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Impact of cypermethrin on stream fish populations under field-use in biotech-soybean production

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Abstract

The impact of biotech-soybean technology on freshwater ecosystems is being evaluated in the Rolling Pampas region, Argentina. The effect of cypermethrin, the main soybean insecticide, on low-order temperate-stream fish populations was investigated for two consecutive crop cycles under field-use conditions in biotech-soybean production. Cypermethrin was unable to induce mortality or behavioral effects on any of the fish species resident in a first-order stream across a crop field (pulsed acute exposure scenario) sprayed according to conventional practices. No spatially or temporally dependent effects were observed on population parameters (size-class structure, abundance, survival, sex and immature/mature ratio, condition factor) of resident or caged *Cnesterodon decemmaculatus* after spraying or rainfall events, not even one year after, at the beginning of the next crop cycle. Although cypermethrin was ''very highly toxic'' to *C. decemmaculatus* in laboratory water (96 h-$LC_{50} = 0.43$ μg/l), its toxicity was reduced in filtered (78%) and unfiltered (92%) stream water. Changes in LC_{50} values were mainly correlated with the OC content of each water fraction ($r^2 = 0.99$; $p < 0.01$; $n = 9$), showing that both DOC and TOC contributed proportionally to toxicity reduction. Protective effects of stream water (12-fold reduction LC_{50} values) explained the lack of effects on fish populations in the field, despite cypermethrin water concentrations after spraying reached values comparable with the 96 h-LC_{50}. Therefore, cypermethrin under field-use conditions in transgenic-soybean production represents a low risk of acute exposure for fish populations inhabiting low-order temperate-streams rich in TOC. The relationship between LC_{50} and TOC could be a convenient way to improve risk estimation based on laboratory toxicity testing.

Keywords: Cypermethrin; Transgenic-soybean crops; Temperate-plains streams; *Cnesterodon decemmaculatus*; Field-laboratory comparison; Organic carbon

1. Introduction

Biotech crops have been sustainedly increasing around the world since 1996, and in recent years, the main increase took place in developing countries. Transgenic-soybean is the principal biotech crop of the world, occupying 54.4 million hectares. Argentina represents 20% of the global crop surface, and together with Brazil, Paraguay, and Uruguay, reaches up to 26 million of hectares of biotech-soybean (James, 2005). The Pampa is the main Argentine agricultural district. Since the mid-1990s the glyphosate-resistant soybean became available and ''no-tillage managerial practices'' were adopted by the farmers, replacing the traditional production system. Soybean crops increased from less than 40 000 ha in the 1970s to 8 300 000 ha at the end of the century (Pengue, 2000), and at present reach up to 15 000 000 ha (James, 2005). This led to the increase of pesticide consumption from 6 to 18 million kg between 1992 and 1997 (Pengue, 2000), and it has continued to rise at slower rates since then. The main soybean insecticide is

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the pyrethroid cypermethrin (followed by chlorpyrifos and endosulfan), representing more than half of the total insecticide consumption.

Although there are numerous reports related to the single-species laboratory toxicity of insecticides, there is a need for effect studies conducted in natural surface waters affected by normal farming practices (Schulz, 2004). Most studies with pyrethroids were conducted mainly in North America and Europe, and little is known about the possible effects on the indigenous biota from other regions of the world. A few publications on the impact of insecticide application on the resident invertebrate communities of local Pampasic streams have recently become available (Jergentz et al., 2004). However, the effect on the fish communities remains unreported. Pyrethroids are known to be extremely toxic to fish under standardized laboratory conditions (Haya, 1989). In the environment, the actual effect of pesticide on resident biota might, however, be rather different due to pyrethroid interaction with suspended matter, bottom sediments, and aquatic plants (Hill, 1989). Effects induced by pyrethroids also depend on field-use conditions. However, recently it has been recognized that most risk assessments are based on data from experiments in freshwater model ecosystems, since descriptive hydrobiological field research is scarce. Additionally, acute effects were considered of special relevance in the assessment of effects induced by pyrethroids due to their relatively low environmental persistence (Van Wijngaarden et al., 2005).

The aim of the present study was to assess the impact of cypermethrin on fish populations exposed under field-use conditions in biotech-soybean production in a representative low-order stream (acute pulsed scenario) of the Rolling Pampas region (Argentina), and to compare the effects observed under field conditions with those expected from laboratory studies, identifying factors that could help to link both levels of analysis.

