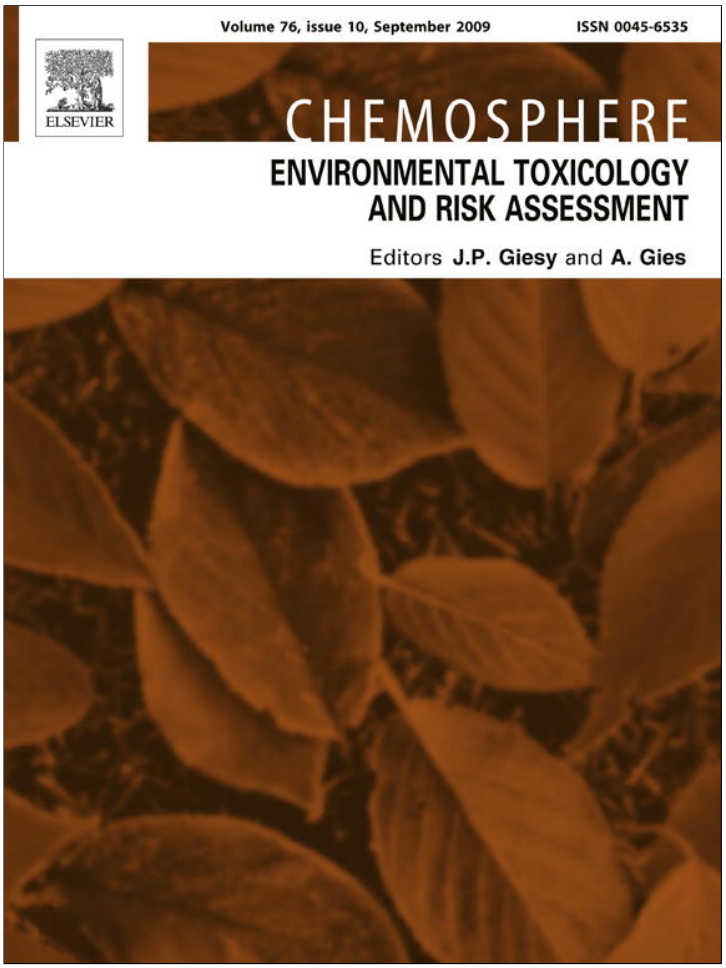

Chemosphere 76 (2009) 1451–1455

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Impact of glyphosate on the development, fertility and demography of *Chrysoperla externa* (Neuroptera: Chrysopidae): Ecological approach

M.I. Schneider [a,*], N. Sanchez [a], S. Pineda [b], H. Chi [c], A. Ronco [d]

[a] Centro de Estudios Parasitológicos y de Vectores (CEPAVE) (CCT La Plata CONICET-UNLP), Calle 2 N° 584 La Plata, Bs. As, Argentina
[b] Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Km.9.5 Carr. Morelia-Zinapécuaro, 58880 Tarímbaro, Michoacán, México
[c] Laboratory of Theoretical Ecology, Department of Entomology, National Chung Hsing University, Taiwan, Republic of China
[d] Centro de Investigaciones del Medio Ambiente (CIMA), Facultad de Ciencias Exactas (UNLP), CONICET, Calle 47 y 115, La Plata, Bs. As, Argentina

ARTICLE INFO

Article history:
Received 7 February 2009
Received in revised form 12 May 2009
Accepted 16 May 2009
Available online 3 July 2009

Keywords:
Chrysoperla externa
Glyphosate formulation
Ecotoxicology
Demographic parameters

ABSTRACT

Few ecotoxicological studies have used life table analysis to evaluate the toxicity of pesticides on beneficial organisms. This study is the first report of the effect of the herbicide glyphosate on a predator insect, *Chrysoperla externa*, using a demographic approach. This predator is associated to soybean pests and has a potential role as a biological control agent in the Neotropical Region. The objective of this work was to evaluate the side-effects of glyphosate on the development, fertility and demography of *C. externa*, treated orally by ingestion of glyphosate-dipped eggs of *Sitotroga cerealella* in laboratory conditions. The data were analyzed using the age-stage, two-sex life table. Development from third larval instar to pupae and adult longevity were shorter in glyphosate-treatment than in the control. Adult pre-reproductive period was longer in glyphosate-treatment than in the control. Fecundity and fertility were deeply reduced, as well, being fertility greater affected. A high important reduction was registered in all population parameters. Most eggs from glyphosate-treated cohort looked abnormal, smaller than control, dehydrated and became black 2 d after oviposition. In addition, adults developed tumours in the abdomen region at 20 d after emergence, being the effect more drastic in females than males. It is beyond the scope of our study to speculate on the effects of this herbicide on *C. externa* field populations. However, it seems likely that populations under continuous use of glyphosate would be exposed at greater detrimental effects in the long term.

