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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*

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#### 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 1<sup>st</sup>-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 1<sup>st</sup>-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 1<sup>st</sup>-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<sup>-1</sup>) 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 1<sup>st</sup>-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  $27 \pm 3$  °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  $24 \pm 1$  °C,  $55 \pm 5$  % 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  $0.23 \times 0.23$ ), 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<sup>®</sup>. Then, they were individually placed inside a Petri dish  $(\emptyset \ 9 \ cm)$  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  $25 \pm 1$  °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<sup>-1</sup>. Each stem was put in a 2 ml plastic tube filled with fertilized water and sealed with Parafilm<sup>®</sup>. 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  $25 \pm 1$  °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 1<sup>st</sup>-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 1<sup>st</sup>-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  $25 \pm 1$  °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, 1<sup>st</sup>- and 4<sup>th</sup>-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  $24 \pm 2$  °C,  $50 \pm 10\%$  RH, and  $150 \pm 10$  lux.

#### 171 **2.6. Statistical analyses**

172 After testing for homogeneity of variance (Levene) and normality (Shapiro-Wilk), data on egg and 173 1<sup>st</sup>-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  $6.4 \pm 1.5$  to  $12.4 \pm 0.7$  eggs day<sup>-1</sup>, 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,  $11.0 \pm 0.7$  and  $8.6 \pm 0.8$  eggs day<sup>-1</sup>, 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 1<sup>st</sup>-instar larvae as preys, except for a small percentage (4%) of predation on 2<sup>nd</sup>-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 1<sup>st</sup>-instar larvae were offered for 24 hours, females 195 were more effective than males, preying on an average of  $2.40 \pm 0.54$  and  $1.30 \pm 0.29$  larvae, 196 respectively (ANOVA: df = 1, 38, F = 7.79, P = 0.008) (Fig. 1). Also on 1<sup>st</sup>-instar larvae, older 197 males (10-days-old) were the least effective predators (Kruskal Wallis: df = 3,  $\chi^2$  = 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 1<sup>st</sup>-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 ( $20.38 \pm 0.75$  days) than with *T. absoluta* eggs ( $24.12 \pm 1.05$ ) (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

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 1<sup>st</sup>- or 4<sup>th</sup>-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 1<sup>st</sup>-instar larvae and 4<sup>th</sup>-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 1<sup>st</sup>-instar larvae, which are less mobile and smaller in size; only one female accepted 2<sup>nd</sup>-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 1<sup>st</sup>-instar (Urbaneja et al., 263 264 2009). Overall, males and females of the indigenous predator preyed on a significantly different number of 1<sup>st</sup>-instar larvae ( $2.4 \pm 0.5$  and  $1.3 \pm 0.3$  larvae day<sup>-1</sup>, 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<sup>-1</sup> (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<sup>-1</sup>) 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 ( $20.4 \pm 0.8$  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 2<sup>nd</sup>-, 3<sup>rd</sup>-, and 4<sup>th</sup>-instar nymphs of 292 D. errans feeding on 1<sup>st</sup>-instar larva of T. absoluta might be explained by the small size of the 293 nymphs compared with the 5<sup>th</sup>-instar nymph and adult. By contrast, the complete survival of 1<sup>st</sup>-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 1<sup>st</sup>-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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#### 322 **References**

