

Trissolcus japonicus foraging behavior: implications for host preference and classical biological control

Robert Malek^{1,2}, Joe M Kaser^{3*}, Gianfranco Anfora^{2,4}, Marco Ciolli^{1,4}, Ashot Khrimian⁵, Donald C Weber⁵, Kim A Hoelmer³

¹ Department of Civil, Environmental and Mechanical Engineering, University of Trento, Trento, TN, Italy

² Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige, TN, Italy

³ Beneficial Insects Introduction Research Unit, USDA Agricultural Research Service, Newark, DE, USA

⁴ Center for Agriculture, Food and Environment (C3A), University of Trento, San Michele all'Adige, TN, Italy

⁵ Invasive Insect Biocontrol and Behavior Laboratory, USDA Agricultural Research Service, Beltsville, MD, USA

* Corresponding author, e-mail: joseph.kaser@usda.gov; Address: 501 S. Chapel St., Newark, DE 19713 USA

Abstract:

The brown marmorated stink bug, *Halyomorpha halys*, is an alien pest native to East Asia, which in the past two decades has invaded USA, Europe and other countries around the globe, causing severe economic losses and public nuisance. The Asian egg-parasitoid *Trissolcus japonicus* is the most promising agent currently under study for the classical biological control of *H. halys*. The foraging behavior of this wasp was investigated in response to chemical traces - 'footprints' - deposited by its host *H. halys* and by a suboptimal predatory host species, the spined soldier bug, *Podisus maculiventris*. Motion tracking software (Ethovision) was employed to record and analyze the behavior of *T. japonicus* on stink bug-contaminated and on control substrates. Wasps exhibited a 'motivated searching' behavior (i.e. longer residence time, slower walking velocity, higher angular velocity and coverage of greater distances) in response to footprints originating from females and from males of both species compared to blank controls. However, this searching behavior was significantly more intense on *H. halys* footprints, compared to those of *P. maculiventris*. Moreover, *T. japonicus* significantly intensified its searching on footprints of all mobile nymphal instars of *H. halys*, but not on those of *P. maculiventris* nymphs. Additionally, the longevity of *H. halys* female trails was assessed, and footprints remained bioactive, eliciting a consistent searching behavior in the wasp for 72 hours after initial deposition. A series of GC-MS chemical analyses revealed components of these trails, with *n*-tridecane and (*E*)-2-decenal found to be the most abundant, and probably the key components of the kairomone utilized by the wasp

32 for short range host location. Solutions of the synthetic compounds were tested both together
33 and singly. The blend of *n*-tridecane and (*E*)-2-decenal (4: 1) significantly prolonged the
34 residence time of *T. japonicus*, as did *n*-tridecane alone, whereas (*E*)-2-decenal significantly
35 shortened the wasp's residence time on filter paper. These results are discussed in the context
36 of host preference and classical biological control.

37 **Keywords:** Biological control; Invasive pest; Kairomone; Risk assessment; Chemical ecology;
38 Trophic interaction

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1. Introduction:

The ecology of information is the study of how organisms provide, acquire, and use information in decision-making to manage their lives and secure current and future reproductive success (Schmidt et al. 2010). Faced with environmental complexity, organisms can acquire information about their physical and biotic environment (or future environment) that reduces uncertainty and improves their likelihood of increasing fitness (Stephens 1989). Upon emergence in a new complex environment, female parasitoids are faced with several challenges related to mate and host finding to ensure offspring survival. Thus, parasitoid fitness is strongly dependent on their ability to locate hosts in a timely manner, before the target life stage becomes unsuitable due to rapid development (Godfray 1994). Parasitoids of the third trophic level often use herbivore-induced plant volatiles (HIPVs) and/or oviposition-induced plant volatiles (OIPVs) as long-range cues to locate their inconspicuous hosts (Mumm and Dicke 2010; Hilker and Fatouros 2015). After landing on a host-infested plant, parasitoids exploit short-range and/or contact kairomones such as gustatory, visual and olfactory cues (Vet and Dicke 1992; Aartsma et al. 2018). During this phase of parasitoid foraging, chemical traces originating from the host (termed indirect host-related cues) elicit a ‘motivated searching’ and play an important role in finding and locating life stages suitable for oviposition (Colazza et al. 1999; Colazza et al. 2007; Peri et al. 2016).

When exotic organisms are introduced into new environments, novel interactions encompassing several trophic levels may arise and lead to unpredictable ecological consequences. Laboratory host range studies are useful in predicting the likelihood of non-target parasitism by introduced biological control agents, but they often overestimate their ecological host range (i.e. the number of host species that are parasitized in the field) (Babendreier et al. 2003; Haye et al. 2005). Such overestimates may hamper the development of successful classical biological control programs, especially because of strict regulatory limitations on the importation and release of natural enemies (Mason et al. 2013). Success of a biological control program utilizing egg parasitoids is largely dependent upon the behavioral decisions made by the biological control agent in searching for and parasitizing host eggs (Mills and Wajnberg 2008). According to Duan and Messing (1997), behavioral tests are as important as suitability tests when assessing host specificity.

89 A relevant system for such an investigation is that of the brown marmorated stink
90 bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), its main egg-parasitoid, the
91 samurai wasp, *Trissolcus japonicus* Ashmead (Hymenoptera: Scelionidae) and an
92 indigenous North American predatory stink bug, the spined soldier bug, *Podisus*
93 *maculiventris* Say (Hemiptera: Pentatomidae). The latter is a generalist predator known to
94 feed on 50 insect species (McPherson et al. 1980), including important agricultural pests,
95 notably *H. halys* eggs (Pote and Nielsen 2017). *Halyomorpha halys* is an invasive pest that
96 feeds on over 170 plants, including economically important fruit trees, vegetables, row
97 crops, as well as ornamentals (Lee et al. 2013). This insect is native to Eastern Asia, but its
98 occupied range expanded outside of its areas of origin, probably due to global anthropogenic
99 forcing (Hulme et al. 2008), thus spreading through most of the United States, and into
100 Canada, Chile, and numerous European and Eurasian countries (EPPO 2019). Feeding by
101 *H. halys* results in deformed, symptomatic produce with indents on the surface and corky
102 spots in the flesh, hampering marketability (Nielsen and Hamilton 2009). It is estimated
103 that over \$21 billion worth of crops in the United States alone are threatened by *H. halys*
104 feeding damage (ODA 2016). Besides being highly polyphagous, *H. halys* is capable of long-
105 distance flight and dispersal (Lee and Leskey 2015) making it a hard-to-control landscape
106 level pest. Phenological models and laboratory studies predicted the capacity for bivoltinism
107 throughout the United States and Italy (Costi et al. 2017; Nielsen et al. 2016), matching
108 field observations (Acebes-Doria et al. 2019), whereas the completion of only one generation
109 per year was suggested by field studies in Switzerland (Haye et al. 2014). Additionally, *H.*
110 *halys* tends to aggregate in man-made structures in late autumn to protect itself from
111 harsh winter conditions (Inkley 2012), rendering it a pervasive residential nuisance.

112 Managing outbreaks and high-density populations of this pest relies mostly on the
113 extensive use of broad-spectrum insecticides that were found to be only partially effective
114 and which disrupted already established IPM programs (Kuhar and Kamminga 2017). An
115 integrated approach has since been in development, with trap crops, attract and kill,
116 insecticidal nets and other techniques being evaluated and adapted for its control.
117 Indigenous natural enemies (predators, parasitoids and entomopathogens) across North
118 America and Europe were found to be incapable of significantly reducing this pest's
119 populations in most cropping systems (Abram et al. 2017). Moreover, an evolutionary
120 mismatch expressed by physiological incompatibilities between native egg-parasitoids and

121 *H. halys* is potentially causing the latter's egg masses to act as an evolutionary trap,
122 reducing the indigenous wasp populations, and potentially increasing that of the native
123 pentatomids (Abram et al. 2014; Kaser et al. 2018). For these reasons, classical biological
124 control may be the best management option for this pest at a landscape scale, based upon
125 *T. japonicus*, the most effective egg parasitoid of *H. halys* in its native range in Eastern
126 Asia (Yang et al. 2009).

127 *Trissolcus japonicus* is a solitary endoparasitoid of *H. halys* eggs throughout its native
128 range. It can complete multiple generations per year and has a highly female-biased sex
129 ratio (Qiu et al. 2007; Yang et al. 2009) with parasitism rates in China reaching up to 90%
130 (Zhang et al. 2017). Based on CLIMEX models, Avila and Charles (2018) projected that *T.*
131 *japonicus* will naturally spread in all places where *H. halys* is known to occur and in most,
132 if not all, areas where *H. halys* has been predicted to expand. Adventive *T. japonicus*
133 populations, presumed to be accidentally introduced, were first detected in the USA in
134 Maryland in 2014 (Talamas et al. 2015) and subsequently recorded in Virginia, West
135 Virginia, Delaware, New Jersey, New York, Oregon, Washington, and the District of
136 Columbia (Leskey and Nielsen 2018), and most recently in Pennsylvania, Ohio, Michigan,
137 California and Utah (NE IPM 2019). It has also been reported in the Canadian provinces of
138 British Columbia (Abram et al. 2019) and Ontario (Gariepy and Talamas 2019). In Europe,
139 the first report of *T. japonicus* populations emerged in 2018 after field surveys in
140 Switzerland detected the wasp for two consecutive years in three different locations (Stahl
141 et al. 2018). Shortly after, reports of the wasp's presence followed from the neighboring
142 Lombardia region in Northern Italy (Sabbatini Peverieri et al. 2018). Field surveys along
143 with choice and no-choice laboratory studies revealed that *T. japonicus* is capable of
144 parasitizing multiple pentatomid species, with a fundamental host range that includes
145 predatory stink bugs such as *Arma custos* F., *A. chinensis* Fallou and *P. maculiventris*
146 (Zhang et al. 2017; Haye et al. 2019). Botch and Delfosse (2018) found that *T. japonicus*
147 exhibited strong preference for its naturally associated host *H. halys* when reared on its
148 eggs. However, when reared on non-target hosts including *P. maculiventris*, the wasp
149 showed reduced host specificity that came with measurable trade-offs with respect to brood-
150 size and fertility, suggesting specialization to *H. halys*. A recent study by Boyle et al. (2019)
151 showed that *T. japonicus* responds more actively to adult *H. halys* footprints deposited on

152 various leaf substrates compared with those of *P. maculiventris*, but that study did not
153 examine the chemical components influencing the wasp's response.