1. Introduction

In Argentina, transgenic soybean crop (Roundup Ready, RR) has undergone a major expansion over the last 15 years (Peruzzo et al., 2008), with the consequent increase of glyphosate [(N-phosphonomethy) glycine] applications, a non-selective, broad-spectrum and post emergence herbicide. It is absorbed by the foliage and translocated to the entire plant, being its mode of action based on binding to the enzyme 5-enolpyruvylshikimic acid-3-phophate synthase (EPSPS) in the biosynthetic pathway of aromatic amino acids (Smith and Ochme, 1992). Soybean crops are inhabited by several arthropods belonging to different trophic levels. Among them, parasitoids and predators are relevant as natural enemies of many herbivore pests. *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) is a predator associated to soybean pests, distributed from the southeast of the United States and the Antilles, to South America (Adams, 1983; Albuquerque et al., 1994). This predator is being considered a potentially biological control agent in South America because it feeds on some important agricultural pest and it has a strong preference for open habitats (Albuquerque et al., 1994; Carvalho et al., 1998). In soybean crops, larvae were observed feeding on eggs and small larvae of lepidoptera and hemiptera pests such as *Rachiplusia nu* larvae and *Nezara viridula* eggs (Schneider and Rimoldi, unpublished data).

The negative impact of pesticides on non-target organisms have been extensively recognised (Stark and Banks, 2003; Desneux et al., 2007; Stark et al., 2007). Due to the very little compatibility of biological control with non-selective pesticide use, concern of practitioners of IPM programs has significantly increased (Stark et al., 2007; Kogan and Jepson, 2007). In addition to death and reduced fecundity, exposure to a toxicant may result in multiple sublethal effects such as shortened life span, offspring mutations, weight loss, and changes in fertility rates, changes in oviposition behaviour, and development times. Demographic parameters estimation through life table analysis is an essential approach for ecological predictions of population growth (Stark et al., 2007) and valuable for pest management. Side-effects of several insecticides (conventional and biorational), commonly used in Argentina for soybean pest control, have been evaluated in natural enemies

* Corresponding author. Tel.: +54 221 4233471x21; fax: +54 221 4232327.
E-mail address: mschneider@cepave.edu.ar (M.I. Schneider).

0045-6535/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2009.05.029

during the last 5 years (Schneider et al., 2006, 2008; Rimoldi et al., 2008). However, few studies have evaluated the effect of insecticides on life history traits and population parameters of natural enemies of pests (Stark and Banks, 2003; Desneux et al., 2007; Stark et al., 2007).

Results of several studies carried out over the past decade showed that glyphosate as well as insecticides may has detrimental effects on vascular plants, fishes, amphibians, snails, earthworms, carabids, etc., by causing development, morphological, physiological, immunological and biochemical alterations (Tate et al., 2000; Smith, 2001; Lajmanovich et al., 2003; Cauble and Wagner, 2005; Glusczak et al., 2006; Sobrero et al., 2007; Yasmin and D'Souza, 2007; Achiorno et al., 2008). However, effects of glyphosate on arthropod biology and ecology have been little documented so far (Paoletti and Pimentel, 2000; Manzoni et al., 2006). Our hypothesis is that as glyphosate affects other organisms, it will adversely affect arthropod natural enemies of pests. We predicted that development time and population parameters will exhibit changes indicating a negative impact of this herbicide on population performance.

The objective of this study was to determine the side-effects of glyphosate on development, fertility and demographic parameters of *C. externa* (Neuroptera: Chrysopidae) treated orally by ingestion of glyphosate-dipped eggs of *Sitotroga cerealella*, in the laboratory.

2. Materials and methods

2.1. Insects rearing

Organisms used in this study were obtained from a colony of *C. externa* collected in La Plata area, Argentina, and maintained in the laboratory. After quarantine, *C. externa* adults were fed *ad libitum* an artificial diet (Vogt et al., 1998) and larvae on *S. cerealella* Olivier (Lepidoptera: Gelechidae) eggs as a "factitious prey" and provided by the insectaria IMYZA-Castelar, Argentina. Colony and experiments were maintained in a growth chamber at 25 ± 0.5 °C temperature, 75 ± 5% RH, and a photoperiod of 16:8 (*L*:*D*) h.

