1 **Natural enemies associated with *Tuta absoluta* and functional biodiversity in vegetable**

2 **crops**

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8 **Abstract**

9 *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is considered one of the main pests

10 threatening tomato production worldwide. In the Mediterranean, the use of predatory mirids is

11 widespread, and although several larval parasitoid species have been recorded in the area, their

12 contribution to the biological control of the pest is often neglected. With the general objective

13 of improving the biological control of *T. absoluta*, our field study aimed to determine the

14 relative abundance of natural enemies associated with *T. absoluta* in tomato fields and to assess

15 whether insectary plants placed in the vicinity of vegetable crops would help to improve

16 functional biodiversity in the farms. The study was conducted during two years in six

17 commercial tomato fields in Northeast Spain that were managed using an integrated pest

18 management program based on predatory mirid conservation. Our results indicated that

19 *Necremnus tutae* Ribes & Bernardo (Hymenoptera: Eulophidae) was by far the main natural

20 enemy responsible for larval parasitism of *T. absoluta.* The flowering plants used in the present

21 study were attractive to several natural enemies that are of interest not only for tomato but also

22 for other vegetable crops coexisting at the farm level, including parasitoid wasps, hoverflies,

23 and other important beneficials, such as *Orius* spp. ladybeetles and predatory thrips.

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25 **Key words:** conservation biological control, tomato, *Lobularia maritima*, *Sinapis alba*,

26 *Achillea millefolium* and *Fagopyrum esculentum*

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Introduction

Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) is considered one of the main tomato pests globally. After invading Spain in 2006, it quickly spread to several countries beyond its area of origin (Desneux et al. 2010, 2011; Biondi et al. 2018; Han et al. 2019), threating tomato production. In the Mediterranean, the use of predatory mirid bugs following either conservation or inoculation strategies is one of the most widely used methods for biological control of the pest (Urbaneja et al. 2012). These predators are polyphagous and feed not only on *T. absoluta* but also on other vegetable pests (Arnó et al. 2018a). In the Mediterranean, several predators have been described as feeding efficiently on *T. absoluta* eggs but as poor predators of *T. absoluta* larvae, such as *Macrolophus pygmaeus* Rambur, *Nesidiocoris tenuis* (Reuter), *Dicyphus errans* (Wolff), and *Dicyphus bolivari* Lindberg (Hemiptera: Miridae) (Arnó et al. 2009; Urbaneja et al. 2009; Ingegno et al. 2019).

Regarding parasitoids, several species have been reported worldwide (Zappalà et al. 2013; Biondi et al. 2018; Mansour et al. 2018; Salas Gervasio et al. 2019). In the Spanish Mediterranean area, Gabarra et al. (2014) described a number of species attacking *T. absoluta* larvae belonging to the five families also recorded in other parts of the Mediterranean (Biondi et al. 2018; Mansour et al. 2018). These authors also reported the occurrence of egg parasitoids of the genus *Trichogramma*. Among the larval parasitoids, the genus *Necremnus* has been found the most abundant and widespread. This genus was reviewed by Gebiola et al. (2015), who found that several of the individuals that had been classified as *Necremnus artynes* (Walker) (Hymenoptera: Eulophidae) or *N.* nr. *artynes* actually corresponded to a new species they called *Necremnus tutae* Ribes & Bernardo.

The biology of *N. tutae* (referred to as *N. artynes*) has been extensively studied (Calvo et al. 2013; Chailleux et al. 2014; Bodino et al. 2016; Calvo et al. 2016). Fitness of this parasitoids benefits from feeding on sugar-reach diet such as honey and honeydew (Arnó et al. 2018b; de

Campos et al. 2020). This species has been considered for inoculative release as a control strategy against *T. absoluta* by several companies that mass rear natural enemies. Although currently it is not commercially available, it occurs spontaneously and abundantly in many tomato fields (Abbes et al. 2014; Crisol-Martínez and van der Blom 2019). Therefore, its promotion could be a good strategy to improve the biological control of *T. absoluta*.

