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Toxicity Persistence in Runoff Water and Soil in Experimental Soybean Plots Following Chlorpyrifos Application

Hernán Mugni · Pablo Demetrio · Ariel Paracampo · Martín Pardi · Gustavo Bulus · Carlos Bonetto

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Abstract Toxicity persistence in runoff water and soil was studied in experimental soybean plots in successive runoff events produced by an irrigation system. Three chlorpyrifos applications throughout the growing period were assayed. Runoff and soil toxicity to the amphipod *Hyalella curvispina* and the fish *Cnesterodon decemmaculatus* was assessed. Toxicity persistence to *H. curvispina* was shorter in the early and midseason applications (23–28 and 21–69 days in runoff and soil, respectively) and longer in the late application (more than 140 days). The same trend was observed for *C. decemmaculatus*: 13 days for early and 56 for the late application.

Keywords Insecticides · Acute toxicity · *Hyalella curvispina* · *Cnesterodon decemmaculatus*

H. Mugni · A. Paracampo · C. Bonetto (✉)
ILPLA, Instituto de Limnología ''Dr. Raúl. A. Ringuelet'' (CONICET-CCT La Plata-UNLP), Av. Calchaquí Km 23.5, 1888 Florencio Varela, Buenos Aires, Argentina
e-mail: bonetto@ilpla.edu.ar

P. Demetrio
CIMA, Centro de Investigaciones del Medio Ambiente, Facultad de Ciencias Exactas, UNLP, Calle 47 y 115, 1900 La Plata, Argentina

P. Demetrio
CONICET, Concejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

M. Pardi
Estación Experimental Julio Hirschhorn, Facultad de Ciencias Agrarias y Forestales, UNLP, Av. 66 y 168, La Plata, Buenos Aires, Argentina

G. Bulus
CIC, Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, La Plata, Argentina

The Argentine Pampa is an extensive plain of mild climate and fertile soils originally covered by grasslands. At present, soybean represents roughly half of the total harvest and cultivated area (50 million tons and 15 million ha, respectively, Mugni 2009), consisting mainly of the genetically modified variety resistant to glyphosate. Argentina is the world's third largest transgenic soybean producer after the USA and Brazil. Pesticide consumption increased from 6 to 18 million kg in the period from 1992 to 1997 alone and has continued to increase, at slower rates, since then (Mugni et al. 2011). Chlorpyrifos is often applied in early crop growth to control soil insects and cutworms (*Agrotis ipsilon, Agrotis malefida, Peridroma saucia, Euxoa sp.). D*uring the growing season, defoliating caterpillars (Lepidoptera) and later, at the fruiting stage, bugs (Hemiptera) and the borer *Epinotia aporema*, (Lepidoptera, Tortricidae) are usually controlled with chlorpyrifos.

Marino ands Ronco (2005) determined the occurrence of chlorpyrifos in several Pampasic rivers. Jergentz et al. (2004a, b) reported toxicity pulses in streams draining intensively cultivated areas. Mugni et al. (2011) studied toxicity of cypermethrin and chlorpyrifos in a first order stream draining plots cultivated under common managerial practices. Toxicity pulses were recorded and related to pesticide applications in the adjacent crop. The importance of runoff events was emphasized. Within the local environment toxicity pulses produced by runoff were stronger than the drift related contamination.

The aim of the present study was to assess the critical period in which chlorpyrifos 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. Three different conditions were assayed; applications were produced early in the soybean growing period, with the soil almost bare, at an intermediate stage, with the soil covered by the foliage, and close to the harvest, soon before the soybean lost the leaves that form a litter layer on the soil surface.

Materials and Methods

This study was performed at the Experimental Field Station of the School of Agronomic Science at La Plata University, located 8 km southwest of La Plata city, Buenos Aires, Argentina (35° 01′ S, 57° 59′ W). Experimental plots of 8 × 30 m were seeded with soybean. Irrigation sprinkler equipment was installed. It consisted of a perimeter pipe, 3 cm in diameter, provided with 9 sprinkler heads mounted at a distance of 15 m from one another. Each impact sprinkler head was a Senninger 7025 model, 9.5 mm in diameter, providing a simulated rain of 16 mm/h with drops of 0.7–1 mm in diameter. The whole system was fed with water from a perforation pumped with a 60,000 L/h pump. The field has a slope of 1 %. At the lower end of each plot a small trench was excavated into the soil in order to capture the runoff water. A 5 liter bucket was buried in the trench.

