A COMPARISON OF RUNOFF- AND SPRAY-DRIFT-RELATED PESTICIDE CONTAMINATION IN AGRICULTURAL SURFACE WATERS: EXPOSURE, EFFECTS AND MITIGATION

By

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Submitted in fulfillment of the requirements for the

Degree of Doctor of Philosophy

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UNIVERSITY OF CAPE TOWN

JUNE 2004

DECLARATION

This thesis reports original research carried out in the Freshwater Research Unit, Department of Zoology, University of Cape Town, between 2001 and 2003. It has not been submitted in part or in whole for a degree at any other university. Data presented here are original, and any other sources of data acquired through collaborative activities are fully acknowledged.

J.M. Dabrowski

DEDICATION

To Ralf Schulz – my mentor, my inspiration and my friend.

ACKNOWLEDGEMENTS

This thesis is the result of studies undertaken during the German-South African collaborative project, ENVIROMAP (Project: I/76 177). I gratefully acknowledge funding received from the Volkswagen Stiftung, Hannover. I am also enormously thankful to Dr. Jenny Day for financial assistance and her supervision of my degree.

I would also like to thank Sue K.C. Peall and Christina Hahn for sample preparation and pesticide analysis and the management of Vergelegen and Lourensford farms, Somerset-West, for permission to enter their properties, for information on spraying programmes and for their helpful co-operation in planning of field experiments. I would also like to thank all my friends and colleagues from the Freshwater Research Unit and those from far and wide, for their companionship and good laughs. In particular I'd like to thank Erin Bennett, Anne Bollen, Charlene Coulsen and Silke Bollmohr, all of whom ably assisted me in field trips, pesticide analysis and general 'admin' and with whom I enjoyed very special friendships.

Thanks also to my parents, Frank and Anita and my sisters Kate and Steph who have all been very supportive throughout the duration of my studies.

Finally I would like to thank Dr. Ralf Schulz, my co-supervisor for this thesis. Thanks for the thousands of e-mails, the odd phone call, the cracking of the whip and your enormous and generous heart. Bru, I dedicate this thesis to you.

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ABSTRACT

Runoff and spray-drift-related pesticide input are important sources of nonpoint-source pesticide pollution in surface waters but few studies have directly compared these routes in a risk assessment scenario. Accordingly a risk assessment approach was instituted to determine differences between runoff and spray-drift based on exposure, effect and mitigation.

An exposure assessment using predictive modelling that was validated by field-based sampling was used to compare relative pesticide inputs associated with runoff and spray-drift at the catchment level. To this end, a runoff formula suggested by the Organisation for Economic and Cooperative Development (OECD) and basic drift values (95^{th} percentiles) were integrated into a Geographical Information System (GIS) to predict runoff and spray-drift-related loading of azinphos-methyl (AZP) in the Lourens River, South Africa. The GIS-integrated calculations were first validated in the tributaries of the river where measured loads were well predicted for both runoff ($r^2 = 0.95$; $p < 0.0001$; $n = 9$) and spray-drift ($r^2 = 0.96$; $p = 0.0006$; $n = 8$).

Through extrapolation to the catchment scale containing 400 ha of orchards, the GIS-integrated calculations predicted similar loads of AZP as measured in the Lourens River mainstream for six runoff (between a factor of 1.03 and 1.86 lower) and six spray-drift (between a factor of 1.1 and 2.4 higher) events. Mean measured loads per event were significantly ($p = 0.004$) higher for runoff (27.8 ± 19.1 g) than for spray-drift (0.69 ± 0.32 g). Based on long-term meteorological data and average application regimes, runoff leads to a higher annual load (47.6 g) than spray-drift (5.5 g) in the Lourens River.

Runoff is clearly a more important source of nonpoint pollution in the studied catchment and mitigation strategies should focus first on addressing this aspect on a catchment scale and secondly on addressing problem areas on a sub-catchment scale.

Runoff and spray-drift typically result in differing exposure scenarios. Pesticides derived from runoff-related input are typically associated with suspended sediment in combination with increased discharge and flow velocity, while spray-drift leads to increased concentrations of pesticide dissolved directly in the water-phase. Accordingly the effects of the pyrethroid insecticide, cypermethrin, increased flow speed and increased suspended particles on drift behaviour and activity of mayfly nymphs (*Baetis harrisoni*) were investigated individually and in combination in a laboratory stream microcosm.

Exposure scenarios were chosen so as to simulate typical runoff and spray-drift conditions in the field. The effect of spray-drift was investigated by exposing the nymphs to 1μg/l cypermethrin (CYP). During runoff trials, 2000 μg/kg contaminated sediment (CYP x PART) was introduced to the microcosm at a concentration of 500 mg/l. Both studies were carried out under high (CYP x FLOW and CYP x PART x FLOW) and low flow (CYP and CYP x PART) conditions and for all cases control experiments were performed: uncontaminated water (Control) and sediment (PART) and increased discharge (FLOW). Drift rate, drift density (for any treatments with increased flow) and activity were used as behavioural endpoints. A multi-factorial analysis of variance showed that CYP exposure significantly increases the drift, while sediment and flow as single factors lead to a significant decrease in drift ($p < 0.05$). In addition, activity decreases significantly under high flow conditions. This indicates that the mayflies show different avoidance reactions; drifting in the case of CYP exposure and reducing drift and activity by searching shelter under the stones in case of high flow and sediment.

CYP x PART treatments resulted in a decrease in drift behaviour; comparing expected and measured responses indicates a significant antagonistic interaction of the two stressors suggesting a reduction of the bioavailability of the pesticide by the sediment. CYP x FLOW treatments resulted in a reduction of drift and again a significant antagonistic interaction was observed. Results indicate that mayflies reacted actively in response to flow conditions and passively in response to pesticide exposure. Reactions to the different exposure treatments indicate that mayflies show a greater behavioural response to spray-drift exposure than to runoff exposure.

Recently increased attention has focused on mitigation strategies designed to reduce nonpoint-source pesticide pollution in non-target surface waters. Intensive research has focused on the potential of aquatic macrophytes to reduce pesticide transport in surface waters. No studies have however compared the relative effectiveness of aquatic macrophytes in reducing runoff- and spray-drift-associated pesticide concentrations. Thus, the potential and effectiveness of aquatic macrophytes in reducing runoff- and spray-drift-induced pesticide input was compared by monitoring the fate and transport of runoff- and spray-drift-induced AZP input in a vegetated and non-vegetated stream.

In a rigorous sampling design, water, sediment and plant samples were taken during runoff and spray-drift events at 5, 20, 45, 90 and 180 m and 5, 45 and 180 m from the point of input in a vegetated and non-vegetated stream, respectively. Monitoring of a 10 mm and 22 mm runoff event (3 and 1 day/s after application, respectively) revealed no reduction in water dissolved AZP concentrations in the non-vegetated stream. During the 10 mm runoff event, peak concentrations of AZP showed a significant exponential decrease (R^2 = 0.99; p < 0.0001; n = 5) from 0.24 μg/l to 0.11 μg/l with increasing distance from the point of input in the vegetated stream. Presumably as a result of greatly increased discharge levels, no reduction of peak or average AZP concentrations took place during the 22 mm event. Decreased AZP concentrations were measurable in both streams after spray-drift input; however, reduction was 40% more efficient in the vegetated stream. During the spray-drift event, peak concentrations of AZP showed a significant linear decrease (R^2 = 0.93; p = 0.0084; n = 5) from 4.3 μg/l to 1.7 μg/l with increasing distance from the point of input in the vegetated stream. Plant samples taken after the spray-drift event showed an increase in AZP concentrations, which decreased with increasing distance from the point of input, suggesting that the plants and/or plant surfaces are an important sink for water dissolved AZP.

