Available online at www.sciencedirect.com
SCIENCE DIRECT
Science of the Total Environment 367 (2006) 418–432
Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind
www.elsevier.com/locate/scitotenv

# Water quality and macroinvertebrate community response following pesticide applications in a banana plantation, Limon, Costa Rica

Luisa Eugenia Castillo [a,b,c,*], Eduardo Martínez [a], Clemens Ruepert [a], Candida Savage [c,f], Michael Gilek [c,d], Margareth Pinnock [a], Efrain Solis [e]

[a] *Central American Institute for Studies on Toxic Substances (IRET), Universidad Nacional, Heredia, Costa Rica*
[b] *Department of Research, Universidad Nacional, Heredia, Costa Rica*
[c] *Department of Systems Ecology, Stockholm University, SE-106 91 Stockholm, Sweden*
[d] *Sodertorns University College, Box 4101, S-141 04 Huddinge, Sweden*
[e] *School of Chemistry, Universidad Nacional, Heredia, Costa Rica*
[f] *Department of Marine Science, University of Otago, PO Box 56, Dunedin, New Zealand*

Received 18 October 2005; received in revised form 24 February 2006; accepted 26 February 2006
Available online 27 April 2006

**Abstract**

Pesticides used in banana production may enter watercourses and pose ecological risks for aquatic ecosystems. The occurrence and effects of pesticides in a stream draining a banana plantation was evaluated using chemical characterization, toxicity testing and macrobenthic community composition. All nematicides studied were detected in the surface waters of the banana plantation during application periods, with peak concentrations following applications. Toxicity tests were limited to the carbofuran application and no toxicity was observed with the acute tests used. However, since pesticide concentrations were generally below the lowest LC50 value for crustaceans but above calculated aquatic quality criteria, there remains a risk of chronic toxicity. Accurate ecological assessments of pesticide use in banana plantations are currently limited by the lack of local short-term chronic toxicity tests and tests using sensitive native species. Relatively constant levels of four pesticides (imazalil, thiabendazole, chlorpyrifos and propiconazole), which had toxic effects according to the 96h hydra and 21d daphnia chronic test, were recorded in the effluent of the packing plant throughout the study, indicating that the solid waste trap used in this facility was not effective in eliminating toxic chemicals. Certain taxa, such as *Heterelmis* sp. (Elmidae), *Heteragrion* sp. (Megapodagrionidae, Odonata), *Caenis* sp. (Caenidae, Ephemerotera), and *Smicridea* sp. (Hidropsychidae, Trichoptera), were more abundant at reference sites than in the banana farm waters, and may be good candidates for toxicity testing. Multivariate analyses of the macroinvertebrate communities clearly showed that the banana plantation sites were significantly different from the reference sites. Moreover, following the pesticide applications, all the banana plantation sites showed significant changes in community composition, with the same genera being affected at all sites and for all pesticides (terbufos, cadusafos and carbofuran). Consequently, the results presented here show that multivariate analysis of community composition was more sensitive in distinguishing pesticide effects than the toxicity tests and richness and composition measures used. We conclude that monitoring macroinvertebrate communities can be a powerful tool in the assessment of ecological effects of banana production.


*Keywords:* Pesticides; Banana plantations; Aquatic ecosystems; Toxicity tests; Macroinvertebrates; Multivariate analysis

* Corresponding author. Tel.: +506 238 0696.
*E-mail address:* lcastill@una.ac.cr (L.E. Castillo).

0048-9697/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2006.02.052

## 1. Introduction

Banana production typically occurs in intensely managed agroecosystems with high inputs of synthetic chemicals. Banana plantations cover approximately 10% of the cultured land of Costa Rica, equating to 42,182 ha in 2002 (Sepsa, 2003). An estimated third of the pesticide volume imported into Costa Rica is used on banana plantations and in their packing plants. The compounds applied in banana production include dithiocarbamate, chlorophenyl, conazole and benzimidazole fungicides; organophosphate and carbamate nematicides and insecticides; and triazine and bipyridyl herbicides. In contrast to traditional organochlorines, most of these compounds are not highly persistent, but some can be highly toxic to aquatic organisms (Castillo et al., 2000a). Intensively managed banana plantations are also characterized by an extensive system of drainage canals where surplus water may flow into local streams and rivers. Consequently, the aquatic ecosystems located downstream of banana plantations are vulnerable due to the intensive pesticide use, drainage systems, and high precipitation that typically occur in tropical areas where banana production occurs. Pesticide pollution from banana plantations may thus endanger the highly diverse wetlands of the Costa Rican coast, among them the Tortuguero Conservation Area, which protects the manatee (*Trichechus manatus*), green sea turtle (*Chelonia mydas*) and the fossil fish (*Atractosteus tropicus*).

The presence of several of the pesticides used in banana production has been identified in surface waters receiving runoff from banana plantations (Castillo et al., 2000a). The most frequently encountered compounds are the fungicides thiabendazole, propiconazole and imazalil; the nematicides terbufos and cadusafos; and the insecticide chlorpyrifos. To assess the ecological risks of this intense pesticide use, knowledge of both chemical exposure and biological effects is required (Henriques et al., 1997). Acute and chronic risk ratios based on observed exposure levels and toxicity values from the literature indicate that some of these pesticides analyzed, including most of the insecticides and nematicides, pose a risk for acute or chronic toxicity to aquatic organisms (Castillo et al., 2000a). However, most of the data in the literature stems from temperate regions and thus assessment of ecological effects based solely on literature data is unreliable. Moreover, to date, only a few studies have been performed on the environmental distribution and ecological effects of pesticides in Central America (see review by Castillo et al., 1997).

The objective of this study was therefore to examine the occurrence and effects of pesticides in surface waters receiving runoff from a Costa Rican banana plantation as well as in the effluent from a banana packing plant by studying environmental concentrations and effects following pesticide applications (terbufos, cadusafos and carbofuran). Effects were assessed both using toxicity testing of sampled water using the microcrustacean *Daphnia magna*, the freshwater cnidarian *Hydra attenuata*, seeds of *Lactuca sativa* and the freshwater shrimp *Macrobrachium rosenbergii*, and by comparing benthic invertebrate communities on artificial substrates in reference and impacted areas. Both toxicity tests and macroinvertebrate benthic communities have been successfully used to assess the degree of contamination of various aquatic habitats (Tucker and Burton, 1999; La Point, 1995; Canfield et al., 1994). The analysis of community structure using biotic indices and multivariate approaches is commonly used for biological monitoring of water quality, with multivariate techniques often being particularly sensitive in elucidating even subtle shifts in community structure (Warwick and Clarke, 1991; Wright et al., 1995; Hewitt et al., 2005). A secondary aim of this study was therefore to assess and discuss the applicability of the various ecological assessment methods to determine the impact of pesticide use in banana plantations on receiving aquatic ecosystems.

## 2. Methods

### 2.1. Study area

The study was conducted in a small (~40 ha) banana plantation in Siquirres, Costa Rica (Fig. 1) between March 1995 and May 1997. Fungicides, herbicides, insecticides and nematicides were used in the banana farm, the latter two types of biocides being the most toxic. Nematicides were applied as granular formulations to the ground 2 or 3 times per year to mature plantations and sometimes also to new plants at a rate of approximately 4–6 kg active ingredient (a.i.) per hectare. Plastic bags impregnated with one percent of the insecticide chlorpyrifos were used to protect the fruit in the field. Several types of fungicides, such as mancozeb, benomyl, bitertanol, chlorothalonil and propiconazole, were applied (rotating one or two fungicides per application) by aeroplane about 45 times per year. Fungicides were also used in the packing plant to protect the bananas prior to export. The packing plant studied operated 2–4 days per week and pesticide use included imazalil and thiabendazole. Some banana plantations have improved environmental standards by using computer-guided

