In: Ecosystem Ecology Research Trends
Editors: J. Chen and C. Guo, pp. 209-239
ISBN: 978-1-60456-183-8

Chapter 6

INTEGRATED APPROACH FOR THE ASSESSMENT OF BIOTECH SOYBEAN PESTICIDES IMPACT ON LOW ORDER STREAM ECOSYSTEMS OF THE PAMPASIC REGION

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Abstract

In the last decades Argentine farmers have massively adopted the genetically modified glyphosate resistant soybean together with no tillage managerial practices. The area cultured with soybeans increased to 15 million ha with consequent higher pesticide consumption. Although recent publications deal with some detailed aspects of pesticide effects on organisms, the overall impact of such vast agricultural intensification remains unreported. The present contribution review and integrate results from laboratory and field studies within the frame of a major project oriented towards an integrated assessment of soybean agrochemicals impacts on aquatic ecosystems from streams of the region. The main conclusions are: i) chemical analysis of water, sediments and soils indicate low impacts on nutrient levels; ii) all the biota components of the studied ecosystems (flora, insects, fish and amphibians) were affected in different extent after pesticide applications, representing an appreciable stress over the systems. Effects were sometimes low, without noticeable impact on resident populations, as observed in fishes, or being more drastic, populations tend to recover after the episodic toxicity pulses, as observed in the resident invertebrate fauna; iii) of the three most commonly used insecticides, endosulphan fumigation caused the most severe effects, followed by the terrestrial chlorpyriphos and cypermethrin applications; iv) regarding spatial extent of impacts, the herbicide glyphosate drift and runoff caused chlorophyll content reduction on adjacent riparian and, at least 250 meters downstream application, on aquatic macrophyte

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vegetation. Although effects were followed by later recovery, studied sites showed a rather poor ruderal composition, suggesting that reiterated applications, together with the history of land use eventually impoverished the regional community originally described for the area. Populations of non target invertebrates were the most sensitive ecosystem components, showing strongest effects, with total disappearance of ephemeroptera, odonata, amphipoda as a response to pesticide runoff, eventually at long distance from spray areas, while fish and amphibian impacts were generally circumscribed to adjacent sectors; v) *in situ* exposed experimental populations of insects, fish and amphibians showed good correspondence with observed responses of resident populations within the same watercourses; vi) finally, complementation between laboratory and field studies resulted of great significance for detecting erroneous predictions from the former and lighten observations from the latter. An integrated approach analysis seems to be a promising option towards the understanding of the overall impact of intense soybean farming on aquatic related environments. Improving managerial practices and riparian habitat restorations seems feasible goals for long term sustainable production.

Introduction

The Argentine Pampa is an area of mild climate and fertile soils developed over deep loessic sediments. It was originally covered by grasslands and for a long time the farmers employed a mixed system of livestock and crops, mainly wheat and corn. The gentle slopes and the occurrence of heavy showers in coincidence with the seeding period made erosion a matter of concern. Although no tillage managerial practices have long been advised it was not until the soybean resistant to glyphosate became available that the direct seeding technique was adopted by the farmers. Several applications of the economical and efficient glyphosate herbicide are needed in order to implement the no tillage practices. During the last few decades, the traditional production system has been replaced by intensive soybean production. The areas cultured with soybeans increased from less than 40,000 ha in the 70s to 8,300,000 ha at the end of the century (Pengue, 2000), reaching at present 15 million ha. Wheat varieties of short growing period allow two harvests per year, wheat followed by soybean. A three year rotation is now a common practice, soybean is cultured the first year, wheat followed by soybean the second, and corn in the third. Livestock was moved to marginal areas or concentrated in feedlots. The fast implementation of new technological developments transformed soybean related products (oil, flour, pellets) into the main Argentine production. Pesticide consumption increased from 6 to 18 million kg only in the period from 1992 to 1997 (Pengue, 2000), and has continued to increase at slower rates since then, being formulations of the herbicide glyphosate and the insecticide cypermethrin the most commonly used. The pyrethroid cypermethrin represents more than half of the total insecticide consumption, followed by the organophosphate chlorpyrifos and the organochlorinated endosulphan. Cypermethrin is sprayed usually twice during the soybean growing period, at a rate of 25 g/ha. Thus, approximately 500 tons of pesticide is introduced annually to the environment. Pesticides are sold freely to farmers in rural areas and applied without supervision or control. Although some recent publications deal with detailed aspects of pesticide effect on the biota, the overall impact of such vast agricultural intensification remains unreported. The present contribution review and integrates the studies performed on the effect of the typical agricultural management in commercial farms on the biota of low order streams draining intensively cultivated rural basins.

Study Sites

Four stream tributaries from the Arrecifes River basin were studied in the main soybean area of the Pampa, 150 to 190 km northwest from Buenos Aires (34º36'S, 58º30' W) throughout the 2000-2001 soybean growing period (Mugni *et al.*, 2006). They are small second- or third order streams in the ranges 2-6 m width, 0.2-0.5 m depth, and 0.1-0.5 m^3/s discharge, draining basins of 380 -500 ha (Figure 1). Two streams, Helves and Luna, have steep slopes and lack floodplains. The other two, Horqueta and Maguire streams developed a 20 to 30 m wide floodplain containing patches of grasses mixed with macrophytes. Maguire developed a natural small wetland of about 100 m long, 20 m wide and 0.2 m deep, colonized by dense stands macrophytes, mainly bulrush (*Schoenoplectus californicus*), and showing luxuriant periphyton growth. Horqueta has a dam for recreational purposes upstream of the sampling site.

Later on, a small first order stream born within a farm close to Arrecifes, 150 km northwest from Buenos Aires, was sampled at two sites, during two successive growing periods from 2002 to 2004 (see detailed insert of Figure 1). The first site was located within the plot in which the stream is born, the second about 250 m downstream, in another plot. The first studied year, the upstream plot was cultivated with soybean, and the downstream plot was maintained with cattle. The second year the upstream plot was cultured with wheat followed by soybean and the downstream plot was cultivated with soybean.

Another stream tributary from the south-eastern coastal sector of the Pampa plains was selected for comparative studies due to the recent introduction of the soybean transgenic culture in the area. The Pescado stream (see detailed insert of Figure 1) is fed by rainwater and groundwater. The stream is 2-5 m width, with a basal depth of about 20 cm, reaching about a maximum of two metres water depth in the event of heavy rain storms. The longitudinal gradient slope varies from 0.4 to 0.1%, running well-drained and developed soils in the upper and middle course (Ronco *et al.*, 2001; Camilión *et al.*, 2006). The studied stream sector runs through cultivated plots mainly with soybean, intercalated with corn or wheat, depending on the year cycle.

Pesticide concentration levels associated to this region have been published by Peruzzo *et al.* (2003), Marino and Ronco (2005), and Berkovic *et al.* (2006). Detectable levels of cypermethrin and clorpyrifos were found in downstream locations of main streams and rivers flowing into the lower Paraná basin during the growing season. Measured concentrations of both insecticides in water from the Arrecifes tributary were mostly below detection limits, occasionally reaching maximum values close to 5 and 10 µg/l, respectively. For the case of sediments, detectable levels were more frequently found, with concentrations within the range of 2 and 1075 µg/kg of cypermethrin; and between 1 and 15 µg/kg of chlorpyrifos. A different mode of cypermethrin partition was observed in Pescado stream. Insecticide application in summer coincided with a drought, resulting in low water levels in the stream. Small pools, some times lacking connection among them, with water containing high levels of suspended material were evident during the field assessments. Minimum and maximum concentrations of cypermethrin here were within the range of 0.3 and 94 µg/l in the water, and below 1 µg/kg in the sediments. The insecticide remained associated to suspended material after reaching the water body from application drifts.

The herbicide levels found in the water streams of the studied areas were often below 1 mg glyphosate/l, in Arrecifes tributary. Although, concentration ranges between 1.8 and

10.9 mg/l, and 2.8 and 10.5 mg/kg were detected during sprays in water and sediments of Pescado tributary.

The climate of the region is mild and humid, with an annual mean temperature of 16°C (mean and extreme temperatures for January until midsummer 24°C and 43ºC, and July until midwinter 9°C and –5°C, respectively, and the mean annual rainfall is 1000 mm, with a fairly uniform distribution.

A detail of the type of assessment studies in relation to pesticide inputs from crop management activities and runoff associated to following rains is given in table 1.