Although peak concentrations of AZP were as effectively mitigated during the 10 mm runoff event as during the spray-drift event (61% reduction from the 5 m to the 180 m sampling point), predictive modelling revealed that maximum concentrations expected during a worst case scenario 10 mm runoff event (0 days after application) are an order of magnitude lower than what can be expected for a worst-case spray-drift and 22 mm runoff event, suggesting that spray-drift derived pesticide concentrations are more effectively mitigated than those of runoff.

Although aquatic macrophytes reduce aqueous-dissolved pesticide concentrations after nonpoint-source events, they may also provide an additional mitagatory function with respect to spray-drift by intercepting pesticide before it lands on the water surface. Accordingly the effectiveness of emergent aquatic vegetation in reducing deposition of spray-drift-derived AZP in an agricultural stream via foliar interception was investigated.

The stream was dominated by three species of emergent aquatic macrophytes; *Juncus capensis, Fuirena hirsuta* and *Pycreus* sp.; resulting in a coverage of almost 80% and the formation of a centrally situated exposed channel. During an application of AZP, drift deposition was determined on the surface of the vegetation, on the surface of the exposed channel of the stream and beneath *J. capensis* and *F. hirsuta* by means of drift collectors (n = 6). Drift deposition on the surface of the vegetation (1.5 mg/m^2 ± 0.3) was well predicted by 90th percentile basic drift values (1.3 mg/m^2), indicating that the sampling devices resulted in an accurate measurement of drift deposition. Drift deposition on the surface of the exposed channel (1.0 mg/m^2 ± 0.3) was lower than measured on the vegetation surface indicating a positive shielding effect by the emergent plants. Drift deposition beneath *J. capensis* was significantly lower (p = 0.005) than on the vegetation surface and the exposed channel (p = 0.048), indicating highly effective interception of AZP.

A simple formula was generated to make predictions of drift deposition reductions based on different percentage macrophyte coverage. Predictions showed that 50% macrophyte coverage in combination with a 5 m buffer strip resulted in as large a reduction in drift deposition as the combination of a 10 m buffer strip with 0% macrophyte coverage. Results thus indicate that emergent aquatic vegetation may be as effective a mitigation strategy for reducing spray-drift induced pesticide input as increasing the width of the no spraying buffer zone.

In summary, the results of this study indicate that, at the catchment scale, runoff leads to higher concentrations of water-dissolved and particle-associated pesticides than spray-drift. This is as a result of the larger spatial and temporal scale associated with runoff contamination. The suitability of vegetated water bodies in reducing the transport of water-dissolved pesticide concentrations is not as great for runoff as it is for spray-drift as a result of decreased sorption to plants during high discharge conditions. This could lead to higher concentrations of pesticide being measured during runoff events in catchments where vegetated ditches are prominent. Furthermore, emergent aquatic macrophytes afford additional protection against spray-drift by effectively intercepting drift before it lands on the water surface, thereby significantly reducing water-dissolved pesticide concentrations.

Thus, in terms of exposure, runoff potentially poses the greatest threat to aquatic systems. The effect study however indicates that increases in flow velocity may reduce the toxic potential of pesticides during runoff events. Thus, even if pesticide concentrations in a given catchment were higher during runoff events than during spray-drift events, the ecotoxicological effect may not be as high as would be predicted by the concentrations present.

CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

The global drive towards environmental protection and sustainable development has led to increased interest in the occurrence and effects of potentially toxic man-made chemicals in non-target areas. The use of agrochemicals (such as pesticides and fertilizers) in particular highlights the problematic dilemma that sustainable development presents. Their benefits in protecting crops from pests and thus ensuring food security for countries and providing trading opportunities, especially for developing countries, are undeniable (Ecobichon 2001). The application of pesticides to crops is however one of the few examples where highly toxic chemicals are intentionally released into the environment, thus presenting a serious environmental risk. Although pesticides are used to intentionally kill a particular pest, they are not species-specific and are thus potentially toxic towards non-target insects, as well as fish, birds, mammals and humans. In this respect the agrochemical industry has made advances towards increasing the specificity of their products. For example pyrethroids are a relatively new class of pesticides which are being increasingly used, partly owing to the fact that they are far less toxic towards fish (Giddings *et al.* 2001), birds and mammals (Breneman & Pontasch 1994; Tyler *et al.* 2000) in comparison to other classes of pesticides, such as organochlorines and organophosphates.

It is essential that the pesticide use be managed in a sustainable manner, so as to ensure that the benefits they provide are not derived at the cost of ecological damage and loss of biological diversity. For this reason it is important to understand the fate of pesticides in agricultural fields and in non-target environments and the potential effects they may have on non-target organisms.

Owing to man's dependence on water, the impact of contaminants on surface waters such as rivers and wetlands has recently come under increased scrutiny. With respect to agricultural and rural environments, nonpoint-source pollution is generally regarded as the most important source of contaminants in surface waters (Loague *et al.* 1998). Runoff (during a heavy rainfall event) and spray-drift (during the application process) in particular, have been highlighted as the most important mechanisms of pesticide input in surface waters (Wauchope 1978; Groenendijk *et al.* 1994; Flury 1996).

Leaching of pesticides through the soil is largely responsible for contamination of groundwater which is generally less contaminated than surface waters (Schiavon *et al.* 1995; London *et al.* 2000). Contamination of surface waters by groundwater is only likely to occur under unique geological conditions (Squillace *et al.* 1996). Whilst many studies have independently focused on the fate, effects and exposure of runoff (Domagalski *et al.* 1997; Leonard *et al.* 1999; Dabrowski *et al.* 2002) and spray-drift (Crossland *et al.* 1982; Ganzelmeier *et al.* 1995) induced pesticide input in surface waters, very few studies have directly compared these two routes of pesticide contamination in a single experimental design. Such comparisons are essential in understanding the relative impacts of these two processes and could result in improved decision making with regard to the sustainable management of pesticide contamination at the edge-of-field and catchment level.

1.2 LITERATURE REVIEW – PESTICIDE EXPOSURE

Runoff

Surface runoff is the lateral movement of water from agricultural fields into adjacent water bodies and occurs when the precipitation rate exceeds the infiltration rate of the soil, or when the length of a rainfall event exceeds the infiltration capacity of a soil. A complex interaction of a multiple number of variables ultimately influences the quantity of pesticide that can be expected to be present in surface runoff. Important factors 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 vegetated buffer strips (Cole *et al.* 1997). Furthermore, runoff is highly dependent on the physico-chemical properties of the pesticides themselves, which ultimately determine the amount of pesticide physically available to surface runoff (Blanchard & Lerch 2000; Capel & Larson 2001).

Pesticides can be found in two forms in surface runoff, in the soluble form (dissolved in runoff water) and in the eroded form (sorbed to suspended solids). The proportion and quantity of a chemical in each form at a given site depends upon the extent of sorption with the associated soil matrix, partitioning between runoff water and the suspended eroded material in transit and the degradation rate in the soil. Thus, important physico-chemical properties to consider include the water solubility, half-life time and K_{OC} (soil sorption index; the tendency of a chemical to bind to organic carbon in the soil). Pesticides with low water solubilities tend to be more associated with suspended sediments as opposed to being dissolved in the water phase of the surface runoff. A study which monitored runoff-induced pesticide levels in the Lourens River (Somerset West, South Africa) (Dabrowski *et al.* 2002), showed that only 9% of water samples taken during runoff events had detectable concentrations of chlorpyrifos (an organophosphate pesticide with low water solubility [1.2 mg/l] and a high K_{OC} [6070]). In contrast, the frequency of detection in sediment samples was 67%, with high concentrations of particle-associated chlorpyrifos being detected (up to 150 μg/kg). Another organophosphate, azinphos-methyl, with a high water solubility (28 mg/l) and low K_{OC} (1000) was detected more frequently in water samples (31%), with very few sediment samples having any measurable levels (20%). Thus the water solubility and K_{OC} of a compound is very important in terms of the fate of the pesticide and its partitioning in the water body.