2. Materials and methods

2.1. Field studies

(a) *Studied streams*: The impact of cypermethrin under field-use conditions in soybean production was assessed during two consecutive crop cycles (September to April) in a representative first-order (mean and maximum flow rates 7.93×10^{-3} and $0.23\ m^3/s$, respectively) tributary of the Arrecifes River, "Stream 1". Its source is in a private farm and crosses a core soybean production region northwest of the Buenos Aires Province (Argentina) within the ecoregion of the Rolling Pampas (Fig. 1). The selected farm followed conventional agricultural practices depending on the technology associated with transgenic-soybean crop in other regions of the world, but was characterized by the direct seeding technique (no-tillage). The studies were carried out along the first 2 km from the stream source. The first kilometer drain a 119-ha plot with a mean slope of 1.5% which was sown with soybeans during the two consecutive years of study. The second kilometer the stream flows though an adjacent pasture free of pesticide spraying. Both rooted and submerged aquatic vegetation were present in small ponds formed along the course. Riparian strips within the soybean plot were narrow (1–2 m) or inexistent. Two sites of similar characteristics were selected along the stream for *in situ* caging experiments and systematic monitoring of the natural population. Site 1 (S1) was a pond placed within the soybean plot, 50 m before the downstream boundary, and site 2 (S2) was a pond located within the pasture plot, 500 m downstream from S1. The S1 was selected as a site of maximum impact of cypermethrin spraying. Considering the small stream discharge, the abundant macrophyte growth and the larger distance donwstream the pesticide source, it was hypothesized that site 2 could eventually show an attenuated toxicity impact on the fish population.

Additionally, water chemistry from seven other streams (see Fig. 1) was studied in order to understand potential impacts of cypermethrin on other watercourses of the region. They were selected within a homogenous area characterized by the presence of typical argiudol soils (Brunizem with B textural) developed over deep loessic deposits.

(b) *Pesticide spraying and rain events*: The soybean crop was sprayed with cypermethrin (Sherpa® Formulation) and glyphosate (Roundup® Formulation) following the usual spray calendar and practices. Pesticides were sprayed by tractor-mounted blowers at a rate of 25 and 1500 g active ingredient/ha of cypermethrin and glyphosate, respectively. Two analogous spraying and the respective first rainfall events were monitored in two consecutive harvests. The first spraying event took place on 27-11-02 and the first rainfall (57 mm), occurred 19 days after the spraying. The second spraying event was on 07-01-04 and the first rainfall (5 mm) 16 days later.

(c) *Survey of local fish population*: The fish community in "Stream 1" was characterized by sampling a sector (100 m) downstream S2 with a 10 m long seine net (15 mm stretched mesh and 5 mm stretched bag mesh) and an upstream blocknet. Resident populations of *Cnesterodon decemmaculatus* at S1 and S2 were sampled 96 h before and 96 h after a spraying event and at the beginning of the next crop cycle, using a 0.6 m diameter net, 0.5 mesh size. The same unit effort was used at each sampling point, and minimum unit effort was established as three nets per sample. One sample from the beginning, middle, and end of each pond (six nets) were collected. The total length (TL), weight (W), maturity and sex (I: immature, M: male, F: female) of each fish was recorded and the M/F and I/Total ratios were calculated from fish caught at each unit effort. The condition factor (K) of male fish measuring between 14 and 20 mm was calculated as $K = [W/(\mathrm{TL})^3] * 100000$ (APHA, 1998). In addition, drift nets (5 mm mesh size) were placed across the stream up and downstream from S1, and downstream from S2. Drift net contents and fish mortalities and behavioral alterations along the studied sector of the stream were checked every 12 h from

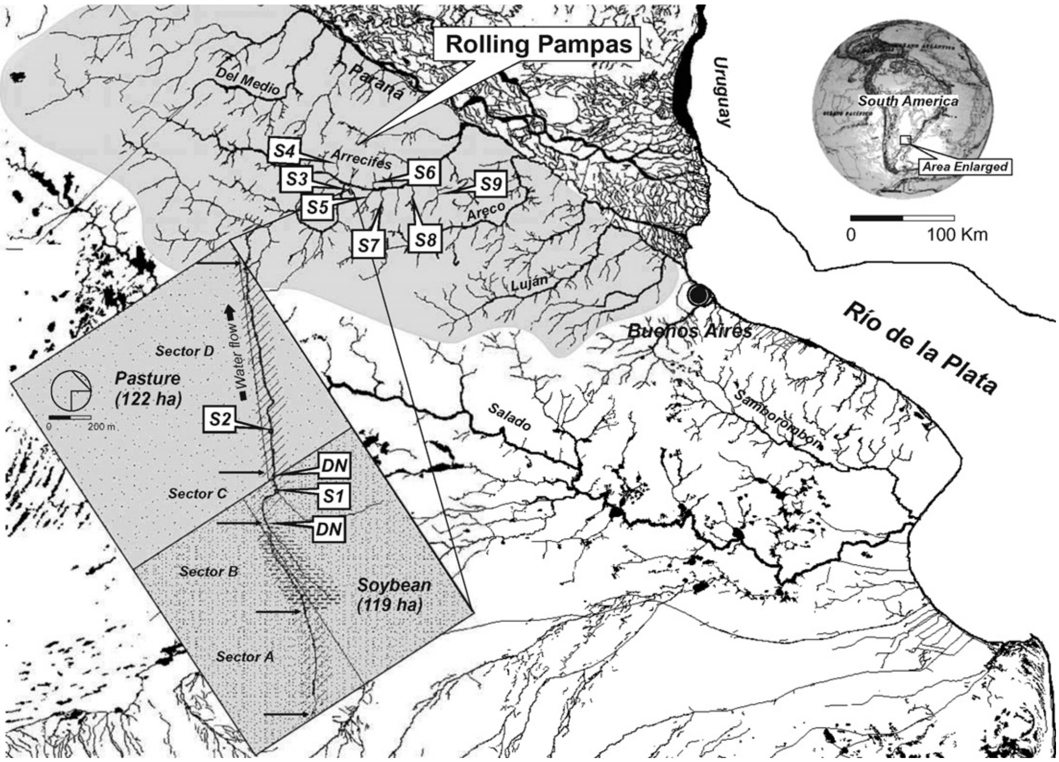

Fig. 1. Map of the Rolling Pampas (shadow) with the sampling sites at representative streams: "Stream 2" (ST), Maguire (MG), Contador (CN), Helves (HL), Horqueta (HR), Luna (LU), Marmol (MR). Area enlarged shows sampling points along "Stream 1" (S1: site 1; S2: site 2; DN: drift nets).

