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Predatory ability of the ant *Crematogaster scutellaris* on the brown marmorated stink bug *Halyomorpha halys*

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1 **Title Page**

2 **Title**

3 Predatory ability of the ant *Crematogaster scutellaris* on the brown marmorated stink bug
4 *Halyomorpha halys*

5

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21

22 **Abstract**

23 The pest *Halyomorpha halys*, is a recent example of severe worldwide biological invasion. In Italy,
24 after the first detection in 2012, it has rapidly spread and economic damage has already been reported
25 in commercial fruit orchards, eliciting enormous concern for Italian agricultural production. As a
26 long-term solution, biological control investigations are focusing on the potential of natural
27 antagonists, such as generalist predators, in the invaded areas. Due to their biology, ecology and
28 behavior, ants represent one such generalist predator potentially suitable for inflicting mortality on
29 *H. halys*. In this research, *Crematogaster scutellaris* was used as a study model. Specifically, the
30 effect of ant predatory behavior on the survival of all *H. halys* pre-imaginal stages was tested in the
31 laboratory environment. The results suggest that *C. scutellaris* have a significant predatory impact on
32 all *H. halys* pre-imaginal stages except for eggs. The reduction of survivorship is between 95% and
33 85% for instars 1 and 2, and between 38% and 52% for instars 3, 4 and 5. The different levels of
34 predation success suggest that these ants could act as useful generalist predators when associated with
35 other antagonists and/or other control strategies. Although further field investigations are needed, the
36 potential of ants as biocontrol agents of invasive species in agroecosystems deserves consideration in
37 integrated pest management programs.

38

39 **Keywords**

40 Generalist predators, invasive species, natural antagonists, cooperative predatory response, Integrated
41 Pest Management, Biological Control

42

43 **Key Message**

- 44 • The invasive *Halyomorpha halys* is threatening Italian agricultural productions.
- 45 • Ants are generalist predators and potentially suitable for pest control.
- 46 • The ant *Crematogaster scutellaris* was used to test the effects of predation on *H. halys* survival
- 47 • Due to its ability to shift from solitary to cooperative foraging, *C. scutellaris* has a significant
48 impact, but it is more efficient against early immature stages.
- 49 • The synergy with other antagonists and/or control strategies is recommended

50

51

52 **Author Contribution Statement**

53 CC, DAG, LM conceived and designed research. GB and DG collected the data. CC and FAS
54 analysed data. CC, DAG, LM, AM wrote the manuscript. All authors read and approved the
55 manuscript.

56

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60 for English editing and to the reviewers for the valuable suggestions and the revision of the
61 manuscript.

62 **Introduction**

63 The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål, 1855) (Hemiptera:
64 Pentatomidae) is a recent example of severe biological invasion. Native to Eastern Asia, it is now a
65 worldwide menace for several economically important crops, including tree fruits, soybean,
66 vegetables and ornamentals (Haye et al. 2015b; Lee 2015).