The use of floral strips around crops is a recommended practice to promote natural enemies close to the fields in conservation biological control (Blaauw and Isaacs 2012). In particular, the use of strips of *Calendula officinalis* L. (Asteraceae) close to tomato fields to boost mirid predator populations and to be their refuge in non-crop periods is a practice that is quickly becoming common in the horticultural farms in Northeast Spain (Arnó et al. 2018a; Ardanuy et al. in press; Agustí et al. 2020). However, this plant does not seem particularly attractive to parasitoids (Arnó et al. 2012). Including other plants that better contribute to conserving and increasing parasitoids of *T. absoluta* and other pests might enhance biological control in tomato and other vegetables. In this regard, Arnó et al. (2018b) and Balzan and Wäckers (2013) demonstrated in the laboratory that some plants, such as *Lobularia maritima* L. (Brassicaceae), could be useful in improving the survival and reproductive potential of *N. tutae* and other *T. absoluta* larval parasitoids. Arnó et al. (2018b) also reported benefits regarding the fitness of the larval parasitoids of *T. absoluta* when fed on *Sinapis alba* L. (Brassicaceae) and *Fagopyrum esculentum* Moench (Polygonaceae). In a horticultural production system with coexisting crops, the functionality of insectary plants must go beyond the promotion of a single group of parasitoid and extend to a wider range of natural enemies. Wäckers and van Rijn (2005) summarized the importance of flowers as food providers for several groups of natural enemies, including those listed in terms of interest in conservation biological control in vegetables (Riudavets et al. 2020). Considering this background and with the general objective of improving the biological control of *T. absoluta*, our field study aimed to determine the relative

79 abundance of natural enemies associated with *T. absoluta* in tomato fields and to assess whether

80 the use of insectary flowering plants close to the crops would help to improve functional

81 biodiversity in vegetable farms.

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83 **Materials and methods**

84 *Study area and experimental set-up*

85 The study was conducted in six tomato open-air fields (three in 2015 and three in 2016) of 0.25

86 ± 0.05 ha located in the Maresme county (Barcelona) from the second half of July to the last

87 week of September. The fields were managed with an integrated pest management (IPM)

88 program based on the conservation of predatory bugs and the use of selective insecticides when

89 necessary (Arnó et al. 2018a). As in Aparicio et al. (in press), four patches (1 m^2) with four

90 plant containers (40 cm Ø) were arranged along one of the sides of each field at approximately

91 0.5–1.8 m apart from the first row of tomatoes. These patches were separated by an approximate

92 distance of 2 m between them. In each container, four to eight plants of the following species

93 were planted: *L. maritima*, *S. alba*, *Achillea millefolium* L. (Asteraceae), and *F. esculentum*.

94 Hence, in each patch, all four plant species were present. These species were chosen according

95 to the previous results of our research group (Arnó et al. 2012; Arnó et al. 2018b). All plants

96 were grown from seed in seed-starting pots with potting soil in a greenhouse, transplanted to

97 the containers, and taken to the field at week 28 (mid-July) in 2015 and 2016 when they were

98 already in bloom. Containers with the insectary plants were kept in the sites until the end of the

99 samplings in week 39 (end of September). Fertigation was provided throughout the experiment

100 simultaneously to the tomato crop. The phenological stage of the different plant species was

101 recorded biweekly (Table 1).

102 *Natural enemies of* Tuta absoluta

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To estimate the abundance of natural enemies associated with *T. absoluta*, samplings were carried out using yellow sticky traps and sentinel plants. Yellow sticky traps (24.5 cm tall and 20 cm wide; ATRAPAXON, Projar S.A, Spain) were biweekly displayed for one week in the field from week 29 to week 39. Eight traps were placed in each field at a distance of approximately 3.5 m inside the crop: four in front of the four patches with the insectary plants (near) and four apart at a distance of 45–240 m (far) depending on the field size. The number of predatory mirid bugs and hymenopteran parasitoids were counted in all the yellow sticky traps in the laboratory. The number of *M. pygmaeus* and *N. tenuis*, the two most abundant mirid predators colonizing tomato fields in the area (Arnó et al. 2018a), was recorded separately. The abundance of the different families of *T. absoluta* parasitoids have been estimated in traps of two fields in weeks 29, 33, and 37 of both years. Hymenoptera were identified at family level when possible using the taxonomic keys of Grissell and Schauff (1990) and Hanson and Gauld (2006). Only families with species previously identified as *T. absoluta* parasitoids in Spain and in the Mediterranean basin were taken into account. Thus, target families were Eulophidae, Braconidae, Chalcididae, Ichneumonidae, Pteromalidae, and Trichogrammatidae (Gabarra et al 2014; Biondi et al. 2018; Mansour et al. 2018).

