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Pesticide testing on adults of the *Chrysoperla carnea*-complex (Neuroptera: Chrysopidae) and the sibling species problem in the toxicology of common green lacewings

András Bozsik¹

¹Department of Plant Protection, University of Debrecen, Pf. 36, H-4015 Debrecen, Hungary; E-mail: bozsik@agr.unideb.hu

Abstract. The chrysopids, referred to as *Chrysoperla carnea* (Stephens, 1836) sensu lato (Chrysopidae), the so-called "common green lacewings" belong to the best tested beneficial insects regarding their pesticide susceptibility. Side-effects of several hundred pesticides have already been tested on their different developmental stages. However, the systematic position of this species has been changing, and it is not possible to learn at present which taxon/taxa of the *Ch. carnea* complex was/were used for the individual testing during a long period, and so it is difficult to apply the old data to the characterisation of a given natural or reared population. Results of new tests performed on adults of *Ch. carnea* s. l. and the stated *Chrysoperla affinis* (Stephens, 1836) (Chrysopidae) will be presented according to the common principles of toxicology. The toxicity of the preparations was determined by measuring the surface contact effects (dried spray on leaves of *Philadelphus coronarius* Linnaeus). Three to nine concentrations were tested, with 20 adults exposed per concentration. Data were analyzed by probit analysis and one-way ANOVA. On the basis of known categories of evaluation Nissorun 10 WP and Match 50 EC seem to be environmentally safe from point of view of chrysopid adults but in case of Karate 5 EC, Mospilan 20 SP, Danitol 10 EC, Ambush C, Decisquick EC and Talstar 10 EC further semi-field or field test is needed to measure their real effects under field conditions. In addition, after collecting in the original sampling area and identifying the caught common green lacewing individuals, an attempt has been made to identify the composition of the original population.

Key words: side-effects, pesticides, *Chrysoperla affinis*, *Chrysoperla carnea* s.l., *Chrysoperla lucasina*, *Chrysoperla carnea* s. str., Neuroptera, Chrysopidae

Introduction

The function and importance of biological control methods in agriculture grow steadily. However, maintaining natural enemies, whether as introduced biocontrol agents or as natural populations of native species, may be difficult where pesticides are used, due to their remarkable pesticide susceptibility. Consequently, successful introduction, colonization, use, augmentation, conservation, or the summary of these parts, can be dubious. Potential response to this issue can be the use of harmless or less harmful pesticides to natural enemies. For achieving this goal, thorough studies of pesticide side-effects on beneficial species are indispensable.

The aim of the present study was to assess the detrimental effects of some pesticides on adult common green lacewings, prime candidates of biological control and IPM (Integrated Pest Management) programs (Canard *et al.*, 1984; Pree *et al.*, 1989; Bay *et al.*, 1993; Tauber *et al.*, 2000). *Chrysoperla carnea* (Stephens, 1836) sensu lato or "the common green lacewings" are among the best tested beneficial organisms regarding their pesticide tolerance. Side-effects of more than 150 formulated pesticide products have been assessed only on their larvae and pupae (Bigler & Waldburger, 1994; Rumpf *et al.*, 1997b), and also the number of preparations tested on the adults is approximately 100 (Bartlett, 1964; Wilkinson *et al.*, 1975; Suter, 1978; Grafton-Cardwell & Hoy, 1985; Bozsik, 1991). This study also discusses new testing results which characterize *Chrysoperla affinis* (Stephens,

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1836) one of the cryptic species of common green lacewings, plus attempts to determine the original composition of animals used for testing and to make available some old data.

Taxonomic remarks and toxicological inference

The taxonomic status of the species in question has been changing, and instead of a particular species, a complex of sibling or cryptic species, the *Chrysoperla carnea* complex or *carnea*-group (Thierry *et al.*, 1992, 1998; Henry *et al.*, 2001), should be taken into account. The complexes systematical status is not well understood (Tauber *et al.*, 2000; Henry *et al.*, 2001). A number of attempts of various approaches such as courtship sonification (Henry, 1983, 1985), genetic studies with multilocus electrophoresis (Cianchi & Bullini, 1992), morphological characterization of adults and larvae (Thierry *et al.*, 1992), and ecophysiological variability (Thierry *et al.*, 1994; Canard *et al.*, 2002) have been made. These studies endorsed the existence of various sibling species: 1) *Ch. carnea* former *Chrysoperla kolthoffi* (Navás, 1927) sensu Cloupeau (*Cc4* as song species), or "motorboat"(as song type) (Henry *et al.*, 2002) or *Ch. affinis* former *Ch. kolthoffi* (Thierry *et al.*, 1998); 2) *Chrysoperla lucasina* (Lacroix, 1912) (Henry *et al.*, 2001) and 3) *Chrysoperla carnea* sensu stricto (Thierry *et al.*, 1998) or *Cc2* ("slow-motorboat") or *Chrysoperla pallida* (Henry *et al.*, 2002).