2.2. Chemicals and treatments

The commercial Glyfoglex 48® (48% glifosato, Gleba S.A., Buenos Aires, Argentina) was used in toxicity tests. Solutions with 192 mg l^{-1} a.i. (maximum field registered nominal concentration) (CASAFE, 2007) were prepared in distilled water adding a tensoactive (Tween80®, 0.01%, Merck, Darmstadt, Germany) to improve the adherence of the herbicide to the chorion of the *S. cerealella* eggs. Eggs ⩽72 h-old were treated by dipping in glyphosate + tensioactive solution for 60 s, according to Pineda et al. (2004) and then placed under fume cupboard until the herbicide dried.

The glyphosate-dipped eggs were offered by ingestion to the predator. The exposure route was "through the contaminated prey" because it is the most common exposure ways at this instar instead of the residues.

2.3. Development and life history data bioassays

Two cohorts of ≈110 *C. externa* eggs (⩽24 h old) each were randomly selected from the laboratory colony and placed individually in tissue culture vials (1 cm $cell^{-1}$, 24 cells each). Larvae of control treatment were daily fed with *S. cerealella* eggs (approximately 100 eggs $larvae^{-1}$), from hatching to pupation, while at early third larval instar in glyphosate-treatment cohort, they were fed with freshly glyphosate-treated prey daily and during 48 h. After that, the preys were replaced by untreated preys until pupation. The third larval instar was chosen because it is the most voracious instar, which ensures the ingestion of the treated prey. The larvae that did not eat the treated prey (more than 90%) were discarded of the experiment.

Development times, age-stage specific survival rates (s_{xj}), age-stage specific fecundity (f_{xj}), age specific survival rate (l_x), where *x* is the age and *j* is the stage, and age-specific fecundity (m_x) were recorded daily until the death of all individuals. After adult emergence, females and males were paired and placed in 7 × 4 cm (length by diameter) plastic vial with gauze in the lid to improve ventilation and fed artificial diet (brushstroke on the walls) and water *"ad libitum"*. Age-stage, two-sex life tables were constructed (Chi, 1988). Because determining the fertility through the whole adult female stage was extremely time-consuming, only the fertility for the first 25 oviposition days of 15 females, randomly selected from each cohort, were recorded. Fertility was calculated as: (eggs hatched/eggs laid) × 100. Non emerged cocoons were dissected under binocular microscope to determine the stage of development reached (larva, pupa or preadult). Sex ratio was calculated as: males/(females + males). Population parameters were calculated taking the fertility (number of offspring per female) values instead of age-specific fecundity (number of eggs per female).

2.4. Statistical analysis

Analysis of variance (ANOVA) or Kruskal–Wallis tests were used to compare development time from third larval instar to pupa, adult longevity, fecundity and fertility of female data. Means or medians were separated by the least significant differences (LSD) multiple range test or Box and Wisker plot method, respectively (Statgraphics, STSC, 1987). In all tests, $P \leqslant 0.05$ was considered significant.

The following population parameters of each cohort were estimated:

Net Reproductive Rate (R_0)

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \tag{1}$$

Intrinsic Rate of Increase (r)

$$1 = \sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x \tag{2}$$

Mean Generation Time (T)

$$T = \frac{\ln R_0}{r} \tag{3}$$

Gross Reproductive Rate (GRR)

$$GRR = \sum_{x=0}^{n} m_x \tag{4}$$

The age-stage life expectancy (e_{xj}) was calculated according to Chi and Su (2006). The intrinsic rate of increase was estimated by using the iterative bisection method from Euler–Lotka equation (Eq. (2)) with age indexed from 0 (Goodman, 1982). The TWOSEX-MS Chart computer program was used to estimate parameters (Chi, 2008). This program includes a routine for the estimation of standard error of population parameters using Jackknife technique (Meyer et al., 1986). Survival, fecundity and reproductive value curves were constructed. Differences in life history traits and population parameters between *C. externa* exposed and unexposed to glyphosate were compared with *t*-tests (Zar, 1996).

Table 1
Side-effects of glyphosate on development time and some life history traits of *C. externa* when third instar larvae were fed glyphosate-treated preys.

