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Impact of Triflumuron on Halyomorpha halys (Hemiptera: Pentatomidae): laboratory and field studies

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**Impact of triflumuron on Halyomorpha halys (Hemiptera:  
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1      **Impact of triflumuron on *Halyomorpha halys* (Hemiptera: Pentatomidae):**  
2                              **laboratory and field studies**

3

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10 **Abstract**

11 *Halyomorpha halys*, (the brown marmorated stink bug, BMSB), is a high-concern invasive species  
12 causing severe damage to orchards in many countries outside its native Asian range. Control  
13 options matching both effectiveness and sustainability are currently lacking. Inhibitors of chitin  
14 biosynthesis might be exploited for integrated management programs because of the overall better  
15 ecotoxicological profile in comparison with most neurotoxic insecticides used so far against BMSB.  
16 In this study, the activity of triflumuron, a benzoylphenyl urea hampering chitin biosynthesis, was  
17 tested on BMSB in laboratory and field conditions. In laboratory bioassays, the insecticide was  
18 sprayed on potted peach plants (30 cm high) and residues were aged in a glasshouse for 0, 7, 14 and  
19 21 days. Then 3<sup>rd</sup> instar bugs were placed on the plants and continuously exposed to residues.  
20 Mortality was scored after 7, 14 and 21 day exposure. Triflumuron caused significantly higher  
21 mortality on BMSB nymphs in comparison with water controls at all aging periods. Moreover,  
22 aging of residues up to 21 days did not cause any significant reduction of activity. Field  
23 experiments were also carried out in 2019 in eight pear orchards. Injuries to fruits at harvest were  
24 compared between plots where triflumuron was added to insecticide sprays against BMSB and  
25 control plots managed exactly in the same way but without any triflumuron treatment. An overall  
26 mean of  $9.99 \pm 1.98\%$  stink bug injured fruits was detected in plots managed with the strategy  
27 including triflumuron, whereas  $19.45 \pm 3.55\%$  of fruits were injured in plots assigned to controls.

28

29 **Keywords:** Brown marmorated stink bug; Invasive species; Inhibitors of chitin biosynthesis;  
30 Integrated pest management; Reduced-risk insecticides.

31

## 32 **Introduction**

33 The brown marmorated stink bug (BSMB) - *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) -  
34 is a serious agricultural pest in most countries where it has been accidentally introduced (Bosco et  
35 al. 2017, Leskey and Nielsen 2018). Current management strategies rely mostly on neurotoxic  
36 broad-spectrum insecticides that are quite effective in preventing BMSB damages but have a short  
37 residual activity (Kuhar and Kamminga 2017). This forces farmers to increase the frequency of  
38 insecticide sprays (Leskey et al. 2012) causing the disruption of previously established integrated  
39 pest management (IPM) programs and the resurgence of secondary pests (Leskey and Nielsen  
40 2018). The identification of more selective insecticides targeting BMSB would significantly reduce  
41 the environmental issues associated to its control and could contribute to IPM principles. Besides  
42 efficacy on BMSB and less severe side effects, residual activity of insecticides could play a key role  
43 in restoring sustainable management programs.

44 Benzoylphenyl ureas (BPUs) are classified by Insecticide Resistance Action Committee (IRAC,  
45 <https://www.irc-online.org/modes-of-action>) into group 15 (Inhibitors of chitin biosynthesis, type  
46 0). These compounds interfere with chitin biosynthesis, thus hindering insect molts. Detrimental  
47 effects on fecundity and egg viability are reported as well (Pener and Dhadialla 2012). Although  
48 BPUs have been used as pesticides since the 1970s (Retnakaran and Wright 1987, Spomer and  
49 Sheets 2019), the exact mode of action is still quite obscure (Zhu et al. 2016). However, Douris et  
50 al. (2016) shed some light on the molecular targets of BPUs by demonstrating a direct interaction  
51 with the enzyme chitin synthase 1. These insecticides are persistent and do not show cross-  
52 resistance with neurotoxic active ingredients (Doucet and Retnakaran 2012, Arruda et al. 2020).  
53 Moreover, BPUs are considered less harmful to beneficial insects and non-target organisms than  
54 most nerve poisons (Sun et al. 2015). For these reasons, BPUs are desirable for inclusion in IPM  
55 programs in several cropping systems (Dhadialla et al. 2005).

56 Several studies focused on the effects of a number of insecticides on BMSB, but the activity of

57 BPU has not been investigated extensively. Kamminga et al. (2012) reported promising effects of  
58 diflubenzuron and novaluron on BMSB nymphs in laboratory assays carried out in Petri dishes, no  
59 more investigations were undertaken. The first objective of this study was to evaluate the activity of  
60 triflumuron (the only BPU currently allowed on orchards in EU) on BMSB nymphs by means of  
61 laboratory assays. The effects of aged residues were also investigated because triflumuron is known  
62 to be persistent on vegetation (Marx 1977, Aplada-Sarlis et al. 1999) and this could be a crucial  
63 factor to reduce the frequency of insecticide sprays (Kuhar and Kamminga 2017). Potted peach  
64 plants were used in the experiments because this is more representative of real conditions for  
65 residues aging. Moreover, the use of living plants allowed a prolonged exposure of insects, which is  
66 needed because of the mode action of BPUs (Dhadialla et al. 2005). A second aim of the study was  
67 to test, by mean of a field experiment set up in 2019 on pear orchards, if the addition of triflumuron  
68 to IPM strategies against BMSB could lead to a decrease in injuries to fruit.

69

## 70 **Materials and methods**

### 71 *Insects*

72 BMSB nymphs were obtained from a laboratory colony established in 2015 at the Department of  
73 Agricultural and Food Sciences (University of Bologna). Bugs were reared in a walk-in climatic  
74 chamber at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , 50%–70% RH, with a 14 L:10 D photoperiod. Adults and nymphs were  
75 fed twice per week with green beans, carrots and soybean seeds; fresh fruits (pears, apples or kiwi  
76 fruits) were provided weekly to adults only.

77

### 78 *Insecticide treatments*

79 The insecticide Alsystin 48SC (triflumuron 39.34% =  $480\text{ g L}^{-1}$ ) was provided by Bayer Crop  
80 Sciences (Milan, Italy) and applied at  $25\text{ mL hL}^{-1}$ , the maximum field recommended concentration  
81 for pome and stone fruits. Potted peach plants (GF-677 rootstock, approximately 30 cm high) were

82 sprayed using a hand sprayer up to dripping and allowed to dry completely before trials. Control  
83 plants were sprayed in the same way with tap water.

84

#### 85 *Laboratory bioassays*

86 From July 2017 to November 2018, four aging periods (0, 7, 14 and 21 days) of insecticide residues  
87 were investigated, each in a separate experiment. Twelve experimental units, each consisting of a  
88 potted peach plant, were arranged for each aging period. Six plants were treated with triflumuron  
89 and the others, which were assigned to control, with tap water. Overall, 48 experimental units were  
90 set up and 480 BMSB nymphs were tested.

91 Once sprayed outdoor, plants and were transferred into a glasshouse box where residues of the  
92 treatments aged. Here the plants were maintained for 7, 14 or 21 days at the following conditions:

93  $T_{\max}$  30° C,  $T_{\min}$  15° C, 16 L:8 D photoperiod, minimum artificial lighting in photophase 70,000 lx.

94 In the experiment of 0-day aging of residues, the peach plants were used immediately after  
95 treatment droplets dried.

