tomato borer 211 212 compared to uninfested tomato. In particular, significant differences in the responses of adults were found when healthy tomato was compared with a previously infested one ($\chi^2 = 4.83$, P = 0.03), and 213 with tomato infested by 1st- or 4th-instar larvae of *T. absoluta* ($\chi^2 = 4.83$, P = 0.03; $\chi^2 = 6.53$, P =

- 0.01) (Fig. 2). On the contrary, no statistically significant differences were found between 215

uninfested tomato versus T. absoluta adults ($\chi^2 = 1.20$, P = 0.27), or versus tomato with T. absoluta 216

- eggs ($\chi^2 = 3.33$, P = 0.07), and between plants infested by 1st-instar larvae and 4th-instar larvae ($\chi^2 =$ 217
- 218 3.33, P = 0.07) (Fig. 2).
- 219

214

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 continental than N. tenuis, being reported mainly in localities with a mild climate along the 226 227 Mediterranean coast (Kerzhner and Josifov, 1999; Aukema, 2005). Regarding the host range, some plants are known as natural hosts for all three species [tomato, pepper *Capsicum annuum* L., 228 229 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 more than 150 plant species (Voigt, 2005). 231 232 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

flora (Tavella and Goula, 2001; Ingegno et al., 2009). In Piedmont, the predominance of D. errans 235 236 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; Perdikis 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 season when it is present in the surroundings (Ingegno et al., 2009). Moreover, since it shares some 241 242 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 $(73.6 \pm 4.3\% \text{ against } 57.6 \pm 5.4\%)$ 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 period of closely related species lasts between 3 and 6 days (Agustí and Gabarra, 2009), and it is 258 259 known that predatory abilities can also change with age (Abrams, 2000).

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

268 Furthermore, in order to assess the potential of a species as a biological control agent it is important to take into account the time from egg to adulthood of both the predator and its prey. When the 269 270 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 cycle of *T. absoluta* depends greatly on environmental conditions, varying from 23.8 days at 27.1°C 273 274 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 year because there is no clear distinction between the generations (about 12 year⁻¹) that overlap 276 277 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 nymphs reached adulthood in 24.1 \pm 1.1 days, a longer time than on tomato infested with E. 279 280 *kuehniella* eggs (20.4 ± 0.8 days) but still shorter compared to the life span of the pest under the same conditions. This difference could be attributed to the smaller size of *T. absoluta* eggs, 360 µm 281 282 long and 220 µm in diameter (EPPO, 2005), in comparison to E. kuehniella eggs, 500–550 µm long and 290–325 µm wide (Moreno et al., 1994), with a consequently reduced protein intake. 283 284 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,

2000; Perdikis and Lykouressis, 2000, Castañé et al., 2002). In previous studies on M. pygmaeus, 286 90% of nymphs reached adulthood preying on E. kuehniella eggs at 24–25°C in 17–18 days 287 288 (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 (Fauvel et al., 1987; Tedeschi et al., 1999). The high mortality of 2nd-, 3rd-, and 4th-instar nymphs of 292 D. errans feeding on 1st-instar larva of T. absoluta might be explained by the small size of the 293 nymphs compared with the 5th-instar nymph and adult. By contrast, the complete survival of 1st-294 295 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 Puysseleyr et al., 2013). However, *T. absoluta* larvae would seem to be more nutritive 298 for the predator, as the development time was shorter. Recently, both commercialised dicyphine species, *M. pygmaeus* and *N. tenuis*, have been used 299 300 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

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- 514

| 515 | Figure 1. Average number $(\pm SE)$ of 1 st -instar <i>T. absoluta</i> larvae preyed on by <i>D. errans</i> . 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 <i>D. errans</i> in a Y-tube olfactometer to the odours of healthy tomato (α), |
| 521 | adults of <i>T. absoluta</i> (β), tomato infested with eggs (π), 1 st -instar larvae (γ) and 4 th -instar larvae (δ) |
| 522 | of <i>T. absoluta</i> , or tomato previously infested with <i>T. absoluta</i> (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.

| odour source 1 | | odour source 2 |
|--|----|---|
| | | T. absoluta 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 1st-instar larvae | VS | tomato infested with 4 th -instar larvae |

Table 1. Comparison of treatments in olfactometric bioassays.

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 or | ı by |
|---------------|---------------------|-------------|
| | females | males |
| 5 | 9.1±1.4 ab | 10.8±1.3 ab |
| 10 | 12.4±0.7 a | 8.7±1.2 ab |
| 15 | 11.6±1.0 a | 6.4±1.5 b |
| tot | 11.0±0.7 A | 8.6±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 aresignificantly different (Kruskal-Wallis test, P < 0.05).</td>

| Larval instar | % of larvae preyed on b | ру |
|-----------------|-------------------------|-----------|
| | females | males |
| 1 st | 3.4±0.5 a | 2.2±0.6 a |
| 2 nd | 0.2±0.2 b | 0.0±0.0 b |
| 3 rd | 0.0±0.0 b | 0.0±0.0 b |
| 4 th | 0.0±0.0 b | 0.0±0.0 b |

1 **Table 4.** Nymphal development time in days per instar and adult length of *Dicyphus errans* (mean ± 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).

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

| DIET |
|------|
|------|



