Adaptation of indigenous larval parasitoids to Tuta absoluta (Lepidoptera: Gelechiidae) in Italy

Adaptation of Indigenous Larval Parasitoids to *Tuta absoluta* in Italy

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Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a serious threat to tomato crops in South America. In Europe, after its first detection in Spain in 2006, it rapidly spread through the Mediterranean basin, reaching Italy two years later. The aim of our work was to find indigenous effective biological control agents and to evaluate their potential role in the control of larval populations of *T. absoluta* (tomato borer) in controlled conditions. Nine species of larval parasitoids emerged from field-collected tomato leaves infested by *T. absoluta*. The most abundant, *Necremnus* near *artynes* (Walker) and *N.* near *tidius* (Walker) (Hymenoptera: Eulophidae), were tested in laboratory parasitism trials. Furthermore, since the species *N. artynes* and *N. tidius* are each reported in literature as an ectoparasitoid of *Cosmopterix pulchrimella* Chambers (Lepidoptera: Cosmopterigidae) on upright pellitory plants, olfactometer bioassays were performed to assess the response of our parasitoids to the odors of tomato and pellitory leaves infested by *T. absoluta* and *C. pulchrimella*, respectively, compared with healthy ones. Both *Necremnus* species showed good adaptation to the invasive pest, and we observed a high larval mortality of *T. absoluta* due to host feeding and parasitism. Even olfactory responses highlighted a preference of both wasps for tomato plants infested by the exotic pest. These preliminary results demonstrated a high suitability of these indigenous natural enemies for controlling the tomato borer. Further investigations are therefore needed to confirm their role as potential biological agents in commercial tomato plantations.

Keywords

biological control, tomato borer, native natural enemy, exotic invasive pest, *Necremnus* spp.

39 Native to Central America, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (tomato
40 borer) has been a pest of tomato crops in South American countries since 1970, and is distributed in
41 Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay and Venezuela
42 (EPPO 2005). In 2006, it was accidentally introduced into Spain, and in the past few years has
43 spread rapidly through many countries bordering the Mediterranean Sea, including Italy where it
44 was first reported in 2008 (Urbaneja et al. 2009, Viggiani et al. 2009, CABI 2011). The species was
45 added to the European and Mediterranean Plant Protection Organization (EPPO) A1 and A2 action
46 lists for regulation as quarantine pests in 2004 and 2009, respectively, and is now considered one of
47 the major pests on tomato crops. In fact, while its main host is tomato, infestation of potato,
48 eggplant, pepper, tobacco, and common bean is also reported. Moreover, various solanaceous
49 species such as *Solanum nigrum* L., *S. elaeagnifolium* Cav., *S. puberulum* Nutt, *Datura stramonium*
50 L., *D. ferox* L. and *Nicotiana glauca* Graham are reported as wild hosts (EPPO 2005, EPPO 2009,
51 CABI 2011).
52 *Tuta absoluta* is a multivoltine species; females lay eggs on epigeal parts of their host plants
53 and a single female can lay up to 260 eggs during its lifetime (EPPO 2005). Through their feeding
54 activity within the mesophyll of the leaves, the larvae produce large mines, thus affecting the
55 plant's photosynthetic capacity; furthermore, they burrow into stalks, apical buds, and fruits. The
56 pest affects tomatoes destined for the fresh market as well as for processing, with larvae causing
57 losses in its area of origin of up to 80–100% (Desneux et al. 2010). Pupation may take place in the
58 soil, on the leaf surface or within the mines, depending on environmental conditions (EPPO 2005).
59 The tomato borer is a very challenging pest to control. Chemical approaches are difficult
60 because of the mine-feeding behavior of larvae, in addition to the resistance developed to many
61 conventional insecticides and the side effects for useful organisms in integrated pest management
62 (IPM) programs (Siqueira et al. 2000, Lietti et al. 2005, Cabello et al. 2009, IRAC 2011). So, as an
63 alternative approach, biological control has been actively pursued. Several natural enemies
64 occurring in its native area have been reported as fully documented by Desneux et al. (2010), and