96 h before to 96 h after each spraying event, and during the 96 h following the first rainfall event. The fish survey was repeated during two consecutive years.

(d) *In situ caging experiment*: Nine cages containing 50 juveniles of *C. decemmaculatus* ($SL = 7.9 \pm 0.2$ mm) transferred from the laboratory were placed at each site (S1 and S2) 96 h before spraying events, and the number of surviving fish was counted just before spraying, 96 h after the spraying, just before and 96 h after the first rainfall event. Fish were transported from laboratory to the field in 20-l well aerated tanks. In the field, laboratory water was gradually replaced by stream water and fish were held in the tanks 24 h prior to transfer to cages. Cages were made of 50×15 cm PVC tubes perforated with eight 11×20 cm windows, completely covered with a 0.9 mm nylon mesh, and the bottom reinforced with a galvanized wire mesh. Cages were placed with the bottom directly in contact with the sediment and the top $\approx$10 cm above water surface.

2.2. Laboratory studies

Acute lethal toxicity of the insecticide cypermethrin for *C. decemmaculatus* was simultaneously evaluated in laboratory water (LW) and in whole (WSW) and filtered (FSW) water samples from the studied stream. In addition, toxicity of the herbicide glyphosate was also evaluated in LW. In-house cultured 15-day old juveniles (TL: 8.9 ± 0.2 mm) were used as test organisms. Acute lethal toxicity was assessed using a standardized static-renewal toxicity test (USEPA, 2002). Test solutions were completely replaced every 24 h. Tests were conducted by triplicate using five and ten fish per replicate in preliminary and definitive tests, respectively. Five or more differing concentrations of each chemical, plus a control group were tested. A solvent control was included in cypermethrin toxicity tests containing 0.1% ethanol and the same ethanol concentration was used for all tested cypermethrin concentrations. Mortality was recorded at 24, 48, 72, and 96 h. Fish were considered dead when no respiratory movements were observed and immediately removed from test chambers. Laboratory water was La Plata city tap water dechlorinated and filtered through activated carbon. The WSW was sampled just before the sowing of soybean and transported to the laboratory in wet ice using 50 l polypropylene containers, and kept at 4 °C overnight. The FSW was obtained by filtering the sampled water through Whatman GF/C filters. Cypermethrin (91% purity technical grade from Gleba Chemical Company) 1000 mg/l stock solutions were prepared in ethanol before testing and kept in the dark at 4 °C. Glyphosate (95% technical grade from Gleba Chemical Company) stock solutions were prepared at a concentration of 10 000 mg/l in deionized water and kept in the dark at 4 °C. Test solutions of both pesticides were prepared daily. Temperature was held at 22 ± 1 °C and photoperiod set at 16 h light and 8 h darkness. Neither aeration nor food was supplied during testing. Dissolved oxygen, pH, conductivity, and temperature were controlled before and after solution replacement. Organic mater content was under the detection limit in LW. In WSW the organic mater content was 15.2 mg TOC/l, 10.0 mg COD/l, and 5.2 mg POC/l. Suspended matter (SS) in WSW was 40.0 mg/l.

2.3. The fish species

C. decemmaculatus (Jenyns, 1842) is a Poecilid fish widely distributed in a large variety of water-bodies within the ''del Plata'' basin of South America. This small ovoviviparous, benthic-pelagic and non-migratory fish (maximum size, ≈25 and 45 mm male and female, respectively) is often the most abundant and sometimes the only species present in small watercourses. In addition, it is also easily reared under laboratory conditions.

2.4. Chemical analysis

Cypermethrin concentration data in water and sediments from ''Stream 1'' during the studied period were reported in separate papers (Jergentz et al., 2005; Marino and Ronco, 2005). Cypermethrin concentration in work solutions used in laboratory acute toxicity tests were analyzed by GC-ECD, using a certified reference standard provided by the Food Health and Quality National Service of Argentina (SENASA). Absolute ethanol used in dilutions was pesticide grade. Chemical analysis of water was performed on whole and filtered samples through Whatman GF/C. Suspended matter was designated as the weight difference after filtration through the Whatman GF/C filters. The filters were heated to 550 °C for two hours prior to use. Particulate organic C was determined, as described by Golterman et al. (1978), by digestion of the filters used for suspended matter determination. Total organic C was measured in unfiltered water samples fixed with sulfuric acid according to APHA-AWWA-WEF (1998).

