Toxicity persistence in runoff and soil from experimental soybean plots following insecticide applications

ARIEL H. PARACAMPO[1], HERNÁN D. MUGNI[1], PABLO M. DEMETRIO[2,4], MARTÍN H. PARDI[3], GUSTAVO D. BULUS[2,5], MARCELO D. ASBORNO[3] and CARLOS A. BONETTO[1]

[1]*Instituto de Limnología "Dr. Raúl. A. Ringuelet" (CONICET-CCT La Plata-UNLP), Buenos Aires, Argentina*
[2]*Centro de Investigaciones del Medio Ambiente, UNLP, La Plata, Argentina*
[3]*Estación Experimental Julio Hirschhorn, UNLP, Buenos Aires, Argentina*
[4]*Concejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*
[5]*Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, La Plata, Argentina*

Persistence of toxicity in runoff water and soil was investigated in experimental soybean plots subjected to successive runoff events following pesticide application. Runoff events were produced by irrigation using a sprinkler system. The pesticides applied were cypermethrin and endosulfan, which are widely used in soy production in Argentina. Toxicity tests were performed on two abundant components of the regional fauna, the amphipod *Hyalella curvispina* and the fish *Cnesterodon decemmaculatus*. Runoffs from two pesticide applications were assayed at different stages of the growing season: an early application when the soil was almost bare and a late one close to harvest, when the ground was covered by vegetation and just before soy leaves fell. Toxicity to *H. curvispina* in runoff ceased almost one month after the early application of the two pesticides, while it persisted for over three months after the late application. Soil toxicity to *H. curvispina* and runoff toxicity to *C. decemmaculatus* followed the same pattern. Higher temperatures and solar radiation are likely to have enhanced insecticide degradation after the early application. Lower temperatures and solar radiation in combination with increased organic matter from litter probably contributed to the longer persistence of toxicity recorded after the late application, as compared with the early application. Cypermethrin caused no mortality to *C. decemmaculatus* after the early application, while endosulfan toxicity persisted for almost four months after the late one.

Keywords: Acute toxicity, cypermethrin, endosulfan, half-life.

Introduction

The Argentine Pampa is an extensive plain with a mild climate and fertile soils originally covered by grasslands. For a long time farmers employed a mixed system of livestock and crop production, mainly wheat and corn. Soy was not a traditional crop until 1996, when the genetically modified soybean resistant to glyphosate was introduced into the Argentine market. Then, with the development of novel technologies, soybean-related products (oil, flour, pellets) soon became the main Argentine output. Currently, soy accounts for approximately half of the total harvest and cultivated area (50 million tons and 15 million ha, respectively), consisting primarily of genetically modified glyphosate-resistant varieties.[1] Argentina is the world's third-largest transgenic soybean producer after the United States and Brazil. Wheat and soy varieties with a short growing period have allowed two harvests per year, wheat followed by soy. Livestock was moved to marginal areas or concentrated in feedlots. Pesticide consumption increased from 6 to 18 million kg between 1992 and 1997 and at a slower rate thereafter.[2]

Soy is attacked by a variety of insects. Soil pests such as cutworms (Agrotis ipsilon, Agrotis malefida, Peridroma saucia, Euxoa *sp.*) are often treated at early developmental stages. Defoliating caterpillars (Lepidoptera) during the growing season and, late, bugs (Hemiptera) at the fruiting stage, represent a serious threat to the crop. Cypermethrin is used for the control of the looper *Rachiplusia nu* (Lepidoptera, Noctuidae), endosulfan for the green bug *Nezara viridula* (Hemiptera, Pentatomidae) and chlorpyrifos for the borer *Epinotia aporema* (Lepidoptera, Tortricidae).

The application of pesticide mixtures is a common practice. Whenever a pesticide is to be applied, farmers usually add other pesticides in the belief that the residual action will prevent late insect infestations, and thus save the cost of gasoline by preventing further insecticide

Address correspondence to Carlos A. Bonetto, Instituto de Limnología "Dr. Raúl. A. Ringuelet" (CONICET-UNLP), Av. Calchaquí Km 23.5, 1888 Florencio Varela, Buenos Aires, Argentina; E-mail: bonetto@ilpla.edu.ar
Received October 19, 2011.

applications. These agricultural practices pose a risk to surface waters.

Marino & Ronco[3] determined the occurrence of insecticides in several Pampasic streams and rivers. Jergentz et al. [4, 5] reported toxicity pulses in streams draining intensively cultivated areas. Mugni et al[2] studied a first-order stream running through a farm cultivated following common management practices. The authors detected the occurrence of toxicity pulses, which were related to applications of cypermethrin, endosulfan and chlorpyrifos in the adjacent crop field. The relevance of runoff events in the local environment was emphasized. Pesticide runoff was more toxic to *Hyalella curvispina* than spray-drift contamination. Some pesticide applications were followed by several toxicity pulses, coincident with successive rainfall events. In contrast, no toxicity was observed after two pesticide applications during a dry period, when the following rain event occurred a month after each application. This suggests that there is a critical period affecting runoff toxicity, which may vary from one pesticide to another. An improved understanding of such critical period would provide a more accurate assessment of environmental impact.