Soil properties also play an important role in terms of the amount of surface runoff and the quantity of transported pesticide that occurs during an event. Soils with higher organic carbon content will tend to bind pesticides more than soils with low organic carbon content (Domagalski & Dubrovsky 1992; Ankley *et al.* 1994; Flury 1996), while for any given precipitation amount, loamy and clay soils will give rise to a greater quantity of surface runoff (and hence pesticide loss) than a sandy soil, which will promote infiltration and leaching (Reus *et al.* 1999). Furthermore, pesticides with long half-lives (i.e. organochlorines such as endosulfan and DDT) can be measured in surface runoff many months after the last application (Dabrowski *et al.* 2002).

Slope has been shown to be the most important factor influencing runoff but can be greatly modified by the presence of vegetation (Wilcox & Wood 1989). The timing of rainfall events in relation to the application date and half-life time of pesticides also plays a significant role in determining the amount of pesticide available in surface runoff. Studies have shown that the first heavy rainfall after application results in the highest quantity of pesticides in surface waters (Domagalski *et al.* 1997; Dabrowski *et al.* 2002).

Large rainfall events occurring a few days after the application of pesticides have resulted in very high concentrations of pesticides being detected in the Lourens River (Schulz 2001a).

Spray-drift

In large-scale agricultural settings, pesticides are typically applied via crop duster aircraft or orchard airblasts (Frank *et al.* 1994). Spray-drift refers to the amount of pesticide that physically drifts from the main application target towards a non-target area or water body. Spray-drift results in the direct deposition of pesticides into the water body, without allowing time for degradation or adsorption to organic matter in the soil. Contamination in surface waters is thus typically very high and spray-drift has thus often been proposed as being more important than runoff in terms of the quantity of pesticide exposure in surface waters (Gilbert & Bell 1988; AEDG 1992).

Factors determining the quantity of pesticide entering a water body via spray-drift are fewer with less complex interactions than in comparison to runoff and include wind speed and direction and the distance of a water body from the point of application. The percentage of an applied substance that lands in a water body decreases with increasing distance from the point of application (Ganzelmeier *et al.* 1995), although this could be modified in the presence of increased wind speed (Bird *et al.* 1996). Physico-chemical properties of pesticides however, also play an important role in determining the quantity of pesticide in drift and the fate of pesticides after deposition into a water body. Highly volatile pesticides are more prone to drift than those that are more stable (Siebers *et al.* 2003). Furthermore, pesticides with low water solubilities and high K_{OC} co-efficients have been shown to rapidly sorb to aquatic macrophytes and sediments (Brock *et al.* 1992a; Hand *et al.* 2001), thereby rapidly reducing water dissolved pesticide concentrations.

1.3 LITERATURE REVIEW – EFFECTS OF PESTICIDES IN SURFACE WATERS

Benthic macroinvertebrates are amongst the most sensitive components of aquatic ecosystems and have thus been widely used in toxicity testing and bioassessment (Dallas

2003). A wide range of experimental designs have been employed to determine the ecotoxicological effects of pesticides on aquatic communities. A large proportion of the knowledge of effects of pesticides on aquatic fauna have been based on standard toxicity tests that measure endpoints (e.g. mortality, immobility or reproduction) of single species (e.g. *Daphnia magna*) exposed to a range of increasing toxicant concentrations (Day 1989; Moore *et al.* 1998). The measured response in relation to the exposure concentration is then used to calculate LC_{50} or EC_{50} values, which have been widely used in the establishment of water quality guidelines (Sunderam *et al.* 1994; DWAF 1996). These values are often extrapolated to the field to provide an indication of the potential toxicity of contaminants measured in the field. In terms of pesticide contamination of surface waters, the field relevance of this standardized approach is questionable, as the duration of exposure as well as the test species employed in the tests may not accurately represent the conditions that occur in the field. Owing to the fact that pesticide contamination is most often associated with discrete nonpoint-source events (e.g. runoff or spray-drift events), the exposure of aquatic to pesticides typically occurs in pulses and not as a continuous exposure (Hosmer *et al.* 1998; Brent & Herricks 1999; Reinert *et al.* 2002), as employed during standardized toxicity testing. Furthermore it has become apparent that single-species toxicity tests do not take into account interactions between and within species and may thus not be adequate for predicting the impact of pesticides at the community and ecosystem level (Breneman & Pontasch 1994; Woin 1998).

As a result increased emphasis has been placed on developing more field relevant toxicity tests so as to better understand the effects of pesticides on aquatic communities. Studies have shown that exposure scenarios employing low pesticide concentrations (< 1 μg/L) and short duration (< 1 hour), that are typical of nonpoint-source pollution events, are capable of eliciting negative ecotoxicological effects (Schulz & Dabrowski 2001; Schulz *et al.* 2002). Experiments range from the intentional contamination of natural streams (Werner & Hilgert 1992), multispecies micro- (Pontasch & Cairns 1991; Van den Brink *et al.* 1995; Schulz & Liess 2001b) and mesocosm (Tourat & Slimak 1989; Farmer *et al.* 1995) studies and *in-situ* bioassays (caging test organisms in contaminated sites in the field). Intentional contamination of natural streams is undesirable, whilst *in-situ* bioassays have successfully linked pesticide contamination of natural systems via nonpoint-sources with negative biological effects (Schulz & Liess 1999b; Schulz 2003), and provide the most realistic estimation of exposure conditions (Brent & Herricks 1999). Field studies have also been able to link changes in community structure and drift behaviour (Liess & Schulz 1999; Schulz & Liess 1999a; Leonard *et al.* 1999; Leonard *et al.* 2000) measured in the field with nonpoint-source pollution events. During runoff events however, particularly when factors such as increased suspended sediment loads and discharge may also influence macroinvertebrate community structure, the relationship between pesticide contamination and community structure response in the field may only be assumed.

In this respect micro- and mesocosm experiments are useful, in that exposure scenarios can easily be manipulated so as to determine pesticide effects on multi-species assemblages. Microcosms (or artificial streams) allow tests to more accurately simulate conditions in the field by incorporating spatial (a longitudinal stretch of stream), flow and habitat (plants, sediments and rocks) characteristics in the experimental design (Palmer & Scherman 2000). Furthermore, microcosm studies can also be used to study the fate of pesticides in terms of their partitioning between water, sediment and plant phases and the resulting effects on macroinvertebrates (Brock *et al.* 1992a; Brock *et al.* 1992b; Brock *et al.* 1993; Hand *et al.* 2001). Microcosm studies have also enabled the study of the influence of pesticide exposure on predator-prey interactions between fish and drifting invertebrates (Schulz & Dabrowski 2001). Thus, a variety of toxicity testing options have been developed and a combination of field and microcosm or laboratory experiments may potentially provide the best understanding of the effects of pesticides on aquatic communities (Baughman *et al.* 1989; Schulz *et al.* 2002).

1.4 LITERATURE REVIEW - PESTICIDE MITIGATION STRATEGIES

Recent research has increasingly focused on identifying mitigation measures capable of reducing the risk of exposure of surface waters to pesticide contamination. Different risk mitigation options and the understanding of the impact of mitigation measures on pesticide exposure are poorly developed in general and it is clear that further research in this area is required (FOCUS 2001; Streloke & Brown 2003). The implementation of best management practices (BMPs) are management driven decisions or precautions that are implemented farmers, in an attempt to reduce chemical transport from crops into non-target areas. With respect to reducing pesticide loss via runoff, conservation tillage has been shown to reduce pesticide loss in runoff, with contour tillage resulting in a 10% reduction compared to up-down-slope tillage (Felsot *et al.* 1990). Other more simple strategies involve planning the application of pesticides in relation to forecasting of significant rainfall events. Application just before a heavy rainfall event will result in large losses of pesticides and may reduce the efficacy of the pesticide as a result of it having been washed off the target crop. This may also result in the pesticide having to be re-applied, leading to increased economic costs and risk to the environment.