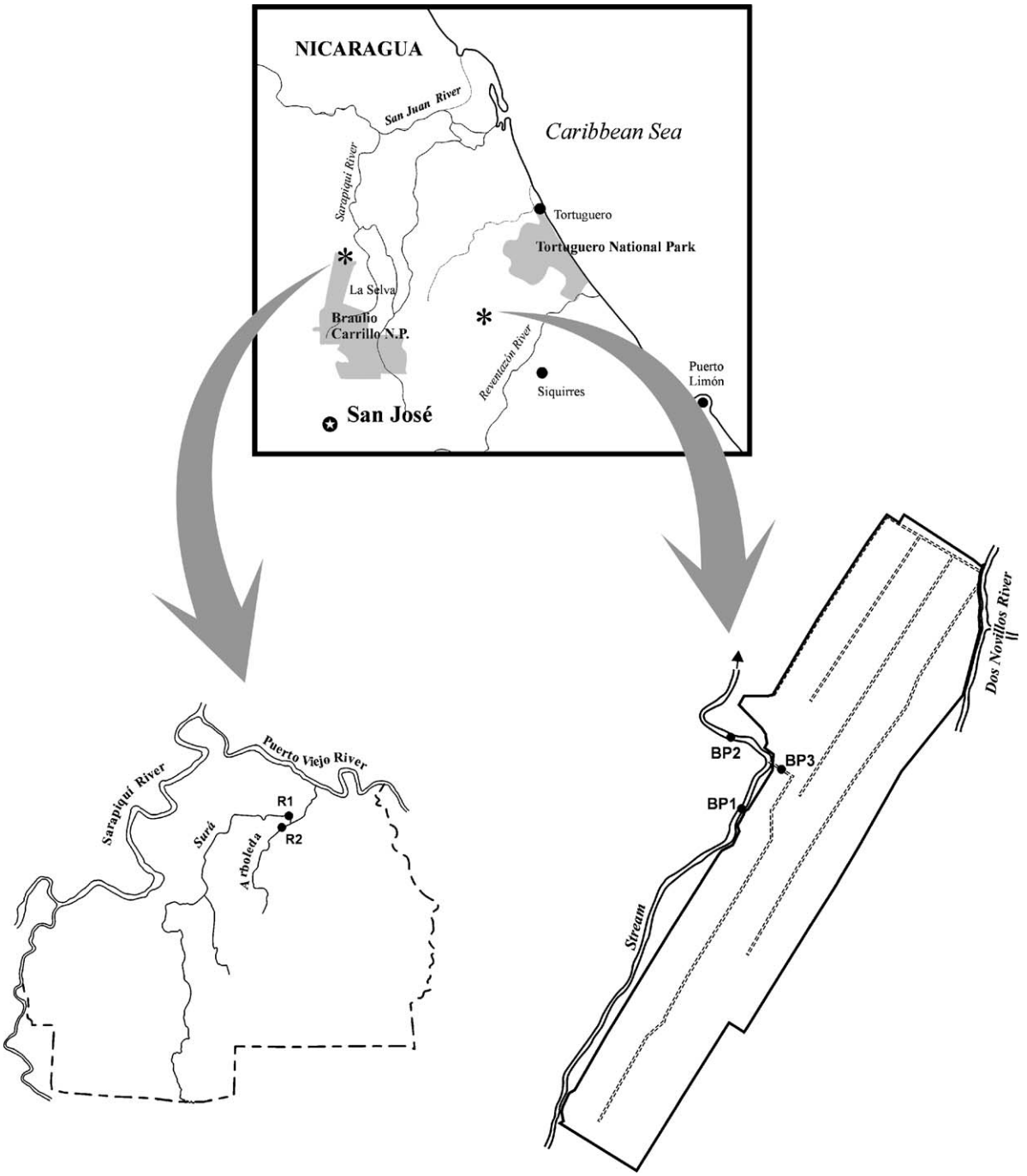


Fig. 1. Location of the banana plantation sites (BP1, BP2 and BP3) and the reference sites (R1 and R2) in the Atlantic Region of Costa Rica.

application of fungicides by helicopter, and preservation of some riparian vegetation alongside sections of the main drainage canal. However, the main difference between the study plantation and other banana plantations in Costa Rica is the size, since most banana farms have an average area of about 250 ha.

A stream bordering the farm was selected that flows through forest, pastures and agricultural land (Fig. 1). The first site (BP1) was located upstream of the drainage canal, the second site (BP2) was 50 m downstream from the inflow of the canal, and the third site (BP3) was located in the canal, which drains approximately 12 ha of

Table 1
Description of the stream characteristics at the banana plantation sites (BP1–BP4) and the reference sites (R1–R2)

| | BP1 | BP2 | BP3 | BP4 | R1 | R2 |
|---|---|---|---|---|---|---|
| Location | Upstream of banana plantation | Downstream of banana plantation | Drainage canal of banana plantation | Downstream of solid waste trap-packing plant | Sura stream, nature reserve | Arboleda stream, nature reserve |
| Width (m) | 3–5 m | 3–5 m | 1–1.5 m | 0.6–0.8 m | 6 m | 4 m |
| Depth (m) | 0.4–1 m | 0.4–1 m | 0.2 m | 0.2 m | 1–1.4 m | 0.3–0.5 m |
| Bottom substrate | Cobbles, sand, silt | Cobbles, sand, silt | Mostly silt | Mostly silt | Cobbles, silt | Sandy-silt |
| Running water | Yes | Yes | Yes | Yes | Yes | Yes |
| Stream borders | Remnants of riparian forest | Remnants of riparian forest | Open canopy | Open canopy | Semi-shaded by open canopy | Semi-shaded by open canopy |

the banana plantation (Table 1). A fourth sampling site (BP4) was in an open canal downstream of a solid waste trap in the packing plant facility. Two sites at Sura (R1) and Arboleda (R2) streams located in the La Selva Biological Station, a private reserve in the lowlands of the Braulio Carrillo National Park in the North Atlantic Region of Costa Rica, were selected as reference sites for the macrobenthic faunal study as well as for the pesticide residue analysis and toxicity tests (Fig. 1). A description of the stream characteristics for all sites is given in Table 1.

### 2.2. Follow-up of pesticide applications in the banana plantation watercourses

Three nematicide applications were studied: 1) 4.2 kg a.i./ha of terbufos in June–July 1995; 2) 3.7 kg a.i./ha of cadusafos in November–December 1995; and 3) 6 kg a.i./ha of carbofuran in February–March 1997. The amount and type of nematicides applied were obtained from the farm register book. The fungicide propiconazole was applied aerially in conjunction with the nematicide applications of terbufos and cadusafos. Water samples were collected for pesticide residue analysis and macrobenthic organisms were sampled concurrently at different intervals after application of the nematicides. Sampling was conducted monthly between March 1995 and April 1997 and as soon as possible after notice from the farm managers that nematicide application had taken place. Sediment samples for residue analysis were collected during the second and third follow-up and water samples for toxicity tests were collected during the third follow-up study in February 1997.

### 2.3. Evaluation of packing plant effluents

Water samples for pesticide residue analysis were collected approximately once per month at BP4 between March 1996 to November 1997, totaling 16 samples. Toxicity tests were carried out on water samples collected at this site from October 1996 to May 1997.

### 2.4. Surface water and sediment sampling

Water samples for residue analysis were collected at all sites in pre-washed 1 l glass bottles and preserved with 25 ml dichloromethane. Samples were kept at <4 °C and extracted within 24 h. Sediment samples of 250 to 500 g were collected manually and kept at <4 °C until extraction. Water samples for toxicity testing were collected in 20 l polycarbonate containers and refrigerated until testing began. Additional water samples were also collected for biochemical oxygen demand (BOD), nitrates, nitrites, ammonia, phosphates and total and suspended solids.

### 2.5. Pesticide residue analysis

The analyses were performed according to the procedures described by Castillo et al. (2000a) using capillary gas chromatography (GC) with electron capture (ECD) and nitrogen–phosphorus detection (NPD) after liquid–liquid extraction. Eleven different pesticides (the fungicides chlorothalonil, propiconazole, thiabendazole and imazalil; the nematicides carbofuran, terbufos, cadusafos and ethoprophos; the insecticides diazinon and chlorpyrifos and the herbicide ametryn) were included in the analysis. Consequently, several of the major pesticides (among them most of the fungicides and herbicides) used in banana plantations were not included in the study due to technical limitations.

Quantification limits in water were between 0.02–0.1 μg/L for most compounds with the exception of carbofuran (0.1–0.5 μg/L ); propiconazole (0.05–0.3 μg/L); imazalil (1 μg/L) and thiabendazole (1–3 μg/L). Recoveries were between 80–112% for the different pesticides except for cadusafos and diazinon, which had lower values (71% and 59%, respectively).