Sentinel plants were used to assess the parasitism of the larval stages of *T. absoluta* during the weeks when no yellow sticky traps were in the field to ensure that the two sampling systems would not interfere with each other. They were potted tomato plants of approximately 50 cm high, which were isolated in aerated cages ($60 \times 75 \times 100$ cm) with adults of *T. absoluta* for 7 days at approximately 25ºC. After removing the adults, the plants were kept in cages for another week in order to obtain plants with second to third instar *T. absoluta* larvae. Then, plants were enclosed in a sleeve (50×30 cm) made of fine net (7×8 threads/cm^2), which was chosen to prevent the exit of *T. absoluta* adults and the colonization by predatory mirid bugs. Preliminary tests done prior to the field experiment confirmed that this net allowed the entrance of *N. tutae*,

128 which is the most abundant larval parasitoid in the Mediterranean area (Gabarra et al. 2014;

129 Biondi et al. 2018). To avoid plant desiccation, potted plants were placed into bigger pots filled

130 with water. The outside of the pots were sprayed with insect-trapping glue to prevent ants and

131 other non-winged predators from climbing the plant. Similar to the yellow sticky traps, eight

132 plants were placed in each field at a distance of approximately 2 m inside the crop, four near to

133 the insectary plants and four far from them. The plants were taken to the fields at 15-day

134 intervals and deployed for 1 week in the fields starting in week 30 and ending in week 38

135 (beginning of October) for both years. In 2016, plants were not available in week 34 due to a

136 failure in the climatic chamber. Therefore, data from this week was missing. Once in the

137 laboratory, galleries were inspected under a stereomicroscope, and the number of live, dead but

138 apparently not parasitized (hereafter "dead"), and ectoparasitized *T. absoluta* larvae was

139 recorded. All larvae were kept in the climatic chambers for at least 4 weeks and parasitoids that

140 reached the adult stage were collected and stored in alcohol. A sample of emerged parasitoids

141 individuals were identified to genus level following morphological characterization, and out of

142 them, a subsample was identified to species level following the taxonomic keys of Askew

143 (1968) and Gebiola et al. (2015).

144 *Presence of natural enemies in flower patches*

145 The attractiveness of the insectary plants to natural enemies of *T. absoluta* and to other insects

146 relevant to the biological control of vegetable pests was evaluated on the same dates that the

147 yellow sticky traps were deployed in the field using two different methods. For hoverflies,

148 which can be easily distinguished during their flight, visual observations of the number of adults

149 that effectively contacted each insectary plant (landing) was recorded for 3 minutes. For insects

150 that could not be seen during their flight and that were usually within the plant foliage or within

151 the flowers, the beating tray method (hereafter "beating") was used. A bunch of each plant

152 species was separately hand-beaten three consecutive times on a white plastic white tray (24

153 ×35 cm). Insects that fell onto the tray were visually classified *in situ* as hymenopteran

154 parasitoids, predatory mirid bugs (Hemiptera: Miridae), *Orius* spp. (Hemiptera: Anthocoridae),

155 ladybeetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), and *Aelothrips*

156 spp. (Thysanoptera: Aeolothripidae). In the same samples, the number of aphids, thrips other

157 than *Aelothrips* spp. (hereafter "thrips"), and *Eurydema* spp. (Hemiptera: Pentatomidae) was

158 also recorded. Other insect groups were discarded. Both methods were conducted biweekly on

159 the same day, between 9:00 and 12:00 hours, avoiding cloudy and rainy days.

160 *Statistical analysis*

161 Data were analyzed for both years, using a generalized linear mixed-effects model (GLMM)

162 with a negative binomial (NB) response distribution using the R function "glmer.nb" and a log

163 link function. The number of insects per beating sample and per yellow sticky trap, and the

164 number of hoverflies landings per insectary plant species were the response variables. The two

165 different distances (treatment factor), nearness to the insectary plants and farness from them,

166 was the main fixed effect. The fields, patches, weeks and the interaction treatment*weeks were

167 the random factors. Statistical inferences for fixed effects were based on Wald-type tests

168 assuming a standard normal distribution. We tested the significance (and contribution) of entire

169 grouping factors of the model using Anova function from package "car" that calculates Wald

170 χ^2 tests (Fox and Weisberg 2019), and pairwise comparisons (post-hoc tests) were conducted

171 with the Tukey's method for multiple comparisons. The statistical analysis was performed using

172 the software R (v3.5.3. 2020), using the packages lme4 (Bates et al. 2015), MASS (Venables et

173 al. 2002), emmeans (Russell Lenth 2020) and ggplot2 (Wickham 2016). For all statistical tests

174 a nominal significance level of 5% ($P < 0.05$) was used for the study.

