Runoff-related short-term pesticide input into agricultural streams: Measurement by use of an in situ bioassay with aquatic macroinvertebrates

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Synopsis

In a stream flowing through agricultural land, *in situ* bioassays were used to investigate the runoff-related pesticide contamination. Runoff events with no insecticide input had no effect on the survival rate of the test organisms. When pesticides were introduced in the runoff (fenvalerate: 6.2 µg l^{-1}, parathion: 0.6 µg l^{-1}) for short time periods, an increased mortality of *L. lunatus* and *G. pulex* was observed. The mortality decreased with increasing distance but even as far as 2 km downstream was greater than that at a control site with no pesticide contamination. The results of the bioassay clearly confirm that insecticide contamination is associated with increased rates of mortality and that the reactions of the test organisms can be used for a detection of short term pesticide contamination.

agriculture, aquatic ecotoxicology, enclosure, fenvalerate, in situ bioassays, macroinvertebrates, nonpoint-source pollution, parathion, pesticides, runoff

Aquatische Ökotoxikologie, Diffuse Einträge, Expositionssysteme, Fenvalerat, Insektizide, Landwirtschaft, Parathion, Oberflächen-Abfluß

1 Introduction

Small headwater streams in cultivated areas are particularly likely to beflanked by fields used for agriculture. The abiotic parameters here are highly dynamic as a result of the surface runoff, exposing the stream inhabitants to hydraulic and chemical stress. Many review articles have emphasized the importance of surface runoff as a route of entry for pesticides (COOPER 1993,WAUCHOPE 1978, WILLIS & MCDOWELL 1982). As runoff-related pesticide input enters agricultural streams only for short time periods (few hours) a measurement and assessment by conventional sampling and analysis is quite difficult. The present paper describes the results of an *in situ* bioassay to test the effect of short-term runoff-related insecticide input into such a stream. In an *in situ* bioassay, test organisms chosen according to the question of interest are experimentally placed at a site so that they are exposed to the actual contamination there under »natural« field conditions, either aquatic or terrestrial. This method provides results considerably more relevant to the field than those of laboratory tests, especially with respect to the contamination scenario (HOPKIN 1993). Unlike chemical analyses in the field, which yield abiotic data, bioassay systems are based on a toxicological reaction (CRANE & MALTBY 1991). Accordingly, their results give considerably better evidence regarding the protection of aquatic communities. *In situ* bioassays amount to a connecting link between ecotoxicological studies in the laboratory and those in the field. In the present experiments, an *in situ* bioassay was employed in a stream flowing through intensively cultivated land. The main objective of the project was to find out whether experimental bioassays are useful for the detection of pesticide effects on the fauna of small streams with special regard to runoff-related short-term input events.

2 Study area and methods

The investigation was carried out in 1995 at a small headwater stream system in northern Germany (N 52 ° 1'; E 10 ° 28'), which consisted of the Ohebach (baseflow 10 l s^{-1}; peak discharge up to 150 l s^{-1}), the Krummbach (base flow 32 l s^{-1}) and a small tributary (base flow 5 l s^{-1}) of the Krummbach. The catchment area of the Ohebach (150 m above sea level) and the Krummbach measures 12 km^2 and has a slope of between 2 % and 4 % (Fig. 1). It is intensively cultivated (sugar beets, winter barley and winter wheat). The catchment area (0.5 km^2) of the small tributary of the Krummbach (Fig. 1) has a slope < 0.5 % and consisted of pasture land without any use of pesticides. The most common soil types in the area are loess loam and clayey marl. Table 1 shows the levels of pesticide contamination measured during the period of the study, by means of runoff-triggered samplers (LIESS 1993). The water samples were tested for various insecticidal substances at the Institute for Ecological Chemistry and Waste Analysis, TU Braunschweig. Extraction of 1000 ml samples was done by solid-phase extraction with C_{18}-columns (Bakerbond). The mea-

surements were made with GC/ECD and confirmed with GC/MS, with the following quantification limits: parathion 0.01 µg l^{-1}; fenvalerate, 0.05 µg l^{-1}.

