Short and Long-Term Effects of Endosulfan, Cypermethrin, Spinosad, and Methoxyfenozide on Adults of *Chrysoperla externa* (Neuroptera: Chrysopidae)

FEDERICO RIMOLDI,[1] MARCELA I. SCHNEIDER,[2] AND ALICIA E. RONCO[1,3]

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ABSTRACT The susceptibility assessment of natural enemies to pesticides is relevant before the use of selective pesticides and biological control agents within the framework of integrated pest management programs. *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) is a predator considered a potential biocontrol agent of agricultural pests in the Neothropical Region. The aim of this study was to evaluate the short and long-term effects of two broad spectrum insecticides (cypermethrin and endosulfan) and two biorational (spinosad and methoxyfenozide) registered in Argentina on young *C. externa* adults under laboratory conditions by ingestion through drinking water. The assessed end-points were: survivorship during preoviposition period, preoviposition time, fecundity and fertility of females, and survivorship of the progeny. Total survivorship of adults was adversely affected only by cypermethrin that reduced the survivorship of adults irrespective of sex. Although endosulfan did not induce significant total mortality, it reduced the survivorship of females. All tested insecticides delayed the reproductive maturity of adults. Cypermethrin, endosulfan, and spinosad reduced the fecundity of females. Fertility was affected only by spinosad. Methoxyfenozide was the insecticide inducing the lowest effects on reproductive parameters. No effects were observed on the survival of progeny with any of the tested compounds. In accordance with the International Organization for Biological Control and Noxious Animals and Plants guidelines the insecticides were classified according to its lethal effects in the following toxicity classes: methoxyfenozide, spinosad, and endosulfan, class 1 (innocuous); cypermethrin, class 2 (moderately toxic). However, if sublethal effects are taken into account, spinosad and endosulfan should not be considered innocuous.

KEY WORDS chrysopid, cypermethrin, endosulfan, methoxyfenozide, spinosad

Biological control by natural enemies is an important strategy for the control of pests in agroecosystems within the framework of integrated pest management (IPM) (Van Driesche et al. 2007). Moreover, in recent years the conservation or augmentation of natural enemies within agroecosystems has been considered an important component in IPM programs, being also compatible with use of low-risk pesticides. During the process of selecting an organism to be used as a biocontrol agent, not only the assessment of the organism bioecological parameters is relevant, but also its susceptibility to pesticides.

For many years the use of generalist predators as biological control agents was relegated by the application of specialist agents such as parasitoids. At present, this trend has reversed in part, and the effectiveness of generalist predators as control agents has found support in both theoretical and practical biological strategies (Symondson et al. 2002).

Use of lacewings in IPM programs has recently increased because of their relative tolerance to several pesticides (Medina et al. 2003; Rimoldi et al. 2007, 2008; Moura et al. 2009). However, there are few studies assessing the insecticidal effects on adults, probably taking into account that larvae feed on the prey, whereas the adults feed on nectar and pollen. The Neotropical green lacewing, *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) is very abundant in Argentinean crops and has been considered a promising candidate as a biocontrol agent within IPM programs in South America (Rodrigues Barbosa et al. 2008). Although pest control is done by the larvae, adults are responsible to increments and dispersion of the population in fields.

The extensive use of broad-spectrum pesticides in agroecosystems could be the cause of pest resurgence, replacement by secondary pests, pesticide resistance development, and environmental contamination (Elzen 2001, Ronco et al. 2008). In this way, the worldwide agricultural tendencies advocate to the use of low-risk pesticides. In Argentina, broad-spectrum in-

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[1] Centro de Investigaciones del Medio Ambiente, Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 115, La Plata, Argentina, CONICET.

[2] Centro de Estudios Parasitológicos y de Vectores, Calle 2 N° 584, La Plata, Argentina (CCT La Plata CONICET–UNLP).

[3] Corresponding author, e-mail: cima@quimica.unlp.edu.ar.