2.5. Data analysis and statistics

Differences between assessed population parameters in S1 and S2, before and after spraying and after the first rain, were evaluated using two-way analysis of variance (ANOVA) followed by the *post hoc* multiple-comparison Tukey Honest Test. Abundance and mean size of each size-class was obtained by polymodal decomposition. Statistical endpoints from toxicity tests were estimated by means of the Probit method using the EPA Probit Analysis Program Version 1.5 (USEPA, 1999). Toxicity of pesticides was ranked according to Kamrin (1997). The time-dependent acute toxicity of cypermethrin was assessed using the equation derived from the critical target occupation model (Legierse et al., 1999).

3. Results

3.1. Sensitivity of C. decemmaculatus to soybean pesticides

Toxicity tests with LW showed that glyphosate was ''not acutely toxic'' for *C. decemmaculatus* (96 h-$LC_{50} > 100$ mg/l). Cypermethrin LC_{10}, LC_{50}, and LC_{90} values at 24, 48, 72, and 96 h, and the incipiency (Newman and Unger, 2003) values obtained in LW are shown in Table 1. The pyrethroid was ''very highly toxic'' for *C. decemmaculatus*. Toxicity significantly changed ($p < 0.05$) with time during the first 72 h but no differences were observed from that time to the incipiency value.

Table 1
Toxicity of cypermethrin to *C. decemmaculatus* in laboratory water (LW), filtered (FSW) and whole (WSW) stream water

	LW		FSW		WSW	
	Value	95% Confidence limits	Value	95% Confidence limits	Value	95% Confidence limits
24 h						
LC_{10}	0.60	0.47–0.65	5.46	4.34–6.04	7.28	5.57–8.00
LC_{50}	0.81	0.72–1.59	6.66	6.01–7.26	8.97	8.23–9.97
LC_{90}	1.10	0.87–4.97	8.12	7.42–9.79	11.05	9.95–15.18
48 h						
LC_{10}	0.35	0.28–0.39	2.57	1.72–3.01	5.14	3.84–5.72
LC_{50}	0.55	0.51–0.61	3.48	2.95–4.02	6.27	5.59–6.84
LC_{90}	0.88	0.76–1.15	4.71	4.07–6.67	7.66	6.99–9.55
72 h						
LC_{10}	0.30	0.22–0.35	1.34	0.60–1.86	4.14	3.05–4.75
LC_{50}	0.47	0.42–0.52	2.55	1.82–3.26	5.54	4.86–6.21
LC_{90}	0.74	0.65–0.92	4.85	3.72–8.49	7.42	6.56–9.59
96 h						
LC_{10}	0.27	0.19–0.32	0.98	0.46–1.39	3.94	2.43–4.66
LC_{50}	0.43	0.37–0.48	2.01	1.42–2.64	5.17	4.20–5.85
LC_{90}	0.69	0.61–0.85	4.12	3.07–7.21	6.77	5.96–8.93
Incipiency	0.42	0.35–0.48	1.87	1.82–1.93	5.10	3.59–6.62

Table 2
Main physicochemical parameters from representative watercourses of the Rolling Pampas

	Stream 1	Stream 2	Maguire	Helves	Luna	Horqueta	Contador	Marmol
Stream order	1	1	2	2	2	2	3	2
Temperature	26 (5.0)	19 (9.8)	22 (4.0)	23 (3.4)	22 (3.2)	22 (3.5)	20 (6.7)	18 (5.0)
(°C)	[20–30]	[11–33]	[19–25]	[18–27]	[18–26]	[10–25]	[14–28]	[15–24]
OD	11.2 (2.6)	12.0 (2.5)	7.6 (2.2)	9.3 (3.0)	7.5 (3.8)	7.4 (2.7)	12.0 (2.0)	10.1 (2.5)
(mg/l)	[5.6–15.2]	[8.6–15.0]	[5.0–11.5]	[3.0–11.1]	[0.4–13.0]	[4.1–12.3]	[11.4–15.1]	[7.7–12.0]
Conductivity	461 (58)	695 (80)	943 (182)	861 (70)	905 (226)	870 (122)	871 (86)	798 (60)
(μS/cm)	[325–550]	[620–790]	[680–1159]	[780–905]	[440–1053]	[750–991]	[770–980]	[740–880]
pH	8.2 (0.4)	7.8 (0.3)	8.1 (0.34)	8.2 (0.3)	8.1 (0.7)	8.2 (0.45)	8.3 (0.5)	8.0 (0.3)
	[7.3–8.7]	[7.5–8.1]	[7.8–8.6]	[7.7–8.4]	[6.7–8.8]	[7.7–8.8]	[7.9–9. 0]	[7.7–8.3]
Suspended matter	29.4 (24.0)	20.2 (5.4)	15.4 (10.7)	5.2 (1.0)	14.7 (4.9)	27.8 (15.9)	13.9 (0.1)	25.0 (8.2)
(mg/l)	[2–104]	[16–24]	[6–30]	[4–6]	[8–19]	[9–38]	[13–14]	[19–31]
TOC	8.2 (3.5)	2.0 (0.5)	4.7 (6.2)	1.95 (1.1)	2.7 (1.9)	8.9 (7.9)	2.0 (1.4)	5.0 (1.4)
(mg C/l)	[4–18]	[1–3]	[1–14]	[<1–6]	[1–3]	[4–18]	[1–3]	[4–6]
DOC	6.9 (3.6)	1.4 (0.25)	4.3 (5.9)	1.8 (0.9)	2.4 (1.9)	7.7 (7.8)	1.6 (0.7)	4.4 (0.9)
(mgC/l)	[3–16]	[1–2]	[1–13]	[<1–3]	[1–5]	[3–17]	[1–3]	[4–5]
POC	1.6 (0.6)	0.6 (0.2)	0.4 (0.3)	0.1 (0.1)	0.3 (0.1)	1.2 (0.8)	0.4 (0.1)	0.5 (0.1)
(mgC/l)	[0.7–3.0]	[0.4–0.8]	[<0.1–0.8]	[<0.1–0.2]	[0.2–0.4]	[0.3–1.9]	[0.3–0.4]	[0.4–0.6]