65 the efficacy of entomopathogenic fungi, bacteria and nematodes have also been evaluated for the
66 implementation of biological control strategies (Batalla-Carrera et al. 2010, González-Cabrera et al.
67 2010, Pires et al. 2010, Hernández-Fernández et al. 2011). Natural enemies have been investigated
68 in many South American countries with the aim of using them in biological control programs; in
69 particular, research has been carried out on egg parasitoids such as *Trichogramma pretiosum*
70 (Riley), *T. nerudai* (Pintureau & Gerding), *T. exiguum* Pinto & Platner and *Trichogrammatoidea*
71 *bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) (Pratissoli and Parra 2000, Zucchi and
72 Querino 2000, Faria et al. 2008, Desneux et al. 2010, Virgala and Botto 2010), and larval
73 parasitoids such as *Apanteles gelechiidivoris* Marsh, *Pseudapanteles dignus* (Muesebeck), *Bracon*
74 spp. (Hymenoptera: Braconidae), *Dineulophus phthorimaeae* (de Santis) (Hymenoptera:
75 Eulophidae), and *Diadegma* spp. (Hymenoptera: Ichneumonidae) (Colomo et al. 2002, Marchiori et
76 al. 2004, Miranda et al. 2005, Bajonero et al. 2008, Sánchez et al. 2009, Luna et al. 2010).
77 However, none of these beneficial organisms seem to have so far been decisive in controlling *T.*
78 *absoluta* and the research is still ongoing.
79 Indigenous predators and parasitoids with the ability to control this exotic leafminer have also
80 been investigated throughout the Mediterranean area. Larval parasitoids such as *Necremnus* spp.
81 and *Stenomesius* spp. (Hymenoptera: Eulophidae) (Arnó and Gabarra 2010, Gabarra and Arnó
82 2010, Rizzo et al. 2011, Zappalà et al. 2011a), and predators such as *Macrolophus pygmaeus*
83 (Rambur), *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), *Nabis pseudoferus* (Remane)
84 (Hemiptera: Nabidae) (Urbaneja et al. 2009, Mollá et al. 2010, Fois et al. 2011, Zappalà et al.
85 2011b), have been reported in Spain and more recently in Italy. Additionally, the shift of other
86 indigenous entomophagous species, especially braconids and ichneumonids, onto the exotic pest
87 has been documented by recent literature (Loni et al. 2011, Rizzo et al. 2011, Zappalà et al. 2011a),
88 revealing a gradual adaptation of these generalist parasitoids to the new host. The egg parasitoid
89 *Trichogramma achaeae* Nagaraja & Nagarkatti, which is however reported in the list of the
90 hymenopteran species alien to Europe (Rasplus et al. 2010), was also tested in inundative biological

control programs, and has become commercially available following its promising potential (Cabello et al. 2009, Desneux et al. 2010).

Due to the huge potential of *T. absoluta* to expand its distribution range, a multidisciplinary approach exploiting natural enemies in several countries is gaining favor. With the aim of searching for indigenous parasitoids able to adapt to the new host, and which are suitable for mass-rearing and augmentation biological control programs, field surveys were firstly carried out in horticultural areas of Italy. The species that most frequently emerged from infested tomato, and that proved to be fit for mass-rearing, were therefore tested in the laboratory to evaluate their effectiveness in controlling *T. absoluta*; to contribute further to the implementation of effective and environmentally friendly control strategies, their ability to recognize and choose new host larvae was also assessed.

Materials and Methods

Field Collection and Rearing of Parasitoids. In the two-year period 2009–2010, surveys were carried out in 24 IPM-protected tomato fields located in four Italian horticultural areas: Liguria (four fields), Campania (six fields), Sardinia (five fields), and Sicily (nine fields), where *T. absoluta* was established on tomato plants. In the surveyed sites, samples of 100 infested leaves were randomly collected from tomato crop once or twice in the mid-growing season, placed in sealed plastic bags, and transferred to the laboratory. All the leaves were carefully checked using a microscope for the presence of tomato borer larvae and pupae, in order to exclude any other insect infestation. The leaves were then placed in cages in climatic chambers at 24 ± 1°C, 60 ± 5% RH, 16:8 h (L:D) photoperiod to detect the emergence of possible larval parasitoids. Each cage consisted of a cardboard box (40 × 60 × 30 cm) with a tight-fitting lid and two clear jars extending from two holes in its side. When the adults emerged, they were attracted to the light entering through the jars where they then accumulated and were easily collected with the aid of an insect aspirator. The emerged adult parasitoids were killed with ethyl acetate and stored in 70% ethanol in glass test tubes (60 mm high × 8 mm diameter) until identification. The parasitoids belonging to the

117 Eulophidae family were then identified at the DIVAPRA (Dipartimento di Valorizzazione e
118 Protezione delle Risorse Agroforestali, University of Torino, Italy) (identifications by co-author P.
119 N.), while all other parasitoid species were sent to the respective specialists. Voucher specimens
120 were deposited at the DIVAPRA.

121 The most abundant species of the emerged parasitoids were selected on the basis of their
122 suitability for mass-rearing, as tested at the Bioplanet laboratories (Bioplanet s.c.a., Cesena, Italy).
123 Therefore, two species of the genus *Necremnus* that had proved to be fit for mass-rearing on *T.*
124 *absoluta* on tomato plants at the Bioplanet laboratories, were tested at the DIVAPRA laboratories to
125 assess their effectiveness in controlling the pest, and to study their behavior and host preference
126 under laboratory conditions. Before using them in the experiments, the parasitoid adults from
127 Bioplanet were sexed, fed every 48 h with drops of honey on cardboard, and kept in individual glass
128 tubes (120 mm high × 18 mm diameter), in a climatic chamber at 24 ± 1°C, 60 ± 5% RH, 16:8 h
129 (L:D) photoperiod.

