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

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Abstract

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

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

Key words: *Eurygaster maura*, *Nezara viridula*, *Triticum aestivum*, LD_{50}, dissipation

Introduction

Pests can reduce the yield and quality of common wheat (*Triticum aestivum* L., Poaceae). Among these, many species belonging to the genera *Aelia* (Heteroptera: Pentatomidae) and *Eurygaster* (Heteroptera: Scutelleridae), known also as sunn pests or cereal bugs, are responsible for significant economic losses in many countries of eastern and southern Europe, western and central Asia and northern Africa (Paulian and Popov 1980; Voegelé 1996; Critchley 1998). Sunn pests feed on wheat stems, leaves and ears; early attacks can cause seedling loss and failed germination of damaged seed, while late attacks during the grain filling period seriously affect its bread-making quality (Konarev et al. 2011). In fact, while feeding on grain the insects inject proteolytic enzymes, which still have a low activity in the flour; their action increases when dough is formed, leading to protein degradation and alteration of gluten structure, without modifying other physic-chemical properties (Aja et al. 2004; Pérez et al. 2005). Maximum damage occurs from bug feeding at the late milk-ripe stage (Vaccino et al. 2006) that corresponds to growth stage (GS) 75, according to Zadoks et al. (1974).

In Europe, cereal bugs are detrimental pests on wheat in southeastern countries (Paulian and Popov 1980), but they could spread also into western and northern Europe with changes in climate (Konarev et al. 2011). In particular, in southeastern Europe, as well as in Asia, *E. integriceps* Puton is considered the most noxious pest and therefore has been the most investigated among cereal bugs (Critchley 1998; Trissi et al. 2006; Kutuk et al. 2010). In southwestern Europe it is replaced by *E. maura* (L.) and *E. austriaca* (Schrank), causing similar cereal damage (Ruiz et al. 2001; Vaccino et al. 2006). Another important species in Europe is *A. rostrata* Boheman (Ruiz et al. 2001). In Italy, severe damage to wheat due to the species *A. acuminata* (L.), *A. rostrata* and *E. maura* was reported in the North-East (Veneto) at the beginning of the 1930s (Malenotti 1931, 1933). Subsequently, sunn pest infestations were reported in Sicily (Genduso and Di Martino 1974) and more recently in North-West (Piedmont), central (Tuscany) and Sardinia (Tavella et al. 2003; Bin et al. 2006; Salis et al. 2010), where the most abundant bug was *E. maura*. Nevertheless, during field surveys in Piedmont other bugs such as *A. acuminata*, *A. rostrata, Carpocoris* sp., *E. austriaca* and *Nezara viridula* (L.) were always observed on wheat (Coppa et al. 2011). The last species has been responsible for reductions in germination, kernel weight, and kernel texture despite infestation with relatively few individuals during the milk-ripe stage in the United States (Viator et al. 1983).

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

In Italy, in areas where sunn pest infestations can be detrimental to wheat bread-making quality, insecticide treatments play an increasing role in crop production practices and several insecticides, mainly synthetic pyrethroids such as λ-cyhalothrin, deltamethrin and τ-fluvalinate, are currently labelled for cereal bug control. In Italy another pyrethroid, α-cypermethrin, is registered for use on wheat but only against aphids. Insecticides should be applied at the wheat milk-ripe stage (GS75) in order to control sunn pests at the point when their feeding activity leads to a severe reduction in baking quality. However, timing insecticide application to wheat between heading (GS58) and anthesis (GS65) could be technically and economically more convenient for farmers. In fact, the practice of applying a fungicide at this stage in order to control *Fusarium* ear blight and foliar diseases is common and broadly recommended for its positive effect on yield and for deoxinivalenol control (Blandino et al. 2006). In a field trial with a medium sunn pest infestation, an application of deltamethrin at heading showed a similar efficacy in preserving grain quality to that observed with application at the milk-ripe stage (Tavella et al. 2007).