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). They also help improve water quality and remove sediment from surface runoff by stabilizing stream-banks and promoting infiltration (Spatz *et al.* 1997). 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, infiltration and adsorption of pesticides to vegetation (Castelle *et al.* 1994).

Mitigation of spray-drift-derived pesticide input is obviously closely related to the application procedure. The most commonly utilized mitigation strategy is the establishment of buffer zones, a strip of uncultivated land lying in between the target crop and a non-target water body, which have been shown to be highly effective in reducing drift deposition and resulting toxicity in adjacent water bodies (De Snoo & De Wit 1998; Lahr *et al.* 2000). Physical wind barriers in the form of hedgerows or wind breaks can also significantly decrease the amount of drift entering a water body (Hewitt 2000; Ucar & Hall 2001). During application the liquid pesticide mixture is forced through nozzles under high pressure, producing a fine spray, which allows for increased biological efficacy by allowing the pesticide to cover a large crop area and present an overall larger exposure potential to pest insects. An important feature of the spray is the droplet size, which can vary according to the type of nozzle used. The smaller the droplet size, the greater the potential for drift to non-target areas (Bird *et al.* 1996). The increased concern over drift of plant protection products into surface areas has resulted in the development of drift reducing nozzles, which electrostatically charge fine droplets or produce coarser, heavier droplets, thereby reducing drift to non-target areas (Matthews 1994).

Fate studies of pesticides in water bodies and microcosms has led to the realization that aquatic macrophytes may serve a highly effective mitigatory function by sorbing aqueous dissolved pesticides (Hand *et al.* 2001) and promoting sedimentation (Braskerud 2001), thereby potentially reducing transport of particle associated pesticides. Accordingly recent research has focused extensively on the ability of vegetated water bodies (i.e. constructed wetlands and agricultural drainage ditches) in reducing the transport of nonpoint-source derived pesticides. Constructed wetlands have been widely used in wastewater treatment works to reduce the input of nutrients and chemicals into receiving waters (Wood 1990; Stottmeister *et al.* 2003; Cameron *et al.* 2003), however their efficiency on reducing pesticide transport has only recently been investigated.

Studies have shown that vegetated wetlands are effective in reducing runoff-(Moore *et al.* 2000; Schulz & Peall 2001) and spray-drift-induced (Schulz *et al.* 2003a) pesticide concentrations thereby reducing toxicity towards macroinvertebrates (Schulz *et al.* 2001c; Schulz *et al.* 2003b; Sherrard *et al.* 2004). Two intensive studies have focused on the mitigation potential of a constructed flow-through wetland that was built into a tributary that flows into the Lourens River, South Africa (Schulz & Peall 2001; Schulz *et al.* 2003a). The wetland has an inlet that receives water from a tributary that flows directly through an intensive fruit orchard area and an outlet, which flows directly into the Lourens River mainstream. Field based sampling showed a decrease in concentrations of runoff-related organophosphate azinphos-methyl contamination (up to 93%), nutrients (75-84%) and suspended sediment (78%) as well as a decrease in toxicity from the inlet to the outlet during a large rainfall event (Schulz & Peall 2001). The same wetland reduced spray-drift derived concentrations of azinphos-methyl by 90% (Schulz *et al.* 2003a). Reduced flow conditions in the wetland promote siltation, resulting in decreased turbidity and thus a decrease in sediment associated pesticide concentrations.

Furthermore, the reduced flow slows the transport to such an extent that there is more time to allow for degradation of pesticides via volatilisation, photolysis, hydrolysis or metabolic degradation (Schulz *et al.* 2003a). Vegetation within the wetland also provides additional substrate for the adsorption of aqueous-dissolved pesticides after which they are rapidly broken down (Moore *et al.* 2002; Schulz *et al.* 2003b). Similarly, vegetated ditches adjacent to agricultural fields have been shown to reduce runoff-induced pesticide transport via adsorption to macrophytes (Moore *et al.* 2001). Constructed wetlands have been shown to be very effective in mitigating nonpoint-source pollution in general and have been suggested as best management options in agricultural areas to ensure protection of sensitive or more vulnerable water bodies (Rodgers Jr. & Dunn 1992).

The influence of factors such as aquatic macrophytes on pesticide mitigation has led to a drive to classify different water bodies based on their biodiversity and vulnerability towards agrochemicals (Williams *et al.* 2004). For example streams, ponds and agricultural ditches may be characterized according to the hydrological and aquatic macrophyte coverage characteristics and may thus vary in their ability to mitigate pesticide contamination. Furthermore, there may be differences in the vulnerability of these systems according to the type of nonpoint-source pollution event they are exposed to.

1.5 LITERATURE REVIEW - EXPOSURE ASSESSMENTS

Accumulating evidence of the detrimental effects of pesticides in aquatic environments has necessitated the need to predict areas and identify areas where there is high risk of pesticide contamination (Black *et al.* 2000). This has led to the development and implementation of risk assessments, which are designed to identify any possible effects of toxic contaminants on workers, consumers, the environment and non-target plants and animals.

Standardised aquatic risk assessments typically involve an assessment of exposure (e.g. fate and behaviour of a substance in the environment) and ecotoxicological effect (based on single species toxicity tests covering the main biological components of aquatic systems: macroinvertebrates, fish and algae). Regulatory bodies and pesticide registration authorities in particular make detailed risk assessments of plant protection products before they can be sold on the market. As a result, exposure assessments in particular have evolved to the point where predictive environmental fate models ultimately determine the exposure risk that a chemical may present. Environmental fate models have been used to describe the behaviour of crop protection products since the early 1980's and the models presently play an integral role in pesticide regulation and registration in Europe and the USA (Jones & Mangels 2002).

In the European Union exposure assessments have become more detailed and have incorporated the use of fate models to calculate predicted environmental concentrations (PECs) in an attempt to determine potential environmental loadings. The registration procedure for plant protection products according to the Council Directive 91/414/EEC includes the use of models for the calculation of PECs in surface waters (FOCUS 1997). Depending on the PECs, further investigations (e.g. toxicity tests) have to be conducted in order to demonstrate acceptable risk to aquatic organisms. Thus models currently exist that make calculations for each of the main nonpoint-sources of pesticide contamination of surface waters; spray-drift (Spray-drift Calculator), drainage (MACRO) and surface runoff (Pesticide Root Zone Model; PRZM) (FOCUS 2001). Additional fate models, such as the Toxic Substances in Water Model (TOXSWA; EU) and the Exposure Analysis Modelling System (EXAMS; USA) are also used to predict the fate of chemicals in surface waters as a result of dissipation, partitioning and prevailing hydrological conditions. Pesticide fate models are also similarly used in the USA according to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Geographical Information Systems (GIS) have played an integral role in the application of these models in analysing various spatial, topographical, pedological, hydrological and land use input variables required for the models.

The combination of land use, meteorological and chemical factors, results in a large number of input variables being required for runoff predictions. Including PRZM, complex models such as Chemicals, Runoff and Erosion from Agricultural Management Systems (CREAMS), Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) and the Agricultural Non-point Source Pollution model (AGNPS) have been developed for this purpose (Donigian Jr. & Huber 1991). These models have been validated in the field with some success (Donigian Jr. & Huber 1991; Solomon *et al.* 1996; Grunwald & Norton 2000; Jones & Mangels 2002), but have disadvantages in that they are complicated and normally require a large number of input variables that may not be generally available (Adriaanse *et al.* 1997a). Minimal data requirements and ease of application are the main advantages of simpler simulation methods (Donigian Jr. & Huber 1991).