96 To set up experimental units, potted plants were placed in plexiglas cylinders (diameter 8 cm,  
97 height 30 cm), sealed with a fine net on the top and with a hole (diameter 8 cm) closed with a fine  
98 net on the base to allow air circulation and avoid mold growth. The soil surface on the pot was  
99 covered with nonwoven fabric, which was tightly wrapped to plant stem. Ten BMSB early 3<sup>rd</sup> instar  
100 nymphs were placed in each cylinder. Given that BMSB nymphs must feed on fruit or vegetables to  
101 complete development, carrots and green beans were provided ad libitum and changed twice per  
102 week. The green beans were hung on the plants to force the nymphs to climb on canopy in order to  
103 feed (Supplementary data Fig. SD1).

104 Experimental units were held at the same conditions reported for BMSB rearing and mortality was  
105 checked after 7, 14 and 21 days of continuous exposure of nymphs to residues. Besides the number  
106 of dead nymphs also their instars, and therefore the number of molts they successfully completed,

107 was recorded. Moribund nymphs (i.e. unable to upright themselves when flipped on their back) found  
108 at the last exposure interval were reared individually until they died or reached the adult stage  
109 (recorded as a recovery). Nymphs were held in ventilated 200-mL plastic jars at the same  
110 conditions and with the same food provisions as previously reported.

111

### 112 *Field trials*

113 From 21 June to 5 September 2019, field experiments were carried out in eight pear orchards  
114 located in Northern Italy, which suffered severe damages by BMSB in the previous years. Each  
115 orchard was considered as an experimental block and two contiguous plots (> 0.5 ha) were  
116 delimited. One plot was managed according to IPM strategies (here after referred as “farmer’s  
117 strategy”) recommended in Northern Italy. The other plot of each orchard underwent the same  
118 management practices as the former, but a treatment with Alstylin 48 SC at 25 mL hL<sup>-1</sup> was also  
119 added (hereafter reported as triflumuron strategy). This treatment was carried out between 21 and  
120 30 June targeting the first-generation nymphs of the BMSB. Of course, no triflumuron treatment  
121 was allowed in plots managed with farmer’s strategy. The sprays of broad-spectrum insecticides,  
122 which included chlorpyrifos-methyl, acetamiprid and pyrethroids (deltamethrin, lambda-cyhalothrin  
123 and tau-fluvalinate), varied slightly among orchards, as farmers were allowed to make minor  
124 changes in order to adjust for pest pressure and phenology. These sources of variation were  
125 accounted in the block factor.

126 Fruit sampling was carried out in a transect of 3 rows x 15 m. The transects were established in the  
127 middle of each plot at least 10 m away from plot edges to avoid border effect from the perimeter of  
128 the orchards and to accommodate for drift of triflumuron spray. External injuries were checked at  
129 harvesting on a sample of 400 fruits per plot. Two hundred fruits were sampled at eye-level and 200  
130 were sampled above 2.5-m height using a fruit picking ladder, because most severe injuries by  
131 BMSB are usually recorded on fruit growing in the upper parts of the trees (Bariselli et al. 2016).



132 Pears were examined directly on the trees and scored as injured if at least one distinct depression or  
133 discoloration on fruit surface compatible with BMSB punctures could be detected (Acebes-Doria et  
134 al. 2016). Although the occasional occurrence of Heteropteran bugs other than BMSB could not be  
135 ruled out, the overwhelming majority of fruit injuries were likely due to feeding activity by BMSBs.  
136 Damages to fruit by Heteropteran bugs have been rarely reported in orchards of Northern Italy and  
137 the symptoms used to score injuries to pears are quite specific to BMSB.

138

### 139 *Statistical analysis*

140 To assess the activity of triflumuron on BMSB nymphs in laboratory experiments, insecticide-  
141 treated plants and relative water controls were compared within each aging period of residues. Data  
142 were analyzed by general linear mixed models (GLMM) with first-order autoregressive covariance  
143 structure, binomial distribution, probit link function and Kenward–Roger method for estimating  
144 degrees of freedom. The number of dead insects out of the total nymphs tested was considered as  
145 dependent variable. The treatments (triflumuron and water control) were used as factors, the  
146 exposure intervals (7, 14 and 21 days) were included as repeated measures and their interaction  
147 (treatment x exposure interval) were tested as well.

148 To study the possible decrease in the activity of triflumuron residues (i.e. to compare mortality only  
149 in insecticide-treated plants among different aging period), nymphs mortality recorded in  
150 triflumuron experimental units was corrected by Schneider-Orelli formula (Schneider-Orelli 1947)  
151 considering as natural the mean mortality recorded in water controls aged for the same period.

152 Corrected percentages of mortality matched the assumptions of parametric tests and were analyzed  
153 by a mixed design ANOVA without any transformation. Aging periods (0, 7, 14 and 21 days) were  
154 considered as between-subject factor and exposure intervals (7, 14 and 21) as within-subject factor.

155 The interaction aging period x exposure interval was tested as well.

156 Pearson  $\chi^2$  was used to test the association between the ages of residues and the number of molts that

157 dead nymphs exposed to triflumuron were able to successfully complete before dying.  
158 A GLMM was carried out to analyze data from the field experiments. Binomial error distribution  
159 and log link function were selected, the number of injured fruits out of the total number of fruits  
160 checked was considered as dependent variable, treatments (farmer's strategy vs triflumuron  
161 strategy) and location of fruits (>2.5 m vs eye level) were considered as fixed factors, and orchards  
162 were included as random block factor.  
163 All the analyses were performed using IBM SPSS Statistics (ver. 26).

164

## 165 **Results**

### 166 *Laboratory bioassays*

167 GLMMs detected significant effects on mortality by both main factors (i.e., treatment and exposure  
168 interval) for all aging periods of residues. Whereas the interaction treatment \* exposure interval was  
169 significant only for 7- and 14 -day aged residues (Table 1). In other word, triflumuron residues  
170 caused overall higher mortality than water controls and longer exposure interval increased mortality  
171 (Fig. 1). Because of significant interactions, treatments were compared within each of the exposure  
172 intervals for 7-and 14-day old residues. For both aging periods, GLMMs did not detect significant  
173 difference between triflumuron and control at 7-day exposure but indicated higher mortality in  
174 triflumuron than in water at 14- and 21-day exposures (Table 1).

175 Mortality increased at longer exposure intervals also in the controls with a certain degree of  
176 variation among aging periods. Overall, the lowest number of dead nymphs was counted in the  
177 controls of residues tested immediately after drying. Whereas for other aging periods the natural  
178 mortality ranged from  $16.06 \pm 3.99\%$  to  $28.60 \pm 9.80\%$ ,  $23.55 \pm 4.99\%$  to  $38.14 \pm 7.30\%$  and  $30.07$   
179  $\pm 4.77\%$  to  $47.79 \pm 6.61\%$  for 7-, 14- and 21-day exposure, respectively.

180 The comparison of corrected percentages of mortality did not show any significant effect of aging  
181 periods on the activity of triflumuron residues ( $F_{(3; 20)} = 1.34$ ;  $p = 0.29$ ). The interaction aging

182 period \* exposure interval was not significant too ( $F_{(6, 40)} = 1.32$ ;  $p = 0.27$ ). Therefore, the aging of  
183 residues up to 21 days did not significantly decrease the activity of the insecticide.

184 The number of molts that nymphs exposed to triflumuron were able to complete before dying did  
185 not differ among aging periods ( $\chi^2 = 6.34$ ;  $df = 6$ ;  $p = 0.39$ ). Therefore, the ages of residues did not  
186 significantly affect the insecticide speed of action. Pooling all aging periods, 29.70% of BMSB  
187 nymphs died without completing any molt, 57.43% of individuals successfully molted from 3<sup>rd</sup> to  
188 4<sup>th</sup> instar but died before or during the next molt, 12.87% reached the 5<sup>th</sup> instar but failed to cast the  
189 exuviae and died during the molt to adult stage (Supplementary data Fig. SD2).