Weather conditions (irradiance, temperature) were recorded with an automatic meteorological station (Davis Instruments station, Groweather Industrial model), located about 300 m from the experimental plots. Soil temperature and moisture were measured with Decagon ETC. and Echo20/ec5 sensors, respectively, stuck 10 cm deep in the soil and recorded every 60 min with a Decagon device model Em5b datalloger.

The Agricultural Station contains Argiudol soils characterized by an A surface horizon, 30 cm deep, of grain-sized silty clay (52 %–64 % silt and 26 %–34 % clay), followed by a B horizon, 80–100 cm deep, with 50 %–60 % clay; organic matter content was 4.5 % and soil pH 7.8 (Mugni 2009).

Two successive soybean crops were studied during the 2008/2009 and 2009/2010 growing periods. Two chlorpyrifos applications were made at the beginning and end of the 2008/2009 growing period, referred to hereinafter as the early and late applications. A single chlorpyrifos application was made in the 2009/2010 growing period, when the crop attained soil cover, referred to hereinafter as the midseason application.

In each application three plots were treated with chlorpyrifos while another three remained as controls. The preparation of the soil for the seedbed was carried out with conventional tillage; two disc harrows and one spike tooth harrow were passed previous to the mouldboard plough. In the 2008/2009 growing period, the soybean was seeded on 28 Nov. 2008. Soon after emergence, on 17 Dec. 2008, the early pesticide application was performed over the almost bare soil. The late application was performed close to the harvest, soon before the soybean leaves fell, on 7 April 2009. In the 2009/2010 growing period, the soybean was seeded on 8 Nov. 2009, in the same way as in the previous year. The midseason chlorpyrifos application was performed on 28 Dec. 2009, when the soybean attained soil cover. Chlorpyrifos was applied using a tractor-mounted sprayer at the doses recommended by the producers: 2,000 ml/ha of Terfos commercial formulation (48 g chlorpyrifos per 100 ml). These doses represent 960 g a.i. per hectare.

The first simulated rain episode was produced immediately after chlorpyrifos application and was successively repeated until toxicity ceased. Each simulated rain event lasted until a surface runoff flux was observed, and stopped soon thereafter, in order to gather the whole runoff excess in the buckets. The runoff was transferred to dark bottles and immediately transported to the laboratory in coolers. The toxicity of the runoff water to the amphipod *Hyalella curvispina* and the fish *Cnesterodon decemmaculatus* was assessed by means of laboratory toxicity tests. Procedures for *H. curvispina* followed standardized protocols described for *H. azteca* (USEPA 2000). Three replicates from each plot were assessed. Ten *H. curvispina* of 5–10 mm length were exposed in 100 ml of runoff water in 250 ml beakers. Tests were carried without feeding, at 22 ± 2°C and natural photoperiod, assessing mortality after a 48 h exposure. As a validity criterion, less than 10 % mortality was considered as no effect.

Soil toxicity tests to *H. curvispina* were performed following USEPA (2000). Ten *H. curvispina* measuring 5–10 mm were exposed to an amount of soil equivalent to 15 g dry weight, and 150 ml of reconstituted fresh water (APHA 1998) in 250 ml beakers, in triplicate. Mortality was assessed after a 10 day exposure. *H. curvispina* were fed every two days with algae obtained from cultures. Mortalities lower than 20 % in soil exposures were considered as no effect.

H. curvispina were originally sampled from an uncontaminated stream, 25 km south from La Plata. They were later bred in the laboratory for several weeks.

Laboratory toxicity tests with the fish *Cnesterodon decemmaculatus* were performed in water samples taken from each plot immediately after every irrigation event, following standardized procedures described by USEPA (2002). Ten adult fish of 15–20 mm length were exposed in 2 L glass beakers during 96 h, in triplicate. Tests were carried without feeding, at 22 ± 2°C and natural photoperiod. The fish were originally sampled from the same stream as the amphipod and reared in laboratory for several weeks until used in the laboratory assays.

In each runoff event water temperature, conductivity (Hanna Instruments 8733) and pH (Orion 250 A) were measured in situ.