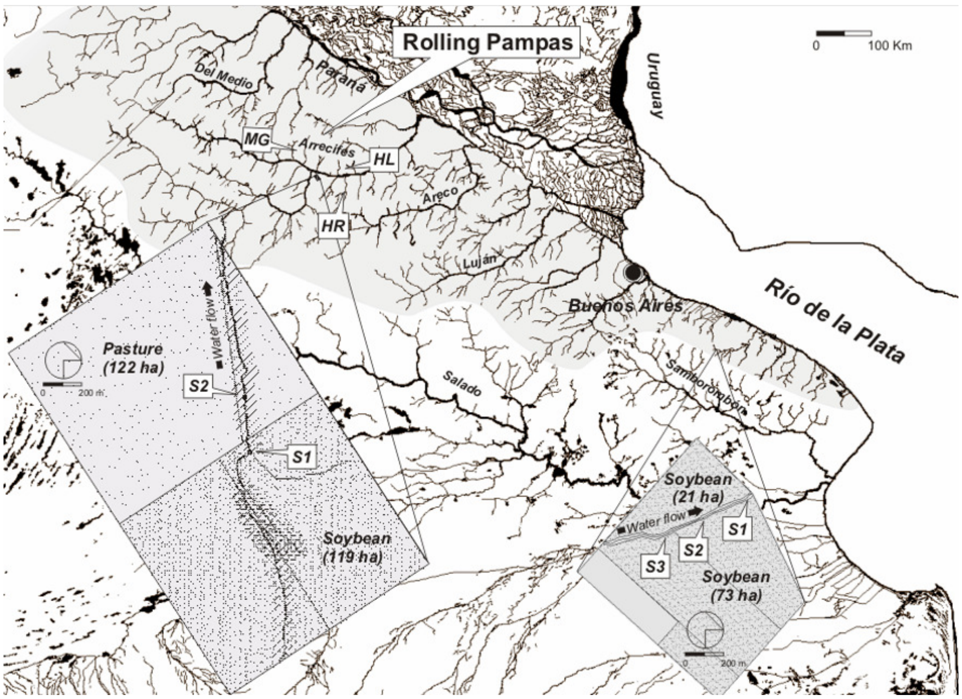

Figure 1. Study areas. MG: Maguire; HL: Helves; HR: Horqueta streams. Insert on the left: Arrecifes stream tributary study area, with sites S1 and S2 locations; insert on the right: Pescado stream tributary_study area, with sites S1, S2 and S3 locations.

Table 1. Type of assessment studies performed associated with specific pesticide ground sprays and rain events in stream tributaries. Used pesticide formulations were: Roundup®Max or Roundup®Full for glyphosate; Sherpa for cypermethrin; and Shooter or Lorsban for chlorpyrifos.

Application events in related stream	**Pesticide application**				**Days to the first rain after spray; mm rain**	**Type of assessment performed**
	glyphosate	**cypermethrin**	**chlorpyrifos**	**endosulphan**		
Arrecifes tributary						
First year						
Spring, post-emergence	1500 g/ha	100 ml/ha	-	-	17; 57	Stream and caged fish and amphibian populations. Caged invertebrate.
Summer, pre-fructification	1000 g/ha	-	1000 ml/ha	-	8; 67	Stream and caged flora, fish and amphibians. Caged invertebrate.
Second year						
Summer, post-emergence	1000 g/ha	100 ml/ha	-	-	20; 80	Stream and caged fish and amphibian populations.
Summer, post-fructification	1000 g/ha	100 ml/ha	800 ml/ha	-	9; 34	Stream and caged flora, fish and amphibian populations.
Pescado tributary						
First year						
Spring, pre-emergence	2000 ml/ha				No runoff assessment	Stream and caged fish populations
Summer, post-emergence			800 ml/ha		No runoff assessment	Stream and caged amphibian populations
Summer, post-emergence	1000 g/ha				No runoff assessment	Stream and caged flora.
Summer, post fructification (*)		150 ml/ha		700 ml/ha	No runoff assessment	Stream and caged fish and amphibian populations
Second year						
Summer, post fructification (*)		150 ml/ha		700 ml/ha	No runoff assessment	Stream fish, and stream and caged amphibian populations

(*) corresponds to an aerial application of the pesticide mixture

Nutrients

Conductivity (670-1100 µS/cm), pH (7.2-9.0) and the dominant ions, except nitrates, did not show differences among streams. Conductivity, pH and the dominant ions, including nitrates, decreased in coincidence with rain events and were lower in the runoff than in the streams (Mugni *et al.*, 2005). Soluble reactive phosphorus (SRP) and ammonium showed the opposite trend, being higher in coincidence with the largest discharges after rain events and in the runoff than in the streams. Such higher concentrations were not provided by the rain itself, since nutrient concentrations in rainwater were rather small.

Experiments performed mixing soil samples taken adjacent to each stream and deionized water with the same pH as rain water showed fast pH increase and release of SRP, calcium and bicarbonate. Soil P fractionation showed that the largest soil P pool was organic P, amounting 57-67% of the total P content, followed by iron (19-22%) and calcium bound P (14-21%). The soil which showed the largest pH increase and SRP, calcium and bicarbonate release was the one which showed the largest calcium bound P content. The concentrations of SRP released in the experiments were similar to the concentrations measured in the runoff samples. Since the calcium-bound P fraction is released in response to acidification, it was suggested that the calcium bound P of the surrounding soils contributed most of the observed SRP in the streams after rain events. Fast release of soluble organic P from soil particles seems another P source to the stream water after rain events. Mean SRP showed very little variation among the studied streams attaining an overall mean of 143 µg/l.

Table 2 shows the nutrient concentration in the first order stream at Arrecifes commercial farm during two successive growing periods. Mean SRP concentrations were significantly lower at the plot with soybean than in the downstream plot with cattle on the 2002-2003 growing period. None of them were fertilized. The following year, wheat followed by soybean was cultured at site 1 and soybean was grown at site 2 (Figure 1). Wheat was ploughed in winter and was fertilized with 100 kg/ha of di-ammonium phosphate and 135 kg/ha of urea. SRP concentration at site 1 was significantly higher the second year than the first one. Mean SRP concentration in the Arrecifes first order stream was very similar to mean SRP concentrations previously recorded in the 4 representative streams of the area.

SRP concentrations resulted comparatively high when compared with reported figures from intensively cultivated areas. Schulz & Liess (1999) reported 29 µg/l in Ohebach, (Germany) and Probst (1985) 33 µg /l in Girou (France). Meybeck (1998) compared nutrient concentrations in streams draining agricultural and forested areas in the Seine watershed (France), without observing significant differences between them, and reporting mean SRP concentrations ranging 12-14 µg/l. Similarly, Miller *et al.* (1997) did not observed significant differences between forested and agricultural steams in the Potomac basin (USA), reporting a mean of 20 µg/l. Within the Pampasic streams agricultural loads result comparatively modest, since only a minor proportion of the farmers fertilize the soybean, the culture representing more than half of the cultivated surface, while wheat and corn are fertilized with doses up to 100 kg/ha, but represent a minor proportion of the cultivated land. Breeding cattle represent a still lower proportion of the land surface. However, SRP were significantly higher at the plot with cattle. Although the plot was not fertilized, the roving behaviour of the cattle might have played a role by exploring the grass abundance within the whole plot but concentrating in the stream to drink, and therefore disturbing the riparian strip and increasing the nutrient load.

Serrano *et al*. (1999) showed that leakages from manure increased nutrient loads in a Mediterranean wetland while Nair *et al*. (1995) observed higher labile soil P concentration in grazed pastures than in adjacent forage cultures, suggesting higher leaching to nearby water bodies. Comparatively high SRP concentrations in the Pampasic streams seem more related with the geochemical nature of the rich loessic sediments than with the comparatively modest fertilizer loads.

Table 2. Mean nutrient concentrations in a first order spring along two successive growing periods.

Harvest	N	Site	Culture	Conductivity	PRS	NO_3^-	NH_4^+
				µS/cm	µg/l	µg/l	µg/l
02-03	10	1	Soybean	325-500	144 (75-208)	381 (45-1993)	36 (5-54)
		2	Cattle	430-525	241 (100-322)	1765 (759-3335)	34 (12-61)
03-04	6	1	Wheat/Soybean	190-236	456 (307-775)	142 (31- 410)	69 (25-33)
		2	Soybean	530-600	317 (136-477)	468 (112-998)	60 (33-60)

Nitrate concentration were significantly higher in the two steams lacking floodplain, Helves and Luna, attaining a mean concentration of 3.6 and 3.1 mg N/l, respectively, and lower in the streams provided with a floodplain, Maguire and Horqueta, attaining mean concentrations of 1.4 and 1.1 µg N/l, respectively (Mugni *et al*., 2005). Nitrate concentrations were higher during dry periods and decreased in coincidence with higher discharges. Nitrate concentrations were significantly lower in runoff than stream samples.

Ammonium stream concentrations were orders of magnitude lower than nitrate concentrations, and were higher in the floodplain streams Maguire and Horqueta attaining means of 30 -31 µg N/l and lower in the Helves and Luna streams lacking floodplain, attaining means of 17 -19 µg N/l. Ammonium concentrations were higher at high water levels and mean concentrations were significantly higher at runoff than stream samples.

Nitrate concentrations at the first order stream at Arrecifes commercial farm were significantly higher at the downstream cattle breeding plot than in the upstream soybean plot (Table 2), during the first studied growing period. None of the plots were fertilized. The following growing period, concentrations were not significantly different at both plots, the upstream one cultivated with wheat followed with soybean and the downstream one cultivated with soybean. As previously stated, wheat was ploughed and fertilized in winter. A lower number of samples concentrated in the soybean growing period were available on the second growing period. Sampling deficiencies might have missed nitrate leaching from the surrounding soils in the first rains after fertilization. Nitrate concentrations at site 2 were significantly lower the second year, when soybean was cultured, than in the first year, when cattle was breaded in the same plot.