In April there was a runoff event in the study stream that produced a marked increase in peak discharge but was not associated with pesticide contamination (1st application in 1995: 28.04.95). The other three events both raised the peak discharge and introduced large amounts of the insecticides fenvalerate and parathion. As had been observed in other years (SCHULZ in press), the contamination of the investigated stream always occurred at the runoff site R1, above the sampling site 1. The level of contamination was therefore expected to decrease progressively at sites 1, 2 and 3 (Fig. 1). At site C no pesticide contamination was ever measurable. At four sampling stations (Nos. 1 and 2 in the Ohebach, No. 3 in the Krummbach and site C in the small tributary), during the period from April 18 to July 8, 1995, four boxes (40 x 17 x 15 cm) containing organisms (30 4th and 5th instar larvae of *Limnephilus lunatus* Curtis (Trichoptera) and 30 *Gammarus pulex* L. (Amphipoda) > 6 mm) were installed in the stream. The boxes floated with the upper third above the water surface; the end walls were made of netting (1 mm mesh) so that the water could flow through. Within each box were 100 g sand, two stones (ca. 5 x 5 x 3 cm) and four water parsnip plants (*Berula erecta* Coville) to provide materials for constructing the larval cases and substrates for pupation, as well as food and shelter. As can be seen in Table 2, important water quality parameters are comparable between sites and are within acceptable limits, so that these parameters would not be expected to have deleterious effects.

Fig. 1

Schematic drawing to show the locations of the sampling stations. R1 identifies the site at which runoff-related insecticide inputs were observed. The circles indicate the sites of the sampling stations, and the decrease in the black area of the circles symbolizes the decreasing insecticide contamination.

Site C is surrounded by pasture land and served as a control with no insecticide contamination.

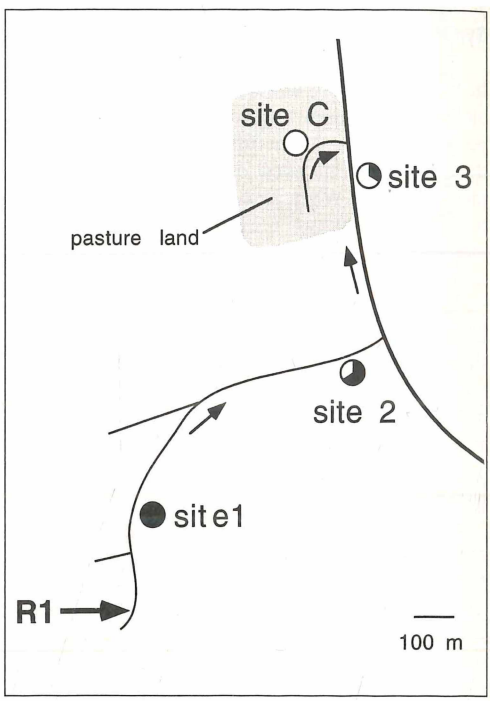

Table 1: **Occurrence of runoff events in the 1995 study period. In the right column are the peak contamination values measured with automatic samplers (LIESS 1993) placed in the stream between site 1 and site 2. At sampling site C no insecticide substances were measured at any time during the year.**

Date	Precipitation (mm d^{-1})	Hourly peak discharge (l s^{-1})	Pesticides in water samples (between sampling sites 1 and 2)
19/20.04.	11.5	25.13	not quantifiable
27.05.	15.6	7.28	fenvalerate: 6.2 µg l^{-1}
			parathion: 0.6 µg l^{-1}
01.06.	26.3	37.90	fenvalerate: 3.3 µg l^{-1}
			parathion: 0.15 µg l^{-1}
02.07.	16.9	6.63	fenvalerate: 0.85 µg l^{-1}
			parathion: 0.08 µg l^{-1}
mean *	1,4 ± 2,4	5.88 ± 4.7	not quantifiable

* mean values for the periods during the study in which no runoff event occurred.

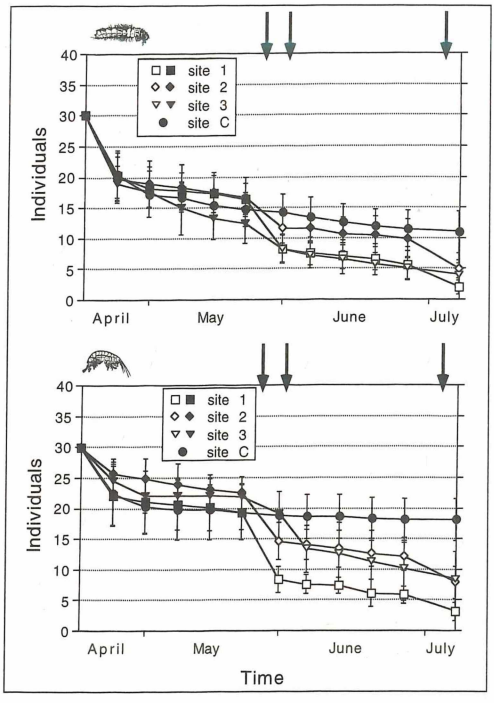

Fig. 2
Survival rate (± SD) of *Limnephilus lunatus* (above) and *Gammarus pulex* (below) in the *in situ* bioassay. The arrows show the times at which runoff-related pesticide inputs occurred. Open symbols (□, ▽, ◇) indicate significant (ANOVA, Fisher's PLSD; $p < 0.05$) differences in the number of surviving test organisms in comparison with the control (site C) at the same time.