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secticides (such as endosulfan and cypermethrin) are widely used for pest control. However, in recent years, new and more selective active ingredients considered safer because of their lower impact on beneficial organisms (such as spinosad and methoxyfenozide) (Williams et al. 2003, Schneider et al. 2008) have been synthesized worldwide, though are very slowly incorporated in the local market, probably for their high cost compared with conventional ones.

Endosulfan is an organochlorinated neurotoxic insecticide, which acts by blocking the chloride channels in postsynaptic cells. This insecticide was banned in 47 countries (Martin et al. 2009); however, in Argentina although it is still in use, is being restricted (Resolution 511/2001 of SENASA). Cypermethrin is one of the most widely used insecticides (Cámara Argentina de Sanidad Agropecuaria y Fertilizantes [CASAFE] 2009). It belongs to the pyrethroid group and also acts on the central nervous system, altering the axonic sodium channels and allowing excessive ion entrance, causing abnormal nervous activity and eventually paralysis (Stenersen 2004). Spinosad is a metabolite derived from the fermentation process of the soil actinomycete *Saccharopolyspora spinosa.* It is also neurotoxic, as it alters the binding of acetylcholine in the specific nicotinic receptors of the postsynaptic cell and may also act on GABA receptors (Salgado 1997). Finally, methoxyfenozide is a molting accelerating compound (MAC) belonging to the insect growth regulator (IGR) insecticides group. MACs directly stimulate the molting hormone receptor by binding to the same natural hormone receptors, causing an anticipated lethal molt (Dhadialla et al. 1998).

Previous studies have evaluated the effects of these insecticides on eggs and larvae of *C. externa* (Rimoldi et al. 2007, 2008); however, there is scarce information on the effects on adult stage. The aim of the current study was to evaluate, under laboratory conditions, the lethal and sublethal effects of the broad-spectrum insecticides cypermethrin endosulfan and the biorational spinosad and methoxyfenozide on adults of the generalist predator *C. externa* by ingestion, and contribute with information for the assessment of compatibility between these insecticides and *C. externa.*

Materials and Methods

Insects. *C. externa* organisms used in the tests were obtained from a laboratory colony initiated and established from adults collected in 2004 in fields of the greater La Plata area (Argentina), without history of pesticide use. After quarantine, *C. externa* adults were maintained and multiplied in laboratory at 25 ± 2°C, with 75 ± 5% relative humidity (RH) and a photoperiod of 16:8 (L:D) h. Annually, the colony was infused with wild stock collected from the same geographical source to help maintaining genetic variability.

Insecticides. The following commercial products were used in tests: Tracer (48% spinosad; Dow Agroscience, Ciudad Autónoma de Buenos Aires, Argentina), Intrepid (24% methoxyfenozide, Dow Agrosciences, Ciudad Autónoma de Buenos Aires, Argentina), Glexin25 (25% cypermethrin, Gleba, La Plata, Buenos Aires Province, Argentina Plata, Argentina), and Endosulfan35Glex (35% endosulfan, Gleba).

Toxicity Testing: Short and Long-Term Effects. Adults $<$24 h old were observed under a binocular microscope for sex determination, and couples were placed in plastic cylindrical boxes (8.5 cm diameter × 9.0 cm depth) for mating. The containers were covered internally with dark cardboard on the sides and with a black net on top as oviposition support and to ease identification, count, and extraction of eggs.

Adults were fed with artificial diet ad libitum according to Nuñez (1998). Each couple was exposed by ingestion (during all life span), given insecticide solutions in drinking water chronically without renewal. Medina et al. (2003) estimated that adults of *Chrysoperla carnea* should drink water at least once in 4 d after emergence for normal development. Maximum field recommended concentrations (MFRCs; registered in Argentina or recommended by manufacturers; CASAFE 2009) were tested for each insecticide: cypermethrin, 25 mg (active ingredient [AI])/liter; endosulfan, 105 mg (AI)/liter; spinosad, 120 mg (AI)/liter; methoxyfenozide, 144 mg (AI)/liter. Distilled water was provided for negative controls. Between four to seven replicates of three pairs of adults were done for each treatment. The experiments were performed under the same environmental conditions as described for the rearing.