Mean nitrate concentrations in the first order stream at the Arrecifes farm was lower than the means of the four previously recorded representative streams of the region, except when cattle was breaded at the downstream plot.

The overall mean nitrate concentrations in the Pampasic streams (2.3 mg N/l) resulted comparatively low when compared with reported figures from intensively cultivated areas in Europe and North America. Mean nitrate concentrations in Ohebach, Germany (Schulz &

Liess, 1999) and Girou, France (Probst, 1985), streams attained 5.9 and 5.7 mg N/l, respectively. Meybeck (1998) compared the nitrate concentrations in forested and agricultural basins of the Seine watershed, the agricultural basins showed an order of magnitude higher nitrate concentration (mean 5 mg N/l) than the forested basins (mean 0.5 mg N/l). Miller *et al.* (1997) studied many streams of the Potomac watershed in Chesapeake (USA) and reported that the highest nitrate concentrations, up to 8 mg N/l, were determined in streams draining basins with intense cropping (mean 3.7 mg N/l), while streams with forested basins showed the lowest (mean 0.4 mg N/l). Boyer & Pasquarell (1995) found that mean nitrate concentrations correlated with the percentage of each basin occupied by agriculture in Appalachian streams (USA). Similarly, Stone *et al*. (1995) and Boyd (1996) found higher nitrates concentrations in streams draining cropland basins in North Carolina (mean 5.6 mg N/l) and Central Nebraska (mean 5 mg N/l), USA, respectively, than in forested (mean 1.1 mg N/l) and rangeland basins. Excess fertilization causes high nitrate concentrations in streams of agricultural basins (Meybec, 1998). It is therefore suggested that low nitrate concentration in Pampasic streams result form the lower fertilizer loads. Soybean is a leguminous symbiotic nitrogen fixer. Since no harvest increase is observed in response to fertilization, it is not fertilized with nitrogen in the Argentine Pampa. However, not all the N present in the biomass pool is attained by fixation, a variable amount is up taken from the soil (Sarandon, 2002). Soybean uptakes available nitrogen from the soil profile resulting in lower nitrate concentrations in the runoff water and adjacent streams, even lower than the concentrations prevailing in cattle breeding plots. Nitrate concentrations were highest at the lowest water levels and became diluted in coincidence with flood events. The opposite trend was observed in Ohebach and Girou streams by Probst (1985) and Schulz and Liess (1999). At high fertilizer loads, nitrate saturates the soil profile and is fast released by rainwater increasing concentrations at flood peaks. In the Pampasic streams the main N contribution comes from the mineralization of the soil organic N pool which is comparatively small and is diluted by the rain water resulting in the lower concentrations observed in the runoff and flood peaks. As previously stated, the roaming behaviour of the cattle likely contributed higher nitrate loads to stream in the corresponding plot. The lower nitrate concentrations in the floodplain streams provided with an upstream marsh (Maguire) and a recreation dam (Horqueta) suggest the influence of these environments on nitrogen transport. Osborne & Kovacic (1993) reviewed the influence that riparian buffer strips might have on the N retention from the adjacent landscape concluding that such environments represent effective N sinks reducing up to 90% of the N leakage from agriculture. N removal by wetlands has repeatedly been reported. Nguyen *et al*. (1999) reported that a riparian wetland removed 54% of the inputs from an adjacent sheep grazed pasture in New Zealand. They also reported higher ammonium downstream leaching consistent with the higher concentrations determined in Maguire and Horqueta streams in the present study. Schulz and Peall (2001) reported that a constructed wetland performed on a small stream of similar characteristics of the Maguire stream reduced the downstream nitrate concentration from a small orchard basin by 70%. El-Habr and Golterman (1990) reported important N losses in marshes and irrigation channels of Camarge (France). Although macrophyte uptake might be important, denitrification seems the main process determining the high observed N sinks in wetlands (Golterman *et al.*, 1988; Minzoni *et al*., 1988).

Satellite images show that rroughly half of the streams in the area showed stretches provided with floodplains or strips occupied with riparian vegetation of variable width. These stretches represent from 10 to 50% of the total stream length. Differences are often found in neighbor fields along the streams, suggesting that most of them originally counted with riparian vegetation and it was removed in roughly half of them by the agricultural practice. Some farmers leave the natural vegetation along the stream side, while others plough over the small first order stream headwaters in the plots of gentle slopes. Riparian restoration along the streams and maintaining natural vegetation strips along the streams therefore seems a promising and inexpensive option for sustainable long-term improvement of water quality.

Invertebrates

Jergentz *et al*. (2004a) studied the occurrence of toxicity and the effect on the resident invertebrate fauna in the three typical Pampasic streams: Maguire, Horqueta and Helves during the 2000-2001 growing period. Water samples were weekly taken and toxicity assays were performed with *Daphnia magna*. Runoff, flood and suspended matter samples were taken by means of passive devices installed in the surrounding fields (runoff samplers) and within the stream itself (flood and suspended matter samplers). The resident macroinvertebrate fauna associated to the macrophytes was sampled at four occasions from February to April 2001. The macroinvertebrate drift was studied with driftnets installed in the stream bed sampled at five occasions throughout the same period.

No toxicity was found in stream water itself. However, after a rain period of 229 mm occurred from march 1 to march 6 endosulphan was measured in suspended matter from Horqueta and Helves (30 and 40 µg/kg, respectively), Horqueta flood suspended matter (318 µg/kg) and runoff sediments from Helves (10 µg/kg). No pesticide was detected in other samples of Helves and Horqueta streams and no pesticide was ever detected in the Maguire stream at the study site, downstream the marsh. The number of species determined in each stream was reduced from 12 and 9, in Horqueta and Helves respectively before the rain, to 8 and 3 respectively, after the rain, while at Maguire, it remain on 8 throughout the studied period. Ephemeroptera density in Horqueta stream decreased from 350 ind/m^2 before the rain to 50 ind/m^2 after the rain, and from 100 ind/m^2 to none in Helves stream. Odonata density in Horqueta stream decreased from 50 ind/m^2 before the rain to 7 ind/m^2 after the rain, and from 60 ind/m^2 to none in Helves. On the other hand Ephemeroptera density in Maguire did not show significant variations and increased after the rain in Maguire stream. A significantly higher number of individuals were caught in the drift net after the rain at Horqueta, being *Hyallela curvispina* and Ephemeroptera the dominant, while no effect was observed in Maguire. This study showed the occurrence of toxicity pulses in the Pampasic streams associated with intense precipitations and thus, pointing to edge-of -field runoff as the main cause. Although cypermethrin is the most widely used pesticide only endosulphan was detected, plausibly because of its longer environmental persistence (Goebel *et al*., 1982). Endosulphan was detected in the particulate fraction, pointing that the hydrophobic nature of the pesticide favours its association to soil particles that are later incorporated to the streams by erosion processes. Ensosulphan concentration in runoff suspended matter up to 12 mg/kg has been reported in the Lourens River, South Africa, draining an orchard area, by Schulz (2001). Leonard *et al*. (2000) determined endosulphan concentrations up to 48 µg/kg in sediments from the Namoi River, Australia. No endosulphan was detected in the Maguire stream downstream the small natural riparian wetland. Similarly, Schulz and Peall (2001) showed strong pesticide reduction downstream a small wetland in an affluent of the Lawrence River in South Africa. Pesticide adsorption to particles retained within the marsh seems the cause. Since a high macrophyte biomass is yearly incorporated to the bottom sediments, pesticide association to organic matter likely contributes to the efficient wetland retention.

The observed decrease in density and species number in coincidence with endosulphan contamination points a cause-effect relation. Toxicity associated to the endosulphan particulate fraction was reported by Leonard *et al*. (2001) determining a 10-day LC_{50} concentration of 162 µg/kg for *Jappa cutera* (Ephemeroptera). Furthermore, Leonard *et al*. (2000) inferred that several Ephemeroptera and Trichoptera species were negatively influenced by runoff associated endosulphan exposure in field studies in the Namoi basin. Schulz and Liess (1999) showed increased drift of various invertebrate species at laboratory experiments with fenvalerate inoculated to soil suspensions simulating runoff events.