The aim of the present study was to assess the critical period in which toxicity is transferred from the crop to the runoff in an experimental soybean crop where rain events were simulated by means of a sprinkler irrigation device. Toxicity was assessed using two organisms widely distributed in South America, the amphipod *Hyalella curvispina* and the fish *Cnesterodon decemmaculatus*. The pesticides, applied at recommended doses, were cypermethrin and endosulfan, which are commonly used in soy production in Argentina. Two pesticide applications under two different conditions were made during the soybean growing season: an early application when the soil was almost bare and a late one close to the harvest when the ground was covered with vegetation, just before soybean leaves fell forming a litter layer.

Materials and methods

Study site

This study was conducted at the Experimental Field Station of the College of Agricultural Sciences, La Plata University, located 8 km southwest of La Plata City, Buenos Aires, Argentina (35° 01′ S, 57° 59′ W). An experimental field of 80 × 60 m was divided into nine 8 × 30-m plots to be used for the experimental crop. The irrigation equipment consisted of a perimeter pipe of 3 cm in diameter, which was installed between every two plots, and 15 impact sprinkler heads (9.5 mm in diameter, Senninger Inc., model 7025) mounted at a distance of 15 m from one another. Simulated rainfall was applied at an intensity of 16 mm/h, with a drop diameter of 0.7–1 mm. Water was supplied from a 65 m-deep well and driven by a 60,000 L/h pump. The field slope was 1 %. The runoff water was collected from a small trench dug at the lower end of each plot. A 5 L bucket was buried in the trench.

The soil of the Agricultural Station was classified as Argiudol, which is characterized by a surface A horizon (30 cm deep) with silty–clay grain size (52–64 % silt and 26–34 % clay) and 4.5 % organic matter, followed by a B horizon (80–100 cm deep), with 50–60 % clay.[6] A composite sample of 10 well-mixed superficial cores taken from the experimental plots had a grain-size composition of 54 % silt, 26 % clay and 20 % sand, an organic matter content of 4.3 % and a pH of 7.8.

Experimental setup

The experimental soybean plots received two applications of cypermethrin or endosulfan, one at the beginning and the other at the end of the growing season 2008–2009, hereinafter referred to as early and late applications, respectively. Three of the experimental plots received cypermethrin, three received endosulfan and the remaining three were left untreated and used as control. Two of the control plots were placed at opposite sides of the field, while treatments were randomly assigned to the plots. However, if two replicates of the same treatment were contiguous, then the procedure was repeated to ensure that the experimental units were adequately interspersed. This approach was selected to eliminate bias in analysis of variance design for field studies that have small numbers of replicates.[7]

Seedbed preparation was carried out with conventional tillage, using two disc harrows and a single spike tooth harrow prior to mouldboard ploughing. The soy was seeded on November 28, 2008, with 45 seeds/m^2 and at a spacing of 35 cm between furrows. The early pesticide application was conducted on December 17, 2008, soon after crop emergence, and covered most of the bare soil surface. The late application was conducted on April 7, 2009, close to harvest and shortly before soy leaves fell.

Pesticides were applied using a tractor-mounted sprayer. The commonly available commercial formulations of cypermethrin (Glextrin) and endosulfan (Master) were used at doses recommended by the manufacturers (75 g a.i./ha and 1,050 g a.i./ha, respectively).

Toxicity tests

The first simulated rain event was produced immediately after pesticide application and was successively repeated until disappearance of runoff toxicity to the test organisms. Each simulated rain event ended soon after the occurrence of surface runoff flow, so that all excess water could be collected in the buckets. Only a negligible amount of runoff leaked slowly from the plots after the buckets were removed. The runoff water was then transferred to dark bottles and immediately transported to the laboratory in coolers with ice.

Toxicity of runoff water to the amphipod *Hyalella curvispina* and the fish *Cnesterodon decemmaculatus* was assessed by means of laboratory toxicity tests. Procedures for *H. curvispina* were similar to standardized protocols for other invertebrate species.[8] Three replicates from each plot were tested. Ten *H. curvispina* individuals 5–10 mm in length were exposed to 100 mL of runoff water in 250-mL beakers. Tests were conducted without feeding, at 22 ± 2°C and under natural photoperiod conditions. Mortality was recorded after 48 h of exposure. As a validity criterion for the negative control, less than 10 % mortality was considered acceptable.