Data is given as mean values with standard error between parenthesis and ranges between brackets.

3.2. Characterization of regional surface water chemistry

Measured physicochemical parameters of water samples from "Stream 1" and seven other representative streams of the region are given in Table 2.

3.3. Effects of cypermethrin on fish populations under field-use conditions in soybean production

The fish community at "Stream 1" was found to be composed at least by five fish species (*Cheirodon interruptus*, *C. decemmaculatus*, *Hoplias malabaricus*, *Rhambdia quelen*, *Synbranchus marmoratus*). During the two consecutive years of study, no fish mortalities or individuals presenting abnormal behavior (loss of equilibrium, synchronic swimming, startle response, lethargy, coloration pattern) were recorded along the stream, and no dead fish were trapped in the drift nets during either the 96 h after cypermethrin spraying or 96 h after the first rainfall event, not even in sectors where the arms of the spraying machine passed directly above the water surface.

C. decemmaculatus represented the most conspicuous and abundant species of the fish community in "Stream 1". The total number of fish captured per effort unit (by triplicate) and the size-class structures (mean length ±SE and abundance) of *C. decemmaculatus* populations observed in S1 and S2 before and after the spraying event and at the beginning of the next crop cycle are shown in Table 3. Although some selectivity of the fishing nets was noted, three well defined size-classes were distinguished in both studied sites. No differences were found in the mean total length of corresponding size-classes between and within sites at any sampled time. Abundance of classes I and II was always significantly higher in S2, and so was the total number of fish (two-way ANOVA, $p < 0.05$). Although an apparent increase and decrease of the abundance of class I was noted after spraying in S1 and S2, respectively, no significant effect of the cypermethrin or pesticide-site combined effect was found on this parameter. No differences were observed in sex ratio (M/F), immature ratio (I/T) or condition index (K) between or within sites before and after spraying, or as a consequence of their combined effect (two-way ANOVA, $p < 0.05$) (Table 3). The survey conducted during the second year showed a very similar pattern, with no significant variations of *C. decemmaculatus* population structure between or within sites, before and after cypermethrin spraying.

The mean number of caged fish showed no statistically significant differences ($p < 0.05$) between sites (S1 and S2) either 96 h after spraying or 96 h after rainfall (Fig. 2a). Associated cypermethrin concentrations in water and sediments are shown in Fig. 2b. Mean survival for caged fish in S1 and S2 were 81.2% and 73.4% after spraying and 32.0% and 26.4% after rainfall, respectively. Mortality rates and SD, observed 96 h before spraying and 96 h after spraying in the 9 cages placed at each site, were 1.20 ± 0.47 and 1.17 ± 0.52 fish per cage per day in S1 and 1.49 ± 0.62 and 1.48 ± 0.55 fish per cage per day in S2. The same parameters but 96 h before rainfall and 96 h after rainfall were 1.39 ± 0.49 and 1.42 ± 0.54 fish per cage per day in S1, and 1.55 ± 0.88 and 1.53 ± 0.96 fish per cage per day in S2. No significant differences ($p < 0.05$) in the mortality rate of caged fish were induced by the cypermethrin either after the spraying or rainfall event, showing no correlation ($r^2 = 0.09$; $p < 0.05$) with the cypermethrin sediment concentration.

The physicochemical parameters registered in "Stream 1" during field studies are shown in Table 2. TOC values fluctuated from 4.4 mg C/l at the start of the second crop cycle sampling period to 14.6 mg C/l just after the spraying event, and then decaying to 7.0 at the end of the study (Fig. 2c). The observed increase of the TOC concentration along the stream after spraying was a consequence of the augmentation of DOC levels, from 6 to 13 mg/l, while

Table 3
Population parameters of wild *C.decemmaculatus* at sites S1 and S2 before and after cypermethrin spraying during first crop cycle