130 **Insect Colonies and Host Plant Management.** Colonies of *T. absoluta* were established
131 starting from an initial culture collected in a tomato commercial plantation in Liguria (NW Italy). A
132 continuous mass-rearing of all development stages was maintained on tomato plants in an open-
133 sided greenhouse, in cages (150 × 150 × 110 cm) that had a stainless steel frame structure
134 supporting an insect-proof net (mesh 0.23 × 0.23). Plants of *Lycopersicon esculentum* Mill.
135 Montecarlo F1 variety were used for both mass-rearing and laboratory trials. In particular, plants
136 used in the parasitism trials were approximately 25 cm high from the soil surface, in pots filled with
137 a mixture of soil and covered with a layer of white sand in order to easily detect possible individuals
138 dropped off during trials.

139 Moreover, for olfactometer bioassays, colonies of *Cosmopterix pulchrimella* Chambers
140 (Lepidoptera: Cosmopterigidae), reported as a natural host of the related *Necremnus* species
141 (Bernardo and Viggiani 2002, The Natural History Museum 2011), were also established, starting
142 from an initial culture collected on upright pellitory [*Parietaria officinalis* L. (Urticaceae)] in

143 Piedmont (NW Italy), and maintained in the cages described above. Upright pellitory plants were
144 collected in wastelands and cuttings were taken in order to obtain new plants for *C. pulchrimella*
145 rearing.

146 All the plants were cultivated in plastic pots (diameter 20 cm), watered daily and fertilized,
147 and kept in an open-sided greenhouse at 27 ± 3°C, 55 ± 23% RH, 16:8 h (L:D) photoperiod.

148 **Parasitism Trials.** Before testing the selected parasitoids for their effectiveness in controlling
149 tomato borer, laboratory trials were performed to assess if the parasitoid females showed any
150 preference for different instar larvae. Potted tomato plants were infested with 20 larvae of *T.*
151 *absoluta* of different instars, and encaged in Plexiglas cylinders (40 cm high × 18 cm diameter).
152 Newly molted larvae (i.e., five for each of the four instars) were chosen and randomly distributed
153 over the plant. Single five-day-old females ready to oviposit, after being mated and fed with honey,
154 were introduced and maintained on the infested tomato plants for 48 h. Following this, the females
155 were removed and the larvae were observed under a stereomicroscope to determine if they were
156 dead or alive. Five replicates were performed for each parasitoid species.

157 To evaluate the effectiveness of the selected parasitoids as biocontrol agents, tomato leaves
158 with *T. absoluta* larvae no older than 24 h were collected from mass-rearing cages, and the portion
159 of the leaf which contained the larva inside the mine was cut off after checking that the mine was
160 not empty using the stereomicroscope. This leaf portion was then fixed onto a leaf of healthy potted
161 tomato plant with the aid of a drop of silicone. On each tomato plant consisting of four leaves, 20
162 leaf sections were fixed homogeneously, so as to obtain a density of five larvae per leaf to ensure a
163 consistent infestation while preventing plant collapse. The potted plant was placed separately inside
164 a Plexiglas cage (20 × 20 × 30 cm) with two sides and a lid of fine gauze (30/10 net) and a mesh
165 sleeve inserted in the middle of a side (diameter 11 cm) to allow access to the plant.

166 Larvae were left for 48 h to allow them to transfer from the leaf section to the plants and
167 establish new galleries. One female parasitoid was then released into each cage and removed five
168 days later. Five-day-old females ready to oviposit were used, after being mated and fed with honey