Runoff water samples were passed though C18 columns (Agilent, solid phase extraction). 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 a HP5 column, 15 m and 0.53 ID, N_2 carrier, ramp and detector temperatures: 190–250°C and 320°C, respectively. Solvents used were J. T. Baker for pesticide analysis. Standards utilized for calibration were Accustanard®. Chlorpyrifos detection limit was 0.01 μg/L.

Mortality data in each runoff event following pesticide application were used to estimate the LT50 by means of the Probit analysis. Whenever the Probit analysis could not be performed, the LT50 was estimated using the trimmed–Spearman–Karber method. Differences among treatments were assessed by means of a t test for independent samples. Whenever the required conditions (homoscedasticity, normality) for *t* test utilization were not attained, equivalent non parametric methods were used, such as the Mann–Whitney tests. Significance level for all the applied tests was 0.1.

Results and Discussion

During the month following the early and midseason applications mean irradiance and soil temperature were about 66 %–68 % and 8–10°C higher than in the late application (Table 1). The irrigation water was alkaline; water pH ranged from 8.0 to 8.8 and conductivity from 578 to 780 μS/cm.

The chlorpyrifos concentrations in successive runoff events after the early application decreased with time following an exponential function ($r = 0.91$). Chlorpyrifos decreased from 0.35 μg/L immediately after spraying to 0.12 μg/L a week later, 0.09 μg/L 2 weeks later and 0.06 μg/L 19 days after application. Runoff events following the early application produced mortality to *H. curvispina* until the fifth event, 28 days after the application (Fig. 1). Mortality in the chlorpyrifos treated plots dropped from 100 % 19 days after application to 0 % 28 days after application. Its LT50 was estimated at 23 days (trimmed, Spearman-Karber). No mortality was observed in the control treatments.

Table 1 Mean values of irradiance and soil temperature measured during the month following each application

Applic. time	Applic. date	Irradiance (Wat./m^2)	Soil temp (°C)
Early	17 Dec. 2008	6,033	24.5
Midseason	28 Dec. 2009	5,926	22.1
Late	7 April 2009	3,570	14.4

Soil toxicity to *H. curvispina* ceased 42 days after the application in the chlorpyrifos treatments (Fig. 1). Mortality in the control treatments fell below the non effect concentration (USEPA 2000). Chlorpyrifos LT50 was estimated at 58.6 days (Trimmed, Spearman-Karber).

In the midseason application, chlorpyrifos concentrations immediately after application were an order of magnitude higher than in the early application (3.6 μg/L), decreased to 0.5 μg/L a week later, to became undetec table 2 weeks after spraying. Runoff toxicity to *H. curvispina* ceased 23 days after application (Fig. 2). Estimated LT50 was 17.4 ± 0.12. Soil toxicity to *H. curvispina* ceased 21 days after application (Fig. 2). Estimated LT50 was 13.1 ± 0.5 days. No mortality was observed in the control treatments.

The soybean lost leaves soon after the late application resulting in a litter layer covering the soil surface. Toxicity persistence to *H. curvispina* in the runoff remained at 100 % mortality until the fifth irrigation event, 42 days after application, and decreased slowly after that (Fig. 3). Chlorpyrifos still produced roughly 30 % mortality at the end of the experiment, 140 days after fumigation. Estimated LT50 was 92.8 ± 8.4 days, significantly longer than in the midseason application ($p < 0.08$, One tailed U Mann–Whitney).

Chlorpyrifos mortality in soil was 100 % until 84 days after spraying, remaining high (80 %) at the end of the experiment, 140 days after application (Fig. 3). The LT50 was thus longer than 140 days, significantly longer than in the midseason application ($p < 0.006$, student test).

Runoff toxicity to *C. decemmaculatus* resulted in 47 % mortality in the first irrigation event, immediately after the early application, and ceased in the second one, 6 days later (Fig. 4). By contrast, runoff toxicity to *C. decemmaculatus* ceased 42 days after the late application.

Throughout the present study, toxicity persistence showed an extended variation range in relation to the application time, highlighting the importance, for the observed persistence, of the environmental conditions prevailing during each application. The early and midseason applications are representative of the conditions prevailing in most of the growing period and provided essentially the same results: runoff toxicity to *H. curvispina* persisted until almost a month after application (23–28 days). By contrast, runoff toxicity remained longer when the application was made close to the end of the growing period, attaining 30 % mortality almost 3 months after the application. Soil toxicity to *H. curvispina* and runoff toxicity to *C. decemmaculatus* followed the same trend, attaining longer persistence in the late application.