Horqueta and Maguire streams were also monitored the following growing period, from November 2001 to February 2002, by Jergentz *et al*. (2004b). Maguire was sampled at two sites, up and downstream the wetland. Two widespread and abundant local invertebrate species, *Hyallela cruvispina* (Amphipoda) and *Macrobrachium borelli* (Decapoda) were *in situ* exposed in small cylindrical enclosures with excavated windows covered with plastic nets. After each exposure, lasting 6-8 days, mortality was determined and new organisms were exposed. The local invertebrate population was monthly assessed four times throughout the study period. Stream and runoff suspended matter was retained with passive samplers installed in the stream bottom and in the adjacent field erosion rills respectively. Insecticides were detected in three occasions in runoff suspended matter samples taken after rain events occurred previous to the samples of 8 and 29 January and 7 February, amounting 94, 34 and 85 mm respectively. In the first runoff event 150 µg/kg chlorpyrifos, 46 µg/kg cypermethrin and 8 µg/kg endosulphan were determined in the runoff particulate fraction of Horqueta stream. In the following 29 Jan and 7 Feb runoff particulate samples chlorpyrifos (43 and 15 µg/kg, respectively) and cypermethrin (53 and 13 µg/kg, respectively) were determined. Only chlorpyrifos was determined in runoff water (0.07-0.3 µg/l) and stream suspended matter (64 µg/kg) after a sampling interval without rain lasting from 14 to 23 Jan. Neither endosulphan nor cypermethrin were detected in the stream or runoff water phase or stream suspended matter samples. No pesticide was detected in Maguire stream. *In situ* exposures of *H. curvispina* showed survival rates of 77-85% in both streams throughout the studied period at times when pesticide exposure was not detected. Only one exposure showed severe toxicity; the exposure lasting from 15 to 23 Jan in Horqueta showed 100% mortality of *H. curvispina* and *M. borelli* in coincidence with the detection of chlorpyrifos in stream suspended matter. The resident *H. curvispina* population showed a rather constant density attaining roughly 100 ind/m^2 at the first samplings, and was significantly reduced to zero following the 23 Jan sampling date in coincidence with the 100% mortality in the in situ exposures and chlorpyrifos detection in the stream suspended matter. Simultaneous chlorpyrifos contamination and complete mortality in exposed and resident organisms resulted very consistent. Since there was no rain in the sampling interval it was suggested that pesticide input was related to the drift of air plane fumigations observed in neighbor fields at the sampling area in the previous days. Later *in situ* exposures showed the background survival rates (77-85%) and consistently no pesticide was detected in the stream, pointing that toxicity represented a short pulse-like episode. However, the local invertebrate population did not recover in the last sampling period, until early march. Although the previous and two following runoff episodes contained detectable amounts of pesticides, mortality was not observed in the *in situ* stream exposures but significantly increased drift of small young organisms following the first runoff event of Jan 8. Runoff from a single plot is diluted by the overall flood pulse simultaneously coming from the whole basin. Sublethal exposures might cause increased drift without drastic effects on the resident population. Organism drift has been reported in various studies in relation to transient insecticide pollution (Cuffney *et al*., 1984; Kreutzweiser and Sibley, 1991). It has been shown that amphipods are present in the organism drift during insecticide exposure, representing an avoidance reaction (Breneman Pontasch, 1994; Schulz and Liess, 1999). It seems likely that the pesticide exposures during the rainfall events caused only a sublethal drift reaction (Taylor *et al*., 1994), not attaining enough intensity to produce drastic measurable changes in the resident population. Sibley *et al*. (1991) described invertebrate drift caused by permethrin concentrations of 0.35 µg/L, while population effects were evident when concentrations surpassed 1.7 µg/L. Several studies point the sensitivity of amphipods as sentinel organisms for water quality assessment. The amphipod *G. pulex* was the most sensitive organism (Brock *et al*., 1992a, b) in outdoor experimental ditches contaminated with chlorpyrifos, showing significant population decrease at 0.9 µg/l (Van den Brink *et al*., 1996). Werner *et al*. (2000) found in stream samples toxicity of chlorpyrifos that caused 100% mortality in 48 h bioassays with *Ceriodaphnia dubia* at a concentration of 0.13 µg/l, from the Sacramento–San Joaquin river delta.

During the following growing period (2002-2003), the Arrecifes tributary was monitored, sampling at two sites, inside the soybean plot (site 1) and at a 250 m downstream plot (site 2) with cattle (Figure 1). On Nov. 27 a mixture of glyphosate and cypermethrin was applied at the beginning of the growing period. Cypermethrin was determined in the bottom sediments attaining 37 and 54 µg/kg at each site, respectively. Eighteen days later (Dec. 15) a 57 mm rain occurred, and soon after (Dec. 17) cypermethrin in the bottom sediments increased to 1,075 and 595 µg/kg at each site, respectively. On Jan. 11 chlorpyrifos was applied to the soybean plot. Although chlorpyrifos was not detected within the stream cypermethrin remained in the bottom sediments on Jan 11, attaining 90 and 120µg/kg at sites 1 and 2, respectively. After the first rain cypermethrin was high and roughly double at the applied plot than the close downstream site. A month later cypermethrin decreased to roughly 10% at the applied plot while concentrations remained in the same order of magnitude at both sites. Present results suggest a comparatively low persistence in the stream environment together with a relatively low downstream transport.

Figure 2 shows the survival rates of *H. curvispina* in the *in situ* exposures at both sites. 100% mortality was observed in coincidence with the exposure from 10 to 17 Dec. when the 57 mm rain caused an important runoff and the maximum cypermethrin concentrations were measured in the bottom sediments. The next exposure, from 17 to 26 Dec. a rainfall event of 80 mm was produced and survival attained 60 and 29% at each site, respectively. The following exposure, from 10 to 12 Jan., included the chlopyrifos application without rain, attaining a survival rate of 45 and 89% respectively. The two following exposures included rains of 17 and 67 mm, with the smaller one, mortality was 100% at site 1 while a 10% survival was attained at site 2. With the later and larger rain, mortality was 100% at both sites. No rains occurred the next two exposures and survival rates increased to 15 and 25% at each site respectively the first one (29 Jan to 5 Feb) and 75-70% the last one. Consistently, almost complete mortality was observed after the first rain following each application while the chlorpyrifos application caused roughly 50% mortality within the applied plot without discernible effect on the downstream plot. Within the managerial practice at the studied farm, edge of the field runoff represents a more important toxicity source than the application drift. Both, cypermethrin and chlorpyrifos are hydrophobic compounds, and therefore are fast retained to the particulate phase. Since the streams produce a fast increase in discharge and water velocity in response to rain events, the presence of pesticides in true solution remains as a sampling challenge. Although brief and sharp ephemeral peaks of dissolved pesticides might eventually occur it would remain extremely difficult to be detected. High concentrations in bottom sediments and the lower effect of drift than runoff inputs on exposed organisms suggest that the applied pesticides are fixed to the soil and introduced to the stream already sorbed to the particulate fraction of the runoff.

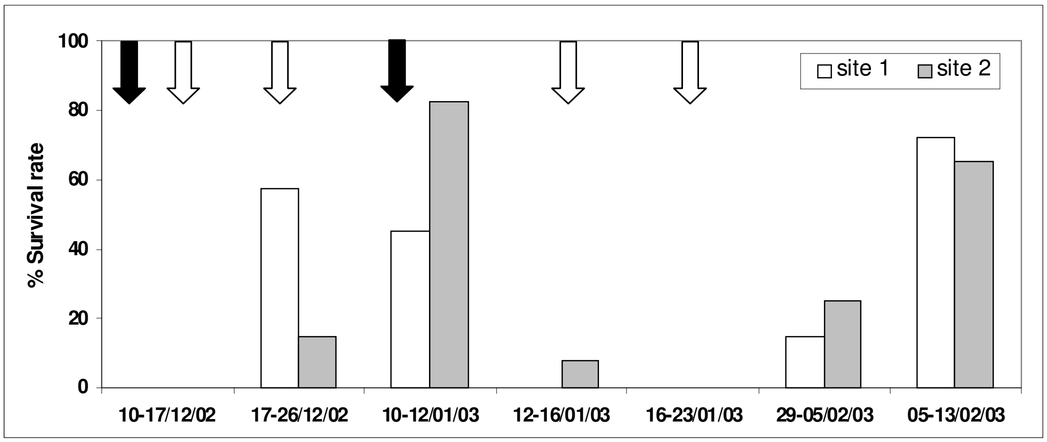

Figure 2. Mortality of *H. curvispina* exposed in field enclosures at two sites of a first order stream born in a soybean plot (site 1) and flowing into a 250 m downstream livestock plot (site 2). Black arrows represent pesticide applications: the left one represent a mixture of cypermethrin and glyphosate applied on 27 Nov. 02, and the right one a mixture of chlorpyrifos and glyphosate applied on 11 Jan. 03. White arrows represent rain events producing runoff.

Vascular Plants

Riparian and aquatic plant communities are essential for maintaining the quality of the aquatic habitat in agro ecosystems. Shoreline vegetation plays critical roles in soil stabilization, and therefore in reduction of erosion (Blackburn and Boutin, 2003). Aquatic macrophytes have shown to be effective in reducing concentrations of pesticides resulting from spray-drift (Dabrowsky *et al*, 2005), regulate water fluxes and provide habitat to aquatic life. Plants of these communities next to agroecosystems are impacted by related activities, particularly the spray of herbicides.

Field assessments from detailed studies of the riparian stream flora carried out monitoring phenological status before and after pesticide (herbicide or herbicide plus insecticides) applications, were performed aiming towards the detection of long term effects on the local flora (Martin *et al*., 2005). This strategy allowed the assessment of potential changes within reproductive and seasonal cycles, with impacts on the composition of the plant community. Effects assessments on phenological status and richness in Pescado stream from one year crop cycle indicated that the herbicide applications during the soy crop pre-emergence coincided with 12% of the flowering and 10% of the fructification periods of the riparian species. Summer crop development coincided with 50% flowering and 25% fructification. Richness evaluation indicated that of a total of 58 species from the area, 71% corresponded to native species, and 36% of them were weeds. Although the shortness of the studied period, the relevance of potential impacts were clearly associated to the simultaneity of herbicide sprays and seeding time of a high percentage of the species from the area (Martin *et al*., 2005).