- Abrams, P.A., 2000. The evolution of predator-prey interactions: theory and evidence. Annu. Rev.
  Ecol. Syst. 31, 79–105.
- 325 Agustí, N., Gabarra, R., 2009. Effect of adult age and insect density of Dicyphus tamaninii Wagner
- 326 (Heteroptera: Miridae) on progeny. J. Pest. Sci. 82, 241–246.
- 327 Alomar, Ò., Albajes, R., 1996. Greenhouse whitefly (Homoptera: Aleyrodidae) predation and
- 328 tomato fruit injury by the zoophytophagous predator *D. tamaninii* (Heteroptera: Miridae), in:
- 329 Alomar, Ò., Wiedenmann, R.N., (Eds.), Zoophytophagous Heteroptera: implications for life history
- and integrated pest management, Thomas Say Publications in Entomology, Lanham, MD, Proc.
- 331 Entomol. Soc. Am., Lanham, Maryland, 155–177.
- Alomar, O., Goula, M., Albajes, R., 2002. Colonisation of tomato fields by predatory mirid bugs
- 333 (Hemiptera: Heteroptera) in northern Spain. Agric., Ecosyst. Environ. 89, 105-115.
- Arnó, J., Alonso, E., Gabarra, R., 2003. Role of the parasitoid *Diglyphus isaea* (Walker) and the
- 335 predator *Macrolophus caliginosus* Wagner in the control of leafminers. IOBC WPRS Bull. 26, 79–
  336 84.
- 337 Arnó, J., Sorribas, R., Prat, M., Matas, M., Pozo, C., Rodriguez, D., Garreta, A., Gómez, A.,
- 338 Gabarra, R., 2009. *Tuta absoluta* a new pest in IPM tomatoes in the northeast of Spain. IOBC
- 339 WPRS Bull. 49, 203–208.
- 340 Arzone, A., Alma, A., Tavella, L., 1990. Ruolo dei Miridi (Rhynchota Heteroptera) nella
- 341 limitazione di Trialeurodes vaporariorum Westw. (Rhynchota Aleyrodidae). Boll. Zool. Agrar.
- 342 Bachic. 22 (1), 43–51.
- 343 Aukema B., 2005. Fauna Europaea: Hemiptera Heteroptera. In: Aukema B. (ed.), Fauna Europaea:
- 344 Hemiptera Heteroptera, Bugs. Fauna Europaea version 2.4,
- 345 http://www.faunaeur.org/full\_results.php?id=452427/403/395.

- 346 Barrientos, Z.R., Apablaza, H.J., Norero, S.A., Estay, P.P., 1998. Temperatura base y constante
- térmica de desarrollo de la polilla del tomate, *Tuta absoluta* (Lepidoptera: Gelechiidae). Cienc.
  Investig. Agrar. 25, 133–137.
- 349 Belda, J.E., Giménez, A., Javier Calvo, F., 2011. Success and extension of biological control
- 350 strategies for managing *Tuta absoluta* (Meyrick) (Lep. Gelechiidae) populations in the
- 351 Mediterranean area. EPPO/IOBC/FAO/NEPPO Abstracts'book of the International Symposium on
- 352 management of *Tuta absoluta* (tomato borer). Agadir, Morocco, November 16-18, 2011, 28.
- Biondi, A., Siscaro, G., Desneux, N., Amiens-Desneux, E., Zappalà, L., 2012. Biology and
- 354 behaviour of the indigenous parasitoid *Bracon nigricans* on the invasive South American tomato
- 355 pinworm *Tuta absoluta*. IOBC/WPRS Bull. 80, 131.
- Boualem, M., Hamadi, R., Allaoui, H.A., 2011. Study of parasitic complex of *Tuta absoluta* in
- 357 Mostaganem area (Algeria). EPPO/IOBC/NEPPO Joint International Symposium on Management
- 358 of *Tuta absoluta* (Tomato Borer), Agadir, Morocco, November 16-18, 2011, 58.
- 359 Cabello, T., Gallego, J.R., Vila, E., Soler, A., del Pino, M., Carnero, A., Hernández-Suárez, E.,
- 360 Polaszek, A., 2009a. Biological control of the South American tomato pinworm, *Tuta absoluta*
- 361 (Lep.: Gelechiidae), with releases of *Trichogramma achaeae* (Hym.: Trichogrammatidae) on
- tomato greenhouses of Spain. IOBC/WPRS Bull. 49, 225–230.
- 363 Cabello, T., Gallego, J.R., Fernandez-Maldonado, F.J., Soler, A., Beltran, D., Parra, A., Vila, E.,
- 364 2009b. The damsel bug *Nabis pseudoferus* as a new biological control agent of the South American
- 365 Tomato Pinkworm, *Tuta absoluta*, in tomato crops of Spain. IOBC/WPRS Bull. 49, 219–223.
- Calabrò, M., Nucifora, A., 1993. Presenza di Miridi zoofitofagi (Rhynchota, Heteroptera) su
  pomodoro e altre piante e loro utilizzabilità come ausiliari. Boll. Accad. Gioenia Sci. Nat. 26 (342),
  115–131.