Treatment		Development time L_3 – pupa (d)[A]	Pre-reproductive period (d)[B]	Adult longevity (d)		Fecundity[E] (eggs female^{-1})	Fertility[H,F] (%)	Effective fecundity[G] (eggs female^{-1})
				Males[C]	Females[D]			
Control	$\bar{x} \pm$ SE	19.4 ± 0.1a	3.06 ± 0.08a	83.0 ± 2.5a	87.0 ± 2.1a	1469.1 ± 39.5a	71.28 ± 0.46a	1047.2 ± 28.2a
	n	95	49	46	49	49	15	49
Glyphosate	$\bar{x} \pm$ SE	17.3 ± 0.3b	5.90 ± 0.23b	33.4 ± 2.9b	49.8 ± 4.4b	681.09 ± 96b	12.78 ± 1.36b	87.04 ± 12.3b
	n	79	32	47	32	32	15	32

Means within a column followed by a different letter are significantly different between control and glyphosate-treated cohorts. ([1,2,3]ANOVA, [4,5,6,7]Kruskall–Wallis, $P \leqslant 0.05$; LSD or Box and Whisker Plot mean/median separation).
n: number of individuals.
[A] $F = 278.82$; df = 1198; $P \leqslant 0.001$.
[B] $F = 176.66$; df = 176; $P \leqslant 0.001$.
[C] $F = 163.41$; df = 190; $P \leqslant 0.001$.
[D] $K = 37.61$; $P \leqslant 0.001$.
[E] $K = 39.19$; $P \leqslant 0.001$.
[F] $K = 35.07$; $P \leqslant 0.001$.
[G] $K = 57.36$; $P \leqslant 0.001$.
[H] Data correspond to % of hatched eggs from 0 to 25 d of the females' lifespan.

3. Results

Glyphosate did not show any short-term effects on third larval instar of predator, recording similar survivorship at this instar in both populations (glyphosate-treatment and control). However, several long-term effects of glyphosate were observed. Development time from third larval instar to pupae, as well as adult longevity of both male and female were significantly shorter in glyphosate-treated population than those in the control treatment (Table 1). Adult pre-reproductive period was 2.9 d longer in glyphosate-treated cohort than that in the control. Both fecundity and fertility were negatively affected by the herbicide, and the fertility was even greater affected.

The significantly negative effect of glyphosate can also be observed in the curves of age-stage survival rate (Fig. 1). The mortality in pupae stage of the glyphosate-treated cohort was of 23% whereas in the control cohort was 0%. As consequence, lower survival curves in adult stages were obtained. The negative effect of glyphosate was revealed during the adult stage of both males and females, and it was more pronounced in young females. By taking the fertility into consideration, the effect of glyphosate on reproduction was evident in both age-specific cohort total fecundity (Fig. 2) and female age-stage fecundity (Fig. 3). In addition, the reproductive period was ≈60 d shorter in glyphosate-treated females. The age-stage life expectancy in glyphosate-treated cohort was shorter than that of the control cohort (Fig. 4), being the reduction more marked in the males. The sex ratio at adult stage, was male biased (0.62:0.38) in glyphosate population whereas in the control (0.48:0.52), was more even. Glyphosate-treatment resulted in a significant reduction in all population parameters (Table 2). The Intrinsic Rate of Increase (r) and the Net Reproductive Rate (R_0) at the glyphosate-treatment exhibited a 41% and 94% of reduction compared to the control, respectively.

Also, several malformations and abnormalities were observed in glyphosate-treatment. Most eggs from glyphosate-treated cohort looked abnormal, smaller than control, dehydrated and became black 2 d after oviposition. In addition, adults developed tumours in the abdomen region at 20 d after emergence and this was more drastic in females than males.