67 Outside the Asian continent, *H. halys* was first reported in the United States in the late 1990s
68 (Hoebeke and Carter, 2003), where it is now established in 43 states (USDA-NIFA SCRI 2017). The
69 colonization of Europe is more recent, with the first established population found in Switzerland in
70 2007 (Wermelinger et al. 2008). Subsequently, *H. halys* was recorded in Greece in 2011 (Milonas
71 and Partsinevelos 2014), France and Italy in 2012 (Callot and Brua, 2013; Maistrello et al., 2014),
72 Hungary in 2013 (VéTek et al. 2014), Russia in 2014 (Mityushev 2016), Romania, Serbia and Austria
73 in 2015 (Macavei et al. 2015; Rabitsch and Friebe 2015; Šeat 2015), Bulgaria, Southern France and
74 Spain in 2016 (Maurel et al. 2016; Simov 2016; Dioli et al. 2016). As suggested by Zhu et al. (2012)
75 in their bioclimatic model, Mediterranean countries of Europe could support the geographic
76 expansion of this pest due to the presence of many favourable climatic conditions. This is particularly
77 true for Italy where suitable average temperatures with a long summer season and high diversity of
78 crops are potentially the main aspects helping the establishment of this insect. After the first detection
79 in Italy in 2012 in Emilia Romagna (Maistrello et al., 2014), established breeding populations were
80 found in 2013 in the same region and also in other regions of Northern Italy (Lombardia and
81 Piemonte) and in the Swiss Canton Ticino (Pansa et al., 2013; Maistrello et al. 2016b). A recent
82 investigation shows that this insect is widespread in the northern regions and it is rapidly spreading
83 in the central ones, with occasional detections in the southern regions, including the islands Sicily
84 and Sardinia (Dioli et al. 2016, Maistrello et al. unpublished data). According to Cesari et al. (2015),
85 there are at least two different populations in Italy, which may have originated from two recent but
86 separate invasions. The population found in Lombardia has a greater genetic diversity and it is
87 probably derived from Switzerland whereas the Emilia Romagna population is more uniform and
88 probably originated from a few specimens that arrived by passive transport from North America or
89 Asia. In Emilia Romagna, one of the most important regions for fruit production in Europe (CREA
90 2015), increasing damage has been reported in fruit orchards since 2014 (Maistrello et al. 2016a).
91 Serious economic damage (more than 50% deformed fruits) was recorded at harvest in commercial
92 pear orchards during summer 2015 (Maistrello et al. unpublished data), which elicited a high concern
93 from Italian growers (Fanfani and Pieri 2016).

94 The development of effective procedures to control the BMSB invasion is still in progress. In the
95 United States, particularly in Mid Atlantic states, *H. halys* is a severe pest of fruit orchards (Leskey

96 et al. 2012a) and its management, based on multiple treatments with high dosage broad spectrum
97 insecticides, has disrupted previous Integrated Pest Management programs for other pests, with a
98 negative impact on natural antagonists (Leskey et al. 2012c). Management of BMSB is even more
99 challenging in organic farming, since authorized products are not as effective as those used in
100 conventional farming (Lee et al. 2014b). Moreover, investigations of synthetic insecticides showed
101 that they do not provide satisfactory results, due to low initial knock-down effects and short residual
102 activity (Leskey et al., 2012c). As a long-term and more sustainable solution for the management of
103 BMSB, biological control investigations are focusing on the potential of natural antagonists in the
104 invaded areas. So far, surveys performed both in the US and in Switzerland showed a very low impact
105 of the native species of egg parasitoids (Haye et al. 2015a; Ogburn et al. 2016; Cornelius et al. 2016).
106 In this scenario, an alternative strategy is to look for generalist predators. In their review of
107 manipulative field studies, Symondson et al. (2002) found that in 75% of cases, generalist predators
108 significantly reduced pest numbers. The main advantages in using generalist predators rather than
109 specialists are: a rapid colonizing ability enhanced by the fact that generalists could be already
110 present, subsisting on non-pest prey; a temporal persistence that allows the predator to live also after
111 the decline of pest invasion; an opportunistic feeding habit that lets the generalists quickly shift to
112 pests when their abundance increases (Ehler 1990; Symondson et al. 2002). Focusing on *H. halys*,
113 there are few studies on the identification of efficient generalist predators. Morrison et al. (2016)
114 found that the most efficient predators for *H. halys* eggs were katydids (Orthoptera: Tettigoniidae),
115 ground beetles (Coleoptera: Carabidae), crickets (Orthoptera: Gryllidae), earwigs (Dermaptera:
116 Forficulidae), and jumping spiders (Araneae: Salticidae). Moreover, Morrison et al. (2017) found that
117 adults of *H. halys* were predated by several families of web-building spiders such as Theridiidae,
118 Pholcidae, and Agelenidae.