Detection limits in sediments were between 6 and 20 mg/kg dry weight (dw) for ametryn, diazinon and terbufos; 20 and 100 mg/kg dw for carbofuran, ethoprophos and propiconazole and between 100 and 200 mg/kg dw for imazalil and thiabendazole. Recoveries were between 70% and >90% except for chlorothalonil (50–70%) and thiabendazole (<50%). The results for sediments are presented as mg/kg dw. Results are not corrected for recovery. Confirmation was obtained by injection on columns of different polarity and some of the extracts were sent for confirmation by GC–MS to the Organic Environmental Chemistry Section, Swedish University of Agricultural Sciences, Sweden. No false-positive samples were found.

### 2.6. Other physical and chemical variables

Dissolved oxygen (DO), temperature (YSI, model 57 or 58, Yellow Springs Instruments Co., Yellow Springs, OH, USA), conductivity (HI 9033, Hanna Instruments, Woonsocket, RI, USA), and pH (Phep 3, Hanna Instruments) or pH-indicator strips (Merck, Darmstadt, Germany), were all measured in situ. The instruments were calibrated before use according to manufacturers specifications. Mean pH values ranged from 6.6 to 7.0 and mean temperatures ranged from 24.2 to 26.1 °C for the different sites. DO values ranged from 6.3 to 7.2 mg/L and conductivity from 67.5 to 260 μS/cm. Conductivity values were higher at the reference site in Arboleda stream (R2), which has been related to high phosphorus levels of geothermal origin (Pringle et al., 1990). Unfiltered water samples were analyzed for biochemical oxygen demand (BOD), nitrates, nitrites, ammonia and phosphates at the Laboratory for Chemical Analysis and Services (Universidad Nacional, Heredia, Costa Rica) using standard methods (APHA, 1989). Total solids were determined by evaporation of residues at 105 °C of unfiltered water.

BOD values were lower at the reference sites (R1 and R2) and BP1, with mean values of 4.4–5.9 mg/L and maxima of 8.4 mg/L, and relatively higher at BP2-BP4, with average values of 7.1 to 11.9 mg/L and maxima of 43 mg/L. Ammonia showed higher values at the packing plant (0.21 mg/L), nitrates and nitrites were higher at BP2 and BP3 with mean values for nitrates of 7.4 and 21.7 mg/L, respectively. Average nitrate values were 1 mg/L at all other sites. Nitrites ranged from 0.01 (BP1) to 0.06 mg/L (BP2) and maximum values of 0.16 mg/L were observed at BP2 and BP3. Soluble phosphates were higher at BP4 with average values of 1.8 mg/L, being higher than those found at the natural phosphate-enriched waters of the reference site (0.8 and 0.34 mg/L at R2 and R1, respectively). Mean total solids were similar for the different sites but had slightly higher maximum values at BP3 and BP4 located in canals with no riparian vegetation.

### 2.7. Toxicity testing

Toxicity tests were performed for several samples from sites BP1–BP4 using the cladoceran *D. magna*, postlarvae of the freshwater shrimp *M. rosenbergii*, freshwater hydra (*H. attenuata*) and lettuce seeds (*L. sativa*) tests. All tests were conducted at ambient temperature conditions (24–28 °C) and a photoperiod of 16:8 h light:dark. Water from R1 was used for controls. Methods for the short-term tests with *D. magna*, *H. attenuata* and *L. sativa* are described in Castillo et al. (2000b) and only a brief description is included here.

### 2.8. D. magna assay (Daphnia test)

Exposures were conducted using 25 ml of solution for this acute bioassay (Dutka, 1989a). Three replicates of ten organisms (24 h old neonates) per vessel were used for each sample and for the control. The test was extended to 96 h with water renewal at 48 h and mortality recorded at 24 h intervals. Daphnids were exposed to 100%, 56% and 32% water collected during the nematicide application (carbofuran) of February 1997. *Daphnia* chronic tests (21 d reproduction test) were conducted with waters collected at the packing plant on 27-2-1997 and 15-5-1997 and with water from the reference site (R1) used as a negative control. Water samples were kept at 4 °C prior to testing. Daphnids were exposed in individual containers to 50 ml of the test solutions renewed three times per week. Ten replicates were used for each sample and control. Temperature, pH and DO were measured before and after solution renewal. The containers were checked daily for possible dead adults and the number of live and dead neonates and adults recorded three times per week.

### 2.9. H. attenuata acute toxicity test (hydra test)

The 96 h static bioassay (Trottier et al., 1997) was performed in 12-well microplates with three replicates for each sample and for the control. Three hydras were transferred to each well containing 4 ml of solution. Sub-lethal and lethal endpoints were recorded daily. Five types of hydras have been identified (Trottier et al., 1997): one is indicative of normal organisms, two are indicative of sublethal effects and two of lethal effects. A control of the *Hydra* growth rate ($k$) was conducted prior to the performance of each set of assays, this was within the normal parameters of $k = 0.3–0.4$.

### 2.10. L. sativa toxicity assay (seed test)

This short-term (120 h) root elongation test was performed according to Dutka (1989b). No attempt was made to select seeds of similar size for the assays. Twenty seeds were placed over filter paper moistened with 6–7 ml of samples or controls in Petri dishes, covered with aluminum foil and kept in the dark for the duration of the test. After 96 or 120 h the number of germinated seeds was counted and the roots were measured (node to tip measurement).

### 2.11. Freshwater shrimp (M. rosenbergii)

This freshwater shrimp is native to Asia and has been introduced to Costa Rica for commercial purposes. Native shrimp from the same genus are found in the rivers and streams of lowland areas in Costa Rica. Postlarvae of this shrimp were obtained from a commercial farm in Filadelfia (Guanacaste, Costa Rica) and were exposed individually to 75 ml of solution in 125 ml containers, using 10 replicates per treatment. The shrimps were exposed in static-renewal toxicity tests for 72 h (samples from BP4, October and November, 1996) or 96 h (samples from BP1–BP3, February 1998 and BP4, February and May 1997) to 100%, 56% and 32% of water samples. Water from the reference site (R1) was used as a negative control. All containers were placed in a water bath at 28 °C. Temperature, pH and DO were measured before and after solution renewal. Mortality was recorded in all treatments at 24 h intervals and the LC50 estimated by the probit method (Stephan, 1977). Results of the reproductive parameter were compared using a one-way analysis of variance (ANOVA) followed by Tukey's test (Statgraphics for Windows, 3.1).

### 2.12. Benthic macroinvertebrate sampling

The structure of the macroinvertebrate benthic community at the various study sites in the banana plantation (BP1–BP3) was investigated using artificial substrates that consisted of concrete blocks (20 × 10 × 8 cm) placed in a plastic mesh bag. Artificial substrates enable standardization of the collection methods and therefore enable sample replication, especially when they are placed in comparable macrohabitats (Cairns and Pratt, 1993). During the baseline monthly sampling (March 1995 to April 1997), four of these substrates were placed every month at each of the different sampling sites at the farm (BP1–BP3) and were collected approximately one month later. Not all substrates were recovered due to losses or manmade damage. On average, three were recovered per month per site. Prior to the nematicide application, twenty of these artificial substrates were placed in each study site allowing a minimum of three weeks before collection. Two to three replicates were recovered at different times after the nematicide applications. The organisms were recovered from the artificial substrates in a 250 mm mesh and preserved in 96% ethanol. Macrobenthic organisms were extracted in the laboratory, sorted and identified to genus level when possible using a stereoscope and taxonomical keys (Springer, 1998; Springer et al., unpublished information; Merritt and Cummins, 1984; Roldán Pérez, 1992; Pennak, 1978; Edmunds et al., 1976). The collection was also checked by Monica Springer, manager of aquatic insect collection, Universidad de Costa Rica. Each taxon was assigned to a functional feeding-group based on Jackson and Sweeney (1995) and Merritt and Cummins (1984).

### 2.13. Benthic metrics

Benthic metrics recorded included richness measures such as the total number of taxa (usually genera), families and orders; EPT richness (number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). Composition measures such as percentage of EPT (percent of Ephemeroptera, Plecoptera, and Trichoptera); EPT to Chironomidae ratio (ratio of the total number of Ephemeroptera, Plecoptera and Trichoptera to the total number of Chironomidae); and percentages of the different orders. Community comparison indices were calculated including the Jaccard coefficient of similarity, which measures the degree of similarity in taxonomic composition between two stations; and the community loss index, which measures the loss of benthic taxa between reference and comparison sites (Barbour et al., 1997).