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176 **Results**

177 *Natural enemies of* Tuta absoluta

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178   During the 2 years of sampling, 3,091 hymenopteran parasitoids were caught in the yellow

179   sticky traps, with number per trap significantly higher near to than far away from the insectary

180   plants patches (12.34 ± 0.66; 9.84 ± 0.60, $Z = 2.89$, $P = 0.0039$). Traps used to estimate the

181   abundance of the different families of *T. absoluta* parasitoids yielded a total of 940 individuals

182   that were identified to family level. Eighty-nine of them belonged to the target families

183   Eulophidae, Braconidae, Ichneumonidae, Pteromalidae, and Trichogrammatidae, whereas no

184   Chalcididae specimen was identified in the traps. Significant differences in abundance were

185   found among the mentioned families ($\chi^2 = 69.05$, df=4, $P < 0.0001$), with Eulophidae being the

186   most abundant (0.69 ± 0.12 individuals per trap) compared to the other target families with

187   similar abundances (between 0.01 ± 0.01 and 0.16 ± 0.06 individuals per trap). However, the

188   number of individuals of these target families recorded in the yellow sticky traps was not

189   significantly different between those placed near to and far from the insectary plant patches for

190   none of the families and for the total (Table 2).

191   A total of 817 predatory mirid bugs were caught in the yellow sticky traps for the entire

192   sampling period. When comparing the abundances of both mirid bugs in the yellow sticky traps

193   placed close and far to the insectary plant patches, no significant differences were observed for

194   any of the two species (0.58 ± 0.15 vs. 0.40 ± 0.07, $Z = 0.869$, $P = 0.3847$ for *M. pygmaeus,*

195   and 2.97 ± 0.56 vs. 1.89 ± 0.33, $Z = 0.486$, $P = 0.6271$ for *N. tenuis*).

196   From the sentinel plants placed within the tomato crop, 3,557 *T. absoluta* larvae were recovered

197   (671 ectoparasitized, 977 alive and 1,909 dead). Regarding the number of ectoparasitized larvae

198   per plant in the sentinel plants placed near to and far from the flower patches, no significant

199   differences were found (2.73 ± 0.23 and 3.07 ± 0.29, respectively; $Z= 1.105$, $P = 0.2691$). A

200   total of 462 hymenoptera adults were reared from the sentinel plants. Four were identified as

201   belonging to the family Braconidae, subfamily Microgastrinae. Of the remaining 458, a

202   randomly selected sample of 159 individuals were identified to genus level, and 155 were

morphologically compatible with *Necremnus* spp. Out of them *Necremnus* spp., 42 individuals were identified to the species level, with all but one being identified as *N. tutae* and the other as *N. artynes*.

Presence of natural enemies in flower patches

The total number of hymenopteran parasitoids and predatory mirid bugs recorded by beating each insectary plant is shown in Figure 1. Results revealed that hymenopteran parasitoids were significantly more abundant on *A. millefolium* than on *S. alba*, whereas *F. esculentum* and *L. maritima* had intermediate values ($\chi^2 = 19.61$, *df= 3, P* = 0.0002). Regarding predatory mirid bugs, recorded populations were very low (< 0.10 individuals/beating sample), and no significant differences were found among the different plant species tested ($\chi^2 = 0.16$, *df= 3, P* = 0. 9845).

Samplings conducted on the different plants also provided information regarding the potential of the insectary plants as a food resource for other biological control agents that, although not abundant in tomato crops and not *T. absoluta* control agents, may be relevant in other vegetable crops. In this vein, out hymenopteran parasitoids, the most abundant natural enemies recorded with the beating method were *Orius* spp. (30%) and ladybeetles (18%). In addition, some predatory thrips (5%) were collected. Altogether, 947 individuals were accounted for. No differences in numbers of natural enemies among the different insectary plant species were found (Table 3). On the contrary, the number of hoverfly landings recorded during the visual sampling was significantly higher on *F. esculentum* than in *L. maritima* and *A. millefollium*. Flowers of *S. alba* registered significantly less landings than any of the other three insectary plants.

Regarding phytophagous insects, no significant differences were found for thrips numbers among the four insectary plants tested. Significantly higher number of aphids were recorded on *A. millefollium* and *F. esculentum* than on *L. maritima* and *S. alba.* By contrast, significantly

228   more *Eurydema* spp. individuals were found on *L. maritima* and *S. alba* than on *A. millefollium*

229   and *F. esculentum* (Table 4).