3 Results and discussion

3.1 Pesticide input and bioassay reaction

As can be seen from Table 1, during the investigation period three runoff related insecticide input events occured. Apart from these contamination peaks which lasted only few hours, no insecticide contamination was measureable in the stream water. Fig. 2 shows the numbers of *L. lunatus* und *G. pulex* surviving at the various sites as the study progressed. The arrows indicate the times at which insecticides were introduced by runoff (Table 1). During the first week of bioassay employment (April 18–25, 1995), which was before the first insecticide application (April 28, 1995), the number of test organisms decreased by as much as 35 %; this mortality was about the same at all sampling stations. In this interval rainfall increased the turbidity of the water at all the sites, with the result that sediment was deposited in the boxes at a higher rate (Table 2). It is possible that these higher rates of sediment deposition, in combination with increased stress, caused the observed mortality immediately after beginning of the experiment on April 18. Following the first insecticide input (Table 1), the number of individuals of both species declined sharply at the contaminated sites, whereas at the control site there was no change in the mortality rate. Because of the increased mortality at this time (decrease by 30–50 %), at the end of the experiment significantly fewer (ANOVA, Fisher's PLSD; $p < 0.05$) test organisms were present at the contaminated sampling sites than at the control site.

Table 2: **Mean and standard deviation of water quality parameters during the study period. The values are derived from monthly measurements (deposition of sediments in the cages was measured weekly).**

Parameter	site 1	site 2	site 3	site C
Nitrite (mg l^{-1})	0.032 ± 0.01	0.035 ± 0.02	0.045 ± 0.01	0.032 ± 0.02
Ammonium (mg l^{-1})	0.005 ± 0.01	0.002 ± 0.005	0.002 ± 0.005	0.002 ± 0.005
Nitrate (mg l^{-1})	14.3 ± 7.7	13.7 ± 4.8	9.4 ± 3.1	10 ± 3.5
Orthophosphate (mg l^{-1})	0.04 ± 0.04	0.05 ± 0.05	0.15 ± 0.03	0.05 ± 0.03
Hardness (mg $CaCO_3$ l^{-1})	391 ± 14	360 ± 6	519 ± 49	391 ± 11
pH	7.9 ± 0.6	8.1 ± 0.1	8.2 ± 0.2	7.9 ± 0.2
Oxygen (mg l^{-1})	9.1 ± 1.1	9.5 ± 1.5	10.5 ± 1.3	8.8 ± 0.5
Temperature (°C)	10.5 ± 2.2	11.1 ± 2.4	11.7 ± 2.6	11.8 ± 2.1
Deposition of sediments (l wk^{-1})				
18.04. – 25.04.*	1.75 ± 0.1	1.81 ± 0.2	1.95 ± 0.2	1.64 ± 0.2
23.05. – 31.05. **	1.45 ± 0.2	1.39 ± 0.3	1.53 ± 0.3	1.45 ± 0.2
18.04. – 08.07.***	1.12 ± 0.3	1.25 ± 0.3	1.35 ± 0.5	1.21 ± 0.2

* during 1st week, runoff without contamination; ** during 1st runoff with insecticide contamination;
*** mean values for the whole study period.

Responses of both species to insecticide inputs had already been demonstrated in laboratory and field experiments (LIESS & al. 1993, SCHULZ in press). *L. lunatus* has been found to be comparatively sensitive (LIESS & SCHULZ 1996, SCHULZ & LIESS 1995), whereas *G. pulex* is quite common in agricultural streams, including those in the region under study, despite severe contamination (LIESS 1993). However, mortality responses of *G. pulex* as a result of severe carbofuran contamination have been documented by *in situ* bioassay (MATTHIESEN & al. 1995). CRANE & al. (1995) suggested that the mortality of *G. pulex* they measured by *in situ* bioassay in agricultural streams was caused by pesticides, but were unable to demonstrate contamination analytically. Because the sediment deposition rates during this period (May 23–31) were no different at the control site than at sites 1, 2 and 3 (Table 2), the influence of sediment on the differential mortality at this time must have been negligible. It follows that the runoff-related insecticide input on May 27 (Table 1) is a likely cause of the elevated mortality. The insecticides were measured both in the runoff collector R1, positioned between cultivated ground and the stream, and in the runoff-triggered water sampler between sites 1 and 2. The crucial significance of pesticide contamination in the runoff, in comparison to hydraulic effects of the water influx, has been established both experimentally (LIESS 1995) and in field studies (SCHULZ 1994). It follows that the *in situ* bioassay used in this study may be a helpful tool for the detection of short-term pesticide input events. Otherwise, a reliable measurement of short-term pesticide pollution needs a considerable technical and financial effort.