### 2.14. Multivariate analysis of macroinvertebrates

The genus-level abundance data collected during the sampling occasions between and including April 1995 and April 1997 were included in the multivariate analyses (Field et al., 1982). The abundance data were averaged for all substrates successfully recovered at each site for each sampling occasion and the data analyzed using PRIMER. Cluster analysis and non-metric multidimensional scaling (MDS) ordination were performed on 4th-root transformed to give equal weighting to rare and abundant genera (Clarke and Green, 1988), and using the Bray–Curtis coefficient of

similarity. Here we present the results of the MDS ordinations of:

(a) all sites combined: the two reference sites (R1 and R2), the site upstream (BP1) and the site downstream of the drainage canal (BP2) and the site located in the canal (BP3),
(b) the sites in the vicinity of the banana plantation only (BP1, BP2 and BP3),
(c) site BP2, downstream of the drainage canal from the banana plant, to assess whether there is any temporal variability within a site.

To test whether the reference sites were statistically different to the sites near the banana plantation in terms of species composition, one-way ANOSIM was performed (Clarke and Green, 1988). Two-way crossed ANOSIM (Clarke and Warwick, 1994) was performed to test for inter-site differences, averaged across pesticide applications, and to test for pesticide differences averaged across all sites. The SIMPER (SIMilarity PERcentages) algorithm in PRIMER was used to identify the genera that were affected by the different pesticide applications.

## 3. Results and discussion

### *3.1. Banana plantation*

#### *3.1.1. Pesticide residues during monthly samplings*

Exclusive of the pesticide application periods, pesticides were generally below detection levels in the stream near the banana plantation, with only one sample in each of the two sites (BP1 and BP2) having pesticides present over detection levels. Pesticides found during the monthly samplings included the herbicide ametryn at BP1 and propiconazole at BP1 and BP2. Terbufos was measured once in BP1 after a nematicide had been applied to a regrowth area. Pesticides were found more frequently in the drainage canal (BP3), 40% of the 17 samples collected in the monthly samplings had at least one pesticide over detection limits, including cadusafos, carbofuran, chlorpyrifos and propiconazole. No pesticides were detected in sediment samples excluding the samples collected for the follow-up of applications.

#### *3.1.2. Pesticide residues during follow-up of applications*

Pesticide concentrations in surface waters peaked soon after the application dates and then generally decreased quickly. The downstream site (BP2) had maximum concentrations of 0.04 μg/L for terbufos, 0.3 μg/L for cadusafos and 0.15 μg/L for carbofuran. The maximum concentrations of the applied nematicides were detected in the drainage canal (BP3): 2.1 μg/L for carbofuran, 0.48 μg/L for cadusafos, and 1.2 μg/L for terbufos, the latter diminished quickly following application but was still above quantification limits after 8 days. Cadusafos was detected at similar concentrations for one week (0.17–0.48 μg/L) and remained above quantification levels one month later at BP3. Similarly, cadusafos remained above quantification levels at BP2 15 days after application, suggesting that this compound is more persistent in the soil/water environment than other nematicides. The frequent occurrence of cadusafos found by Castillo et al. (2000a) has been attributed to its greater use however it could also be related to a higher persistence in the soil/water environment. The higher peak of carbofuran could be related to its solubility. This is compatible with findings of other studies (Mortensen et al., 1998; Castillo et al., 2000a), which report peak occurrences of this compound after rain events. Although the highest concentrations detected in BP3 were during the application of carbofuran, the concentration levels of this compound decreased quickly over the first five days, and at BP2 it was detected only once.

The concentrations of nematicides detected in surface waters were generally below the LC50 value for crustaceans (0.3, 1.6 and 15 μg/L for terbufos (Extoxnet, 1996), cadusafos (Tomlin, 1994), and carbofuran (Tomlin, 1994), respectively). This value was exceeded only in one sample collected in the drainage canal (BP3) during the terbufos application. However, terbufos and cadusafos were present in all samples collected at BP3 during the first eight days after applications at an average concentration of 0.09 μg/L (excluding the peak value of 1.2 μg/L) and 0.36 μg/L, respectively, which exceed calculated aquatic quality criteria (Castillo et al., 2000a). Carbofuran was also detected at an average concentration of 0.7 and 0.16 μg/L for the first five days after application at BP3 and the first three days at BP2, respectively, which also exceeds aquatic quality criteria for this compound (Teunissen-Ordelman and Schrap, 1997).

Cadusafos was detected in sediments at BP2 and BP3 at concentrations ranging from 9 to 18 mg/kg dw during the first two days after application but not thereafter. No residues were found above quantification limits in sediment samples collected during the carbofuran application period.

The aerially applied fungicide, propiconazole, was detected at a maximum concentration of 0.15 μg/L at BP1 and 0.18 μg/L at BP3 during June–July 1995; and 13 μg/L at BP2 and 1.5 μg/L at BP3 during November

1995. In the first case, the samples were collected two days after application, while samples were collected during the day of application on the second occasion. Following the November 1995 application, the concentrations diminished but were still above quantification limits 12 days after application at both BP2 and BP3. These concentrations are well under the toxicity levels of propiconazole for crustaceans (LC50:2.2 mg/L, Kemi, 1998) and within the calculated aquatic quality criteria (22 μg/L, Castillo et al., 2000a). Propiconazole is used frequently over an extensive area and the data from the Suerte River Basin shows a constant presence of this compound (Castillo et al., 2000a). The detection of this fungicide in the upstream site (BP1) in the monthly samplings and in one of the follow-up periods verifies the potential of aerially applied fungicides to pollute streams, even when there is some riparian vegetation, as in this case.

### 3.2. Toxicity tests

Only samples collected at the banana plantation during the February 1997 application of carbofuran were tested for toxicity. No toxicity was observed with the seed elongation, shrimp and daphnia acute toxicity tests, which agrees with the acute toxicity reference values for carbofuran. More extensive use of toxicity tests, would be necessary to evaluate toxicity following applications, including chronic toxicity since concentrations of some of the pesticides were in the range where this could be expected. Other studies in agricultural areas have demonstrated that concentrations well below acute toxicity data correlated with long-term changes of community composition (Liess and Von der Ohe, 2005).

### 3.3. Macroinvertebrate community structure

The reference site, the stream and the drainage canal in the banana plantation exhibited similar taxonomic richness and benthic invertebrate densities (mean abundance), although the number of taxa, families and orders were lower for BP3 (Table 2). Abundance was greater in the upstream site of the banana plantation (BP1) than in the downstream site (BP2), with dipterans including Simuliidae and *Pseudochironomus* sp., the coleopteran *Microcylloepus* sp. and mayflies of the genus *Leptohyphes* mainly responsible for this increased abundance. Gastropods composed 40% of the abundance at BP3.

In general the benthic community was dominated by the orders Ephemeroptera, Trichoptera and Coleoptera in the reference sites, whereas Ephemeroptera, Diptera, Coleoptera, Gastropoda and Trichoptera were the main groups in the banana plantation sites (Fig. 2). These results agree with data from the Caribbean lowlands of Costa Rica (Pringle and Ramirez, 1998; Ramirez et al., 1998; Ramirez and Pringle, 1998a,b). The percentage of Ephemeroptera, Plecoptera and Trichoptera (EPT) and the ratio of abundance of EPT to Chironomidae were significantly higher in the reference sites, however the number of EPT taxa was similar for all sites (Table 2). The family Trichorytidae accounted for most of the mayflies at all sites. Caenidae were more abundant at the reference sites, while Baetidae (considered a more tolerant family of mayflies) accounted for 21% of ephemeropterans at BP3, and this percentage varied from 3% to 10% at all other sites. Trichoptera were more abundant at BP3 than at BP1 and BP2, but were less abundant than at the reference site. Also, while *Smicridea* sp. was the main genus at the reference sites (18.4 and 27.8% in R1 and R2, respectively), it only constituted 5.6% at BP3, where the main genus was *Leptonema* (13.2%). The gastropods *Potamopyrgus* sp.

