Pest Management Science
Pest Manag Sci 62:651–654 (2006)

Susceptibility of adult *Coccinella septempunctata* (Coleoptera: Coccinellidae) to insecticides with different modes of action

András Bozsik*
Plant Protection Department, Faculty of Agricultural Sciences, University of Debrecen, Böszörményi út 138, H-4032 Debrecen, Hungary

Abstract: Five insecticides (pyriproxifen, imidacloprid, deltamethrin + heptenophos, lambda-cyhalothrin and *Bacillus thuringiensis* Berliner subsp. *tenebrionis*) were examined in the laboratory for their acute detrimental side-effects at field rates on adult seven-spot ladybird beetle, *Coccinella septempunctata* L. The toxicity of the preparations was determined by measuring the acute surface contact effects (dried spray on leaves of *Philadelphus coronarius* L.), except for *B. thuringiensis* where mixed pollen was treated. Four to six concentrations were tested (pyriproxifen 12.5, 25, 50, 100, 200, 400 mg AI litre^{-1}; imidacloprid 62.4, 125, 250, 500 mg AI litre^{-1}; deltamethrin + heptenophos 26.4, 53.1, 106.3, 212.5 mg AI litre^{-1}; lambda-cyhalothrin 1.1, 3.4, 10, 30 mg AI litre^{-1}; *B. thuringiensis* 1.5, 3.0, 12.0, 48, 192, 768 mg AI litre^{-1}), with 22 adults exposed per concentration. All tests were conducted in the laboratory of the Plant Protection Department (University of Debrecen, Hungary) at 22–25 °C, 40–60% RH, under a 16:8 h light:dark photoperiod in 1998–1999. Data were analyzed by probit analysis, probit transformation and analysis of variance. According to different categories of evaluation, pyriproxifen, imidacloprid and *B. thuringiensis* subsp. *tenebrionis* seem to be safe for *C. septempunctata* adults but the other two preparations were moderately harmful to them, which requires further semi-field or field tests to measure their real effect under field conditions.

Keywords: *Coccinella septempunctata*; Coleoptera; Coccinellidae; insecticides; side-effects

1 INTRODUCTION

To use beneficial arthropods as biocontrol agents or preserve their local natural populations in integrated pest management, their susceptibility to the pesticides used must be taken into account. In order to save released or local beneficials and to augment and exploit their performance, several well-known strategies have been proposed:

- looking for preparations harmless to biological agents among the existing pesticides;[1]
- developing novel selective active ingredients;[2]
- finding (collecting/selecting) tolerant or resistant strains of natural enemies.[3]

All of these strategies assume systematic and detailed toxicological studies on interactions between pesticides and natural enemies. Until now, the first proposition has most commonly been employed as it is regarded as being closest to practical application.[4,5] The testing of pesticides on natural enemies has become practice in the last 20 years.[5–10] However, the number of natural enemies introduced into testing and the number of preparations tested on a particular species are variable. Coccinellids are supposed world-wide to be one of the most effective and best-studied natural enemy groups and in Europe the widely occurring seven-spot ladybird beetle, *Coccinella septempunctata* L., is considered to be one of the most important species of these. *Coccinella septempunctata* larvae and adults prey mainly on aphids and coccids, have a high searching capacity, their density can be considerable even under natural conditions and they arrive very soon after the aphids settle in agricultural crops, which enables them to be effective controllers of sucking pests.[11,12] However, their successful use, either naturally or by colonization, presupposes the availability of extensive, reliable data on their tolerance to pesticides in order to be able to establish relevant control regimes. When surveying the data on pesticide testing on *C. septempunctata*, it became evident that even those species considered as thoroughly studied, and the seven-spot ladybird beetle was reported as the fifth best-studied beneficial organism in the frame of pesticide registration in Germany,[13] investigations have been relatively restricted. One of the gaps is that in most cases only the larvae of coccinellids have been investigated and, despite the fact that the adults are also effective predators in addition to being the most important base of reproduction, the latter have

* Correspondence to: András Bozsik, Plant Protection Department, Faculty of Agricultural Sciences, University of Debrecen, Böszörményi út 138, H-4032 Debrecen, Hungary
E-mail: bozsik@helios.date.hu
(*Received 22 February 2005; revised version received 8 November 2005; accepted 16 November 2005*)
Published online 28 April 2006; DOI: 10.1002/ps.1221

not been involved in testing.[6,8,12–15] Therefore, the objective of this study was to obtain new information on the tolerance of *C. septempunctata* adults to some commonly used insecticides.