In the Arrecifes tributary no detectable levels of variations in biomass, richness, surface cover and abundance in relation to pesticide applications were seen during a single crop cycle. Three species, *Anthemis cotula* (Compositae), *Trifolium repens* (Leguminosae) and *Echinochloa crusgalli* var. mitis (Gramineae), represented more than 33% of the total plant cover in spring. In summer, *E. crusgalli* replaced *A. cotula* as dominant in the taller stratum, while *T. repens* remained as dominant in the lower stratum. *Cyperus reflexus*, *Bromus unioloides*, *Paspalum dilatatum*, *Setaria geniculata*, *Eleusine tristachya*, *Verbena montevidensis*, *Verbena rigida*, *Verbena gracilescens*, *Melilotus indicus*, *Rumex crispus* and *Polygonum hydropiperoides* represented 10% of the total surface cover. These species coincided with a previous description, performed roughly 10 years ago, and corresponded to the *P. dilatatum* grassland community described by Faggi (1966). However, some characteristic elements of the association were missing (*Stipa hyalina, S. papposa, Bothriochloa laguroides, Bromus catharticus*). A reduction of the species richness was evident, with the presence of only 20% of the 76 species described for the association in the area (Martin *et al*., 2003). Present evidence suggests an impoverishment in species richness of the assemblage, plausibly related to the agricultural practice. Further work is needed on this subject.

Different type of injuries such as chlorosis, or decreased biomass on riparian and aquatic plants were detected as an immediate response to pesticide spray events on natural and enclosed populations adjacent to the applied culture (Sobrero *et al*., 2007). Effects on total chlorophyll content (TCC) of a riparian community from the Arrecifes tributary in relation to herbicide sprays indicated significant decrease in TCC for *P. dilatatum* and *R. cripus*. Effects were evident four days after herbicide sprays with 32 and 29% reduction, respectively. Plant recovery was observed 20 and 3 days later, respectively. Measurements of TCC for *T. repens* did not show significant level of variation in relation with spray applications, possibly due to the protection offered by plants from taller strata. For the case of aquatic plants, *Sagittaria montevidensis, Hydrocotyle ranunculoides* and *Pontederia roundifolia* also evidenced TCC reduction at the level of 25, 71 and 12%, respectively. The herbicide reached at least 250 meters downstream application sites, with detectable effects on aquatic macrophyte vegetation. *P. dilatatum, H. ranunculoides, R. crispus* and *S. montevidensis* were sensitive and represented indicators of impacts on riparian and aquatic non-target species in the region, for a rapid assessment of effects at the level of chlorophyll content (Martin *et al*., 2003). Although no noticeable differences could be observed in biomass in the short term measurement performed, inhibition of TCC gives evidences of potential long term effects on growth and reproduction, depending on the stage of plant development.

Field tests with *L. gibba* or *Spirodella intermedia* within enclosed devices (Sobrero *et al*., 2007) in stream sectors of the Arrecifes tributary adjacent to soy bean fields, showed inhibition in biomass production (dry weight per frond) and in the TCC of *L. gibba*, with 28 and 75% reduction, respectively, for an application of glyphosate with cypermethrin.

Assessments from two later applications also indicated effects on these parameters (35.5 and 75.5% reduction of biomass and TCC, respectively, for an application of glyphosate with chlorpyrifos; and 53.9 and 0% reduction for an application of glyphosate, chlorpyrifos and cypermethrin). Enclosures were protected from the application drift by covering them with transparent nylon. It was shown that the main effect was related to exposure by runoff. Other reports from tests performed in Pescado tributary with *S. intermedia* also indicated significant effects of pesticide runoff on TCC and population growth reduction (multiplication rate and number of frond per colony) between two and seven days after exposure, as observed for *L. gibba* (Martin *et al*., 2005).

Field glyphosate chemical analyses reported by Peruzzo *et al*. (2003) and Sobrero *et al*. (2007) ranged from 0.5 to 2.1 mg/l, and were consistent with the expected environmental concentrations estimated from recommended doses, following Boutin *et al.* (1995).

Comparisons between the effects actually observed in the field exposures associated to these concentrations with those from laboratory standardized tests (Environment Canada, 1999; Sobrero *et al*., 2007), indicates that it would be necessary higher concentrations of glyphosate for equivalent effects in laboratory toxicity tests. Taking into account that the studied field sceneries were associated with the application of mixtures of the herbicide with insecticides, a possible explanation for higher effects in the field could be associated to additional or synergistic toxicity in mixtures. Furthermore, limitations in extrapolations between laboratory and field conditions should be also considered.

Martin and Ronco (2006) studied the impact of mixtures of pesticides on vascular plants assessing the effects of single pesticides formulations and their mixtures on the inhibition of germination and seedling root elongation with laboratory bioassays. A comparative assessment of the effects of a glyphosate formulation using a standardized battery of plant seeds showed that sensitivity was largely variable among the assayed families. The IC50 values for root elongation inhibition ranged between 8.89 and 1164.3 mg/L, with a sensitivity rank as follows: *Lactuca sativa*> *Lolium perenne* > *Medicago sativa* > *Allium cepa* > *Brassica napus*. Additionally, a detailed study performed with *L. sativa* showed a slightly higher sensitivity to the Roundup® Max formulation (IC50= 7.3 [6.5-9.4] expressed as mg/L of a.i.), in comparison with glyphosate, the active principle (IC50= 9.8 [8.2-11.8] mg/L). The mixtures of formulated glyphosate, with cypermethrin and chlopyrifos using the most sensitive plant seed, *L. sativa*, indicated higher toxicity of those containing the herbicide with cypermethrin or with both insecticides together when compared to the herbicide alone. Toxicity increased up to seven times when mixed with the insecticide cypermethrin. These results are consistent with the observed higher plant sensitivity in the field than in laboratory exposure.

Results of toxicity tests performed by Martin and Ronco (2006) with a stream sediment elutriate (1:4, sediment: water) sampled after pesticide application (a mixture of the herbicide plus the two insecticides), showed significant effects on root elongation inhibition of *L. sativa* seeds (13 % inhibition at the direct sample elutriate). The detected effects were in agreement with the field study with caged experiments performed simultaneously in the same area (see results previously given in this section), indicating that laboratory tests with environmental samples were capable of detecting toxic effects from exposure to a sediment elutriate sampled at a time and in the place where field tests with aquatic plants were also affected.

We conclude that vascular plants can therefore be affected by pesticide drift or runoff concentrations that are orders of magnitude lower than levels applied in target areas. These results are in accordance with observation done by Ferenec (2001) for low-dose herbicide exposures. Authors also found that adult riparian and aquatic plants could experience effects from a few centimetres distance to as far as 250 m from spray areas. Boutin and Rogers (2003) pointed out that lethal effects on some plants clearly change the composition of and affected community, however, it is unknown to what extent the plant community could be affected when sub-lethal effects are observed (i.e. reduction in the competitiveness or reproductive output of some species). The observed effects on vascular plants from the riparian and aquatic sectors in the Pampasic areas were at the sublethal levels. Long term impacts of sublethal effects on non target plant species growing along margins will strongly depend on their phenological stages (some species may have mature seeds, others may not have reproduced at that moment) during pesticide applications. Since vegetation provide structure to riparian and aquatic communities (including invertebrate, fish and amphibians), changes in plant species may result in an impact on other _trophic levels.

Phytoplancton and Periphyton

Pérez *et al*. (2007) studied the effects of Roundup® addition on phytoplankton and periphyton communities in earthen outdoor mescoscosms of 25 m^2 and 1.2 m depth. Effects of added glyphosate at initial concentrations of 5.5 and 12.7 mg/l, by triplicate, were compared with controls. Glyphosate fast disappeared with a mean half life of 6.6 days. No significant differences were observed in nutrient concentration among treatments except for total phosphorus concentrations which increased in the glyphosate treatments. As expected, significant differences in micro and nano phytoplankton densities were observed among treatments. By the end of the experiment, mesocosms treated with both doses of Roundup showed a 2.5-fold decrease in micro- and nanophytoplankton mean abundances. The abundance of the heterotrophic bacteria and picoplanktonic eukaryotes did not exhibit significant differences among treatments. However, picocyanobacteria (Picy) showed the opposite trend. By the end of the experiment, mean Picy abundance increased roughly 40 times in the high-dose treatment as compared to the initial values. Correspondingly, the mesocosms treated with the higher dose experienced a significant two fold increase in phytoplankton primary production.

The periphyton assemblages did not show significant differences in chlorophyll concentration or density abundances among treatments. Similarly as for the phytoplankton assemblage, the periphytic cyanobacteria (particularly *Cyanothece* spp.) appeared to increase after the addition of the herbicide, but observed differences among treatments were not statistically significant.