	S1	S2
	Mean ± SE	Mean ± SE
N Total		
BS	64.0 ± 5.9[a]	235.0 ± 8.8[b]
AS	102.0 ± 9.5[a]	235.0 ± 29.8[b]
YA	94.3 ± 5.7[a]	209.7 ± 4.1[b]
N Class 1		
BS	10.2 ± 8.4[a]	112 ± 13.1[b]
AS	46.5 ± 15.4[a]	79.4 ± 18.4[b]
YA	46.5 ± 15.4[a]	112.5 ± 4.9[b]
N Class 2		
BS	49.1 ± 8.7[a]	118 ± 17.8[b]
AS	53.6 ± 18.0[a]	142 ± 12.1[b]
YA	36.1 ± 5.4[a]	81.7 ± 17.1[b]
N Class 3		
BS	4.4 ± 2.8	6.1 ± 4.4
AS	2.1 ± 1.8	14.1 ± 7.6
YA	12.1 ± 3.9	17.7 ± 1.9
LT Class 1		
BS	8.6 ± 0.2	10.4 ± 0.1
AS	9.2 ± 0.2	9.9 ± 0.2
YA	9.3 ± 1.4	10.1 ± 1.4
LT Class 2		
BS	17.7 ± 0.3	16.2 ± 0.2
AS	17.7 ± 0.3	16.9 ± 0.2
YA	16.7 ± 2.0	15.5 ± 1.6
LT Class 3		
BS	26.1 ± 0.6	27.8 ± 1.6
AS	27.8 ± 0.4	23.8 ± 0.4
YA	22.8 ± 1.7	22.5 ± 2.4
M/F		
BS	1.4 ± 0.1	1.61 ± 0.3
AS	1.1 ± 0.1	1.26 ± 0.5
YA	1.4 ± 0.3	1.09 ± 0.1
I/T		
BS	0.1 ± 0.1	0.57 ± 0.0
AS	1.3 ± 0.5	0.48 ± 0.1
YA	0.5 ± 0.1	0.60 ± 0.1
K		
BS	1.9 ± 0.1	1.65 ± 0.1
AS	1.8 ± 0.0	1.87 ± 0.1
YA	1.9 ± 0.0	1.85 ± 0.0

M/F: male/female ratio; *I/T*: inmature/total ratio; *K*: condition index; BS: before spraying; AS: after spraying; YA: year after; Different letters mean statistically significant differences ($p < 0.05$) between sites.

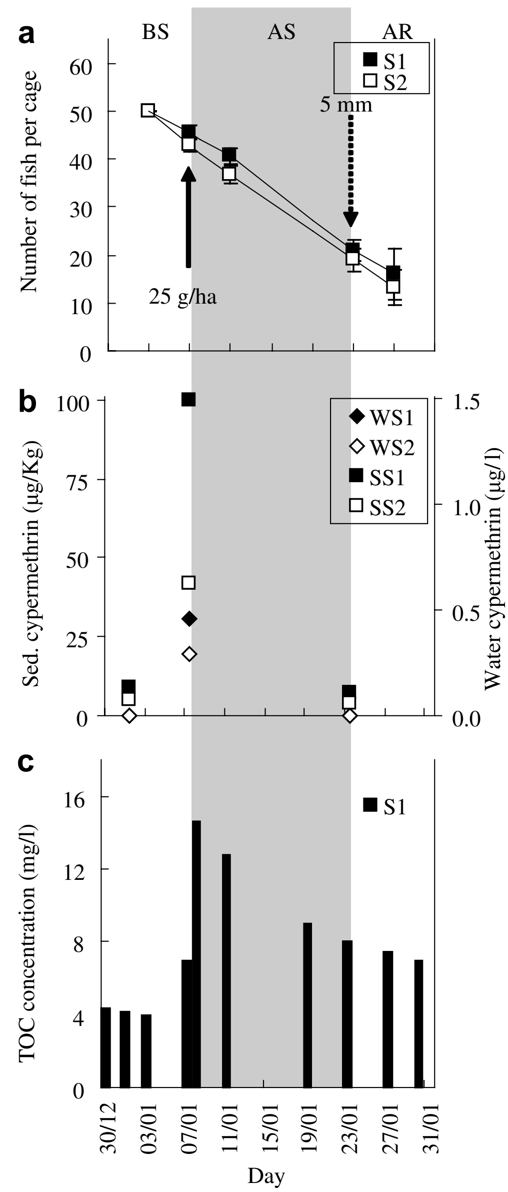

Fig. 2. Field experiment with caged *C. decemmaculatus* during second crop cycle. (a) Mean number of caged fish. (b) Cypermethrin concentrations in sediments and water. (c) Total organic carbon (TOC) in stream water. BS: before spraying; AS: after spraying; AR: after rain (in white, gray, and white background, respectively); S1: site 1; S2: site 2; SS1 and SS2, and WS1 and WS2: cypermethrin concentration in sediments and water at S1 and S2, respectively; solid arrow: spraying time and ratio; dotted arrow: rainfall event.

POC remained almost constant (1–2 mg/l) during the whole period.

3.4. Toxicity of cypermethrin in natural waters

The cypermethrin LC_{50} values for fish exposed for 24 h in FSW and WSW (Table 1) were 8.2- and 11.1-fold greater than for those exposed in LW, respectively. After 96 h exposure, LC_{50} values were 4.7 and 12.0-fold greater. The time-dependent acute toxicity was similar in the three different tested waters, and no differences were observed between 96 h-LC50 and the incipiency value, indicating no expected further mortalities. There was a marked difference in the LC_{10} and LC_{90} range values for WSW and LW, being this interval 7 to 10 times wider for WSW (Table 1).