119 Thanks to several peculiar features of their biology, ecology and behavior, ants represent a model of
120 generalist predators potentially suitable for pest management. In terms of biomass and species
121 richness, they are extremely abundant in most ecosystems and this leads to a high number of
122 interactions with virtually all the other components of their environment, especially the plants and
123 the multitrophic systems based on them (Hölldobler and Wilson 2009, Grasso et al. 2015). They are
124 present in most natural environments but they are able to also colonize urban areas and
125 agroecosystems (Castracani et al. 2010, 2015; Lucky et al. 2014). Due to their efficient recruitment
126 system, ants are able to recruit nest mates to high prey densities, showing the potential for a fast
127 numerical response to pest invasion. In addition, recruitment and other ant behaviors can be
128 artificially manipulated and used to attract/repel ants to focal points (Hölldobler and Wilson 1990;
129 Castracani et al. 2005, 2008; Maňák et al. 2013). Most species are polyphagous and sometimes have

130 polymorphic workers allowing them to deal with a wide range of prey types (Cerdà and Dejean 2011;
131 Campolo et al. 2015). Aggressiveness, territoriality and group defense are the main features that allow
132 ants to attack and deter pests that are far beyond the size of potential prey typical of their predator
133 size (Goheen and Palmer 2010). Territoriality and eusociality lead to stable colonies, both in time and
134 space, which can strongly affect the surrounding environment and provide predictable ecosystem
135 services (Solida et al. 2010, 2011; Del Toro et al. 2012). Finally, their social organization in colonies
136 with many adults and offspring induces the ants to forage beyond the requirements of solitary
137 generalist predators since they need large amounts of resources to feed both adults and larvae
138 (Hölldobler and Wilson 1990).

139 For these reasons, ants have been suggested as control agents against several pests in different
140 agricultural systems all around the world (Rosumek et al. 2009; Campolo et al. 2015; Offenberg
141 2015). *Oecophylla* spp. are a well-documented example of cost-efficient biological control programs
142 producing cascading effects from ants to pest damages and ultimately leading to increased yields (see
143 Offenberg 2015 and references within). In Brazilian sugarcane, species of the genera *Solenopsis*,
144 *Crematogaster* and *Pheidole* are important predators of sugarcane borers as they feed on eggs and
145 preimaginal stages (De Oliveira et al. 2012). In Sweden, species of the genera *Formica* and
146 *Myrmica* were successfully used to control the damage of the pine weevil, *Hylobius abietis* on
147 coniferous seedlings, by attracting the ants with sugar baits (Maňák et al. 2013). Lastly, Baraibar et
148 al. (2009) found that *Messor barbarus* is able to remove up to approximately 80% of artificially
149 placed weed seeds in Spanish fields, suggesting an important role in weed control.

150 The present research is a first attempt to verify the feasibility of ants as possible control agent of *H.*
151 *halys* in Mediterranean regions. *Crematogaster scutellaris* (Olivier, 1792) was used as study model
152 since this species is very common in Italy (both in natural and agricultural systems), arboreal, quite
153 aggressive and can be defined as generalist predator (Ottonetti et al. 2008). Hence, *C. scutellaris* is a
154 good candidate to be tested for *H. halys* control. Specifically, we tested, in a laboratory environment,
155 the effect of ant predatory behavior on the survival of all *H. halys* pre-imaginal stages.

156 **Material and Methods**

157

158 *Test species and breeding conditions*

159 Study prey insects

160 Adults and pre-imaginal stages of *H. halys* were collected by hand and beat sampling from several
161 locations near the city of Parma (Italy) in 2016 (from July to September). Insects were kept in six
162 different ventilated breeding cages (10 dm³) according to their developmental stage: pre-imaginal *H.*
163 *halys* instars (I1 – I2 – I3 – I4 – I5) and adults. Eggs were collected from the adults' cage. Cages were
164 checked daily to spot nymphs that had entered the next instar. All nymphs and adults were reared
165 with carrots, green beans, peanuts, tomatoes and water, in a room where it was possible to control
166 environmental parameters. Rearing conditions were: 25±1°C (Temperature), 50±5% relative
167 humidity and 16:8 hour light:dark photoperiod.