Despite numerous studies revealing the taxonomic position of Palaearctic *Ch. Carnea*, the present situation of species separation is still not clear because there is no agreement in reliable criteria (Tauber *et al.*, 2000; Henry *et al.*, 2001, 2002; Canard *et al.*, 2002; Canard & Thierry, 2007). There is a deep disagreement between the two groups of researchers in criteria for distinguishing the cryptic species of *carnea*-group. One group applies the substrate-born vibrational songs and certain morphological features like shape of certain male genital characteristics, another favours ecophysiological traits and fine morphological differences, like distribution and colour of hairs on the abdomen, pigment cover of the stipes, etc. The first group concluded that the true *Ch. carnea* described by Stephens in 1836 must be *Cc4* (Henry *et al.*, 2002) which according to the other group is another species, the *Ch. affinis* (Canard & Thierry, 2007). The other candidate species for being the "true" *Ch. carnea* may be *Cc2* above mentioned like *Ch. carnea* s. str. (Canard, 2003, personal communication) but in contrast with it, this taxon was assigned a new name, *Chrysoperla pallida* by Henry *et al.* (2002). Regarding the lack of faultless proofs, the validity of these names, however, has not yet been discussed and consented by the neuropterists.

Hence, it seems to be impossible to know at present, which taxa of the *Ch. carnea* complex were used for the individual screenings for a long period and it is uncertain to apply the "old" data for a given natural or reared population whose origin is unknown. Under "old" data should be regarded not only the above cited excellent sources but also essentially all of the recently published toxicological studies and testing results (Vogt & Viñuela, 2001; Hilbeck & Bigler, 2001; Bozsik, 2001; Cisneros *et al.*, 2002; Dutton *et al.*, 2002, 2003; Medina *et al.*, 2002, 2003; Huerta *et al.*, 2003a,b; Güven & Göven, 2003) where as test animals in most cases merely the denomination *Chrysoperla carnea* has been given.

Generally, the testing data are carried out to ameliorate direct biological control, to enhance conservation and augmentation of natural populations of this beneficial insect or simply to study the direct or indirect impact of a new plant protection product or agent. When starting such a programme it is extremely important to be clear with the most correct taxonomical status of the animals to be studied because it is probable that between similar but different species important ecophysiological (Thierry *et al.*, 1994; Canard *et al.*, 2002), behavioural (Duelli *et al.*, 1996), as well as toxicological differences exist. As to the toxicological differences no comparative study has been published yet but there are some preliminary data that prove the different susceptibility of the cryptic species of Palaearctic *carnea*-group. The response of in-vitro susceptibility of acetylcholinesterase of the three most frequent Belgian and Hungarian species (*Ch. affinis, Ch. carnea* s. str. and *Ch. lucasina*) to several characteristic inhibitors (some of them are well known insecticides) was investigated and significant differences have been found between the species in most cases (Bozsik, Haubruge & Gaspar, 2002, unpublished data).

Material and Methods

Ch. carnea s. 1. adults were collected in 1991, 1992 in an uncultivated area in Gödöllő (30 km north-east of Budapest) and adult *Ch. affinis* specimens were caught in 1998 and 1999 in the botanical garden of the Debrecen University in Debrecen (Hungary). Captures were obtained by sweeping net. Individuals were identified according to the descriptions of Thierry *et al.* (1992) and also samples of various morphological types (courtesy of D. Thierry) and song morphs (courtesy of P. Duelli) have been used. In the case of *Ch. affinis* atypical specimens were excluded. Table 1 contains the list of chemicals and also the concentrations examined.