1.6 FORMULATION OF RESEARCH HYPOTHESIS

As a result of the direct nature of input, spray-drift has often been presumed to be the route of pesticide input that presents the highest risk to surface waters (Groenendijk *et al.* 1994). In spite of the numerous studies performed on the exposure, fate, effects and mitigation of runoff- and spray-drift-induced pesticide contamination in surface waters, very few of these studies have however directly compared these two routes in a risk assessment scenario.

As runoff results in a high proportion of sediment associated pesticide input, comparative studies of the effects of typical runoff and spray-drift exposures have typically compared water-dissolved (spray-drift) and particle-associated (runoff) pesticide concentrations. Erstfeld (1999) exposed fathead minnows (*Pimphales promelas*) to typical runoff and spray-drift exposure scenarios, using the pyrethroid insecticide deltamethrin and showed that accumulation was directly related to aqueous concentrations, with spray-drift exposures resulting in bioavailability an order of magnitude higher than runoff exposures. Schulz and Liess (2001a) exposed *Limnephilus lunatus* (Trichoptera) to field relevant water-dissolved and sediment particle-associated concentrations of the pyrethroid fenvalerate so as to compare the effect of typical spray-drift and runoff exposure scenarios on endpoints such as mortality and emergence. Lethal and sub-lethal effects were observed as a result of aqueous-phase contamination whilst only sub-lethal effects were observed after particle-associated contamination. Toxicity during the runoff scenario trials were between a factor of 10 and 100 lower than during spray-drift trials, once again indicating that the bioavailability of the pesticide is strongly reduced in the presence of fine sediment.

The effect of increased current velocity on toxicity during runoff conditions is a factor that has largely been ignored in experimental microcosm studies. Although studies have employed field relevant pesticide concentration and exposure durations, differences in current velocity may also influence the toxicity of a chemical towards benthic invertebrates (Lowell *et al.* 1995). For example drift behaviour of aquatic macroinvertebrates has been shown to be affected by sub-lethal pesticide concentrations and is commonly used as a sensitive endpoint in microcosm studies (Mitchell *et al.* 1993; Hose *et al.* 2002). In most cases where declines in macroinvertebrate abundance has been measured in the field it is likely that the declines occur as a result of increased drift (Kreutzweiser & Sibley 1991; Breneman & Pontasch 1994) as opposed to increased mortality as a result of acute toxicity. Increased current velocity has also been shown to affect drift behaviour (Ciborowski *et al.* 1977; Ciborowski 1982). As runoff and spray-drift are typically complimented by high and low flow velocity regimes respectively, it is possible that synergistic or antagonistic reactions between contamination and flow velocity may occur, which could determine the relative toxic or disruptive potential of runoff and spray-drift.

As runoff and spray-drift typically rely on different mitigatory strategies, a direct comparison is difficult to make. The reduction of pesticide concentrations as a result of sorption to aquatic macrophytes in vegetated wetlands and ditches is a mitigatory strategy that has however been shown to be effective in reducing runoff- and spray-drift-related pesticide concentrations, although no direct comparison in a single wetland or ditch has been made. An increase in current velocity and discharge during runoff conditions may also influence the potential of aquatic macrophytes in mitigating runoff-induced pesticide concentrations, particularly in faster flowing water bodies, such as ditches or streams. Most studies investigating the potential of aquatic macrophytes in mitigating runoff-induced pesticide concentrations have been performed under simulated conditions, and may not have accurately incorporated the increase in discharge in the experimental design. Thus the inherent hydrological differences typical of runoff and spray-drift events may influence the potential of vegetated water bodies in mitigating against their respective pesticide inputs.

Many fate studies (including predictive modelling) have been performed at the edge-of-field scale and would suggest that for any given water body, spray-drift would result in higher aqueous dissolved pesticide concentrations in comparison to runoff events. Indeed-spray drift is regarded as the worst-case scenario in many regulatory exposure assessments (Groenendijk *et al.* 1994; FOCUS 1997; FOCUS 2001). No studies have however performed a detailed exposure assessment comparing runoff and spray-drift pesticide input at the catchment level.

Percentage losses of applied pesticide have been calculated to be approximately between 0.5 and 1 % of the total applied pesticide for runoff (Wauchope 1978), while as much as 10 % of the applied substance can land in surface waters from a distance of 5m during spray-drift (Ganzelmeier *et al.* 1995). Very few studies have however investigated the relative inputs at the catchment scale. In this respect, broader landscape assessments are becoming increasingly important and can help put preliminary edge-of-field assessments into perspective within the agricultural landscape (Brown *et al.* 2003). As rain can fall over an entire catchment, each orchard adjacent to a tributary can potentially contribute towards pesticide input. Spray-drift input on the other hand is dependent on a number of specific wind conditions, which may mean that not all sprayed orchards in a catchment may contribute towards pesticide loading of the catchment. Thus an exposure assessment of the contributions of each source of pesticide at a larger spatial scale may provide a more holistic view on the relative importance of runoff and spray-drift.

1.7 RESEARCH OBJECTIVES

A detailed comparison of runoff and spray-drift could provide valuable insight into their relative threats to the environment and could help ensure that the use of pesticides in agricultural catchments is managed in an environmentally sustainable manner. Central to this thesis is the question of which of runoff and spray-drift poses the greatest risk towards aquatic systems in terms of pesticide contamination and what are the essential mechanisms that determine their relative threats to the environment. This question is addressed by monitoring, modelling and experimental studies designed so as to compare exposure and effects of runoff- and spray-drift pesticide contamination within a risk assessment context. Key processes that are investigated include the following:

- *The influence of different spatial and temporal scales on the exposure risk of runoff and spray-drift.*
- *The ability of a predictive model to predict differences in pesticide contamination resulting from runoff and spray-drift.*
- *The influence of flow speed on the toxicity of runoff- and spray-drift-induced pesticide contamination.*
- *The ability of aquatic macrophytes to mitigate against the transport of runoff- and spray-drift-derived pesticide transport.*
- *The influence of flow speed on the potential of aquatic macrophytes to mitigate against runoff- and spray-drift-derived pesticide transport.*
- *The use of modelling techniques in demonstrating the potential effectiveness of mitigation strategies.*

1.8 THESIS STRUCTURE

Each aspect of the assessment (exposure, effects and mitigation) is examined in separate chapters and is presented as individual papers, which shall be individually submitted for publication. The combined results of the different chapters are discussed so as to provide a broad comparison of the relative impacts of runoff- and spray-drift-induced pesticide contamination in an agricultural catchment. The chapters are as follows:

Chapter 1: General Introduction

Chapter 2: Materials and Methods. Details are provided on sampling methods used and chemical analysis of pesticide concentrations.

Chapter 3: An exposure assessment of runoff- and spray-drift-related pesticide input at the catchment level to compare inputs on a specific event and annual basis.

Chapter 4: The effect of differences in current velocity in conjunction with typical runoff and spray-drift exposure scenarios on the drift behaviour of mayfly nymphs is investigated through the use of microcosms.

Chapters 5: The potential of emergent aquatic macrophytes in reducing water-dissolved and sediment-associated pesticide concentrations resulting from runoff and spray-drift events is compared in an agricultural ditch.

Chapter 6: Interception of spray-drift derived pesticide input by emergent aquatic vegetation is investigated.

Chapter 7: General Discussion.

Chapter 8: Summary of Conclusions.