The soil toxicity tests using *H. curvispina* were based on standardized protocols for testing sediment toxicity to *Hyalella azteca*.[8] Ten individuals 5–10 mm in length were exposed to an amount of soil equivalent to 15 g dry weight, and 150 mL of reconstituted freshwater,[9] in 250-mL beakers, in triplicate. Mortality was recorded after 10 d of exposure. *H. curvispina* individuals were fed laboratory-cultured algae *ad libitum* every two days. As a validity criterion for the negative control, less than 20 % mortality was considered acceptable.

Specimens of *H. curvispina* were originally collected from an uncontaminated stream located 25 km south of La Plata City and transported to the laboratory, where they were reared for several weeks. They were kept in large plastic containers with stream water, which was gradually replaced with dechlorinated tap water. The macrophyte *Lemna* sp. was placed on the surface of the water. *H. curvispina* individuals fed on the periphytic community of the *Lemna* rhizosphere and received a supplement of a mixture of fresh lettuce leaves and dried algae twice a week.

Laboratory toxicity tests with the fish *Cnesterodon decemmaculatus* were performed on water samples taken from each plot immediately after every irrigation event, following standardized procedures described by the US EPA.[10] Ten adult fish 15–20 mm in length were exposed to 1 L of runoff water in 2 L glass beakers for 96 h, in triplicate. Tests were conducted without feeding, at 22 ± 2°C and under natural photoperiod conditions. The fish were originally collected from the same stream as the amphipod, reared in the laboratory for several weeks and fed with commercial food until used in the bioassays.

Physical and chemical analysis

Weather conditions (solar radiation, humidity, and temperature) were recorded at an automatic meteorological station (Industrial Groweather, Davis Instruments) located about 300 m from the experimental plots. Soil temperature and moisture were measured with sensors ETC and ECH2O EC-5 (Decagon Devices, Inc.), respectively, buried at a depth of 10 cm. Data were recorded every 60 min with an Em5b data logger.

Temperature, conductivity (model HI 8733, Hanna Instruments) and pH (model 250A, Orion Research, Inc.) in the irrigation and runoff water were measured in situ throughout the experiments.

Runoff water samples were passed through C18 columns (Agilent, solid phase extraction) and frozen until pesticide analysis. Extracts were eluted from the C18 columns with 5 mL hexane followed by 5 mL dichloromethane. The sample extracts were injected into a GC-ECD (Carlo Erba 6,000), equipped with an HP5 column, 15 m and 0.53 ID, N_2 carrier, ramp and detector temperatures of 190–250 and 320°C, respectively. The solvents used for pesticide analysis were obtained from J.T. Baker. Standards used for calibration were obtained from Accustandard®. The endosulfan standard consisted of a mixture of approximately 45 and 55 % of alpha and beta isomers respectively; the detection limit was 0.01 μg/L for both isomers. Alpha and beta endosulfan signals were joined together and reported as a single measurement. Endosulfan sulphate was not measured. The detection limit for cypermethrin was 0.025 μg/L.

Statistical analysis

For each test organism (*H. curvispina* and *C. decemmaculatus*), the persistence of toxicity following rainfall events was expressed as the 50 % lethal time (LT50), estimated from mortality data for each replicate of each treatment (cypermethrin and endosulfan). Values of LT50 were calculated by Probit analysis or the trimmed Spearman-Karber method, where appropriate.

Differences between treatments were determined by the t-test for independent samples when data met the assumptions of homoscedasticity and normality; otherwise the equivalent non-parametric Mann-Whitney test was used. The significance level was set at $P < 0.1$.

The values of pesticide concentrations obtained from the successive runoff events were fitted to an exponential function of time since application by a non-linear estimation procedure. The half-life of each pesticide was estimated as ln 2/k, k being the exponent of the decay function.[11] Differences between the half-lives of the assayed pesticides were evaluated by comparing the slopes obtained with log-linear transformed data, using the t-test.

Results and discussion

Table 1 summarizes results of the environmental variables measured after each application. Mean solar radiation and soil temperature during the month following the early application were about 69 % and 10°C higher, respectively, than the corresponding values after the late application. The irrigation water was alkaline; water pH and conductivity ranged from 8.0 to 8.8 and from 578 to 806 μS/cm, respectively.

After the early application, the concentrations of the pesticides in the first runoff event remained proportional to the doses applied, with higher values for endosulfan than for

Table 1. Mean values of the environmental variables measured during the month following the early and late applications.

Application date	*Application time*	*Solar radiation Wat/m²*	*Soil temp. °C*	*Soil humidity % sat.*	*Air temp. °C*	*Air humidity % sat.*
December 17, 2008	Early	6033	24.5	68	24.1	58
April 7, 2009	Late	3570	14.4	75	16.3	70

cypermethrin (Table 2). Pesticide concentrations over successive rainfall events decreased with time since application and were fitted with exponential decay functions of time ($r = 0.99$ and 0.96 for cypermethrin and endosulfan, respectively). Estimated half-lives ranged from 19 to 20 days, with no significant differences between pesticides.