169 inside the rearing glass tubes. Ten replicates were performed for each parasitoid species and 10
170 plants were also set up as control.
171 Cages were checked for parasitoid and moth emergence every day, and all the emerged
172 individuals were aspirated off the cage through the sleeve and counted; the parasitoids were then
173 sexed and sex ratio was provided. Collection continued for two weeks after initial emergence. After
174 30 days, each experimental cage was dismantled and all the leaves observed under a
175 stereomicroscope.
176 All the trials were performed in a climatic chamber at 24 ± 1°C, 60 ± 5% RH, 16:8 h (L:D)
177 photoperiod.
178 Moreover, single females of each parasitoid species were offered tomato leaves with five
179 first-instar larvae, placed on a wet filter paper inside a Petri dish (diameter 10 cm), in order to
180 obtain preliminary data on their behavior in relation to parasitism and host feeding. So, females,
181 mated, fed with honey, and not experienced with a host were used for the experiments. Their
182 behavioral sequence (e.g., host location and acceptance, oviposition, host-feeding) on larvae of *T.*
183 *absoluta* was observed using a steromicroscope for 30 min, and 10 replications were carried out for
184 each parasitoid species.
185 **Olfactometer Bioassays.** In the olfactometer bioassays, five day-old females of the selected
186 parasitoid species were used to assess their olfactory responses to the odors of tomato plants
187 uninfested and infested by *T. absoluta*, and to the odors of *P. officinalis* uninfested and infested by
188 *C. pulchrimella* as an alternative host. In particular, three comparisons were carried out for each
189 parasitoid species: a) upright pellitory leaves infested by *C. pulchrimella* compared to tomato leaves
190 infested by *T. absoluta*; b) healthy upright pellitory leaves compared to healthy tomato leaves; and
191 c) healthy tomato leaves compared to tomato leaves infested by *T. absoluta*. Each odor source
192 consisted of five tomato leaflets or pellitory leaves, uninfested or infested with a density of three
193 larvae per leaf, for a total of 15 larvae. Before trials, insects were kept at 15°C without any host or
194 plant in a glass tube for 18 h with a humid cotton cap and microdrops of honey. The bioassays were

195 carried out in a Y-shaped Pyrex tube (internal diameter 1.2 cm) formed by an entry arm and two
196 side arms, each 10 cm long (70° angle), and positioned horizontally. Each side arm was connected
197 to a round modified beaker (250 mL volume capacity, diameter 9 cm) as an odor-source container.
198 Airflow was provided by an air pump (Air 275R, Sera, Germany), then filtered in an activated CO_2
199 filter, regulated with a flow meter at 2.5 L min^{-1} (EK-2NRK, Comer, Italy) and humidified in a 1-L
200 water bubbler half-filled with deionized water. After the flow was established, a single parasitoid
201 female was introduced into the entry arm. Each female was observed until she had moved at least 2
202 cm up one of the side arms or until 10 min had elapsed. Females that did not choose a side arm
203 within 10 min were considered as "no choice" and were not counted in the subsequent data analysis.
204 For each test, a female was evaluated only once to prevent any behavior conditioned by experience.
205 The odor sources chosen by females that responded were recorded. Thirty responses were recorded
206 for each pair of odor sources. After testing five females, odor sources were switched between the
207 left-hand and right-hand side arms to minimize any spatial effect on choices. The Y-tube and
208 cameras were cleaned with mild soap and alcohol (70%$_v$) and sterilized in an autoclave at 120°C for
209 20 min. The olfactory bioassays were conducted at 24 ± 2°C, 50 ± 10% RH and 150 ± 10 lux.
210 **Statistical Analyses.** Significance of mean values of the data was analyzed by one-way
211 analysis of variance (ANOVA), after ascertaining the homogeneity of variance (Levene's test). In
212 the olfactory bioassays, responses of parasitoid females were analyzed by a Chi-square test. The
213 null hypothesis was that parasitoid females had a 50:50 distribution across the two odor sources. All
214 analyses were performed using the software SPSS version 17.0 (SPSS, Chicago, IL, USA).
215
216 **Results**
217 Nine species of indigenous parasitoids emerged from tomato leaves infested by *T. absoluta*
218 collected in Arma di Taggia (43°50'54" N - 07°50'27" E), Ceriale (44°06'0" N - 08°13'60" E), Pula
219 (39°00'36" N - 09°00'6" E), Portopalo (36°40'24" N - 15°05'20" E), Sampieri (36°46'60" N -
220 14°41'60" E), namely: *Necremnus* near *artynes* (Walker), *N.* near *tidius* (Walker), *Neochrysocharis*

221 *formosa* (Westwood), *Pnigalio* (=*Ratzeburgiola*) *cristatus* (Ratzeburg), *Pnigalio* sp. *soemius*
222 complex (Hymenoptera: Eulophidae), *Diadegma ledicola* Horstmann (Hymenoptera:
223 Ichneumonidae), *Bracon osculator* (Nees), *B. hebetor* Say, and *Agathis* sp. (Hymenoptera:
224 Braconidae) (Table 1). The presence of these parasitoids varied in relation to the surveyed area;
225 however the most abundant species were *N.* near *artynes* coming from Sardinia and Sicily, and *N.*
226 near *tidius* and *D. ledicola* from Liguria (Table 1).

227 In preliminary multiplication trials performed at the Bioplanet, *D. ledicola* was found to be
228 not suitable for wasp offspring and was subsequently discarded, whereas both *Necremnus* species
229 proved to be fit for mass-rearing on *T. absoluta* on tomato plants (data not shown). Therefore,
230 populations of *N.* near *artynes* and *N.* near *tidius*, obtained from tomato leaves collected in Pula and
231 Arma di Taggia respectively, and mass-reared at the Bioplanet, were used for parasitism and
232 olfactory bioassays.