Every 24 h and throughout the rest of their life cycle, total mortality (irrespective of sex considered) and mortality per sex during the preoviposition period, length of preoviposition period, and the fecundity and fertility of females were evaluated. Preoviposition period was taken from the time of the emergence of female adults to the first oviposition. Additionally, length of egg period (time from oviposition to hatching, including the embryo development inside of egg) was assessed for the first three ovipositions. Furthermore, 30 eggs from the fourth oviposition (96 h) were collected from each treatment, larvae were fed with the prey, and survival of this progeny (F2) was evaluated.

Fecundity was assessed by counting the number of eggs laid by each female at 24, 48, and 72 h since the first oviposition (first three ovipositions). Fertility was calculated as the proportion of emerged larvae/eggs laid.

Statistical Analysis. Results are presented as the mean ± SE. The mortality in each treatment was corrected with the values observed in the control according to Abbott (1925). A Shapiro–Wilk test was used to assess the distribution of data. If data showed normal distribution, one-way analysis of variance (ANOVA) or repeated measures ANOVA was used to observe differences between treatments. When proportional data required normalization, the arc-sine square-root transformation was done before analysis. The Kruskal–Wallis test was used for the set of data not reaching normality. After ANOVA and repeated measures ANOVA, the means were separated by applying the least significant difference (LSD) test to assess differences among treatments or Dunnett's test to

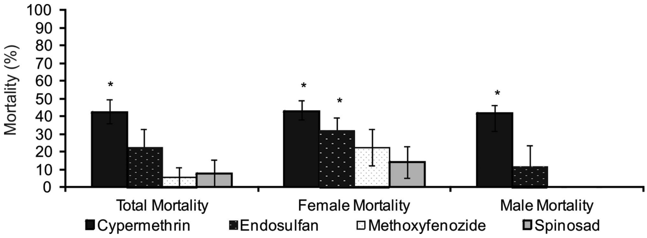

Fig. 1. *C. externa* adult mortality during the preoviposition period. Values correspond to the mean ± SE, corrected according Abbott (1925). A one-way ANOVA with $\alpha = 0.05$ was performed to assess differences between treatments: total mortality, $F = 3.157$, $P = 0.038$, df = 4, 23; female dead, $F = 2.595$, $P = 0.069$, df = 4, 23; male dead, $F = 2.283$, $P = 0.098$, df = 4, 23. Asterisk indicates significant differences of each treatment respect to control.

compare treatments respect to control ($P \leq 0.05$). After the Kruskal–Wallis test, Dunn's test was used for multiple pairwise comparisons. The program Xlstat (ADDINSOFT 2004, XLstat for Excel, ver. 7.5, Addinsoft, New York, NY) was used in analysis.

Results

Short-Term Effects on Young Adults. Figure 1 shows the total mortality of *C. externa* adults during preoviposition period, as well as the differential mortality by sex. Cypermethrin caused the highest mortality of adults, inducing 42.3% total mortality in immature adults. This was significantly higher than control treatment for both males and females (Fig. 1).

Endosulfan did not cause any detrimental effects on total survival of *C. externa* adults. However, when the relative mortality by sex was analyzed, this insecticide caused higher percentages of female mortality than those observed in the control treatment (Fig. 1). Tests with methoxyfenozide and spinosad showed no significant effects on survival of exposed organisms with respect to control treatments.

Long-Term Effects. *Length of Preoviposition Period.* Results for this assessment endpoint are shown in Fig. 2. All tested insecticides lengthened the average time required by females to initiate oviposition.