154 Therefore, in this study we investigated the behavioral preferences of *T. japonicus* in
155 response to indirect host-related and-suboptimal-host-related cues, namely to the
156 "footprints" (chemical residues deposited on substrates after passage of adults and nymphs)
157 of *H. halys* and *P. maculiventris* respectively. We also assessed the longevity of female *H.*
158 *halys* footprint: the duration for which it remains bioactive. Finally, we identified the
159 underlying chemicals shaping *T. japonicus* foraging behavior and decoded the role of the
160 two main detected compounds.

161 **2. Materials and Methods:**

162 *2.1 Insects:*

163 Field-collected and laboratory-reared *H. halys* and *P. maculiventris* adults and
164 nymphs were kept in walk-in rearing chambers at the USDA ARS Beneficial Insects
165 Introduction Research Unit in Newark, DE, under controlled environmental conditions (24
166 $\pm 2^\circ\text{C}$, 60% R.H., 16:8 L:D). *Halyomorpha halys* were reared in screened cages (35 x 42 x 47
167 cm) containing fresh (2-3 weeks) green bean plants, *Phaseolus vulgaris* L., as substrates for
168 egg deposition. They were also provided with hulled sunflower seeds, fresh green beans,
169 grapes, slices of apples and carrots for food and water-soaked pieces of cotton. *Podisus*
170 *maculiventris* were provided with green beans, greater wax worms, *Galleria mellonella* L.,
171 and water-soaked pieces of cotton. Pieces of foam were hung from the lid served as
172 substrates for oviposition. All plant and food material were changed bi-weekly or as needed.
173 For footprint extracts, *H. halys* used were obtained from the USDA ARS Invasive Insect
174 Biocontrol and Behavior Laboratory colony in Beltsville, MD, reared under similar
175 conditions and provided with fresh green beans hulled raw sunflower seeds and buckwheat
176 seed as well as water ad libitum. *Podisus maculiventris* used for extracts also came from
177 the Beltsville IIBBL laboratory colony and were reared similarly as above. Starter colonies
178 from a population of *Trissolcus japonicus* originally collected in Beijing in 2007 were reared
179 and maintained at the USDA ARS Newark quarantine facility under controlled conditions
180 ($24 \pm 2^\circ\text{C}$, 40% R.H., 16:8 L:D). Wasps were reared on *H. halys* egg masses attached to card
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183 stock using sand-sprinkled double-sided sticky tape in 10-dram snap cap vials and provided
184 with a drop of honey on the lid.

185 2.2 *Footprint foraging bioassay* 186

187 All experiments were carried out in the quarantine facility of USDA-ARS laboratories
188 in Newark DE, at 23±1 °C and 35±10 % RH. Step (1): to collect its chemical footprints, a
189 single stink bug was placed in an arena consisting of a petri dish, confined in a plastic
190 container and motivated to walk on a filter paper (4.25 cm ø) for 30 minutes. Whenever the
191 insect rested and stopped walking, the container was gently rotated to stimulate continued
192 movement. Step (2): the contaminated filter paper was then transferred to a sterile glass
193 petri dish (8 cm ø) which was placed on a LED light pad (MEDALight LP-400N, Technical
194 Lamps Ltd, Middlesex, United Kingdom) providing illumination from below to optimize
195 visibility and contrast. Directly above the center of the light pad, a camera (ICD-49,
196 Ikegami Tsushinki Co., Ltd., Tokyo, Japan) was mounted on a portable tripod and
197 connected to a video monitor and desktop PC. Step (3): a single 2-4 day-old, mated, naïve
198 (i.e. had no previous experience of stink bug chemicals except for the *H. halys* egg mass
199 from which it emerged) *T. japonicus* female was placed in the arena and confined under a
200 plastic lid (0.5 cm height). Using the motion tracking software EthoVision XT 8.0 (Noldus
201 Information Technology, Wageningen, The Netherlands) the behavior of the wasp was
202 recorded, tracked and processed in real-time. Step (1) was repeated using single gravid
203 females, males, 5th instars, 4th instars, 3rd instars and 2nd instars of either *H. halys* and *P.*
204 *maculiventris*. The variables measured included the wasp's residence time (time in seconds
205 spent on the filter paper), mean linear velocity (mm/s), angular velocity (°/s) and distance
206 moved (mm). These variables are characteristic of a wasp's motivated searching, which
207 starts with a motionless period with the antennae kept in contact with the surface, followed
208 by drumming of the substrate and alteration in the orthokinetic and klinotaxic locomotion,
209 and increased turning frequency (Gardner and van Lenteren 1986; Peri et al. 2006; Colazza
210 et al. 2007). Recording commenced 2 seconds after the wasp was detected on the filter paper
211 by the software and stopped after a maximum of 10 minutes or after the wasp left the
212 substrate for a cumulative 15 seconds. Each wasp was used for only one replicate, and 30
213 replicates were conducted for each treatment. Control treatments were conducted using

214 non-contaminated filter paper. For all treatments, each filter paper was used for 5
215 replicates.

216 2.3 *Footprint longevity* 217

218 The duration for which *H. halys* female footprints elicit a motivated searching
219 behavior in *T. japonicus* was assessed up to 4 days after initial deposition. In total, 44 filter
220 papers were contaminated following the same above-described method in step (1). A subset
221 of 11 treated filter papers were exposed to 11 unique 2-4-day-old naïve, mated *T. japonicus*
222 females at day 1 (within 1 hour after footprint-contamination) and treated filter papers
223 were alternated with untreated controls. The remaining footprint-contaminated filter
224 papers were individually stored in closed plastic petri dishes inside a climatic chamber
225 (24°C, 16h L: 8h D and 70% RH) until needed. At each consecutive treatment (days after
226 exposure), 11 treated filter papers were utilized at day 2, 3 and 4 (24, 48 and 72 hours after
227 initial footprint-deposition, respectively).

228 2.4 *Chemical extraction and identification* 229

230 For extraction of the chemical footprints of the different life stages of *P. maculiventris*
231 and *H. halys*, we slightly modified the exposure method described in step (1). We used
232 sterile Pyrex petri dishes (150 x 15 mm) that were washed, cleaned with acetone and placed
233 into an oven at 140 °C to remove any remnants of volatile compounds. Afterwards, instead
234 of exposing the 4.25 cm ø filter paper to one bug, we contaminated a 150 mm ø filter paper
235 by confining 20 bugs and allowing them to walk for 30 minutes. Filter papers were then
236 removed with sterile forceps, inspected for fecal deposits and if any were observed, the
237 contaminated section was cut out with sterile scissors rinsed with acetone. Five filter
238 papers, on which no stink bug walked, were similarly enclosed in a petri dish and
239 considered as controls. Filter papers were then cut into four equal parts, rolled, inserted
240 into the anterior part of a sterile glass tube and washed by applying 0.5 ml
241 dichloromethane, of which 100 µl were kept for GC-MS analyses and the rest stored at -80
242 °C and later utilized for behavioral bioassays. The chemical analyses were conducted using
243 HP-5 MS UI (30 m x 320 µm x 0.25 µm) column on Agilent technologies 7890B GC System
244 coupled to a 5977B GC/MSD in EI mode. Samples were handled by Agilent technologies
245 7693 Autosampler and injected in a pulsed-splitless mode. Inlet temperature was 200°C

246 and the transfer line was set at 270°C. Oven temperature was set at 40 °C, held for 5 min,
247 then programed to 270 °C at 10 °C/min; the carrier gas was He at 2 ml/min.

248 2.5 *Behavioral responses of T. japonicus to n-tridecane and (E)-2-* 249 *decenal*

251 For testing the effects of synthetic *n*-tridecane and (*E*)-2-decenal (Sigma-Aldrich,
252 Atlanta, GA), aliquots containing 0.2 nl of each compound in 100 µl of dichloromethane
253 were individually applied on sterile filter papers (4.25 cm ø) and allowed to dry for 2 min.
254 Afterwards, 2-4 days old naïve, mated *T. japonicus* females were placed on the filter paper
255 and their behavior was recorded as described above. The two compounds were tested alone
256 and in two blends of opposite ratios (4: 1 and 1: 4), with *n*-tridecane: (*E*)-2-decenal (1.6: 0.4
257 and 0.4: 1.6 nl/ml), respectively, using the same above-mentioned concentration. Controls
258 consisted of 100 µl aliquots of the solvent dichloromethane.