The most interesting effect observed in this study is the large 40 fold increase in Picy density. Cyanobacteria are known to have a comparatively higher tolerance to glyphosate by either one of two mechanisms: the overproduction of synthases, or the production of glyphosate-tolerant enzymes (Powell *et al*., 1991, 1992). The total P increase observed in the glyphosate treatments agreed with the 14% contribution of P to the molecular weight of glyphosate. Roundup contains glyphosate, water, and POEA, a complex mixture of fat from cattle or sheep tissues, which is not supposed to contain phosphorus. Since glyphosate is fast metabolized within the water-body its P content enhance the advantage of the comparatively more resistant cyanobacteria species and has important potential implications for water quality, since cyanobacteria represent potential hazard due to bloom forming nuisance and toxic cyanotoxin production and release. It is therefore not only conceivable, but also quite likely that current agricultural practices, which heavily relays upon continual additions of glyphosate at regional scales, may alter the structure and function of many natural aquatic environments leading to enhanced eutrophication levels.

Fish

The fish community of Arrecifes tributary was found to be composed at least by five fish species (*Cheirodon interruptus, Cnesterodon decemmaculatus, Hoplias malabaricus, Rhambdia quelen, Synbranchus marmoratus)* (Carriquiriborde *et al.* (2007). *C. decemmaculatus* represented the most conspicuous and abundant species of the fish community

The effect of cypermethrin-glyphosate mixture (ground applications) on the local resident and caged populations was investigated in two consecutive crop cycles under field-use conditions by Carriquiriborde *et al*. (2007). No fish mortalities or individuals presenting abnormal behaviour were recorded along the stream, and no dead fish were trapped in drift nets after pesticide application or after the first rainfall event during the two consecutive years of study. The total number of fish captured per effort unit, the size -class structures and several other parameters measured (immature: total ratio, male: female ratio and condition index) of *C. decemmaculatus* populations did not show significant differences before and after pesticide application at both sites (Table 3).

A similar trend was observed on population parameters in a later ground application of a mixture containing cypermethrin, chlorpyrifos and glyphosate. However, moderate fish kills in the stream between four and 24 h after spraying were detected in this case. In accordance with these results, observed fish survival within *in situ* caged exposed *C. decemmaculatus* indicated no significant differences in survival rates respect to controls (percent survival: 86.2±4.0) after application of cypermethrin- glyphosate, but a significant reduction (percent survival: 78.8±7.4) after application of the cypermethrin- chlorpyrifos- glyphosate mixture (Carriquiriborde *et al*., 2005).

Fish community of Pescado stream tributary was composed by the same species as those found in Arrecifes tributary. Additionally, *Corydoras paleatus* was also observed (Carriquiriborde *et al*., 2005). Studied pesticide spray impacts in this water body included a ground spray of the herbicide and aerial applications of a mixture containing endosulphan and glyphosate (see table 1). The authors found neither fish kills in the stream and no significant mortalities in caged experiments (survival: 88.7±12.5) in relation with the spray of glyphosate. This was also in agreement with the results of laboratory toxicity tests using *C. descemmaculatus* showing that glyphosate was not acutely toxic to the species (Carriquiriborde *et al*., 2007). The opposite result was observed for the applications of endosulphan in Pescado stream tributary. Field observations and drift nets allowed detecting generalized fish kills 24 and 72 h after fumigation. Only 17.0±5.9 % survival was detected in the exposed *C. descemmaculatus* caged population during the fumigation of the mixture containing endosulphan and glyphosate.

The absence of acute lethal effects observed for cypermethrin under field conditions contrasted with the high toxicity showed by this pesticide under laboratory conditions. The cypermethrin 96 h-LC50 for *C. decemmaculatus* following standardized protocols with dechlorinated laboratory tap water (USEPA, 2002) was 0.46 µg/l (Carriquiriborde *et al*., 2007), similar to reported figures for other fishes, as *Oncorhynchus mykiss* (0.5 µg/l) and *Scardinius erythropthalmus* (0.4 µg/l), and lower than *Cyprinus carpio* (0.9–1.1 µg/l) and *Tilapia nilotica* (2.2 µg/l) (Stephenson, 1982). The 96 h-LC50 of cypermethrin for C. *decemaculatus* increased to 2 µg/l in filtered stream water and to 5 µg/l when unfiltered stream water was used. A correlation was found between stream organic matter content and cypermethrin LC50.

Although cypermethrin was shown to be very highly toxic to *C. decemmaculatus* at the standardized laboratory tests and that during the second crop cycle the pesticide reached water concentrations of 0.46 and 0.29 µg/l at sites 1 and 2, respectively (Carriquiriborde *et al*., 2007), higher than the 96 h LC50 determined following standardized protocols, no mortality or behavioral changes were observed in the resident fish population throughout the two successive crop cycles. No differences in the mortality within *in situ* exposed enclosures were observed in both sites comparing the periods previous and following the application.

Reduced toxicity of pyretroid exposure under field conditions has been previously reported (Haya, 1989; Hill, 1989) and attributed to the tendency of pyrethroids to bind to suspended particulate matter, sediments, and aquatic plants. Surface waters in the Pampas are moderately rich in suspended solids with medium to high organic matter contents. Total organic carbon (TOC) values ranged from 2 to 8.2 mg/l in eight typical pampasic streams (Carriquiriborde *et al*., 2007); showing a mean content close to the mean reported figures for warm temperate-streams (Spitzi and Leenheer, 1991). Total OC concentration in the stream at the Arrecifes farm was the largest of the studied Pampasic streams. Moreover TOC concentrations increased from 4.4 mg C/l at the start of the second crop cycle to 14.6 mg C/l just after the application, and later decreased to 7.0 mg C/l at the end of the sampling period. It seems likely that glyphosate application enhanced organic carbon leakage from the decaying vegetation to the stream, therefore contributing to the observed decrease cypermethrin toxicity for fish.

Risk assessment evaluations relay on toxicity exposure ratios (TER) estimated with standardized procedures for measuring the lethal concentrations. When the procedure was applied to the Arrecifes stream an effect was predicted. If the TER ratios are calculated using the LC50 values actually measured with stream water no effect is expected, in 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 stream water 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).

Table 3. Effects of soybean pesticides on fish population under current used field conditions in the Pampas region

	Pesticide spraying events			
	glyphosate	[1]glyphosate+cypermethrin	glyphosate+ cypermethrin+ chlorpyriphos	glyphosate+ endosulphan
Field observations and drift nets				
Generalized fish kills in the stream	Not observed	Not observed	Moderate (from 4 to 24 h after spraying)	Severe (from 24 to 72h after spraying)
C. decemmaculatus caged population				
96h-Survival	88.7±12.5 NSD	86.2±4.0 NSD	78.8±7.4 % SD	17.0±5.9 % SD
C. decemmaculatus wild population parameters[†]				
Size class structure	---	NSD	NSD	---
Immature/total ratio	---	NSD	NSD	---
Male/female ratio	---	NSD	NSD	---
Condition index	---	NSD	NSD	---

SD: significantly different respect before spraying; NSD: Not significantly different before and after spraying; ---: not assessed.

[1] Assessed during two consecutive years

Multy Organism Tests

Laboratory studies with mescosm were developed by Mugni *et al*. (2006) in order to detect toxicity thresholds of pesticide exposures at field realistic conditions by simulating runoff events on two representative components of the resident fauna, the amphipod *H. curvispina* and the fish *C. decemaculatus*. Three replicates and controls were used to assess the toxicity of cypermethrin, chlorpyrifos and endosulphan in aquariums containing stream sediment and water. A realistic runoff scenario was simulated by mixing soil and water and adding pesticide to attain a concentration of 10 µg/l in the suspension, giving an initial 1µg/l concentration in the aquariums. Water was taken out of each aquarium at different times and 48 h exposures assays performed.

Chlorpyrifos and cypermethrin were highly toxic for *H. curvispina* attaining 100% mortality at the beginning of the experiment (Figure 3), while endosulphan did not cause any mortality. A significant decrease in water toxicity to *H. curvispina* was observed at the second day for chlorpyrifos and no toxicity was observed from the forth day onward. The treatment exposed to cypermethrin showed decreased mortality from the forth day onward reaching a minimum at the end of the experiment, after nine days incubation. No mortality of fish was observed for any for the three assayed pesticides. A second assay was immediately performed with a higher pesticide concentration of 5 µg/l added in the same way. At such concentration 100% mortality was attained by endosulphan while no mortality was observed by cypermethrin or chlorpyrifos. In spite that 50% lethal concentrations for *C. decemaculatus* following standard laboratory protocols lye in the order of 1 µg/l (Carriquiliborde *et al*., 2007), our simulated runoff with realistic, field relevant concentrations did not cause any observable effect on the common endemic fish *C. decemaculatus*, in agreement with no observed fish mortality in streams following farm applications. Decreased toxicity due to strong pesticide adsorption to the particulate and dissolved organic mater must be invoked in order to account for such result. *H. curvispina* resulted much more sensitive to cypermethrin and chlorpyrifos exposure than the fish, confirming the suitability of using it as sentinel organism for toxicity assessments.

Toxicity was fast depleted in the local water and sediment environment characterized by alkaline water of comparatively high conductivity, hardness, and organic and suspended matter contents. Toxicity to *H. curvispina* was higher for cypermethrin and chlorpyrifos than endosulphan and decreased faster for chlorpyrifos. *C. decemaulatus* resulted more sensitive to endosulphan than cypermethrin and chlorpyrifos.