Considering the strong qualitative damage to the wheat market, especially for improved and superior bread making wheat, consequent to sunn pest infestations, it is necessary to quantify the effectiveness of different insecticides on these insects, evaluating both their direct toxicity and their persistence. Therefore, the aim of this study was to investigate the toxicity and persistence on foliage of three pyrethroids commonly used on wheat (λ-cyhalothrin, α-cypermethrin and τ-fluvalinate) against the wheat bug *E. maura* and the green stink bug *N. viridula* in both laboratory and semi-field conditions.

Materials and Methods

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

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

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

<u>Insecticide trials</u>

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

Laboratory trials

In the laboratory, direct mortality due to pyrethroid insecticides at the label rate indicated by companies, and lethal dose (LD_{50}) (mg L^{-1} or μL L^{-1}) using four progressive dilutions (1:5 for *N. viridula* and 1:2 for *E. maura*) were evaluated. Adult bugs were introduced into glass Petri dishes (diameter 140 mm, height 30 mm, five adults $dish^{-1}$) previously treated with the tested insecticide at the label rate and its progressive dilutions. The active ingredient (a.i.) was applied by spraying 1 mL of solution into each dish to have an even layer of insecticide on the whole surface. The bugs were introduced when the dishes were dried. One hour after bug introduction, a green bean pod for *N. viridula* and some wheat kernels for *E. maura*, plus a small vial filled with water, were added as food into each dish. For each a.i. and concentration, five dishes were treated, as well as five dishes were left as untreated control.

In June 2010, for α-cypermethrin and λ-cyhalothrin, lethal doses were also evaluated using wheat leaves and spikes as substratum. Wheat plants were treated in a field sited in Carignano (Piedmont, 44°53'N 7°41'E, 231 m a.s.l.) using the insecticides at the label rate and at four progressive dilutions (1:5 for *N. viridula* and *E. maura*). The insecticides were applied by slow walking (0.5 m s^{-1}) with a four-nozzle (XR Tee-Jet® 11004-VK, TeeJet® Technologies, Denmark) precision backpack sprayer (Honda® WJR 2525, Honda Italia Industriale, Italy) producing a fine mist to ensure an effective coverage. The delivery pressure at the nozzle was 324 kPa. For each product and concentration, a wheat plot (2×10.5 m) was treated while another wheat plot was left as untreated control. One hour after treatment, leaves and spikes were collected from each plot and immediately transferred to the laboratory to arrange the trial. Ten leaves and two spikes collected from the same plot were placed into an untreated glass Petri dish (diameter 140 mm, height 30 mm). For each product and concentration, and for the untreated control, five replications were done.

All bioassays were conducted in growth chambers at 25±1°C and 70±5% RH with a 16L:8D photoperiod. The bug's mortality was evaluated at 1, 24, 48 and 72 h from their insertion into the dishes. Mortality percentages were corrected according to Abbott's formula (1925). Moreover, data on bug mortality at 48 h were compared between the products using the non-parametric Kruskal-Wallis analysis because they were inhomogeneous; values were separated by Mann-Whitney *U*-test ($P<0.05$) when the Kruskal-Wallis analysis was significant. LD_{50}, upper and lower confidence limits, regression equations and chi-square (χ^2) values were calculated by probit analysis. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 2008).

Field cage trials

Semi-field trials on wheat were conducted on *N. viridula* in 2009, and on *E. maura* in 2010 at Carignano (Piedmont, 44°53'N 7°41'E, 231 m a.s.l.). In both years, the cultivar 'Aubusson' (Limagrain Italia is in charge of seed conservation; classified in the Italian bread-making quality grading system as ordinary bread making wheat) was grown. Wheat was seeded after an autumn ploughing (30 cm) and disk harrowing to prepare a proper seedbed, following a previous crop of maize. Planting was conducted in 12 cm wide rows at a seeding rate of 450 seeds m^{-2} on November 4 in 2008 and October 10 in 2009. Each year, the experimental field received 150 and 115 kg ha^{-1} of N and K_2O, respectively. Weed control was conducted with iodosulfuron-methyl-sodium, mefenpyr-diethyl and mesosulfuron-methyl (Hussar® Maxx, Bayer CropScience, Italy) at wheat tillering (GS 31). At wheat heading, prothioconazole and tebuconazole (Prosaro®, Bayer CropScience, Italy) were applied to control foliar and head disease. On May 19 in 2009 and June 1 in 2010, the field was divided into plots of 21 m^2 (2×10.5 m), following a randomised complete block design with three replicates for each of four treatments. At wheat heading (GS 55), the insecticides were applied as described above, by slow walking (0.5 m s^{-1}), at the manufacturers' recommended field rates (Table 1) with a four-nozzle (XR Tee-Jet® 11004-VK, TeeJet® Technologies, Denmark) precision backpack sprayer (Honda® WJR 2525; Honda Italia Industriale, Italy). The delivery pressure at the nozzle was 324 kPa.

