PREDICTION AND ECOTOXICOLOGICAL EFFECTS OF RUNOFF INDUCED PESTICIDE CONTAMINATION IN AGRICULTURAL SURFACE WATERS: A RISK ASSESSMENT USING GIS AND MICROCOSMS.

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature **Date**

ABSTRACT

Runoff is generally regarded as one of the most important routes of nonpoint source pesticide pollution in agricultural surface waters. Of major concern is the fact that low, sub-lethal levels of pesticide exposure are responsible for negative ecotoxicological effects, stressing the need for methods capable of identifying problem areas where populations could be at risk. Predicted average losses of three pesticides in tributaries of nine sub-catchments of the Lourens River were calculated through use of a GIS-based runoff model. There was a significant ($p < 0.005$) positive correlation between the predicted average loss and mean measured concentrations of the insecticides both in water and suspended sediments (R^2 between 0.75 and 0.9), indicating that the model could serve as a powerful tool for the risk assessment and management of surface waters in South African orchard areas. Based on field relevant exposure scenarios, the potential effects of azinphos-methyl on macroinvertebrate communities were evaluated in a combined microcosm and field approach. Microcosms were contaminated for 1 h with AZP (control, 0.2; 1, 5 and 20 µg/L; three replicates each) and acute effects on survival were evaluated 6 days after exposure. The sensitivity or tolerance of 12 core taxa was determined based on their response to the exposure scenarios and compared to field tolerance/sensitivity as was established by a field investigation at a control and contaminated site of the Lourens River. The sensitivity/tolerance of ten of the 12 taxa corresponded to that which was found in the field. Thus microcosm studies employing a field relevant design can be successfully linked to field studies and indicate that transient pesticide contamination affects the aquatic communities of the Lourens River.

OPSOMMING

Afloop word oor die algemeen beskou as een van die belangrikste roetes van nie-puntbron pestisiedbesoedeling in landbou oppervlakwaters. Die feit dat lae, sub-letale vlakke van pestisiedblootstelling negatiewe ektoksikologiese gevolge kan hê, is van groot belang. Dit beklemtoon die behoefte aan metodes om probleemgebiede te kan identifiseer waar bevolkings aan risiko onderhewig is. 'n GIS-gebaseerde afloopmodel is gebruik om die gemidddelde verlies van drie pestisiede in die sytakke van nege sub-opvangsgebiede van die Lourensrivier te voorspel. Daar was 'n beduidende ($p < 0.005$) positiewe korrelasie tussen die voorspelde gemiddelde verlies en gemete konsentrasies van insektisiede in beide die water en sediment (R^2 between 0.75 and 0.9) fases, wat aandui dat die model as 'n kragtige hulpmiddel vir risikobestuur van oppervlakwaters in Suid Afrikaanse boord-gebiede kan dien. Die potensiële gevolge van azinfos-metiel (AZP) op makroinvertebraat gemeenskappe is deur middel van 'n gekombineerde mikrokosmos (wat op veldrelevante blootstellings gebaseer is) en veldbenadering bepaal. Mikrokosmosse is vir 1 h met AZP gekontamineer (kontrole; l; 0.2; 1; 5 en 20 µg/L; drie replikate elk), en die akute gevolge op oorlewing is ge-evalueer na ses dae van blootstelling. Die sensitiwiteit of toleransie van 12 sleutel taksa is deur middel van hulle respons op die blootstellingsreeks bepaal, en met hulle veldtoleransie/sensitiwiteit vergelyk wat in 'n veldstudie by 'n kontrole- en gekontamineerde gebied in die Lourensrivier bepaal is. Die sensitiwiteit/toleransie van 10 van die 12 taksa in die mikrokosmos eksperimente het ooreengestem met die wat in die veld gevind is. Mikrokosmosstudies wat op 'n veldrelevante ontwerp gebaseer is, kan dus suksesvol aan veldstudies gekoppel word, en dui aan dat oorgedraagde pestisiedkontaminasie die akwatiese gemeenskap van die Lourensrivier beinvloed.

DEDICATION

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INTRODUCTION

Nonpoint source pollution in surface waters

Nonpoint source agricultural pollution is generally considered one of the major threats to surface water quality in rural areas (Loague *et al.*, 1998). Nutrients, sediments and pesticides potentially enter aquatic environments and thus pose a risk to the communities that inhabit them (Cooper, 1993). Of all nonpoint source pollutants, insecticides are among the most crucial chemical stressors, simply because of their extremely high toxicity to many non-target aquatic organisms (Baier *et al.*, 1985).

In comparison to point sources, our knowledge of the extent of nonpoint-source pollution is still quite restricted (Line *et al.*, 1997). The main factors contributing to this situation are presumably the number and characteristics of variables (land use, meteorology, soil types) that must be taken into account to understand and predict the occurrence and extent of nonpoint-source pollution (Walklate, 1992; Wauchope, 1996).

Non-point source pesticide pollution can enter streams and rivers via three main routes; leaching, spray drift and runoff (Antonious and Byers, 1997; Fawell, 1991; Merkle and Bovey, 1974). Since many insecticides have a relatively low water solubility, their transport via leaching is negligible (Flury, 1996). Moreover, leaching would contribute preferentially to contamination of ground water and thus might affect surface waters only under specific geological conditions (Squillace *et al.*, 1996; Zullei-Seibert and Skark, 1993).

Spray drift and edge-of-field runoff are the most important routes of entry for agricultural nonpoint source insecticide pollution into surface waters (Groenendijk *et al.*,

1994) and may result in considerably different exposure scenarios (Erstfeld, 1999). Whereas spray drift leads to input of pesticides dissolved in the water phase, the contamination during runoff is sometimes largely as a result of pesticides associated with suspended particles (Mian and Mulla, 1992). Although pesticides dissolved in the water phase pose more of an immediate toxicological threat, sediment associated pesticides have also been shown to adversely affect macroinvertebrate communities (Schulz and Liess, 2001).

Importance of runoff

Because of the direct input and bio-availability of pollutants so introduced, spray drift is commonly regarded as the worst case scenario for pesticide exposure in aquatic risk assessment (AEDG, 1992; Ganzelmeier *et al.*, 1995; Gilbert and Bell, 1988). In the few studies that have however directly compared these routes of entry in the same catchment, runoff has been shown to be the most important source of nonpoint pollution (Kreuger, 1998; Schulz, 2001a) and is regarded as the most important factor in terms of contamination of surface waters in arid areas (Everts, 1997).

One potential reason is related to land use factors and the fact that the spatial and temporal scale of runoff contamination is far greater than spray drift (Schulz, 2001a). Many pesticides have long half-life times and previous studies (Kreuger, 1998; Williams *et al.*, 1995) have shown that runoff-induced pesticide loading can occur long after the previous application, indicating that runoff integrates chemical input over a large time span. Runoff can also influence an entire catchment simultaneously and as such, a river can collect surface runoff from a large geographical area. In comparison, spray drift is instantaneous and contamination can only occur during application, in combination with specific meteorological requirements (downwind orientation of surface waters to the point of application), which further restricts the potential for contamination (Schulz, 2001a). Furthermore spray deposition in surface waters is considerably reduced with increasing distance from the point of application (Ganzelmeier *et al.*, 1995; USEPA, 1999)

The quantity of pesticides that enter surface waters via runoff are dependent on a number of factors and include the time interval between the application of pesticides and the first heavy rainfall event, the slope and soil types of the catchment, the quantity of applied pesticide and the size and characteristics of buffer strips (Cole *et al.*, 1997; Merkle and Bovey, 1974). Furthermore, runoff is highly dependent on the physico-chemical properties of the pesticides themselves as they determine the amount of pesticide available to surface runoff (Capel and Larson; 2001; Blanchard and Lerch, 2000). For instance, pesticides with low water solubilities or high Koc (tendency of chemicals to bind to organic carbon) values will tend to be more associated with sediments as opposed to dissolved in the water phase. Thus, when evaluating the risk of runoff, it is important to sample for pesticides in both the water and suspended sediment phases.

Runoff induced pesticide input in South Africa

Previously only a few studies have dealt with pesticide levels in farm dams (Davies and Peall, 1997; Hassett *et al.*, 1987), river ecosystems (Greichus *et al.*, 1977; Grobler, 1994; Roux *et al.*, 1994) or groundwater (John Weaver, CSIR, pers.

communication) in South Africa. None of these studies attempted to establish a direct link between agricultural use of chemicals and contamination of the aquatic environment. Moreover, they have not addressed the problem of runoff as a potential route of entry into the freshwater ecosystem. A factor that has contributed to the lack of research is the shortage of laboratories with the equipment and expertise to carry out complex analyses (Dallas and Day, 1993).

The Lourens River, South Africa

More recently, extensive research has focused on nonpoint source pollution by pesticides in the Lourens River, Western Cape, South Africa. After leaving a naturally vegetated fynbos area, the river runs through intensive farming areas (orchards and vineyards) in its middle reaches before flowing through the town of Somerset West. The upper reaches of the river are in a relatively pristine state and are currently under a Class 2 conservation status (Tharme *et al.*, 1997). During recent decades a decrease in water quality in Western Cape rivers has been observed. This shift has also occurred in the middle and lower reaches of the Lourens River, and is attributed to intensified agriculture, sediment input and loss of indigenous vegetation (Tharme *et al.*, 1997). However, no information exists about the extent to which toxic substances from the orchard plots in the surroundings are responsible for the degradation of the Lourens River.

It has now been established that runoff is the most important source of nonpoint pesticide pollution in this catchment (Schulz, 2001a) and that most contamination of the

Lourens River mainstream is as a result of runoff activity in the tributaries (Dabrowski *et al.*, 2001; Schulz, 2001b).

Studies have shown that the first heavy rainfall after application results in the highest quantity of pesticides in surface waters (Domagalski *et al.*, 1997). Thus, in the context of the Western Cape, important periods for determining runoff-related contamination are at the beginning of April, when the first heavy rains normally fall after the end of the spraying season in late February (Dabrowski *et al.*, 2001; Schulz *et al.*, 2001; Schulz, 2001a), and after heavy rainfall events during intense spraying (October to December) (Schulz, 2001a; Schulz, 2001b).

Modelling runoff induced pesticide input

As evidence of the detrimental effects of pesticides in aquatic environments accumulates, so the need to predict areas of risk becomes more and more urgent (Black *et al.*, 2000). While extensive sampling in river catchments can identify 'hotspots' and areas of concern, the ability to predict problem areas is very valuable in implementing and prioritising mitigation strategies and in cutting costs associated with the analysis of samples.

Studies have linked agricultural land use to pesticide contamination in surface waters (Munn and Gruber, 1997), but few have attempted to predict problem areas encompassing agricultural land use only. Assessment of potential side effects of pesticide use includes an estimation of exposure, which is usually based on predicted environmental concentrations (PEC) (Bascietto *et al.*, 1990). However, it has also been pointed out that modelling approaches in exposure assessment should also be validated through measurement of real contamination in the field (Solomon *et al.*, 1996; Wauchope, 1996). Such was the main aim of the first part of this thesis, which is described in detail in Chapter 2.

Because of the combination of land use, meteorological and chemical factors, the prediction of runoff requires a great number of input variables, and complex models such as GLEAMS, PRZM and AGNPS have been developed for this purpose (Donigian Jr. and Huber, 1991). These models have been validated in the field with varying degrees of success (Solomon *et al.*, 1996; Donigian Jr. and Huber, 1991; Grunwald and Norton, 2000), but have disadvantages in that they are complicated and normally require a large amount of input variables that may not be generally available (Adriannse *et al.*; 1997). Minimal data requirements and ease of application are the main advantages of simpler simulation methods (Donigian Jr. and Huber, 1991).