168 Study predator insects

169 Ants of *C. scutellaris* were collected from 10 different field nests located near Parma and Fornoli
170 (Northern Italy). In order to reduce the effects of captivity, ants (200 workers) were collected 1-3
171 days before each experimental session and kept in artificial plastic nests (Ø: 20 cm; H: 10.5 cm) with
172 natural nest fragments and wet cotton balls. Ants were starved for at least 24 hours before the
173 experiments. Rearing conditions were: 25±1 °C (Temperature), 50±5% relative humidity and 16:8
174 hours light:dark photoperiod. The ants were reared separately from prey, in a second room where it
175 was possible to control environmental parameters.

176

177 *Experimental Arena*

178 An open plastic box (Ø: 20 cm; H: 10.5 cm) was used as the experimental arena. The arena and the
179 ant nest were connected by a removable plastic tube (Ø: 2.5 cm; L: 7 cm) where it was possible to
180 control the access of the ants. In the center of the arena, we placed a bean plant (*Phaseolus vulgaris*)
181 (H: 14 cm) with at least two well-developed leaves. Plants were cultivated in our laboratory from
182 seeds (cultivation period: 13±3 days). Inside the arena, plants were located in a plastic pot (Ø: 8 cm)
183 with topsoil (©Compo Sana – Universal Soil). A wood stick (L: 12 cm) was used as bridge allowing
184 the ants to easily reach the plant.

185

186 *Experimental Protocol*

187 For each test, and 20 min. before the beginning of data recording, we connected the experimental
188 arena to the ant nest. During this interval, the ants were not allowed to enter the arena. In the
189 meantime, eggs or nymphs were placed on the leaves of the bean plant. In each test we used one of

190 the six types of prey: eggs (E) and the five pre-imaginal instars (I1 – I2 – I3 – I4 – I5). A unique mass
191 of 28 eggs was removed from the sides of rearing cages and then stitched on the abaxial surface of
192 one leaf in the arena. Alternatively, six individuals of the same pre-imaginal stage were randomly
193 placed on the adaxial surface of the two leaves. We performed a total of 60 tests (10 replicas for each
194 prey). Since ants and all prey pre-imaginal stages were not available simultaneously, replicates were
195 performed over time. The same individuals (prey or predators) and plants were used for only one
196 replicate.

197 At the beginning of the test (t_0), the ants were allowed to enter the arena and a continuous observation
198 was carried out for 30 min. During this continuous sampling, we recorded the latency of the first ant-
199 plant contact (Plant Latency), the time from t_0 to the first ant climbing the plant. Additionally, we
200 recorded the latency of the first ant-prey contact (Prey Latency), the time from t_0 to the first ant
201 touching the prey. Continuous sampling was set at 30 min because this was the highest Prey Latency
202 value recorded.

203 Three scan-samplings were performed, respectively after 2hrs (t_1), 24hrs (t_2) and 48 hrs (t_3). In each
204 scan sampling (duration: 2 min), the number of survived prey was recorded. Eggs were considered to
205 have survived predation if they were not damaged (the chorion was not pierced/opened) or not moved
206 from the original position. Pre-imaginal stages were considered to have survived predation if they
207 were still alive or moribund according to definitions in Leskey et al. (2012b).

208 To test the possible effect of the experimental setup on prey's survival, an additional control arena
209 connected to the nest was used in each test, but in this case, the ants were not allowed to enter. After
210 48 hrs, the number of surviving prey was recorded (see above for survival conditions). To confirm
211 that surviving eggs were not fatally damaged, they were reared for two additionally weeks. For both
212 treatment (with ants) and control (without ants) arenas, the number of hatched eggs was recorded.