CHAPTER 2

MATERIALS & METHODS

2.1 PESTICIDE SAMPLING

2.1.1 Runoff samples

Runoff-associated pesticide input is often a short-term event, and thus difficult to detect. Sampling thus needs to co-incide with expected peak contamination. Runoff samples were collected according to a method described in Schulz *et al.* (1998). Samples representing the pesticide levels during runoff were accomplished using high water level samplers. At each sampling site metal stakes were driven into the river/stream bed. The sampling device consisted of a 750 ml glass wine bottle, which was attached to the stake. A small glass pipe (Pasteur pipette with the tip broken off) was tied in the opening of the bottle, using synthetic nylon, which enabled a free flow of water into the bottle while air could flow out via the glass pipe. During rainfall induced surface runoff, the bottles were filled passively as a result of the rising water level. A minimum of four high water samplers, each with the opening of the bottle placed at continuously increasing heights above the water surface (i.e. 3, 6, 9 and 12 cm above the water level), was placed at each sampling site so as to ensure a measurement of peak pesticide levels. In order to prevent contamination of the samples, all bottles were thoroughly rinsed with acetone prior to collection of water samples. Retrieval and extraction of water samples took place within 24 hours of the runoff event. Normally, timed automated sampling devices are used for collection of runoff event samples. These devices are however very expensive and the method described above has been used in a number of previously published studies (Schulz & Liess 1999a; Schulz 2001a; Schulz *et al.* 2001a)

Sediment associated pesticides samples were obtained using a suspended sediment sampler (Liess *et al.* 1996), which consisted of a plastic container (500-ml) with a screw-on lid with a hole (2 cm in diameter) cut in the center. An open glass jar was installed within the container such that any suspended sediment falling through the hole in the lid would be collected in the glass jar. The samplers were attached to the base of a metal stake driven into the river/stream bed. The sampling devices provide integrated values for the pesticide contamination of suspended particles, which are often highly contaminated because of the low water solubility of many pesticides.

2.1.2 Spray-drift sampling

Unless otherwise described, spray-drift samples were taken by dipping closed sampling bottles (700 ml) into the water column and opening the jars approximately 10 cm below the water surface, so as to avoid contamination with surface film. The samples thus represent subsurface pesticide concentrations.

2.2 TURBIDITY

Turbidity was measured using a turbidity meter (Dr. Lange, Germany). To calibrate the turbidity measurements as described by Gippel (1995), certain samples were filtered through pre-weighed Whatman GF/F (0.45 µm pore-size) glass microfibre filters and dried at 60°C for 48 h. The filter paper was then re-weighed to determine TSS (total suspended solids).

2.3 DISCHARGE

Flow velocity was measured using a 1210 "AA current meter" (Scientific Instruments Inc. Milwaukee, WN, USA). A top-setting wading rod was used to set the flow meter at 60% depths. Velocity and depth readings where taken at 1 m intervals in the Lourens River mainstream and at 20 cm intervals in the tributaries. The velocity-area method (Gordon *et al.* 1992) was used to calculate total discharge.

In cases where discharge was measured in a vegetated stream (Chapter 5), flow velocity readings where taken in a stretch of stream where vegetation was removed in a line immediately behind upstream vegetation.

2.4 PESTICIDE ANALYSIS

Analysis of pesticide levels in water and sediment samples was performed by the Forensic Chemistry Laboratory of the Department of National Health, Cape Town. Water samples (500 to 900 ml) were first filtered (to remove suspended solids) and then solid-phase extracted (SPE) using C18 columns (Chromabond) which had been previously prepared with 6 ml methanol and then 6 ml water. The columns were air-dried for 30 minutes and kept at –18°C until analysis. Suspended sediment and plant samples were placed in 250 ml polypropylene bottles and centrifuged. The supernatant water was discarded and 50 ml methanol was added. The sediment and plant extracts and methanol were shaken until well mixed. The polypropylene bottles were then placed in an ultrasonic bath for 30 minutes and centrifuged. The supernatant methanol was filtered through glass fibre filter paper into 500 ml measuring cylinders. Another 50 ml methanol was added to the sediment and the samples were again mixed well, placed in the ultrasonic bath and centrifuged. The methanol extracts for each sample were pooled and made up to 350 ml with pure water. A 50 ml aliquot of the extract was passed through a C18 column.

The pesticides were eluted with 2 ml hexane and then 2 ml dichloromethane. These extracts were dried in a stream of nitrogen and then dissolved in 1 ml hexane. The extracted sediments and plants were transferred into previously weighed beakers and dried at 150 °C. Concentrations for sediments and plants were expressed as μg/kg dry weight (dw). Water samples were eluted from defrosted SPE columns and then dissolved in 0.5 ml hexane.

Hexane solutions of water, sediment and plant samples were analysed using gas chromatograph/electron-capture/nitrogen-phosphorous detector (GC/ECD/NPD), ^{63}Ni ECD temperature: 300 °C with nitrogen as make up gas, NPD temperature: 300 °C. The gas chromatograph HP 5890 (Series II; Hewlett-Packard) was equipped with an HP 7673 auto sampler (Hewlett-Packard) and a split/splitless injector and capillary column, HP 5 (15 m length, 0.32 mm i.d., 0.25 μm film thickness; HP) and with nitrogen as carrier gas (1.1 ml min^{-1}), temperature programmes: 170 °C (1 min) → 20 °C min^{-1} → 300 °C → (1 min), 5 μl was injected with the splitter closed for 0.75 min. Measurements were confirmed using a gas chromatograph/flame-photometric detector (GC/FPD), FPD temperature: 250 °C. The gas chromatograph HP 5890 (Series II; Hewlett-Packard) was equipped with an HP 7673 auto sampler (Hewlett-Packard) and a split/splitless injector and capillary column, DB 210 (30 m length, 0.32 mm i.d., 0.25 μm film thickness; J&W) and with nitrogen as carrier gas (1 ml min^{-1}), temperature programmes: 150 °C (0.5 min) → 30 °C min^{-1} → 210 °C → (1 min) → 30 °C min^{-1} → 240 °C → (1 min), 5 μl was injected with the splitter closed for 1 min.

Identity of the pesticides were established by asking farmers which pesticides they had used and then confirming the identity by matching retention times on 3 different stationary phases in the case of the organophosphates and two stationary phases for the organochlorines. Method validation was conducted on water, sediment and plant matrices that were determined to have no detectable levels of the investigated pesticides. The validation consisted of spiking water at 8 spiking levels over the range of concentrations found in the actual samples. Overall mean recoveries were between 79 and 106%. For quality control, a matrix blank was analysed with each extraction set. The investigated pesticides were never detected in matrix blanks. The detection limits were 0.01 μg/L for water, 0.1 μg/kg for sediments, and 0.1 μg/kg for plants.

CHAPTER 3

PREDICTED AND MEASURED LEVELS OF AZINPHOS-METHYL IN THE LOURENS RIVER, SOUTH AFRICA: COMPARISON OF RUNOFF AND SPRAY-DRIFT

Published:

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(Environmental Toxicology and Chemistry, Vol. 22, No. 3, pp. 494-500, 2003)

*R. Schulz appears as a co-author in his capacity as co-supervisor and provided valuable assistance in developing the study design

3.1 INTRODUCTION

Nonpoint-source pesticide pollution from agricultural areas is widely regarded as one of the greatest threats to contamination of natural surface waters, necessitating the need to predict areas of risk (Loague *et al.* 1998). Spray-drift and runoff are considered to be important routes of entry for pesticides (Groenendijk *et al.* 1994) and as such, in terms of a risk assessment scenario, it is vital to compare these two processes with regard to their threat to water quality.