259 2.6 *Statistical analysis*

261 All statistical analyses were conducted in R (v. 3.4.3). For *T. japonicus* foraging
262 behavior, the distributions of all response variable values for each treatment were
263 evaluated for normality using a Shapiro Wilk test ($\alpha = 0.05$), and if required, appropriate
264 transformations were made. The behavior of *T. japonicus* in response to footprints of *H.*
265 *halys* females and *P. maculiventris* females were compared with controls, interspersing the
266 treatments that correspond to each tested life stage (i.e. 5 trials with *H. halys* followed by 5
267 trials with *P. maculiventris* followed by 5 controls). The same was done for the remaining
268 treatments, such as male stink bugs, as well as 5th, 4th, 3rd and 2nd instar nymphs. A non-
269 parametric Wilcoxon rank sum test (Mann-Whitney *U* test) was used to compare different
270 treatments, when data were not normally distributed under any transformation utilized.
271 When data were normally distributed or became so after logarithmic or square root
272 transformations, Student's t-test was utilized.

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275 3. Results

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277 3.1 *Trissolcus japonicus* foraging behavior on adult footprints

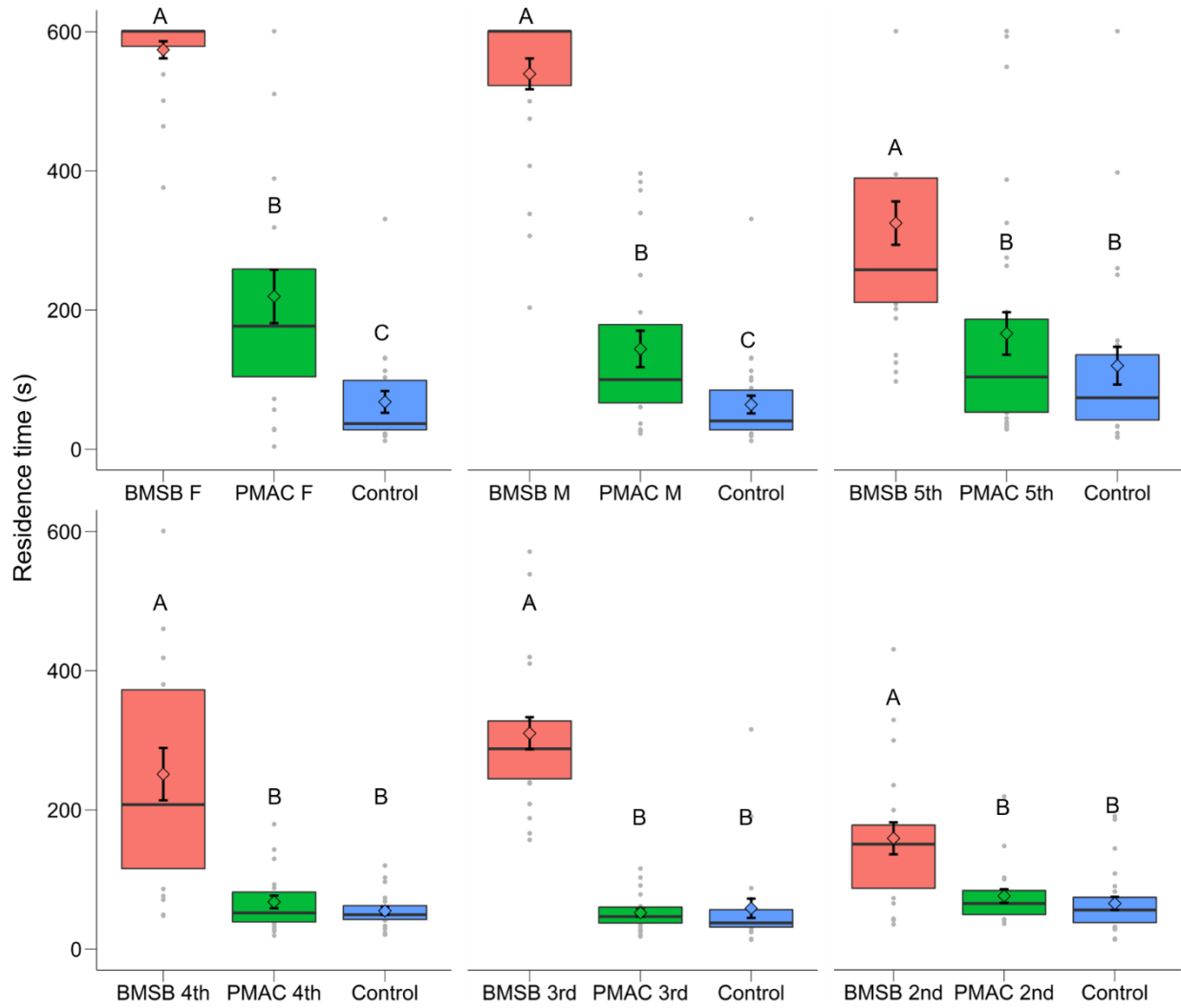
278

279 In general, wasps resided for a significantly longer time, had a slower walking
280 velocity, higher angular velocity and covered greater distances on filter papers
281 contaminated by *H. halys* footprints compared with *P. maculiventris* and with controls.

282 3.1.1 Residence time

283

284 In response to adult footprints, wasps were retained for significantly longer durations
285 on *H. halys* females (574.03 ± 12.24 s Mean \pm SEM) vs *P. maculiventris* females ($219.75 \pm$
286 38.37 s; $W= 34$, $P<0.001$) and vs controls (68.08 ± 15.67 ; $W= 462$, $P<0.001$). The wasps also
287 spent significantly more time on footprints of *P. maculiventris* females vs controls ($W=80$,
288 $P<0.001$). The residence time of *T. japonicus* was significantly longer on footprints from *H.*
289 *halys* males (539.57 ± 22.07 s) vs controls (64.26 ± 12.77 ; $W= 648$, $P<0.001$), *H. halys* males
290 vs *P. maculiventris* males (144.16 ± 26.26 s; $W= 13$, $P<0.001$) also on *P. maculiventris* males
291 vs controls ($W= 154.5$, $P= 0.006$). Results are summarized in Figure 1.



292
 293 **Fig. 1.** Residence time of female *T. japonicus* on footprint-contaminated substrate from different life
 294 stages of both *H. halys* (BMSB) and *P. maculiventris* (PMAC) females, males, 5th, 4th, 3rd and 2nd
 295 instars. Different letters express statistical difference between groups. Box plots show interquartile
 296 range with horizontal bar as median. Diamonds and whiskers indicate mean \pm SEM. Gray dots
 297 represent data points that fell outside the interquartile range. N=25 individual wasps per treatment.

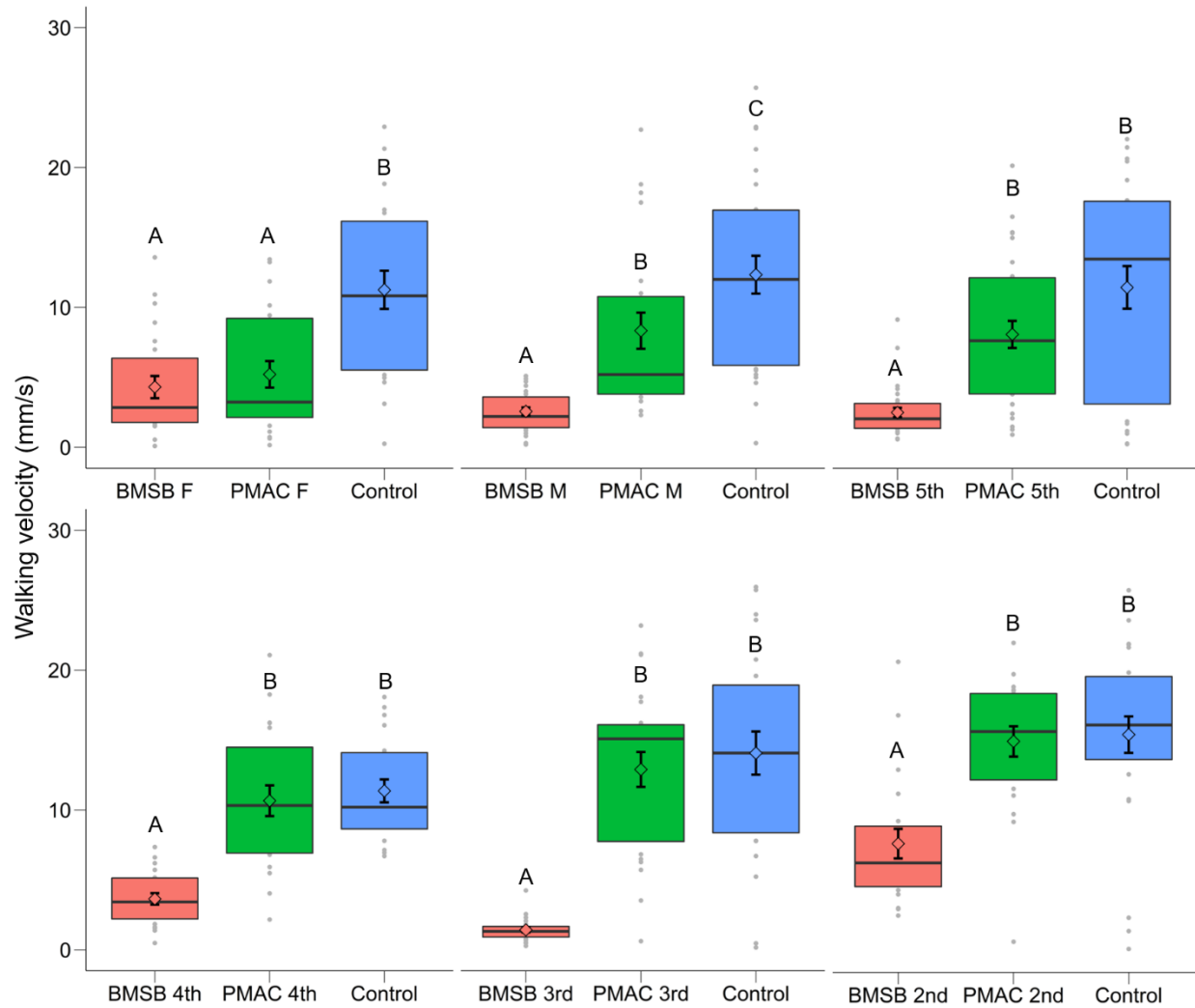
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299 3.1.2 Walking velocity