Following the early application, mortality of *H. curvispina* exposed to runoff was detected until the fifth event, 28 days after the application of both pesticides (Fig. 1). The LT50 was significantly longer ($P < 0.021$, t-test) for cypermethrin (21.2 ± 3.1 days) than for endosulfan (10.4 ± 4.0 days). In regard to the late application, mortality of *H. curvispina* exposed to pesticide runoff remained at 100 % mortality until 42 days after application with a gradual decrease thereafter. Mortality of *H. curvispina* ceased 98 days after the application of cypermethrin and endosulfan in the plots. The estimated LT50 values were 68.0 ± 11.2 and 67.8 ± 4.2 days for endosulfan and cypermethrin, respectively, which were significantly longer than those recorded for the early application ($P < 0.003$ and $P < 0.0007$, respectively, t- test).

Toxicity of runoff to *C. decemmaculatus* showed a quite different pattern from that of *H. curvispina* (Fig. 2). After the early application, the runoff from cypermethrin-treated plots caused no significant mortality to *C. decemmaculatus*, and no mortality was observed 14 days after the late application. In contrast, the mortality of fish exposed to the runoff from endosulfan-treated plots remained at 100 % for as long as 56 days and decreased gradually to 23 % in the last sampling, 84 days after the early application, while it remained at 100 % until the end of the experiment, 140 days after the late application. The LT50 of fish exposed to the runoff from endosulfan-treated plots was 76.2 ± 6.6 days for the early application. Assuming a LT50 of 140 days for the late application, toxicity persistence was significantly longer than that after the early application ($P < 0.08$, Mann-Whitney one tailed U-test). Persistence of toxicity in runoff from endosulfan-treated plots was longer for *C. decemmaculatus* than for *H. curvispina* ($P < 0.0001$, t test).

Results of the soil toxicity tests using *H. curvispina* showed that mortality ceased 42 days after the early application of cypermethrin and 119 days after the late one (Fig. 3). After the early application, the LT50 of *H. curvispina* exposed to soil from cypermethrin-treated plots was 23.6 ± 0.6 days, a value not significantly different from that obtained for the runoff ($P < 0.51$, Mann Whitney test). Soil toxicity to *H. curvispina* ceased 69 days after the early application of endosulfan. The LT50 of *H. curvispina* exposed to the soil from the endosulfan-treated plots was 55.3 days for the three replicates (trimmed Spearman-Karber method), which is significantly longer than that obtained for the runoff ($P < 0.04$, Mann Whitney test). The LT50 of *H. curvispina* exposed to the soil from

Table 2. Mean pesticide concentrations (± standard deviation) in runoff events following the early application.

Runoff event	*Days after application*	*Cypermethrin (μg/L)*	*Endosulfan (μg/L)*
17 Dec. 2008	0	0.10 (±0.01)	0.66 (±0.34)
23 Dec. 2008	6	0.09 (±0.04)	0.62 (±0.24)
30 Dec. 2008	13	0.08 (±0.02)	0.41 (± 0.13)
05 Jan. 2009	19	0.04 (±0.03)	0.35 (±0.07)

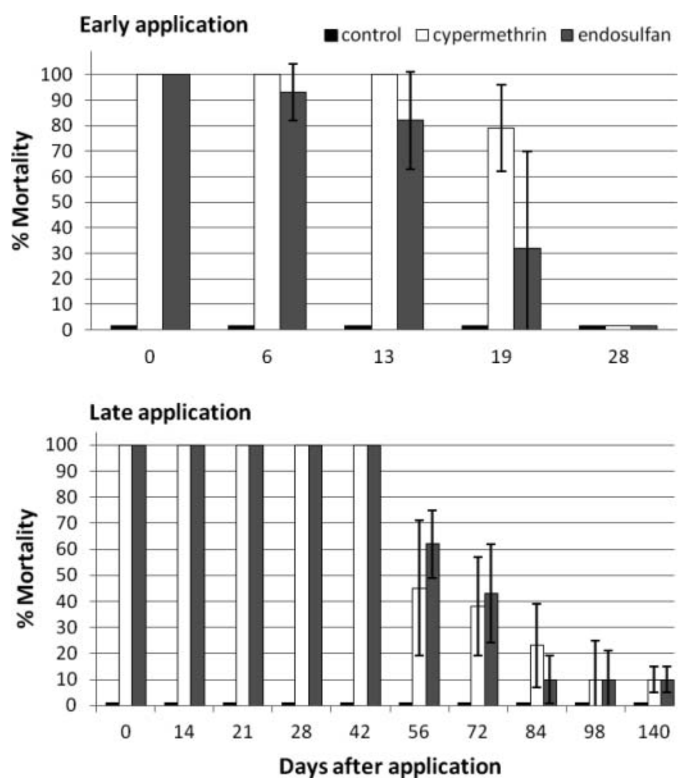

Fig. 1. Toxicity of cypermethrin and endosulfan to the amphipod *H. curvispina* exposed to runoff from successive simulated rain events occurring after an early (December 17, 2008) and a late (April 7, 2009) application. Error bars represent the standard deviation.