- 369 Cano, M., Vila, E., Janssen, D., Bretones, G., Salvador, E., Lara, L., Tellez, M.M., 2009. Selection
- 370 of refuges for Nesidiocoris tenuis (Het.: Miridae) and Orius laevigatus (Het.: Anthocoridae): virus
- 371 reservoir risk assessment. IOBC/WPRS Bull. 49, 281–286.
- 372 Carvalho, P., Mexia, A., 2000. First approach on the potential role of *Dicyphus cerastii* Wagner
- 373 (Hemiptera: Miridae), as natural control agent in Portuguese greenhouses. IOBC/WPRS Bull. 23
- 374 (1), 261–264.
- 375 Cassis, G., 1984. A systematic study of the subfamily Dicyphinae (Heteroptera: Miridae). Oregon
  376 State University Thesis.
- 377 Castañé, C., Iriarte, J., Lucas, E., 2002. Comparison of prey consumption by *Dicyphus tamaninii*
- 378 reared conventionally, and on a meatbased diet. BioControl 47, 657–666.
- 379 Castañé, C., Alomar, Ò., Goula, M., Gabarra, R., 2004. Colonization of tomato greenhouses by the
- 380 predatory mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii*. Biol. Control 30, 591–597.
- 381 Castañé, C., Zapata R., 2005. Rearing the predatory bug Macrolophus caliginosus on a meat-based
- 382 diet. Biol. Control 34 (1), 66–72.
- 383 De Puysseleyr, V., De Man, S., Höfte, M., De Clercq, P., 2013. Plantless rearing of the
- 384 zoophytophagous bug *Nesidiocoris tenuis*. Biocontrol 58, 205-213.
- 385 Desneux, N., Wajinberg, E., Wyckhuys, K.A.G., Burgio, G., Arpaia, S., Nárvaez-Vasquez, C.A.,
- 386 González-Cabrera, J., Catálan Ruescas, D., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello,
- 387 T., Urbaneja, A., 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology,
- 388 geographic expansion and prospects for biological control. J. Pest. Sci. 83, 197–215.
- EPPO, 2005. Data sheets on quarantine pests *Tuta absoluta*. EPPO Bull. 35, 434–435.
- 390 Faraci, F., Rizzotti Vlach, M., 2003. Heteroptera. In: Checklist of the species of the Italian fauna.
- 391 Stoch, F. (ed.). On-line version 2.0. http://checklist.faunaitalia.it.
- 392 Fauvel, G., Malausa, J.C., Kaspar, B., 1987. Etude en laboratoire des principales caracteristiques
- 393 biologiques de *Macrolophus caliginosus* (Heteroptera: Miridae). Entomophaga 32, 529–543.

- 394 Ferracini, C., Ingegno, B.L., Navone, P., Ferrari, E., Mosti, M., Tavella, L., Alma, A., 2012a.
- 395 Adaptation of Indigenous Larval Parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. J.
- 396 Econ. Entomol. 105 (4), 1311–1319.
- 397 Ferracini, C., Ingegno, B.L., Mosti, M., Navone, P., Tavella, L., Alma, A., 2012b. Promising native
- 398 candidates for biological control of *Tuta absoluta* (Meyrick) in Italy. IOBC/WPRS Bull. 80, 51–55.
- 399 Fois, F., Porcu, M., Sau, S., Carrusci, P., Deiana, M., Nannini, M., 2011. Valutazioni preliminari
- 400 del predatore Macrolophus pygmaeus (Hemiptera, Miridae) nel contenimento di Tuta absoluta
- 401 (Lepidoptera, Gelechiidae). Atti XXIII Congr. Naz. Entomol. Genova, 337.
- 402 Gillespie, D.R., McGregor, R.R., 2000. The functions of plant feeding in the omnivorous predator
- 403 *Dicyphus hesperus*: water places limits on predation. Ecol. Entomol. 25, 380–386.
- 404 Grenier, S., Guillaud, J., Delobel, B., Bonnot, G., 1989. Nutrition et élevage du prédateur polyphage
- 405 Macrolophus caliginosus (Heteroptera: Miridae) sur milieux artificiels. Entomophaga 34, 77-86
- 406 Guenaoui, Y., Bensaad, R., Ouezzani, K., 2011. Importance of native polyphagous predators able to
- 407 prey on *Tuta absoluta* Meyrich (Lepidoptera: Gelechiidae) on tomato crop.
- 408 EPPO/IOBC/FAO/NEPPO Abstracts'book of the International Symposium on management of *Tuta*
- 409 *absoluta* (tomato borer). Agadir, Morocco, November 16-18, 2011, 30.
- 410 Hamdi, F., Chadoeuf, J., Chermiti, B., Bonato, O., 2013. Evidence of cannibalism in *Macrolophus*
- 411 *pygmaeus*, a natural enemy of whiteflies. J. Insect Behav., DOI 10.1007/s10905-013-9379-3.
- 412 Harpaz, L.S., Graph, S., Rika, K., Azolay, L., Rozenberg, T., Yakov, N., Alon, T., Alush, A.,
- 413 Stinberg, S., Gerling, D., 2011. IPM of *Tuta absoluta* in Israel. EPPO/IOBC/FAO/NEPPO
- 414 Abstracts'book of the International Symposium on management of *Tuta absoluta* (tomato borer).
- 415 Agadir, Morocco, November 16-18, 2011, 32.
- 416 Ingegno, B.L., Goula, M., Navone, P., Tavella, L., 2008. Distribution and host plants of the genus
- 417 *Dicyphus* in the Alpine valleys of NW Italy. Bull. Insectol. 61 (1), 139–140.
- 418 Ingegno, B.L., Pansa, M.G., Tavella, L., 2009. Tomato colonization by predatory bugs
- 419 (Heteroptera: Miridae) in agro-ecosystems of NW Italy. IOBC/WPRS Bull. 49, 287–291.