213

214

215 *Statistical analysis*

216 To test the effect of ants on prey survival, *survival* was calculated by dividing the number of live prey
217 at the end of the scan sampling by the number of initial prey. For eggs only, *hatching* was calculated
218 by dividing the number of hatched eggs (after 15 days) by the number of undamaged eggs at the end
219 of the experiment (t_3 scan).

220 In order to test the ant predatory ability, a first database was created from trials where ants were
221 present (N=180: 10 replicas*6 prey types*3 scans). *Survival* was used, as dependent variable, in a
222 two-way ANOVA, followed by a Tukey post-hoc test. Prey (Eggs – I1 – I2 – I3 – I4 – I5) and scans
223 (t_1 – t_2 – t_3) were considered fixed factors. Interaction between the two factors was also considered in

224 the model. In this analysis, a transformed version of *survival* was used according to the following
225 formula: $TrSurvival = 2 * \arcsine(\sqrt{Survival})$ (Lehner 1996).

226 To test the possible effect of experimental setup, a second database was created from data on *survival*
227 after 48 hrs, both in treatment (with ants) and control (without ants) trials (N=120: 10 replicas*6 prey
228 types*2Treatments). *Survival* was used, as dependent variable, in a two-way ANOVA, followed by
229 a Tukey post-hoc test. Prey (Eggs – I1 – I2 – I3 – I4 – I5) and treatment (ants vs control) were
230 considered fixed factors. Interaction between the two factors was also considered in the model. In this
231 analysis, the transformed *survival* data were used according to the following formula: $TrSurvival =$
232 $2 * \arcsine(\sqrt{Survival})$ (Lehner 1996). For eggs only, *hatching* data were used to perform a one-
233 way ANOVA, comparing trials where ants were present (treatment) with trials without ants (control).
234 In this analysis, transformed *hatching* data were used according to the following formula:
235 $TrHatching = 2 * \arcsine(\sqrt{Hatching})$ (Lehner 1996).

236 Plant Latency and Prey Latency, recorded during the initial continuous sampling, were used as
237 dependent variables in a one-way ANOVA followed by a Tukey post-hoc test. Prey (Eggs – I1 – I2
238 – I3 – I4 – I5) were considered as fixed factor.

239 All tests were run with the software IBM SPSS Statistics, Italian Version 24 and in all Tukey post-
240 hot tests the alpha level was fixed at 0.05.

241

242

243 **Results**

244 Concerning the predator ability, the two-way ANOVA (fixed factors: prey, scans) and following post-
245 hoc tests on *Survival* showed that ants have a different effect on prey survival according to prey type,
246 scan period and their interaction (Prey: $F_{(5,162)}=30.12$, $P<0.001$; Scan: $F_{(2,162)}=49.39$, $P<0.001$;
247 Prey*Scan: $F_{(10,162)}=2.72$, $P=0.004$; see Fig.1). According to prey type, three different clusters were
248 found: the first one refers to eggs that have the highest levels of *survival* during the whole experiment;
249 the second cluster includes I1 and I2 that show the most rapid decrease in *survival* and the lowest
250 values of *survival*; the third cluster includes I3, I4 and I5 and shows values in between the previous
251 two. According to scan period, a decrease in the *survival* rate from the beginning to the end of the
252 experiment was found.

253 Concerning the effect of the experimental setup, the two-way ANOVA (fixed factors: prey, treatment)
254 and following post-hoc tests on *survival* showed that there is a statistically significant effect of both
255 factors and their interaction (Prey: $F_{(5,108)}=20.73$, $P<0.001$; Treatment: $F_{(1,108)}=222.53$, $P<0.001$;
256 Prey*Treatment: $F_{(5,108)}=21.51$, $P<0.001$; see Fig.2). According to prey type, three different clusters
257 were found as shown previously. According to treatment, the presence of ants reduce prey survival

258 except for eggs. The one-way ANOVA on *hatching* showed that the presence of ants has no effect on
259 egg *hatching* (Treatment – Ants: n=10, Mean±SE=0.95±0.01; Control – No Ants: n=10,
260 Mean±SE=0.85±0.05; $F_{(1,18)}=4.14$, P=0.057).