230

231   **Discussion**

232   The main groups of natural enemies of *T. absoluta* described in the literature (Biondi et al.

233   2018), namely, predatory mirid bugs and larval parasitoids, were present in the sampled tomato

234   fields. The presence of mirids was foreseeable, since the IPM program used in the sampled

235   fields was based in the conservation of generalist predatory mirids, mainly *M. pygmaeus* and

236   *N. tenuis* that contributed to the control of several tomato pests (Arnó et al. 2018a). Soon after

237   the invasion of this pest in Spain, Arnó et al. (2009) and Urbaneja et al. (2009) had already

238   determined the great impact of these predators on *T. absoluta* eggs. More than three times as

239   many *N. tenuis* than *M. pygmaeus* were recorded. The relative abundance of *N. tenuis* over *M.*

240   *pygmaeus* has increased in recent years in the tomato crops in the area of study (RG, JA, JR

241   unpublished data; Matas 2014), a phenomenon probably associated with climate change since

242   *N. tenuis* is a clearly thermophilic species (Sánchez et al. 2009).

243   The number of hymenopteran parasitoids was by far the highest among the potential natural

244   enemies caught in the sticky traps. However, the percentage of individuals belonging to the

245   target families that include known *T. absoluta* parasitoids (i.e., Eulophidae, Braconidae,

246   Ichneumonidae, Pteromalidae, and Trichogrammatidae) represented less than 9% of total

247   hymenopterans. Eulophidae were the most abundant followed by Braconidae, which is in

248   accordance with Zappalà et al. (2013) and Biondi et al. (2018). These reviews indicated that

249   these two families were the best represented among parasitoids naturally attacking *T. absoluta*

250   in the Mediterranean in terms of both the number of species and their geographical distribution.

251   Most of the parasitoids recruited with the sentinel plants were *Necremnus* spp., and according

252   to the sample of individuals identified to the species level, 98% were *N. tutae*. The higher

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prevalence of *N. tutae* in the sentinel plants was consistent with the higher prevalence of Eulophidae in the yellow sticky traps over other families with potential parasitoids of *T. absoluta* larvae. The net sleeve around the sentinel plants might have biased the results in regards to the parasitoid guild. Preliminary studies to verify the permeability of the net were only conducted with *N. tutae*. Therefore, it cannot be ruled out that parasitization by other bigger hymenopterans was prevented. Additionally, plants were taken to the field when second to third instar larvae were present, which are the preferred instars for *N. tutae* (Calvo et al. 2013). Therefore, the bias if any, does not seem to have substantially modified the parasitoid species composition obtained with the sentinel plants. In fact, our results are consistent with previous studies that suggest that *N. tutae* is widely distributed in the Mediterranean (Ferracini et al. 2012; Zappalà et al. 2013; Gabarra et al. 2014; Gebiola et al. 2015; Biondi et al. 2018). This species was considered as a potential biological control agent to be released for pest control (Calvo et al. 2013; Chailleux et al. 2014; Bodino et al. 2016; Calvo et al. 2016). Although it is not currently commercialized for inoculation, several authors have acknowledged its contribution to the conservation biological control of *T. absoluta*. In the present study, the parasitism rate in the sentinel plants was close to 20%. In the same area, a survey in tomato crops conducted in 2018 yielded a parasitism rate of approximately 14% (authors, unpublished), and parasitism rates were up to 73% in tomato greenhouses in the south of Spain (Crisol-Martínez and van der Blom 2019). Similarly, Abbes et al. (2014) reported an average parasitism rate of 26% in Tunisian open field crops using sentinel plants and between 11% and 15% when directly sampling tomato leaves from the crop.

The number of captures on yellow sticky traps placed close to the insectary plants were approximately the same than those placed far from the blooming plants, both in the case of parasitoids and predators. Likewise, the number of ectoparasitized *T. absoluta* larvae, and therefore the presence of *T. absoluta* parasitoids, were similar in sentinel plants located near to

278 and far away from insectary plants placed in one side of the field. Both results indicated that in

279 our experiment set up, the distribution of natural enemies within the fields was uniform. This

280 is probably because the size of the commercial fields in the area hampered the ability to

281 differentiate between the effects of nearness to versus farness from the insectary plants due to

282 the operational range of some parasitoids as has been suggested by Heimpel (2019).

283 Attractiveness of natural enemies on flowers is a first step to achieve biocontrol-related benefits

284 from habitat manipulation. Among the insectary plants tested, *A. millefolium* was the most

285 attractive for parasitoids, followed by *F. esculentum* and *L. maritima*, whereas *S. alba* registered

286 the lowest populations. Attraction of parasitoids to *A. millefolium* has been previously reported

287 in field experiments (Dib et al. 2012; El-Nabawy et al. 2015; Arnó et al. 2012; Gibson et al.

