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1 Title: Toxicity and persistence of three pyrethroids for the control of cereal bugs on common
2 wheat

3 Marco G. Pansa, Massimo Blandino, Barbara L. Ingegno, Ester Ferrari, Amedeo Reyneri,
4 Luciana Tavella

5

6 Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino, via
7 Leonardo da Vinci 44, I-10095 Grugliasco (TO), Italy

8

9 Corresponding author: Luciana Tavella

10 Phone +39 011 6708533, fax +39 011 6708535, luciana.tavella@unito.it

11

12 Abstract

13 Sunn pests are responsible for serious kernel damage on wheat (*Triticum aestivum* L.,
14 Poaceae), affecting bread-making quality from their feeding at the late milk-ripe stage. In this
15 study the toxicity of three pyrethroids usually applied to this crop (λ -cyhalothrin, α -
16 cypermethrin, τ -fluvalinate) was evaluated on two bug species, the wheat bug *Eurygaster*
17 *maura* (L.) and the green stink bug *Nezara viridula* (L.) in both laboratory and semi-field
18 conditions, to implement an effective and sustainable control strategy. Laboratory trials
19 showed small differences between the insecticides, whereas field cage trials gave more
20 information on toxicity and persistence of the three pyrethroids. The most toxic and with good
21 persistence on plants was α -cypermethrin, followed by λ -cyhalothrin, which had an
22 intermediate persistence. By contrast, τ -fluvalinate showed a high persistence on plants, but
23 seemed unsuited for wheat bug control because of its low toxicity, as clearly demonstrated in
24 both laboratory and semi-field trials. The results provide clear information on the application
25 strategy of the tested pyrethroids. In this context, α -cypermethrin proved to be the most

26 promising active ingredient for wheat bug control, particularly for early application of the
27 insecticide in a mixture with a fungicide at the wheat heading stage, eliminating the need for a
28 separate additional treatment by the farmer.

29

30 Key words: *Eurygaster maura*, *Nezara viridula*, *Triticum aestivum*, LD₅₀, dissipation

31

32 **Introduction**

33 Pests can reduce the yield and quality of common wheat (*Triticum aestivum* L., Poaceae).

34 Among these, many species belonging to the genera *Aelia* (Heteroptera: Pentatomidae) and

35 *Eurygaster* (Heteroptera: Scutelleridae), known also as sunn pests or cereal bugs, are

36 responsible for significant economic losses in many countries of eastern and southern Europe,

37 western and central Asia and northern Africa (Paulian and Popov 1980; Voegelé 1996;

38 Critchley 1998). Sunn pests feed on wheat stems, leaves and ears; early attacks can cause

39 seedling loss and failed germination of damaged seed, while late attacks during the grain

40 filling period seriously affect its bread-making quality (Konarev et al. 2011). In fact, while

41 feeding on grain the insects inject proteolytic enzymes, which still have a low activity in the

42 flour; their action increases when dough is formed, leading to protein degradation and

43 alteration of gluten structure, without modifying other physico-chemical properties (Aja et al.

44 2004; Pérez et al. 2005). Maximum damage occurs from bug feeding at the late milk-ripe

45 stage (Vaccino et al. 2006) that corresponds to growth stage (GS) 75, according to Zadoks et

46 al. (1974).

47 In Europe, cereal bugs are detrimental pests on wheat in southeastern countries (Paulian and

48 Popov 1980), but they could spread also into western and northern Europe with changes in

49 climate (Konarev et al. 2011). In particular, in southeastern Europe, as well as in Asia, *E.*

50 *integriceps* Puton is considered the most noxious pest and therefore has been the most

51 investigated among cereal bugs (Critchley 1998; Trissi et al. 2006; Kutuk et al. 2010). In
52 southwestern Europe it is replaced by *E. maura* (L.) and *E. austriaca* (Schrank), causing
53 similar cereal damage (Ruiz et al. 2001; Vaccino et al. 2006). Another important species in
54 Europe is *A. rostrata* Boheman (Ruiz et al. 2001). In Italy, severe damage to wheat due to the
55 species *A. acuminata* (L.), *A. rostrata* and *E. maura* was reported in the North-East (Veneto)
56 at the beginning of the 1930s (Malenotti 1931, 1933). Subsequently, sunn pest infestations
57 were reported in Sicily (Genduso and Di Martino 1974) and more recently in North-West
58 (Piedmont), central (Tuscany) and Sardinia (Tavella et al. 2003; Bin et al. 2006; Salis et al.
59 2010), where the most abundant bug was *E. maura*. Nevertheless, during field surveys in
60 Piedmont other bugs such as *A. acuminata*, *A. rostrata*, *Carpocoris* sp., *E. austriaca* and
61 *Nezara viridula* (L.) were always observed on wheat (Coppa et al. 2011). The last species has
62 been responsible for reductions in germination, kernel weight, and kernel texture despite
63 infestation with relatively few individuals during the milk-ripe stage in the United States
64 (Viator et al. 1983).