- 420 Ingegno, B.L., Pansa, M.G., Tavella, L., 2011. Plant preference in the zoophytophagous generalist
- 421 predator *Macrolophus pygmaeus* (Heteroptera: Miridae). Biol. Control 58 (3), 174–181.
- 422 Jacobson, R., 2011. Tuta absoluta: A season- long IPM strategy based on predatory bugs.
- 423 EPPO/IOBC/FAO/NEPPO Abstracts'book of the International Symposium on management of *Tuta*
- 424 *absoluta* (tomato borer). Agadir, Morocco, November 16-18, 2011, 33.
- 425 Kerzhner, I.M., Josifov, M., 1999. Cimicomorpha II: Miridae. Vol 3 in Aukema B., Rieger C.:
- 426 Catalogue of the Heteroptera of the Palaearctic Region. Wageningen, The Netherlands, 577.
- 427 Kindlmann, P., Dixon, A.F.G., 1999. Generation time ratios-determinants of prey abundance in
- 428 insect- predator-prey interactions. Biol. Control 16, 1333–1138.
- 429 Kindlmann, P., Dixon, A.F.G., 2001. When and why top-down regulation fails in arthropod
- 430 predator-prey systems. Basic Appl. Ecol. 2, 333–340.
- 431 Kindlmann, P., Dixon, A.F.G., 2002. Insect predator-prey dynamics and the biological
- 432 control of aphids by ladybirds. Proc. 1<sup>st</sup> Int. Symp. Biol. Control Arthropods, Honolulu, Hawaii,
- 433 USA, January 14-18, 2002, 118–124.
- 434 Lietti, M.M.M., Botto, E., Alzogaray, R.A., 2005. Insecticide resistance in Argentine populations of
- 435 *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotrop. Entomol. 34, 113–119.
- 436 Loni, A., Rossi, E., van Achterberg, K., 2011. First report of *Agathis fuscipennis* in Europe as
- 437 parasitoid of the tomato leafminer *Tuta absoluta*. Bull. Insectol. 64 (1), 115–117.
- 438 Lykouressis, D., Perdikis, D., Tsagarakis, A., 2000. Polyphagous mirids in Greece: host plants and
- 439 abundance in traps placed in some crops. Boll. Lab. Entomol. Agrar. Filippo Silvestri 56, 57–68.
- 440 Lykouressis, D., Giatropoulos, A., Perdikis, D.C., Favas, C., 2008. Assessing the suitability of non-
- 441 cultivated plants and associated insect prey as food sources for the omnivorous predator
- 442 *Macrolophus pygmaeus* (Hemiptera: Miridae). Biol. Control 44, 142–148.
- 443 Malausa, J.C., 1989. Lutte intégrée sous serre: les punaises prédatrices Mirides dans les cultures de
- 444 Solanacées du sud-est de la France. Rev. Hortic. 298, 39–43.