190

### 191 *Field trials*

192 The addition of triflumuron to insecticide treatments allowed in IPM strategy for BMSB led to a  
193 significant decrease in the percentage of injured fruits ( $F_{(1; 28)} = 106.93$ ;  $p < 0.001$ ). Fruit location  
194 had also a significant effect ( $F_{(1; 28)} = 137.92$ ;  $p < 0.001$ ), and the percentage of injured fruits was  
195 higher above 2.5 m than at eye level. The interaction strategy \* fruit location was not significant  
196 ( $F_{(1; 28)} = 0.81$ ;  $p = 0.78$ ). A mean of  $9.99 \pm 1.98\%$  injured fruits was detected in plots managed with  
197 triflumuron strategy, whereas  $19.45 \pm 3.55\%$  of fruits were injured in plots assigned to farmer's  
198 strategy. Overall,  $20.19 \pm 3.42\%$  of pears showed symptoms of feeding activity by BMSB above  
199 2.5 m. This percentage dropped to  $9.25 \pm 1.95$  at eye level (Fig. 2).

200

### 201 **Discussion**

202 Triflumuron residues on potted peach plants showed a significant activity on BMSB nymphs that  
203 died increasingly between progressive nymphal instars. These results agreed with laboratory assays  
204 by Kamminga et al. (2012) who reported 90% and 65% mortality of BMSB nymphs fed for 7 days  
205 with fresh beans dipped in solutions of novaluron and diflubenzuron, respectively. Significant  
206 reduction in the abundance of BMSB and other stink bugs was also described for diflubenzuron

207 applications to soybean in open field conditions (Herbert et al. 2013). On the other hand, Gradish et  
208 al. (2019) found neither contact nor residual toxicity of novaluron on BMSB nymphs. However, in  
209 this study, mortality was checked only in the short term, whereas prolonged exposure to BPU  
210 residues is necessary to detect activity of this group of insecticide (Dhadialla et al. 2005).  
211 The activity of triflumuron on BMSB nymphs persisted up to 21 days of residue aging in  
212 glasshouse. Overall, the mortality of bugs exposed to the insecticide was higher than in controls for  
213 all ages of residues. Moreover, no significant decrease in mortality could be detected on insecticide-  
214 treated plants at increasing periods of residues aging. The environmental persistence of BPU has  
215 been well documented and the slow decrease in residue concentration of triflumuron over time  
216 (Bayer Crop Sciences, unpublished data) is in line with the steady insecticidal activity of this active  
217 ingredient up to the longest aging period. Given that treated plants were maintained in a glasshouse,  
218 the only relevant insecticide degradation factor was photolysis. Rainfall, which was not considered  
219 in this study, seems indeed a minor issue as BPU are known to adhere to plant waxes and to  
220 undergo to slight wash off (Retnakaran and Wright 1987, Doucet and Retnakaran 2012).  
221 A relatively high mortality in the controls of some aging periods was related to the long-term  
222 exposure used in the bioassays. Fluctuations in nymphal survival has been often observed in BMSB  
223 laboratory colonies. Our findings were in line with Medal et al. (2013) who reported a juvenile  
224 mortality from 23% to 50% in BMSB rearing and with Fisher et al. (2020) who recorded overall  
225 low nymphal survival when testing the influence of temperature and humidity on BMSB.  
226 Progressive poisoning through tarsal adsorption seemed the main way of insecticide contamination  
227 because most of BMSB nymphs did not die at the first molt, but many insects completed one or  
228 even two molts before succumbing. Ingestion has been usually considered as the major way of  
229 uptake for BPU, but slow penetration through cuticle was also described (Hammann and  
230 Sirrenberg 1980, Retnakaran and Wright 1987, Sun et al. 2015). This second way of triflumuron  
231 uptake was likely prevalent in our experiments, given that untreated food was used and the bug

232 probing on treated peach plants was negligible.

233 Given that intoxicated nymphs could survive for a couple of weeks, the damage they may cause  
234 before succumbing needs to be taken into account. In this context, sub lethal effects may be worth  
235 of consideration as secondary impacts on pest population (Cira et al. 2017, Depalo et al. 2017). In  
236 particular, BPU's could reduce hatch rate, impair feeding activity and increase susceptibility to  
237 pathogens on intoxicated BMSB (Catchot et al. 2021).

238 Early BMSB instars are more susceptible to insecticides than adults and several insecticides have  
239 demonstrated a significant activity on nymphs (Kuhar and Kamminga 2017, Gradish et al. 2019).  
240 Mostly broad-spectrum neurotoxic active ingredients have been so far investigated and, in  
241 comparison with these insecticides, BPU's are more compatible with IPM (Dhadialla et al. 2005,  
242 Doucet and Retnakaran 2012). Nevertheless, BPU's do not lack any impact on beneficials. Lethal  
243 effects have been found on nymphs of predatory bugs (Soares et al. 2019) and on larvae of  
244 ladybeetles (Cabrera et al 2017). Some sub-lethal effects have been reported on adult parasitoids  
245 either after direct contact with residues (Matioli et al. 2019) or when parasitized eggs of the hosts  
246 were treated (Goulart et al. 2012). Therefore, the possible detrimental effects of BPU's on natural  
247 enemies of BMSB seem worth of investigations to draw a comprehensive picture of the possible  
248 role of these insecticides in IPM scenario.

249 Besides the environmental side effects and the disruption of IPM practices, the overuse of a few  
250 neurotoxic insecticides to control BMSB often impaired the rotation of insecticide chemistries upon  
251 which insecticide resistance management strategy should rely (Hurley and Mitchell 2014). The  
252 potential for insecticide-resistance development in BMSB populations has not been established yet,  
253 but it would be wise to avoid over-reliance on neurotoxic active ingredients (Alford et al. 2020).  
254 From this standpoint, the inclusion of BPU's in IPM practices could help to restore active ingredient  
255 rotation as a strategy to delay the insurgence of resistance (Spomer and Sheets 2019).

256 The long-lasting activity of triflumuron, and possibly other BPU's, could be exploited to reduce

257 damages by BMSB nymphs without the need of frequent treatments. The short residual activity,  
258 which lasts less than three days for most insecticides used against BMSB (Leskey et al. 2014), is  
259 widely recognized as a major problem (Kuhar and Kamminga 2017). This pest is polyphagous and  
260 very mobile; both nymphs and adults have big dispersal abilities and move across the landscape  
261 following phenology of crops and uncropped plants (Martinson et al. 2015, Hamilton et al. 2018).  
262 Repeated and unpredictable infestations of orchards are common and frequent sprays of short-  
263 lasting insecticides are needed.

264 The field experiments, which showed a significant decrease in the percentages of injured pears  
265 where triflumuron was added, reinforced the findings of laboratory assays. Field trials pointed out  
266 also that in 2019 strategies mainly relying on neurotoxic active ingredients did not achieve a  
267 satisfactory control of BMSB. The mean percentage of injured fruit recorded in plots managed by  
268 farmer's strategy ( $19.45 \pm 3.55$ ) far exceeded the economic threshold. Although the percentage of  
269 injured fruits dropped to  $9.99 \pm 1.98$  in the plots where triflumuron was added, this was still not  
270 completely acceptable from an economic standpoint. A refinement of management strategies for  
271 BMSB seems therefore urgently needed and the development of treatment thresholds for this stink  
272 bug would be a relevant step forward (Short et al. 2017).