65 For the control of sunn pest infestations, chemical treatments are usually performed. In field
66 trials conducted in Turkey, the pyrethroid λ -cyhalothrin was very effective, causing total
67 mortality of *E. integriceps* (Koçak and Babaroğlu 2006). In laboratory trials other substances
68 have also demonstrated insecticidal potential on *E. integriceps*, such as the insect growth
69 regulators pyriproxyfen, methoxyfenozide and buprofezin, the metabolic compound produced
70 by *Artemisia annua* L. (Asteraceae), the entomopathogenic fungus *Beauveria bassiana*
71 (Balsamo-Crivelli) Vuillemin (Ascomycetes) and the natural insecticide azadirachtin (Kivan
72 2005; Zibae and Bandani 2009, 2010; Mojaver and Bandani 2010).

73 In Italy, in areas where sunn pest infestations can be detrimental to wheat bread-making
74 quality, insecticide treatments play an increasing role in crop production practices and several
75 insecticides, mainly synthetic pyrethroids such as λ -cyhalothrin, deltamethrin and τ -

76 fluvalinate, are currently labelled for cereal bug control. In Italy another pyrethroid, α -
77 cypermethrin, is registered for use on wheat but only against aphids. Insecticides should be
78 applied at the wheat milk-ripe stage (GS75) in order to control sunn pests at the point when
79 their feeding activity leads to a severe reduction in baking quality. However, timing
80 insecticide application to wheat between heading (GS58) and anthesis (GS65) could be
81 technically and economically more convenient for farmers. In fact, the practice of applying a
82 fungicide at this stage in order to control *Fusarium* ear blight and foliar diseases is common
83 and broadly recommended for its positive effect on yield and for deoxinivalenol control
84 (Blandino et al. 2006). In a field trial with a medium sunn pest infestation, an application of
85 deltamethrin at heading showed a similar efficacy in preserving grain quality to that observed
86 with application at the milk-ripe stage (Tavella et al. 2007).
87 Considering the strong qualitative damage to the wheat market, especially for improved and
88 superior bread making wheat, consequent to sunn pest infestations, it is necessary to quantify
89 the effectiveness of different insecticides on these insects, evaluating both their direct toxicity
90 and their persistence. Therefore, the aim of this study was to investigate the toxicity and
91 persistence on foliage of three pyrethroids commonly used on wheat (λ -cyhalothrin, α -
92 cypermethrin and τ -fluvalinate) against the wheat bug *E. maura* and the green stink bug *N.*
93 *viridula* in both laboratory and semi-field conditions.

94

95 **Materials and Methods**

96 Collection and mass rearing of *N. viridula* and *E. maura*

97 In spring-summer 2009 and 2010, *N. viridula* and *E. maura* were collected in soya bean and
98 wheat fields, respectively, in Piedmont (NW Italy). Field-collected individuals were used to
99 start stock colonies. *N. viridula* and *E. maura* were reared on French bean pods (*Phaseolus*
100 *vulgaris* L., Fabaceae) and on young plants of wheat, respectively, inside 3L plastic boxes.

101 Box lids (265mm×175mm) were cut in the middle and closed with net. Mass rearing was
102 conducted in growth chambers maintained at 25±1°C and 70±5%RH with a 16L:8D
103 photoperiod.

104 Insecticide trials

105 Direct toxicity and persistence of the three pyrethroids λ-cyhalothrin, α-cypermethrin and τ-
106 fluvalinate (Table 1) were evaluated on *N. viridula* and *E. maura* under laboratory and semi-
107 field conditions. All three pyrethroids are registered on wheat and used for aphid control in
108 Italy; however, only λ-cyhalothrin and τ-fluvalinate are labelled also for cereal bugs.

109 *Laboratory trials*

110 In the laboratory, direct mortality due to pyrethroid insecticides at the label rate indicated by
111 companies, and lethal dose (LD₅₀) (mg L⁻¹ or μL L⁻¹) using four progressive dilutions (1:5 for
112 *N. viridula* and 1:2 for *E. maura*) were evaluated. Adult bugs were introduced into glass Petri
113 dishes (diameter 140 mm, height 30 mm, five adults dish⁻¹) previously treated with the tested
114 insecticide at the label rate and its progressive dilutions. The active ingredient (a.i.) was
115 applied by spraying 1 mL of solution into each dish to have an even layer of insecticide on the
116 whole surface. The bugs were introduced when the dishes were dried. One hour after bug
117 introduction, a green bean pod for *N. viridula* and some wheat kernels for *E. maura*, plus a
118 small vial filled with water, were added as food into each dish. For each a.i. and
119 concentration, five dishes were treated, as well as five dishes were left as untreated control.
120 In June 2010, for α-cypermethrin and λ-cyhalothrin, lethal doses were also evaluated using
121 wheat leaves and spikes as substratum. Wheat plants were treated in a field sited in Carignano
122 (Piedmont, 44°53'N 7°41'E, 231 m a.s.l.) using the insecticides at the label rate and at four
123 progressive dilutions (1:5 for *N. viridula* and *E. maura*). The insecticides were applied by
124 slow walking (0.5 m s⁻¹) with a four-nozzle (XR Tee-Jet® 11004-VK, TeeJet® Technologies,
125 Denmark) precision backpack sprayer (Honda® WJR 2525, Honda Italia Industriale, Italy)