Land use, meteorological and application characteristics directly influence both spray-drift (Ganzelmeier *et al.* 1995; USEPA 1999) and runoff (Cole *et al.* 1997) and are thus important factors to consider when assessing the risk of these routes of nonpoint pollution to surface waters. Furthermore, runoff is highly dependent on the physicochemical properties of the pesticides themselves as they determine the amount of pesticide available to surface runoff (Blanchard & Lerch 2000; Capel & Larson 2001).

Nonpoint-source pollution models incorporate all of these variables in an attempt to predict contamination levels and could thus be valuable in comparing runoff and spray-drift as important sources of pollution in a river catchment. Such was the focus of this study, which was carried out in the Lourens River catchment, Western Cape, South Africa. Based on intensive studies of nonpoint-source pesticide pollution in this catchment (Schulz 2001a; Schulz *et al.* 2001a; Schulz *et al.* 2001b; Dabrowski *et al.* 2002), a modelling approach was now implemented using a simple runoff formula by Reus *et al.* (1999) and basic spray-drift values by Ganzelmeier *et al.* (1995).

Standardized drift studies for orchards as summarized by Ganzelmeier *et al.* (1995) are similar to those recommended by the Spray-drift Task Force (SDTF) (USEPA 1999) and are proposed for use in exposure assessments. Prediction of runoff requires a great number of input variables, and complex models such as Groundwater Loading Effects of Agricultural Management Systems (GLEAMS), Pesticide Root Zone Model (PRZM) and Agricultural Nonpoint-source models (AGNPS) have been developed for this purpose (Donigian Jr. & Huber 1991). The formula by Reus *et al.* (1999) however, 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 (OECD 1999). This formula was used as almost all input variables are easily available from digital maps and soil databases. The organophosphate pesticide azinphos-methyl [*O,O*-dimethyl-*s*-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl)]phosphorodithioate], in comparison to other insecticides, has a relatively low K_{OC} of 1000 L/kg and a high water solubility of 29 mg/L at 25 °C (Hornsby *et al.* 1995). It has been shown to persist in pond water with a half-life of about 2.4 days (Tanner & Knuth 1995). AZP is frequently applied to apple, pear and plum orchards in the catchment and has been regularly detected following runoff and spray-drift activity in the mainstream and tributaries (Schulz 2001; Dabrowski *et al.* 2002). The estimated total application in fruit orchards of the Western Cape is 52000 kg active ingredient (a.i.) per year. It is also one of the most heavily applied pesticides in the United States, and in 1997, almost 950000 kg a.i. were applied throughout the entire country (NCFAP 1997).

The main aim of the present study is to compare spray-drift and runoff as routes of nonpoint-source AZP pollution of the Lourens River based on predicted and measured values. Existing predictive approaches were implemented using a GIS and validated using measured loads from sub-catchments of the Lourens River. The ultimate comparison of runoff and spray-drift was done at the catchment level by comparing the results of the validated GIS-models with measurements of pesticide loads from the field during spray-drift and runoff events. Finally, the importance of spray-drift and runoff at the catchment level was compared on an annual basis using long-term meteorological data and average application characteristics.

3.2 MATERIALS & METHODS

3.2.1 Study Area

The Lourens River emerges from a natural sclerophyllous vegetation area (fynbos) after which it runs through forestry and farming areas in its middle reaches before flowing through the town of Somerset West (34° 06´ S; 18° 48´ E) (Schulz 2001a). The total catchment area is approximately 44 km^2, consisting of ten sub-catchments (A to J), each of which is drained by a tributary that discharges into the Lourens River mainstream, with site LR representing the catchment outlet (Fig. 3.1). The annual mean rainfall is 915 mm, most of which occurs during the winter months between April and October, as is characteristic of the region's Mediterranean climate

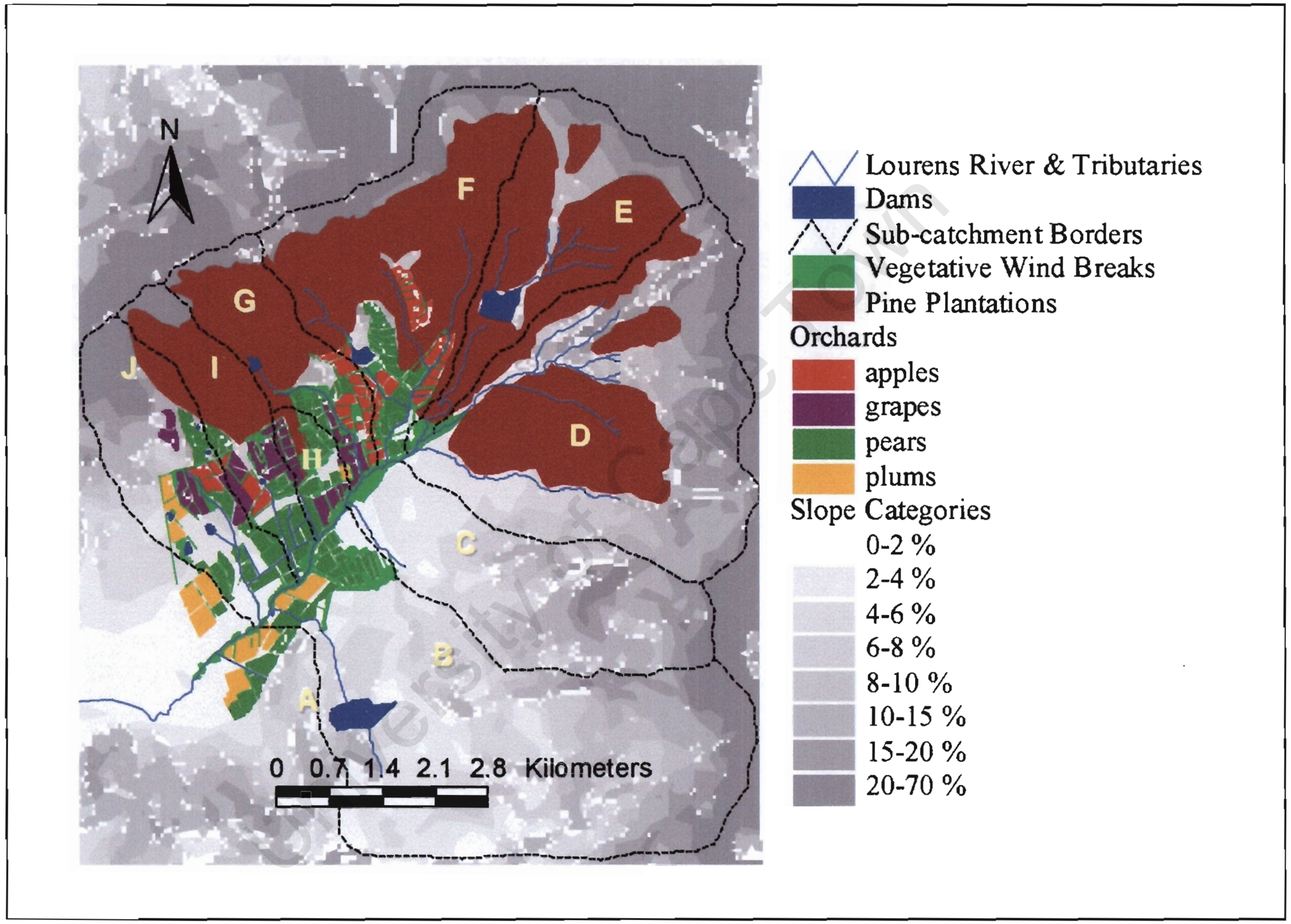

Fig. 3.1: Scale diagram of the land use of the Lourens River (LR) catchment, South Africa, with sub-catchments A-J.