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

Direct toxicity and persistence on wheat plants were evaluated by introducing bugs into five cages at three times: immediately (t0), and at 7 (t7) and 14 (t14) days after application. Mortality of bugs was assessed 2 and 8 days after their insertion. Mortality percentages were corrected according to Abbott's formula (1925). Data on bug mortality evaluated after 2 and 8 days were compared between the products at t0, and within each product at t0, t7 and t14 using one-way analysis of variance (ANOVA) after performing tests for homogeneity of variance (Levene) and normality (Shapiro-Wilk); means were then separated by Tukey's test ($P<0.05$) (SPSS 2008).

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

The acetate-buffered QuEChERS sample preparation method for pesticides (AOAC Official Method 2007.01) was applied to all the samples, according to Anastassiades et al. (2003). Briefly, wheat leaf samples were chopped in a house-hold mixer (equipped with stainless steel blades). A 15 g portion of the homogenized sample was weighed into a 50 mL polytetra fluoro ethylene (PTFE) tube and 100 mL of 50 mg mL^{-1} triphenyl phosphate (TPP) surrogate standard solution in acetonitrile was added, followed by 15 mL of acetonitrile containing 1% acetic acid. Then, 6 g MgSO4 and 2.5 g sodium acetate trihydrate (equivalent to 1.5 g of anhydrous form) were added and the sample was shaken forcefully for 1 min. Afterward, 150 mg anhydrous $MgSO_4$ and 25 mg primary secondary amine (PSA) sorbent were mixed with 1 mL acetonitrile extract. Analysis was performed by the CG/MS method. The recovery of all three active ingredients was higher than 90%; this level can be considered as satisfactory for residue determinations.

Results

<u>Insecticide trials</u>

Laboratory trials

The toxicity of pyrethroids at 2 days after treatment was high, with both *N. viridula* and *E. maura* showing mortality rates of about 100%, except with τ-fluvalinate at the label rate (Fig. 1). At 1 h after bug insertion, dead adults were found for both bug species in the dishes treated with λ-cyhalothrin, and only for *N. viridula* in the dishes treated with α-cypermethrin. In particular, α-cypermethrin caused a lethal action in *E. maura* only after 2 days; before this, most adults were not able to move and remained on their backs. By contrast, in the dishes treated with τ-fluvalinate the first dead adults were detected 24 and 48 h after the insertion of *N. viridula* and *E. maura*, respectively.

LD_{50}, upper and lower confidence limits, regression equations and chi-square (χ^2) values of pyrethroids for the two bugs are shown in Tables 2 and 3. All pyrethroids were more active on *N. viridula* than on *E. maura*. In the trials conducted in treated Petri dishes, α-cypermethrin was the most insect-toxic pyrethroid for *N. viridula*, with a very low LD_{50} (0.29 mg L^{-1}). Indeed, the LD_{50} of α-cypermethrin for *N. viridula* was about 30 times lower than for *E. maura* (9.02 mg L^{-1}) (Table 2). On the contrary, λ-cyhalothrin was the most insect-toxic for *E. maura*, even with an LD_{50} (4.46 µL L^{-1}) about 10 times higher than for *N. viridula* (0.43 µL L^{-1}) (Table 2). For both bug species, τ-fluvalinate was the least insect-toxic a.i., with a very high LD_{50} compared with those of the other two pyrethroids (31.47 µL L^{-1} for *N. viridula* and 43.34 µL L^{-1} for *E. maura*) (Table 2).