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301 The wasps walked significantly more slowly on *H. halys* female footprints (4.30 ± 0.79
 302 mm/s) compared to controls (11.25 ± 1.36 ; $t_{38.64} = -4.36$, $P < 0.001$), *H. halys* females vs *P.*
 303 *maculiventris* females (5.21 ± 0.94 mm/s; $t_{39.89} = -0.63$, $P < 0.001$), also when comparing *P.*
 304 *maculiventris* females with controls ($t_{39.72} = -3.53$, $P = 0.001$) (Figure 2). They also walked
 305 significantly more slowly on footprints of *H. halys* males (2.56 ± 0.28 mm/s) vs control

306 (12.33 ± 1.35; W= 37.5, P<0.001), *H. halys* males vs *P. maculiventris* males (8.33 ± 1.29
 307 mm/s; W= 69.5, P<0.001) and *P. maculiventris* males vs control (W= 175, P=0.022).



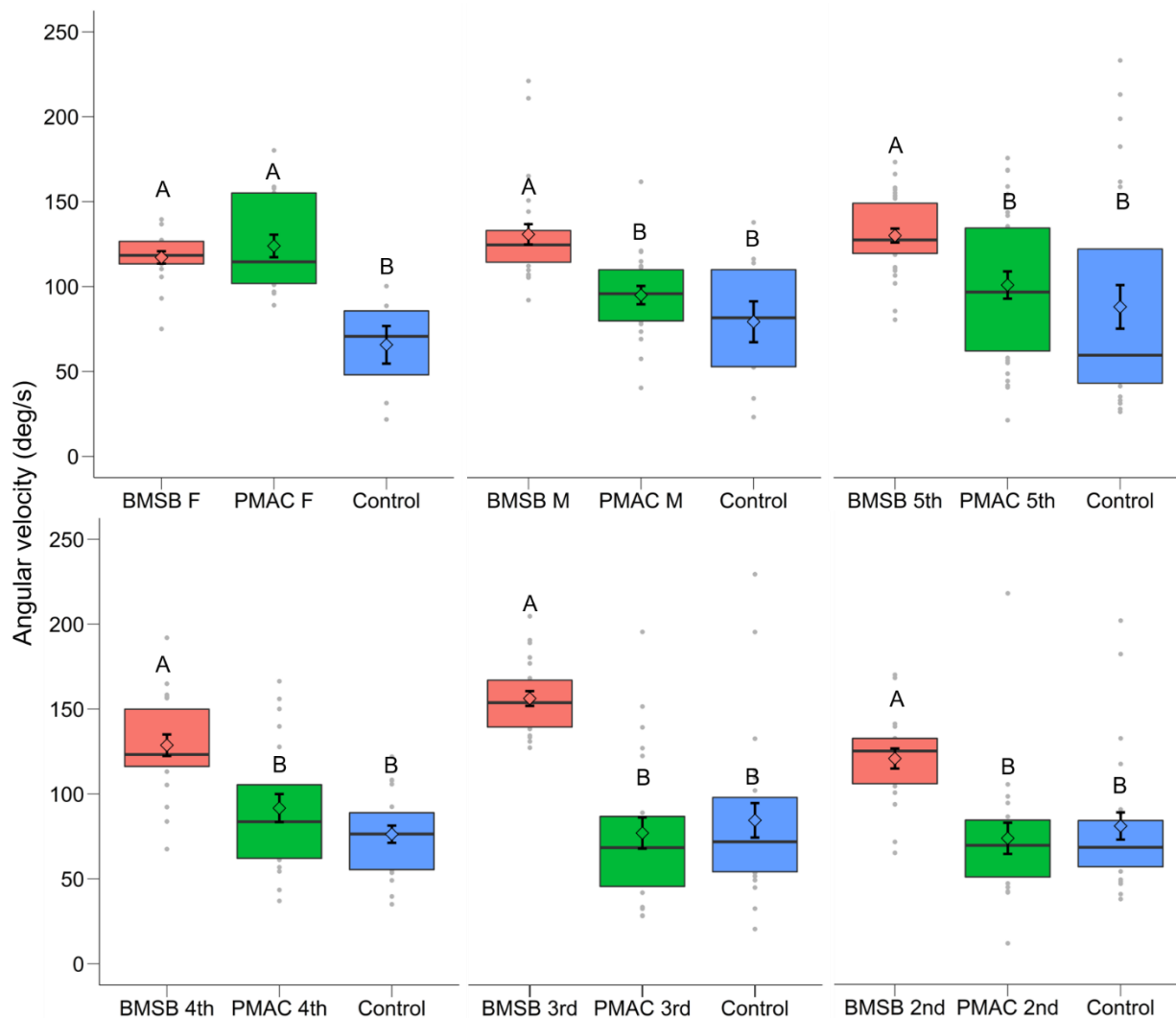
308
 309 **Fig. 2.** Walking velocity (mm/s) of female *T. japonicus* on substrate contaminated by footprints of *H.*
 310 *halys* and *P. maculiventris* life stages. Different letters express statistical difference between groups.
 311 Box plots show interquartile range with horizontal bar as median. Diamonds and whiskers indicate
 312 mean ± SEM. Gray dots represent data points that fell outside the interquartile range. N=25
 313 individual wasps per treatment.

314 3.1.3 Angular velocity

315

316 Angular velocity of *T. japonicus* was higher on footprints from *H. halys* females
 317 (117.14 ± 3.58 deg/s) vs controls (65.70 ± 11.06; $t_{7.29}=4.42$, P=0.002) and on *P. maculiventris*
 318 vs controls (123.91 ± 6.65 deg/s, $t_{10.6}=4.5$, P<0.001); no statistical difference was detected
 319 when comparing *H. halys* to *P. maculiventris* footprints (P>0.05) (Figure 3). The wasps had
 320 a higher angular velocity on footprints from *H. halys* males (130.74 ± 5.98 deg/s) vs controls

321 (79.24 ± 12.01; W= 215, P<0.001), *H. halys* males vs *P. maculiventris* males (94.93 ± 5.41
 322 deg/s, W= 483, P<0.001), but not on *P. maculiventris* males vs controls (P>0.05).