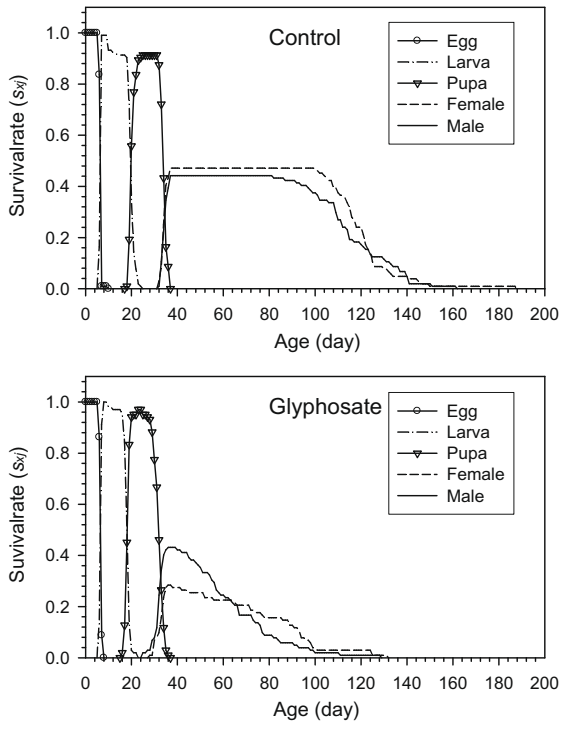

Fig. 1. Survival rate curves (s_{xj}) of *C. externa*: control (without pesticide) and glyphosate: population exposed to this herbicide at third larval instar (L_3). To simplify the graphs, all three larval instars were grouped as a single larval stage.

4. Discussion

As predicted, our results showed the side-effects of glyphosate on life history traits and demographic parameters of the predator

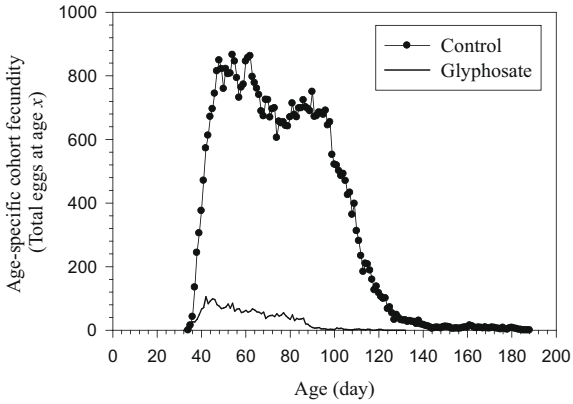

Fig. 2. Age-specific cohort fecundity of *C. externa* females exposed and unexposed to glyphosate during the third larval instar (L_3).

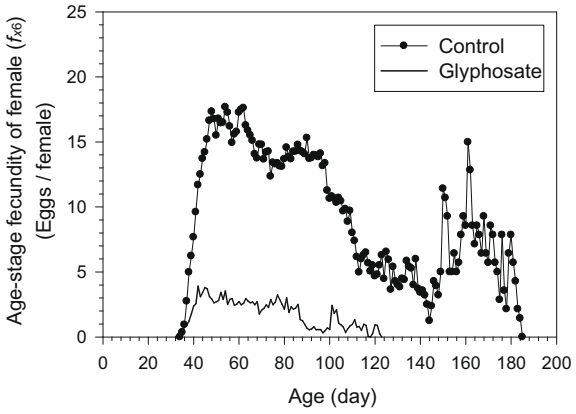

Fig. 3. Age-stage specific fecundity of female adult (f_{x6}) of *C. externa* exposed and unexposed to glyphosate at third larval instar (L_3). Female adult is the sixth life stage.

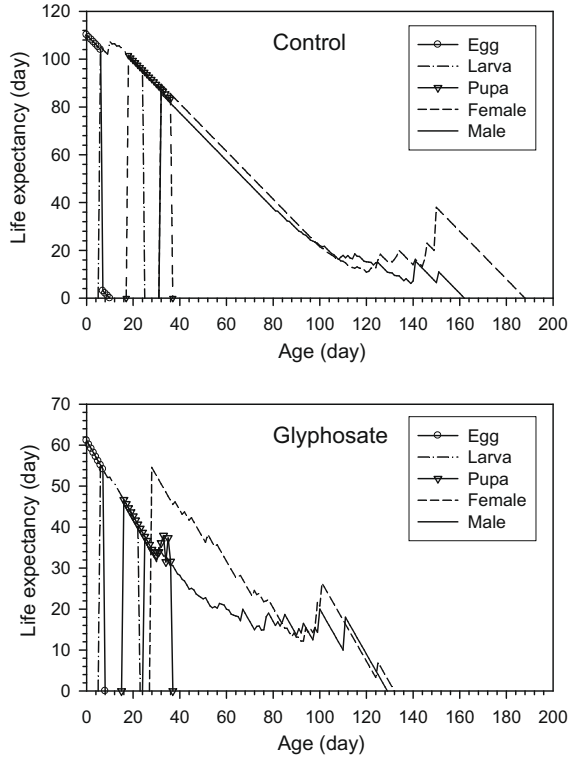

Fig. 4. Effects of glyphosate on the life expectancy of *C. externa*: (A) control population and (B) population exposed to glyphosate at third larval instar (L_3). To simplify the graphs, all three larval instars were grouped as a single larval stage.