Fecundity and Fertility of Females. Data on fecundity and fertility for the first three ovipositions (24, 48, and 72 h) are shown in Table 1. Cypermetrhin, endosulfan, and spinosad induced the highest effects on fecundity of adults, significantly reducing the number of oviposited eggs in the three first ovipositions, whereas methoxyfenozide inhibited this end point only during the first oviposition.

With respect to the assessment of the effects of the tested insecticides on fertility, the results were disparate. The fertility in the endosulfan treatment was significantly higher than in the control treatment in the first and second ovipositions. Similarly, this effect was observed in the methoxyfenozide treatment in the first oviposition. To the contrary, a significant reduction in fertility was seen in the third ovipositions in the spinosad treatment. In the cypermethrin treatment, no differences were observed in the fertility of females with respect to the control.

Effects on the Progeny (F2) and Length of Egg Period. Methoxyfenozide shortened the egg period in the first and second ovipositions; however, no effects were observed in the third one. The rest of the insecticides did not induce significant effects on this endpoint.

However, none of the tested insecticides induced a significant effect on total mortality (from egg to adult) for the offspring of the adults (F2) originally exposed via drinking water ($F = 0.760$; $P = 0.574$; df = 14, 4).

Discussion

IPM paradigm includes within its control strategies the joint use of natural enemies and selective insec-

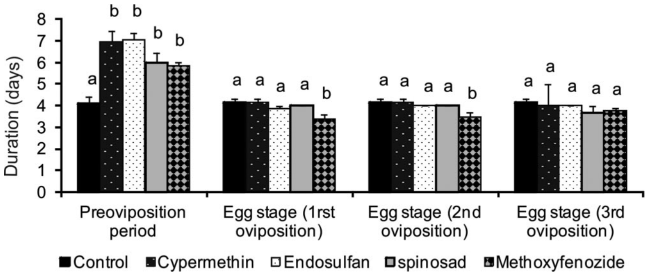

Fig. 2. Effect of cypermethrin, endosulfan, spinosad, and methoxyfenozide on the duration of the preoviposition time (F1) and the duration of egg stage laid 24, 48, and 72 h from the time of the first oviposition for exposed adults. Statistical analysis was performed separately for each end point. Given values correspond to mean ± SE. A one-way ANOVA with $\alpha =$ 0.05 was performed to assess differences between treatments: preoviposition period, $F = 9.844$, $P < 0.0001$, df = 4, 23; egg stage (first oviposition), $F = 3.864$, $P = 0.031$, df = 4, 16; egg stage (second oviposition), $F = 3.988$, $P = 0.028$, df = 4, 16; egg stage (third oviposition), $F = 0.469$, $P = 0.758$, df = 4, 15. Different letters indicate significant differences from the control.

Table 1. Effects of cypermethrin, endosulfan, spinosad, and methoxyfenozide on the fecundity and fertility of *C. externa* females

Treatment	24 h		48 h		72 h	
	Fecundity	Fertility (%)	Fecundity	Fertility (%)	Fecundity	Fertility (%)
Control	16.0 ± 3.2b	63.9 ± 10.1ef	16.3 ± 3.0b	66.3 ± 10.3cdef	21.4 ± 3.4a	76.0 ± 8.9b
Cypermethrin	6.1 ± 1.6de	63.9 ± 13.3def	6.9 ± 2.1de	58.0 ± 16.4fg	11.3 ± 2.8bcd	77.1 ± 3.8ab
Endosulfan	8.4 ± 1.6cde	78.4 ± 9.3ab	9.5 ± 2.9cde	86.3 ± 2.5a	10.3 ± 2.9cde	71.2 ± 12.1bcde
Spinosad	5.5 ± 1.4e	64.1 ± 12.2def	7.3 ± 2.6de	67.1 ± 11.9bcdef	10.3 ± 2.7cde	53.5 ± 11.1g
Methoxyfenozide	10.2 ± 1.6cde	73.7 ± 6.8bcd	13.5 ± 2.2bc	74.3 ± 6.2bc	16.5 ± 2.5ab	76.7 ± 5.5ab

Given values correspond to mean ± SE Repeated measures ANOVA was used for treatment comparisons ($\alpha = 0.05$). Fecundity: $F = 3.216$; $P < 0.0001$; fertility: $F = 0.830$; $P < 0.0001$.