Preparation	Registered concentrations (%)	Test concentrations (%)
Ambush C (100 mg/l cypermethrin)	0.04	0.0004-0.004-0.01-0.04
Danitol 10 EC (10 % fenpropathrin)	0.05-0.1	0.0012-0.0037-0.011-0.033-0.1
Decisquick EC (25 g/l deltamethrin +	0.03	0.0125-0.025-0.050
400 g/l heptenophos)		
Karate 5 EC (5 % lambda-cyhalothrin)	0.03-0.05	0.0002-0.0006-0.0024-0.012-0.015-0.03-0.06
Match 50 EC (50 g/l lufenuron)	0.1	0.0125-0.025-0.100
Mospilan 20 SP (20 % acetamiprid)	0.0125-0.0400	0.005-0.010-0.020-0.040-0.080-0.160-0.32-
		0.64–1.28
Nissorun 10 WP (10 % hexythiazox)	0.05–0.1	0.025-0.050-0.100-0.200-0.400
Talstar 10 EC (100 g/l bifenthrin)	0.025-0.067	0.00156-0.00312-0.00625-0.0125-0.05-0.20

Table 1. Chemicals and their concentrations used in screening

Leaves of *Philadelphus coronarius* Linnaeus, 1758 were immersed in the test solutions then air dried for about one hour. The leaf was placed into a glass Petri dish (10 cm diameter) and a small plastic dish with food (1:1:1 mixture of honey, yeast and pollen) and a little ball of wet cotton were put on it. 10 adults were placed in each dish. There were two dishes per concentration. The test animals remained in the dish until a stable mortality resulted. The number of paralyzed or dead individuals was recorded after 1, 5, 10, 20, 40 minutes, 1, 2, 4, hours, 1, 2,...days. Data were analyzed by probit analysis with a program that incorporates Abbot's (1925) correction for natural mortality and one way ANOVA (Sváb, 1981). All tests were conducted in the laboratory at 22–25 °C, 40–60 % RH, and under a L16:D8 photoperiod.

Results

The results showing the detailed effects of the eight pesticides are summarized in Tables 2 and 3. Table 4 shows the detrimental effects of all preparations examined according to some known categories of evaluation.

Match 50 EC and Nissorun 10 WP were classified as being harmless, Karate 5 EC and Mospilan 20 SP as slightly harmful but Danitol 10 EC was moderately harmful and Ambush C, Decisquick EC and Talstar 10 EC were harmful to adult *Ch. carnea* s. l. in terms of the IOBC categories. As to the effect of hexythiazox (mite growth regulator, specific mode of action is unknown) and lufenuron (chitin synthesis inhibitor), there was no significant difference (at P= 0.1 level) either between the concentrations administered of the insecticides themselves or between the treatments and the check (Table 3). Despite their different penetration and mode of action the systemic acetamiprid (pyridylmethylamine insecticide) and the contact pyrethroid ester lambda-cyhalothrin caused similar detrimental effects, furthermore Karate 5 EC proved to be the least toxic pyrethroid. Comparing the effects observed for Danitol 10 EC (synthetic pyrethroid ester) with those of Decisquick EC (a combination of a pyrethroid ester and an organophosphate active ingredient), Ambush C and Talstar 10 EC (contact pyrethroids), the tests demonstrated fenpropathrin to be a safer insecticide for adult chrysopids than these and the other pyrethroids (except lambda-cyhalothrin).

Three preparations (Match 50 EC, Mospilan 20 SP, Talstar 10 EC) were tested on *Ch. affinis* but the other toxicological data were gained on *Ch. carnea* s. 1. Therefore, it cannot be known which sibling species' tolerance was measured and classified in case of the other treatments. Learning the origin of the test lacewings by further sampling of the collecting areas and identifying the specimens might lead to identifying indirectly the tested chrysopids. Table 5 shows such a characterization of the areas' sibling species assemblages. Almost all of the tested lacewings were caught in Gödöllő in September 1991. Only the specimens used for screening Ambush C were captured in May 1992 at the same site. Rough estimations for identifying the original populations can be made on the basis of the mean of four consecutive years (1996, 1997, 1998, 1999) or on the strength of mean of the four years' relevant months (Table 5) if a relatively stable lacewing assemblage could be presumed at the site in question. Regarding the version of the month, in the case of Ambush C *Ch. affinis*, individuals may have constituted the crucial majority (93%) of the test lacewings. For the other testing the conclusions cannot be so

Preparat (time of eval		LC ₅₀ (95% FL) %	LT ₅₀ of the registered concentration days	Effect of the registered concentration % of mortality
Ambush C	(10 days)	0.0098 (0.006–0.015)	6.47	91.1
Danitol 10 EC	(10 days)	0.052 (0.012-0.224)	3.52	62.4
Decisquick EC	(6 days)	0.011 (0.002–0.066)	2.87	99.0
Karate 5 EC	(7 days)	0.056 (0.024–0.414)	7.45	39.4
Mospilan 20 SP*	(10 days)	0.359 (0.201–0.841)	>15.00	10.5
Talstar 10 EC*	(9 days)	0.0023 (0.001-0.003)	4.48	100.0

Table 2. Side effects of pesticides on adult Chrysoperla carnea sensu lato

Legend: FL – fiducial limits; * = Chrysoperla affinis specimens were treated.