288 2019; Lundin et al. 2019). This statement disagrees with findings by Wäckers (2004), who in

289 laboratory experiments demonstrated the repellency of *A. millefolium* flowers for two species

290 of Braconidae and one Ichneumonidae; this repellent effect prevented these species from

291 feeding nectar. In fact, Arnó et al (2018b) demonstrated that access to *A. millefolium* flowers

292 did not provide any benefit to the survival and reproduction of three *T. absoluta* parasitoids, i.

293 e. *N. tutae*, *Stenomesius* nr. *japonicus* Ashmed (Hymenoptera: Eulophidae), and *Bracon* nr.

294 *nigricans* Szépligeti (Hymenoptera: Braconidae). Further studies will be necessary to clarify

295 the divergent results from field and laboratory experiments regarding the role of *A. millefolium*

296 as an insectary plant.

297 *Sinapis alba*, a spring oilseed crop typically harvested in early summer (Sáez-Bastante et al.

298 2016), registered the lower parasitoid abundance, which might be linked to the low flower

299 presence during the sampling period (July–September). However, the presence of this plant

300 when in bloom might be of interest. Laboratory experiments have proven its positive effects on

301 the egg load of *S.* nr. *japonicus* (Arnó et al. 2018b) and the longevity and fecundity of *Cotesia*

302 *glomerata* (L.) (Hymenoptera: Braconidae) and *Diadegma semiclausum* Hellén (Hymenoptera:

Ichneumonidae) (Winkler et al. 2009). Furthermore, field surveys have corroborated the value

of this flowering plant to enhance parasitism of beetles (Manojlovic et al. 2000) and cereal

aphids by *Aphidius* spp., especially when hosts and other food resources are limited (Damien

et al. 2017).

During our samplings, *F. esculentum* and *L. maritima* had an intermediate position related to

the attractiveness for parasitoids. These are two of the most commonly used insectary plants to

enhance natural enemy populations, due to the good results on parasitoid fitness obtained when

tested in laboratory experiments (Fiedler et al. 2008; Badenes-Pérez 2019). Arnó et al. (2018b)

reported these two species to be the most suitable to enhance survival and reproduction of *T.*

absoluta parasitoids. Similarly, Balzan and Wäckers (2013) reported survival increases for *N.*

artynes.

None of the tested insectary plants was more attractive than others for the predatory mirid bugs

considered in the survey, namely, *M. pygameus* and *N. tenuis*, and number of individuals was

low (<0.10 individuals/sample) although the insectary plants were close to tomato fields were

mirid populations were well established. By contrast, in one of the farms in 2015, *C. officinalis*

yielded an average of 4.3 ± 0.7 adults of *M. pygmaeus* using the same beating method (authors,

unpublished). This suggests that plants tested in our field experiments were much less attractive

than the tomato crop for these two species of predatory mirids although scientific reports have

suggested that their fitness benefits when feeding on pollen, nectar, or other sugary foods

(Vandekerkhove and De Clercq 2010; Portillo et al. 2012; Put et al. 2012; Urbaneja-Bernat et

al. 2015). Therefore, none of the four insectary plants examined in this study can be considered

as a candidate to replace *C. officinalis*, but they could complement the floral margins of this

plant to promote the presence of a more varied guild of natural enemies, in particular

parasitoids.

327 Regarding other predators that are interesting biological control agents in vegetables other than

328 tomato and that were found in the insectary plants during the samplings, only hoverflies have

329 shown preference for some species over others: namely, *F. esculentum*, which was the most

330 preferred, and *S. alba*, which was the less preferred. *Fagopyrum esculentum,* and to a lesser

331 extent, *A. millefolium*, were ranked as suitable flowers to feed zoophagous hoverflies due to

332 their nectar accessibility, which influences fitness and attractiveness (van Rijn and Wäckers

333 2016). On the other hand, *L. maritima* has been previously described as very attractive for adult

334 hoverflies (reviewed by Badenes-Pérez 2019). The low number of visits to the yellow *S. alba*

335 flowers was probably related to the poor blooming during the sampling period, which might

336 have biased the preference among different insectary plant species as discussed by Ambrosino

337 et al. (2006). Carreck and Williams (1997) reported *S. alba* being attractive to syrphid adults

338 when present in flower mixtures. On the other hand, yellow flowers elicited landing more than

339 other colors (Rodríguez-Gasol et al. 2019).