Treatments with different letters are significantly different.

ticides. In this way, *Chrysoperla* species have been the more studied chrysopids in relation with their compatibility within biological control strategies. The use of ecosystem services by means of natural enemies becomes relevant in the last years. Hence, *C. externa* adults could play an important role in the conservation of this depredator in the agroecosystems (Pappas et al. 2011). However, there is scarce information on the effects of pesticides on adult chrysopids, mainly because of the regular sequential testing scheme given by the IOBC for the assessment of secondary effects, established for larvae (Vogt et al. 2000).

Scientific literature indicates that most conventional broad spectrum insecticides are more harmful to beneficial insects than biorational spectrum compounds (Legaspi et al. 2000, Rimoldi et al. 2008). In this sense, considering that cypermethrin and endosulfan interfere with metabolic processes common for a large group of organisms; a low selectivity would be expected. According to the present results, the two tested broad spectrum insecticides were also more harmful than the two biorational ones. However, while cypermethrin reduced survival of all exposed adults, affecting both males and females, no effects on the total survival of adults were observed with endosulfan. However, when survival was evaluated according to sex, endosulfan-treated females showed lower survival than controls. Modes of action involved to explain this differential response are unclear, and should be elucidated in future studies.

Despite our results indicating effects of cypermetrhin on survival of *C. externa* adults, Huerta et al. (2004) did not find significant mortality of adults of *C. carnea* (Neuroptera: Chrysopidae) exposed to natural pyrethrins. Cypermethrin, unlike natural pyrethroids, has a cyano group in the molecule that favors lethal effect on pests, and consequently may induce higher effects on nontarget organisms. However, deltamethrin, also with the cyano group in its molecule, did not induce mortality on *C. carnea* adults under residual exposure (Giolo et al. 2009). In previous studies we detected high delayed lethality of neonate larvae of *C. externa* when exposing ≤24-h-old eggs by immersion to MFRCs of endosulfan and cypermethrin (Rimoldi et al. 2008). There are several reports alerting about different levels of toxicity of endosulfan on beneficial insects (Grundy et al. 2000, Symington 2003, Jones et al. 2008).

In addition to lethal effects, in the current study we observed that the broad spectrum insecticides evaluated reduced the fecundity of female survivors, though fertility was not affected. Even in some cases endosulfan treated organisms showed higher fertility than nonexposed control ones. This result could be associated to an overestimation of the fertility because of the low fecundity. Huerta et al. (2004) and Giolo et al. (2009) also did not observe significant effects on the fecundity or fertility of *C. carnea* exposed to other pyrethroids. Endosulfan is considered an endocrine disruptor to vertebrates and invertebrates (Wirth et al. 2002), so the reduction of the fertility could be attributed to its potential effects on the endocrine system of exposed adults. Moreover, Haynes (1988) has reviewed the sublethal effects of neurotoxic insecticides on insect reproduction, attributing these effects to the alteration of Central Nervous System (CNS), which governs the functioning of the endocrine system.

Spinosad is classified as an environmentally and toxicologically reduced-risk insecticide by the United States Environmental Protection Agency (U.S. Environmental Protection Agency [USEPA] 1997). In the current study, although no direct effects on the survival of adult organisms were detected, a significant reduction of fecundity in the three first ovipositions and fertility of the third oviposition were detected. Despite that mode of action involved in altering reproductive parameters are not well known, we can hypothesize that could derive from the action of spinosad on the CNS, affecting the natural brain stimulation on the production centers of molting hormones and juvenile hormones essential for spermatogenesis and oogenesis (Pineda et al. 2007). From a practical perspective, these effects are important because they would affect the progeny of treated individuals, allowing long-term maintenance populations below the economic injury level (EIL).