273 BPU's lack any lethal effect on adult insects and have no chance to decrease the pressure exerted by  
274 highly damaging adult bugs that migrate into crops from surrounding areas (Acebes-Doria et al.  
275 2016). Therefore, triflumuron and other BPU's might be considered for field applications only as a  
276 tool in a wider management strategy. Control methods aimed at preventing the invasion of crops by  
277 adult bugs such as exclusion nets (Candian et al. 2018), insecticide netting screens and sprays at  
278 orchard perimeter (Blaauw et al. 2015) might take major benefits from few and long-lasting BPU  
279 treatments aimed decreasing nymphal populations and hampering reproduction cycle that can occur  
280 within the orchards.

281

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286

287

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400 **Figure Caption**

401 **Fig. 1.** Mortality of *Halyomorpha halys* nymphs exposed for 7, 14 and 21 days to residues of  
402 triflumuron (black line) or control (gray line) on potted peach plants. Each panel represent a  
403 different aging period of residues in glasshouse conditions. Vertical bars indicate standard errors of  
404 the means. Asterisks indicate significant differences between treatments, which were compared  
405 within exposure intervals, in the case of significant interaction of treatment \* exposure interval  
406 detected by GLMM.

407

408 **Fig. 2.** Percentages of fruit with feeding injuries by *Halyomorpha halys* recorded in 2019 in pear  
409 orchards in Northern Italy. GLMM detected significant differences between strategies ( $p<0.001$ )  
410 and between fruit locations ( $p<0.001$ ). Vertical bars indicate standard errors of the means.



10 **Abstract**

11 *Halyomorpha halys*, (the brown marmorated stink bug, BMSB), is a high-concern invasive species  
12 causing severe damage to orchards in many countries outside its native Asian range. Control  
13 options matching both effectiveness and sustainability are currently lacking. Inhibitors of chitin  
14 biosynthesis might be exploited for integrated management programs because of the overall better  
15 ecotoxicological profile in comparison with most neurotoxic insecticides used so far against BMSB.  
16 In this study, the activity of triflumuron, a benzoylphenyl urea hampering chitin biosynthesis, was  
17 tested on BMSB in laboratory and field conditions. In laboratory bioassays, the insecticide was  
18 sprayed on potted peach plants (30 cm high) and residues were aged in a glasshouse for 0, 7, 14 and  
19 21 days. Then 3<sup>rd</sup> instar bugs were placed on the plants and continuously exposed to residues.  
20 Mortality was scored after 7, 14 and 21 day exposure. Triflumuron caused significantly higher  
21 mortality on BMSB nymphs in comparison with water controls at all aging periods. Moreover,  
22 aging of residues up to 21 days did not cause any significant reduction of activity. Field  
23 experiments were also carried out in 2019 in eight pear orchards. Injuries to fruits at **harvest** were  
24 compared between plots where triflumuron was added to insecticide sprays against BMSB and  
25 control plots managed exactly in the same way but without any triflumuron treatment. An overall  
26 mean of  $9.99 \pm 1.98\%$  **stink bug** injured fruits was detected in plots managed with the strategy  
27 including triflumuron, whereas  $19.45 \pm 3.55\%$  of fruits were injured in plots assigned to controls.

28

29 **Keywords:** Brown marmorated stink bug; Invasive species; Inhibitors of chitin biosynthesis;  
30 Integrated pest management; Reduced-risk insecticides.

31

## 32 **Introduction**

33 The brown marmorated stink bug (BSMB) - *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) -  
34 is a serious agricultural pest in most countries where it has been accidentally introduced (Bosco et  
35 al. 2017, Leskey and Nielsen 2018). Current management strategies rely mostly on neurotoxic  
36 broad-spectrum insecticides that are quite effective in preventing BMSB damages but have a short  
37 residual activity (Kuhar and Kamminga 2017). This forces farmers to increase the frequency of  
38 insecticide sprays (Leskey et al. 2012) causing the disruption of previously established integrated  
39 pest management (IPM) programs and the resurgence of secondary pests (Leskey and Nielsen  
40 2018). The identification of more selective insecticides targeting BMSB would significantly reduce  
41 the environmental issues associated to its control and could contribute to IPM principles. Besides  
42 efficacy on BMSB and less severe side effects, residual activity of insecticides could play a key role  
43 in restoring sustainable management programs.

44 Benzoylphenyl ureas (BPUs) are classified by Insecticide Resistance Action Committee (IRAC,  
45 <https://www.irc-online.org/modes-of-action>) into group 15 (Inhibitors of chitin biosynthesis, type  
46 0). These compounds interfere with chitin biosynthesis, thus hindering insect molts. Detrimental  
47 effects on fecundity and egg viability are reported as well (Pener and Dhadialla 2012). Although  
48 BPUs have been used as pesticides since the 1970s (Retnakaran and Wright 1987, Spomer and  
49 Sheets 2019), the exact mode of action is still quite obscure (Zhu et al. 2016). However, Douris et  
50 al. (2016) shed some light on the molecular targets of BPUs by demonstrating a direct interaction  
51 with the enzyme chitin synthase 1. These insecticides are persistent and do not show cross-  
52 resistance with neurotoxic active ingredients (Doucet and Retnakaran 2012, Arruda et al. 2020).  
53 Moreover, BPUs are considered less harmful to beneficial insects and non-target organisms than  
54 most nerve poisons (Sun et al. 2015). For these reasons, BPUs are desirable for inclusion in IPM  
55 programs in several cropping systems (Dhadialla et al. 2005).

56 Several studies focused on the effects of a number of insecticides on BMSB, but the activity of

57 BPU has not been investigated extensively. Kamminga et al. (2012) reported promising effects of  
58 diflubenzuron and novaluron on BMSB nymphs in laboratory assays carried out in Petri dishes, no  
59 more investigations were undertaken. The first objective of this study was to evaluate the activity of  
60 triflumuron (the only BPU currently allowed on orchards in EU) on BMSB nymphs by means of  
61 laboratory assays. The effects of aged residues were also investigated because triflumuron is known  
62 to be persistent on vegetation (Marx 1977, Aplada-Sarlis et al. 1999) and this could be a crucial  
63 factor to reduce the frequency of insecticide sprays (Kuhar and Kamminga 2017). Potted peach  
64 plants were used in the experiments because this is more representative of real conditions for  
65 residues aging. Moreover, the use of living plants allowed a prolonged exposure of insects, which is  
66 needed because of the mode action of BPUs (Dhadialla et al. 2005). A second aim of the study was  
67 to test, by mean of a field experiment set up in 2019 on pear orchards, if the addition of triflumuron  
68 to IPM strategies against BMSB could lead to a decrease in injuries to fruit.

69

## 70 **Materials and methods**

### 71 *Insects*

72 BMSB nymphs were obtained from a laboratory colony established in 2015 at the Department of  
73 Agricultural and Food Sciences (University of Bologna). Bugs were reared in a walk-in climatic  
74 chamber at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , 50%–70% RH, with a 14 L:10 D photoperiod. Adults and nymphs were  
75 fed twice per week with green beans, carrots and soybean seeds; fresh fruits (pears, apples or kiwi  
76 fruits) were provided weekly to adults only.

77

### 78 *Insecticide treatments*

79 The insecticide Alsystin 48SC (triflumuron 39.34% =  $480\text{ g L}^{-1}$ ) was provided by Bayer Crop  
80 Sciences (Milan, Italy) and applied at  $25\text{ mL hL}^{-1}$ , the maximum field recommended concentration  
81 for pome and stone fruits. Potted peach plants (GF-677 rootstock, approximately 30 cm high) were



82 sprayed using a hand sprayer up to dripping and allowed to dry completely before trials. Control  
83 plants were sprayed in the same way with tap water.