Based on previous studies of runoff-induced pesticide pollution in the Lourens River catchment (Dabrowski *et al.*, 2001; Schulz, 2001a; Schulz, 2001b; Schulz *et al.*, 2001) a modelling approach was implemented using a runoff formula by Reus *et al.* (1999). This formula is designed as a simple tool for prediction of pesticide loss in runoff and has been proposed as a risk indicator for runoff by the OECD.

Assessment of effects

From the lack of information about the extent of agricultural nonpoint-source pollution it follows that the resulting effects on aquatic communities are also not fully understood (Cooper, 1993; Willis and McDowell, 1982). It has been stated by many authors that conventional toxicity testing is often based on exposure scenarios with questionable field relevance, since concentrations and/or exposure times are not based on conditions in the field (Brent and Herricks, 1998; Hosmer *et al.*, 1998; Liess and Schulz, 1996). Moreover, type and composition of test species do not necessarily reflect the complexity that prevails in the field (Breneman and Pontasch, 1994; Pontasch and Cairns, 1991).

Ecotoxicological investigations can be classified according to their level of complexity: experimental micro- and mesocosm studies in laboratory and field systems, purposeful contamination of natural streams, in situ bioassays and field studies about the effects of the "natural" contamination (Buikema Jr. and Voshell Jr., 1993). Studies including experimental insecticide input into surface waters (Davies and Cook, 1993; Dermott and Spence, 1984; Dosdall and Lehmkuhl, 1989; Kreutzweiser and Sibley, 1991; Lugthart *et al.*, 1990; Yasuno *et al.*, 1981) or the use of in situ bioassays (Baughman *et al.*, 1989; Matthiesen *et al.*, 1995) reveal a distinct correlation of contamination and biological effect. When investigations are based on the determination of abundance and drift in the stream itself, the relationship can only be assumed or approximately assessed (Heckman, 1981; Lenat and Crawford, 1994; Sallenave and Day, 1991; Sályi and Csaba, 1994; Tada and Shiraishi, 1994).

To sum up these studies, it can be concluded that correlation between contamination by insecticides applied at commonly used rates and effects on the aquatic communities in headwater streams is rarely investigated or established. As can be seen from various review articles (Cooper, 1993; Willis and McDowell, 1982), very little information is available about the impact of insecticide contamination on aquatic communities in the field. It can be concluded that although a considerable amount of general ecotoxicological information has been published, field studies of relevant contamination and their biological effects on aquatic communities are very scarce.

The regulatory assessment of pesticide effects is generally based on experimental investigations in laboratory or outdoor microcosms and mesocosms (Setac, 1991; Setac, 1993; Setac, 1998; Setac, 1999). As a consequence, there is an obvious conflict between chemical testing in an experimental system and the derivation of proper risk assessment for the conditions in the field (Cairns Jr. *et al.*, 1994; Crossland, 1994; Koeman, 1982; Pontasch *et al.*, 1989).

One concern is that the duration of exposure and observation periods in ecotoxicological studies do not always reflect field-relevant conditions. For example, the episodic nature of pollution events cannot adequately be addressed by conventional toxicity testing methods with fixed-duration continuous exposure (Hosmer *et al.*, 1998). To obtain toxicity data relevant to the field situation, different short-term exposure scenarios must be compared and assessed (Abel, 1980; Parsons and Surgeoner, 1991). Furthermore, recent ecotoxicological studies have increasingly emphasized the importance of long-term observations of effects after short-term contamination (Liess and Schulz, 1996; Woin, 1998).

The release of pesticides into aquatic ecosystems generally impacts entire communities. As a result, multispecies tests have been developed to reduce uncertainties when extrapolating from the laboratory to the field (Crane, 1997; Pontasch and Cairns, 1991). In addition to direct lethal effects, sublethal and indirect reactions play an important role, and the resulting ecological effects are often difficult to assess (Anderson, 1989; Day, 1989; Hurlbert, 1975). In nature the dynamics of invertebrate populations are influenced by a number of environmental factors simultaneously (Peckarsky and I., 1980; Townsend *et al.*, 1983). However, studies of these environmental factors have usually been single-factor studies. In nature, the population dynamics are likely to be influenced by many factors simultaneously. The input of pollutants into aquatic surface waters also produces a potential for combined effects of introduced chemicals and already existing biological interactions, such as predation or competition (Schulz and Dabrowski, 2001).

In conclusion, experimental studies form an important part of the effect assessment within an ecotoxicological risk assessment. However it is important that they are field relevant in terms of length and concentration of exposure and represent the communities that occur naturally in the field. Chapter 3 of this thesis addresses this aspect.

Objectives of this study

A typical ecotoxicological risk assessment includes an assessment of exposure and biological effect, both of which are encompassed by the following two chapters of this thesis. The two main objectives are:

1. An assessment of exposure, based on the prediction and field-based validation of levels of runoff induced pesticide contamination in sub-catchments of the Lourens River (Chapter 2).
2. An assessment of biological effect, based on ecotoxicological studies at the microcosm level on multispecies communities at concentration and exposure

scenarios relevant to field conditions (1 hour exposure at concentrations previously reported in field studies) (Chapter 3).

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PREDICTING RUNOFF INDUCED PESTICIDE INPUT IN AGRICULTURAL SUB-CATCHMENT SURFACE WATERS: LINKING LAND USE AND CONTAMINATION

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Abstract-An urgent need exists for applicable methods to predict areas of risk of pesticide contamination within agricultural catchments. As such, an attempt was made to predict and validate contamination in nine separate sub-catchments of the Lourens River, South Africa, through use of a GIS-based runoff model, which utilizes land use and physico-chemical characteristics of applied pesticides as its variables. The results of the prediction were compared with measured contamination in water and suspended sediment samples collected during runoff conditions in tributaries discharging these sub-catchments. The most common insecticides applied and detected in the catchment over a 3-year sampling period were azinphos-methyl (AZP), chlorpyrifos (CPF) and endosulfan (END). AZP was preferably found in water samples, while CPF and END were detected at higher levels in the suspended particle samples. Significant ($p < 0.005$) positive correlations were found between the predicted average loss and the concentrations of the three insecticides both in water and suspended sediments (R^2 between 0.75 and 0.9). Two sites in the sub-catchment were identified as posing the greatest risk to the Lourens River mainstream. It is assumed that lack of buffer strips, presence of erosion rills and high slopes are the main variables responsible for the high contamination at these sites. It can be concluded that this approach to predict runoff related surface water contamination may serve as a powerful tool for risk assessment and management in South African orchard areas.

INTRODUCTION

Much attention has focused on the impact of insecticides in aquatic ecosystems as evidence accumulates of their detrimental effects to community structure and reproductive and developmental processes in several taxa, including macroinvertebrates (Schulz and Liess, 1999; Leonard *et al.*, 2000), amphibians (Berrill *et al.*, 1998), birds, fish and other wildlife (Alho and Vieira, 1997; Gruber and Munn, 1998). Of major concern is the fact that low, sub-lethal levels of pesticide exposure are responsible for negative ecotoxicological effects, stressing the need for methods capable of identifying problem areas where populations could be at risk.

A few studies have attempted to evaluate and predict the impact of different land uses on pollution in stream systems, and many have linked agricultural land use to poor water quality as a result of nutrient (Heidtke and Auer, 1993; Basnyat *et al.*, 2000) and bacterial contamination (Gilliland and Baxter-Potter, 1987). Munn and Gruber (1997) and Black *et al.* (2000) showed that the presence of organochlorine pesticides in streambed sediment and fish tissue was correlated to agricultural land use. All of these studies focused on a large-scale regional level and compared different types of land use such as forestry, urban and agriculture and correlated these practices to contamination in stream systems. This research is valuable in comparing large areas for risk mitigation but do not identify 'hotspots' within a catchment area encompassing one type of land use. As agriculture has been shown to be responsible for much of the pollution occurring in river systems, this study focuses specifically on the investigation of an agricultural area and identifying problem areas within this specific land use.

Runoff has been shown to be a major nonpoint source of pesticides to surface waters in agricultural areas (Kuivila and Foe, 1995; Domagalski *et al.*, 1997; Schulz, 2001a) and is dependent on the application and physico-chemical properties (such as half-life and KOC) of the pesticides and the land use characteristics of the surrounding catchment (Merkle & Bovey, 1974). Land use factors influencing runoff include, the gradient of the lands on which pesticides have been sprayed, crop type, organic carbon in the soil, the size of the cropped area and the type and density of buffer strips that lie between agricultural lands and water bodies (Cole *et al.*, 1997).

Several attempts have been made at predicting the level of runoff induced pesticide contamination in surface waters, but few of these have ever been validated in the field. This is the main objective of this present study, which was performed in the Lourens River catchment, in the Western Cape of South Africa. This catchment has been intensely studied since 1998 till present, over which time much research has been dedicated to the occurrence of pesticides in the river and its tributaries. Contamination of the mainstream is primarily as a result of runoff activity in the tributaries (Dabrowski *et al.*, 2001; Schulz, 2001a) and in comparison to spray drift, has been shown to be responsible for the majority of pesticide contamination in this catchment (Schulz, 2001b).

The aim of the present study was to predict relative runoff induced insecticide loss from sub-catchments of the Lourens River using a GIS-based runoff formula (Reus *et al.*, 1999) and to validate the results by comparison to measured levels from field samples taken in the tributaries of the sub-catchments. Through using the formula the intention was not to accurately predict the concentration of pesticides in the tributaries. Rather, it was to determine whether the formula predicts differences in the average loss of pesticides during a runoff event, based on land use and the physico-chemical characteristics of applied pesticides in the sub-catchments (which are incorporated into the formula), and whether these differences are reflected in water samples taken at all of the sites.

MATERIALS AND METHODS

Study Area

The Lourens River originates at an altitude of 1080 m in the Hottentots Holland Mountain range and flows in a south-westerly direction for about 20 kilometers before discharging into False Bay at Strand (34° 06′ S; 18° 48′ E). After leaving a naturally vegetated fynbos area, the river runs through intensive forestry (pine plantations) and farming areas (orchards and vineyards) in its middle reaches before flowing through the town of Somerset West. The study area has a total catchment area of approximately 44 km^2, consisting of eight sub-catchments (LL1 to LL6, V1 and V2), each of which is drained by a tributary that discharges into the Lourens River mainstream (Fig. 1). LR1 represents the Lourens River upstream of any agricultural activity, which was considered as the ninth sub-catchment for prediction, and LR2 represents the site comprising the entire catchment of the study area. The annual mean rainfall is 915 mm most of which occurs during the winter months between April and October (Tharme *et al.*, 1997), as is characteristic of the region's Mediterranean climate. A more detailed description of the study area can be viewed in Dabrowski *et al.* (2001).