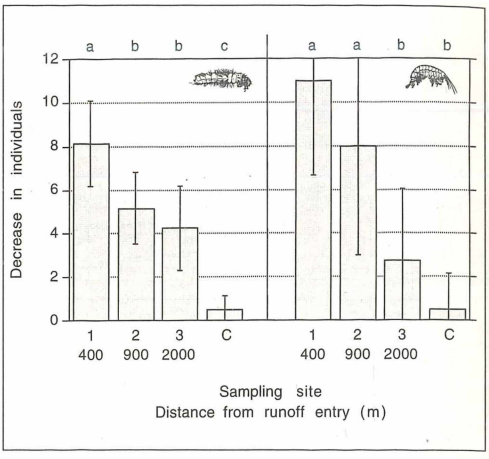

Fig. 3
Decrease in number of individuals (±95 % confidence interval) of *L. lunatus* (left) and *G. pulex* (right) along the study stream during the period of the first insecticide input, from May 23 to May 31. Differences between the letters above adjoining columns denote significant differences (ANOVA, Fisher's PLSD; $p < 0.01$) in the amount of reduction at the associated sampling sites.

3.2 Reaction along the longitudinal gradient

Since several studies have demonstrated that flowing water becomes progressively less contaminated with increasing distance downstream from the site of pesticide introduction (SIBLEY & al. 1991, SUNDARAM 1991), the levels of contamination in the stream under study presumably varied in the order site 1 > site 2 > site 3. Fig. 3 shows the decrease in number of the two test species at the time of the first insecticide input, between the 23rd and the 31st of May, in order of increasing distance between sampling site and input site. For both species, the decrease was greatest at the closest site and became smaller further away. Mortality in comparison to the control site was significantly higher at all three test sites in the case of *L. lunatus* and at the upper two sites in the case of *G. pulex* (ANOVA, Fisher's PLSD; $p < 0.01$). The reduction of the biotic effects with increasing distance downstream might possibly be due to reduction of the effective concentration of the pesticide. For instance, it has been suggested that pesticides may be adsorbed to solid particles (HILL 1989), and their concentration may be diluted by the influx of uncontaminated water (WILLIAMS & al. 1995). In either case, a likely result would be that near the input site the contamination is brief but intense, whereas organisms further downstream are exposed to lower concentrations for a longer time (DENDY 1983). Investigations of the toxicity of heavy metals or pesticides presented with a constant product of concentration & time have shown that brief exposure to high concentrations has stronger effects (ABEL 1980, MCCAHON & PASCOE 1991, SCHULZ 1995). In the present example, toxicity was distinctly diminished over a distance of about 2000 m along the stream, but still was well above the negligible toxicity at the control site. The decline in the biological response provides further evidence that the factor responsible for the mortality was something that became progressively less effective downstream, such as insecticide contamination. SIBLEY & al. (1991) artificially polluted a stream with permethrin (16 µg l^{-1}) and found that the number of species was reduced by 47 % at a position 100 m below the input site, but only by 17 % at a distance of 230 m. The present results do not conclusively establish the distance downstream at which a pesticide input remains effective. However, the marked mortality at site 3 implies that when insecticides are introduced to relatively small streams, it is entirely possible for the aquatic community to be

negatively affected as far as a few kilometers away from the input site. On the other hand, precautionary measures (e.g. buffer zones) for a reduction of non-point-source pesticide input seems to be most important at the upper first kilometers. These informations may be of great importance for a cost-effective planning of water quality management in agricultural regions.

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References

ABEL, P. D., 1980: Toxicity of hexachlorcyclohexane (Lindane) to *Gammarus pulex*; mortality in relation to concentration and duration of exposure. – Freshwat. Biol. 10: 251–259.