C. externa, in laboratory bioassays. Indeed, results of the present study support the hypothesis that glyphosate will decrease arthropod population performance. The major detrimental effect observed on *C. externa* was on fecundity and fertility. Although these traits were recorded during the first 25 d from adult female emergence, it is important to take into account the relevance of the oviposition of the younger females in determining the future population growth (Lewontin, 1965). Furthermore, tumours were observed in females and males abdomen from 20 d after adult emergence. The dissected females showed abnormal ovaries with fat granules around the follicles and hyperplasia were detected in the tumours region for the both sexes. Likewise, Lajmanovich et al. (2003) reported malformations and abnormalities in larvae

Table 2
Effects of glyphosate on population parameters of *C. externa* when third instar larvae were fed glyphosate-treated preys.

Population parameter	Control	Glyphosate	*P*
Intrinsic Rate of Increase (r)	0.1051 ± 0.0022a	0.0624 ± 0.0035b	0
Net Reproductive Rate (R_0)	493.4 ± 53.2a	30.7 ± 5.8b	0
Gross Reproductive Rate (GRR)	948.4 ± 241.3a	85.9 ± 16.1b	0.0005
Mean Generation Time (T)	59.1 ± 0.4a	55.1 ± 1.0b	0.0003

Data are mean ± SE obtained by Jackknife method. Means within the file followed by a different letter are significantly different by using the Student's *t*-test ($P < 0.01$).

(craniofacial and mouth deformities, eye abnormalities and bent curved tails) and tadpoles (hyrobranchial skeletons alterations) of *Scinax nasicus* exposed by glyphosate formulations. Reductions of female fecundity by glyphosate were reported previously in the parasitoid *Trichogramma pretiosum* (Manzoni et al., 2006) and in the earthworm *Eisenia fetida* (Yasmin and D'Souza, 2007).

Biotech-soybean RR technology includes the use of glyphosate during several physiological crop stages (James, 2005). This led to an increase in herbicide consumption from 12 to 118 million litres (1996–2006) (SAGPYA, 2008). Recent ecotoxicological considerations about side-effects of pesticides on natural enemies associated to crop pests have pointed out the need of more ecological relevant endpoint as a measure of the impact of pesticides on beneficial species (Desneux et al., 2007; Stark et al., 2007). Stark and Banks (2003) have reported that results obtained from demographic studies provide better estimates than acute mortality measures and the short-term pesticides effects. Using demographic approach, our results demonstrate the detrimental effect of glyphosate on development, fecundity, and fertility of the predator *C. externa*.

All these traits have profound effects on population fitness. The deep decrease in population growth rates indicated an intense reduction in the next generation population.

A natural mortality between 1% and 15% is typical during immature stage (at first larval instar, generally), because this stage is the most sensitive one (Carvalho et al., 1998). However the mortality registered at pupal stage in the glyphosate-treatment could be related to the herbicide action because low mortalities (<3%) are often recorded in control populations. Our results agree with those reported for other organisms. Lajmanovich et al. (2003) observed an increment in the mortality of the frog *Scinax nasicus* during its immature stages. Likewise, higher larval mortalities and fail to undergo metamorphosis in *Rana cascadae* (Cauble and Wagner, 2005) and in other frogs, such as *Pseudacris triseriata* and *Rana blairi* (Smith, 2001) were also reported.

Although the role of natural enemies as biological control agents has been deeply documented and recognized (Barbosa, 1998; Symondson et al., 2002), the controversy over side-effects of glyphosate on non-target organisms is still ongoing and practices to preserve natural enemies in agricultural landscapes are receiving little attention.

Taking into consideration the studies detailed previously concerning the side-effects of this herbicide on non-target organism by causing development, morphological, physiological, biochemical and immunological alterations should alert us about a potential toxicity risk in the natural enemies associated to the pest in an agro-ecosystem. Moreover, in vitro studies in animals classified to glyphosate as an endocrine disruptor, increasing the mortality of placental cells (Walsh et al., 2000). Accordingly, it could hypothesized that similar pathway could be occurring in the arthropods and relating with reductions found in the fecundity and fertility of *C. externa*.

It is beyond the scope of our study to speculate on the effects of this herbicide on C. *externa* field populations. However, it seems

likely that populations under continuous use of glyphosate would be exposed at greater detrimental effects in the long term. Future studies should be undertaken including other development stages and other exposure routes for further knowledge on this research.