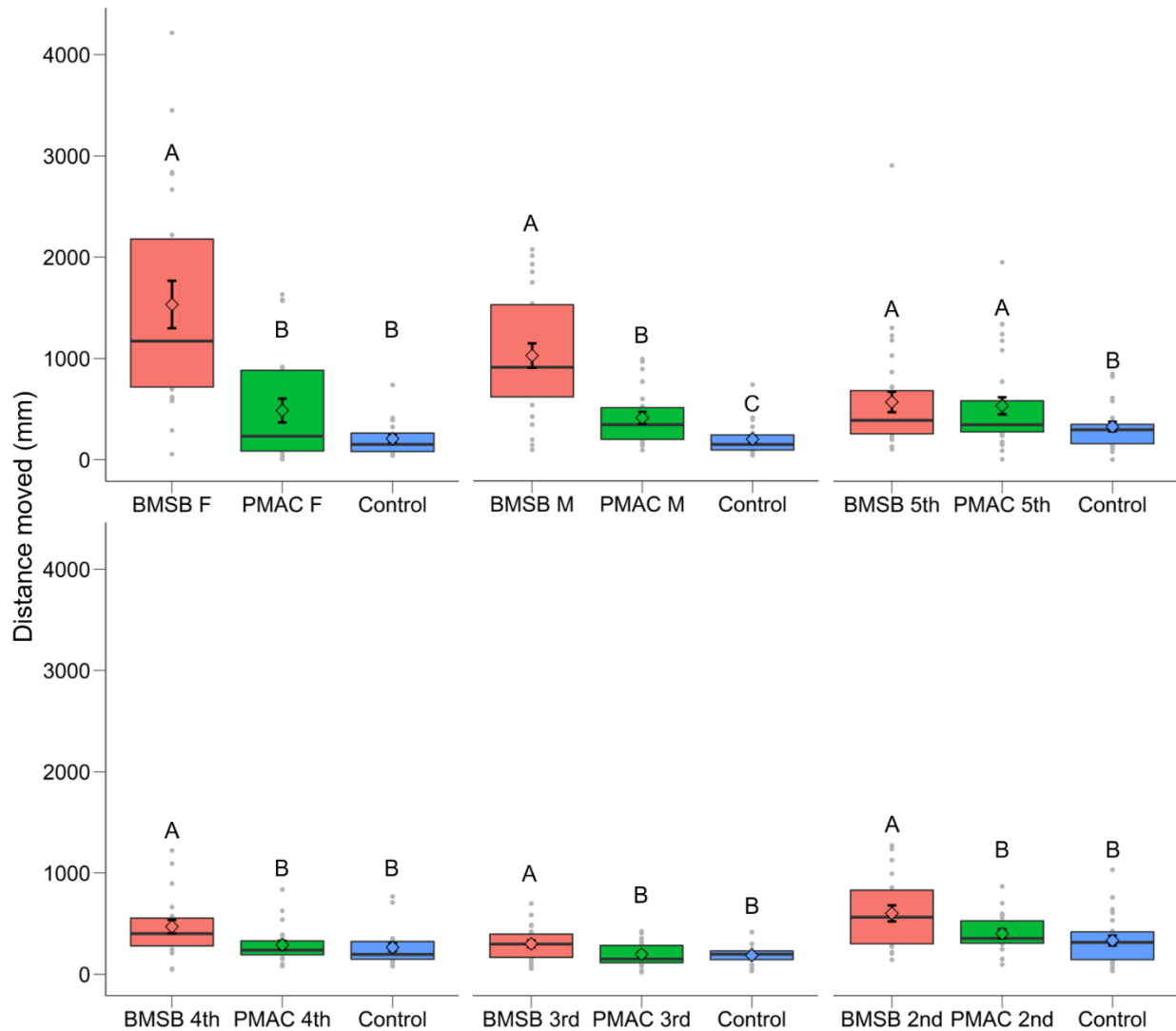


323
 324 **Fig. 3.** Angular velocity (deg/s) of female *T. japonicus* on substrate contaminated by footprints of *H.*
 325 *halys* and *P. maculiventris*. Different letters express statistical difference between groups. Box plots
 326 show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean ±
 327 SEM. Gray dots represent data points that fell outside the interquartile range. N=25 individual
 328 wasps per treatment.

329 *3.1.4 Distance moved*
 330

331 *Trissolcus japonicus* covered significantly greater distances when exposed to
 332 footprints originating from *H. halys* females (1532.65 ± 234.08 mm) vs controls (207.68 ±
 333 37.37; $t_{40.46}=7.31$, P<0.001), *H. halys* females vs *P. maculiventris* females (485.82 ± 117.95
 334 mm; $t_{32.83}=4.11$, P<0.001), but not to footprints from *P. maculiventris* females vs controls

335 ($t_{30.04}=0.87$, $P=0.38$) (Figure 4). Greater distances were covered on footprints from *H. halys*
 336 males (1026 ± 120.85 mm) vs controls (199.73 ± 30.55 ; $t_{47.9}=7.4$, $P<0.001$), *H. halys* males vs
 337 *P. maculiventris* males (410.12 ± 58.98 mm; $t_{44.8}=4.02$, $P<0.001$) and *P. maculiventris* males
 338 vs controls ($t_{45.6}=3.7$, $P<0.001$).



339
 340 **Fig. 4.** Distance moved (mm) by female *T. japonicus* on substrate contaminated by footprints of *H.*
 341 *halys* and *P. maculiventris*. Different letters express statistical difference between groups. Box plots
 342 show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean \pm
 343 SEM. Gray dots represent data points that fell outside the interquartile range. N=25 individual
 344 wasps per treatment.

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3.2 *Trissolcus japonicus* foraging behavior on nymphal footprints

In response to footprints from 5th, 4th, 3rd and 2nd nymphal instars of both species, the wasps exhibited a significantly stronger arrestment response on those footprints originating from *H. halys* compared with *P. maculiventris* and with controls. However, no statistical difference was observed when comparing all the studied variables of *T. japonicus* behavior on *P. maculiventris* footprints vs controls. Results presented in Figures 1, 2, 3 and 4.

3.2.1 5th nymphal instars

The wasps' residence time was significantly longer on *H. halys* (325.02 ± 31.18 s) vs controls (120.15 ± 27.01 s; $W= 651$, $P<0.001$), it walked significantly more slowly on *H. halys* (2.48 ± 0.33 mm/s) vs controls (11.42 ± 1.52 mm/s; $W= 154$, $P<0.001$), it had a higher angular velocity on *H. halys* (130.02 ± 4.08 deg/s) vs controls (88.02 ± 12.86 deg/s; $t_{28,84}= 3.11$, $P=0.004$) and covered greater distance on *H. halys* (569.27 ± 100.6 mm) vs controls (327.13 ± 47.31 mm; $W= 508$, $P= 0.031$). Similarly, the wasp spent significantly more time on *H. halys* vs *P. maculiventris* (166.37 ± 30.53 s; $W= 728,5$, $P<0.001$), it walked significantly more slowly on *H. halys* vs *P. maculiventris* (8.06 ± 0.96 mm/s; $W= 140$, $P<0.001$), it had a higher angular velocity on *H. halys* vs *P. maculiventris* (100.88 ± 8.03 deg/s; $t_{43,06}= 3.23$, $P=0.002$), with no difference in the distance covered ($P>0.05$).

3.2.2 4th nymphal instars

The residence time of *T. japonicus* was significantly longer on substrate contaminated with *H. halys* (251.39 ± 37.66 s) vs controls (55.01 ± 5.56 ; $W= 408$, $P<0.001$), the wasp had significantly slower velocity on *H. halys* (3.64 ± 0.41 mm/s) vs controls (11.36 ± 0.82 ; $t_{39,85}= 8.93$, $P<0.001$), it had a higher angular velocity on *H. halys* (128.79 ± 6.33 deg/s) vs controls (76.39 ± 5.07 deg/s; $t_{38,18}= 6.45$, $P<0.001$), and covered greater distance on *H. halys* (470.29 ± 66.17 mm) vs controls (264.97 ± 38.65 mm; $t_{35,46}= 2.26$, $P= 0.029$). The wasp also spent significantly more time on *H. halys* vs *P. maculiventris* (67.77 ± 9.06 s; $W= 384$, $P<0.001$), it walked significantly more slowly on *H. halys* vs *P. maculiventris* (10.66 ± 1.1 mm/s; $t_{34,4}= 6.34$, $P<0.001$), had a higher angular velocity on *H. halys* vs *P. maculiventris* (91.74 ± 8.28

379 deg/s; $t_{37.42} = 3.55$, $P = 0.001$), with no significant difference in the distance covered on *H.*
380 *halys* vs *P. maculiventris* footprints.

381 3.2.3 3rd nymphal instars

382

383 *Trissolcus japonicus* resided for significantly more time on *H. halys* (310.25 ± 23.04 s)
384 vs controls (58.65 ± 13.86 s; $W = 510$, $P < 0.001$), it walked significantly more slowly on *H.*
385 *halys* (1.42 ± 0.17 mm/s) vs controls (14.06 ± 1.54 mm/s; $W = 45$, $P < 0.001$), it had a higher
386 angular velocity on *H. halys* (156.31 ± 4.37 deg/s) vs controls (84.53 ± 10.13 deg/s; $W = 482$,
387 $P < 0.001$) and covered greater distance on *H. halys* (300.69 ± 33.94 mm) vs controls (186.41
388 ± 17.27 mm; $t_{38.6} = 2.81$, $P = 0.007$). With regard to the wasp's behavior on *H. halys* vs *P.*
389 *maculiventris* footprints, it spent significantly more time on *H. halys* vs *P. maculiventris*
390 (52.31 ± 5.2 s; $W = 529$, $P < 0.001$), it walked significantly more slowly on *H. halys* vs *P.*
391 *maculiventris* (12.89 ± 1.24 mm/s; $W = 21$, $P < 0.001$), it had a higher angular velocity on *H.*
392 *halys* vs *P. maculiventris* (77.07 ± 9.15 deg/s; $W = 490$, $P < 0.001$), also covered greater
393 distances on *H. halys* vs *P. maculiventris* footprints (197.66 ± 25.52 mm; $t_{43.75} = 2.42$, $P =$
394 0.019).