Another laboratory experiment was performed in long and narrow aquariums containing sediments and water from the first order stream in the Arrecifes farm, in which a water flux was induced with pumps, and chlorpyrifos was added as a mixture of soil and water, in order to simulate the effect of a runoff event on representative organisms of the local stream fauna (Argemi *et al*., 2003). Each aquarium contained bottom sediments and water from the stream. A runoff event was simulated by pouring into the aquarium a suspension of soil and water spiked with chlorpyrifos, resulting in a measured initial concentration in the aquariums of 15 and 73 µg/l for the lower and higher dose treatments, assayed by triplicate. Chlorpyrifos disappeared fast from the water in the aquariums attaining a final concentration of 0.6 and 0.4 µg/l after nine days, at the end of the experiment. Bottom sediment concentration at the end of the experiment were conspicuous attaining 83 and 212 µg/kg for each dose respectively, representing 30 and 16% of the total amount of chlorpyrifos initially added. An initial 100% mortality was attained for the amphipod *H. curvispina* and the decapod *Macrobrachium* *borelli*. However, at the end of the experiment, exposed *H. curvispina* did not show mortality at all. Fishes showed initial symptoms of poisoning such as swallowed gills and spastic swimming movements. Nevertheless, there were no mortality in any replicate and they recover to the end of the experiment.

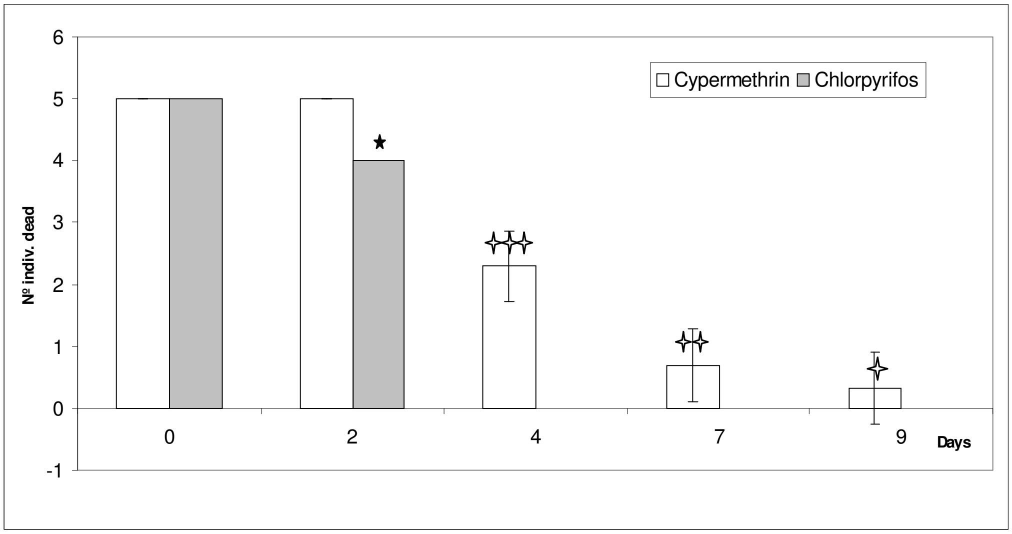

Figure 3. *H. curvispina* mortality in exposure assays following pesticide addition to laboratory aquariums simulating field realistic runoff concentrations. Stars denote significant differences with the previous sampling day, bars represent standard deviation.

Amphibians

Cajade and Natale (2003), reported 15 species of anurans in the study area of Arrecifes (*Chaunus arenarum, Chaunus fernandezae, Hypsiboas pulchellus, Scinax nasicus, Scinax granulatus, Scinax berthae, Leptodactylus latinasus, Leptodactylus gracilis, Leptodactylus ocellatus, Leptodactylus mystacinus, Odontoprhynus americanus, Physalaemus biligonigerus, Physalaemus albonotatus, Pseudopaludicola falcipes, Elachistocleis bicolor*), representing 55.5% of the species present in the Province of Buenos Aires (Gallardo, 1974; Lavilla *et al*., 2000; Lavilla and Cei, 2001). Cajade and Natale (2003) also found higher richness than expected according to vouchers existent in Academic and Scientific herpetological collections for the area. The anuran fauna contained forms belonging to the families Hylidae, Bufonidae, Leptodactylidade and Microhylidae. They have aquatic larvae and different morphological type of adults, with diverse reproductive strategies. Although the anuran community was well represented, only few species reproduced within the study site of the Arrecifes tributary.

Natale and Ronco (2003) and Agostini *et al*. (2005a) reported the impact of several pesticide applications in Arrecifes and Pescado stream tributaries on field sampled anuran larvae. Mortalities within the stream and drift nets located at the sampling sites of the Arrecifes tributary in relation with the applications of a cypemethrin and glyphosate mixture were low (effects were observed only on caged organisms during the first year, Table 4), pointing to subtle acute effects on tadpoles of this type of ground spray. Sublethal short and long term effects were not assessed in these reports. Associated runoff after the first rain also had an attenuated impact on mortalities (Table 4, Figure 4). Simultaneous field experiments with caged *H. pulchellus* and *C. arenarum* tadpoles detected significant mortalities only in a test group of tadpoles from the former species adjacent to the spray patches in the first year. Mortalities after application and runoff of chlorpyrifos (in its mixture with glyphosate) in the same sites were moderate, with higher mortalities in comparison with the cypermethrin-glyphosate spray (Table 4). Figure 4 shows tadpole mortalities for both types of pesticide spray mixtures before and after application and after the first rain (Natale and Ronco, 2003). Severe effects were observed in the field, assessed by means of observations and drift nets, associated to a ground spray of a mixture containing cypermethrin with chlorpyrifos and glyphosate in the Arrecifes tributary, and also significant mortalities in caged tadpole populations of *L. gracilis* and not significant for *H. pulchellus* (Table 4).

The comparison of the spatial extent of impacts indicate that amphibian and fish impacts were generally circumscribed to adjacent sectors, possibly reaching the water body from pesticide drifts of applications, while the herbicide glyphosate drift and runoff caused chlorophyll content reduction not only on the adjacent riparian vegetation, but at least 250 meters downstream application.

The description of the anuran community from the Pescado stream reported by Agostini *et al*. (2005b) was represented by seven species (*C. arenarum, C. fernandezae, H. pulchellus, L. latinasus, L. gracilis, L. ocellatus, P. falcipes*), constituting 46% of the species present in the region, and only 23% of those reported for the Buenos Aires Province (Gallardo, 1974; Lavilla *et al*., 2000; Lavilla and Cei, 2001). The field assessments in this study area indicated severe effects of an endosulphan plus glyphosate application, detected immediately after fumigation and throughout the following 24 h. Additionally, high mortality in caged populations of *H. pulchellus* 24 h after fumigation (Agostini *et al*., 2005a) was detected. The impacts of these aerial applications were also severe on the fish as reported by Carriquiriborde *et al*. (2005). Hence, fumigation of endosulphan determined a high acute impact on these populations, providing additional evidence on the risk associated to the use of this insecticide.

Amphibians occurring in soy bean areas from the province of Entre Ríos and Santa Fe, a few hundred kilometers of distance from the Arrecifes tributary study sector, showed a similar richness as the one found in Arrecifes (Lajmanovich and Peltzer, 2004, Attademo *et al*., 2005, Peltzer *et al*., 2006). These authors examined the related landscapes in the studied areas, pointing to the relevance of the presence of a close protected forest, and also at the recent introduction of this type of agricultural activity. The observed higher diversity was compared with another impoverished agricultural area in the province of Córdoba, where agriculture had expanded more extensively. Peltzer *et al*. (2006) proposes that management to prevent recent anuran declines will require defining high-quality habitats for individual species or group of species, followed by efforts to preserve or restore these aquatic habitats. The maintenance of shore vegetation in ponds and hedgerows may increase the number of species and diversity of anurans within agricultural landscapes. Lajmanovich and Peltzer (2004), indicate that these studies will help in our understanding of the status and conservation of amphibians in coastal fluvial areas and will serve to help coordinate research and conservation efforts.

Even though the Arrecifes tributary had a much longer history of soybean cultivation, the presence of several small vegetated patches with tall grasses and typical allochthonous trees could represent eventual refuges for maintaining species richness. Although the community of anurans was well represented, it was only for a few of these species that was possible to detect breeding sites (Cajade and Natale, 2003). It is important to point out, as indicated by Hayes *et al*. (2006) for observations in cornfields in the mid-western of the United States of America, that ponds or streams within agricultural fields could not be predictable year after year, and not even day after day. Results obtained by Piha (2006), pointed on the essentiality of the existence of enough wetlands adjacent to the breeding sites for amphibian survival. The author found that in the studied areas of Sweden increased isolation of breeding ponds from expanding agriculture partly explained the absence of some species.

Although soybean pesticides exhibit low bioaccumulation and short environmental life times, some of them show high toxicity levels, such as the ones referred in this chapter or those reported by other scientists (Smith and Stratton, 1986; Kamrin, 1997). There is an extended amount of reports from the literature on the effects of these insecticides on wildlife, and at present is widely accepted the vulnerability of amphibians to these toxicants. Particularly, sensitivity of early stages of life, lethal and sublethal effects, and different response patterns according to specific life stages have been reported in the last decade (Power *et al.*, 1989; Carey and Bryant, 1995; Hill, 1995; Cowman and Mazanti, 2000; Pauli *et al.*, 2000).