84

#### 85 *Laboratory bioassays*

86 From July 2017 to November 2018, four aging periods (0, 7, 14 and 21 days) of insecticide residues  
87 were investigated, each in a separate experiment. Twelve experimental units, each consisting of a  
88 potted peach plant, were arranged for each aging period. Six plants were treated with triflumuron  
89 and the others, which were assigned to control, with tap water. Overall, 48 experimental units were  
90 set up and 480 BMSB nymphs were tested.

91 Once sprayed outdoor, plants and were transferred into a glasshouse box where residues of the  
92 treatments aged. Here the plants were maintained for 7, 14 or 21 days at the following conditions:

93  $T_{\max}$  30° C,  $T_{\min}$  15° C, 16 L:8 D photoperiod, minimum artificial lighting in photophase 70,000 lx.

94 In the experiment of 0-day aging of residues, the peach plants were used immediately after  
95 treatment droplets dried.

96 To set up experimental units, potted plants were placed in plexiglas cylinders (diameter 8 cm,  
97 height 30 cm), sealed with a fine net on the top and with a hole (diameter 8 cm) closed with a fine  
98 net on the base to allow air circulation and avoid mold growth. The soil surface on the pot was  
99 covered with nonwoven fabric, which was tightly wrapped to plant stem. Ten BMSB early 3<sup>rd</sup> instar  
100 nymphs were placed in each cylinder. Given that BMSB nymphs must feed on fruit or vegetables to  
101 complete development, carrots and green beans were provided ad libitum and changed twice per  
102 week. The green beans were hung on the plants to force the nymphs to climb on canopy in order to  
103 feed (Supplementary data Fig. SD1).

104 Experimental units were held at the same conditions reported for BMSB rearing and mortality was  
105 checked after 7, 14 and 21 days of continuous exposure of nymphs to residues. Besides the number  
106 of dead nymphs also their instars, and therefore the number of molts they successfully completed,

107 was recorded. Moribund nymphs (i.e. unable to upright themselves when flipped on their back) found  
108 at the last exposure interval were reared individually until they died or reached the adult stage  
109 (recorded as a recovery). Nymphs were held in ventilated 200-mL plastic jars at the same  
110 conditions and with the same food provisions as previously reported.

111

### 112 *Field trials*

113 From 21 June to 5 September 2019, field experiments were carried out in eight pear orchards  
114 located in Northern Italy, which suffered severe damages by BMSB in the previous years. Each  
115 orchard was considered as an experimental block and two contiguous plots (> 0.5 ha) were  
116 delimited. One plot was managed according to IPM strategies (here after referred as “farmer’s  
117 strategy”) recommended in Northern Italy. The other plot of each orchard underwent the same  
118 management practices as the former, but a treatment with Alsystem 48 SC at 25 mL hL<sup>-1</sup> was also  
119 added (hereafter reported as triflumuron strategy). This treatment was carried out between 21 and  
120 30 June targeting the first-generation nymphs of the BMSB. Of course, no triflumuron treatment  
121 was allowed in plots managed with farmer’s strategy. The sprays of broad-spectrum insecticides,  
122 which included chlorpyrifos-methyl, acetamiprid and pyrethroids (deltamethrin, lambda-cyhalothrin  
123 and tau-fluvalinate), varied slightly among orchards, as farmers were allowed to make minor  
124 changes in order to adjust for pest pressure and phenology. These sources of variation were  
125 accounted in the block factor.

126 Fruit sampling was carried out in a transect of 3 rows x 15 m. The transects were established in the  
127 middle of each plot at least 10 m away from plot edges to avoid border effect from the perimeter of  
128 the orchards and to accommodate for drift of triflumuron spray. External injuries were checked at  
129 harvesting on a sample of 400 fruits per plot. Two hundred fruits were sampled at eye-level and 200  
130 were sampled above 2.5-m height using a fruit picking ladder, because most severe injuries by  
131 BMSB are usually recorded on fruit growing in the upper parts of the trees (Bariselli et al. 2016).

132 Pears were examined directly on the trees and scored as injured if at least one distinct depression or  
133 discoloration on fruit surface compatible with BMSB punctures could be detected (Acebes-Doria et  
134 al. 2016). Although the occasional occurrence of Heteropteran bugs other than BMSB could not be  
135 ruled out, the overwhelming majority of fruit injuries were likely due to feeding activity by BMSBs.  
136 Damages to fruit by Heteropteran bugs have been rarely reported in orchards of Northern Italy and  
137 the symptoms used to score injuries to pears are quite specific to BMSB.

138

### 139 *Statistical analysis*

140 To assess the activity of triflumuron on BMSB nymphs in laboratory experiments, insecticide-  
141 treated plants and relative water controls were compared within each aging period of residues. Data  
142 were analyzed by general linear mixed models (GLMM) with first-order autoregressive covariance  
143 structure, binomial distribution, probit link function and Kenward–Roger method for estimating  
144 degrees of freedom. The number of dead insects out of the total nymphs tested was considered as  
145 dependent variable. The treatments (triflumuron and water control) were used as factors, the  
146 exposure intervals (7, 14 and 21 days) were included as repeated measures and their interaction  
147 (treatment x exposure interval) were tested as well.

148 To study the possible decrease in the activity of triflumuron residues (i.e. to compare mortality only  
149 in insecticide-treated plants among different aging period), nymphs mortality recorded in  
150 triflumuron experimental units was corrected by Schneider-Orelli formula (Schneider-Orelli 1947)  
151 considering as natural the mean mortality recorded in water controls aged for the same period.

152 Corrected percentages of mortality matched the assumptions of parametric tests and were analyzed  
153 by a mixed design ANOVA without any transformation. Aging periods (0, 7, 14 and 21 days) were  
154 considered as between-subject factor and exposure intervals (7, 14 and 21) as within-subject factor.  
155 The interaction aging period x exposure interval was tested as well.

156 Pearson  $\chi^2$  was used to test the association between the ages of residues and the number of molts that

157 dead nymphs exposed to triflumuron were able to successfully complete before dying.  
158 A GLMM was carried out to analyze data from the field experiments. Binomial error distribution  
159 and log link function were selected, the number of injured fruits out of the total number of fruits  
160 checked was considered as dependent variable, treatments (farmer's strategy vs triflumuron  
161 strategy) and location of fruits (>2.5 m vs eye level) were considered as fixed factors, and orchards  
162 were included as random block factor.  
163 All the analyses were performed using IBM SPSS Statistics (ver. 26).

164

## 165 **Results**

### 166 *Laboratory bioassays*

167 GLMMs detected significant effects on mortality by both main factors (i.e., treatment and exposure  
168 interval) for all aging periods of residues. Whereas the interaction treatment \* exposure interval was  
169 significant only for 7- and 14 -day aged residues (Table 1). In other word, triflumuron residues  
170 caused overall higher mortality than water controls and longer exposure interval increased mortality  
171 (Fig. 1). Because of significant interactions, treatments were compared within each of the exposure  
172 intervals for 7-and 14-day old residues. For both aging periods, GLMMs did not detect significant  
173 difference between triflumuron and control at 7-day exposure but indicated higher mortality in  
174 triflumuron than in water at 14- and 21-day exposures (Table 1).

175 Mortality increased at longer exposure intervals also in the controls with a certain degree of  
176 variation among aging periods. Overall, the lowest number of dead nymphs was counted in the  
177 controls of residues tested immediately after drying. Whereas for other aging periods the natural  
178 mortality ranged from  $16.06 \pm 3.99\%$  to  $28.60 \pm 9.80\%$ ,  $23.55 \pm 4.99\%$  to  $38.14 \pm 7.30\%$  and  $30.07$   
179  $\pm 4.77\%$  to  $47.79 \pm 6.61\%$  for 7-, 14- and 21-day exposure, respectively.