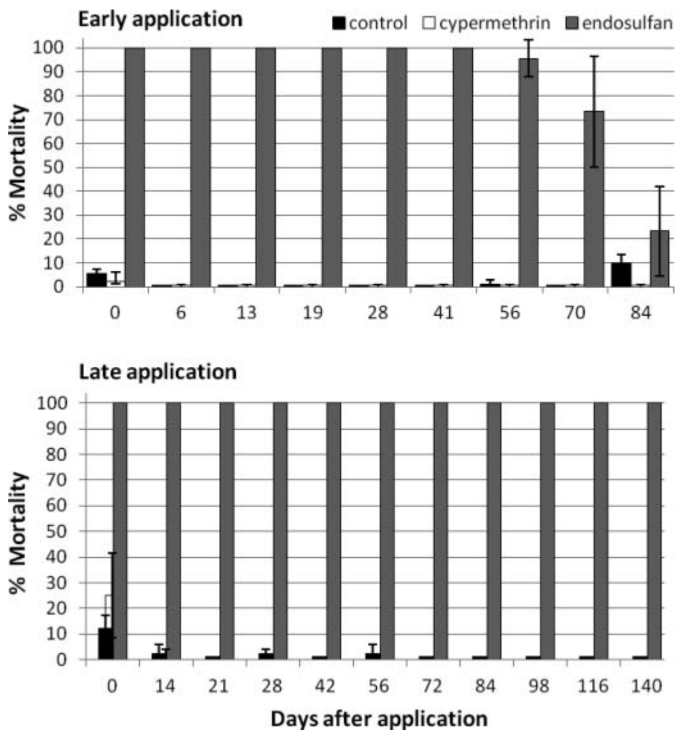

Fig. 2. Toxicity of cypermethrin and endosulfan to the fish *C. decemmaculatus* exposed to runoff from successive simulated rain events occurring after an early (December 17, 2008) and a late (April 7, 2009) application. Error bars represent the standard deviation.

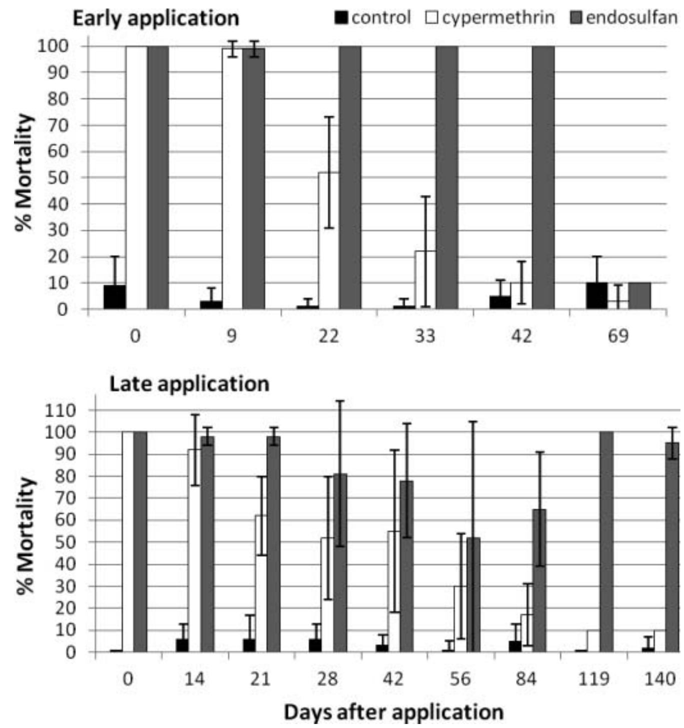

Fig. 3. Toxicity of cypermethrin and endosulfan to the amphipod *H. curvispina* exposed to soil following successive simulated rainfall events occurring after an early (December 17, 2008) and a late (April 7, 2009) application. Error bars represent the standard deviation.

the endosulfan-treated plots was significantly longer than that from the cypermethrin-treated plots ($P < 0.04$, Mann Whitney test). In the late application, mortality exhibited a gradual decrease followed by an increase, attaining high values until the end of the experiment (140 days later). The border between the recently formed litter layer and the soil was initially sharp but it became progressively blurred at the last samplings. There was a high variability among replicates for both pesticides studied.