The widespread local agricultural practice makes use of pesticide mixtures in different combinations and proportions, and there is scarce information on their combined effects; additionally, the range of responses observed for anurans from exposure to agricultural mixtures determines a complex approach for undertaking their study. While some extensive reviews about toxicity of pesticides on amphibians are available (Power *et al*., 1989; Devillers and Exbrayat, 1992), there are there are only few reports of the effects of pesticide mixtures on anurans (Sparling *et al*., 2000). Since it has been suspected that the effect of chemical pollutants is being underestimated within the multiple stress factors contributing to numerical amphibian decline (Burkhart *et al*., 2003), there is increasing concern on evidences about the role of organic chemicals involved in retardation of growth and development, time and size to reach metamorphosis, endocrine disruption and related immunosuppressive effects are likely to be highly relevant (Hayes *et al*., 2006).

The clear impact of some of the pesticides on tadpole mortalities associated to sprays and runoff reported here evidence the toxicity of soybean mixtures of pesticides, associated to different management practices and variable exposure conditions. The observed effects in the field were directly related to pesticide applications reaching the water bodies by drift or runoff. The authors agree that habitat fragmentation, and long term effects of pesticides on populations should be carefully analyzed towards the understanding of the impacts of biotech pesticides on amphibian communities.

Table 4. Effects of soybean pesticides on species of anuran tadpoles under current used field conditions in the Pampas region

	Pesticide spraying events			
	[1]cypermethrin+ glyphosate	[2]chlorpyrifos+ glyphosate	cypermethrin+ chlorpyrifos+ glyphosate	endosulphan+ glyphosate
Field observation and drift nets				
Generalized tadpole kills in the stream	Subtle (through 24h)	Moderate (through 24h)	Severe (through 24h)	Severe (through 24h)
Tadpole caged population				
24h survival after application	*H. pulchellus* 55.0±6.42*/ 100±0 *C. arenarum* 91.7±3.57	*H. pulchellus* 100±0/ 96.5±2.00 *C. arenarum* 90.0±3.87	*H. pulchellus* 83.3±6.80 *L. gracilis* 50.0±8.94*	*H. pulchellus* 27.1±2.80*
24h survival after first rain	*H. pulchellus* 37.0±6.42* / 73.3±8.07 *C. arenarum* 88.3±4.14	*H. pulchellus* 58.3±14.23* *C. arenarum* 73.3±5.71		

Values are given as mean ± standard deviation of percentage of survivals.
*: significantly different ($p<0.05$) before and after spraying.
[1]Assessed during two consecutive years in the same sites; [2]Assessed during two consecutive years in different sites.

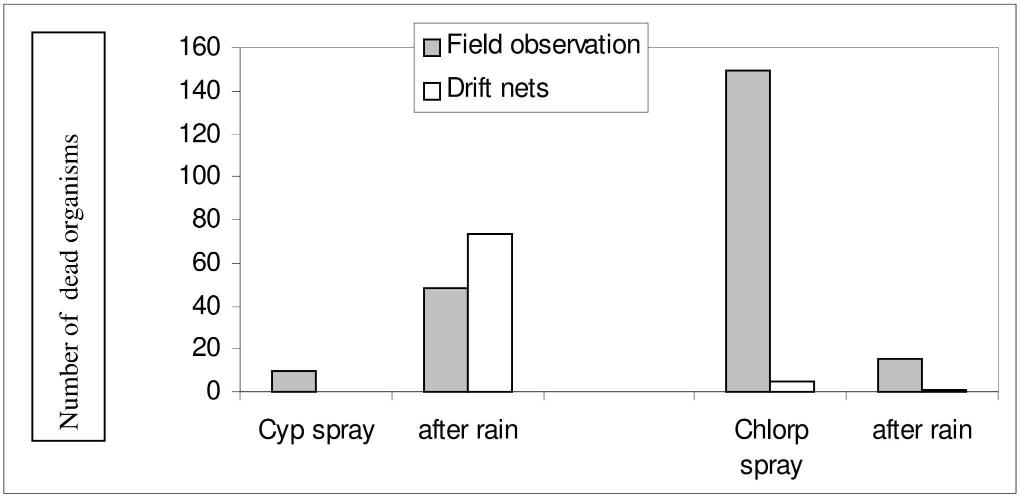

Figure 4. Observed tadpole mortalities in field observations and drift nets in the Arrecifes tributary in relation with cypermethrin (Cyp) and chlorpyrifos (Chlorp) sprays and runoff after the first rain.

Conclusion

Present information, sustained on the studies performed on several typical streams throughout several successive years, suggests that the Pampasic streams contain comparatively low nitrate and comparatively high SRP concentrations. The former results from the relatively low fertilization loads presently employed, since the soybean has no agronomic response to nitrogen fertilization. The latter seems related with the naturally high P content of the deep and rich Argiudol soils developed over the loessic sediments deposited during the Holocene. Available information suggests that agricultural intensification in the Pampas has no evident impact on stream nutrient concentrations at the present fertilizer loads and managerial practices employed. The regional nutrient budget shows that the fertilizer loads do not replace the amount of harvested nutrients. The present agricultural practice is exhausting the soil available pool of nutrients and is therefore not sustainable on the long term. Fertilizer loads, although comparatively low, have shown a significant increase in recent times and it is expectable that such trend will be increased with time leading to enhanced nutrient concentrations in the future.

Results of effects assessments indicate that all the biota components of the studied ecosystems (flora, invertebrates, fish and amphibians) were affected in different extent after pesticide applications, representing an appreciable stress over the systems. Sometimes the effects were comparatively subtle, particularly on the vegetation, and others quite drastic, such as the observed on the invertebrate populations. Evidence from laboratory and field conditions were very consistent, showing high sensitivity of non target invertebrates, resulting in toxicity pulses mainly associated with runoff events that produced mortality in the resident populations with total disappearance of some groups of the resident fauna (ephemeroptera, odonata, amphipoda) in response to toxic pulse events occurring several times per year. Runoff seemed more important than spray drift in the local environment and managerial practice. The toxic pulses were ephemeral as shown by *in situ* exposures, and local populations recover with time. Toxicity persistence in the local environment was quite short. Chlorpyrifos showed faster toxicity dissipation than cypermethrin in laboratory simulated runoff exposures. Chlorpyrifos toxicity disappeared from water-sediment systems within a few days and cypermethrin in about a week. These experiments showed the combined effect of partition between water and sediment and the half life in each phase. Being highly hydrophobic compounds, fast adsorption to the particulate phase and interaction with organic matter seems to contribute the most to the fast observed toxicity disappearance. Soil persistence is longer than that of water and therefore a single field application resulted in several, albeit short, toxic pulses in coincidence with the following rain events.

Simultaneous exposures of the amphipod *H. curvispina* and the fish *C. decemaculatus* showed the larger tolerance of the latter. Of the three most commonly used insecticides, toxic loads associated to endosulphan fumigation caused the most severe effects for amphibians and fishes, followed by the applied mixtures of chlorpyriphos, cypermethrin and glyphoste. Also, severe effects on amphibian mortalities and higher effects on plant chlorophyll were associated to the application of cypermethrin and chlorpyrifos mixture with the herbicide. Information from laboratory toxicity tests with seeds also showed enhanced toxicity of mixtures, in accordance with field observations.

Several environmental aspects influence the impact of each application. Density and height of the riparian and aquatic vegetation, together with the stage of development of the crop, represent protective barriers against pesticide transfer to the water bodies. Larger crop and riparian biomass were coincident with lower effects on the stream biota. Another point to be taken into account in relation to the magnitude of the pesticide impact during application is the water level of the stream. The climatic conditions prevailing at the applications seem also important. The largest effect observed in the studied period was coincident with an endosulphan plane application in the Pescado stream in coincidence with an intense drought. Although endosulphan was more toxic to amphibians and fishes than cypermethrin or chlorpyrifos, drought condition enhanced their effect. The insecticide concentration levels detected in major watercourses of the region, although biological effects were not measured, indicate potential pulsed acute impacts on the local biota.

Complementation between laboratory and field studies resulted of great significance for detecting erroneous predictions from the former and lightens observations from the latter. For example, cypermethrin toxicity in laboratory tests performed with fish in laboratory water point to higher effects than those observed in the field, explained by the protective effect given by the organic matter from stream water. Conversely, lower sublethal effects on vascular plants were detected in the laboratory when compared to those detected in the field for equivalent herbicide concentrations, probably associated to enhanced toxicity of mixtures.

An integrated approach analysis seems to be a promising option towards the understanding of the overall impact of intense soybean farming on associated aquatic ecosystems. Improving managerial practices and riparian habitat restorations seems feasible goals for long term sustainable production.

Streams counting with floodplains and riparian habitats showed less nitrate concentrations and less pesticide contamination, to such an extent that not any single toxic event was observed downstream Maguire wetland. Restoration of riparian habitats and leaving buffer zones between the cultures and the streams would largely improve present hazard condition. Education represents the most important tool for achieving long term environmental improvement.

Acknowledgements

The authors thank the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) for financial support.

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