395 3.2.4 2nd nymphal instars

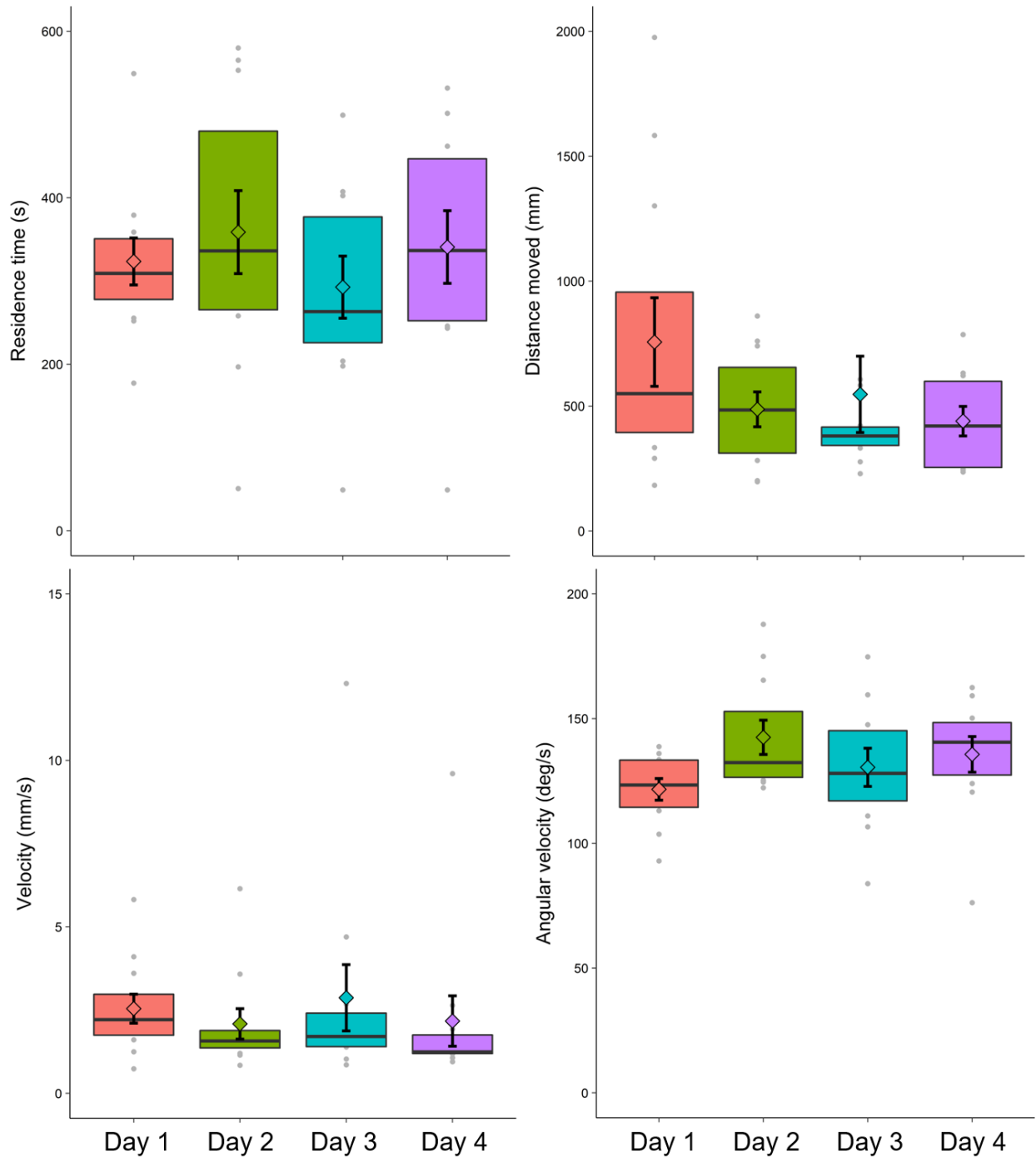
396

397 The residence time of *T. japonicus* was significantly longer on substrate contaminated
398 by *H. halys* (158.99 ± 22.91 s) vs controls (65.36 ± 9.45 s; $t_{40.28} = 4.38$, $P < 0.001$), its walking
399 velocity was significantly slower on *H. halys* (7.67 ± 1.05 mm/s) vs controls (15.44 ± 1.30
400 mm/s; $W = 92$, $P < 0.001$), its angular velocity was higher on *H. halys* (120.74 ± 5.91 deg/s) vs
401 controls (80.93 ± 8.05 deg/s; $t_{40.89} = 4.39$, $P < 0.001$) and covered greater distances on *H. halys*
402 (601.31 ± 78.86 mm) vs controls (333.73 ± 48.02 mm; $t_{38.75} = 3.06$, $P = 0.003$). When comparing
403 the wasp's behavior on *H. halys* and *P. maculiventris* footprints, it spent significantly more
404 time on *H. halys* vs *P. maculiventris* (76.08 ± 9.75 s; $t_{33.43} = 3.45$, $P = 0.0015$), had significantly
405 slower walking velocity on *H. halys* vs *P. maculiventris* (14.95 ± 1.08 mm/s; $W = 58$,
406 $P < 0.001$), had a significantly higher angular velocity on *H. halys* vs *P. maculiventris* (73.67
407 ± 9.2 deg/s; $t_{30.11} = 4.61$, $P < 0.001$) and covered greater distances on *H. halys* vs *P.*
408 *maculiventris* (401.41 ± 43.25 mm; $t_{33.45} = 2.05$, $P = 0.047$).

409 3.3 Footprint longevity

410

411 Using *T. japonicus* horizontal mobility and arrestment as a proxy for the longevity of
412 *H. halys* female footprints, we recorded an almost unchanging behavior when comparing
413 the wasp's arrestment response on treated filter papers up to 4 days after initial exposure
414 (Figure 5). There was no significant difference ($P>0.05$) in the wasp's residence time,
415 walking velocity, angular velocity and distance moved between the four different
416 treatments (Day 1, 2, 3 and 4).



417

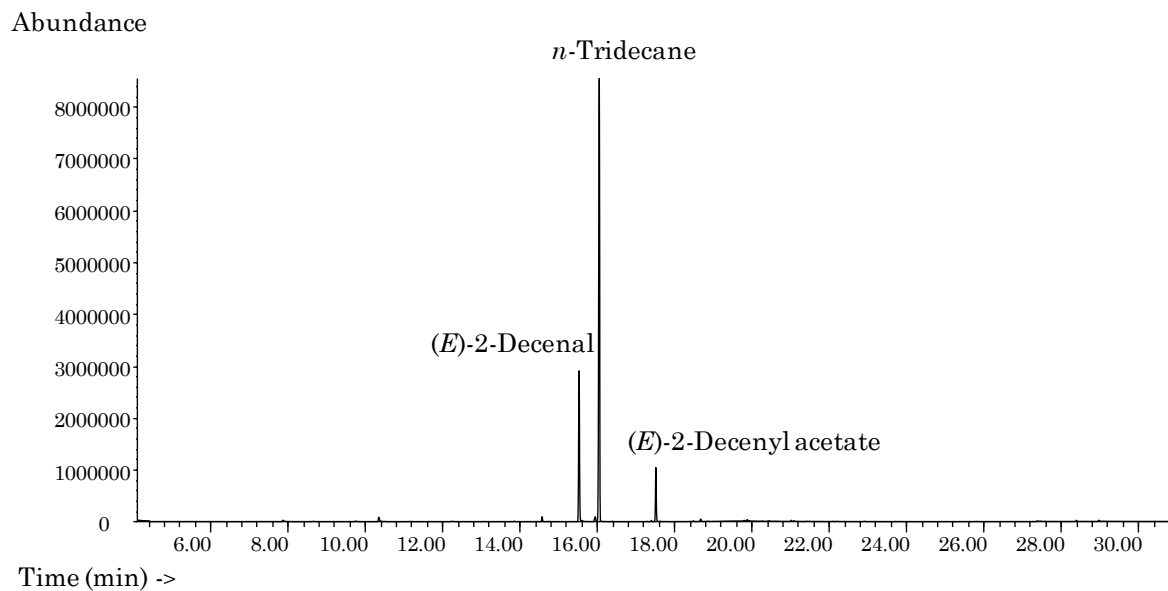
418 **Fig. 5.** Box plots showing the four studied variables of female *T. japonicus* arrestment response up to
 419 4 days after initial exposure. Boxplots represent the interquartile range with horizontal bar as
 420 median. Diamonds and whiskers indicate mean \pm SEM. Gray dots represent data points that fell
 421 outside the interquartile range. N= 11 individual wasp per treatment.

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3.4 Chemical footprint identity



426 Time (min) ->

427 **Fig. 6.** GC-MS total ion chromatogram of *H. halys* female footprints. *n*-tridecane and (*E*)-2-decenal
428 were identified by matching retention times and mass-spectra those of authentic standards. (*E*)-2-
429 Decenyl acetate was identified using NIST mass-spectral library

430

431 Chemical analyses revealed the presence of *n*-tridecane and (*E*)-2-decenal in all tested
432 life stages of *H. halys*, while much smaller amounts of these compounds were detected in *P.*
433 *maculiventris* footprints. Both *n*-tridecane and (*E*)-2-decenal were most abundant in male
434 *H. halys* extracts compared with those of other life stages from both species (table 1).

435 **Table 1.** Mean \pm SEM of absolute *n*-tridecane and (*E*)-2-decenal in *H. halys* and *P.*
436 *maculiventris* extracts.