When the relative contribution of dissolved or particulate fractions for reducing the toxicity of cypermethrin (expressed as toxic units) was compared, greater effect was attributed to the dissolved fraction. Fig. 3 shows the contribution of dissolved and particulate fractions to cypermethrin toxicity reduction. Maximum cypermethrin

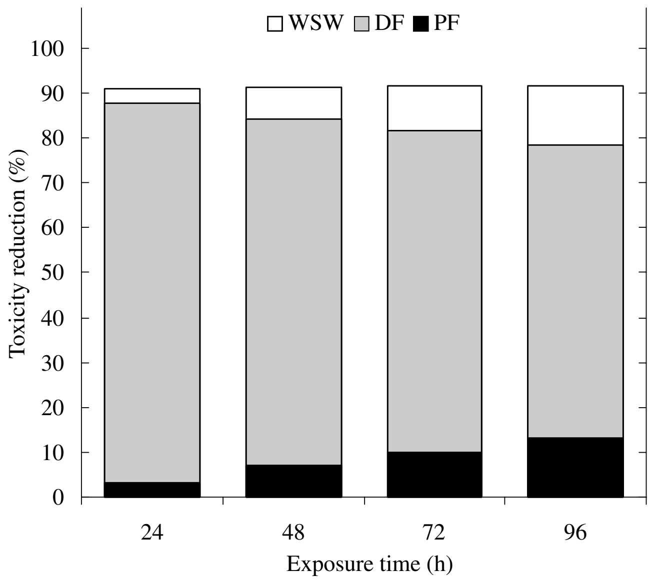

Fig. 3. Percentage of toxicity reduction of cypermethrin to *C. decemmaculatus* in stream water (WSW) with respect to laboratory water at different exposure times, and relative contribution of the dissolved (DF) and particulate (PF) fractions.

toxicity reduction caused by the dissolved fraction was 87.4% at 24 h exposure, then diminishing during the course of the experiment to 78.6% after 96 h exposure. Conversely, the minimum mitigating effect of particulate fraction was seen at 24 h, then increasing with time from 3.2% to 13.1%. However, reduction of cypermethrin toxicity caused by whole stream water remained almost constant during experiment, varying between 91.0% to 91.8%. A good correspondence was obtained between cypermethrin LC_{50} values and the OC content in LW, FSW, and WSW. The correlation was higher at 24 h ($r^2 = 0.99$, $p < 0.05$, $n = 9$) than at 96 h ($r^2 = 0.86$, $p < 0.05$, $n = 9$) of exposure as a consequence of a relative reduction of the dissolved fraction protective action observed at the end of the experiment.

4. Discussion

In the present study, the toxicity of the soybean pesticides glyphosate and cypermethrin was reported for a widely distributed South American fish. The herbicide glyphosate was "not acutely toxic" to *C. decemmaculatus*, presenting an 96 h-LC_{50} comparable with that reported for example for *Carassius auratus* (Antón et al., 1994). Conversely, cypermethrin was "highly toxic" to *C. decemmaculatus* exposed under laboratory conditions using organic-matter-free laboratory water. This result was consistent with previous studies which demonstrated that fish are the most sensitive group of vertebrates to this pesticide (Edwards et al., 1986), generally presenting 96 h-LC_{50} values lower than 10 μg/l (Coats et al., 1989). Moreover, the 96 h-LC_{50} found for *C. decemmaculatus* was under the 10th percentile value reported for all of the vertebrates (Solomon et al., 2001). Particularly, the juveniles of the studied species showed a sensitivity equivalent to that reported for *Oncorhynchus mykiss* ($LC50_{96h}$ 0.5 μg/l) and *Scardinius erythropthalmus* (96 h-LC_{50} 0.4 μg/l), and higher than that reported for *Cyprinus carpio* (96 h-LC_{50} 0.9–1.1 μg/l) and *Tilapia nilotica* (96 h-LC_{50} 2.2 μg/l) (Stephenson, 1982).

Field observations in a typical private farm of the Pampas region showed that conventional practices involve a ground spraying of a mix of cypermethrin and glyphosate (after a month of a first pre-emergent treatment with glyphosate), using track-mounted blowers at a rate of 25 and 1000 g/ha, respectively. Commonly, no attenuation strips are kept near watercourses. During the experiments of the fist year it was not observed detectable cypermethrin concentrations in water samples from S1 and S2, neither after spraying nor after rain. Although, cypermethrin concentrations in sediment samples from S1 and S2 were, respectively, 36.7 and 53.5 μg/Kg after spraying and 1075 and 595 μg/Kg after a rainfall event of 57 mm occurring 19 days after spraying. Concentrations observed during the second year indicated detectable levels in water only after spraying at both sites, S1 (0.46 μg/l) and S2 (0.29 μg/l). Levels in sediments from S1 and S2 were, respectively, 100 and 42.1 μg/Kg after spraying and 7.5 and 4.0 μg/Kg after a rainfall event of 5 mm occurring 16 days after the application (Marino and Ronco, 2005). This study showed that the pesticide reached the stream by both spray drift after applications and runoff after rainfalls. In the first year predominated the runoff and in the second mainly the drift. The pesticide concentrations in sediments from S1 were approximately twice those in S2 suggesting fast downstream decrease. Cypermethrin concentration in "Stream 1" showed a pulsed behavior responding to spraying and rainfall events, quickly decreasing to undetectable levels in less than a week during inter-application periods.