340 Insectary plants behaved differently as host of aphids and *Eurydema* spp., but not of thrips.

341 Aphids were mostly abundant on *A. millefollium* and *F. esculentum*, and the pentatomid on the

342 two Brasicaceae species. The presence of herbivores in insectary plants placed in the vicinity

343 of crops may have an ambivalent role in biological control. On the one hand, they may act as

344 refuges for pest to further colonize the crop, and on the other, as alternative prey or hosts to

345 boost natural enemy populations. Furthermore, they may also act as trap plants that attract and

346 retain target insects and reduce damage in the main crop. This might be the case for *S. alba* and

347 *L. maritima* and *Eurydema* spp. in our study. Badenes-Pérez et al. (2017) found another

348 Brasicaceae, *Barbarea vulgaris,* useful to reduce damage produced by *Eurydema ornata* L.

349 (Hemiptera: Pentatomidae) in cauliflower. Therefore, their negative or positive role for the

350 crops nearby deserve further investigation.

To summarize, results obtained in the present study indicate that 17 years after the invasion of *T. absoluta* in Northeast Spain, the eulophid *N. tutae* is the main species responsible for larval parasitism of *T. absoluta*, a phenomenon which has been recorded in other Mediterranean production areas (Gebiola et al. 2015). According to our results, the role of parasitoids belonging to other target families in the control of the pest was minimal: they were scarcely present in the fields and were not found parasitizing *T. absoluta* larvae, apart from a few Braconidae. Similarly, the low number of Trichogrammatidae captured in the yellow sticky traps is in accordance with Gabarra et al. (2014), which found egg rates of parasitism to be very low. Therefore, in the area of study, *T. absoluta* biological control is the result of egg predation by *M. pygmaeus* and *N. tenuis* and parasitation of larvae by *N. tutae*.

Moreover, the flowering plants used in the present study were attractive to several natural enemies that are of interest not only for tomatoes but also for other vegetable crops. Although *A. millefollium* was the most attractive for parasitoid wasps and *F. esculentum* for hoverflies, all of them were visited by these two groups of beneficials and other important predators (i.e., *Orius* spp., ladybeetles, and predatory thrips) in vegetable production systems characterized by the coexistence of several crops. Therefore, inclusion of these insectary plants in the *C. officinalis* margins that are already used in the area will surely contribute to increasing functional biodiversity in the farms and benefiting biological control of several pests in different crops. Considering the number of hymenopteran parasitoids in the insectary plants as a proxy for attractiveness to *T. absoluta* parasitoids, our results together with those of Arnó et al. (2018a), which reported benefits of these plant species in the fitness of *T. absoluta* larval parasitoids, suggest that the presence of these insectary plants might benefit the control of this pest. Therefore, it would be necessary to define methods of incorporating these plants into the horticultural agro-ecosystem in order to improve the biological control of pests.

Declarations:

Conflicts of interest. The authors declare that they have no conflicts of interest.

Ethical approval: This article does not contain any study with human participants or vertebrate animals.

Availability of data and material: The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors contributions: JA, RG, and JR conceived and designed the research. All authors conducted experiments and analyzed data. JA and JR wrote the manuscript. All authors read and approved the manuscript.

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574

575 **Table 1**. Number of containers with insectary plants on bloom for each plant species during the

576 sampling periods of 2015 and 2016 out of 12 containers per each insectary plants polling all

577 fields together.

Insectary plants	Week of the year 2015						Week of the year 2016					
	29	31	33	35	37	39	29	31	33	35	37	39
A. millefolium	12	12	12	12	11	12	12	12	12	11	11	10
F. esculentum	12	12	12	12	12	12	12	12	12	12	12	12
L. maritima	12	12	12	12	9	12	12	12	12	10	10	10
S. alba	12	8	6	5*	3*	3	12	12	12	0	0	0

578 *The total number of containers were 11 instead of 12

579

580 **Table 2.** Number of hymenopteran individuals (mean ± standard error) of the different target

581 families per yellow sticky trap placed near to and far from the insectary plant patches and results

582 of the generalized linear mixed-effects model based on the Wald test. The P value > 0.05

583 indicates no significant differences between values in a row. N = 48 traps in each treatment.