233 **Parasitism Trials.** In the experiments, when different instar larvae were simultaneously
234 offered, both *N.* near *artynes* and *N.* near *tidius* females were shown to prefer and set on larvae of
235 the first two instars, which were well-accepted as hosts, both for feeding and oviposition ($F = 87.55$;
236 $df = 3$; $P < 0.001$ for *N.* near *artynes*; $F = 195.85$; $df = 3$; $P < 0.001$ for *N.* near *tidius*). In fact, high
237 percentages of larvae of the first- and of the second-instar were killed by the wasps, whereas no
238 larvae of the third- and fourth-instar were attacked (Table 2).

239 Therefore, the effectiveness of *N.* near *artynes* and *N.* near *tidius* as biocontrol agents was
240 evaluated on larvae of the first two instars on potted tomato plants in cages. In these experiments, a
241 high mortality of *T. absoluta* larvae was recorded when they were exposed to the parasitoids, with
242 statistically significant differences between control and treated plants. The average number of adults
243 of the emerged moths (± SE) was 2.6 ± 0.9 and 1.2 ± 0.5 in the presence of *N.* near *artynes* and *N.*
244 near *tidius*, respectively, in contrast to 17.2 ± 0.6 in the control ($F = 180.28$; $df = 2$; $P < 0.001$).

245 Females of both *Necremnus* species were able to parasitize host larvae, and the average
246 offspring (± SE) obtained was 2.7 ± 1.1 and 1.5 ± 0.4 with a sex ratio of 1:3.5 and 1:1♀1:♂) for

247 *N.* near *artynes* and *N.* near *tidius*, respectively. The developmental time from the introduction of
248 the wasps in the cage to the emergence of the progeny was shorter for *N.* near *artynes* at 10.2±0.1
249 days and longer for *N.* near *tidius* at 14.3±0.2 days, while at least three weeks were needed for the
250 adult moths to emerge. In the leaves observed with the stereomicroscope, 64.8% and 78.6% of the
251 larvae exposed to *N.* near *artynes* and *N.* near *tidius*, respectively, were found dead inside the mines.
252 Considering the death rate in control plants (14.0%), the previous mortalities can be ascribed to
253 host-feeding or to failed larval development of the parasitoid.

254 Females of both *Necremnus* species showed the same behavior when they were introduced
255 into the arena with tomato leaves infested by first-instar larvae of *T. absoluta*. First, the females
256 walked and searched at random on the leaves until they located the mine. Then, they explored the
257 mine inserting the ovipositor repeatedly at different points; they were guided by the host larva's
258 movement until they could sting to inject venom and paralyze the larva. The ovipositing females
259 laid one or more eggs per larva at different points of the mine, whenever reinserting the ovipositor.
260 The location of eggs was related to the size and shape of the mine. Since the larva became
261 paralyzed many hours after being injected by venom from the parasitoid female, the eggs were
262 generally laid far from the host to be safe from its movement. After oviposition, the parasitoid
263 females did not touch nor brush the point of insertion with the tip of the gaster. The females showed
264 a more aggressive behavior when they killed the larva for host-feeding. In fact, they pierced the
265 body of the larva with their ovipositor repeatedly, causing an irreversible paralysis followed by
266 death. Drilling was accomplished by inserting the ovipositor, and swinging the valves up and down
267 against each other. Once the ovipositor was completely withdrawn, females began to suck the
268 haemolymph exuded at the puncture. This behavior lasted for up to 2–3 min.

269 **Olfactometer Bioassays.** Since the species *Necremnus artynes* (Walker) and *N. tidius*
270 (Walker) are reported in literature as ectoparasitoids of *C. pulchrimella* on upright pellitory
271 (Bernardo and Viggiani 2002, The Natural History Museum 2011), the olfactometer bioassays were
272 performed to testing the attractiveness both of tomato and of pellitory plants for the wasp females.