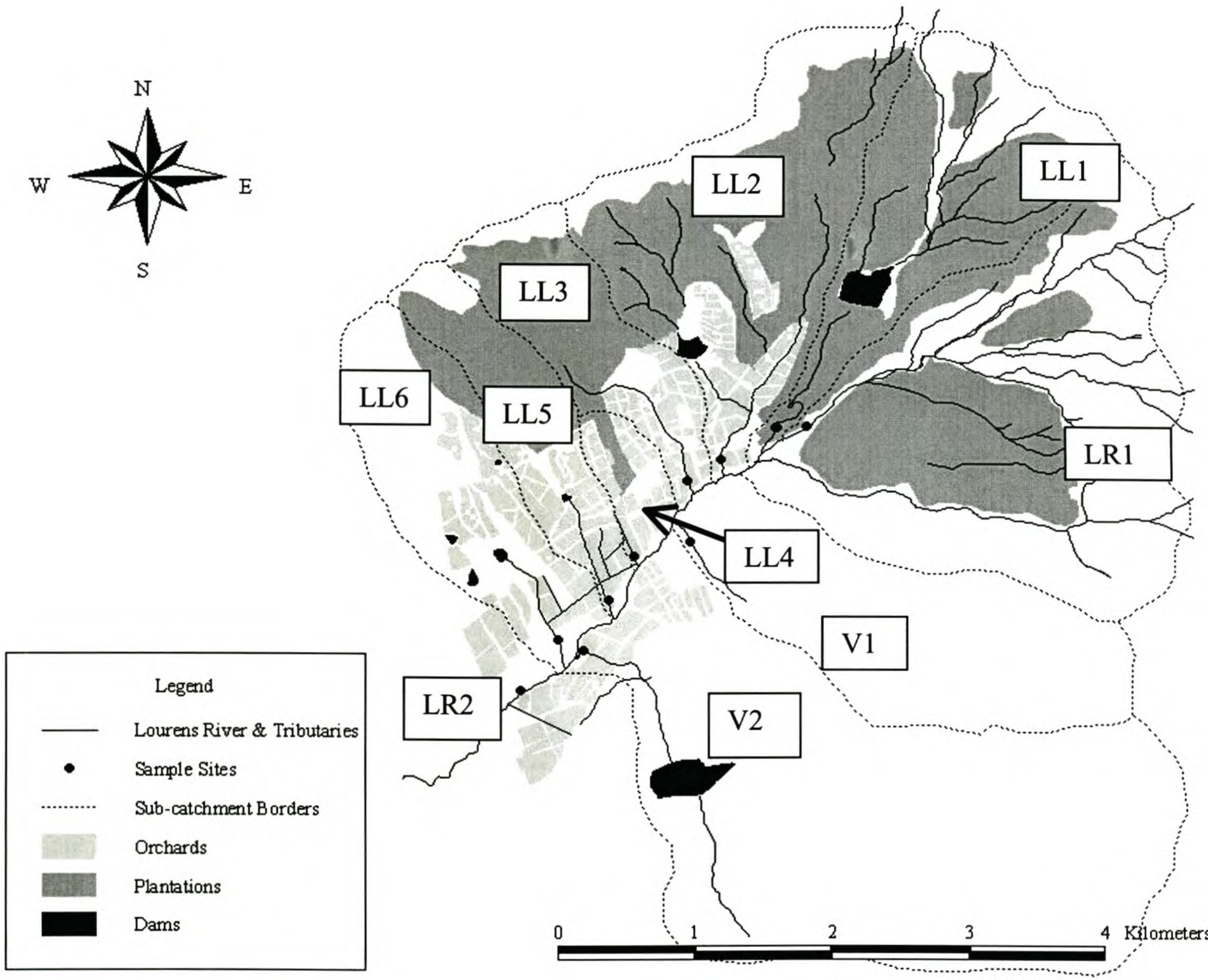

Fig. 1: Scale diagram of the land use in the nine sub-catchments used for prediction (LL1 to LL6, V1, V2 and LR1) and of the total agricultural catchment of the Lourens River, South Africa, represented by site LR2.

Agricultural crops consist exclusively of pear, plum and apple orchards (total growing area: 4 km^2), on which pesticide application takes place between August and mid February before fruit harvest. The three pesticide active ingredients predominantly applied on the orchards and studied are endosulfan (END), chlorpyrifos (CPF) and azinphos-methyl (AZP) (Table 1). CPF and AZP are used frequently between October and February on pears and plums, up to about one application every two weeks on each single plot. END is applied mainly onto apple and plum orchards.

Table 1. Physico-chemical properties and application rates of the most commonly used pesticides in the Lourens River catchment.

	Azinphos-methyl	Chlorpyrifos	Endosulfan
Water Solubility (mg/L)[a]	28 (20°C)	1.2 (25°C)	0.32 (22°C)
DT_{50}soil (days)[b]	10	30	50
K_{OC}[b]	1000	6070	12400
Application rate (g/m^2)[c]	0.0525	0.1008	1.425

[a] USDA ARS database, 1989.

[b] Hornsby *et al.* 1995

[c] Application rates represent the amount applied in one application and were calculated according to the local farmers spraying programme.

Sampling Programme

The sampling area comprised an approximately 6-km stretch of the Lourens River and its side streams running between two farms (Fig. 1). The orchard plots are separated from the Lourens River itself by a strip of vegetation (Eucalyptus trees, shrubs and grasses) between 30 and 100 m in width, making direct input of edge-of-field runoff into the river highly unlikely. In contrast, most of the tributaries (eight in total) are directly adjacent to orchard plots (distance: about 5 m). Ten sampling sites (nine sub-catchments plus LR2) were selected for measurement of aqueous and particle-associated pesticide levels (Fig. 1). Samples were collected at the end of each tributary, ensuring that the streams were well mixed at the point of collection and that the samples were representative of the land use in the respective sub-catchments. Three sub-catchments contained no agricultural activity and acted as reference sites (V1, LL1 and LR1), while all of the other sub-catchments contained some degree of agricultural activity. All water and sediment samples were collected during runoff conditions according to the methods described in Schulz *et al.* (1998); Dabrowski *et al.* (2001); Schulz (2001a) and Liess *et al.* (1996), respectively.

Sampling took place from December 1998 until May 2001 and represented varying application scenarios (Fig. 2). The first heavy rainfall events of the season have been shown to be very important for the entry of pesticides into the river (Schulz *et al.*, 2001), and as such, samples were collected from 1999 until 2001 during this period. Samples collected during November and December of 1998 and 1999, were taken during intensive spraying in the catchment, while those samples taken from July till October 1999, represented the beginning of the spraying season, when application is less intensive.

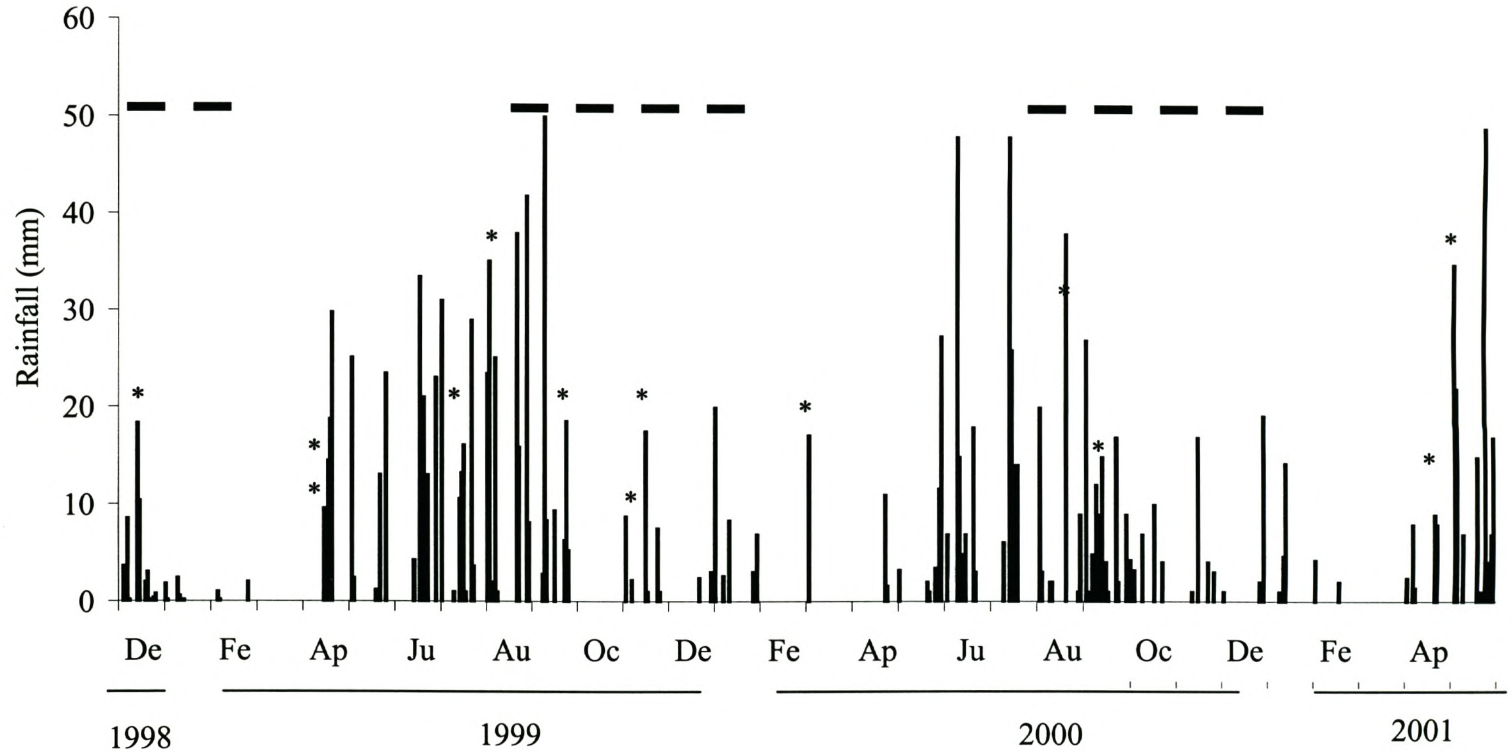

Fig. 2: Daily rainfall in the Lourens River catchment from December 1998 to May 2001. The dashed lines indicate the pesticide application period and the asteriks indicate the runoff events that were sampled.

Water and sediment samples were extracted and analysed for the selected pesticides used in the study area according to the methods described in Dabrowski *et al.*, (2001) and Schulz *et al.* (2001). Analysis was performed by the Forensic Chemistry Laboratory of the Department of National Health, Cape Town. Water samples (500 to 900 ml) were solid-phase extracted (SPE) within 10 h after sampling, using C18 columns (Chromabond). Pesticides from suspended sediment samples were extracted with methanol and concentrated using C18 columns. The columns were air-dried for 30 minutes and kept at –18°C until analysis. Measurements were done using gas-chromatographs (HP 5890′s) fitted with standard HP electron-capture, nitrogen-phosphorus and flame-photometric detectors. The following detection limits were obtained for water and suspended sediments: 0.01 µg/l and 0.1 µg/kg dry wt. Spiked recovery efficiencies were between 79 and 106%.

Land use

Geographical and land use features of the study area were digitised from 1:10 000 orthophoto maps, converted to shapefiles and analysed in the ArcView 3.1 Geographical Information Systems (GIS) programme (ESRI™). A detailed shapefile of slope categories (%) was provided by the Department of Geography, University of Stellenbosch, and a contour line shapefile was provided by Stewart Scott Consulting Services, Cape Town. The entire catchment was divided into sub-catchments based on the contour line shapefile (Fig. 1) and orchards were identified according to their crop type and slope category and assigned to their respective sub-catchments. The following land use information (for every sub-catchment) was then extracted using GIS: area of different fruit types; area of slope categories of each fruit type; length of tributary; and length of tributary bordered by orchards. Buffer strip width and the number of erosion rills adjacent to each tributary was determined through field-based measurements.

Prediction

A GIS-based runoff model (Reus *et al.*, 1999) was used to attempt to predict contamination in the tributaries of each sub-catchment based on land use, pesticide application characteristics and physico-chemical properties of applied pesticides. This formula is designed as a simple method for predicting indirect runoff induced pesticide loading in surface waters, as existing runoff formulas or models are very complex for the purposes of indicating risk and comparison of sub-catchments on a relative basis. The formula is as follows:

$$L\%_{runoff} = (Q/P) \times f \times \exp(-3 \times \ln 2/DT_{50soil}) \times 100/(1+Kd)$$

where:

$L\%_{runoff}$ = Percentage of application dose being available in run-off water as a dissolved substance.

Q = Runoff amount (mm) calculated according to the model of Lutz (1984) and Maniak (1992).