- Malausa, J.C., Trottin-Caudal, Y., 1996. Advances in the strategy of use of the predaceous bug *Macrolophus caliginosus* (Heteroptera: Miridae) in glasshouse crops in: Alomar, Ò., Wiedenmann,
  R.N., (Eds.), Zoophytophagous Heteroptera: implications for life history and integrated pest
  management, Thomas Say Publications in Entomology, Lanham, MD, Proc. Entomol. Soc. Am.,
  Lanham, Maryland, 178–189.
- McGregor, R.R., Gillespie D.R., Park C.G., Quiring D.M.J., Foisy, M.R.J., 1999. Potential use of *Dicyphus hesperus* Knight (Heteroptera: Miridae) for biological control of pests of greenhouse
  tomatoes. Biol. Control 16, 104–110.
- 453 Mollá, Ó., Montón, H., Vanaclocha, P.; Beitia, F.; Urbaneja, A., 2009. Predation by the mirids
- 454 *Nesidiocoris tenuis* and *Macrolophus pygmaeus* on the tomato borer *Tuta absoluta*. IOBC/WPRS
  455 Bull. 49, 209–214.
- Mollá, Ó., González-Cabrera, J., Urbaneja, A., 2011. The combined use of *Bacillus thuringiensis*and *Nesidiocors tenuis* against the tomato borer *Tuta absoluta*. Biocontrol 56: 883-891.
- 458 Moreno, J., Barry, P., Jimenez, R., 1994. Morphological changes on the egg surface of *Ephestia*
- 459 *kuehniella* Zeller (Lepidoptera: Pyralidae) after parasitization by *Phanerotoma (Phanerotoma)*
- 460 *ocularis* Kohl (Hymenoptera: Braconidae). Appl. Entomol. Zool. 29 (2), 282–284.
- 461 Perdikis D.C., Lykouressis D.P., 2000. Effects of various items, host plants, and temperatures on
- 462 the development and survival of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). Biol.
- 463 Control 17, 55–60.
- 464 Perdikis, D.C., Kapaxidi, E., Papadoulis, G., 2008. Biological control of insect and mite pests in
  465 greenhouse solanaceous crops. Eur. J. Plant Sci. Biotechnol. 2 (1), 125–144.
- 466 Pereyra, P.C. and Sánchez, N.E., 2006: Effect of two solanaceous plants on developmental and
- 467 population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera:
- 468 Gelechiidae). Neotrop. Entomol. 35, 671–676.
- 469 Pignatti, S., 1982. Flora d'Italia. Vol. secondo, Bologna, Edagricole, 514.

- 470 Roditakis, E., Skarmoutsou, C., Staurakaki, M., 2013. Toxicity of insecticides to populations of
  471 tomato borer Tuta absoluta (Meyrick) from Greece. Pest Manag. Sci. 69, 834–840.
- 472 Sanchez, J.A., Gillespie, D.R., McGregor, R.R., 2003. The effects of mullein plants (Verbascum
- *thapsus*) on the population dynamics of *Dicyphus hesperus* (Heteroptera: Miridae) in tomato
  greenhouses. Biol. Control 28, 313–319.
- 475 Sanchez, J.A., Gillespie, D.R., McGregor, R.R., 2004. Plant preference in relation to life history
- 476 traits in the zoophytophagous predator *Dicyphus hesperus*. Entomol. Exp. Appl. 112, 7–19.
- 477 Seguret, J., Maignet, P., Ridray, G., 2011. Efficacy of the combination of two beneficials to control
- 478 Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) in tomato glasshouses in southern
- 479 France. EPPO/IOBC/FAO/NEPPO Abstracts'book of the International Symposium on management
- 480 of *Tuta absoluta* (tomato borer). Agadir, Morocco, November 16-18, 2011, 47.
- 481 Servadei, A., 1967. Rhynchota (Heteroptera, Homoptera Auchenorrhyncha). Catalogo topografico e
  482 sinonimico, in: Fauna d'Italia, 9. Calderini, Bologna, 1–851.
- 483 Shipp, J.L., Whang, K., 2006. Evaluation of *Dicyphus hesperus* (Heteroptera: Miridae) for
- 484 biological control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse tomato. J.
- 485 Econ. Entomol. 99 (2), 414–420.
- 486 Siqueira, H.A.A., Guedes, R.N.C., Picanco, M.C., 2000. Insecticide resistance in populations of
- 487 *Tuta absoluta* (Lepidoptera: Gelechiidae). Agric. For. Entomol. 2, 147–153.
- 488 Tavella, L., Alma, A., Sargiotto, C., 1997. Samplings of Miridae Dicyphinae in tomato crops of
- 489 Northwestern Italy. IOBC/WPRS Bull. 20, 249–256.
- 490 Tavella, L., Goula, M., 2001. Dicyphini collected in horticultural areas of north-western Italy
  491 (Heteroptera Miridae). Boll. Zool. Agrar. Bachic. 33 (1), 93–102.
- 492 Tedeschi, R., Clercq, P., de Veire, M. van de Tirry, L., 1999. Development and predation of
- 493 Macrolophus caliginosus (Heteroptera: Miridae) on different prey. Proc. 51<sup>st</sup> Int. symp. on crop
- 494 prot., Gent, Belgium, 4 May 1999. Part I, 64 (3a): 235–240.