Differences between application timings

The persistence of toxicity was highly variable depending on the application timing. For both cypermethrin and endosulfan, runoff toxicity to the amphipod *H. curvispina* and the fish *C. decemmaculatus* and soil toxicity to *H. curvispina* persisted roughly 2–3 times longer after the late application as compared to the early application. Environmental conditions prevailing after each application are likely to have influenced pesticide persistence. Mean solar radiation was 69 % higher and mean soil temperature was 10°C higher in the month following the early application (late spring and early summer), than in the month following the late application (autumn) (Table 1).

Volatilization, photodegradation, and chemical and microbial degradation represent major routes of pesticide loss.[12] Field studies have shown that the dissipation rates of pesticides from soil were faster in tropical Brazil than in temperate regions; all the studied pesticides showed a faster dissipation, which was attributed to increased volatilization and microbial and chemical degradation resulting from high soil temperatures.[12] A fast dissipation of endosulfan from the soil was also recorded in sub-tropical India, which was explained by increased volatilization rates due to high temperatures.[13] Likewise, the dissipation of endosulfan from cotton soils was found to be faster in Australia than in most of the cold temperate regions. [14] The dissipation pattern of endosulfan from the soil was better described by a second-order kinetic function. The initial rapid dissipation was assumed to be dependent on volatilization and photolysis; it was followed by a slower second- phase decline related to steady microbial and chemical degradation.[14] Soil and water pH may also affect degradation rates. High pH is known to increase the loss of cypermethrin[15] and endosulfan. [13] The half-life of endosulfan in water at pH 5, 7, and 9 was 28, 5.7, and 0.7 days, respectively.[14] In the present study, high pH in the soil and irrigation water (8.0–8.8) favored a faster dissipation of pesticides from runoff water. The lower solar radiation and temperatures prevailing after the late application may have contributed to the longer persistence of toxicity by reducing volatilization, photolysis and degradation losses. In the late application, the spray settled on the soy foliage. Soy leaves fell soon after the application and the organic litter layer on the ground probably had a scavenging effect on the hydrophobic pesticides, thus contributing to their longer persistence as compared with the early application. The fact that the toxicity of endosulfan in soil showed an initial decrease followed by an increase could be accounted for by pesticide leaching from the litter layer and subsequent incorporation of litter into the soil. The high variability among replicates and sampling dates seems likely to be related to substantial spatial heterogeneity of the accumulated organic debris. Kennedy et al[14] reported that the mean half-life of endosulfan in litter and trash from several cotton plots located on commercial farms was 61 days, an order of magnitude higher than that in soils (7 days). This evidence suggests that extensive sorption of pesticides to the organic substrate contributed to the observed delay in pesticide mineralization after the late application in the present study.

Differences between test organisms

Cypermethrin toxicity persisted longer for *H. curvispina* than for *C. decemmaculatus*, likely due to a higher sensitivity of the former. Giddings et al,[16] who conducted mesocosm and field studies focused on the toxic effects of cypermethrin, showed a sensitivity ranking for test organisms, with amphipods being among the most sensitive species and fish among the least sensitive species. Amphipods populations declined and failed to recover in mesocosms exposed to 0.030 μg/L cypermethrin, while exposure to 0.1 μg/L had no effect on the fish *Cyprinus carpio*. The reported mean LC50 for different amphipod species was 0.021 μg/L, orders of magnitude smaller than the reported mean value for fish species (2.7 μg/L).[16] In the present study, there was no mortality to the fish *C. decemmaculatus* exposed to runoff following the early application of cypermethrin, while the late application caused 25 % mortality in the worst-case scenario corresponding to rainfall occurring immediately after application and ending a few days later. Carriquiriborde et al[17] studied the effect of cypermethrin applications on a stream-resident population of *C. decemmaculatus*. Fish, which were caged in a first-order stream born within a soybean plot, experienced no mortality associated with applications of cypermethrin during the 2-year study. Results of the toxicity tests carried out by these authors revealed that the LC50 values were higher for stream water than for laboratory synthetic water, the difference being related to the organic and suspended matter content of the former.[17] In the present study, the concentrations of cypermethrin in runoff water were below the LC50 value for stream water (5 μg/L) reported by Carriquiriborde et al [17].