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

Field cage trials

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

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

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

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

The second-most effective pyrethroid for bug control was λ-cyhalothrin. For *N. viridula*, mortality of adults introduced just after treatment (t0) was 50 and 89% 2 days and 8 days after bug insertion, respectively, significantly higher than those obtained when bugs were introduced at t7 (0% 2 days and 8 days after bug insertion) and at t14 (0% 2 days and 26% 8 days after bug insertion) (ANOVA, df = 2, 12; mortality at 2 days, F = 6.70, P = 0.011; mortality at 8 days, F = 7.36, P = 0.008) (Table 4). For *E. maura*, mortality was lower compared to that of *N. viridula* at t0 (25 and 80% 2 days and 8 days after bug insertion, respectively) while it remained higher at t7 (25 and 56% 2 days and 8 days after bug insertion, respectively). No mortality was observed for individuals introduced at t14; consequently it was significantly lower than the mortality obtained at t0 and t7 (ANOVA, df = 2, 12; mortality at 2 days, F = 5.00, P = 0.026; mortality at 8 days, F = 41.60, P < 0.000) (Table 4).

The third pyrethroid, τ-fluvalinate, demonstrated a low mortality for both bug species and no significant differences were observed between the bug insertion periods (ANOVA, df = 2, 12; *N. viridula*: mortality at 2 days, F = 0.069, P = 0.934; mortality at 8 days, F = 0.113, P = 0.894; *E. maura*: mortality at 2 days, F = 2.923, P = 0.092; mortality at 8 days, F = 1.057, P = 0.378) (Table 4).

Discussion and Conclusions

The effectiveness of pyrethroids for bug control was shown to be related to the active ingredient and to the bug species. Indeed, all tested pyrethroids were more toxic to *N. viridula* than to *E. maura*. The latter species appeared to be more resistant to contact insecticides such as pyrethroids, probably due to its morphology (i.e., external structures). In fact, *E. maura*, compared to *N. viridula*, presents a robust and wide scutellum covering almost the whole body, a typical morphological trait of the family Scutelleridae (Schuh and Slater 1995), even if in our laboratory trials the route of exposure is more likely via absorption through tarsi and mouthparts.

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

For successful chemical control it is crucial to know the best time of application and thus the persistence of the insecticides on the crop, which depends on many factors including the species cultivated and the weather conditions. Field cage trials allowed us to obtain more information on the actual effectiveness of the three pyrethroids over time. Although, theoretically, persistence is generally expected to be one of the most important characteristics of insecticides used in cereal defence, where it is not economical to apply more than a single treatment from heading to harvest, there have been few field studies to support results obtained in laboratory tests. Both laboratory and field cage experiments were performed to compare three pyrethroids and yielded similar and consistent results, showing a different efficacy of the tested products against wheat bugs. In particular, the field cage experiments provided a method of determining potential insecticide impact. The difference in mortality caused by the three insecticides was not related to the persistence of the active ingredient on the leaves. Therefore, this method could be suggested for evaluating the efficacy of products with a high knockdown, such as pyrethroids. In fact, this approach allows to determine an insecticide's effectiveness over time after spraying, taking into account both its relative persistence and toxicity under field conditions.

Among the tested pyrethroids, α-cypermethrin demonstrated the most potential against both bug species under field cage conditions, still showing the highest toxicity one and two weeks after treatment for *E. maura* and *N. viridula*, as a consequence of the good persistence of this active ingredient on plants. However, α-cypermethrin must be applied rationally because of the incipient resistance to this active ingredient found in some Turkish strains of *E. maura* (Karaagac et al. 2011). Due to its low LD_{50} α-cypermethrin should be effective even at lower concentrations. Westcott and Reichle (1987) reported a lower half-life (6.2 days) of α-cypermethrin on wheat plants. This fact could be due to the environment but also to the possible influence of so-called "inert" ingredients (i.e., adjuvants, solvents, emulsifiers, surfactants, and/or preservatives) which sometimes represent about 90% of the volume of an insecticide formulation, and are rarely identified on the product label (Cox and Surgan 2006). Moreover, these substances are known to alter the bioavailability and toxicity of the active ingredients and should be considered in risk assessment (Beggel et al. 2010).