Table 2
Richness and composition measures of benthic invertebrates in reference (R1 and R2) and banana plantation sites (BP1, BP2 and BP3)

| | R1 *n*: 78 | R2 *n*: 75 | BP1 *n*: 96 | BP2 *n*: 88 | BP3 *n*: 98 |
|---|---|---|---|---|---|
| Total taxa | 78 | 76 | 86 | 88 | 72 |
| Total families | 47 | 46 | 46 | 46 | 39 |
| Total orders | 16 | 15 | 16 | 14 | 12 |
| Mean abundance[a] | 239 | 213 | 330 | 164 | 355[b] |
| % EPT[c] | 76.1 | 81.1 | 37.8 | 35.5 | 41.5 |
| EPT richness[d] | 22 | 22 | 22 | 21 | 19 |
| EPT/ Chironomidae[e] | 14.8 | 27.5 | 2.3 | 1.8 | 5.8 |
| Taxa in common with control[f] | 63 | 63 | 54/53 | 54/51 | 46/46 |
| Community loss[g] | 0.17 | – | 0.27 | 0.28 | 0.42 |
| Jaccard coefficient[h] | 0.69 | – | 0.49 | 0.45 | 0.45 |

[a] Average of the number of organisms per substrate throughout the study period.
[b] Although BP3 had the highest mean abundance 40% of it was composed by gastropods.
[c] Percentage of the composite of Ephemeroptera, Plecoptera and Trichoptera.
[d] Number of taxa in the orders Ephemeroptera, Plecoptera and Trichoptera.
[e] Ratio of the total number of Ephemeroptera, Plecoptera and Trichoptera to the total number of Chironomidae.
[f] Comparing each site with the reference site R1/R2.
[g] Measures the loss of benthic taxa between reference and the station of comparison, R2 (Arboleda) was used as reference.
[h] Measures the degree of similarity in taxonomic composition between two stations. Each site was compared to the reference site R2 (Arboleda).

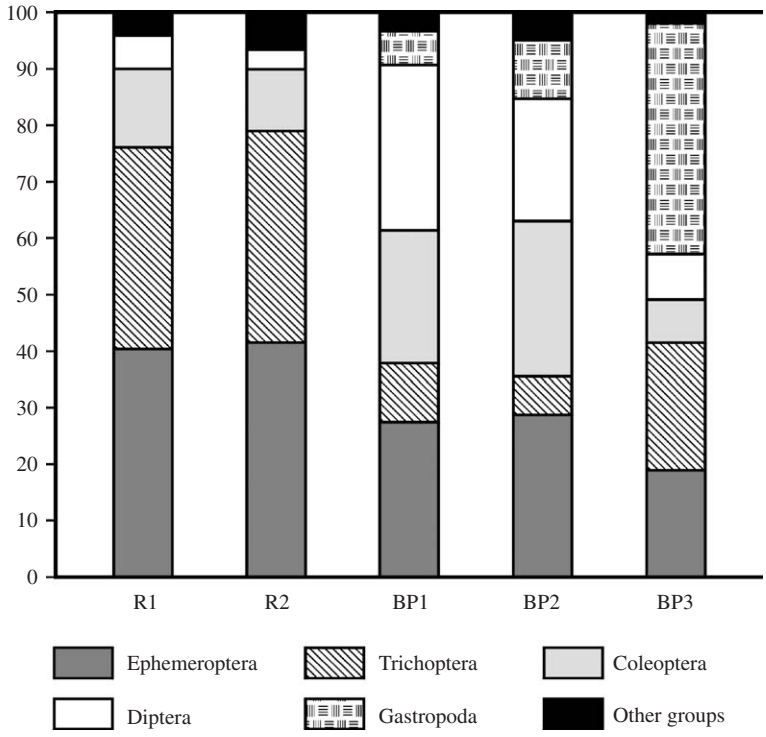


Fig. 2. Proportion of major invertebrate groups at reference (R1 and R2) and banana plantation sites (BP1, BP2 and BP3).

and *Bythinella* sp. were the most dominant taxa at BP3 (23% and 17%, respectively). While *Bythinella* sp. was also abundant at BP2 and BP1, this taxa was absent at the reference sites.

Community loss was higher in the drainage canal (BP3) and showed similar values for BP1 and BP2 (Table 2). Using the Jaccard coefficient of similarity the two reference sites were the most similar followed by the two sites in the stream (BP1 and BP2). The lowest similarity was between the reference sites and BP2 and BP3. Composition measures such as % EPT, ratio of EPT to Chironomidae abundance, community loss and the Jaccard similarity index showed a difference between the benthic community in the undisturbed streams of the reference sites and that of the disturbed stream of the banana plantation area. Pringle and Ramirez (1998) observed lower taxon richness and diversity in streams draining banana plantations. The drainage canal (BP3) was the most polluted site in terms of the occurrence of pesticides in its waters and exceeding aquatic quality criteria. This site had the highest occurrence of gastropods and the highest community loss (Table 2).

Certain taxa such as *Heterelmis* sp. (Elmidae), *Heteragrion* sp. (Megapodagrionidae, Odonata), *Caenis* sp. (Caenidae, Ephemerotera), *Smicridea* sp. (Hidropsychidae, Trichoptera) were more prevalent in both reference sites than in the banana farm waters. These native tropical species could prove to be more sensitive to chemical stress and might be good candidates for toxicity testing.

Since there is very little known about feeding strategies and trophic dynamics of benthic macroinvertebrates and their relationship with chemical stress in the Central American region, the classification of feeding groups was based mostly on literature from other geographical areas. Collectors/filterers (35–37%) and collectors/gatherers (30–37%) were the most abundant groups at the reference sites. Gatherers were also important at BP1 and BP2 (38–39%) but accounted for only 17.5% at BP3, where the most important group was scrapers (mainly gastropods).

High variability in the abundance of benthic organisms and of key families and taxa was observed during this study at both the reference sites and in the banana farm waters. Other studies in the Caribbean lowlands of Costa Rica have also reported strong temporal fluctuations. Paaby et al. (1998) found low similarity indices between sampling times indicating a macroinvertebrate community subject to frequent disturbance events. Fluctuations have been related to rainfall and water discharge (Ramirez and Pringle, 1998a,b; Paaby et al., 1998; Leonard et al., 2000)) and to the presence of predatory fish and shrimps (Flowers and Pringle, 1995). Annual precipitation in the study area (banana farms and reference sites) average

4000 mm with daily values up to 170 mm. However, other studies have not found evidence of a decrease in benthic invertebrates due to hydraulic stress (Liess and Von der Ohe, 2005; Liess and Schulz, 1999). It is likely that in our study the mesh bag reduced predation by fish, although some predation by shrimps could have occurred. This natural fluctuation of the benthic macroinvertebrate community obscures the identification of changes in relation to chemical stress. The use of in situ and laboratory toxicity assays might be useful to clarify this relationship.

Recovery of the macroinvertebrate community to different stressors is an important factor in the analysis of the ecological impact of pesticide pollution (Sherratt et al., 1999; Liess and Schulz, 1999; Liess and Von der Ohe, 2005) and should be addressed in the future. However, more information is needed on the life-history of macroinvertebrates for this region as well as toxicity to native species and environmental fate of pesticides in tropical conditions.

### 3.4. Multivariate analysis

Multivariate analysis of all sites combined showed a significant difference (Global $R=0.741$, $p<0.001$) between the reference sites (R1, R2) and the sites near the banana plantation (BP1–BP3). Furthermore, when analysed separately, the reference sites (R1 and R2) were significantly different from the drainage canal, BP3 (Global $R=0.974$, $p<0.001$), and from BP1 and BP2 (Global $R=0.795$, $p<0.001$). The MDS plot of all sites and all sampling times combined shows overlap between the different sampling occasions that coincide with the different pesticide applications and the intervening periods, and between the different banana plantation sites, due to the polarity of the reference and banana plantation sites (Fig. 3). At this level of resolution it is difficult to discern any clear temporal or spatial patterns in the benthic communities and therefore a non-metric MDS ordination was performed using only the samples near the banana plantation, namely BP1–BP3.