180 The comparison of corrected percentages of mortality did not show any significant effect of aging  
181 periods on the activity of triflumuron residues ( $F_{(3; 20)} = 1.34; p = 0.29$ ). The interaction aging

182 period \* exposure interval was not significant too ( $F_{(6, 40)} = 1.32; p = 0.27$ ). Therefore, the aging of  
183 residues up to 21 days did not significantly decrease the activity of the insecticide.

184 The number of molts that nymphs exposed to triflumuron were able to complete before dying did  
185 not differ among aging periods ( $\chi^2 = 6.34; df = 6; p = 0.39$ ). Therefore, the ages of residues did not  
186 significantly affect the insecticide speed of action. Pooling all aging periods, 29.70% of BMSB  
187 nymphs died without completing any molt, 57.43% of individuals successfully molted from 3<sup>rd</sup> to  
188 4<sup>th</sup> instar but died before or during the next molt, 12.87% reached the 5<sup>th</sup> instar but failed to cast the  
189 exuviae and died during the molt to adult stage (Supplementary data Fig. SD2).

190

### 191 *Field trials*

192 The addition of triflumuron to insecticide treatments allowed in IPM strategy for BMSB led to a  
193 significant decrease in the percentage of injured fruits ( $F_{(1; 28)} = 106.93; p < 0.001$ ). Fruit location  
194 had also a significant effect ( $F_{(1; 28)} = 137.92; p < 0.001$ ), and the percentage of injured fruits was  
195 higher above 2.5 m than at eye level. The interaction strategy \* fruit location was not significant  
196 ( $F_{(1; 28)} = 0.81; p = 0.78$ ). A mean of  $9.99 \pm 1.98\%$  injured fruits was detected in plots managed with  
197 triflumuron strategy, whereas  $19.45 \pm 3.55\%$  of fruits were injured in plots assigned to farmer's  
198 strategy. Overall,  $20.19 \pm 3.42\%$  of pears showed symptoms of feeding activity by BMSB above  
199 2.5 m. This percentage dropped to  $9.25 \pm 1.95$  at eye level (Fig. 2).

200

### 201 **Discussion**

202 Triflumuron residues on potted peach plants showed a significant activity on BMSB nymphs that  
203 died increasingly between progressive nymphal instars. These results agreed with laboratory assays  
204 by Kamminga et al. (2012) who reported 90% and 65% mortality of BMSB nymphs fed for 7 days  
205 with fresh beans dipped in solutions of novaluron and diflubenzuron, respectively. Significant  
206 reduction in the abundance of BMSB and other stink bugs was also described for diflubenzuron

207 applications to soybean in open field conditions (Herbert et al. 2013). On the other hand, Gradish et  
208 al. (2019) found neither contact nor residual toxicity of novaluron on BMSB nymphs. However, in  
209 this study, mortality was checked only in the short term, whereas prolonged exposure to BPU  
210 residues is necessary to detect activity of this group of insecticide (Dhadialla et al. 2005).

211 The activity of triflumuron on BMSB nymphs persisted up to 21 days of residue aging in  
212 glasshouse. Overall, the mortality of bugs exposed to the insecticide was higher than in controls for  
213 all ages of residues. Moreover, no significant decrease in mortality could be detected on insecticide-  
214 treated plants at increasing periods of residues aging. The environmental persistence of BPU has  
215 been well documented and the slow decrease in residue concentration of triflumuron over time  
216 (Bayer Crop Sciences, unpublished data) is in line with the steady insecticidal activity of this active  
217 ingredient up to the longest aging period. Given that treated plants were maintained in a glasshouse,  
218 the only relevant insecticide degradation factor was photolysis. Rainfall, which was not considered  
219 in this study, seems indeed a minor issue as BPU are known to adhere to plant waxes and to  
220 undergo to slight wash off (Retnakaran and Wright 1987, Doucet and Retnakaran 2012).

221 A relatively high mortality in the controls of some aging periods was related to the long-term  
222 exposure used in the bioassays. Fluctuations in nymphal survival has been often observed in BMSB  
223 laboratory colonies. Our findings were in line with Medal et al. (2013) who reported a juvenile  
224 mortality from 23% to 50% in BMSB rearing and with Fisher et al. (2020) who recorded overall  
225 low nymphal survival when testing the influence of temperature and humidity on BMSB.

226 Progressive poisoning through tarsal adsorption seemed the main way of insecticide contamination  
227 because most of BMSB nymphs did not die at the first molt, but many insects completed one or  
228 even two molts before succumbing. Ingestion has been usually considered as the major way of  
229 uptake for BPU, but slow penetration through cuticle was also described (Hamman and  
230 Sirrenberg 1980, Retnakaran and Wright 1987, Sun et al. 2015). This second way of triflumuron  
231 uptake was likely prevalent in our experiments, given that untreated food was used and the bug

232 probing on treated peach plants was negligible.

233 Given that intoxicated nymphs could survive for a couple of weeks, the damage they may cause  
234 before succumbing needs to be taken into account. In this context, sub lethal effects may be **worth**  
235 of consideration as secondary impacts on pest population (Cira et al. 2017, Depalo et al. 2017). In  
236 particular, BPU's could reduce hatch rate, impair feeding activity and increase susceptibility to  
237 pathogens on intoxicated BMSB (Catchot et al. 2021).

238 Early BMSB instars are more susceptible to insecticides than adults and several insecticides have  
239 demonstrated a significant activity on nymphs (Kuhar and Kamminga 2017, Gradish et al. 2019).  
240 Mostly broad-spectrum neurotoxic active ingredients have been so far investigated and, in  
241 comparison with these insecticides, BPU's are more compatible with IPM (Dhadialla et al. 2005,  
242 Doucet and Retnakaran 2012). Nevertheless, BPU's do not lack any impact on beneficials. Lethal  
243 effects have been found on nymphs of predatory bugs (Soares et al. 2019) and on larvae of  
244 ladybeetles (Cabrera et al 2017). Some sub-lethal effects have been reported on adult parasitoids  
245 either after direct contact with residues (Matioli et al. 2019) or when parasitized eggs of the hosts  
246 were treated (Goulart et al. 2012). Therefore, the possible detrimental effects of BPU's on natural  
247 enemies of BMSB seem worth of investigations to draw a comprehensive picture of the possible  
248 role of these insecticides in IPM scenario.

249 Besides the environmental side effects and the disruption of IPM practices, the overuse of a few  
250 neurotoxic insecticides to control BMSB often impaired the rotation of insecticide chemistries upon  
251 which insecticide resistance management strategy should rely (Hurley and Mitchell 2014). The  
252 potential for insecticide-resistance development in BMSB populations has not been established yet,  
253 but it would be wise to avoid over-reliance on neurotoxic active ingredients (Alford et al. 2020).  
254 From this standpoint, the inclusion of BPU's in IPM practices could help to restore active ingredient  
255 rotation as a strategy to delay the insurgence of resistance (Spomer and Sheets 2019).

256 The long-lasting activity of triflumuron, and possibly other BPU's, could be exploited to reduce

257 damages by BMSB nymphs without the need of frequent treatments. The short residual activity,  
258 which lasts less than three days for most insecticides used against BMSB (Leskey et al. 2014), is  
259 widely recognized as a major problem (Kuhar and Kamminga 2017). This pest is polyphagous and  
260 very mobile; both nymphs and adults have big dispersal abilities and move across the landscape  
261 following phenology of crops and uncropped plants (Martinson et al. 2015, Hamilton et al. 2018).  
262 Repeated and unpredictable infestations of orchards are common and frequent sprays of short-  
263 lasting insecticides are needed.