Finally, this is the first report on side-effects of glyphosate on the development and demography of a predator insect.

Acknowledgments

This research was supported by a PICT 0115150 BID 1728 OC-AR project from the Argentinean National Agency for the Promotion of Science and Technology (ANPCyT). M.S., N.S. and A.R. are members of the research career of CONICET. The contribution of Hsin Chi to this paper is partly supported by Bureau of Animal and Plant Health Inspection and Quarantine, Taiwan, Republic of China [96AS-14.2.1-BQ-B4(6) and 97AS-14.2.1-BQ-B3(2)]. The authors thank to Gleba S.A. for the donation of Glicoglex 48® samples and Damian Aliardi for assistance with the *C. externa* rearing and experiments. We are grateful to Dr. Elisa Viñuela (UPM University, Madrid, Spain) for reviewing an earlier version of manuscript and her suggestions for the improvement of it.

References

Adams, P.A., 1983. A new subspecies of *Chrysoperla externa* (Hagen) from Cocos Island, Costa Rica (Neuroptera: Chrysopidae). Bull. South. Calif. Acad. Sci. 82, 42–45.

Achiorno, C.L., de Villalobos, C., Ferrari, L., 2008. Toxicity of the herbicide glyphosate to *Chordodes nobilii* (Gordiida, Nematomorpha). Chemosphere 71, 1816–1822.

Albuquerque, G.S., Tauber, C.A., Tauber, M.J., 1994. *Chrysoperla externa* (Neuroptera: Chrysopidae): life history and potential for biological control in Central and South America. Biol. Control 4, 8–13.

Barbosa, P., 1998. Agroecosystem and conservation biological control. In: Barbosa, P. (Ed.), Conservation Biological Control. San Diego, California, USA, pp. 39–53.

Carvalho, C.F., Souza, B., Santos, T.M., 1998. Predation capacity and reproduction potential of *Chrysoperla externa* (Hagen) (Neuroptera, Chrysopidae) fed on *Alabama argillacea* (Hübner) eggs. Acta Zool. Fenn. 209, 83–86.

Cámara Argentina de Sanidad Agropecuaria y Fertilizantes: Guía de productos fitosanitarios. 2007. Tomo 1. CASAFE. Buenos Aires. Argentina.

Cauble, K., Wagner, R.S., 2005. Sublethal effects of the herbicide glyphosate on amphibian metamorphosis and development. Bull. Environ. Contam. Toxicol. 75, 429–435.

Chi, H., 1988. Life-table analysis incorporating both sexes and variable development rates among individuals. Environ. Entomol. 17, 26–31.

Chi, H., Su, H.Y., 2006. Age-stage, two sex life tables of *Aphidius gifuensis* (Ashmead) (Hymenoptera: Braconidae) and its host *Myzus persicae* (Sulzer) (Homoptera: Aphididae) with mathematical proof of the relationship between female fecundity and the net reproductive rate. Environ. Entomol. 35, 10–21.

Chi, H., 2008. TWOSEX-MSChart: computer program for age-stage, two-sex life table analysis. <http://140.120.197.183/Ecology>.

Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106.

Goodman, D., 1982. Optimal life histories, optimal notation, and the value of reproductive value. Am. Nat. 119, 803–823.

Glusczak, L., Dos Santos Miron, D., Crestani, M., Braga da Foncesa, M., De Araujo Pedron, F., Duarte, M.F., Pimentel Vieira, L., 2006. Effects of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). Ecotox. Environ. Safety 65, 237–241.

Manzoni, G.C., Grützmacher, A.D., Pinheiro Giolo, F., da Roza Härter, W., Müller, C., 2006. Selectividade de agrotóxicos usados na produção integrada de maçã para adultos de *Trichogramma pretiosum*. Pesq. Agropec. Bras., Brasilia 41, 1461–1467.

James, C., 2005. Executive Summary of Global Status of Commercialized Biotech/GM crops: 2005. ISAAA Briefs No. 34: ISAAA: Ithaca, NY.

Kogan, M., Jepson, P., 2007. In: Kogan, M., Jepson, P. (Eds.), Perspectives in Ecological Theory and Integrated Pest Management. Cambridge University Press, USA.

Lajmanovich, R.C., Sandoval, M.T., Peltzer, P.M., 2003. Induction for mortality and malforamation in *Scinax nasicus* tadpoles exposed to Glyphosate formulations. Bull. Environ. Contam. Toxicol. 70, 612–618.