COOPER, C. M., 1993: Biological effects of agriculturally derived surface-water pollutants on aquatic systems – a review. – J. envir. Qual. 22: 402–408.

CRANE, M., P. DELANEY, C. MAINSTONE & S. CLARKE, 1995: Measurement by *in situ* bioassay of water quality in an agricultural catchment. – Wat. Res. 29: 2441–2448.

CRANE, M. & L. MALTBY, 1991: The lethal and sublethal responses of *Gammarus pulex* to stress: Sensitivity and sources of variation in an *in situ* bioassay. – Envir. Toxicol. Chem. 10: 1331–1339.

DENDY, F. E., 1983: Runoff from a small watershed – a selected storm. – Journal of the Mississippi Academy of Sciences 28: 1–7.

HILL, R., 1989: Aquatic Organisms and Pyrethroids. – Pest. Sci. 27: 429–465.

HOPKIN, S.P., 1993: *In situ* biological monitoring of pollution in terrestrial and aquatic ecosystems. – In: P. Calow (ed.): Handbook of Ecotoxicology. – Blackwell, Oxford: 397–427.

LIESS, M., 1993: Zur Ökotoxikologie der Einträge von landwirtschaftlich genutzten Flächen in Fließgewässer. – Cuvillier, Göttingen, 133 S.

LIESS, M., 1995: Insektizid-Belastung landwirtschaftlicher Gewässer: Erfassung und Bewertung. – Erweiterte Zusammenfassungen, Deutsche Gesellschaft für Limnologie (Berlin): 877–881.

LIESS, M. & R. SCHULZ, 1996: Chronic effects of brief contamination with the pyrethroid insecticide fenvalerate on the caddisfly *Limnephilus lunatus*. – Hydrobiologia 324: 99–106.

LIESS, M., R. SCHULZ & U. WERNER, 1993: Macroinvertebrate Dynamics in Ditches as Indicator for Surface Water Runoff – An Ecological Aspect for Assessment of Agricultural Impact on Running Water Ecosystems. – Modelling of Geo-Biosphere Processes 2: 279–292.

MATTHIESEN, P., D. SHEAHAN, R. HARRISON, M. KIRBY, R. RYCROFT, A. TURNBULL, C. VOLKNER & R. WILLIAMS, 1995: Use of a *Gammarus pulex* bioassay to measure the effects of transient carbofuran runoff from farmland. – Ecotox. envir. Safety 30: 111–119.

MCCAHON, C. P. & D. PASCOE, 1991: Brief-exposure of first and forth instar *Chironomus riparius* larvae to equivalent assumed doses of Cadmium: Effects on adult emergence. – Water, Air, and Soil Pollution 60: 396–403.

SCHULZ, R., 1994: Freilandeffekte von insektizidkontaminiertem Oberflächen-Runoff auf aquatische Makroinvertebraten. – Erweiterte Zusammenfassungen, Deutsche Gesellschaft für Limnologie (Hamburg): 893–897.

SCHULZ, R., 1995: Feststoffgebundene Pyrethroide (Insektizide) in landwirtschaftlichen Fließgewässern – Gibt es Effekte auf aquatische Makroinvertebraten? – Erweiterte Zusammenfassungen, Deutsche Gesellschaft für Limnologie (Berlin): 887–891.

SCHULZ, R., in press: Macroinvertebrate dynamics in a stream receiving insecticide-contaminated runoff. – Proc. intern. Assoc. Theor. Appl. Limnol.

SCHULZ, R. & M. LIESS, 1995: Chronic effects of low insecticide concentrations on freshwater caddisfly larvae. – Hydrobiologia 299: 103–113.

SIBLEY, P. K., K. N. KAUSHIK & D. P. KREUTZWEISER, 1991: Impact of a Pulse Application of Permethrin on the Macroinvertebrate Community of a Headwater Stream. – Envir. Pollut. 70: 35–55.

SUNDARAM, K. M. S., 1991: Fate and Short-Term Persistence of Permethrin insecticide injected in a northern Ontario (Canada) Headwater Stream. – Pest. Sci. 31: 281–295.

WILLIAMS, R. J., D. BROOKE, P. MATTHIESEN, M. MILLS, A. TURNBULL & R. M. HARRISON, 1995: Pesticide transport to surface waters within an agricultural catchment. – J. Inst. Wat. envir. Man. 9: 72–81.

WILLIS, G. H. & L. L. MCDOWELL, 1982: Pesticides in agricultural runoff and their effects on downstream water quality. – Envir. Toxicol. Chem. 1: 267–279.

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