2 EXPERIMENTAL

Coccinella septempuctata adults were collected in 1998–1999 in the experimental area of Debrecen Agricultural University (in the north-east of Hungary). Captures were obtained by sweeping net. Table 1 lists the chemicals and the concentrations examined. The chemicals were purchased from or placed at our disposal by Summit-Agro Hungaria (Budapest) and Bayer (Budapest).

Leaves of mock orange (*Philadelphus coronarius* L.) were immersed in the test solutions of each preparation, except that containing *Bacillus thuringiensis* Berliner subsp. *tenebrionis* toxin, then air dried for about 1 h. A treated and dried leaf was placed in a plastic Petri dish (9 cm diameter) and a small plastic dish with a small ball of wet cotton was put on it. For the *B. thuringiensis* subsp. *tenebrionis* treatment, 0.5 g of mixed pollen (Varga Méhészet, Debrecen, Hungary) per Petri dish was treated by spraying with the test concentrations and dried. Eleven *C. septempunctata* adults were placed in each dish. There were two dishes per concentration and four to six concentrations per preparation were used (Table 1). The beetles exposed to *B. thuringiensis* subsp. *tenebrionis* received *Aphis craccivora* Koch as a food on the third, seventh and 11th days of the testing. The test beetles remained in the dish until a stable mortality resulted or the number of living individuals stabilized. The number of harmed or dead individuals was recorded after 1, 2 and 4 h and each day for 14 days using a binocular microscope (20×) to estimate the acute efficiency or mortality. Ladybird beetles were considered to be dead if they did not move either their legs and mouth parts or abdominal segments when they were prodded with a chrome-plated needle probe. Data were analyzed using probit analysis[15] with a program that incorporates Abbot's[16] correction for natural mortality and one-way analysis of variance (ANOVA).[17] The LT_{50} values and the mortality of the registered concentrations were calculated by probit transformation.[18] All tests were conducted in the laboratory of the Plant Protection Department (University of Debrecen, Hungary) at 22–25 °C, 40–60% RH and under a 16:8 h light:dark photoperiod in 1998–1999.

3 RESULTS AND DISCUSSION

The results showing the detailed effects of the insecticides are summarized in Tables 2–4. Table 5 shows the detrimental effects, using two classification

Table 2. Acute surface contact side-effects of pyriproxifen on field-collected adult *Coccinella septempunctata*

Treatment		
Formulation (% v/v)	AI (mg litre^{-1})	No.[a] of surviving individuals 9 days after treatment
Control		9.0
0.0125	12.5	9.0
0.025	25	9.0
0.05	50	8.0
0.1	100	9.0
0.2	200	9.5
0.4	400	9.5
$SD_{5\%}$		2.28

[a] Average of three repetitions.

Table 3. Side-effects of *Bacillus thuringiensis* subsp. *tenebrionis* applied to diet on field-collected adult *Coccinella septempunctata*

Treatment		
Formulation (% v/v)	AI (mg litre^{-1})	No.[a] of surviving individuals 9 days after treatment
Control		8.5
0.005	1.5	8.0
0.01	3.0	9.5
0.04	12	9.5
0.16	48	9.5
0.64	192	10.0
2.56	768	8.5
$SD_{5\%}$		3.41

[a] Average of three repetitions.

Table 1. Insecticide preparations and their concentrations used in screening

Preparation	Registered concentration (% v/v formulation) [mg AI litre^{-1}]	Test concentrations (% v/v formulation) [mg AI litre^{-1}]
Decisquick EC (25 g litre^{-1} deltamethrin + 400 g litre^{-1} heptenophos)	0.03 [127.5]	0.0062, 0.0125, 0.025, 0.05 [26.4, 53.1, 106.3, 212.5]
Karate 5 EC (50 g litre^{-1} lambda-cyhalothrin)	0.025 [12.5]	0.0022, 0.0067, 0.02, 0.06 [1.1, 3.35, 10, 30]
Confidor 200 SL (200 g litre^{-1} imidacloprid)	0.06 [120]	0.0312, 0.0625, 0.125, 0.25 [62.4, 125, 250, 500]
Admiral 10 EC (100 g litre^{-1} pyriproxifen)	0.05 [50]	0.0125, 0.025, 0.05, 0.1, 0.2, 0.4 [12.5, 25, 50, 100, 200, 400]
Novodor FC (3% *Bacillus thuringiensis* subsp. *tenebrionis*)	0.01 [3.0]	0.005, 0.01, 0.04, 0.16, 0.64, 2.56 [1.5, 3.0, 12, 48, 192, 768]