261 Although the aim of the present study was not focused on the ethological aspects of ant-*H. halys*
262 interactions, we verified that all instars were fiercely attacked by one or more ants simultaneously.
263 The ants were often observed biting the bugs, sometimes dragging the prey to the nest, but seldom
264 raising the gaster or using venom (Fig. 3).

265 The one-way ANOVAs on Plant and Prey Latencies show no differences among the six types of prey
266 (Plant Latency: $F_{(5,54)}=1.86$, P=0.116; Prey Latency: $F_{(5,54)}=2.06$, P=0.085; see Tab.1).

267

268 Discussion

269 In this research, a laboratory approach was used for the first time to evaluate the role of ant predatory
270 behavior on survival rates of *H. halys* pre-imaginal stages. The use of ants as pest control agents in
271 agriculture has already been suggested and several examples are present in literature (see Offenberg
272 2015 and references within). However, this is the first attempt to test the suitability of a native ant
273 species (*Crematogaster scutellaris*) to manage the recent invasion of the brown marmorated stink
274 bug "*H. halys*" in Italy and to provide useful information for further development of biological control
275 of this pest.

276 Data on the survival rates at the end of the whole observation window suggested that ants have a clear
277 positive effect in reducing the survival rates of all stages, except for eggs. After a 48hr period of
278 permanence in a close system with ants, the survival rates of *H. halys* is reduced to a value around
279 50% for instars 3-5 and 9% for instars 1-2. If we consider the first instar alone, the presence of ants
280 reduced the bug survival to 3%, indicating the potential for a dramatic impact on the *H. halys*
281 population soon after hatching. These results suggest that *C. scutellaris* can consume *H. halys* and
282 quickly respond to this pest. To our knowledge, this is one of the first examples where the success of
283 a generalist predator is demonstrated for all the pre-imaginal stages, whereas most previous studies
284 had focused on the susceptibility of *H. halys* eggs (Abram et al. 2015; Morrison et al. 2016).
285 According to our observations, the ants seem to employ physical rather than chemical aggression
286 towards *H. halys*. In fact, interactions with the bugs were followed by biting and grasping on the prey
287 but no gaster rising or use of venom was recorded as it normally happens during alarm for danger or
288 aggressive encounters with competitor ants (Marlier et al. 2004).

289 Tests on egg survival and hatching rates showed that ants have no effect on them. These results are
290 similar to those found by Abram et al. (2015). They tested the capacity of three generalist predators,
291 *Chrysoperla carnea* (Neuroptera: Chrysopidae), *Coleomegilla maculata* (Coleoptera: Coccinellidae),
292 and *Podisus maculiventris* (Hemiptera: Pentatomidae), to attack *H. halys* eggs in a laboratory
293 environment concluding that none of them were able to consistently consume eggs. A screening of
294 native generalist predators of *H. halys* in the United States demonstrated that the most frequent and
295 efficient predators were members of the family Tettigoniidae (katydids) and Carabidae (ground
296 beetle) (Morrison et al. 2016). It was shown that chewing (opposed to sucking) was the feeding
297 damage most frequently documented in the field and most efficient in terms of number of eggs
298 consumed. In the same study, it was also documented that ants showed no interest in eggs. Studies
299 on the survival of the American populations of the pest *Nezara viridula* (Hemiptera: Pentatomidae)
300 showed that the ant *Solenopsis invicta*, an invasive species native of South America, is an important
301 predator of *N. viridula* eggs (Tillman 2010, 2011, 2015; Olson and Ruberson 2012). According to our