Although cypermethrin demonstrated to be very highly toxic to *C. decemmaculatus* and during the second crop cycle the pesticide reached water concentrations in streams comparable to the estimated 96 h LC_{50}, no mortality or behavioral changes attributable to the insecticide were detected in either *C. decemmaculatus* or in the other resident fish species along "Stream 1," not even in sectors where the spraying machine blower-arms passed circumstantially directly above the water surface (differently to observations from other field studies with chlorpyriphos and endosulfan; unpublished results). In addition, albeit insecticide concentrations were different in the study sites, no relative changes in the population parameters between or within sites, before and after spraying or rainfall events were observed at any sampling time throughout the two successive crop cycles. Although mortality was observed in caged fish in both sites, it could not be correlated to cypermethrin concentrations, and should therefore be attributed to other sources of mortality. Predation by arthropod larvae and presence of fungus in the gill and body flanks of fish were commonly observed in relation to dying fish inside the cages.

The absence of measurable effects induced by cypermethrin on "Stream 1" fish populations exposed under field-use conditions in biotech-soybean production was consistent with the response of population parameters for different fish species observed in mesocosm studies using up to 1 μg/l of cypermethrin (Crossland et al., 1982; Getty et al., 1983; Palmeiri et al., 1992). Toxicity reduction found here in laboratory experiments with stream water helps to interpret the lack of effects evidenced during field studies. This is in agreement with a recognized fact that exposure of aquatic organisms under field conditions is reduced through the tendency of pyrethroids to bind to suspended particulate matter, sediments, and aquatic plants (Haya, 1989; Hill, 1989). In the present study, the magnitude of the toxicity reduction induced by particulate and dissolved components in natural waters was quantified in a single laboratory experiment. Results also showed good correlation between LC_{50} and TOC content in the tested water. Reduction of cypermethrin bioavailability and toxicity by OC to fish has been scarcely reported and was found controversial. For example, trout exposed to 0.1 μg/l of the pesticide in synthetic water, 239-lake filtered water (6.7 mg DOC/l), and filtered (6.7 mg DOC/l) and unfiltered (6.7 mg DOC/l plus 6 mg POC/l) Aldrich Humic acid (AHA) solution, showed significant reduction of the uptake rate and BCF of cypermethrin in unfiltered AHA solution, but little influence of the natural and AHA DOC on the overall bioaccumulation (Muir et al., 1994). The discrepancy among results obtained by Muir et al. (1994) and the present study could be due to differences in the chemical nature of DOC, to lower DOC concentration used by Muir et al. (1994), or methodological problems discussed by these authors. Conversely, a clear contribution of both, POC and DOC, in the reduction of the cypermethrin toxicity in natural waters was evidenced in the present study. Surface waters in the Pampas are moderately rich in suspended solids with medium and high contents of organic matter (see Table 2). Particularly, "Stream 1" exhibited the highest levels when compared with other streams in the area. DOC values were close to the mean values reported for warm temperate-streams (Spitzi and Leenheer, 1991) and the DOC/POC ratio within the expected levels, according to suspended matter content (Ittekkot and Laane, 1991).

When the LC50 values obtained for *C. decemmaculatus* exposed in laboratory water (Table 1) are related to cypermethrin field water concentrations for the highest impact site (S1), following a conventional approach in first tier for risk assessment by means of the toxicity exposure ratio (TER), values are 0.57 and 1.07 (no safety factor was considered) for 24 h and 96 h, respectively. These indicate that effects should have been observed in the field, although this was not the case for the studied scenario. Now, if the TER ratios are calculated using the 24 h and 96 h LC_{50} values obtained in WSW, values drop to 0.051 and 0.089, in better agreement with the lack of effects observed on fish populations in the studied stream. Considering that the main factor explaining the reduction of cypermethrin toxicity in WSW was the TOC, it would be useful to include the TOC for the adjustment of LC50 values, in a similar way as DOC is being used to adjust toxic effects of some metals (Welsh et al., 1996).

5. Conclusions

The South American fish *C. decemmaculatus* was very highly sensitive to cypermethrin. However, this pyrethroid was unable to induce measurable adverse effects on resident and transferred fish populations exposed under field-use conditions in transgenic-soybean production in the pampas. The lack of effects was in correspondence with the buffering capacity of natural waters to reduce up to one order of magnitude cypermethrin toxicity to fish. Protective capacity was mainly associated with the organic mater content in the dissolved and particulate fractions. Therefore, the use of cypermethrin in relation to this practice represents low risk of acute exposure for fish populations inhabiting low-order temperate-streams moderately rich in suspended matter with high organic matter content. Organic matter should be included to adjust the endpoint values for a better prediction of cypermethrin effects, and probably other pyrethroids, in LC_{50}-based risk assessment.

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