126 producing a fine mist to ensure an effective coverage. The delivery pressure at the nozzle was
127 324 kPa. For each product and concentration, a wheat plot (2×10.5 m) was treated while
128 another wheat plot was left as untreated control. One hour after treatment, leaves and spikes
129 were collected from each plot and immediately transferred to the laboratory to arrange the
130 trial. Ten leaves and two spikes collected from the same plot were placed into an untreated
131 glass Petri dish (diameter 140 mm, height 30 mm). For each product and concentration, and
132 for the untreated control, five replications were done.

133 All bioassays were conducted in growth chambers at 25±1°C and 70±5% RH with a 16L:8D
134 photoperiod. The bug's mortality was evaluated at 1, 24, 48 and 72 h from their insertion into
135 the dishes. Mortality percentages were corrected according to Abbott's formula (1925).
136 Moreover, data on bug mortality at 48 h were compared between the products using the non-
137 parametric Kruskal-Wallis analysis because they were inhomogeneous; values were separated
138 by Mann-Whitney *U*-test ($P < 0.05$) when the Kruskal-Wallis analysis was significant. LD₅₀,
139 upper and lower confidence limits, regression equations and chi-square (χ^2) values were
140 calculated by probit analysis. All statistical analyses were performed using the Statistical
141 Package for the Social Sciences (SPSS 2008).

142 *Field cage trials*

143 Semi-field trials on wheat were conducted on *N. viridula* in 2009, and on *E. maura* in 2010 at
144 Carignano (Piedmont, 44°53'N 7°41'E, 231 m a.s.l.). In both years, the cultivar 'Aubusson'
145 (Limagrain Italia is in charge of seed conservation; classified in the Italian bread-making
146 quality grading system as ordinary bread making wheat) was grown. Wheat was seeded after
147 an autumn ploughing (30 cm) and disk harrowing to prepare a proper seedbed, following a
148 previous crop of maize. Planting was conducted in 12 cm wide rows at a seeding rate of 450
149 seeds m⁻² on November 4 in 2008 and October 10 in 2009. Each year, the experimental field
150 received 150 and 115 kg ha⁻¹ of N and K₂O, respectively. Weed control was conducted with

151 iodosulfuron-methyl-sodium, mefenpyr-diethyl and mesosulfuron-methyl (Hussar[®] Maxx,
152 Bayer CropScience, Italy) at wheat tillering (GS 31). At wheat heading, prothioconazole and
153 tebuconazole (Prosaro[®], Bayer CropScience, Italy) were applied to control foliar and head
154 disease. On May 19 in 2009 and June 1 in 2010, the field was divided into plots of 21 m²
155 (2×10.5 m), following a randomised complete block design with three replicates for each of
156 four treatments. At wheat heading (GS 55), the insecticides were applied as described above,
157 by slow walking (0.5 m s⁻¹), at the manufacturers' recommended field rates (Table 1) with a
158 four-nozzle (XR Tee-Jet[®] 11004-VK, TeeJet[®] Technologies, Denmark) precision backpack
159 sprayer (Honda[®] WJR 2525; Honda Italia Industriale, Italy). The delivery pressure at the
160 nozzle was 324 kPa.

161 After insecticide or water application, white sleeve cages in polythene net (1.5 m length, 0.4
162 m diameter, 60 mesh), supported by one pole set in the ground, were installed in each plot (15
163 cages treatment⁻¹). In each cage a group of about 20 wheat ears was included. Then, one cage
164 end was anchored on vegetation and the other end was closed over the vegetation to prevent
165 pest entry and escape. Before cage closing, four individuals of *N. viridula* or *E. maura* were
166 introduced into each cage.

167 Direct toxicity and persistence on wheat plants were evaluated by introducing bugs into five
168 cages at three times: immediately (t0), and at 7 (t7) and 14 (t14) days after application.

169 Mortality of bugs was assessed 2 and 8 days after their insertion. Mortality percentages were
170 corrected according to Abbott's formula (1925). Data on bug mortality evaluated after 2 and 8
171 days were compared between the products at t0, and within each product at t0, t7 and t14
172 using one-way analysis of variance (ANOVA) after performing tests for homogeneity of
173 variance (Levene) and normality (Shapiro-Wilk); means were then separated by Tukey's test
174 (P<0.05) (SPSS 2008).

175 Two hundred flag leaves, to be analysed for each a.i. at the label rate, were collected from
176 each plot at 0, 3, 7, 14 and 25 days after insecticide application in 2009 and at 0, 3, 7 and 14
177 days after insecticide application in 2010. Until analysis, leaf samples were immediately
178 stored at -18°C . Collection times at 0, 7 and 14 days corresponded with insect insertions into
179 cages. Data on local weather conditions were recorded throughout the field experiments in
180 both years.