302 observations, some hypotheses can be suggested to explain the failure of ants on *H. halys* eggs. The
303 egg structure and the deposition system could make them less vulnerable to the attacks of relatively
304 small predators like *C. scutellaris*. In fact, in this species, worker's mandibles are smaller than *H.*
305 *halys* eggs and this could complicate their manipulation. Moreover, in our experiments, the eggs were
306 placed underneath the leaves in order to simulate the natural situation (Lee et al. 2013). This
307 deposition site could have influenced the foraging of the predators, preventing optimal access and
308 grasping of the eggs. Interestingly, data on prey latency, although not statistically significant, showed
309 a trend where workers needed more time to locate eggs in comparison with the nymphal stages. This
310 may be due to visual stimulation or accidental substrate vibrations produced by the instars 1-5 during
311 their movements and attracting the ants. However, chemical cues (or their absence) may be involved.
312 Volatiles and surface chemicals, such as cuticular hydrocarbons or other lipids, play a major role in
313 modulating ant behavior towards potential prey (Schatz and Hossaert-McKey 2010; Cerdá and
314 Dejean, 2011). Concerning eggs, the presence (or absence) of specific odors can allow the ants to
315 recognize or discard them as a possible food source. Mechanisms similar to “chemical insignificance”
316 (i.e. the lack of external chemical substances) typical of newborn ants and suggested for some ant
317 parasites could be involved in this context (Lenoir et al. 2001). Otherwise, eggs could have repellents
318 influencing the ant behavior as demonstrated for the parasitoid wasps *Trissolcus erugatus* and
319 *Telenomus podisi* (Tognon et al. 2016). Further field and laboratory investigations are needed to
320 answer these questions.

321 Data on nymphal stages showed that *C. scutellaris* predator ability changed according to instar and
322 time. In fact, ants had the strongest and most rapid effect on *H. halys* early nymphal stages (I1, I2),
323 reducing their survival rates to 3% and 15%, respectively. However, they were able to attack also the
324 older stages with a survival rate of nearly 55% (I3, I4, I5). Similar findings were obtained by Krispyn
325 and Todd (1982), who found that the severe reduction caused by *S. invicta* in populations of caged *N.*
326 *viridula* was mainly due to the highest vulnerability of nymphal stages, especially I1-3. They
327 hypothesized that the tendency of the bugs to remain aggregated during early instars made them easy
328 victims of the foraging methods of fire ants, which utilize recruitment and trail following whenever
329 prey clusters are discovered. In *H. halys*, I1 and I2 are smaller and have a reduced mobility (Lee et
330 al. 2013; Lee et al. 2014a) and this suggests that a single worker of *C. scutellaris* can easily succeed
331 in attacking and killing them. Since older nymphs are bigger and more mobile, it can be assumed that
332 the predatory efficiency of a single worker would be reduced. In contrast to other generalist predators,
333 ants, being social insects, can build up a cooperative predatory response. A cooperative response is
334 clearly slower than a solitary one, since ants need to recruit their nestmates, but it can still be efficient.

335 Our hypothesis can explain the differences we found on survival rates among nymphal stages, but
336 further laboratory and field researches are needed to verify it.

337 Our data on plant and prey latencies seem to suggest that ant-prey encounters are more connected to
338 chance than to other factors. However, these preliminary data deserve further investigation because
339 chemical, visual and mechanical stimuli from the prey or the combination of these factors may be
340 more effective to address ant behavior and elicit predation (Campolo et al. 2015). This may have
341 significant implications, as understanding the nature of stimuli eliciting specific reactions
342 (aggression, attraction to specific areas, recruitment) could be used to direct the behavior of ants. In
343 this context, since cooperative predation based on chemical recruitment is a basic strategy adopted
344 by many ants including *C. scutellaris* (Hölldobler and Wilson 1990), this may be used to increase ant
345 presence towards the prey.