Conversely, the persistence of endosulfan toxicity was longer for *C. decemmaculatus* than for *H. curvispina,* probably due to higher sensitivity of the former. The toxicity of endosulfan in runoff to the fish *C. decemmaculatus* was extremely persistent, with mortalities occurring within 3 months after the early application (Fig. 2) and remaining at 100 % at the last sampling, almost 5 months after the late application. Sunderam et al [18] reported low LC50 values of endosulfan for several fish species as follows: 0.1 μg/L for *Cyprinus carpio* (Cypriniformes Cyprinidae), 0.2 μg/L for *Nematolosa erebi* (Clupeiformes Clupeidae), 0.5 μg/L for *Macquaria ambigua* (Perciformes Percichthyidae) and 1.6 μg/L for *Oncorhynchus mykiss* (Salmoniformes Salmonidae). The LC50 values of endosulfan reported for *Jenynsia multidentata* (Cyprinodontiforme Anablepidae) were 0.72 μg/L for males and 1.33 μg/L for females. [19] Repeated fish kills in New South Wales, Australia, were related to contamination of runoff with endosulfan.[20] The LC50 value of endosulfan for the Australian fish fauna fell within the range of 0.2 to 11 μg/L, while the concentrations of endosulfan in runoff from irrigated cotton farms typically ranged between 2 and 10 μg/L. [14] Pablo & Hyne[20] studied the effect of endosulfan loads on the golden perch *Macquaria ambigua*, a native Australian fish species, using a stream mesocosm system and simulating runoff events. The authors found that caged juveniles exposed to short pulses of endosulfan in natural waters experienced significant mortalities at a concentration as low as 0.09 μg/L.

Runoff toxicity

Conventional management practices for soybean production result in pulses of runoff toxicity following pesticide applications. In the present study, toxicity to *H. curvispina* ceased 28 days after the early application of cypermethrin and endosulfan. These results are consistent with previous field observations. Mugni et al[2] investigated a small first-order stream running through a cultivated farm, which was close to and under similar environmental conditions as the experimental plots studied here.

The researchers evaluated four pesticide applications. On two occasions, the first rain events occurred 4 (65 mm) and 10 days (38 mm) after spraying. A mixture of cypermethrin and chlorpyrifos was applied before the former rainfall event and a mixture of cypermethrin and endosulfan before the latter one. Tests of toxicity to *H. curvispina* in runoff from both events resulted in 100 % mortality. On the other two occasions, the first rain events occurred 30 (130 mm) and 33 days (32 mm) after spraying. A mixture of cypermethrin and endosulfan was applied before the former rain event, and a mixture of cypermethrin plus several herbicides before the latter one. No mortality occurred among *H. curvispina* exposed to the runoff from both events.

Pesticide mixtures are commonly applied to soybean crops 2–3 times per growing season. In the studied area, runoff occurs when rainfall exceeds about 20 mm. In the last 10 years, the average frequency of rainfall events greater than 20 mm was 1.3 per month between November and

April (unpublished). These data suggest that water bodies adjacent to crops receive several pulses of runoff toxicity to the invertebrate fauna per growing season.

On the other hand, the longer persistence of endosulfan toxicity to *C. decemmaculatus* obtained in the present study suggests a larger effect on fish than on resident invertebrate assemblages. If endosulfan is applied early and late in the crop's growth cycle, the toxic runoff events last for most of the year. Kennedy et al[14] analyzed the concentrations of endosulfan in irrigated cotton farms in Australia. Endosulfan, which was applied 3–4 times per season, was found in all the runoff water samples from the field throughout the entire cotton-growing season. After each application, endosulfan concentration in runoff samples was 5–10 μg/L, decreasing to about 2 μg/L after one month. The concentrations recorded were toxic to local fish fauna throughout the whole growing period.

Conclusions

Conventional pesticide applications to soy caused pulses of runoff toxicity following spraying. The persistence of toxicity varied considerably depending on the environmental conditions prevailing at the time of spraying. For the two pesticides studied, toxicity to the amphipod *H. curvispina* persisted for almost one month after the early application. A late application performed at the end of the growing period resulted in much longer persistence, up to roughly three months following spraying.

Soil toxicity to the amphipod *H. curvispina* and runoff toxicity to the fish *C. decemmaculatus* showed a similar trend. High solar radiation and temperatures prevailing in summer are likely to have contributed to the fast toxicity dissipation because of increased volatilization and photo and microbial degradation. Soy defoliation took place at the end of the growing period before harvest, forming a litter layer. Toxicity persisted longer after the late application likely due to insecticide sorption to the newly formed organic debris along with the prevailing lower temperatures and solar radiation during autumn.

Toxicity of cypermethrin and endosulfan in runoff to *C. decemmaculatus* persisted for a short and a long time, respectively. No fish mortality was observed after the early application of cypermethrin and its toxicity ceased shortly after the late application. In contrast, endosulfan toxicity persisted for almost three months after the early application and almost five months after the late one. The overall effect of spraying soybean crops on the adjacent water bodies depends on rainfall frequency and management practices involving choice of pesticide, dosage and timing of applications. The present results confirm previous studies reporting that cypermethrin is the most environmentally friendly pesticide used currently, while endosulfan poses a serious threat to the fish fauna inhabiting surface waters at the region level.

Acknowledgments

Thanks are due to Dr. Alicia Ronco for her assistance in the analysis of the pesticides and to Ing. Barreiro, director of the Experimental Field Station, for his support and encouragement in the field work. Thanks are also due to the unknown reviewers for their valuable comments and suggestions. This research was supported by grants from the National Scientific and Technical Research Council (CONICET), Argentina and the National Agency for Scientific and Technological Promotion (ANPCyT), Argentina.