Table 3. Effects of Nissorun 10 WP and Match 50 EC on adult Chrysoperla carnea sensu lato

Treatments	No. of living individuals			
	1 day	3 days	6 days	10 days
	after treatment			
Check	10	10	9.5	
Nissorun 10 WP 0.025 %	10	10	10	
Nissorun 10 WP 0.05 %	10	10	10	
Nissorun 10 WP 0.10 %	10	10	10	
Nissorun 10 WP 0.20 %	10	9.5	9.5	
Nissorun 10 WP 0.40 %	10	10	10	
$DS_{5\%}$	*	0.65	1.00	
Check	10		9.0	9.0
Match 50 EC 0.0125 %	10		9.0	9.0
Match 50 EC 0.025 %	10		10	10
Match 50 EC 0.10 %	10		9.0	9.0
$DS_{5\%}$	*		2.78	2.78

Legend: * – parameter could not be computed;

 $DS_{5\%}$ – significant difference at P = 0.05.

likely as that because *Ch. affinis* individuals could made out only 82 % of the total number of specimens in September of the sampled years. Since, this kind of estimation is presumably affected with multiple mistakes as well as the pesticide susceptibility of the different sibling species has not been characterized, the former data based on mixed sibling species cannot be relevant to one taxon, in this case *Ch. affinis*.

Discussion

After thoroughly studying the methods of some of the most recent papers, it seems that the majority of authors did not attach adequate importance to the taxonomical exactness and the origin of test animals. E.g., Sterk *et al.* (1999) cited only sources about the lacewings' rearing and testing methods; Dutton *et al.* (2002, 2003) and Huerta *et al.* (2003a,b) mentioned that the *Ch. carnea* individuals used for testing were permanently/routinely reared in their laboratory; Rumpf *et al.* (1997a,b), Hilbeck *et al.* (1998, 1999), Viñuela *et al.* (2000), Medina *et al.* (2002, 2003) indicated the origin of their common green lacewings strains; Senior *et al.* (1998) added to the origin of the chrysopids that the tested sibling species was unknown and Güven & Göven, (2003) took the trouble to have their lacewings identified – not at sibling species level – by the Colin Plant Associates (UK). Most of the testing (except the investigation in Great Britain and Turkey) was made on three European populations, two German ones and a Swiss one. This choice could have interesting consequences. Spanish authors (Viñuela *et al.*, 2000; Medina *et al.*, 2002, 2003) used German strain for testing which originated from

Preparation	Categories of evaluation		
	А	В	С
Ambush C	L	3	4
Danitol 10 EC	М	2	2
Decisquick EC	М	4	4
Karate 5 EC	L	1	1
Match 50 EC*	0	1	0
Mospilan 20 EC*	L	1	1
Nissorun 10 WP	0	1	0
Talstar 10 EC*	L	4	4

Table 4. Susceptibility of adult Chrysoperla carnea sensu lato to the chemicals examined.

Legend: * = *Chrysoperla affinis* specimens were treated. A: categories of Bartlett (1964): 0 = no kill, L = LT $_{50} > 100$ hours, M = LT $_{50} > 24$ hours and < 100 hours, H = LT $_{50} < 24$ hours. B: categories of the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms" (Hassan, 1989): 1 = harmless (< 50 % mortality = M), 2 = slightly harmful (50–79 % M), 3 = moderately harmful (80–99 % M), 4 = harmful (>99 % M). C: categories of Bigler (1984): 0 = no effect, 1 = low effect (< 40 % M), 2 = moderate effect (41–70 % M), 3 = high effect (71–90 % M), 4 = extremely high effect (91–100 % M).

Table 5. Dominance values of sibling species of Chrysoperla carnea complex at Gödöllő (means are followed by SD).