Within the banana plantation sites, BP1 and BP2 were significantly different from the canal BP3 (Global $R=0.454$, $p<0.001$) and BP1 was significantly different from BP2 (Global $R=0.402$, $p<0.001$). An ordination plot distinguishes between the drainage canal samples, the samples from the upstream site and those from the downstream site, however, the macroinvertebrate communities from upstream and downstream sites

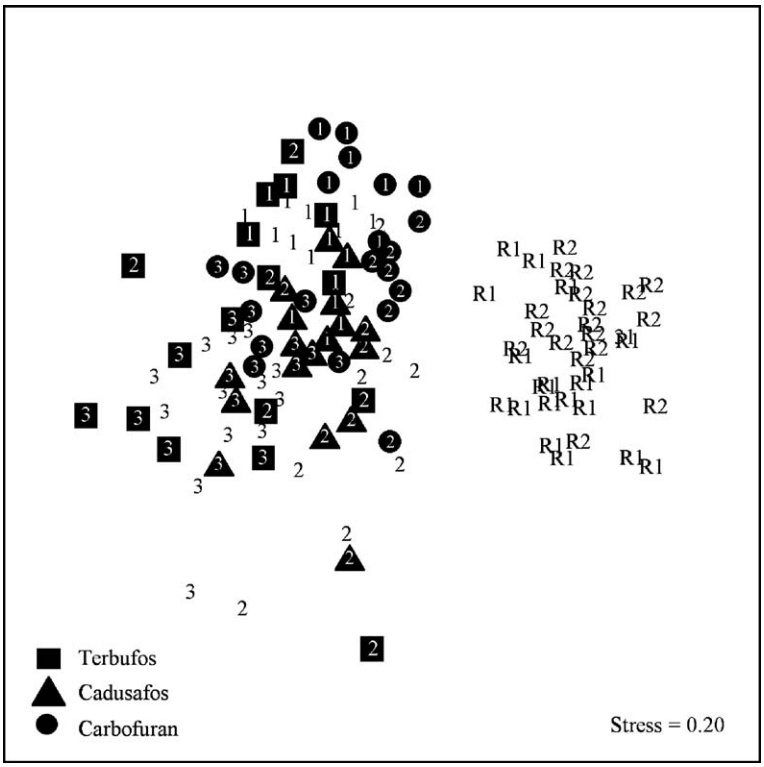


Fig. 3. MDS ordination plot of all sites combined, where R1 = Reference site 1, Sura; R2 = Reference site 2 (Arboleda); 1 = BP1; 2 = BP2 and 3 = BP3 (drainage canal).

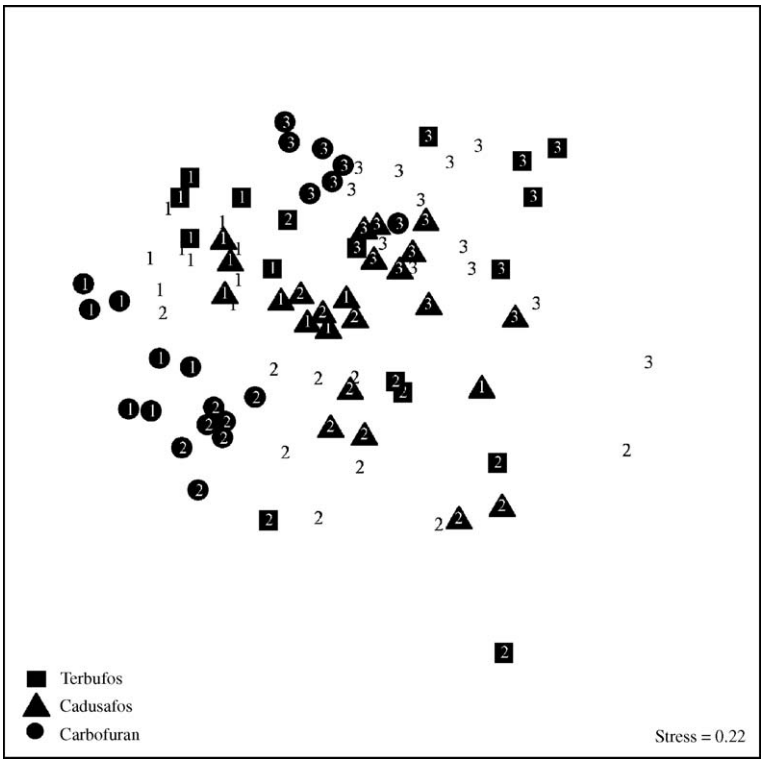


Fig. 4. MDS ordination of samples from upstream (BP1), downstream (BP2) and drainage canal (BP3) sites, where 1=BP1; 2=BP2 and 3=BP3.

tended to be similar during the cadusafos (November/December 1995) and the carbofuran application (February/March 1997) (Fig. 4). One possible explanation may be that the fungicide bitertanol was applied aerially during this same period. Bitertanol is more toxic to crustaceans than any of the other fungicides applied aerially and is also more toxic than carbofuran with an LC50 of 4.8 μg/L (Van Rijn et al., 1995). Although bitertanol was not analyzed in this study, the fact that propiconazole was detected in the upstream site indicates the potential of aerially applied fungicides to reach the surface waters of this site.

There are inherent site differences (two-way crossed ANOSIM: Global $R=0.671$, $p<0.001$), nevertheless, it is possible to detect a significant effect of the different pesticide applications (two-way crossed ANOSIM: Global $R=0.751$, $p<0.001$), which would indicate that the macroinvertebrate communities in the different areas exhibit a similar response to each of the pesticide applications. Although there is a slight difference in the order of importance in terms of how the different genera respond to the different pesticides, the same genera appear to change in abundance for all the application periods (Table 3). These taxa would also be good candidates for the development of toxicity tests appropriate for risk evaluation in tropical areas.

Within each sampling station, there is temporal variability that most likely reflects natural seasonal species replacement. Samples collected between April 1995 and April 1997 from the downstream site (BP2) showed that there was a natural succession in the

Table 3
Results of SIMPER analysis showing the most important genera that respond to the different pesticide applications

| Terbufos | Cadusafos | Carbofuran |
|---|---|---|
| *Microcylloepus* sp. (C) | *Bythinella* sp. (G) | *Microcylloepus* sp. (C) |
| Cf. *Pseudochironomus* sp. (D) | *Tricotythodes* sp. (E) | Cf. *Pseudochironomus* sp.(D) |
| *Bythinella* sp. (G) | Cf. *Pseudochironomus* sp. (D) | *Bythinella* sp. (G) |
| *Leptohyphes* sp. (E) | *Microcylloepus* sp. (C) | *Tricotythodes* sp. (E) |
| *Leptonema* sp. (T) | *Leptohyphes* sp. (E) | *Leptohyphes* (E) |
| | | Pentaneura sp. (D) |
| | | Farrodes sp. (E) |

The genera are listed in decreasing order of importance to a 50% similarity cut-off level. C=Coleoptera, D=Diptera, G=Gastropoda, E=Ephemeroptera, T=Trichoptera.

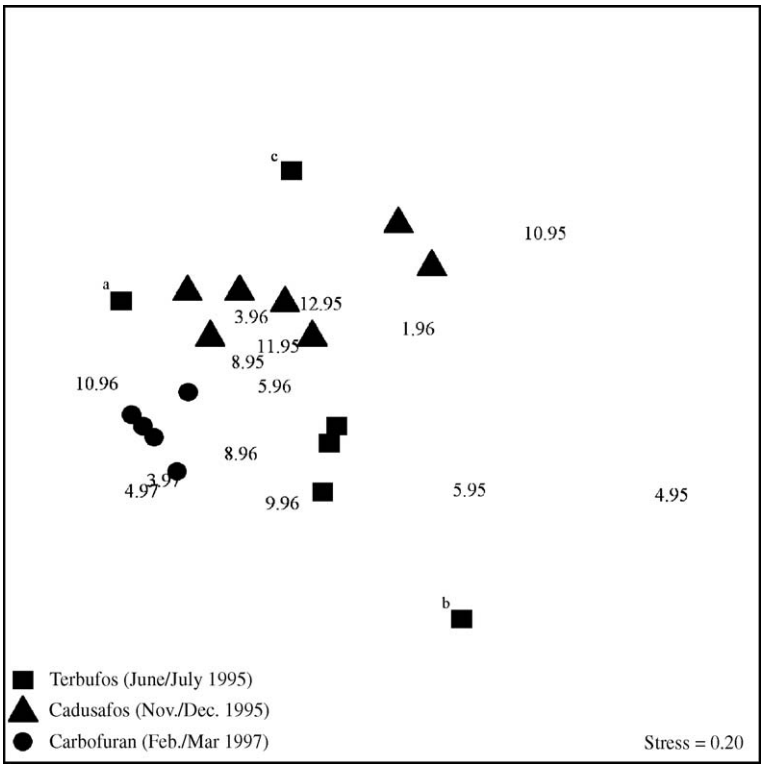


Fig. 5. MDS ordination of samples from the downstream site, BP2, where the monthly sampling occasions are represented by the month and year of sampling (e.g., 1.96 = January 1996), and those coinciding with a pesticide application are represented by the symbols in the key. Pesticide application produced pronounced changes in community structure as can be observed for consecutive samples collected before and after the terbufos application (annotated 5.95 to (a), to (b), to (c)).