273 Almost all the females tested in olfactometer bioassays responded by making a choice within the
274 fixed time. In the experiment with *N.* near *artynes*, females proved to be more attracted by infested
275 tomato compared to infested upright pellitory plants (χ^2 = 19.20; df= 1; P < 0.001), and by healthy
276 tomato compared to healthy upright pellitory plants (χ^2 = 4.80; df= 1; P = 0.028). Significant
277 differences in responses of *N.* near *artynes* females were also found when comparing infested with
278 healthy tomato plants (χ^2 = 10.80; df= 1; P = 0.001) (Figure 1). In the experiment with *N.* near
279 *tidius*, no significant preference of females was detected between healthy tomato and healthy
280 upright pellitory plants (χ^2 = 0.53; df= 1; P = 0.465), and between infested tomato and infested
281 upright pellitory plants (χ^2 = 3.33; df= 1; P 0.068). By contrast, results for infested tomato plants
282 showed them to be largely attractive in comparison with healthy plants (χ^2 = 8.53; df= 1; P = 0.003)
283 (Figure 2).
284
285 **Discussion**
286 *Tuta absoluta* is considered one of the major pests on tomato and other solanaceous crops,
287 both in the field and under protected conditions (EPPO 2005). Because of the ongoing spread of this
288 exotic moth throughout Europe and the lack of totally satisfactory effective management options,
289 most control attempts have moved towards a biological control approach. Several natural enemies
290 have been reported, in particular Eulophidae, Braconidae and Trichogrammatidae as parasitoids,
291 and Miridae, Nabidae and Pentatomidae as predators (Desneux et al. 2010). Nevertheless, some
292 species that we obtained from field-collected samples are recorded here for the first time as larval
293 parasitoids of *T. absoluta*. In fact, although species of the genera *Agathis*, *Bracon* (Hymenoptera:
294 Braconidae), and *Diadegma* (Hymenoptera: Ichneumonidae) are already known as larval parasitoids
295 of the tomato borer in South America (Colomo et al. 2002, Marchiori et al. 2004, Miranda et al.
296 2005), *B. hebetor* and *D. ledicola* have not been previously reported, while *A. fuscipennis*
297 (Zetterstedt) and *B. osculator* along with *D. pulchripes* (Kokujev) have been recently observed in

298 Tuscany (Loni et al. 2011) and Sicily (Zappalà et al. 2011a). However, these species are larval
299 parasitoids of many Lepidoptera species (Milonas 2005).
300 At the same time, several species belonging to the Eulophidae family have been reported
301 among larval parasitoids of *T. absoluta* in South America, and now also in the Mediterranean area
302 (Desneux et al. 2010). These data are not surprising because this family includes several parasitoids
303 of leafminers and gall-making larvae, often able to adapt to exotic hosts. Although records of
304 *Neochrysocharis formosa*, *Pnigalio cristatus* and a species of the *P. soemius* complex have also
305 been recently reported (Luna et al., 2011; Zappalà et al. 2011a), parasitoids of the genus *Necremnus*
306 emerged from larvae of tomato borer in our studies as well as in others (Mollá et al. 2008, 2010;
307 Gabarra and Arnó 2010, Fois et al. 2011, Rizzo et al 2011, Zappalà et al. 2011a). Thus, when
308 considering their suitability for mass-rearing as assessed at the Bioplanet, these species appear to be
309 promising native biological control agents. In contrast, *D. ledicola* proved not to be fit for mass-
310 rearing in spite of its abundance on *T. absoluta* in one of the surveyed tomato fields.
311 The genus *Necremnus* includes solitary and gregarious ectoparasitoids of larvae of
312 coleopteran, lepidopteran and dipteran species (Coudron et al. 2000, Bernardo and Viggiani 2002,
313 Dosdall et al. 2007), but most species have been poorly investigated so far and very little
314 information, not always reliable, is available in the literature on their life history, behavior, and
315 above all their hosts. For example, *N. tidius* is a solitary ectoparasitoid of coleopteran larvae with a
316 Holarctic distribution (Gibson et al. 2005); Diptera and Lepidoptera have also been listed among its
317 hosts but the non-coleopteran host association probably resulted from incorrect identification of the
318 parasitoid (Dosdall et al. 2007). This misidentification would seem to be further confirmed by the
319 different behavior of females after oviposition. In fact, unlike what has been observed for females
320 of *N. tidius* (Dosdall et al. 2007), in our experiments females did not touch and brush the mine after
321 oviposition. Additionally, some characters of *N. artynes*, described in the keys of Boucek (1958),
322 Graham (1959) and Askew (1968), did not match completely those of our individuals. Hence, we

323 chose to name the species emerged from *T. absoluta* and here studied as *N.* near *tidius* and *N.* near
324 *artynes* to avoid any misidentification while awaiting their systematic clarification.
325 Despite their specific identification requiring further investigation, the results obtained from
326 laboratory experiments demonstrated that both *Necremnus* species effectively recognized and
327 parasitized *T. absoluta* in our caged experiments. Therefore, these parasitoids are able not only to
328 accept this new host, as also observed in other studies (Gabarra and Arnó 2010, Mollá et al. 2010),
329 but can also reduce significantly infestations of the tomato borer under laboratory conditions.
330 Parasitoid females exhibited a preference for larvae of the first two instars, on which they could
331 oviposit or feed. Moreover, females of both species proved to be able to recognize and choose
332 tomato leaves infested by *T. absoluta* larvae in the olfactometer bioassays. In particular, females of
333 *N.* near *artynes* showed a very strong response to tomato leaves, both uninfested and infested, which
334 could explain the frequent record of this native parasitoid on the exotic pest. It now remains to be
335 assessed what stimuli form the trigger for behavioral responses of these parasitoids in order to
336 obtain a better understanding of their behavior.
337 In our experiments the impact of the parasitoids was calculated as all dead hosts resulting
338 from the presence of the parasitoids, not only hosts utilized for parasitoid reproduction. Females of
339 both *Necremnus* species were observed to kill *T. absoluta* larvae and feed on their haemolymph;
340 they probably need to feed on the host for maintenance and/or egg production, as do other
341 synovigenic parasitic wasps (Giron et al. 2004). Destructive host-feeders can be considered to be
342 better biological control agents even if host-feeding must not be used as the sole selection criterion
343 (Jervis et al. 1996). However, in our experiments the impact of host-feeding by both *Necremnus*
344 species could be overestimated. In fact, the females were kept in contact with the same larvae in the
345 cages for five days, and consequently the probability that they could feed on the larvae in the mines,
346 where they had previously laid eggs, was increased. When new larvae were offered, on *C.*
347 *pulchrimella* approx 20% and 80% of host larvae were killed by females of *N.* near *tidius* for
348 feeding and oviposition respectively, whereas host-stinging behavior was very rarely observed (P.