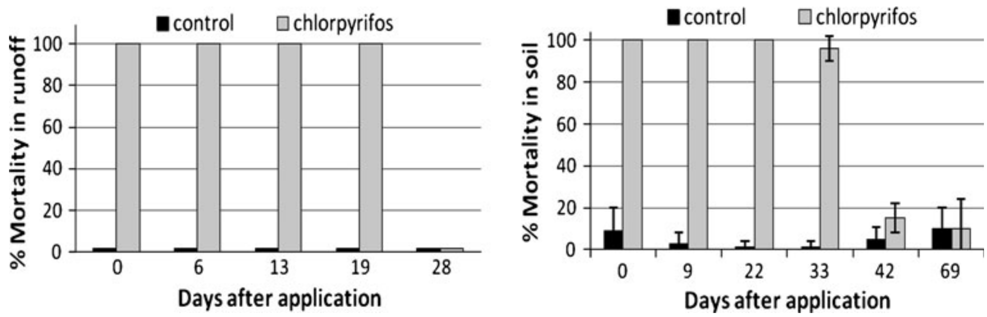

Fig. 1 Toxicity to *Hyalella curvispina* in runoff water and soil after the early application (17 Dec. 2008). *Bars* represent standard deviation

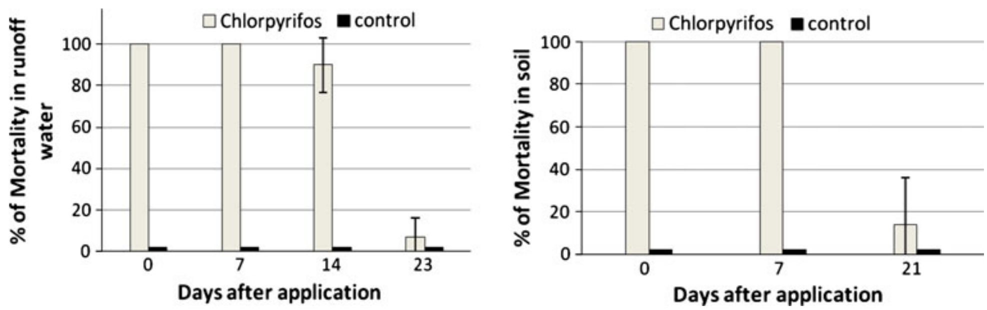

Fig. 2 Toxicity to *H. curvispina* in runoff water and soil following the midseason chlorpyrifos application (28 Dec. 2009). *Bars* represent standard deviation

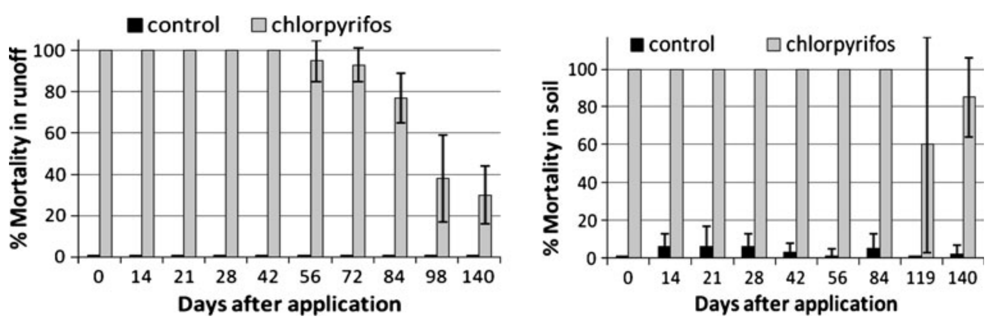

Fig. 3 Runoff and soil toxicity to *H. curvispina* after the late application (7 April 2009). *Bars* represent standard deviation