P = Precipitation amount (mm)

DT_{50soil} = Half-life time of active ingredient in soil.

f = Correction factor, with f = f1 x f2 x f3

f1 = Factor that reflects the influence of field slope on L% if slope is < 20%, where: f1 = 0.02153 x slope + 0.001423 x s24

f2 = Factor that reflects the influence of plant interception (PI) percentage on L%, where: f2 = 1-PI/100

f3 = Factor that reflects the influence of a densely covered buffer zone on L%, where: $f3 = 0.83^{WBZ}$ and WBZ is the width of buffer zone (meters); if the buffer zone is not densely covered with plants, in which case the width is set to zero.

Kd = K_{OC} x %OC/100 where K_{OC} is the sorption coefficient of the active ingredient to organic carbon and OC% is the organic carbon content of the soil

For every pesticide the $L\%_{runoff}$ was calculated for each slope category in each sub-catchment and an average loss (%) was then obtained. The loss of contaminated sediment in surface runoff was predicted by adapting the last expression of the formula to 100/[1-(1+Kd)]. This assumes that contamination of sediments is equal to the difference between the total pesticide in runoff and the amount lost as a dissolved substance in surface runoff (predicted by the original version of the formula). When calculating the predicted average loss values, a rainfall value (P) of 18 mm (the average rainfall for all of the measured runoff events) was used with a corresponding Q value of 1.83 (Reus *et al.*, 1999). The formula was applied to predict the average loss of AZP, CPF and END in each sub-catchment for the water and suspended sediment phases.

Data analysis and model validation

T-tests were performed on the mean frequency of detection for each of the pesticides in the water and sediment phase to determine whether significant differences exist in the partitioning of pesticides, as would be assumed from their physico-chemical properties. For each sub-catchment the mean concentration of each pesticide detected in the tributary over the entire study period was calculated so as to compare contamination between sites. Although both isomers of END and the breakdown product END-sulphate were detected in samples, only the α- and β- isomers of END were used in the calculation of the mean concentration, ensuring that the calculated concentrations represent input of recently applied END into the tributaries. Linear regression analyses were used to determine whether the pesticide losses calculated by the GIS-based runoff model could predict the observed mean concentration of pesticides measured in the tributaries of the nine sub-catchments. This validation procedure was performed on all of the three pesticides, for water and suspended sediment samples.

RESULTS

Land use

All of the sub-catchments were analysed in detail through GIS and field-based measurements. In all sub-catchments containing fruit orchards, the dominant land use is still forest or fallow (76 to 95%). LL5 and V2 contain the lowest percentage orchard area (< 5%), while LL4 has the highest (24%). All of the other catchments have similar percentage orchard land utilization (13 to 15%). LL2 has the largest area covered by fruit orchards and contains the largest covered area of apples in the entire catchment (Table 2). LL6 is the only sub-catchment that contains every fruit type and contains the largest area of pears and plums out of all the sub-catchments. The average slope of land covered by orchards in the entire catchment is 4%, with LL2 and LL6 having the highest average slopes of 5.4 and 4.9% respectively. In general apple orchards are associated with high slopes, up to 5.2 and 7.7% in LL2 and LL6 respectively. All sub-catchments containing orchards are extensively bordered by the orchards along their banks, except for V2. LL5 and V2 have no buffer strips between orchards and tributaries and LL5 and LL6 have the highest number of erosion rills in terms of meter bank length per erosion rill.

Pesticide contamination

Over the three-year sampling period AZP, CPF and END were frequently detected in water and sediment samples. AZP was detected at the significantly highest frequency of detection in the water phase. CPF was detected at significantly higher frequencies in the sediment phase than the other two insecticides (Fig. 3). At 81% detection in the sediments, CPF was the most frequently detected pesticide during the study period. For all pesticides, in both the water and sediment phases, LL6 and LL5 were generally the most heavily contaminated sub-catchments (Table 3), while LL1, V1 and LR1 were the least contaminated. Sub-catchments LL2, LL3, LL4 and V2 showed intermediate to moderately high contamination. Site LR2, which encompasses the entire catchment, showed high levels of contamination for all of the studied pesticides.

Table 2. Land use characteristics for the six sub-catchments of the Lourens River containing orchards and for LR2 representing the land use of the entire catchment. No values are given for LL1, V1 and LR1, since these catchments are not containing any orchards.

Sub-Catchment	Orchard Area (km^2)	Orchard Composition (km^2)			Ave. Slope (%)	Max. Slope (%)	Ave. slope per fruit type (%)[a]			Buffer Width (m)	Bank length bordered by orchards (%)	Bank Length Per Erosion Rill (m)
		Apple	Pear	Plum			Apple	Pear	Plum			
LL2	0.9	0.5	0.4	0	5.0	12.5	5.2	4.7	-	5	93	340.8
LL3	0.5	0.3	0.2	0	4.3	7.0	3.8	5.5	-	5	68	340.8
LL4	0.2	0	0.2	0	1.8	7.0	-	3.4	-	6	100	312.3
LL5	0.6	0.1	0.5	0	3.7	12.5	5.2	3.3	-	0	94	68.9
LL6	0.7	0.2	0.5	0.1	5.4	17.5	7.7	5.1	4.5	1	73	93.4
V2	0.3	0	0.2	0.1	1.0	2.0	-	2.0	2.0	0	32	221.1
LR2	3.2	1.0	1.9	0.3	4.0	17.5	5.0	3.5	1.7	30-100[b]	64[b]	-[b]

[a] Sub-catchments denoted with a "-" do not contain these orchard types and as such have no slope values.

[b] Refers to the Lourens River main channel.

Table 3. Mean concentrations (± standard error) of the most common insecticides detected in water and sediment samples taken during runoff conditions in the Lourens River (LR2) and the surface water discharging the nine sub-catchments from December 1998 to May 2001 (n = number of samples taken during the study period; n.d. = not detected).

Sample	Water (µg/L)				Sediment (µg/kg)			
Site	Azinphos-methyl	Chlorpyrifos	Endosulfan	n	Azinphos-methyl	Chlorpyrifos	Endosulfan	n
LL1	n.d.	n.d.	n.d.	6	n.d.	n.d.	n.d.	8
LL2	0.13 (0.06)	n.d.	n.d.	10	2.28 (1.3)	11.4 (3.9)	6.2 (3.7)	12
LL3	0.01 (0.003)	n.d.	n.d.	3	2.7	12.9	n.d.	3
LL4	n.d.	n.d.	n.d.	3	n.d.	5.9 (3.8)	n.d.	7
LL5	0.19(0.09)	0.02 (0.02)	0.05 (0.02)	11	5.23 (3.7)	11.4 (3.9)	5.63 (3.0)	10
LL6	0.13 (0.08)	0.02 (0.01)	0.05 (0.03)	9	19.46 (17.8)	43.6 (19.0)	34.75 (18.4)	12
V1	n.d.	n.d.	n.d.	8	n.d.	2.0 (1.0)	n.d.	8
V2	0.03 (0.02)	n.d.	0.01 (0.01)	12	3.3	4.3 (2.3)	n.d.	13
LR 1	0.01 (0.01)	n.d.	n.d.	13	n.d.	1.4 (0.7)	n.d.	12
LR 2	0.2 (0.13)	0.03 (0.02)	0.3 (0.25)	13	27.9 (22.0)	21.8 (6.5)	21.1 (13.4)	11

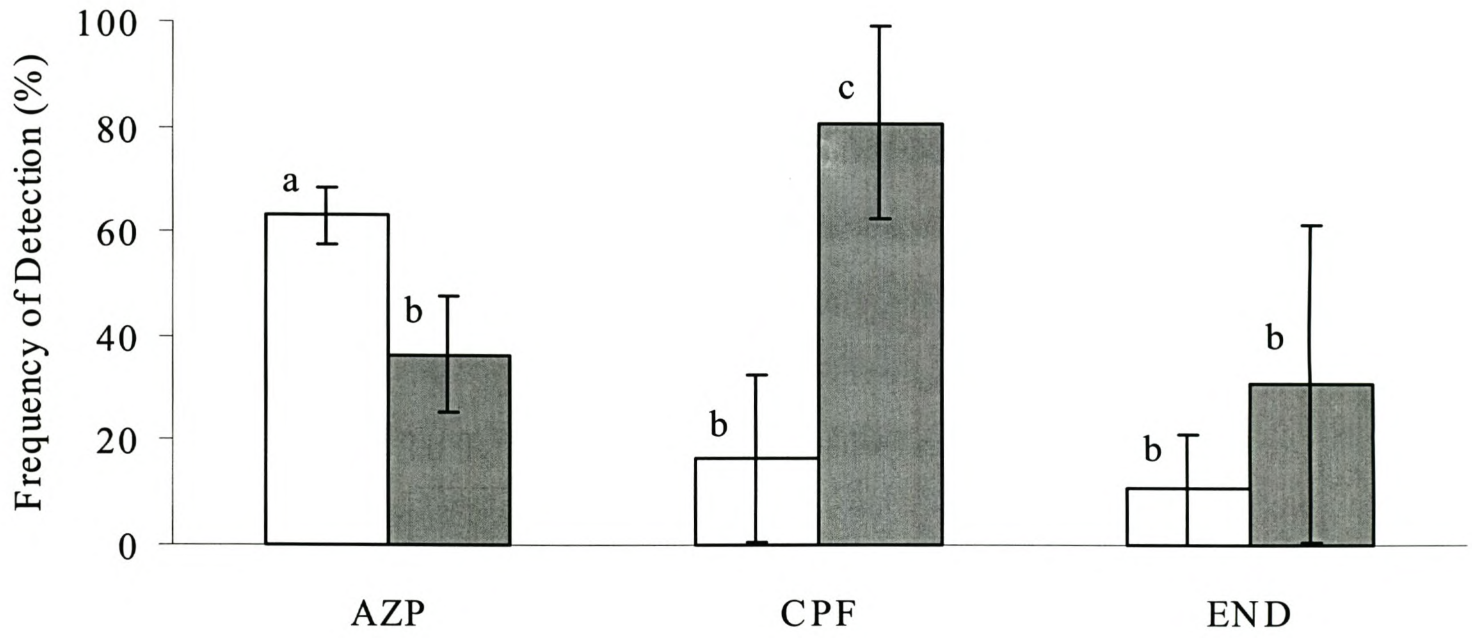

Fig. 3: Mean frequency of detection (± standard error) for azinphos-methyl (AZP), chlorpyrifos (CPF) and endosulfan (END) in water (white bars) and suspended sediment (grey bars) samples. Bars with different letters are significantly different (t-test; d.f. = 9; $p < 0.05$).

Validation of the GIS-based runoff model

For all three of the studied pesticides, in both the water and sediment phases, the GIS-based runoff model predicted LL5 and LL6 as being the most heavily contaminated sub-catchments (Fig. 4), while LR1, LL1 and V1 were predicted as being those sites where contamination would be zero due to the lack of orchards. Of the sub-catchments containing orchards, LL4 and V2 were predicted as having a low contamination. Regression analysis showed a significant positive correlation between measured concentrations and predicted average loss (%) in each sub-catchment for AZP, CPF and END in the water and sediment phase. The squared regression co-efficients (R^2) were between 0.75 and 0.9, indicating strong correlations between predicted loss and measured concentration levels.