- 495 Urbaneja, A., Montón, H., Mollá, O., 2009. Suitability of the tomato borer *Tuta absoluta* as prey for
  496 *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J. Appl. Entomol. 133, 292–296.
- 497 Urbaneja, A., González-Cabrera, J., Arnó, J., Gabarra, R., 2012. Prospects for the biological control
- 498 of *Tuta absoluta* in tomatoes of the Mediterranean Basin. Pest. Manag. Sci. 68, 1215–1222.
- 499 Vandekerkhove, B., Van Baal, E., Bolckmans, K., De Clercq, P., 2006. Effect of diet and mating
- 500 status on ovarian development and oviposition in the polyphagous predator *Macrolophus*
- 501 *caliginosus* (Heteroptera: Miridae). Biol. Control 39, 532–538.
- 502 Vercher, R., Calabuig, A., Felipe, C., 2010. Ecologia, muestreos y umbrales de *Tuta absoluta*
- 503 (Meyrick). Phytoma Esp. 217, 23–26.
- 504 Viggiani, G., Filella, F., Delrio, G., Ramassini, W., Foxi, C., 2009. Tuta absoluta, nuovo lepidottero
- 505 segnalato anche in Italia. Inf. Agr. 2, 66–68.
- 506 Voigt, D., 2005. Untersuchungen zur Morphologie, Biologie und Ökologie der räuberischen
- 507 Weichwanze *Dicyphus errans* Wolff (Heteroptera, Miridae, Bryocorinae). Dissertation, TU
- 508 Dresden, Germany.
- Voigt, D., Gorb, E., Gorb, S., 2007. Plant surface–bug interactions: *Dicyphus errans* stalking along
  trichomes. Arthropod-Plant Interact. 1 (4), 221–243.
- 511 Zappalà, L., Bernardo, U., Biondi, A., Cocco, A., Deliperi, S., Delrio, G., Giorgini, M., Pedata, P.,
- 512 Rapisarda, C., Tropea Garzia, G., Siscaro, G., 2012. Recruitment of native parasitoids by the exotic
- 513 pest *Tuta absoluta* in Southern Italy. Bull. Insectol. 65 (1), 51–61.
- 514

515	<b>Figure 1.</b> Average number $(\pm SE)$ of 1 <sup>st</sup> -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	<b>Figure 2.</b> Responses of <i>D. errans</i> in a Y-tube olfactometer to the odours of healthy tomato ( $\alpha$ ),
521	adults of <i>T. absoluta</i> ( $\beta$ ), tomato infested with eggs ( $\pi$ ), 1 <sup>st</sup> -instar larvae ( $\gamma$ ) and 4 <sup>th</sup> -instar larvae ( $\delta$ )
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. $\chi^2$ 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 <sup>st</sup> -instar larvae
		tomato infested with 4 <sup>th</sup> -instar larvae
		tomato previously infested with larvae
tomato infested with 1 <sup>st</sup> -instar larvae	vs	tomato infested with 4 <sup>th</sup> -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 on 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 by					
	females	males				
1 <sup>st</sup>	3.4±0.5 a	2.2±0.6 a				
2 <sup>nd</sup>	0.2±0.2 b	0.0±0.0 b				
3 <sup>rd</sup>	0.0±0.0 b	0.0±0.0 b				
4 <sup>th</sup>	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* 1<sup>st</sup> instar larvae). Means followed by different letters are significantly different (Kruskal-Wallis test P <

3 0.05).

	E. kuehniella eggs			T. absoluta eggs			<i>T. absoluta</i> 1 <sup>st</sup> instar larvae		
instar	N	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 <sub>a</sub>	0.0%	56	3.64±0.22 a	0.0%
II-III	31	3.42±0.26 <sub>b</sub>	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 <sub>ab</sub>	0.0%	29	7.72±0.81 a	3.3%	17	4.47±0.45 <sub>b</sub>	54.1%
IV-V	29	3.76±0.23 a	6.5%	26	4.15±0.44 <sub>a</sub>	10.3%	6	4.17±1.19 <sub>a</sub>	64.7%
V-A	29	3.24±0.25 a	0.0%	25	3.52±0.34 <sub>a</sub>	3.8%	6	2.67±0.21 a	0.0%
tot	29	20.38±0.75 <sub>b</sub>	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 <sub>a</sub>	

DIET
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