181 The acetate-buffered QuEChERS sample preparation method for pesticides (AOAC Official
182 Method 2007.01) was applied to all the samples, according to Anastassiades et al. (2003).
183 Briefly, wheat leaf samples were chopped in a house-hold mixer (equipped with stainless steel
184 blades). A 15 g portion of the homogenized sample was weighed into a 50 mL polytetra
185 fluoro ethylene (PTFE) tube and 100 mL of 50 mg mL⁻¹ triphenyl phosphate (TPP) surrogate
186 standard solution in acetonitrile was added, followed by 15 mL of acetonitrile containing 1%
187 acetic acid. Then, 6 g MgSO₄ and 2.5 g sodium acetate trihydrate (equivalent to 1.5 g of
188 anhydrous form) were added and the sample was shaken forcefully for 1 min. Afterward, 150
189 mg anhydrous MgSO₄ and 25 mg primary secondary amine (PSA) sorbent were mixed with 1
190 mL acetonitrile extract. Analysis was performed by the CG/MS method. The recovery of all
191 three active ingredients was higher than 90%; this level can be considered as satisfactory for
192 residue determinations.

193

194 **Results**

195 Insecticide trials

196 *Laboratory trials*

197 The toxicity of pyrethroids at 2 days after treatment was high, with both *N. viridula* and *E.*
198 *maura* showing mortality rates of about 100%, except with τ -fluvalinate at the label rate (Fig.
199 1). At 1 h after bug insertion, dead adults were found for both bug species in the dishes treated

200 with λ -cyhalothrin, and only for *N. viridula* in the dishes treated with α -cypermethrin. In
201 particular, α -cypermethrin caused a lethal action in *E. maura* only after 2 days; before this,
202 most adults were not able to move and remained on their backs. By contrast, in the dishes
203 treated with τ -fluvalinate the first dead adults were detected 24 and 48 h after the insertion of
204 *N. viridula* and *E. maura*, respectively.

205 LD₅₀, upper and lower confidence limits, regression equations and chi-square (χ^2) values of
206 pyrethroids for the two bugs are shown in Tables 2 and 3. All pyrethroids were more active on
207 *N. viridula* than on *E. maura*. In the trials conducted in treated Petri dishes, α -cypermethrin
208 was the most insect-toxic pyrethroid for *N. viridula*, with a very low LD₅₀ (0.29 mg L⁻¹).
209 Indeed, the LD₅₀ of α -cypermethrin for *N. viridula* was about 30 times lower than for *E.*
210 *maura* (9.02 mg L⁻¹) (Table 2). On the contrary, λ -cyhalothrin was the most insect-toxic for *E.*
211 *maura*, even with an LD₅₀ (4.46 μ L L⁻¹) about 10 times higher than for *N. viridula* (0.43 μ L L⁻¹)
212 (Table 2). For both bug species, τ -fluvalinate was the least insect-toxic a.i., with a very high
213 LD₅₀ compared with those of the other two pyrethroids (31.47 μ L L⁻¹ for *N. viridula* and
214 43.34 μ L L⁻¹ for *E. maura*) (Table 2).

215 In the trials conducted on treated wheat leaves and spikes, λ -cyhalothrin (1.17 μ L L⁻¹ for *E.*
216 *maura* and 2.10 μ L L⁻¹ for *N. viridula*) was confirmed to be more toxic than α -cypermethrin
217 for both species (Table 3). The latter a.i. showed a similar insect-toxicity for both bug species
218 (3.47 mg L⁻¹ for *N. viridula* and 3.99 mg L⁻¹ for *E. maura*) (Table 3).

219 *Field cage trials*

220 In both years, during the field experiments the mean temperatures were on average 21°C,
221 fluctuating from 14°C to 29°C in 2009, and from 15°C to 28°C in 2010. Rainfalls were higher
222 in 2010 (9.9 mm) than in 2009 (2.5 mm), as well as the mean relative humidities were higher
223 in 2010 (78%) than in 2009 (66%).

224 Comparing the toxicity caused by the three pyrethroids on bugs introduced at t₀, after 8 days
225 α -cypermethrin and λ -cyhalothrin showed significantly higher mortality rates than τ -
226 fluvalinate for both *N. viridula* and *E. maura*. Moreover, already after 2 days α -cypermethrin
227 showed a significantly higher mortality than τ -fluvalinate and λ -cyhalothrin, but only for *E.*
228 *maura* (ANOVA, df = 2, 12; *N. viridula*: mortality at 2 days, F = 3.268, P = 0.074; mortality
229 at 8 days, F = 11.565, P = 0.002; *E. maura*: mortality at 2 days, F = 13.778, P = 0.001;
230 mortality at 8 days, F = 8.968, P = 0.004).