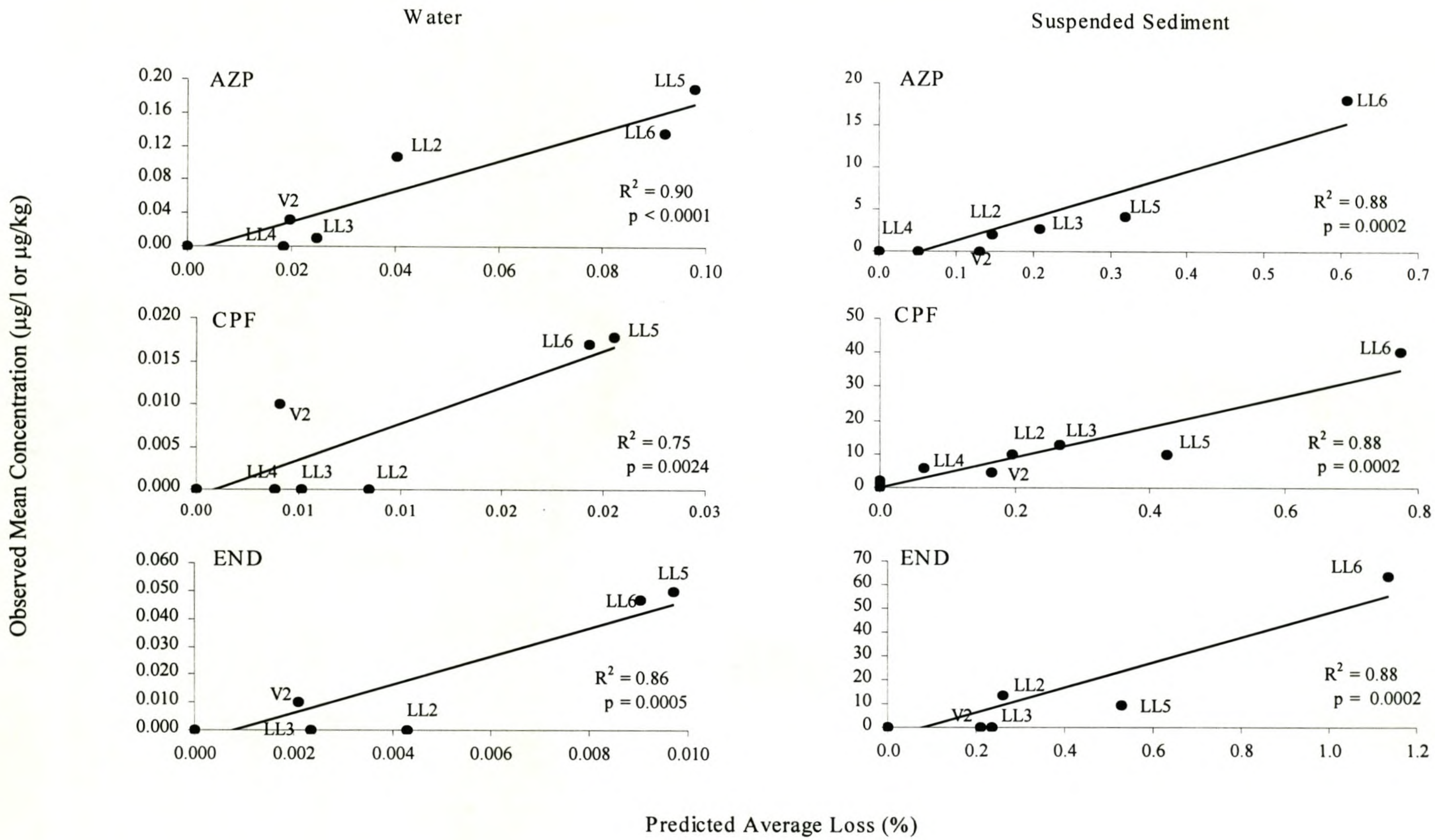

Fig. 4: Correlation of the measured mean concentration and the predicted average loss of azinphos-methyl (AZP), chlorpyrifos (CPF) and endosulfan (END) in sub-catchments of the Lourens River (n = 9). Seperate correlations are given for water (left) and sediment phase (right)

DISCUSSION

Land use and pesticide contamination in the sub-catchments

All of the sub-catchments are dominated by forest, fallow or fynbos. With the exception of LL4, the percentage orchard land use was always the least dominant of the present land uses. In spite of this relationship, pesticides are detected at high concentrations in both the water and sediment phases at LR2 (Table 3), highlighting the impact that a small percentage agricultural land use can have on a running water system. Munn and Gruber (1997) found that the presence of agriculture rather than the percentage of agricultural land use determines the concentrations of organochlorines in bed sediment or fish tissue, further indicating that rivers in catchments with a small percent agricultural land use can still be exposed to high concentrations of pesticides.

In most cases, land use and differences in pesticide application could explain the difference in the level of contamination between sub-catchments. The results clearly indicate that LL5 and LL6 are the most heavily contaminated sites in the study area, and all of the studied pesticides were detected at these sites at high mean concentrations in the water and suspended sediment phases (Table 3). In LL5 the main parameter contributing to this high contamination is most probably as a result of the absence of any vegetated buffer strip, which together with high average slopes, promotes rapid access of surface runoff into the tributary (Table 2). A combination of narrow buffer strip width and a high average slope of cropped land (with a maximum slope of 17.5 %) are the most likely cause of high contamination levels in the tributary of LL6 (Table 2). More importantly, these two tributaries have the smallest amount of bank length per erosion rill, resulting in a high number of entry points for the direct entry of surface runoff.

LL2 and V2 are similar to LL6 and LL5 respectively, in that orchards in LL2 are associated with high average and maximum slopes and V2 lacks a buffer strip (Table 2). However, LL2 is well buffered and orchards in V2 are situated on relatively flat slopes (maximum 2% slope), resulting in the comparatively low contamination detected at these two sites. All of the studied pesticides were detected at V2 in both phases, but at low mean concentrations (Tables 4). This indicates that there is relative easy access for runoff water into the tributary, but that the low slope percentage prevents excessive high contamination.

Of all the sub-catchments containing fruit orchards, LL4 was the least contaminated, with only CPF being detected at a low mean concentration in the suspended sediment phase. The presence of only one fruit type, a dense buffer strip, and low number of erosion rills are presumably responsible for the low contamination in this sub-catchment.

As was expected, no contamination, or at the most, very low levels of contamination were detected in those sub-catchments where no orchards were present – LL1, V1 and LR1. It is unexplainable at this stage as to why low levels of END and CPF were detected at some of these sites.

The high frequency of detection of AZP in water and CPF in the sediment phase (Fig. 3) reflects the different K_{OC} values for both pesticides (Table 1). Studies of single runoff events in this catchment (Dabrowski *et al.*, 2001; Schulz, 2001) and other catchments (Kuivila and Foe, 1995) showed similar partitioning of pesticides according to their water solubility. Out of all the pesticides, CPF was detected in sediments at the highest frequency of 81%. This may be partly due to the fact that this pesticide is applied to all orchard types, and is thus applied in all agricultural sub-catchments at very high quantities. Kreuger (1998) found that the occurrence of pesticides in runoff samples in Southern Sweden was correlated to the amounts used in the catchment.

Validation of GIS-based runoff model

Regression analysis showed a significant positive correlation between the predicted average loss and the observed mean concentration for all pesticides in both the water and sediment phase in each sub-catchment (Fig. 4). This indicates that the loss of pesticides calculated by the formula is well linked to the level of contamination in the tributaries of the sub-catchments. For all sub-catchments containing orchards, LL5 and LL6 had the highest predicted values for all pesticides, while LL4 and V2 had the lowest, which correlates well to observed mean concentrations. Therefore the formula predicts differences in the loss of pesticides associated with surface runoff and assumes that these losses are ultimately responsible for the concentrations detected in the tributaries of the sub-catchments, despite other differences in the sub-catchments that may also influence concentration, such as the hydrology of the tributaries. The physico-chemical properties of the pesticides are ubiquitous to all of the sub-catchments, and as such it is ultimately the differences in the land use variables that are responsible for the observed differences in contamination of the sub-catchments. Thus, results from this study have validated the use of the selected GIS-based runoff model, indicating that the land use variables utilized in the formula are suitable for predicting differences in runoff induced contamination between sub-catchments.

Such a direct link between predicted loss and real contamination resulting from runoff in agricultural sub-catchments has not been documented for pesticides so far. A few other studies have attempted to predict the influence of land use on contamination of agricultural surface waters. Fisher *et al.* (2000) were able to determine which of a number of large-scale agricultural practices (such as grazing land, dairy and poultry operations) were responsible for poor water quality in the Oconee Watershed of Georgia, based on analysis of land use through GIS. Munn and Gruber (1997) used a Geographical Information and Analysis Retrieval System (GIRAS), and showed that organochlorine contamination of streambed sediment and fish was directly linked to the presence of dryland farming. More predictive models have also been developed. Huber *et al.* (1998) developed a model based on land use characteristics and application scenarios to predict the loss of pesticides in surface runoff in the whole of Germany. Fuzzy expert systems have also been developed to predict the potential environmental impact of the application of a particular pesticide in a field crop (van Der Werf and Zimmer, 1998). However validation of these models is required before they can be utilized as a predictive risk assessment tool. Furthermore, most of these models are very complex and require a large amount of input variables that are quite difficult to obtain on a national or supranational level.

Most importantly, our results show that a simple GIS-based runoff model is able to predict differences in the degree of contamination of surface runoff between sub-catchments, which is then validated by samples taken in the field during runoff events.

Such an approach would be very valuable in the planning and execution of mitigation measures in agricultural catchments. The amount of time and money spent on determining real contamination in sub-catchments is great and could be greatly reduced by use of such a formula. All that is required is a detailed analysis of the land use and pesticide application regimes, and problem areas can then be identified, speeding up and prioritising mitigation measures. The formula can be used to predict concentrations in tribuatries by using the $L\%_{runoff}$ to calculate the amount of pesticide lost (g) according to how much is applied in the sub-catchment and combining this with discharge data. However, this would require measurement of TSS and discharge with high temporal resolution over the course of a runoff event for each tributary as well as the length (in time) of the runoff event. While the formula used in this manner does not predict concentrations of pesticides in the tributaries, it is very useful for the relative comparison of sub-catchments, which can then aid farmers or environmentalists in management planning and in identifying areas of risk. The original use of the formula is intended to predict the loss of insecticide during a specific event at a specific site, but in the context of this study it was used as a general predictive tool for comparisons between sub-catchments.

The two most important land use variables utilized in this formula are the slope and vegetated buffer width. Slope has been shown to be the most important factor influencing runoff but can be greatly modified by the presence of vegetation (Wilcox and Wood, 1989). Vegetated buffer strips bordering agricultural plots have been shown to be effective at reducing the level of pesticides in surface runoff (Patty *et al.*, 1997) and help improve the water quality and remove sediment from surface runoff by stabilizing stream-banks and promoting infiltration (Castelle et al., 1994). The vegetation helps to resist the formation of channels (erosion rills), resulting in low flow rate sheet-flow as opposed to rapid channelised flow, which allows more time for the settling of sediments and infiltration. This is reflected in this study, where high-average slope sub-catchments with a narrow buffer width (< 1m) such as LL5 and LL6 had a high number of erosion rills per bank length (Table 2). In comparison, LL2 and LL3 both have large orchard areas situated on steep slopes and wider buffer strips (5 m), but have a low number of erosion rills per bank length and show relatively lower contamination.

The formula predicted a high average loss for AZP and lower values of average loss for CPF and END (END having the lowest predicted loss per sub-catchment) in the water phase (Fig. 4). This is in agreement with the partitioning of AZP according to the samples taken during runoff and to the low K_{OC} of this pesticide. The predictions for the sediments were generally much lower than the measured levels in the field. However, for a direct comparison, TSS and discharge values with high temporal resolution would be necessary, as discussed above.