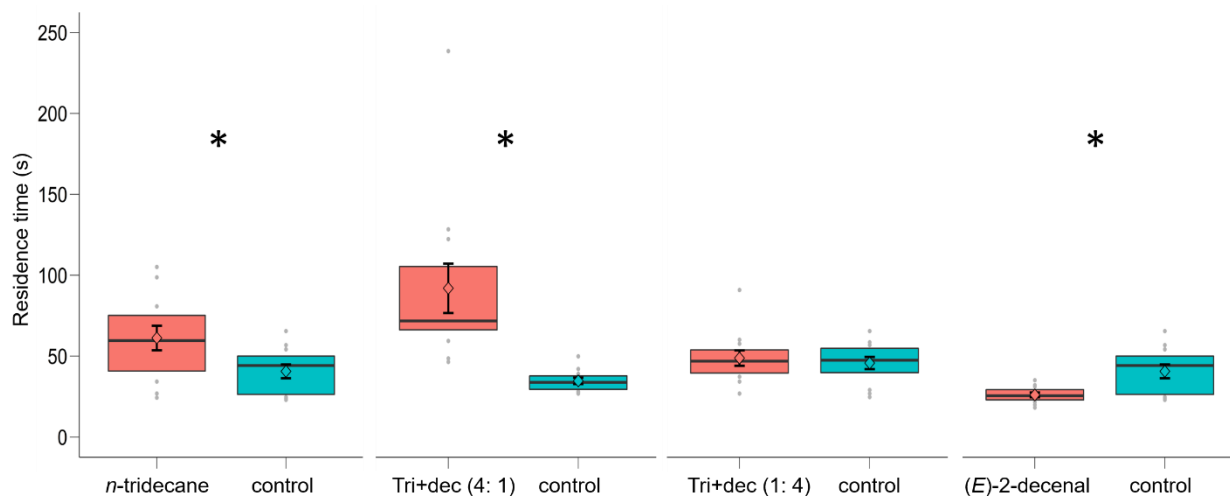
Life stage	N	<i>n</i> -tridecane (μg)	(<i>E</i>)-2-decenal (μg)
<i>H. halys</i> female	10	9.50 \pm 3.49	2.70 \pm 0.97
<i>H. halys</i> male	10	44.01 \pm 13.17	14.14 \pm 4.54
<i>H. halys</i> 5 th instar	10	5.79 \pm 1.16	3.18 \pm 0.87
<i>P. maculiventris</i> female	10	0.26 \pm 0.24	0.025 \pm 0.025
<i>P. maculiventris</i> male	10	0.13 \pm 0.13	0.019 \pm 0.019
<i>P. maculiventris</i> 5 th instar	10	2.40 \pm 1.83	0.004 \pm 0.002

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3.5 Response to synthetic compounds

440 According to Zhong et al. (2017) (*E*)-2-decenyl acetate did not elicit an
441 electrophysiological response in female *T. japonicus*, therefore it was excluded from our
442 behavioral tests. Only residence time was measured for experiments involving *T. japonicus*
443 response to synthetic *n*-tridecane and (*E*)-2-decenal (Figure 6). The average time spent by
444 the wasp on *n*-tridecane (61.24 ± 7.60 s) treated filter papers was significantly longer
445 compared with the control (40.62 ± 4.22 s; $t_{17,20}=2.36$, $P=0.029$). The residence time of *T.*
446 *japonicus* was shortened on (*E*)-2-decenal (26.06 ± 1.45 s) compared with controls ($40.62 \pm$
447 4.22 s; $t_{16,44}=-3.23$, $P=0.005$). The blend of *n*-tridecane: (*E*)-2-decenal (4:1) significantly
448 prolonged the residence time of the wasp (91.95 ± 15.29 s) compared with the controls
449 (34.73 ± 1.95 ; $t_{14,51}=6.12$, $P<0.001$). No statistical difference was found when comparing the
450 residence time of the wasp on *n*-tridecane: (*E*)-2-decenal blend (1:4) with controls ($P>0.05$).



451
452 **Fig. 7.** Box plots representing the effect of *n*-tridecane: (*E*)-2-decenal blends and single compounds
453 on the residence time of female *Trissolcus japonicus*. Asterisks indicate significant difference
454 according to Student's t-test. Box plots show interquartile range with horizontal bar as median.
455 Diamonds and whiskers indicate mean \pm SEM. Gray dots represent data points that fell outside the
456 interquartile range. N= 12 individual wasps per treatment

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4. Discussion

Female parasitoids are motivated to optimize their host-selection behavior because it is directly linked to their reproductive success, since the host is the only food source for their offspring during their immature development (van Alphen and Vet 1986). Therefore, studies on parasitic wasps have played a major role in shaping and testing foraging theory (Godfray 1994).

Samurai wasp behavior in our studies differed greatly on treated compared to untreated substrates. Parasitoids observed on control substrate walked fast in relatively straight lines interspersed with erratic turns and exited the arena fairly quickly. When wasps experienced target or nontarget stimuli, they altered their orthokinetic (i.e. speed of movement dependent upon the intensity of the stimulus) and locomotory behavior, and clearly initiated an arrestment response. The latter is characterized by a decrease in linear walking velocity, an increase in residence time, angular velocity and distance covered on footprint-contaminated substrate (Peri et al. 2006; Rostás and Wölfling 2009; Konopka et al. 2018). Peri et al. (2013) showed that *Trissolcus* egg parasitoids only responded positively to footprints of hosts with which they are normally associated. *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) exhibited an arrestment response to footprints of its host *Nezara viridula* L. (Heteroptera: Pentatomidae) (Colazza et al. 1999), while the generalist *Ooencyrtus telenomicida* Vassiliev (Hymenoptera: Encyrtidae) did not respond to footprint contact kairomones of *N. viridula* (Peri et al. 2011). Rostás et al. (2008) also demonstrated that *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) parasitoids respond to chemical footprints of its host *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), with a characteristic host recognition behavior, displayed as antennal drumming of the host-contaminated substrate. In a recent study, *Anastatus orientalis* Yang and Gibson (Hymenoptera: Eupelmidae) exhibited a motivated searching in response to footprints from females of the invasive spotted lanternfly, *Lycorma delicatula* White (Hemiptera: Fulgoridae) (Malek et al. 2019). In our bioassays, *T. japonicus* exhibited a significantly stronger arrestment in response to footprints originating from its optimal host *H. halys*, compared with the suboptimal *P. maculiventris*. For example, when on *P. maculiventris* contaminated substrate *T. japonicus* resided for 1/3 the time it spent on *H. halys* footprints. Moreover, in response to nymphal footprints, *T. japonicus* displayed a motivated searching

494 only to *H. halys* but not to *P. maculiventris*. Such a behavior would seem to indicate
495 detection of a negligible cue, which translates into an efficient strategy for moving between
496 patches of hosts (Bell and Tobin 1982). Thus, the weaker arrestment to *P. maculiventris*
497 footprints suggests low current profitability, which may become potentially higher by
498 searching elsewhere in the habitat (Thiel and Hoffmeister 2004).

499 Studies aimed at determining *T. japonicus* physiological and ecological host range
500 revealed its capacity to recognize, accept and successfully develop in several species of stink
501 bugs. Efforts in China, North America and Europe found that closely related species in the
502 pentatomid subfamily Asopinae such as *Arma chinensis*, *Podisus maculiventris* and *Arma*
503 *custos* respectively, were highly suitable developmental hosts for *T. japonicus* (Zhang et al.
504 2017; Hedstrom et al. 2017; Haye et al. 2019; Garipey and Talamas 2019). The nontarget
505 effects on these predatory stink bugs, classified according to Kuhlmann et al. (2006) as
506 “safeguard species”, raises more concern than effects on other herbivores. Bertoldi et al.
507 (2019) tested the attraction of *T. japonicus* to tomato plants subjected to feeding and
508 oviposition by either *H. halys* or *P. maculiventris*. They showed that female wasps were
509 significantly more attracted to plant odors induced by *H. halys* feeding and egg laying,
510 whereas no significant attraction was detected when comparing clean plants with those
511 subjected to *P. maculiventris* herbivory. Their results suggest that naïve *T. japonicus*
512 reared on *H. halys* exploit cues associated with its coevolved host but not those related to
513 the novel *P. maculiventris* association. Haye et al. (2019) further investigated the
514 fundamental host range of *T. japonicus* in Europe, revealing that of the twelve nontarget
515 European Pentatomidae species tested, all were accepted by *T. japonicus* and eleven were
516 suitable for parasitoid development in no-choice tests. When paired choice-tests in semi-
517 natural large arenas were performed, egg masses of *Arma custos* F. (Heteroptera:
518 Pentatomidae), *Graphosoma lineatum* and *Acrosternum heegeri* Fieber (Heteroptera:
519 Pentatomidae) were significantly less parasitized when exposed together with *H. halys* egg
520 masses. Their results confirm those of Bertoldi et al. (2019), that additional complexity
521 (host plants with naturally laid egg masses, feeding damage and stink bug footprints) can
522 substantially reduce the degree of nontarget host parasitism in controlled laboratory
523 conditions. When reared on *P. maculiventris*, the residence time of *T. japonicus* on
524 footprints of *H. halys* was not different than on those of *P. maculiventris*, however, egg
525 masses of *P. maculiventris* were significantly less parasitized than those of *H. halys* (Boyle

2017). Botch and Delfosse (2018) found that when reared on *H. halys*, *T. japonicus* showed a stronger preference for it compared with the nontarget stink bugs *P. maculiventris* and *Thyanta custator acerra* McAtee (Heteroptera: Pentatomidae). However, when reared on these same nontarget hosts, *T. japonicus* females showed reduced host specificity, and not only were their progeny significantly smaller in size, but also of a lower number, indicating significant fitness costs. It remains unclear whether habitat preference, competition with native egg-parasitoids or phenology of nontarget species may limit nontarget parasitism; therefore, risk assessment studies dealing with these aspects are warranted. Although it is predicted that *T. japonicus* may detect and parasitize *P. maculiventris* or other native pentatomid species, the aforementioned findings suggest that female wasps will adopt a searching strategy in the field that brings about optimal fitness gains, and consequently reduced probability of nontarget parasitization.