231 Pyrethroid residue concentration on whole flag wheat leaves obtained from the dissipation
232 study and the corresponding first-order decay fits are reported in Fig. 2. As expected, residues
233 at t₀ were proportional to the rate applied. The degradation kinetics of α -cypermethrin were
234 similar in 2009 and 2010. The half-life of α -cypermethrin was 11.9 and 11.7 days in 2009 and
235 2010, respectively. On the other hand, the degradation kinetics of λ -cyhalothrin and τ -
236 fluvalinate deposits was higher in 2010 than in 2009. In each trial, τ -fluvalinate (half-life of
237 16.2 and 10.4 days in 2009 and 2010, respectively) showed a higher persistence than λ -
238 cyhalothrin (half-life of 11.9 and 6.9 days in 2009 and 2010, respectively).

239 In field cages, bug mortality was considerably higher at 8 days after insertion compared to
240 that at 2 days after insertion for both *N. viridula* and *E. maura* (Table 4). The pyrethroid that
241 caused the highest bug mortality was α -cypermethrin: 100% for *N. viridula* and 95% for *E.*
242 *maura* 8 days after insertion of adults just after treatment (t₀). Mortality due to α -
243 cypermethrin remained high when bugs were introduced 7 days (t₇) and 14 days (t₁₄) after
244 treatment for *N. viridula* (71 and 81%, respectively, 8 days after insertion) (Table 4). By
245 contrast, for *E. maura* mortality was still high at t₇ (72%) but halved at t₁₄ (32%). In fact,
246 mortality rates 2 days and 8 days after bug insertion at t₀ and at t₇ were significantly higher
247 than those 2 days and 8 days after insertion at t₁₄ (ANOVA, df = 2, 12; mortality at 2 days, F
248 = 6.00, P = 0.016; mortality at 8 days, F = 8.96, P = 0.004) (Table 4).

249 The second-most effective pyrethroid for bug control was λ -cyhalothrin. For *N. viridula*,
250 mortality of adults introduced just after treatment (t0) was 50 and 89% 2 days and 8 days after
251 bug insertion, respectively, significantly higher than those obtained when bugs were
252 introduced at t7 (0% 2 days and 8 days after bug insertion) and at t14 (0% 2 days and 26% 8
253 days after bug insertion) (ANOVA, df = 2, 12; mortality at 2 days, F = 6.70, P = 0.011;
254 mortality at 8 days, F = 7.36, P = 0.008) (Table 4). For *E. maura*, mortality was lower
255 compared to that of *N. viridula* at t0 (25 and 80% 2 days and 8 days after bug insertion,
256 respectively) while it remained higher at t7 (25 and 56% 2 days and 8 days after bug insertion,
257 respectively). No mortality was observed for individuals introduced at t14; consequently it
258 was significantly lower than the mortality obtained at t0 and t7 (ANOVA, df = 2, 12;
259 mortality at 2 days, F = 5.00, P = 0.026; mortality at 8 days, F = 41.60, P < 0.000) (Table 4).
260 The third pyrethroid, τ -fluvalinate, demonstrated a low mortality for both bug species and no
261 significant differences were observed between the bug insertion periods (ANOVA, df = 2, 12;
262 *N. viridula*: mortality at 2 days, F = 0.069, P = 0.934; mortality at 8 days, F = 0.113, P =
263 0.894; *E. maura*: mortality at 2 days, F = 2.923, P = 0.092; mortality at 8 days, F = 1.057, P =
264 0.378) (Table 4).

265

266 **Discussion and Conclusions**

267 The effectiveness of pyrethroids for bug control was shown to be related to the active
268 ingredient and to the bug species. Indeed, all tested pyrethroids were more toxic to *N. viridula*
269 than to *E. maura*. The latter species appeared to be more resistant to contact insecticides such
270 as pyrethroids, probably due to its morphology (i.e., external structures). In fact, *E. maura*,
271 compared to *N. viridula*, presents a robust and wide scutellum covering almost the whole
272 body, a typical morphological trait of the family Scutelleridae (Schuh and Slater 1995), even

273 if in our laboratory trials the route of exposure is more likely via absorption through tarsi and
274 mouthparts.

275 In laboratory trials the differences among insecticides were slight: at label rate α -cypermethrin
276 and λ -cyhalothrin caused almost complete mortality of both bug species whereas τ -fluvalinate
277 was less effective, causing a mortality of 76% and 92% in *E. maura* and *N. viridula*,
278 respectively. Furthermore, mortality in laboratory trials was always higher than in semi-field
279 trials, consistent with other studies. For example Rea et al. (2003) reported a mortality for *N.*
280 *viridula* of 100% and 87% using λ -cyhalothrin on beans in the laboratory and on sweet corn
281 in field conditions, respectively. In our semi-field trials, the mortality due to the three
282 pyrethroids was almost always under 100%. Moreover, the mortality was always much higher
283 at 8 days than at 2 days after bug insertion, showing that to be maximally harmful pyrethroids
284 need several days. This delayed efficacy of pyrethroids was also reported in laboratory tests
285 on stored wheat, where greater toxicity to the rice weevil *Sitophilus oryzae* (L.) (Coleoptera:
286 Curculionidae) was observed after 7 days compared to 1 and 2 days after treatment
287 (Athanassiou et al. 2004).