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A COMBINED MICROCOSM AND FIELD APPROACH TO EVALUATE THE AQUATIC TOXICITY OF AZINPHOS-METHYL TO STREAM COMMUNITIES

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Abstract— Potential ecotoxicological effects of the orchard insecticide azinphos-methyl (AZP) were evaluated in a combined microcosm and field approach. The upper regions of the Lourens River, South Africa are free of contamination (control site), while the subsequent stretches flowing through a 400-ha orchard area receive transient insecticide pollution (e.g. 0.82 µg/L AZP, 344 µg/kg chlorpyrifos) following spray drift and runoff (contaminated site). Stones taken from the control site were transferred to outdoor microcosms (1.5 x 0.2 x 0.2 m) resulting in 12 core species and approximately 350 individuals per microcosm. Microcosms were contaminated for 1 h with AZP (control, 0.2; 1, 5 and 20 µg/L; three replicates each) and acute effects on survival were evaluated 6 days following exposure. The two highest treatments led to a significant (ANOVA, Fisher´s PLSD) reduction in abundance of various insect groups, such as *Demoreptus* sp., *Castanophlebia* sp., Simuliidae and Chironomidae. In contrast, *Aeshna* sp., *Dugesia* sp., Ceratopogonidae and *Cheumatopsyche* sp. were not affected. In parallel, a quantitative macroinvertebrate survey was conducted at the control site and the contaminated site of the Lourens River. Both sites contained a similar number of species but differed considerably in their species composition. Six out of the eight species that reacted sensitively to AZP in the microcosm study occurred in the field at significantly lower densities or were absent at the contaminated site in comparison to the control site. All of the four species that were tolerant in the microcosm occurred at significantly higher densities at the contaminated field site. Two out of the twelve species reacted differently in the microcosm and the field study. It can be concluded that microcosm studies employing a field relevant design could be linked successfully to field studies and that transient pesticide contamination affects the aquatic communities of the Lourens River.

INTRODUCTION

Nonpoint source agricultural pollution is regarded as the greatest threat to contamination of surface waters in rural areas (Loague *et al.*, 1998). Of all nonpoint source pollutants, insecticides are among the most crucial chemical stressors, because of their extremely high toxicity to many non-target aquatic organisms (Baier *et al.*, 1985). While present knowledge of ecotoxicological effects of pesticides is based largely on standardised single species toxicity tests, their field relevance is questionable, as the type and composition of test species does not necessarily reflect the complexity that prevails in the field (Breneman and Pontasch, 1994; Pontasch and Cairns, 1991). Furthermore, the episodic nature of nonpoint source pollution events cannot be adequately addressed by conventional toxicity testing methods with fixed-duration continuous exposure (Brent and Herricks, 1998; Hosmer *et al.*, 1998).

The release of pesticides into aquatic ecosystems generally impacts entire communities and therefore evaluating the effects of pesticides on aquatic communities is difficult to assess due to the ecological complexity at higher levels of organisation (Shaw and Manning, 1996). As a result, multi-species microcosm tests have been developed to reduce uncertainties when extrapolating from the laboratory to the field (Crane, 1997; Pontasch and Cairns, 1991). Only a few of these studies have employed field relevant exposure scenarios typical of nonpoint source pollution events (Liess *et al.*, 1999; Schulz and Liess, 2001a; Schulz and Liess, 2001b). Runoff and spray drift are the most important routes of nonpoint source pesticide pollution (Groenendijk *et al.*, 1994) and typically result in brief periods of exposure (Liess *et al.*, 1999) at concentrations which are normally not acutely toxic to aquatic fauna (Schulz and Liess, 1995). Short-term exposure can result in long-term effects which are apparent only after an extended period of time (Liess and Schulz, 1996; Woin, 1998), necessitating the need to compare different short-term exposure scenarios (Abel, 1980; Parsons and Surgeoner, 1991). Most importantly, despite the increased ecological relevance of multi-species microcosm experiments, very few studies have been able to establish a causal link between field relevant microcosm studies and community structure in the field.

Such was the aim of this study, which was to establish the long-term effects of different field relevant AZP exposures on the structure of microcosm communities. The community structure of the microcosms was then compared to that which prevails in the Lourens River, South Africa, which is frequently contaminated by nonpoint source pollution events. The main objective of the study was thus to link the microcosm and field-based studies in an attempt to establish a causal relationship between nonpoint source pesticide pollution and its effect on macroinvertebrate communities.

Azinphos-methyl is an organophosphate insecticide with a high water solubility and has been shown to be highly prevalent in surface waters as a result of runoff and spray drift activity in the Lourens River (Dabrowski *et al.*, 2001; Schulz, 2001a; Schulz *et al.*, 2001b; Schulz *et al.*, 2001c) and other surface waters (Granovsky *et al.*, 1996; Smith *et al.*, 1983). This is the most commonly applied pesticide in the catchment and the biological effects of this pesticide on aquatic communities has so far not been demonstrated.

MATERIALS AND METHODS

Study area and sampling sites

The Lourens River originates at an altitude of 1080 m in a naturally sclerophyllous vegetation area (fynbos) and flows in a southwesterly direction for approx. 20 kilometres before discharging into False Bay at Strand (S34°06′; E18°48′). The catchment region is characterized by intensive farming, with orchards and vineyards in its middle reaches. The Lourens River has a total catchment area of approx. 92 km^2 and receives an annual mean rainfall of 915 mm. Approximately 87% of its $35x10^6$ m^3 mean annual discharge occurs during the winter months between April and October, as is characteristic of the region's Mediterranean climate. The 400-ha orchard area consists mainly of pears, plums and apples. The pesticide application period in the study area′s orchards proceeds from early August and continues until end of March. Organophosphorous insecticides, such as azinphos-methyl and chlorpyrifos, are applied between October and February quite frequently to pears and plums (Schulz, 2001a).

Two sites were selected for this study. The control site was situated upstream of all agricultural activity and is free of pesticide contamination (Schulz *et al.*, 2001b). A second site situated in the orchard area approximately 1500 m downstream of the control site was chosen. This site is exposed to pesticides that are transported to the Lourens River via the tributaries (Dabrowski *et al.*, 2001) as a result of runoff (Schulz *et al.*, 2001a) and spray drift (Schulz *et al.* 2001b). Transient peak levels measured at this site reached 0.82 μg/L AZP in water and 344 μg/kg chlorpyrifos in suspended particles (Schulz *et al.*, 2001a; Unpublished Data). Both sites were characterized by rocky substrate and were similar in structural and physico-chemical water quality parameters (Table 1).

Table 1. Mean (± standard error, n = 4) of structural characteristics in riffle areas and physico-chemical water quality parameters measured at the two study sites in the Lourens River

Parameter	Control site	Contaminated site
Bottom substrate composition		
Rocks, > 2cm (%)	65.0±5.4	66.2±5.1
Sand, < 2 cm (%)	32.5±4.3	26.2±6.6
Silt, > 0.6 mm (%)	2.5±1.4	7.5±3.2
Current velocity (m/s)	0.18±0.01	0.16±0.02
Nitrite (mg/L)	0.004±0.004	0.006±0.003
Ammonium (mg/L)	0±0	0.001±0.001
Orthophosphate (mg/L)	0.07±0.05	0.12±0.09
pH	7.0±0.05	7.0±0.1
Oxygen (mg/L)	9.5±0.2	9.4±0.1
Temperature (°C)	19.8±0.2	20.9±0.2
Total suspended solids (mg/L)	3.7±2.2	19.1±2.6

Outdoor stream microcosms

The open outdoor artificial stream system consisted of 15 stainless steel microcosms (1.5 × 0.2 × 0.2 m). Each microcosm contained a longitudinal middle wall forming two channels, each 10 cm in width (Schulz and Dabrowski, 2001). Water taken from the control site in the Lourens River was used in the microcosms. Each stream contained a volume of 30 L. Current (0.2 ± 0.05 m/s) was provided by a 100-W electric motor turning stainless steel paddlewheels attached to a rod.

Experimental procedure

The stream microcosms were established two days before introduction of test organisms. Each stream was equipped with 18 rocks taken randomly from shallow (water depth: 25 to 30 cm) riffle areas at the control site in the Lourens River and containing the associated macroinvertebrate fauna. Each rock (diameter: 8-10 cm) was removed from the stream bed and macroinvertebrates drifting downstream were caught in a handnet (mesh size: 500 μm) that was placed downstream of the rock. Each set of 18 rocks were transported in seperate plastic containers and the rocks and animals were transferred into the microcosms within 3 hours. Temperature remained constant at 20 ± 2°C during transport. The rocks were orientated in the microcosms in the same way as in the stream with the upper side covered by algae. They were entirely submerged in water. Following introduction into the microcosms, the current was stopped for 30 minutes to allow the invertebrates to settle on the rocks.

On the 22.2.2001, one day after introduction of the rocks we exposed the microcosms to the pesticide. Azinphos-methyl [*O,O*-dimethyl-*s*-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl)]phosphorodithioate] was applied as an emulsifiable concentrate (Azin 200SC®, Sanachem, Durban, South Africa) containing 20% (weight/volume) of active ingredient. Apart from the active ingredient the emulsifiable concentrate contains emulsifier and water. All concentrations given here refer to the active ingredient. The emulsifiable concentrate was used rather than the pure substance (technical grade) in order to approximate the conditions in the field.

The nominal concentrations of AZP (0, 0.2, 1, 5, and 20 μg/L) were obtained by serial dilution of a stock solution with 1 g/L of AZP. Each of the five treatments was replicated three times, giving a total of 15 microcosms. One hour following the start of the exposure, the water of each microcosm was replaced with 30 L of pesticide-free water of the same temperature.

During the experiment, the microcosms were covered with gauze (1-mm mesh) to catch emerging insects. Emergent adult insects were aspirated from emergence traps every 24 h and preserved in 70% ethanol. Six days following exposure, the rocks were removed from the microcosms and all animals attached to the rocks were counted. The contents of each microcosm were washed through a sieve (500 μm), and all animals were counted as well. The macroinvertebrates were sorted out in white plastic tubs, preserved in 70% ethanol and identified. Survival data for all species was obtained by summing up the surviving larvae with the emerged adults. Only very few individuals emerged during the short-term experimental period (less than 5% of total density).

Water Quality and Toxicant Analyses

Physico-chemical parameters of the microcosm water were measured with test kits from Macherey & Nagel, Düren, Germany or electronic meters from Wissenschaftliche Technische Werkstaetten, Weilheim, Germany. The means (±SE, n = 6) for pH, temperature, and oxygen were 7.2±0.8, 21.4±0.4 °C, and 8.7±0.1 mg/L, respectively. Nitrite and ammonium levels never exceeded 0.1 mg/L. The metal content of the test water (aluminium, copper, zinc, mercury and lead) was below detection limits (0.005-0.25 mg/L), as were the pesticide (fenvalerate, deltamethrin, chlorpyrifos, azinphos-methyl, prothiofos and endosulfan) levels (0.01 μg/L). All measured water quality parameters would be expected to have no deleterious effects on the test species.

Real exposure concentrations were measured at the Forensic Chemistry Laboratory, Department of Health, Cape Town. Water samples (0.5 L) were solid-phase extracted with C_{18}-columns (Chromabond). The measurements were made with gas chromatographs (Hewlett Packard 5890) fitted with standard Hewlett Packard electron-capture, nitrogen-phosphorus and flame-photometric detectors, with a quantification limit of 0.1 μg/L, and spiked recovery efficiencies were between 79 and 106%. The results for the analytical measurements taken at different times during the experiment are summarized in Table 2. In the following, nominal concentrations are used.

Table 2. Mean azinphos-methyl levels (μg/L) in the stream microcosms (nd = not detectable)

	Nominal concentration				
Time	Control	0.2 μg/L	1 μg/L	5 μg/L	20 μg/L
During exposure (± standard error, n = 3)	nd	0.2±0.01	1.0±0.08	4.9±0.3	19.2±1.0
24 h following water exchange (n = 1)	nd	nd	nd	0.11	0.91
6 d following water exchange (n = 1)	nd	nd	nd	nd	nd

Field sampling

Between the 27.2.2001 and the 5.3.2001 a quantitative macroinvertebrate survey was performed at the control site and at the contaminated site of the Lourens River. A total of 103 rocks of the same size as those used for the microcosms were investigated at the control site and a total of 83 rocks at the contaminated site. Sampling took place at both sites in shallow riffle areas. The same procedure for sampling and identification of invertebrates as described in the experimental section above was used for the field sampling. Macroinvertebrate densities are expressed in number of individuals per 18 rocks to allow direct comparison of densities from field samples with those in the microcosms.