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

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

Finally, the results with τ-fluvalinate showed it is unsuitable for bug control on wheat because of its low toxicity, clearly demonstrated in both laboratory and field cage trials. Although its persistence on wheat was higher compared to α-cypermethrin and λ-cyhalothrin (Fig. 2), this active ingredient at the label rate was not effective, as its high LD_{50} indicated. The low toxicity of τ-fluvalinate for wheat bug control is consistent with Moreby et al. (2001), who showed that among six insecticides tested in wheat fields τ-fluvalinate was the least toxic to beneficial non-target invertebrate groups, including Heteroptera.

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

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Table 1 – Characteristics of the applied pyrethroids. * WG = water dispersible granule; CS = 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 µL L^{-1}	14.2	9.5
τ-fluvalinate	Megic	Sariaf Gowan	EC	770 µL L^{-1}	60.5	11.0

Table 2 – Toxicity of pyrethroids to *Nezara viridula* and *Eurygaster maura* in treated glass 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	*Nezara viridula*	125	2.99±0.61	0.289 (0.20-0.41)	0.780 (0.52-1.70)	11.14	23
	Eurygaster maura	150	2.40±0.33	9.02 (7.04-11.66)	30.817 (21.744-53.23)	26.36	28
λ-cyhalothrin	*Nezara viridula*	125	2.28±0.40	0.428 (0.29-0.64)	1.559 (0.98-3.53)	16.24	23
	Eurygaster maura	150	3.52±0.48	4.464 (3.46-5.79)	10.336 (7.624-17.21)	38.26	28
τ-fluvalinate	*Nezara viridula*	125	2.29±0.36	31.466 (24.18-42.75)	114.266 (74.82-240.25)	23.65	23
	Eurygaster maura	125	2.07±0.36	43.339 (32.29-64.65)	180.910 (106.39-498.92)	20.38	23

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

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	*Nezara viridula*	125	0.86±0.14	3.47 (1.77-7.59)	107.18 (34.95-821.77)	19.91	23
	Eurygaster maura	125	1.21±0.18	3.99 (2.33-7.15)	45.60 (21.11-162.27)	25.12	23
λ-cyhalothrin	*Nezara viridula*	125	1.70±0.26	2.10 (1.34-3.32)	11.94 (6.81-29.91)	13.42	23
	Eurygaster maura	125	0.99±0.15	1.17 (0.64-2.29)	23.04 (9.09-113.58)	26.80	23

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

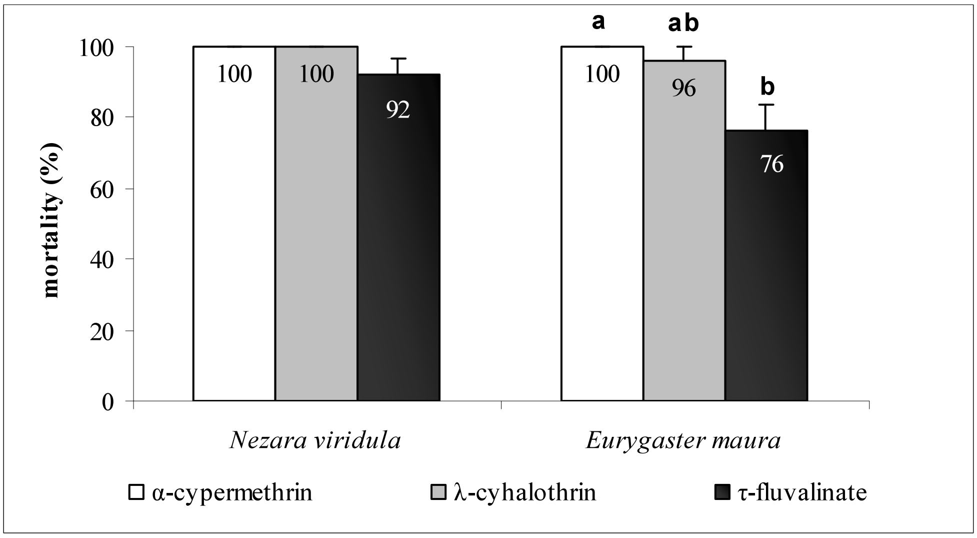
Insertion time[1]	α-cypermethrin			λ-cyhalothrin			τ-fluvalinate		
	Mortality[2] (%)		Persistence[3]	Mortality[2] (%)		Persistence[3]	Mortality[2] (%)		Persistence[3]
	2 d	8 d	(% a.i.)	2 d	8 d	(% a.i.)	2 d	8 d	(% a.i.)
Nezara viridula									
0	60	100	100	50**a**	89**a**	100	15	28	100
7	8	71	62	0**b**	0**b**	49	0	0	62
14	47	81	50	0**b**	26**b**	43	0	20	66
Eurygaster maura									
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