288 For successful chemical control it is crucial to know the best time of application and thus the
289 persistence of the insecticides on the crop, which depends on many factors including the
290 species cultivated and the weather conditions. Field cage trials allowed us to obtain more
291 information on the actual effectiveness of the three pyrethroids over time. Although,
292 theoretically, persistence is generally expected to be one of the most important characteristics
293 of insecticides used in cereal defence, where it is not economical to apply more than a single
294 treatment from heading to harvest, there have been few field studies to support results
295 obtained in laboratory tests. Both laboratory and field cage experiments were performed to
296 compare three pyrethroids and yielded similar and consistent results, showing a different
297 efficacy of the tested products against wheat bugs. In particular, the field cage experiments

298 provided a method of determining potential insecticide impact. The difference in mortality
299 caused by the three insecticides was not related to the persistence of the active ingredient on
300 the leaves. Therefore, this method could be suggested for evaluating the efficacy of products
301 with a high knockdown, such as pyrethroids. In fact, this approach allows to determine an
302 insecticide's effectiveness over time after spraying, taking into account both its relative
303 persistence and toxicity under field conditions.

304 Among the tested pyrethroids, α -cypermethrin demonstrated the most potential against both
305 bug species under field cage conditions, still showing the highest toxicity one and two weeks
306 after treatment for *E. maura* and *N. viridula*, as a consequence of the good persistence of this
307 active ingredient on plants. However, α -cypermethrin must be applied rationally because of
308 the incipient resistance to this active ingredient found in some Turkish strains of *E. maura*
309 (Karaagac et al. 2011). Due to its low LD₅₀ α -cypermethrin should be effective even at lower
310 concentrations. Westcott and Reichle (1987) reported a lower half-life (6.2 days) of α -
311 cypermethrin on wheat plants. This fact could be due to the environment but also to the
312 possible influence of so-called "inert" ingredients (i.e., adjuvants, solvents, emulsifiers,
313 surfactants, and/or preservatives) which sometimes represent about 90% of the volume of an
314 insecticide formulation, and are rarely identified on the product label (Cox and Sorgan 2006).
315 Moreover, these substances are known to alter the bioavailability and toxicity of the active
316 ingredients and should be considered in risk assessment (Beggel et al. 2010).

317 The second pyrethroid with potential effective bug control was λ -cyhalothrin, according to
318 what observed also on other insect pests belonging to different orders (Echeverri-Molina and
319 Santolamazza-Carbone 2010; Latif et al. 2010). Under field cage conditions this insecticide
320 caused 89 and 80% mortality of *N. viridula* and *E. maura*, respectively, but with a higher
321 persistence on the latter species. At any rate, persistence in our field cage trials was less than

322 30 days, as observed for *E. integriceps* under field conditions in Turkey (Koçak and
323 Babaroğlu 2006).

324 Finally, the results with τ -fluvalinate showed it is unsuitable for bug control on wheat because
325 of its low toxicity, clearly demonstrated in both laboratory and field cage trials. Although its
326 persistence on wheat was higher compared to α -cypermethrin and λ -cyhalothrin (Fig. 2), this
327 active ingredient at the label rate was not effective, as its high LD₅₀ indicated. The low
328 toxicity of τ -fluvalinate for wheat bug control is consistent with Moreby et al. (2001), who
329 showed that among six insecticides tested in wheat fields τ -fluvalinate was the least toxic to
330 beneficial non-target invertebrate groups, including Heteroptera.

331 In conclusion, our results give useful information on the best strategy for correct application
332 of the three pyrethroids to successfully manage bug infestations. For an early application at
333 wheat heading, in a mixture with a fungicide, which saves farmers an additional treatment, α -
334 cypermethrin appeared to be the most suitable active ingredient. On the other hand, λ -
335 cyhalothrin, which showed a similar toxicity to α -cypermethrin but with a lower persistence,
336 could be better applied after anthesis at the late milk-ripe, when bug feeding on wheat can
337 severely affect its baking quality (Vaccino et al. 2006).

338

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342

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447 Scutelleridae). J Plant Prot Res 50 (1): 79-85.
- 448

449 Table 1 – Characteristics of the applied pyrethroids. * WG = water dispersible granule; CS =
 450 capsule suspension; EC = emulsifiable concentrate.