Data analysis

Differences in the survival of macroinvertebrates in the microcosms were analysed using oneway analysis of variance (ANOVA) followed by Fisher's protected least significant difference test (PLSD). Differences in the abundance of macroinvertebrates at the control site and the contaminated site of the Lourens River were analysed using t-Tests. Data was transformed using $\ln(x + 1)$ prior to statistical analysis to satisfy the assumptions of the tests.

RESULTS

Microcosm experiment

Samples taken from the microcosms contained 17 taxa, representing 9 orders. Analysis including all 17 taxa revealed a significant reduction in the mean number of species from 14 in the control microcosms to 9.7 in the 20-µg/L treatment ($p = 0.0018$). Total mean densities were significantly reduced from 279.3 individuals in the control microcosms to 141.3 and 49.7 in the 5-µg/l ($p = 0.042$) and in the 20-µg/L ($p = 0.003$) treatment, respectively. The 12 core taxa with a mean density greater than or equal to one in the control treatment were included in the following analysis. They represent 99% of the total macroinvertebrate density in the microcosms. The number and kinds of these 12 core taxa present in the stream microcosms were very similar to those present in the source ecosystem ($R^2 = 0.86$, $p < 0.0001$; df = 11), at the control site of the Lourens River (Fig. 1 and Table 3).

Various taxa were present at significantly reduced densities in the 20-µg/L treamtent (Fig. 1). Simuliidae (Fig. 1a) were present at significantly decreased densities even in the 5-µg/L treatment. Eight out of the total of 12 species were classified as "sensitive" with respect to the applied contamination scenario (Fig. 1a to 1h). Four of these species (Fig. 1a to 1 d) showed a concentration dependent decline in densities over a range of at least the three highest treatments (1- to 20-µg/L). *Demoreptus* sp., *Castanophlebia* sp. and Chironomidae (Fig. 1e to 1g) were all significantly reduced in the highest treatment and Petrothrincidae (Fig. 1h) showed a concentration-dependent decline in the two highest treatments, however the abundances were not significantly different from the control.

In contrast, we classified *Aeshna* sp., *Dugesia* sp., Ceratopogonidae and *Cheumatopsyche* sp. (Fig. 1i to 1l) as "tolerant" species with respect to the applied contamination scenario. They neither showed any concentration dependent decline, nor any significant alterations of densities in comparison to the control

Field investigation

The total species richness was 15 at the control site and 13 at the contaminated site. The same twelve core taxa that were analysed in the microcosms represented 98% of the total macroinvertebrate densities at the control site and 91% at the contaminated site (Table 3). Six out of the 12 species showed a significantly lower density at the contaminated site than at the control site (Table 3a to 3c and 3f to 3h). *Baetis* sp., *Aeshna* sp., *Dugesia* sp., Ceratopogonidae and *Cheumatopsyche* sp. (Table 3e and 3i to 3l) occurred at significantly increased densities at the contaminated site in comparison to the control site. Noteridae (Table 3d) were present at lower densities at the contaminated site, however the differences were not significant.

Combination of microcosm and field results

We directly compared the reaction of macroinvertebrates in the microcosm experiment with their abundances at the two sites in the Lourens River (Table 4). To do so, we classified taxa, that occurred at significantly lower densities at the contaminated site as "sensitive" and taxa that occurred at similar or significantly higher densities at the contaminated site as "tolerant".

Ten out of the 12 core taxa showed a corresponding reaction in the microcosm and distribution in the field. Six out of these ten taxa were classified as "sensitive" in both microcosm and field approach (Table 4a to 4c and 4f to 4h) and for all of the four taxa classified as "tolerant" (Table 4i to 4l), this classification was in agreement between microcosm and field. Only two taxa that were classified as "sensitive" in the microcosm, represented "tolerant" taxa in the field samples (Table 4d and 4e).

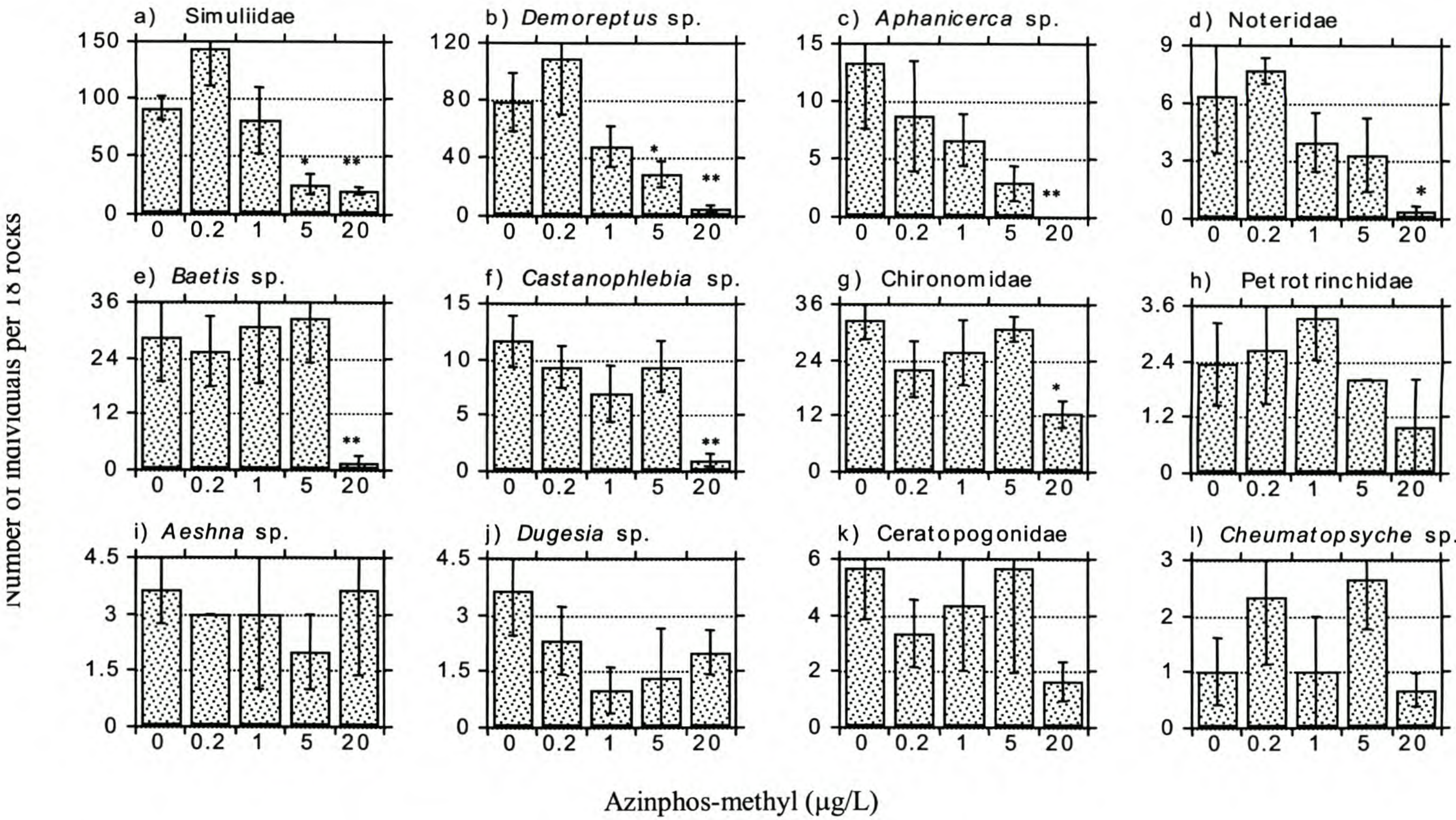

Fig. 1: Mean (±SE, n = 3) densities of the 12 core taxa six days following short-term exposure to different concentrations of azinphos-methyl. Asterisks indicate significant (ANOVA, Fisher´s PLSD: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) differences from the control treatment. The taxa 1a to 1h were classified as "sensitive" and the taxa 1i to 1l as "tolerant" with respect to the applied contamination scenario.

Table 3. Mean (individuals per 18 rocks ± SE) density of the 12 core taxa at the control site (n = 103 rocks) and at the contamianted site (n = 83 rocks) of the Lourens River, South Africa. Asterisks indicate significant (t-test: * p < 0.05; ** p < 0.01; *** p < 0.001) differences between the two sites. The order of the taxa is the same as used in Fig. 1 for the microcosms.

	Taxa	Control	Contaminated	Significance +	Significance -
a.	Simuliidae	68.7 ± 18.0	21.7 ± 7.9		*
b.	*Demoreptus* sp.	105.4 ± 17.3	31 ± 5.6		**
c.	*Aphanicerca* sp.	6.3 ± 1.35	0		***
d.	Noteridae	4.0 ± 1.2	2.6 ± 0.9	ns	ns
e.	Baetis sp.	42.6 ± 3.7	114.5 ± 8.1	***	
f.	*Castanophlebia* sp.	19.2 ± 3.3	0		***
g.	Chironomidae	56.5 ± 5.2	24.5 ± 3.1		***
h.	Petrothrincidae	8.6 ± 1.3	0		***
i.	*Aeshna* sp.	2.1 ± 0.7	5.2 ± 1.0	**	
j.	*Dugesia* sp.	2.8 ± 0.95	10.2 ± 2.1	***	
k.	Ceratopogonidae	20.2 ± 1.8	30.6 ± 3.3	**	
l.	*Cheumatospyche* sp.	1.2 ± 0.45	3.9 ± 1.1	*	

+: significant increasing abundance

-: significant decreasing abundance

Table 4. Classification of the 12 core taxa according to their reaction in the microcosm experiment and their distribution in the field samples taken from the Lourens River. Taxa were classified as "sensitive" in the microcosm study if their density is either reduced significantly at higher treatments or showed a concentration-dependent decrease over at least three concentrations. Taxa were classified as "sensitive" in the field samples if their abundance at the contaminated site was significantly lower than at the control site. The order of the taxa is the same as used in Fig. 1 for the microcosms.

	Taxa	Microcosm	Field
a.	Simuliidae	sensitive	sensitive
b.	*Demoreptus* sp.	sensitive	sensitive
c.	*Aphanicerca* sp.	sensitive	sensitive
d.	Noteridae	sensitive	tolerant
e.	Baetis sp.	sensitive	tolerant
f.	*Castanophlebia* sp.	sensitive	sensitive
g.	Chironomidae	sensitive	sensitive
h.	Petrothrincidae	sensitive	sensitive
i.	*Aeshna* sp.	tolerant	tolerant
j.	*Dugesia* sp.	tolerant	tolerant
k.	Ceratopogonidae	tolerant	tolerant
l.	*Cheumatospyche* sp.	tolerant	tolerant

DISCUSSION

Microcosm experiment

Reduction in species abundance is regarded as one of the most sensitive endpoints in multi-species microcosm toxicity tests (Niederlehner and Cairns Jr., 1994). The 20 µg/L and 5 µg/L treatment significantly reduced the total mean densities and number of species (20 µg/L only) and of the 12 core taxa, 8 reacted sensitively to the applied contamination scenario as a result of significant or concentration dependant increases in mortality (Fig. 1). The results thus indicate that short-term pesticide exposure typical of nonpoint source pollution events can lead to long-term acute effects on macroinvertebrate community structure and species composition. Similar long-term effects have been established based on single species tests (Liess and Schulz, 1996), as well as multi species tests (Schulz and Liess, 2001b; Woin, 1998). Furthermore, while studies have been able to show a correlation between decreased abundance and diversity as a result of pesticide contamination in microcosms (Breneman and Pontasch, 1994; Brock *et al.*, 1993; Van den Brink *et al.*, 1995), only a few have been able to show the same structural changes following exposure scenarios typical of nonpoint pollution events (Liess and Schulz, 1999; Schulz and Liess, 2001a; Schulz and Liess, 2001b).