Dates	affinis	carnea s.str.	lucasina
Gödöllő Year	86.62 ± 10.38	11.30 ± 8.79	2.03 ± 1.85
Gödöllő May	93.18 ± 7.84	6.82 ± 7.84	_
Gödöllő Sep	82.85 ± 11.94	13.53 ± 9.98	3.63 ± 2.49

Legend: Year – mean of total values of the years 1996, 1997, 1998, 1999; May – mean of values of May of the four years; Sep – mean of values of September of the four years.

the Institute for Plant Protection in Orchards (Dossenheim, Germany). It seems to be good for comparing their data with the German ones but not e.g., with the Swiss ones. However, even when supposed stable the sibling species composition of the German population in Spanish colonies, their landsman IPM specialists (e.g., in olive orchards) can hardly use these data because the natural Spanish *Ch. carnea* sibling species composition might differ remarkably from that of Germany or Switzerland, and of course, that can be true for other European countries with dissimilar ecological conditions. Regarding some recent data in the south of Spain (Jaén), the dominant sibling species might be *Ch. agilis* (about 62 %), followed by *Ch. carnea* s. str (22 %), *Ch. lucasina* (11 %) and *Ch. affinis* (four %) (Bozsik & Ruiz, 2003, unpublished data). In Germany like in Belgium *Ch. affinis* predominates and are followed by *Ch. carnea* s. str. and *Ch. lucasina* (Thierry *et al.*, 1994; Bozsik *et al.*, 2003). Taking into consideration the dissimilarities in the regional species composition and also the possible different ecological conditions, it is not impossible either that the formerly observed pyrethroid tolerance difference between Swiss and German *Ch. carnea* strains (Sterk *et al.*, 1999) could be attributed also to the sibling species problem.

Regarding the former testing results concerning the *Ch. carnea* s.l. adults there is practically no information. In the case of pyrethroids the majority of data characterize only their larvae or eggs: cypermethrin (Singh & Varma, 1986; El Maghraby *et al.*, 1994; Rumpf *et al.*, 1997a; Reddy & Manjunatha, 2000), deltamethrin (El Maghraby *et al.*, 1994; Deole *et al.*, 2000), fenpropathrin (Rao *et al.*, 1990; El Maghraby *et al.*, 1994). The only exception is lambda-cyhalothrin. Vogt & Viñuela (2001) examined the impact of Karate 5 EC on *Ch. carnea* s.l. but their results cannot be compared with those presented here because they used topical application in order to compare the susceptibility of adults and larvae and not to categorize the pesticides' harmfulness to adults. As to bifenthrin no data have been found. The case of acetamiprid and hexythiazox is similar to that of the pyrethroids:

the available data concerns larvae (Vogt, 1992; Toda & Kashio, 1997). One can speculate that information gained on larvae might be adapted also to adults. However, that could not be proper because of different efficiency, penetration of pesticides (e.g., IGRs, pyrethroids) on larvae and adults, different possibility of exposure following divergent ecological demands and behaviour (feeding, locomotion, etc.) in nature.

Match 50 EC and Nissorun 10 WP seem to be environmentally safe preparations from point of view of *Ch. carnea* s. l. adults but in case of Ambush C, Danitol 10 EC, Decisquick EC, Karate 5 EC, Mospilan 20 SP and Talstar 10 EC further semi-field or field test is needed to determine their real effects under field conditions because environmental factors may effect the hazard posed by a pesticide to beneficial arthropods. The results of Ambush C, Match 50 EC, Mospilan 20 SP and Talstar 10 EC concern *Ch. affinis* which is the most dominant cryptic species of the European *carnea*-group. To my best knowledge, this is the first report that presents pesticide side-effect data concerning one of the sibling species of the *Ch. carnea* complex. Indirect estimation of originally unknown sibling species composition of previous screening procedures has been attempted. Because of the complexity of the problem more data or more sophisticated methods of collecting data are needed for subsequent correction of species identifies. The most preferable solution though, is that the tests are remade on well identified sibling species of the *Ch. carnea* species complex.

Conclusions

The interpretation and citation of the former toxicological (side-effects of pesticides) data concerning the common green lacewing need sober precaution because nobody knows on which taxa those tests were carried out.

In order to continue the routine testing of pesticide preparations on the cryptic species of the *Ch. carnea* complex it would be crucial to establish new, relatively easily usable taxonomic keys, or if it is not possible, the testing should be made on individuals identified by lacewing specialists.

Unsuitable interpretation of references and the use of poorly determined specimens can cause troubles in the biological control application of the sibling species of *Ch. carnea* (Canard *et al.*, 2002).

The existence of various geographical populations of *Ch. carnea* cryptic species, with presumably different tolerances to pesticides, should be considered. The tests should be carried out on individuals (collected and reared) from various sites of Europe, when working in European context.

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