Active ingredient (a.i.)	Trade name	Firm	Formulation*	Label dose (commercial formulation)	Label rate (g a.i. ha ⁻¹)	A.i. content (%)
α -cypermethrin	Contest	BASF	WG	280 mg L ⁻¹	27.7	14.5
λ -cyhalothrin	Karate zeon	Syngenta Crop Protection	CS	210 μ L L ⁻¹	14.2	9.5
τ -fluvalinate	Megic	Sariaf Gowan	EC	770 μ L L ⁻¹	60.5	11.0

451

452 Table 2 – Toxicity of pyrethroids to *Nezara viridula* and *Eurygaster maura* in treated glass

453 Petri dishes.

Active ingredient	Species	No.	Slope ± SE	LD ₅₀ (mg-μL L ⁻¹) (95% CL)	LD ₉₀ (mg-μL L ⁻¹) (95% CL)	χ ²	d.f.
α-cypermethrin	<i>Nezara viridula</i>	125	2.99±0.61	0.289 (0.20-0.41)	0.780 (0.52-1.70)	11.14	23
	<i>Eurygaster maura</i>	150	2.40±0.33	9.02 (7.04-11.66)	30.817 (21.744-53.23)	26.36	28
λ-cyhalothrin	<i>Nezara viridula</i>	125	2.28±0.40	0.428 (0.29-0.64)	1.559 (0.98-3.53)	16.24	23
	<i>Eurygaster maura</i>	150	3.52±0.48	4.464 (3.46-5.79)	10.336 (7.624-17.21)	38.26	28
τ-fluvalinate	<i>Nezara viridula</i>	125	2.29±0.36	31.466 (24.18-42.75)	114.266 (74.82-240.25)	23.65	23
	<i>Eurygaster maura</i>	125	2.07±0.36	43.339 (32.29-64.65)	180.910 (106.39-498.92)	20.38	23

454

455

456 Table 3 – Toxicity of pyrethroids to *Nezara viridula* and *Eurygaster maura* on treated wheat
 457 leaves and spikes.

Active ingredient	Species	No.	Slope \pm SE	LD ₅₀ (mg- μ L L ⁻¹) (95% CL)	LD ₉₀ (mg- μ L L ⁻¹) (95% CL)	χ^2	d.f.
α -cypermethrin	<i>Nezara viridula</i>	125	0.86 \pm 0.14	3.47 (1.77-7.59)	107.18 (34.95-821.77)	19.91	23
	<i>Eurygaster maura</i>	125	1.21 \pm 0.18	3.99 (2.33-7.15)	45.60 (21.11-162.27)	25.12	23
λ -cyhalothrin	<i>Nezara viridula</i>	125	1.70 \pm 0.26	2.10 (1.34-3.32)	11.94 (6.81-29.91)	13.42	23
	<i>Eurygaster maura</i>	125	0.99 \pm 0.15	1.17 (0.64-2.29)	23.04 (9.09-113.58)	26.80	23

458

459

460 Table 4 – Mortality of *Nezara viridula* and *Eurygaster maura* caged on treated wheat and
 461 insecticide persistence on flag leaves. In each column, values followed by different bold
 462 letters are significantly different (Tukey’s test, P<0.05).

Insertion time ¹	α -cypermethrin			λ -cyhalothrin			τ -fluvalinate		
	Mortality ² (%)		Persistence ³ (% a.i.)	Mortality ² (%)		Persistence ³ (% a.i.)	Mortality ² (%)		Persistence ³ (% a.i.)
	2 d	8 d		2 d	8 d		2 d	8 d	
<i>Nezara viridula</i>									
0	60	100	100	50 a	89 a	100	15	28	100
7	8	71	62	0 b	0 b	49	0	0	62
14	47	81	50	0 b	26 b	43	0	20	66
<i>Eurygaster maura</i>									
0	75 a	95 a	100	25 a	80 a	100	15	30	100
7	50 ab	72 a	60	25 a	56 a	40	0	56	66
14	21 b	32 b	46	0 b	0 b	30	21	37	40

463 ¹ Bugs were introduced in net cages, expressed in days after insecticide treatment in the field.

464 ² Mortality values were corrected according to Abbott’s formula (1925)

465 ³ Residual concentration of each active substance with respect to the one measured at the time of treatment.