Pest Manag Sci **62**:651–654 (2006)
DOI: 10.1002/ps

Table 4. Acute surface contact side-effects of synthetic insecticides on field-collected adult *Coccinella septempunctata*

Insecticide (time of evaluation)	LC_{50} (% v/v formulation) [95% FL][a]	Effect of the registered concentration	
		LT_{50} (days)	Mortality (%)
Deltamethrin + heptenophos (7 days)	0.0041 [0.0003–0.0077]	0.4	89.8
Lambda-cyhalothrin (7 days)	0.0010 [0.0001–0.0028]	2.8	88.3
Imidacloprid (7 days)	0.1332 [0.0120–0.2240]	15.1	30.9

[a] Fiducial limits.

Table 5. Susceptibility of adult *Coccinella septempunctata* to surface contact-applied insecticides

Insecticide	Category of evaluation	
	A[a]	B[b]
Pyriproxifen	0	1
*Bacillus thuringien*sis subsp. *tenebrionis*[c]	0	1
Imidacloprid	L	1
Lambda-cyhalothrin	M	3
Deltamethrin + heptenophos	H	3

[a] A, categories of Bartlett:[21] 0 = no kill, L = $LT_{50} > 100$ h, M = $LT_{50} > 24$ h and < 100 h, H = $LT_{50} < 24$ h.
[b] B, categories of the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms':[5] 1 = harmless (<30% mortality = M), 2 = slightly harmful (30–79% M), 3 = moderately harmful (80–98% M), 4 = harmful (>98% M).
[c] Treatment through diet.

schemes, of all preparations examined. Preparations of pyriproxifen and *B. thuringiensis* subsp. *tenebrionis* were classified as harmless to adult *C. septempunctata* (Tables 2, 3 and 5). Pyriproxifen is an insect growth regulator based on juvenile hormone, which means that it harms mainly juvenile insects. As expected, no side-effects were observed on the treated seven-spot ladybird beetle adults. This corresponds to the results of Olszak *et al.*,[19] according to whom pyriproxifen had only a relatively low influence on the adults of a *C. septempunctata* population in Poland. *Bacillus thuringiensis* subsp. *tenebrionis* δ-endotoxin acts after solubilization and enzymatic cleavage in the midgut of the target organism, where it disrupts midgut membranes. The adults fed with the δ-endotoxin-treated pollen did not show any symptoms of disfunction. In addition, they fed vigorously on the aphids offered them post-treatment, indicating the integrity of their midgut epithelial layer. A preparation of *B. thuringiensis* subsp. *tenebrionis* (under the same commercial name) has already been tested on seven-spotted ladybeetle adults and it has been classified as harmless.[10] However, in that work it was administered as a dry surface residue and thus the penetration of the active ingredient into the midgut was unlikely. Furthermore, young larvae of *C. septempunctata* fed on aphids contaminated with *B. thuringiensis* subsp. *tenebrionis* were susceptible and the preparation was listed as moderately harmful.[20]

Imidacloprid is a systemic, chloronicotinyl (nitroguanidine) insecticide acting on the cholinergic acetylcholine receptors of the target organisms. It was classified as being harmless (IOBC categories),[5] or slightly harmful (categories of Bartlett[21]), to adult *C. septempunctata* (Tables 4 and 5). Earlier investigations were carried out only on young larvae of the test insect and in these the same preparation proved to be harmful – in terms of IOBC – causing 100% mortality.[22] The present mortality rate (30%) seems to be inversely proportional with the more robust life stage of the test animals, i.e. the adults were more tolerant to imidacloprid than were the larvae.