In general the tolerance or sensitivity of taxa to the exposure scenario can be explained by results of previous studies. Of the eight taxa sensitive to the pesticide exposures, four showed concentration dependant decline in densities, indicating that short-term azinphos-methyl exposure can potentially disrupt community structure from as low as 1 µg/L. It has been well established that ephemeropterans and plecopterans are highly sensitive to pesticide pollution (Fischer and Hall Jr., 1992; Sibley *et al.*, 1991; Siegfried, 1993) and of the eight sensitive taxa, four belonged to these orders (3 mayfly species and 1 stonefly species), of which two, *Demoreptus* sp. and *Aphanicerca* sp., showed concentration dependant declines. Caddisfly species have been shown to be generally very sensitive to pesticide pollution (Heckman, 1981; Liess and Schulz, 1996; Schulz and Liess, 1995), which was exhibited by the concentration dependant decline of the family Petrothrincidae. However *Cheumatopsyche* sp. was not affected by any AZP exposure, indicating that sensitivity of Trichoptera to pesticides is species specific. *Dugesia sp.* are regarded as being tolerant of pesticide pollution, whereas Coleoptera (which includes the Noteridae) are generally in the sensitive category (Heckman, 1981; Schulz, 1998). In contrast to this study, Simuliidae and Chironomidae have been shown to be more tolerant of pesticides than other macroinvertebrate taxa (Kreutzweiser and Sibley, 1991), however, sensitivity may also be dependant on the type of pesticide used (Phippis *et al.*, 1995; Taylor *et al.*, 1991).

Assuming microcosm experiments are representative of field situations one would expect to find similar macroinvertebrate community distributions in natural systems that are affected by nonpoint AZP pollution.

Field investigation

As expected, the downstream Lourens River site, R2, showed a decrease in species richness and abundance of six of the 12 core taxa in comparison to the control site

R1 (Table 3). Noteridae were also present at lower densities, although the difference was not significant, while the other 5 taxa were present at significantly higher densities. Biodiversity is generally higher in less intensely cultivated habitats (Duelli *et al.*, 1999) and field studies have been able to link decreased species abundance and biodiversity to runoff induced pesticide contamination (Leonard *et al.*, 2000a; Leonard *et al.*, 2000b; Liess and Schulz, 1999; Tada and Shiraishi, 1994). Runoff induced pesticide contamination has been shown to occur at R2 (Schulz *et al.*, 2001a, Unpublished Data), however it is impossible to draw a clear causal relationship between pesticide contamination and changes in community structure based on the field data of this study alone. Other biological stressors associated with agricultural nonpoint source pollution, such as nutrients and sediments have been shown to occur at this site (Schulz *et al.*, 2001a), and can also negatively influence macroinvertebrate diversity and community structure (Carpenter *et al.*, 1998; Chessman *et al.*, 1987; Schulz, 1996; Taylor and Roff, 1986).

The fact that certain species were present at increased densities is typical of contaminated sites where a small number of tolerant species tend to thrive under such conditions (Heckman, 1981). *Aphanicerca* sp (Plecoptera) and *Castanophlebia* sp. (Ephemeroptera) comprised two of the three taxa that did not occur at R2, which is indicative of their sensitivity towards poor water quality (Fischer and Hall Jr., 1992; Sibley *et al.*, 1991; Siegfried, 1993). In contrast to *Demoreptus* sp., *Baetis* sp. increased in abundance at R2, indicating that the latter species may be more tolerant to pesticide pollution than the former.

Combination of microcosm and field results

The main objective of the microcosm experiments was to employ a design that was relevant to conditions that macroinvertebrate communities experience during typical exposure scenarios associated with nonpoint source AZP pollution. The number and type of core taxa used in the microcosm experiments were very similar to those that were present at the control site in the Lourens River, indicating that the community structure of the microcosms was representative of that in the field. Concentrations of AZP applied in the microcosm experiments were similar to concentrations reported in field studies. Runoff induced transient AZP concentrations between 1.5 and 2.3 µg/L have been measured in the Lourens River (Schulz, 2001b) and other surface waters (Smith *et al.*, 1983; Wan *et al.*, 1995), while Scott *et al.* (1999) measured as much as 7 µg/L AZP in an estuary. The same studies showed that sediment associated AZP concentrations ranged from 216 to as much as 1247 µg/kg.

The sensitivity or tolerance of the taxa in the microcosms was closely linked to that of the taxa in the field at R2 (Table 4). All four taxa that showed tolerance in the microcosm study occurred at higher densities at the contaminated site, while six of the eight sensitive taxa occurred at significantly lower densities at the same site. Thus the results strongly indicate that field relevant microcosm studies can be successfully linked to field studies and demonstrates a causal relationship between nonpoint source pesticide pollution events and their deleterious consequences to the aquatic macroinvertebrate communities of the Lourens River. Potential increases in mortality of key species due to short-term, sub-lethal pesticide exposure have already been demonstrated in this catchment (Schulz and Dabrowski, 2001), however a direct link between pesticide contaminated multi-species microcosm experiments and the prevailing community in a natural environment has so far not been shown.

Liess and Schulz (1999) were able to show a similar relationship between field relevant pesticide exposure in microcosms and communities in a stream in Germany, although the microcosms contained only two different macroinvertebrate species. Based on responses to a complex effluent, Niederlehner *et al.* (1990) used multi-species microcosm tests to predict decreases in species richness in a stream in the mid-Atlantic, USA. The results of this study highlight the importance of microcosms for use in regulatory assessment of pesticides (Harrass and Sayre, 1989).

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CONCLUSION

Once potential for contamination by an ecological toxicant has been identified, the assessment of exposure and biological effect are the most important factors in determining its risk to the environment (Landis and Yu, 1995). Owing to the high toxicity of pesticides and their relatively high persistence in the field, much attention has been focused on the presence of pesticides in natural environments; particularly in aquatic ecosystems. Surface runoff has been established as possibly the most important route of entry for pesticides in agricultural surface waters (Schulz, 2001a). Thus, models which can identify areas responsible for high levels of runoff induced pesticide pollution are extremely important in risk assessment scenarios.

Recent developments in geographic information systems (GIS) provide useful techniques for handling large amounts of spatial data for modeling nonpoint source pollution problems (Tim and Jolly, 1994). The GIS-based runoff model utilised in this study could be of great benefit in the implementation of mitigation strategies and management of agricultural orchard areas in South Africa. The model successfully identified three tributaries (LL6, LL5 and LL2) as being responsible for the high concentrations of the three most commonly used pesticides in the Lourens River catchment (azinphos-methyl, chlorpyrifos and endosulfan), which was validated via field based sampling. Although the model predicted losses of pesticides as a percentage of what was applied on orchards, it did not predict concentrations in the tributaries themselves. Thus, field-based measurements are important to supplement the predictions of the model and highlights the importance of using a variety of methods in assessing the risk of exposure in an aquatic environment (Wauchope, 1996). The concentrations of pesticides are the most ecotoxicologically relevant parameters with respect to the model and future research should focus on an attempt to predict in-stream concentrations based on a single event or average annual basis. In this respect, azinphos-methyl is probably the most relevant pesticide to model in this catchment as it is often detected at relatively high concentrations in the water phase during runoff conditions (Schulz, 2001b; Schulz *et al.*, 2001). By comparison, chlorpyrifos and endosulfan were detected at very low concentrations in all tributaries making it more difficult to do a relative comparison based on measured and predicted concentrations. The model does still however have a very valuable application in management practices as it can identify problem areas in a catchment encompassing agricultural land use only and can thus prioritise these areas for further investigation.

While the model was very successful in predicting contamination in the Lourens River catchment, it remains to be seen whether the same model can be applied to other orchard dominated catchments in South Africa, and this aspect requires further investigation.

The microcosm experiments performed in this study addressed many of the emerging trends in ecotoxicological testing. Most importantly, in the microcosms a multi-species design was used, which comprised of communities directly comparable to those which occur naturally in the Lourens River. Furthermore, exposure levels of azinphos-methyl in the microcosms were field relevant in terms of duration and concentration of exposure. The field relevance of the tests makes them more comparable to situations in the field and also enables easier and more reliable extrapolation from the laboratory to the field. Accordingly, the results of this study strongly suggest that field relevant microcosm studies can be successfully linked to field studies and demonstrate a causal relationship between nonpoint source pesticide pollution events and their deleterious consequences to the macroinvertebrate communities of the Lourens River.

Based on the pesticide concentrations measured so far at the contaminated site of the Lourens River, it is not possible to simply interpret the differences in community composition soley as a result of the effects of azinphos-methyl. Based on measurements in the field, surface runoff commonly results in the input of chlorpyrifos and endosulfan as well as azinphos-methyl. Thus macroinvertebrate communities can be potentially exposed to a multiple number of pesticides simultaneously. As a result synergistic, additive or antagonistic effects can possibly occur depending on which pesticides are present and how they interact with each other (Bocquene *et al.*, 1995; Kungolos *et al.*, 1999; Pape-Lindstrom and Lydy, 1997; Thompson, 1996). Although research has focused on mixed toxicity using standardised single species toxicity tests, further similar work using more field relevant microcosm experiments is still required. In particular, microcosm experiments based on exposure scenarios using a combination of azinphos-methyl, chlorpyrifos and endosulfan would further improve our knowledge of the effects of current use pesticides on the macroinvertebrate structure in the Lourens River.

Apart from pesticides associated with the water phase, runoff also leads to input of sediment, much of which can be contaminated by current use pesticides. Although sediment associated pesticides are generally less bio-available (Mian and Mulla, 1992), studies have indicated that their presence can also lead to negative changes in macroinvertebrate community structure (Schulz and Liess, 2001a; Schulz and Liess, 2001b). Thus the high levels of pesticides associated with the sediment could also lead to the change in community structure observed downstream in the

Lourens River. Once again, potential synergistic effects could occur as a result of simultaneous multiple contamination by different sediment associated pesticides.

To conclude, this study highlights the importance of modelling approaches and microcosm experiments as being valuable tools for the risk assessment of runoff-induced pesticide exposure in agricultural surface waters. The results of this study strongly indicate that runoff induced insecticide input poses a risk to aquatic insect communities and lays the foundations for the implementation of appropriate mitigation strategies. As such, mitigation measures should focus primarily on preventing excessive runoff, with special attention being paid to the tributaries responsible for the highest level of contamination.

Contour cropping as opposed to up-down slope cropping has been shown to successfully reduce excessive runoff (Felsot *et al.*, 1990) and can thus reduce the transport of pesticides towards surface waters. However, this fact should be considered in the process of establishing orchards. Once orchards have been established, mitigation measures should focus firstly on reducing the amount of pesticide entering tributaries and secondly on reducing the amount of pesticide that has entered a tributary from reaching the mainstream.

The lack of well vegetated buffer strips between the orchards and the tributary in sub-catchments LL5 and LL6 was discussed as being one possibility for high levels of pesticide contamination and this aspect demands attention. Once appropriate prevention measures have been introduced, methods can be applied to reduce the amount of pesticide that eventually enters a tributary before entering the Lourens River mainstream itself. In this respect, wetlands constructed in the tributaries, prior to their junction with the mainstream have been shown to be very effective in retaining nonpoint pesticide pollution in this (Schulz and Peall, 2001) and other (Brix, 1994; Moore *et al.*, 2000; Rodgers Jr. and Dunn, 1993) catchments.

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