community during the two years of biological monitoring (Fig. 5). However, pesticide applications tended to produce pronounced changes in the community structure, as shown by the dramatic shifts for communities sampled consecutively in May 1995 (5.95 in Fig. 5) to June 1995 (annotated as (a) to (b) to (c) in Fig. 5) when terbufos was applied. This finding is consistent with other research, which showed that increased shifts in community composition are indicative of increased disturbance (Clarke et al., 1993).

### 3.5. Packing plants

Pesticide residues detected in the packing plant effluent included the two fungicides used in the packing plant imazalil and thiabendazole with a frequency of 88% of samples over detection limits (mean concentrations of 160 and 125 μg/L, respectively). Additionally, chlorpyrifos and propiconazole were detected in 44% of the samples with means of 0.05 and 2.5 μg/L, respectively. Since these compounds are only used in the field, it is possible that some residues remain in the banana skin and are released in the packing plant washing basins.

Samples of the packing plant effluent collected in April and May 1997 were found to be toxic using the 96 h hydra test, the LC50 was calculated as 9.2–9.8% of the effluent concentration. Samples evaluated with the 21 day *Daphnia* reproduction test showed decreased survival in the highest concentrations tested as well as a decrease in the number of neonates at the lowest concentrations tested (Table 4). These results agree with the risk factor for chronic exposure obtained by comparing the average pesticide residues in the packing plant with aquatic toxicity reference values for imazalil, thiabendazole and chlorpyrifos (Castillo et al., 2000a). Other contaminants are released by packing plants such as aluminum sulfate, disinfectants and banana latex (Hernandez and Witter, 1996). No toxicity was observed with the seed and shrimp tests. These results indicate that chemicals present in the packing plant wastewater pose a risk for the receiving aquatic ecosystems especially where there are several packing plants draining into the same water body. The results of

Table 4
Analysis of chronic toxicity (reproduction endpoint) using *Daphnia magna* (21 d)

| Sample | % of sample | Survival (%) | Total number of neonates | Replicates |
|---|---|---|---|---|
| BP4 — 27-2-97 | 100 | 40 | 2 | – |
| A | 56 | 60 | 99 | 16, 12, 24, 18, 18, 11 |
| A | 32 | 50 | 147 | 9, 18, 36, 54, 30 |
| Control[a] | 100 | 90 | 595 | 75, 53, 74, 57, 59, 58, 87, 67, 65 |
| BP4 — 15-5-97 | 100 | 0 | – | – |
| A | 56 | 100 | 0 | – |
| A | 32 | 100 | 11 | 3, 2, 2, 4 |
| Control[a] | 100 | 90 | 290 | 33, 33, 37, 27, 32, 33, 9, 22, 30, 34 |

[a] Surface water from reference site R1 (Sura).

pesticide residues analysis and of toxicity tests indicate that the solid waste trap used in this plant was not effective in eliminating toxic substances released during the packing process.

## 4. Conclusion

Pesticides used in banana plantation farms and packing plants may enter watercourses and pose ecological risks. All nematicides studied were detected in the surface waters draining the banana plantation during application periods. With a relatively simple method, we were able to detect the occurrence of peak concentrations after applications and observe some general differences in the behavior of the different pesticides. More sophisticated equipment (an autosampler) would be necessary to relate pesticide concentrations to discharge more strictly and to calculate pesticide loads over time in order to extrapolate the data from the small farm in this study to bigger farms and watersheds using models.

Relatively constant levels of four pesticides were observed in the effluent of the packing plant (BP4) throughout the study period. The hydra short-term test (96 h) and the daphnia chronic test (21 d) were able to detect toxic effects of this effluent. These tests could therefore be useful for monitoring the quality of the discharges of these plants and to evaluate the performance of treatment facilities.

The concentrations of the nematicides found in the drainage canal of the banana plantation suggest a risk for aquatic organisms based on literature values. Toxicity tests in this study were limited to the carbofuran application and no toxicity was observed with the acute tests used. However, since observed pesticide concentrations at the banana plantation sites were generally below the lowest LC50 value for crustaceans but above calculated aquatic quality criteria, there remains a risk of chronic toxicity, such as has been demonstrated in other studies in agricultural areas (Liess and Von der Ohe, 2005). Consequently, the lack of locally available short-term chronic toxicity tests and especially tests using sensitive native species limits an accurate ecological effects assessment of pesticide use in Costa Rican banana plantations.

Richness and composition measures showed differences between sites, with the percentage of EPT, ratio EPT/Chironomidae abundance, community loss and the Jaccard similarity index showing a difference between the benthic community in the forested streams of the reference sites and that of the disturbed banana plantation area. Community loss seemed to be one of the most sensitive indices decreasing steadily from the reference sites to the farmland stream and to the drainage canal. The multivariate methods for analysis of community composition revealed large inter-site variability as well as substantial temporal changes in the benthic communities. However, despite these inherent problems in recognizing and interpreting site differences and effects of pesticide applications, several significant differences in community composition were found. There was a clear difference in the composition of the benthic communities at the banana plantation sites compared to the reference sites. Furthermore, the upstream and downstream banana plantation sites were also significantly different from each other. Thus, while no clear conclusions on the effects of pesticides on macroinvertebrate communities can be drawn, the results clearly showed that the banana plantation sites were significantly different from the reference sites. Moreover, following the pesticide applications, all the banana plantation sites (BP1–BP3) showed significant changes in community composition, with the same genera being affected across sites and for all pesticides (terbufos, cadusafos and carbofuran).

Consequently, the results presented here show that the multivariate methods used for analysis of community composition were more sensitive in distinguishing pesticide effects than the toxicity tests and richness and biotic indices used. Community studies, however, would benefit from a better understanding of natural variability of macroinvertebrates in wet tropical areas. Since these community-based methods are also ecologically relevant and assess time-integrated effects, we conclude that monitoring macroinvertebrate communities

can be a powerful tool in the assessment of ecological effects of banana production.

## Acknowledgements

Funding for this study was provided by the Swedish Agency for Research Cooperation, Sweden. The authors gratefully acknowledge the assistance of Gilberto Piedra, Ana Ruth Alfaro, Diego Arias, Lilliam Soto in the laboratory and Francisco Hodgson for the illustrations. We also thank two anonymous reviewers who greatly improved the manuscript.

## References

APHA. Standard Methods for the Examination of Water and Waste Water. 17th edition. American Public Health Association, American Water Works Association. Water Environment Federation, Washington D.C., USA, 1989.

Barbour MT, Gerritsen J, Snyder BD, Stribling JB. Revision to rapid bioassessment protocols for use in streams and rivers-Periphyton, benthic macroinvertebrates, and fish: U.S. Environmental Protection Agency, Office of Water, EPA 841-D-97-002. Washington D.C., USA, 1997.

Canfield TJ, Kemble NE, Brumbaugh WG, Dwyer FJ, Ingersoll CG, Fairchild JF. Use of benthic invertebrate community structure and the sediment quality triad to evaluate metal-contaminated sediment in the Upper Clark Fork River. Montana. Environ Toxicol Chem 1994;13:1999–2012.

Cairns J, Pratt R. A history of biological monitoring using benthic macroinvertebrates. In: Rosenberg DM, Resh VH, editors. Freshwater Biomonitoring and Benthic Macroinvertebrates. New York: Chapman & Hall; 1993. p. 10–27.