The other two preparations (deltamethrin + heptenophos and lambda-cyhalothrin) were found to be moderately harmful (IOBC categories), but, according to their LT_{50} values, lambda-cyhalothrin was moderately harmful whereas the deltamethrin + heptenophos combination was highly detrimental (Tables 4 and 5). The pyrethroid components of these preparations rest on the treated leaf surface and act through contacting the target insect. These compounds are sodium channel modulators and act as axonal poisons. The more detrimental effect of the last product may be due to its more rapid action (Table 4), which may result from the combined action of the deltamethrin (synthetic pyrethroid ester) and heptenophos (organophosphate insecticide, acetylcholinesterase inhibitor) combination on different target sites in the nervous system of *C. septempunctata*. The reported efficiency of lambda-cyhalothrin on a reared coccinellid population of presumably French origin was practically identical with the results reported here and this may indicate that the effects of pesticide pressure and the susceptibility of the natural populations to certain insecticides are similar across Europe.[10] The reported tolerance of a Chinese *C. septempunctata* population to the same active ingredient is greater since the mortality rate was much lower (42%).[23] The deltamethrin + heptenophos combination has not been tested elsewhere, but there are some results concerning the toxicity of the individual active ingredients. Thus, heptenophos was moderately harmful (51–75% mortality or reduction in beneficial capacity; IOBC category) to seven-spotted ladybird beetle adults in semi-field testing, which correspond approximately to the present results (Table 2).[10] Data from trials where an Indian population of *C. septempunctata* was treated with deltamethrin show a much lower tolerance (LC_{50} 0.0021%) in that population than was observed for the Hungarian population treated with the deltamethrin + heptenophos combination.[24] This may reflect continental or regional differences in pesticide use. The LC_{50} value of imidacloprid differed significantly from those of lambda-cyhalothrin and of the combination of deltamethrin and heptenophos, but there was no significant difference between the two last treatments.

4 CONCLUSIONS

Pyriproxifen, *B. thuringiensis* subsp. *tenebrionis* and imidacloprid proved to be safe for the population of coccinellid adults used in this work. However, regarding the long-term insecticidal efficiency of imidacloprid to aphids and other soft-bodied insects, it can be indirectly detrimental by removing the food from the predators. Further semi-field or field testing is not needed because, according to the experiences of the IOBC/WPRS Pesticides and Beneficial Organisms Working Group, environmental conditions can influence the hazard of a pesticide and under field conditions the preparations' toxicity to natural enemies might be less harmful than under laboratory conditions.[25] It is strongly recommended that both pyriproxifen (a juvenile hormone mimic IGR) and *B. thuringiensis* subsp. *tenebrionis* (a toxin acting on midgut) are now tested on the larval stages of *C. septempunctata*. For to the deltamethrin + heptenophos combination and lambda-cyhalothrin, further semi-field or field tests are necessary to determine their real effects under field conditions. The results can be applied mainly to the *C. septempunctata* populations of Hungary or to those of other regions with similar plant protection practice (Central and Eastern Europe), because field-collected individuals were used for testing. However, it should be noted that geographical differences in the tolerance of test organisms are possible and that this should be taken into account when considering side-effect results.

REFERENCES

1 Franz JM, Zur Prüfung von Nebenwirkungen von Pflanzenschutzmitteln auf entomophage Arthropoden. *Gesunde Pflanzen* **27**:28–31 (1975).

2 Plapp FW Jr and Bull DL, Modifying chemical control practices to preserve natural enemies, in *Increasing the Effectiveness of Natural Enemies,* ed. by King EG and Jackson RD, Aspect, London, pp. 537–546 (1985).

3 Grafton-Cardwell EE and Hoy MA, Genetic improvement of common green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae): selection for carbaryl resistance. *Environ Entomol* **15**:1130–1136 (1986).

4 Franz JM, Bogenschütz H, Hassan SA, Huang P, Naton E, Suter H, *et al*, Results of a joint pesticide test programme by the Working Group 'Pesticides and Beneficial Arthropods'. *Entomophaga* **25**:231–236 (1980).

5 Hassan SA, Bigler F, Bogenschütz H, Boller E, Brun J, Calis JNM, *et al*, Results of the fifth pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms'. *Entomophaga* **36**:55–67 (1991).