264 The field experiments, which showed a significant decrease in the percentages of injured pears  
265 where triflumuron was added, reinforced the findings of laboratory assays. Field trials pointed out  
266 also that in 2019 strategies mainly relying on neurotoxic active ingredients did not achieve a  
267 satisfactory control of BMSB. The mean percentage of injured fruit recorded in plots managed by  
268 farmer's strategy ( $19.45 \pm 3.55$ ) far exceeded the economic threshold. Although the percentage of  
269 injured fruits dropped to  $9.99 \pm 1.98$  in the plots where triflumuron was added, this was still not  
270 completely acceptable from an economic standpoint. A refinement of management strategies for  
271 BMSB seems therefore urgently needed and the development of treatment thresholds for this stink  
272 bug would be a relevant step forward (Short et al. 2017).

273 BPUs lack any lethal effect on adult insects and have no chance to **decrease** the pressure exerted by  
274 highly damaging adult bugs that migrate into crops from surrounding areas (Acebes-Doria et al.  
275 2016). Therefore, triflumuron and other BPUs might be considered for field applications only as a  
276 tool in a wider management strategy. Control methods aimed at preventing the invasion of crops by  
277 adult bugs such as exclusion nets (Candian et al. 2018), insecticide netting screens and sprays at  
278 orchard perimeter (Blaauw et al. 2015) might take major benefits from few and long-lasting BPU  
279 treatments aimed decreasing nymphal populations and hampering reproduction cycle that can occur  
280 within the orchards.

281



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286

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400 **Figure Caption**

401 **Fig. 1.** Mortality of *Halyomorpha halys* nymphs exposed for 7, 14 and 21 days to residues of  
402 triflumuron (black line) or control (gray line) on potted peach plants. Each panel represent a  
403 different aging period of residues in glasshouse conditions. Vertical bars indicate standard errors of  
404 the means. Asterisks indicate significant differences between treatments, which were compared  
405 within exposure intervals, in the case of significant interaction of treatment \* exposure interval  
406 detected by **GLMM**.

407

408 **Fig. 2.** Percentages of fruit with feeding injuries by *Halyomorpha halys* recorded in 2019 in pear  
409 orchards in Northern Italy. GLMM detected significant differences between strategies ( $p<0.001$ )  
410 and between fruit locations ( $p<0.001$ ). Vertical bars indicate standard errors of the means.

**Table 1.** Percentages of mortality (mean  $\pm$  standard error) of *Halyomorpha halys* nymphs recorded for the different aging periods of residues at increasing exposure intervals. **GLMMs** were carried separately for each aging period and tests of fixed effects are reported. In the case of significant interaction of treatment \* exposure interval, treatments were compared within each exposure interval and the *p* level is reported below mortality means.

<b>0-day aged residues</b>		<b>Exposure intervals</b>		
<b>Treatment</b>	7 days	14 days	21 days	
Triflumuron	36.67 $\pm$ 14.06	65.00 $\pm$ 9.22	80.00 $\pm$ 7.72	
Control	5.00 $\pm$ 3.42	11.67 $\pm$ 4.77	20.00 $\pm$ 6.83	
<b>GLMM effects</b>	<b>F</b>	<b>df</b>	<b>p</b>	
Treatment	14.35	1; 9.89	0.004	
Exposure interval	10.58	2; 21.12	0.001	
Treatment * Exposure interval	0.42	2; 21.12	0.661	

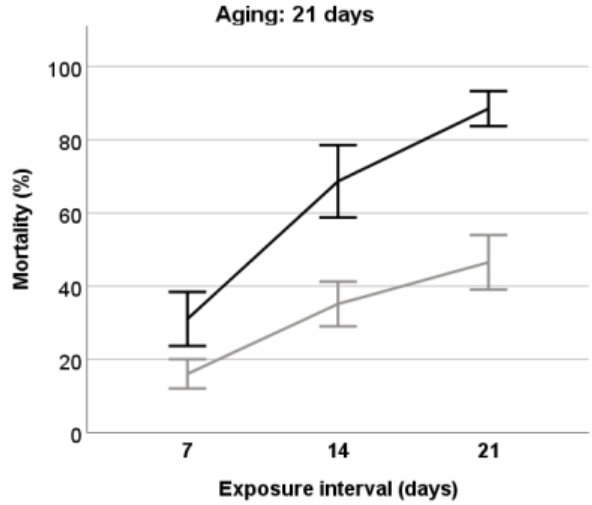
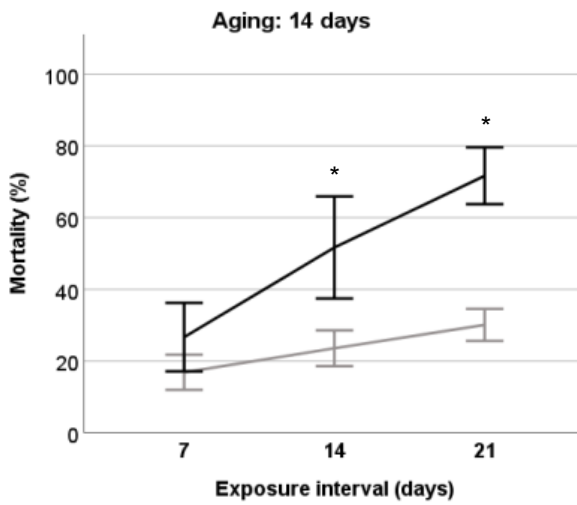
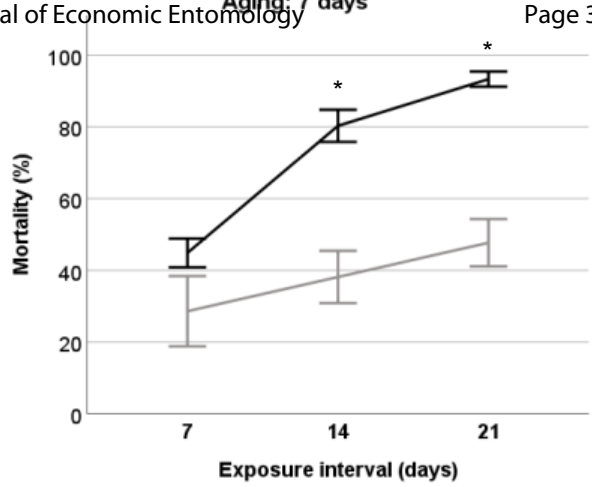
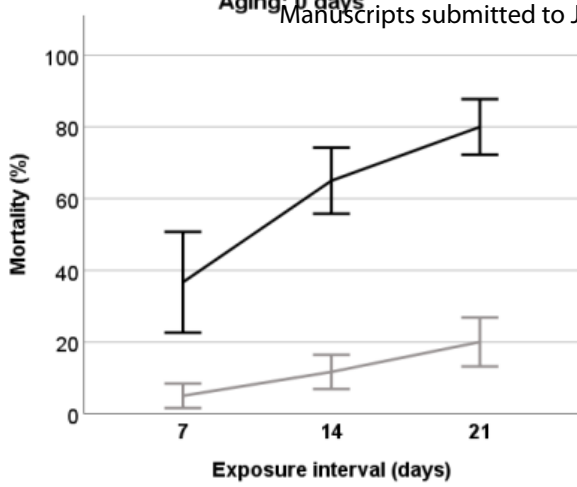
<b>7-day aged residues</b>		<b>Exposure intervals</b>		
<b>Treatment</b>	7 days	14 days	21 days	
Triflumuron	44.85 $\pm$ 4.01	80.30 $\pm$ 4.48	93.33 $\pm$ 2.11	
Control	28.60 $\pm$ 9.80	38.14 $\pm$ 7.30	47.69 $\pm$ 6.61	
	<i>p</i> = 0.064	<i>p</i> < 0.001	<i>p</i> < 0.001	
<b>GLMM effects</b>	<b>F</b>	<b>df</b>	<b>p</b>	
Treatment	21.03	1; 10.33	0.001	
Exposure interval	27.88	2; 20.84	<0.001	
Treatment * Exposure interval	7.50	2; 20.84	0.004	