Lewontin, R.C., 1965. Selection for colonizing ability. In: Baker, H.G., Stebbins, G.L. (Eds.), The Genetics of Colonizing Species. Academic Press, San Diego, California, pp. 77–91.

Meyer, J.S., Ingersoll, C.G., McDonald, L.L., Boyee, M.S., 1986. Estimating uncertainty in population growth rates: Jackknife vs; bootstrap techniques. Ecology 67, 1156–1166.

Paoletti, M.G., Pimentel, D., 2000. Environmental risk of pesticides versus genetic engineering for agricultural pest control. J. Agric. Environ. Ethics 12, 279–303.

Peruzzo, P.J., Porta, A.A., Ronco, A.E., 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. Environ. Pollut. 156, 61–66.

Pineda, S., Budia, F., Schneider, M., Gobbi, A., Viñuela, V., Valle, J., Del Estal, P., 2004. Effects of Two Biorational insecticides: spinosad and methoxyfenozide on cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera:Noctuidae) under laboratory conditions. J. Econ. Entomol. 97, 1906–1911.

Rimoldi, F., Schneider, M., Ronco, A., 2008. Susceptibility of *Chrysoperla externa* eggs (Neuroptera: Chrysopidae) to conventional and biorational insecticides. Environ. Entomol. 37, 1252–1257.

Schneider, M.I., Pineda, S., Smagghe, G., 2006. Side effects of conventional and non-conventional insecticides on eggs and larvae of *Chrysoperla. externa* (Hagen) (Neuroptera: Chrysopidae) in Argentine. Commun. Agric. Appl. Biol. Sci. 71 (2b), 425–427 (Ghent University).

Schneider, M.I., Smagghe, G., Pineda, S., Viñuela, E., 2008. The ecological impact of four IGR insecticides in adults of Hyposoter didymator (Hym., Ichneumonidae). Pharmacokinetics approach. Ecotoxicology 17, 181–188.

Secretaría de Agricultura, Ganadería y Pesca (SAGPYA)., 2008. Statistics of Oleaginous Crops. Available via DIALOG. http://www.sagpya.mecon.gov.ar (accessed 12.12.08).

Smith, E.A., Ochme, F.W., 1992. The biological activity of glyphosate to plants and animals: a literature review. Vet. Hum. Toxicol. 34, 531–544.

Smith, G.R., 2001. Effects of acute exposure to a commercial formulation of glyphosate on the tadpoles of two species of anurans. Bull. Environ. Contam. Toxicol. 67, 483–488.

Sobrero, M.C., Rimoldi, F., Ronco, A.E., 2007. Effects of the glyphosate active ingredient and a formulation on *Lemma gibba* L. at different exoposure levels and assessment end-points. Bull. Environ. Contam. Toxicol. 79, 537–543.

Stark, J.D., Banks, J.E., 2003. Population-level effects of pesticides and other toxicants on arthropods. Annu. Rev. Entomol. 48, 505–519.

Stark, J.D., Sugayama, R.L., Kovaleski, A., 2007. Why demographic and modeling approaches should be adopted for estimating the effects of pesticides on biocontrol agents. BioControl. 52, 365–374.

STSC., 1987. User's Guide Statgraphics. Graphic software system STSC Inc., Rockville, MD, USA.

Symondson, W.O., Sunderland, K.D., Greenstone, M.H., 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47, 561–594.

Tate, T.M., Jackson, R.N., Christian, F.A., 2000. Effects of glyphosate and dalapon on total free amino acids profiles of *Pseudosuccinea columella* snails. Bull. Environ. Contam. Toxicol. 64, 258–262.

Vogt, H., Degrande, P., Just, J., Klepka, S., Kühner, D., Nickless, A., Ufer, A., Waldburger, M., Walterdorfer, A., Bigler, F., 1998. Side-effects of pesticides on larvae of *Chrysoperla carnea*: actual state of the laboratory method. In: Haskell, PT., McEwen, P. (Eds.), Ecotoxicology: Pesticides and Beneficial Organisms. Kluwer Academic Publishers, Dordrecht, pp. 123–138.

Yasmin, S., D'Souza, D., 2007. Effect of pesticides on the reproductive output of *Eisenia fetida*. Bull. Environ. Contam. Toxicol. 79, 529–532.

Walsh, L.P., McCormick, C., Martin, C., Stocco, D.M., 2000. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environ. Health Perspect. 108, 769–776.

Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, New Jersey, USA.