346 In traditional agricultural practice, ants have often been considered as harmful due to their possible
347 trophobiotic relationships with sap-sucking hemipterans and their eventual interference with
348 beneficial insects. However, there is increasing evidence of their positive impact in many key aspects
349 of biological control (see Rosumek et al. 2009; Campolo et al. 2015; Grasso et al. 2015; Offenberg
350 2015). Finally, ants are quite common in agroecosystems but not all the species have the same
351 ecology, behaviour and impact on their multitrophic networks (Castracani et al. 2010). In this context,
352 field investigations are required to study the multifaceted aspects of this trophic interaction and to
353 assess, by a cost/benefit approach, the actual impact of *C. scutellaris* and other ant species on *H. halys*
354 in Mediterranean regions.

355 In conclusion, these first experiments on ant-BMSB interactions suggest that *C. scutellaris* may have
356 an effective predatory impact. Further research is needed to verify if the impact on *H. halys* nymphs
357 is enhanced when ants are shifting from solitary to cooperative foraging, overcoming large and
358 difficult prey. Moreover, this pilot study was performed in laboratory conditions and a validation in
359 the field is needed to test the potential of *C. scutellaris* for biological control where several factors
360 can affect the ants as, for example, the presence of alternative prey. However, different levels of
361 predation success suggest that ants alone probably cannot control *H. halys* populations and that they
362 could only be effective when combined with other control measures.

363

364 **Table 1 . Mean Plant ant Prey Latency calculated during the continuous sampling (30 min) and shown**
 365 **according to prey type (Eggs – I1 – I2 – I3 – I4 – I5).**

Plant Latency				Prey Latency			
	N	Mean (Sec.)	SD		N	Mean (Sec.)	SD
Egg	10	564	435,6	Eggs	10	1200	555,0
I1	10	624	313,9	I1	10	1152	688,1
I2	10	871	480,5	I2	10	945	512,8
I3	10	931	559,7	I3	10	762	547,0
I4	10	638	438,2	I4	10	520	314,2
I5	10	1086	604,3	I5	10	766	718,8
Total	60	786	498,5	Total	60	891	596,5
F(5,54)= 1.86, p=0.116				F(5,54)= 2.06, p=0.085			

366

367 For both latencies, the number of replicates (N), the mean value of latency (Mean) and the Standard
 368 Deviation (SD) are shown. F and p values refer to one-way ANOVA calculated separately for each
 369 latency (see text for further details).

370

371 **Figure Captions**

372 **Fig. 1**

373 Mean *survival* rates (n=10) calculated for prey type (Eggs – I1 – I2 – I3 – I4 – I5) and shown according
374 to the three scan samplings ($t_1=02\text{hrs}$; $t_2=24\text{hrs}$; $t_3=48\text{hrs}$) when ants were present. For each bar, SE
375 interval is shown. Bars with the same letter are not statistically different (Two-way ANOVA, see text
376 for further details).

377 **Fig. 2**

378 Mean *survival* rates (n=10), after 48hrs, calculated for prey type (Eggs – I1 – I2 – I3 – I4 – I5) and
379 shown according to treatment (ants vs no ants). For each bar, SE interval is shown. Bars with the
380 same letter are not statistically different (Two-way ANOVA, see text for further details).

381 **Fig. 3**

382 Attacks by *Crematogaster scutellaris* workers on different instars of *Halyomorpha halys*. (a) A
383 worker chewing a first instar bug; (b) A worker biting and transporting a second instar bug; (c)
384 Cooperative attack on an instar 5.

385 **Conflict of Interest**

386 All authors declare that they have no conflict of interest.

387 **Ethical approval**

388 This article does not contain any studies with human participants or animals (vertebrate) performed
389 by any of the authors.

390 **Informed consent**

391 Informed consent was obtained from all individual participants included in the study.

392

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