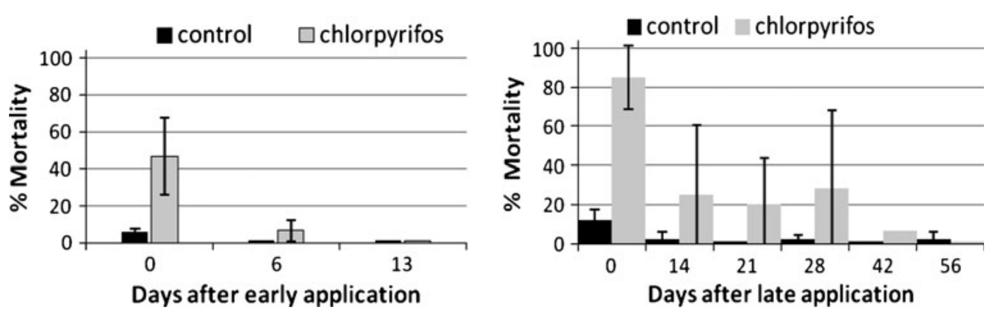

Fig. 4 Toxicity to the fish *C. decemmaculatus* in successive runoff events after the early and late application. *Bars* represent standard deviation

Mean irradiances and soil temperature were higher in the month following the early and midseason applications, in summer, than after the late application, in fall. It seems likely that higher volatilization and photodecomposition contributed to the observed shorter persistence in the former applications. Pesticide soil dissipation in tropical Brazil was shorter than most reported figures for temperate regions, this being attributed to enhanced volatilization, microbial and chemical degradation resulting from the high prevailing soil temperatures (Laabs et al. 2000).

The intermediate application was made when soybean foliage covered the soil; the sprayed pesticide settled on the soybean canopy. The first runoff contained a high pesticide concentration because of extensive pesticide leaching, a fraction of which was retained by the soil, explaining the observed soil toxicity in the first two samplings. However, that amount must have been comparatively modest because soil toxicity shortly disappeared, faster than in the early and late application, the former performed over the bare soil, and the later performed soon before the litter formation. Juraske et al. (2008) reviewed the reported degradation half lives of pesticides on plant surfaces concluding that it was four times faster than in soils. Present evidence suggests that runoff and soil toxicity persistence is short when the application is received by the foliage because of faster pesticide dissipation from the canopy.

The lower irradiances and temperatures prevailing after the late application likely contributed to the longer persistence by decreasing volatilization, photolysis and degradation losses. In the late application, the spray settled on the soybean canopy, but soybean leaves fell down soon after the application resulting in an organic litter formation over the soil surface. It seems likely that the scavenge effect of the organic fraction on the hydrophobic pesticide also contributed to the observed persistence increase. Vandervoort et al. (1997) detected chlorpyrifos residues up to 128 days after application in composted turf grass, similar to the toxicity persistence determined in the runoff of the late application in the present study (140 days). Crossan and Kennedy (2008) studied pesticide concentrations in decaying cotton gin turf. Both the pesticides themselves and their organic substrate simultaneously decomposed, though at different rates; chlorpyrifos concentration increased with time for more than 250 days and was still detectable for almost 400 days. Present evidence therefore suggests that extensive pesticide sorption to the organic substrate delayed mineralization rates in the late application of our study.

Toxicity persistence was longer for *H. curvispina* than for *C. decemmaculatus*, likely due to a higher sensitivity by the former. Trimble et al. (2006) reported a chlorpyrifos LC50 96 h to *Hyalella azteca* of 0.065 μg/L and Moore et al. (1998) of 0.1 μg/L. Chlorpyrifos LC50 96 h to the fish *Gasterosteus aculeatus* and *Pungitius pungitius* were 13.4 and 5.7 μg/L respectively (Van Wijngaarden et al. 1993) and that to *P. promelas* was 162.7 μg/L (Moore et al. 1998).

Conventional pesticide application to soybean caused toxicity pulses in the runoff events following application, affecting the adjacent environments. Toxicity persistence showed an extended variation range depending on the environmental conditions prevailing at the spraying time. Under the conditions prevailing in most of the growing period runoff toxicity to the amphipod *H. curvispina* lasted almost a month. Persistence was greatly increased close to the harvest, likely because of slower volatilization and degradation losses. Chlorpyrifos application in soybean represented a risk for the invertebrate community of the adjacent waterbodies while fish showed limited sensitivity. The overall effect depends on rain frequency and application time. Careful managerial practices may largely reduce the environmental risk.

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