<b>14-day aged residues</b>		<b>Exposure intervals</b>		
<b>Treatment</b>	7 days	14 days	21 days	
Triflumuron	26.67 $\pm$ 9.55	51.67 $\pm$ 14.24	71.67 $\pm$ 7.92	
Control	16.85 $\pm$ 4.90	23.55 $\pm$ 4.99	30.07 $\pm$ 4.77	
	<i>p</i> = 0.327	<i>p</i> = 0.023	<i>p</i> = 0.002	
<b>GLMM effects</b>	<b>F</b>	<b>df</b>	<b>p</b>	
Treatment	6.05	1; 11.09	0.032	
Exposure interval	18.05	2; 21.03	<0.001	
Treatment * Exposure interval	3.82	2; 21.03	0.038	

<b>21-day aged residues</b>		<b>Exposure intervals</b>		
<b>Treatment</b>	7 days	14 days	21 days	
Triflumuron	31.06 $\pm$ 7.38	68.64 $\pm$ 9.88	88.48 $\pm$ 4.79	
Control	16.06 $\pm$ 3.99	35.15 $\pm$ 6.11	46.52 $\pm$ 7.56	
<b>GLMM effects</b>	<b>F</b>	<b>df</b>	<b>p</b>	
Treatment	13.23	1; 11.31	0.004	
Exposure interval	29.13	2; 20.92	<0.001	
Treatment * Exposure interval	2.51	2; 20.92	0.105	



I = triflumuron    I = control



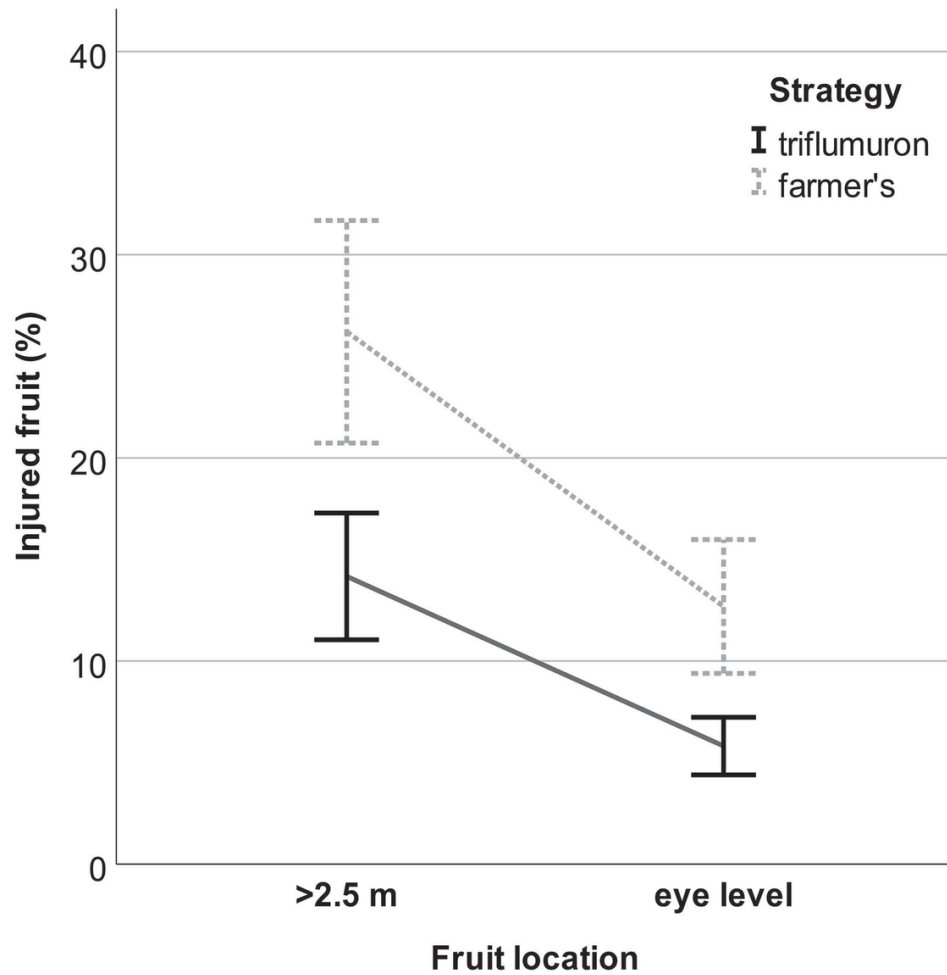


Fig. 2. Percentages of fruit with feeding injuries by *Halyomorpha halys* recorded in 2019 in pear orchards in Northern Italy. GLMM detected significant differences between strategies ( $p < 0.001$ ) and between fruit locations ( $p < 0.001$ ). Vertical bars indicate standard errors of the means.

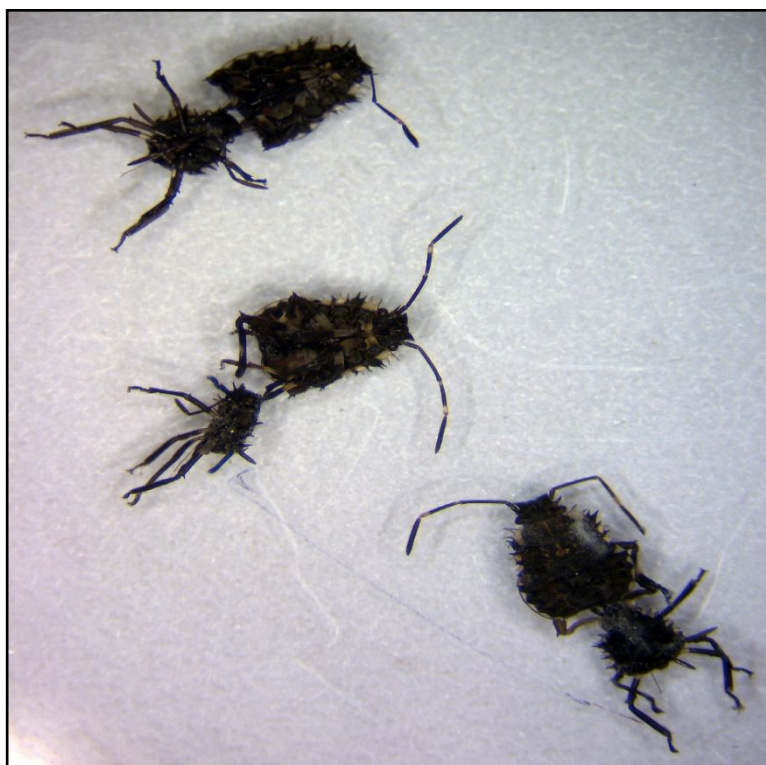
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**SUPPLEMENTARY DATA****Impact of triflumuron on *Halyomorpha halys* (Hemiptera: Pentatomidae):****laboratory and field studies**

Antonio Masetti, Laura Depalo and Edison Pasqualini



**Fig. SD1.** Experimental units with potted peach plants inserted in plexiglas cylinders.

**N3 / N4****N4 / N5****N5 / adult**

**Fig. SD1.** Individuals of *Halyomorpha halys* died at the ecdysis. Unsuccessful molting is a typical symptom of the activity of benzoylphenyl ureas.