466

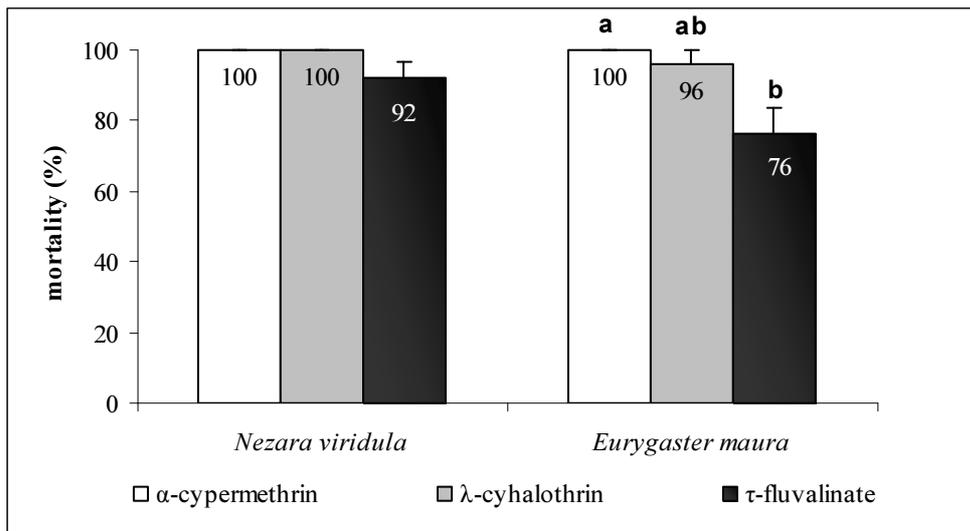
467

468 **Figure captions**

469 Fig. 1 – Mortality of *Nezara viridula* and *Eurygaster maura* [mean percentages + SE
470 corrected according to Abbott's formula (1925)] in laboratory trials 2 days after treatment
471 with the three pyrethroids at the label rate. Values followed by different letters are
472 significantly different (Mann-Whitney *U*-test, $P < 0.05$).

473 Fig. 2 – Dissipation of pyrethroids from whole flag wheat leaves in semi-field trials in 2009
474 and 2010. Active ingredients: (A) α -cypermethrin, (B) λ -cyhalothrin, (C) τ -fluvalinate.

475



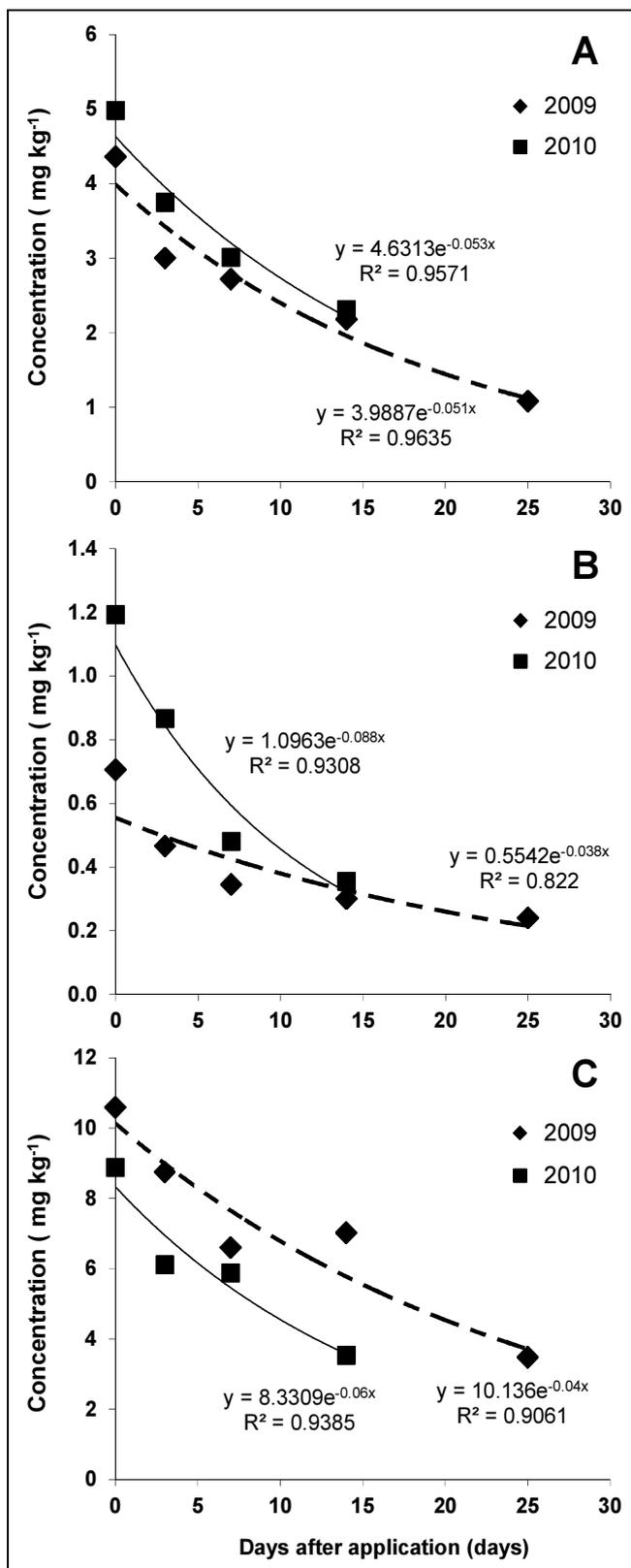
476

477 Fig. 1 – Mortality of *Nezara viridula* and *Eurygaster maura* [mean percentages + SE
 478 corrected according to Abbott's formula (1925)] in laboratory trials 2 days after treatment
 479 with the three pyrethroids at the label rate. Values followed by different letters are
 480 significantly different (Mann-Whitney *U*-test, $P < 0.05$).

481

482

483



484

485 Fig. 2 – Dissipation of pyrethroids from whole flag wheat leaves in semi-field trials in 2009

486 and 2010. Active ingredients: (A) α -cypermethrin, (B) λ -cyhalothrin, (C) τ -fluvalinate.