349 N., unpublished data), unlike other eulophid parasitoids of leafminers such as *Pnigalio soemius*
350 (Walker) (Bernardo et al. 2006). Therefore, the high larval mortality of *T. absoluta* could be due
351 partly to host-feeding and partly to the failure of parasitoid larval development on this new host
352 under laboratory conditions, and needs to be further investigated.

353 Since conditions in the open field are much more varied than those in the laboratory and,
354 among other factors, temperature is observed to play an important role in the development of
355 arthropods, further investigations are needed to assess the behavior of both *Necremnus* species at
356 different thermal conditions. Moreover, our preliminary findings about the host location and
357 acceptance suggest that both *Necremnus* species have several behavioral traits that positively
358 influence their performance as biological control agents. Hence, studies should be continued to
359 investigate the suitability of the selected host for the parasitoid immature development with the aim
360 of improving the efficiency of mass production and implementing augmentation of biological
361 control programs.

362 The ability of *Necremnus* species to find and parasitize *T. absoluta* larvae makes them
363 potential candidates for mass production and biological control, adding to the list another example
364 of the adaptation of an indigenous parasitoid to an exotic pest and highlighting the importance of a
365 rich and variegated biodiversity in finding new associations with harmful pests, as already reported
366 in Nicoli and Burgio (1997). In the light of these satisfactory results, additional studies on both
367 *Necremnus* species will be required to clarify their systematic position, detect their primary hosts,
368 and to evaluate them in biological control and IPM programs in commercial tomato plantations.
369

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521 Figure captions

522

523 **Fig. 1.** Responses of *N.* near *artynes* (no. of responding females in bars) in a Y-tube olfactometer
524 and, when present, number of non-responding individuals (NS) to the odors of infested (δ), or
525 uninfested (π) tomato plants with *T. absoluta* and of infested (α), or uninfested (β) upright pellitory
526 plants with *C. pulchrimella* for each compared pair. Numbers in bars represent individuals that
527 moved toward the volatiles. χ^2 statistics (*P<0.10; **P<0.05; ***P<0.01; df=1) tested the
528 hypothesis that the distribution of side-arm choices deviated from a null model where odor sources
529 were chosen with equal frequency.

530

531 **Fig. 2.** Responses of *N.* near *tidius* (no. of responding females in bars) in a Y-tube olfactometer and,
532 when present, number of non-responding individuals (NS) to the odors of infested (δ), or uninfested
533 (π) tomato plants with *T. absoluta* and of infested (α), or uninfested (β) upright pellitory plants with
534 *C. pulchrimella* for each compared pair. Numbers in bars represent individuals that moved toward
535 the volatiles. χ^2 statistics (*P<0.10; **P<0.05; ***P<0.01; df=1) tested the hypothesis that the
536 distribution of side-arm choices deviated from a null model where odor sources were chosen with
537 equal frequency.

538

Table 1 Indigenous larval parasitoid species emerged from tomato leaves infested by *T. absoluta*, collected in Italian horticultural areas

Species	Site	No. of emerged individuals	Date
Necremnus near *artynes* (Walker)	Portopalo (SR), Sicily	11	9 July 2009
	Pula (CA), Sardinia	27	21 July 2009 22 April 2010
Necremnus near *tidius* (Walker)	Arma di Taggia (IM), Liguria	30	7 Oct 2009
Neochrysocharis formosa (Westwood)	Ceriale (SV), Liguria	4	26 Aug 2010
Pnigalio (=*Ratzeburgiola*) *cristatus* (Ratzeburg)	Ceriale (SV), Liguria	3	26 Aug 2010 10 Oct 2010
Pnigalio sp. *soemius* complex	Ceriale (SV), Liguria	3	10 Oct 2010
Diadegma ledicola Horstmann	Arma di Taggia (IM), Liguria	25	7 Oct 2009
Agathis sp.	Sampieri (RG), Sicily	3	10 July 2009
Bracon osculator (Nees)	Ceriale (SV), Liguria	6	26 Aug 2010 10 Oct 2010
Bracon hebetor Say	Pula (CA), Sardinia	2	21 July 2009