584

Families	Treatment		Z	P
	Far	Near		
Eulophidae	0.62 ± 0.15	0.75 ± 0.19	0.454	0.650
Braconidae	0.23 ± 0.11	0.08 ± 0.04	1.395	0.163
Pteromalidae	0.04 ± 0.03	0.04 ± 0.03	0.000	1.000
Ichneumonidae	0.04 ± 0.03	0.02 ± 0.02	0.566	0.571
Trichogrammatidae	0.00 ± 0.00	0.02 ± 0.02	0.002	0.998
Total of target families	0.94 ± 0.19	0.92 ± 0.22	0.225	0.822

585

586

587   **Table 3.** Number of natural enemies (mean ± standard error) per beating sample and hoverfly

588   landings recorded during the visual observations of the four insectary plants species included

589   in the study, and results of the Wald χ^2 tests. Within a row, different letters indicate significant

590   differences between the different plants. If no letters are indicated in a row, the plant

591   comparisons were not significant ($P > 0.05$). Number of samples for each plant is 144.

| Natural enemies	*A. millefollium*	*F. esculentum*	*L. maritima*	*S. alba*	χ^2	*df*	*P*
			Beating sampling				
Orius spp.	0.54 ± 0.13	0.44 ± 0.08	0.57 ± 0.10	0.37 ± 0.09	3.087	3	0.3784
Ladybeetles	0.32 ± 0.09	0.26 ± 0.06	0.19 ± 0.04	0.40 ± 0.14	1.613	3	0.6564
Predatory thrips	0.06 ± 0.02	0.09 ± 0.03	0.10 ± 0.03	0.04 ± 0.02	6.321	3	0.0969
			Visual sampling				
Hoverflies	1.06 ± 0.25b	5.58 ± 0.85a	1.45 ± 0.37b	0.31 ± 0.09c	73.172	3	<0.0001

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594   **Table 4**. Number of phytophagous insects (mean ± standard error) per beating sample of the

595   four insectary plants species included in the study, and results of the Wald χ^2 tests. Within a

596   row, different letters indicate significant differences between the different plants. If no letters

597   are indicated in a row, the plant comparisons were not significant ($P > 0.05$). Number of

598   samples for each plant is 144.

Phytophagous	*A. millefollium*	*L. maritima*	*S. alba*	*F. esculentum*	χ^2	df	P
Thrips	0.70 ± 0.17	0.65 ± 0.15	0.33 ± 0.10	0.73 ± 0.16	0.7528	3	0.8607
Aphids	1.26 ± 0.56a	0.08 ± 0.04b	0.09 ± 0.05b	1.49 ± 0.48a	11.478	3	0.0094
Eurydema spp.	0.29 ± 0.08b	2.67 ± 0.33a	1.60 ± 0.25a	0.49 ± 0.10a	11.636	3	0.0087

599

600
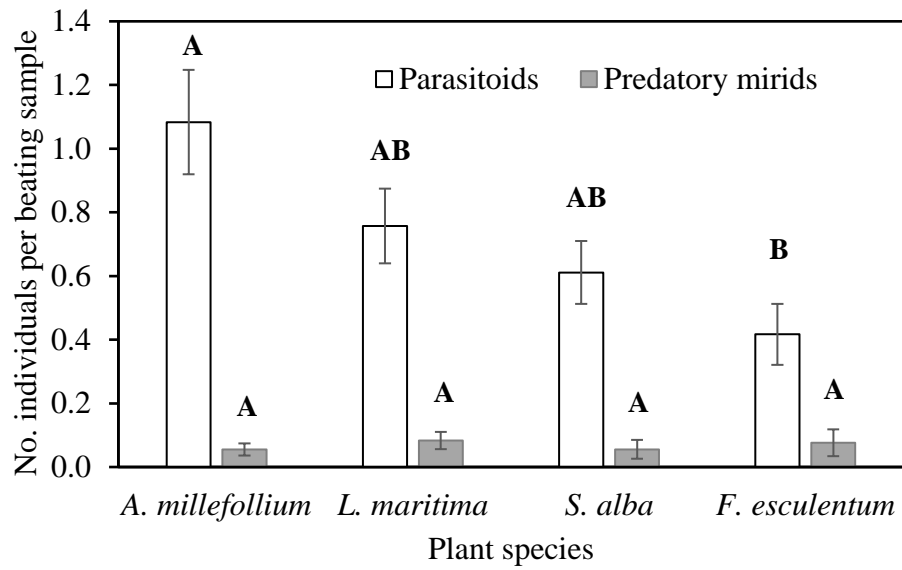
Figure 1. Number of parasitoids and predatory mirids (mean ± standard error) per beating sample found in the different plants included in the study. Different capital letters above the bars indicate significant differences between the different plants for the two types of natural enemies separately ($P<0.05$). The number of samples for each plant species is 144.