References

[1] MAGyP Ministerio de Agricultura, Ganadería y Pesca. Series y Estadísticas. Available at http://190.220.136.179/index.php/series-por-tema/agricultura (retrieved July 2009).

[2] Mugni, H.; Ronco, A.; Bonetto, C. Insecticide toxicity to *Hyalella curvispina* in runoff and stream water within a soybean farm (Buenos Aires, Argentina). Ecotoxicol. Environ. Saf. **2011**, *74* (3), 350–354. DOI 10.1016/j.ecoenv.2010.07.030.

[3] Marino, D.; Ronco, A. Cypermethrin and chlorpyrifos concentration levels in surface water bodies of the Pampa Ondulada, Argentina. Bull. Environ. Contam. Toxicol. **2005**, *75* (4), 820–826.

[4] Jergentz, S.; Mugni, H.; Bonetto, C.; Schulz, R. Runoff-related endosulfan contamination and aquatic macroinvertebrate response in rural basins near Buenos Aires, Argentina. Arch. Environ. Contam. Toxicol. **2004**, *46* (3), 345–353.

[5] Jergentz, S.; Pessacq, P.; Mugni, H.; Bonetto, C.; Schulz, R. Linking in situ bioassays and dynamics of macroinvertebrates to assess agricultural contamination in streams of the Argentine Pampa. Ecotoxicol. Environl. Saf. **2004**, *59*, 133–141.

[6] Mugni, H. Concentración de nutrientes y toxicidad de pesticidas en aguas superficiales de cuencas rurales. 2009, Tesis doctoral, Universidad de La Plata: Buenos Aires, Argentina; 140 pp.

[7] Farmer, D.; Hill, I.R.; Maund, S.J. A comparison of the fate and effects of two pyrethroid insecticides (lambda-cyhalothrin and cypermethrin) in pond mesocosms. Ecotoxicology **1995**, *4* (4), 219–244.

[8] USEPA. *Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, second ed*, EPA 600/R-99/064; USEPA Office of Water: Washington, DC, 2000.

[9] American Public Health Association. *Standard methods for the examination of water and wastewater*. American Public Health Association Washington, D.C. 1998; 1193 pp.

[10] USEPA *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, fifth ed*, EPA-821-R-02–012; USEPA Office of Water: Washington, DC, 2002.

[11] Zar, J. *Biostatistical Analysis,* 5th Edition; Prentice Hall: Englewood Cliffs, 2009; 718 pp.

[12] Laabs, V.; Amelung, W.; Pinto, A.; Altstaedt, A.; Zech, W. Leaching and degradation of corn and soybean pesticides in an Oxisol of the Brazilian Cerrados. Chemosphere **2000**, *41*, 1441–1449.

[13] Kathpal, T.S.; Singh, A.; Dhankhar, J.S.; Singh, G. Fate of endosulfan in cotton soil under sub-tropical conditions of northern India. Pestic. Sci. **1997**, *50*, 21–27.

[14] Kennedy, R.; Sánchez-Bayo, F.; Kimber, S.W.; Hugo, L.; Ahmad, N. Off-Site Movement of endosulfan from irrigated cotton in New South Wales. J. Environ. Qual. **2001**, *30*, 683–696.

[15] Laskowski, D. Physical and chemical properties of pyrethroids. Rev. Environ. Contam. Toxicol. **2002**, *174*, 49–177.

[16] Giddings, J.M.; Solomon, K.R.; Maund, S.J. Probabilistic risk assessment of cotton pyrethroids: II. Aquatic mesocosm and field studies. Environ. Toxicol. Chem. **2001**, *20* (3), 660–668.
[17] Carriquiriborde, P.; Díaz, J.; Mugni, H.; Bonetto, C.; Ronco, A. Impact of cypermethrin on stream fish populations under field use in biotech-soybean production. Chemosphere **2007**, *68*, 613–621.
[18] Sunderam, R.I.; Cheng, D.M.; Thompson, G.B. Toxicity of endosulfan to native and introduced fish in Australia. Environl. Toxicol. Chem. **1992**, *11*, 1469–1476.
[19] Ballesteros, M.L.; Bianchi, G.E.; Carranza, M.; Bistoni, M.A. Endosulfan acute toxicity and histomorphological alterations in Jenysia multidentata (Anablepidae, Cyprinodontiformes). J. Environ. Sci. Health. B. **2007**, *42*, 351–357.
[20] Pablo, F.; Hyne, R.V. Endosulfan application to a stream mesocosm: studies on fate, uptake into passive samplers and caged toxicity test with the fish M. ambigua. Arch. Environ. Contam. Toxicol. **2009**, *56*, 525–535.