6 Suter H, Prüfung der Einwirkung von Pflanzenschutzmitteln auf die Nutzarthropodenart, *Chrysopa carnea* Steph. (Neuroptera: Chrysopidae) – Methodik und Ergebnisse. *Schweiz Landwirtsch Forsch* **17**:37–44 (1978).

7 Hassan SA, Bigler F, Bogenschütz H, Brown JU, Firth SI, Huang P, *et al*, Results of the second joint pesticide testing programme by the IOBC/WPRS Working Group 'Pesticides and Beneficial Arthropods'. *Z Angew Entomol* **95**:151–158 (1983).

8 Hassan SA, Albert R, Bigler F, Blaisinger P, Bogenschütz H, Boller E, *et al*, Results of the third pesticide testing programme by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. *Z Angew Entomol* **103**:92–107 (1986).

9 Hassan SA, Bigler F, Bogenschütz H, Boller E, Chiverton P, Edwards P, *et al*, Results of the fourth pesticide testing programme carried out by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. *Z Angew Entomol* **105**:321–329 (1988).

10 Sterk G, Hassan SA, Baillod M, Bakker F, Bigler F, Blümel S, *et al*, Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. *BioControl* **44**:99–117 (1999).

11 Hodek I, Food relationships, in *Ecology of Coccinellidae,* ed. by Hodek I and Honěk A. Kluwer, Dordrecht, pp. 143–238 (1996).

12 Honěk A and Hodek I, Distribution in habitats, in *Ecology of Coccinellidae*, ed. by Hodek I and Honěk A. Kluwer, Dordrecht, pp. 95–141 (1996).

13 Forster R, Auswirkungen von Pflanzenschutzmitteln auf Nutzorganismen – Kennzeichnung im Rahmen des Zulassungsverfahrens. *Nachrichtenbl Dtsch Pflanzenschutzdienstes* **47**:233–236 (1995).

14 Hassan SA, Bigler F, Blaisinger P, Bogenschütz H, Brun J, Chiverton P, *et al*, Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. *EPPO Bull* **15**:214–255 (1985).

15 Finney DJ, *Probit Analysis.* Cambridge University Press, Cambridge, pp. 1–66 (1971).

16 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).

17 Sváb J, *Biometriai Módszerek a Kutatásban (Biometric Methods in Research)*. Mezogazdasági Kiadó, Budapest, pp. 1–557 (1981).

18 Weber E, *Grundriss der Biologischen Statistik.* VEB Gustav Fischer, Jena, pp. 1–322 (1964).

19 Olszak RW, Pawlik B and Zajac RZ, The influence of some insect growth regulators on mortality and fecundity of the aphidophagous coccinellids *Adalia bipunctata* L. and *Coccinella septempunctata* L. (Col., Coccinellidae). *J Appl Entomol* **117**:58–63 (1994).

20 Keller B and Langenbruch GA, Control of coleopteran pests by *Bacillus thuringiensis*, in *Bacillus Thuringiensis, an Environmental Biopesticide: Theory and Practice*, ed. by Entwistle PF, Cory JS, Bailey MJ and Higgs S. Wiley, New York, pp. 171–191 (1993).

21 Bartlett BR, Toxicity of some pesticides to eggs, larvae and adults of the green lacewing, *Chrysopa carnea*. *J Econ Entomol* **57**:366–369 (1964).

22 Schmidt HW and Hartmann J, Beneficial–friendly use of Confidor for integrated crop protection in fruit cultivation. *Pflanzenschutz-Nachr Bayer* **52**:337–349 (1999).

23 Liu B and Şengonca C, Investigations on side-effects of the mixed biocide GCSC–BtA on different predators of *Plutella xylostella* (L.) (Lep., Plutellidae) in southeastern China. *Anz Schädlingskd Pflanz Umweltschutz* **75**:57–61 (2002).

24 Dhingra S, Effect of some important insecticides on the adults of *Coccinella septempunctata* Linn. predating on different aphid species. *J Entomol Res* **23**:127–131 (1999).

25 Hassan SA, Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group 'Pesticides and Beneficial Organisms'. *EPPO Bull* **15**:214–255 (1985).

Pest Manag Sci **62**:651–654 (2006)
DOI: 10.1002/ps