The reported information about the effects of spinosad on natural enemies is disparate. Mandour (2009) and Medina et al. (2003) detected deleterious effects on the life span and fecundity of *C. carnea* adults via diet exposure to spinosad, while other authors did not observe any alteration of those reproductive parameters for this and other predators exposed to spinosad (Elzen 2001, Medina et al. 2001, Viñuela et al. 2001, Mahdian et al. 2007). Additionally, spinosad has shown high toxicity on Hymenoptera, with reduction in the time of adult emergence and longevity for the parasitoid *Hyposoter didymator* (Thunberg) (Schneider et al. 2004). Galvan et al. (2005) did not detect significant mortality of *Harmonia axyridis* (Pallas) (Coleotera: Coccinellidae) adults exposed to spinosad, but a reduction of fertility and time of development were registered.

The mode of action of methoxyfenozide could be associated to highly selective insecticidal compounds. There is literature supporting this type of behavior (Schneider et al. 2004, 2008, Rimoldi et al. 2008, Giolo et al. 2009). The present results also indicate that methoxyfenozide is innocuous to *C. externa.* However, registration of significant mortality of exposed *C. carnea* adults has been reported (Quiñones Pando et al. 2009). Although, these authors did not observe effects on the survival of *Harmonia axyridis* (Pallas) (Coleptera: Coccinellidae), *Olla v-nigrum* (Mulsant) (Coleoptera: Coccinellidae), *C. rufilabris* (Burmeister), or *Chrysopa nigricornis* (Burmeister) (Neuroptera: Chrysopidae). Medina et al. (2003) assessed the toxicity of the IGRs tebufenozide and pyriproxyfen on *C. carnea* adults, with no detection of effects on survival. Regarding our sublethal effects, methoxyfenozide induced reduction of fecundity only in the first oviposition, but fertility was not affected. Previous studies showed similar effects of this insecticide on adults that had been exposed during the egg stage (Rimoldi et al. 2008).

All insecticides tested in the current study induced a significant lengthening of the preoviposition time, which, although it does not correspond to a developmental stage, indicates that adults take longer to reach reproductive maturity since emergence. The sensitivity of *C. externa* to this assessment endpoint is evident even in the absence of lethal effects. Time to reproductive maturity appears being a useful indicator of exposure to insecticides to be considered for the assessment of impacts.

The assessment of effects on later generations (F2) shows no significant differences with respect to controls for all insecticides tested. Only a reduction in the percentage of hatched larvae from adults exposed to spinosad in the third lay could be detected. Similarly, Schneider et al. (2008) reported the absence of long-term effects of methoxyfenozide on progeny of *Hyposoter didymator* (Thunberg), though detected effects for the insecticides diflubenzuron and piriproxifen.

Taking into account the lethal effects of the MFRCs for the studied insecticides, the compounds can be classified in the following IOBC toxicity classes: methoxyfenozide, spinosad, and endosulfan, class 1 (innocuous); cypermethrin, class 2 (moderately toxic). However, if sublethal effects are included, the following order of toxicity can be expected: cypermethrin $\gg$ endosulfan $>$ spinosad $>$ methoxyfenozide. The relevance of subltethal effects on natural enemies for categorizing pesticide toxicity has been demonstrated once more in the current study. According our results broad spectrum insecticides should not be included in IMP strategies, whereas more studies are needed to complete the toxicity profile of spinosad to reach conclusions on the compatibility with *C. externa.* Lastly, methoxyfenozide could be used jointly with this natural enemy for lepidopteran pest control. The study pointed out the relevance of the assessment of side effects of pesticides on adult chrysopids.

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