Walking by *H. halys* females for 30 minutes left sufficient amounts of infochemicals on the filter papers to induce a strong arrestment response even four days after initial deposition. This persistence of female footprints is comparable to the period during which *H. halys* eggs are still viable for parasitization, but further studies in natural conditions are required to confirm this result. Hemptinne et al. (2001) reported that females *Adalia bipunctata* L. (Coleoptera: Coccinellidae) avoid laying eggs on filter papers contaminated by conspecific larval tracks for at least ten days. In contrast, the response of the parasitic wasp *Cephalonomia tarsalis* Ashmead (Hymenoptera: Bethyridae) to trail traces of its host, the saw-toothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) vanished 30 min after larvae had been removed from the filter paper (Collatz and Steidl 2008). Therefore, as suggested by Rostás and Wölfling (2009), the bioactivity of kairomonal footprints is expected to vary with the perceiving insect, substrate, storage conditions, and nature and concentration of deposited chemicals.

Pentatomids are commonly known as “stink bugs” because they respond to disturbances or aggression by producing irritating defensive secretions with a disagreeable odor from dorsal abdominal glands or metathoracic glands (Aldrich, 1988). Our present study showed that *n*-tridecane and (*E*)-2-decenal were the two main compounds extracted from footprints of females, males and 5th instar nymphs of *H. halys*, while only trace amounts were detected in *P. maculiventris* extracts. These two compounds, along with (*E*)-2-decenyl acetate, dodecane and (*E*)-2-octenal have been previously identified from *H. halys*

558 volatiles and from other stink bug metathoracic secretions (Kitamura et al. 1984; Fávvaro et
559 al. 2011; Solomon et al. 2013; Harris et al. 2015; Fraga et al. 2017; Zhong et al. 2017). They
560 are thought to have defensive roles, mediating behaviors among individual pentatomids,
561 predators, and natural enemies (Borges and Aldrich 1992; Weber et al. 2017). The variable
562 amounts secreted by the different sexes and life stages of *H. halys* may be linked to their
563 differing functions at the given life stages and physiological states of the bugs. Nixon et al.
564 (2018) sampled diapausing and diapause-disrupted aggregations of *H. halys* and found that
565 the predominant compounds included *n*-tridecane, 4-oxo-(*E*)-2-hexenal, and (*E*)-2-decenal,
566 which suggests that these aggregations secrete and possibly passively exude these
567 compounds even in the absence of any disturbance (Harris et al. 2015).

568 In our behavioral studies, synthetic *n*-tridecane alone and in a blend with (*E*)-2-
569 decenal (4: 1 respectively) significantly prolonged the residence time of *T. japonicus*. In
570 contrast, (*E*)-2-decenal alone shortened it and a blend of *n*-tridecane and (*E*)-2-decenal (1: 4)
571 had no effect on the wasp's behavior compared with the solvent control. Our findings
572 suggest that *n*-tridecane acts as an arrestant, while (*E*)-2-decenal is functioning as a
573 deterrent. Our results are in accordance with those of Zhong et al. (2017), who showed that
574 *T. japonicus* females and *H. halys* males were attracted by *n*-tridecane but strongly repelled
575 by (*E*)-2-decenal. In another study, (*E*)-2-decenal was also repellent to *N. viridula* 1st instar
576 nymphs, suggesting an alarm function (Fucarino et al. 2004). (*E*)-2-decenal not only
577 exhibited fungistatic effect and inhibited spore germination of entomopathogenic fungi in
578 the genera *Metarhizium*, *Beauveria*, and *Isaria* (Pike 2014), but was also found to display
579 antibacterial activity as both *Staphylococcus aureus* and *Escherichia coli* were significantly
580 inhibited by it (Sagun et al. 2016).

581 A study by Fraga et al. (2017) found that *n*-tridecane is a major compound released
582 by *H. halys* adults and nymphs, and by *H. halys*-damaged bean pods. They also confirmed
583 that it attracted *Orius insidiosus* Say (Hemiptera: Anthocoridae) in olfactometer assays and
584 field trials but contributed only to arresting the predator rather than increasing prey
585 consumption. Harris et al. (2015) stated that *n*-tridecane inhibited aggregation pheromone
586 production by individual *H. halys* males, while Lockwood and Story (1985, 1987) suggested
587 that it functions as nymphal aggregation pheromone at low doses but as an alarm
588 pheromone at high doses in *N. viridula*. Other authors have stated that *n*-tridecane and
589 similar hydrocarbons function as solvents or carriers rather than inherently bioactive

590 compounds (Calam and Youdeowei 1968; Kment and Vilimova 2010). Interestingly,
591 coupling (*E*)-2-decenal with *n*-tridecane strongly deterred the feeding activity of *H. halys* in
592 laboratory trials (Zhang et al. 2016).

593 **5. Conclusions**

594

595 Our bioassays indicate that the samurai wasp *T. japonicus* employs an infochemical
596 detour (sensu Vet and Dicke 1992), responding to host cues that might lead to the target
597 life stage. Wasps reacted to contact kairomones by activating a significantly stronger
598 arrestment to *H. halys* footprints compared with *P. maculiventris* footprints. While this
599 preference for *H. halys* cues might indicate lower chances of *P. maculiventris* parasitization
600 in the field, negative nontarget effects cannot be excluded. We found that *H. halys* female
601 footprints persist on contaminated filter papers for at least four days, meaning that *T.*
602 *japonicus* can use these cues for the period when eggs are still suitable for parasitoid
603 development, which declines significantly after host eggs are more than about three days
604 old (Yang et al. 2018). Further research is required to understand whether this timeline
605 varies under natural conditions and on various plant substrates. Our chemical analyses
606 revealed that *n*-tridecane and (*E*)-2-decenal are the two major compounds of *H. halys* and *P.*
607 *maculiventris* footprints. The former prolonged *T. japonicus* residence time, whereas the
608 latter shortened it, suggesting that wasps are eavesdropping on *n*-tridecane for host
609 location, while (*E*)-2-decenal performs its presumed defensive role and repels the
610 parasitoid. These findings shed new light on the potential applications of *n*-tridecane in
611 agroecosystems, where it could be used to attract or arrest natural enemies, to improve
612 parasitoid surveys or to provide refugia that may assist in their conservation or
613 augmentation. Further studies are required in this regard to pinpoint the optimal ratios
614 and concentrations that can be utilized for field testing. As *T. japonicus* appears to be
615 following *H. halys* through its invasive journey, first to North America (Talamas et al. 2015)
616 then to Europe (Stahl et al. 2018), efforts to relocate and redistribute it in the USA are
617 already underway (Jentsch 2017). Risks and rewards resulting from introducing this
618 biocontrol agent or assisting its establishment in *H. halys* invaded territories need to be
619 weighed with care, also accounting for the risks of not releasing it and instead waiting for it
620 to disperse on its own. If this agent can help reduce *H. halys* populations, this probably
621 means that the use of broad-spectrum insecticides—that surged under *H. halys* outbreaks,

622 are environmentally harmful, disruptive to IPM programs, cause extensive nontarget
623 effects and linked to global insect decline—would also be reduced.

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638

639 **Competeting interests statement**

640 None declared.

641

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911 **Figure Captions**

912 **Fig. 1.** Residence time of female *T. japonicus* on footprint-contaminated substrate from different life
913 stages of both *H. halys* (BMSB) and *P. maculiventris* (PMAC) females, males, 5th, 4th, 3rd and 2nd
914 instars. Different letters express statistical difference between groups. Box plots show interquartile
915 range with horizontal bar as median. Diamonds and whiskers indicate mean \pm SEM. Gray dots
916 represent data points that fell outside the interquartile range. N=25 individual wasps per treatment.

917

918 **Fig. 2.** Walking velocity (mm/s) of female *T. japonicus* on substrate contaminated by footprints of *H.*
919 *halys* and *P. maculiventris* life stages. Different letters express statistical difference between groups.
920 Box plots show interquartile range with horizontal bar as median. Diamonds and whiskers indicate
921 mean \pm SEM. Gray dots represent data points that fell outside the interquartile range. N=25
922 individual wasps per treatment.

923

924 **Fig. 3.** Angular velocity (deg/s) of female *T. japonicus* on substrate contaminated by footprints of *H.*
925 *halys* and *P. maculiventris*. Different letters express statistical difference between groups. Box plots
926 show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean \pm
927 SEM. Gray dots represent data points that fell outside the interquartile range. N=25 individual
928 wasps per treatment.

929

930 **Fig. 4.** Distance moved (mm) by female *T. japonicus* on substrate contaminated by footprints of *H.*
931 *halys* and *P. maculiventris*. Different letters express statistical difference between groups. Box plots
932 show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean \pm
933 SEM. Gray dots represent data points that fell outside the interquartile range. N=25 individual
934 wasps per treatment.

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Fig. 5. Box plots showing the four studied variables of female *T. japonicus* arrestment response up to 4 days after initial exposure. Boxplots represent the interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean \pm SEM. Gray dots represent data points that fell outside the interquartile range. N= 11 individual wasp per treatment.

Fig. 6. GC-MS total ion chromatogram of *H. halys* female footprints. *n*-tridecane and (*E*)-2-decenal were identified by matching retention times and mass-spectra those of authentic standards. (*E*)-2-Decenyl acetate was identified using NIST mass-spectral library

Fig. 7. Box plots representing the effect of *n*-tridecane: (*E*)-2-decenal blends and single compounds on the residence time of female *Trissolcus japonicus*. Asterisks indicate significant difference according to Student's t-test. Box plots show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean \pm SEM. Gray dots represent data points that fell outside the interquartile range. N= 12 individual wasps per treatment

957 **Table captions**

958 **Table 1.** Mean \pm SEM of absolute *n*-tridecane and (*E*)-2-decenal in *H. halys* and *P.*
959 *maculiventris* extracts.

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