Table 2 Mean number of *T. absoluta* larvae (±SE) killed by single females of *N.* near *artynes* and *N.* near *tidius*. Each female was simultaneously offered 20 larvae, five for each instar, for 48 h

Species	Mean no. of *T. absoluta* larvae killed by the parasitoid			
	1st-instar larvae	2nd-instar larvae	3rd-instar larvae	4th-instar larvae
N. near *artynes*	4.20±0.37 a	3.80±0.20 a	0.00±0.00 b	0.00±0.00 b
N. near *tidius*	4.80±0.20 a	4.60±0.24 a	0.00±0.00 b	0.00±0.00 b

Within the same line, data (mean±SE) followed by a different letter are significantly different ($P<0.001$; ANOVA)

α

***

3	27

β

**

9	21

π

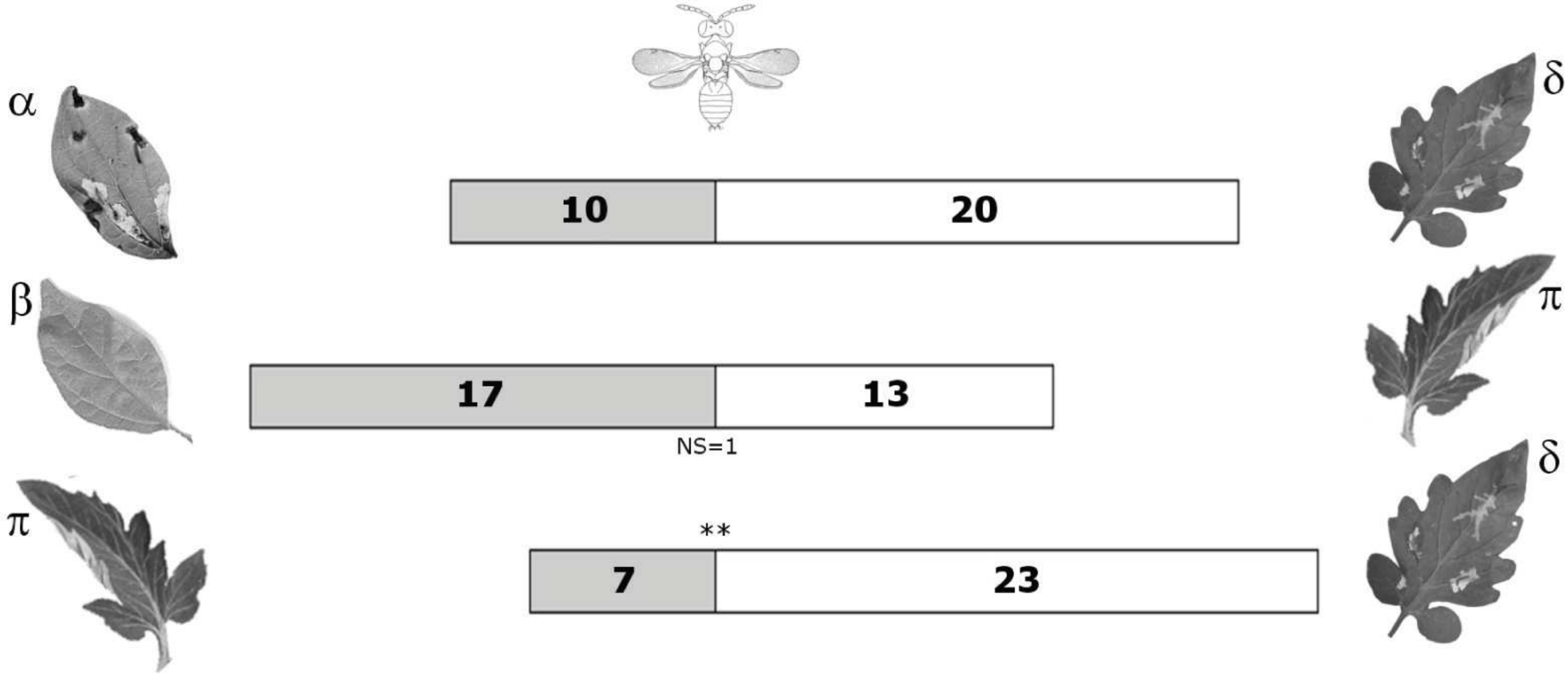
**

6	24

δ

π

δ


α
10
20
δ
β
17
13
π
NS=1
π
**
7
23
δ