Castillo LE, delaCruz E, Ruepert C. Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. Environ Toxicol Chem 1997;16:41–51.

Castillo LE, Ruepert C, Solis E. Pesticide residues in the aquatic environment of banana plantation areas in the North Atlantic Zone of Costa Rica. Environ Toxicol Chem 2000a;19:1942–50.

Castillo LE, Pinnock M, Martínez E. Evaluation of a battery of toxicity tests for use in the assessment of water quality in a Costa Rican laboratory. Environ Toxicol 2000b;15(4):312–21.

Clarke KR, Green RH. Statistical design and analysis for a 'biological effects' study. Mar Ecol Prog Ser 1988;46:213–26.

Clarke KR, Warwick RM. Similarity-based testing for community pattern: the two-way layout with no replication. Mar Biol 1994;118: 167–76.

Clarke KR, Warwick RM, Brown BE. An index showing breakdown of seriation, related to disturbance, in a coral-reef assemblage. Mar Ecol Prog Ser 1993;102:153–60.

Dutka BJ. *Daphnia magna* 48h static bioassay method for acute toxicity in environmental samples. In: Methods for Toxicological Analysis of Waters, Wastewaters and Sediments. National Water Research Institute (NWRI). Environment Canada, Burlington, Ontario, Canada, 1989a.

Dutka BJ. Short-term root elongation toxicity bioassay. In: Methods for Toxicological Analysis of Waters, Wastewaters and Sediments. National Water Research Institute (NWRI). Environment Canada, Burlington, Ontario, Canada, 1989b.

Edmunds GF, Jenson SL, Berner L. The mayflies of North and Central America. Minneapolis, U.S.A.: University of Minnesota Press; 1976. 330 pp.

Extoxnet. Pesticide information profiles. Corvallis, OR, USA: Oregon State University; 1996.

Field JG, Clarke KR, Warwick RM. A practical strategy for analysing multispecies distribution patterns. Mar Ecol Prog Ser 1982;8: 37–52.

Flowers RW, Pringle CM. Yearly fluctuations in the mayfly community of a tropical stream draining lowland pasture in Costa Rica. In: Corkum LD, Ciborowski JJH, editors. Current directions in research on Ephemeroptera. Toronto: Canadian Scholars Press; 1995. p. 131–50.

Henriques W, Jeffers RD, Lacher Jr TE, Kendall RJ. Agrochemical use on banana plantations in Latin America: perspectives on ecological risk. Environ Toxicol Chem 1997;16:91–9.

Hernandez CE, Witter SG. Evaluating and managing the environmental impact of banana production in Costa Rica: a systems approach. Ambio 1996;25:171–7.

Hewitt JE, Anderson MJ, Thrush SF. Assessing and monitoring ecological community health in marine systems. Ecol Appl 2005;15: 942–53.

Jackson JK, Sweeney BM. Egg and larval development times for 35 species of tropical stream insects from Costa Rica. J N Am Benthol Soc 1995;14:115–30.

Kemi. Ämnen mot swampangrepp. PM 6/98. Stockholm, Sweden: The National Chemicals Inspectorate; 1998.

La Point TW. Signs and measurements of ecotoxicity in the aquatic environment. In: Hoffman KJ, Rattner BA, Burton Jr GA, Cairns Jr J, editors. Handbook of Ecotoxicology. Boca Raton, Florida: CRC Press; 1995.

Leonard AW, Hyne RV, Lim RP, Pablo F, Van den Brink PJ. Riverine endosulfan concentrations in the Namoi River, Australia: link to cotton field runoff and macroinvertebrate population densities. Environ Toxicol Chem 2000;19:1540–51.

Liess M, Schulz R. Linking insecticide contamination and population response in an agricultural stream. Environ Toxicol Chem 1999;18:1948–55.

Liess M, Von der Ohe P. Analyzing effects of pesticides on invertebrate communities in streams. Environ Toxicol Chem 2005;24:954–65.

Merritt RW, Cummins KW. An introduction to the aquatic insects of North America. 2nd edition. Iowa, U.S.A.: Kendall/Hunt Publishing Co.; 1984. 722 pp.

Mortensen SR, Johnson KA, Weisskopf CP, Hooper MJ, Kendall RJ. Avian exposure to pesticides in Costa Rican banana plantations. Bull Environ Contam Toxicol 1998;60:562–8.

Paaby P, Ramirez A, Pringle CM. The benthic macroinvertebrate community in Caribbean Costa Rican streams and the effect of two sampling methods. Rev Biol Trop 1998;46(Supl. 6):185–99.

Pennak RW. Fresh-water invertebrates of the United States. 2nd edition. New York, U.S.A.: Wiley-InterScience Publication; 1978. 803 pp.

Pringle CM, Ramirez A. Use of both benthic and drift sampling techniques to assess tropical stream invertebrate communities along an altitudinal gradient, Costa Rica. Freshw Biol 1998;39: 359–73.

Pringle CM, Triska FJ, Browder G. Spatial variation in basic chemistry of streams draining a volcanic landscape on Costa Rica's Caribbean slope. Hydrobiologia 1990;206:73–85.

Ramirez A, Pringle CM. Invertebrate drift and benthic community dynamics in a lowland neotropical stream, Costa Rica. Hydrobiologia 1998a;386:19–26.

Ramirez A, Pringle CM. Structure and production of a benthic insect assemblage in a neotropical stream. J N Am Benthol Soc 1998b;17: 443–63.

Ramirez A, Paaby P, Pringle CM, Aguero G. Effect of habitat type on benthic macroinvertebrates in two lowland tropical streams, Costa Rica. Rev Biol Trop 1998;46(Supl. 6):201–13.

Roldán Pérez G. Guía para el Estdio de los Macro-invertebrados Acuáticos del Departamento de Antioquía. Colombia: Universidad de Antioquía; 1992. 217 pp.

SEPSA. Información básica del sector agropecuario de Costa Rica. Boletín Estadístico No. 14, Secretaría Ejecutiva de Planificación Sectorial Agropecuaria, Ministerio de Agricultura y Ganadería, San José, Costa Rica, 2003.

Sherratt T, Roberts G, Williams P, Whitfield M, Biggs J, Shillabeer N, et al. A life-history approach to predicting the recovery of aquatic invertebrate populations after exposure to xenobiotic chemicals. Environ Toxicol Chem 1999;18:2512–8.

Springer M. Genera of aquatic insects from Costa Rica, deposited at the Museo de Zoologia, Universidad de Costa Rica. Rev Biol Trop 1998;46:137–41.

Springer M, Ramirez A, Hanson P, editors. Guía para la identificación de artrópodos de agua dulce de Costa Rica. (Manuscript).

Stephan CE. Methods in calculating an LC50. In: Mayer FL, Hamelink JL, editors. Aquatic toxicology and hazard evaluation. ASTM STP 634. Philadelphia, USA: American Society for Testing and Materials; 1977.

Teunissen-Ordelman HGK, Schrap SM. 1996 aquatic outlook. An analysis of issues pertaining to aquatic environments. Pesticides. Riza Policy document No. 97.038. The Netherlands; 1997.

Tomlin C. The Pesticide Manual, 10th ed. Crop Protection Publication. British Crop Protection Council and The Royal Society of Chemistry. Farnham, Surrey, UK, 1994.

Trottier S, Blaise C, Kusui T, Johnson EM. Acute toxicity assessment of aqueous samples using a microplate based *Hydra attenuata* assay. Environ Toxicol Water Qual 1997;12:265–71.

Tucker K, Burton Jr GA. Assessment of nonpoint-source runoff in a stream using in situ and laboratory approaches. Environ Toxicol Chem 1999;18:2797–803.

Van Rijn JP, Van Straalen NM, Willems J. Handboek Bestrijdingsmiddelen Gebruik and Milieu-effecten. Amsterdam, The Netherlands: Free University of Amsterdam (VU); 1995.

Warwick RM, Clarke KR. A comparison of some methods for analysing changes in benthic community structure. J Mar Biol Assoc 1991;71:225–44.

Wright IA, Chessman BC, Fairweather PG, Benson LJ. Measuring the impact of sewage effluent on the macroinvertebrate community of an upland stream: the effect of different levels of taxonomic resolution and quantification. Aust J Ecol 1995;20:142–9.