[1] Bugs were introduced in net cages, expressed in days after insecticide treatment in the field.
[2] Mortality values were corrected according to Abbott's formula (1925)
[3] Residual concentration of each active substance with respect to the one measured at the time of treatment.

Figure captions

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

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



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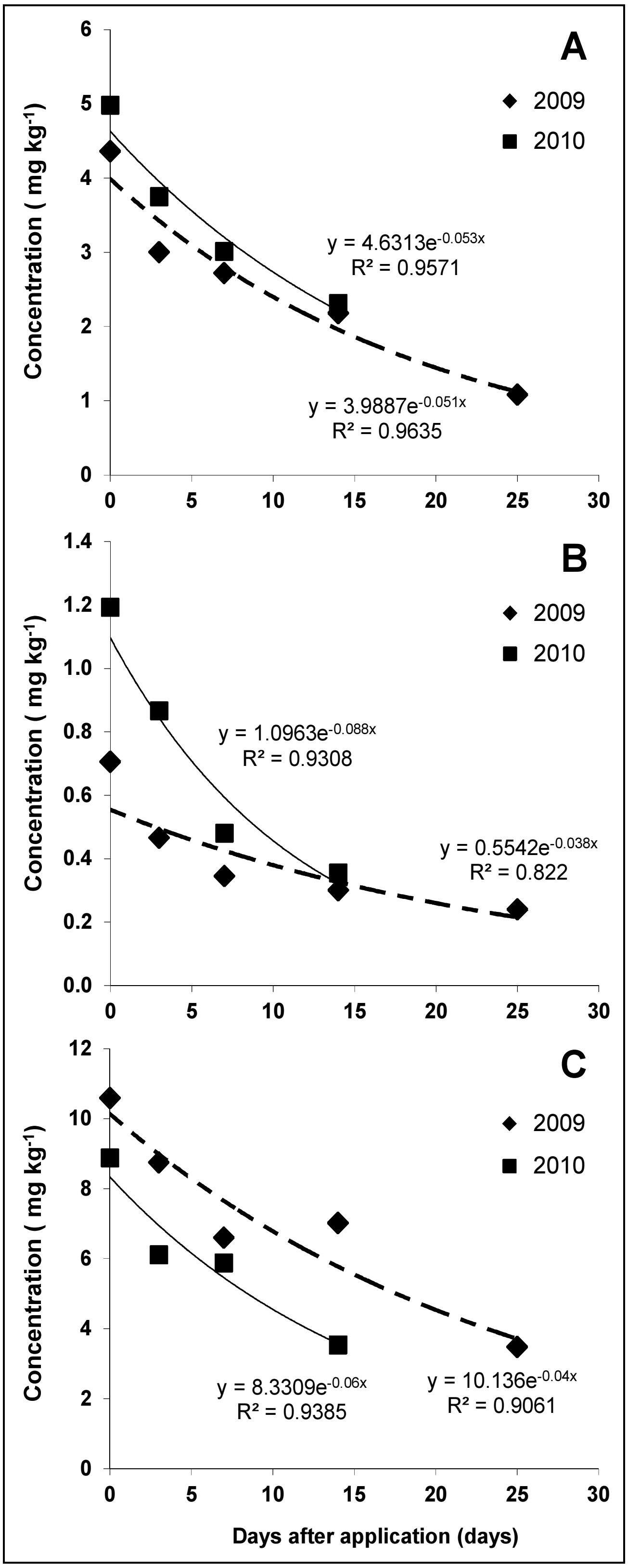

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