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. AZP is the most commonly applied insecticide and is used frequently on all orchard types between October and February; at up to about one application every two weeks on each single plot. During each application, AZP is applied at 0.15 kg a.i./ha on sub-catchments A and B and at 0.525 kg a.i./ha in sub-catchments E to J (Schulz 2001; Dabrowski *et al.* 2002).

3.2.2 General Concept

It has been well established that due to the dense buffer strip (30-100m wide) lining the mainstream, the Lourens River receives pesticides only via the tributaries (Schulz 2001; Dabrowski *et al.* 2002). Thus, the models would first have to be applied in the tributaries in order to predict contamination in the mainstream. Accordingly, the initial validation of the runoff and spray-drift models was done using the tributaries situated in sub-catchments A to J. As a second step. the direct comparison of spray-drift and runoff based on predicted and measured AZP levels was performed at the catchment level so as to reflect the total contamination at site LR. The predicted AZP loads at this site were represented by the sum of the predicted loads for all affected sub-catchment tributaries during a runoff or spray-drift event. Finally, annual loads were evaluated by extrapolating average runoff and spray-drift predictions to a yearly basis using spraying programmes and long-term meteorological data. Predictions were based on levels in the water phase of runoff, as AZP has a relatively low K_{OC} and thus preferably occurs in the water phase (Dabrowski *et al.* 2002).

3.2.3 Runoff Prediction and Measurement.

The runoff formula by Reus *et al.* (1999) was designed to calculate pesticide loads in runoff water. In the present paper, the formula was applied to predict AZP loads in the water phase for the tributaries discharging the ten sub-catchments A to J. The runoff formula is as follows:

$$L\%_{\text{runoff}} = \frac{Q}{P} \cdot f \cdot e^{-t \cdot \frac{\ln 2}{DT_{50soil}}} \cdot \frac{100}{1 + K_d}$$

where; $L\%_{runoff}$ = percentage of application dose being available in run-off water as a dissolved substance; Q = runoff amount (mm) calculated according to hydrological models (Lutz 1984; Maniak 1992); P = precipitation amount (mm); DT_{50soil} = half-life of active ingredient in soil (10 d for AZP) (Hornsby *et al.* 1995); f = f1 x f2 x f3; correction factor reflecting the influence of slope (f1 = 0.02153 x slope + 0.001423 x slope^2), plant interception (PI), the percentage of applied pesticide intercepted by trees in the orchards (f2 = 1-PI/100) and buffer width (f3 = 0.83^{WBZ} and WBZ is the width of buffer zone (meters); if the buffer zone is not densely covered with plants, the width is set to zero); t = time between application and rainfall in days; K_d = (K_{OC} x OC); factor reflecting the tendency of the pesticide to bind to organic carbon in the soil, where K_{OC} is the sorption coefficient of the active ingredient to organic carbon (1000 L/kg for AZP) and OC% is the organic carbon content of the soil (0.75%, F. Ellis, Department of Soil Science, University of Stellenbosch, South Africa, personal communication).

All land use and catchment variables of the study area representing the input variables for the runoff formula were analysed using GIS ArcView 3.1 (ESRI™, Redlands, CA). Predictions were based on the assumption that tributaries would only receive runoff related pesticide input from orchards lying directly adjacent to the tributary. Using GIS, each sub-catchment was divided into slope categories (Fig. 3.1) and the total area of each slope category covered by relevant orchards was determined. The $L\%_{runoff}$ for each slope category was calculated and based on the amount of applied pesticide (g/m^2), the loss of AZP (g) per total area of each slope category was calculated. The total loss of AZP (g) per sub-catchment was then calculated by summing the loss of AZP in each slope category within the sub-catchment. Buffer strip characteristics (WBZ values) and the number of erosion rills (an eroded drainage channel leading directly from the edge of an orchard plot to the bank of a tributary) per tributary were obtained via field observations. The average rainfall for all the 15 measured runoff events (16 mm per event) between December 1998 and May 2001 was used in the formula to predict the input of AZP for an average runoff event in each sub-catchment.

In order to validate the runoff formula, the predicted average loads were compared to average loads measured in each tributary during 3 to 12 runoff-related peak discharge events from December 1998 to May 2001 (Schulz 2001; Dabrowski *et* *al.* 2002). Measured average loads in the nine tributaries B to J were derived from dissolved AZP concentrations and discharge levels measured during runoff events that were assumed to last 1 h based on detailed monitoring of earlier events (Schulz 2001a). The tributary of sub-catchment A was excluded from the regression, as only one runoff event was measured.

3.2.4 Spray-drift Prediction and Measurement

Prediction and validation of spray-drift related AZP loads in the drift-receiving tributaries discharging sub-catchments of the Lourens River was done using basic drift values (95^{th} percentiles) by Ganzelmeier *et al.* (1995) for a total of eight measured spray-drift events. Measured loads were derived from replicate (n = 6 per event) discrete tributary water samples, representing short-term peak concentrations of AZP present directly after spray deposition, relative to the water volume (Schulz *et al.* 2001b). The spray-drift events were monitored during conditions in which the wind (speed: 1.7 to 2.6 m/s) was blowing from the orchards in a perpendicular direction to the tributaries, according to the methods described in Schulz *et al.* (2001b). Distances between the sprayed orchards and the tributaries varied between 10 and 15 meters.

3.2.5 Comparison of Spray-drift and Runoff

The direct comparison of runoff and spray-drift was done using six runoff and six spray-drift events during which AZP was monitored at site LR (Fig. 3.1) between December 1998 and May 2001. Predicted loads (which were based on the validated runoff and spray-drift models) as well as measured loads were used for this comparison.

The predicted load for each of the six runoff events measured at LR was calculated by totalling the total loss of pesticide in each of the ten sub-catchments A to J. For each prediction the rainfall that was measured on the particular day and the corresponding Q value were inserted into the formula. The percentage plant interception was adjusted according to the growth stage at that particular time of year (Linders *et al.* 2000). Composite water samples were collected at site LR during runoff-related peak discharge events according to previously published methods (Schulz 2001; Dabrowski *et al.* 2002). For all runoff events a load was calculated according to the measured discharge and the assumption that an event lasted 1 hour, based on information from detailed runoff event monitoring (Schulz 2001a). Sediment samples were also collected during runoff events according to methods described by Liess *et al.* (1996).

Spray-drift related contamination at LR was predicted for each of the six spray-drift events between January 1999 and February 2001. The land use of the catchment in relation to potential spray-drift contamination via the tributaries was first analysed using GIS. It was assumed that vegetative windbreaks, such as a dense line of trees would prevent spray-drift from entering the tributaries (Mander 1989). The distance between plots and tributaries and the length of orchards bordering tributaries was determined using GIS. The predicted load of AZP in each tributary was calculated separately for each spray deposition event according to the basic drift values (Ganzelmeier *et al.* 1995) and then extrapolated to the total length of orchards adjacent to the tributary. In order to accommodate the influence of wind direction on the exposure of tributaries during each spray-drift event, only those sections downwind of sprayed orchard plots were considered in the prediction. The total load of AZP at site LR was determined by adding the loads for the sub-catchment tributaries.

Spray-drift sampling was accomplished by collecting discrete water samples every two hours for a total of eight hours during all of the six spraying days. Previous experiments showed this sampling design to be appropriate (Schulz *et al.* 2001b). These samples were analysed and an average concentration was calculated, representing the concentration in the mainstream during the 8-h spraying period. Estimated loads were calculated by multiplying the integrated 8-h average pesticide concentration by the mean discharge for the same time interval.

3.2.6 Pesticide analysis

All runoff and spray-drift samples were analysed for AZP 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 Chromabond® C18 columns (Macherey-Nagel, Düren, Germany). The columns were air-dried for 30 minutes and kept at –18°C until analysis. Measurements were done using gas-chromatographs (HP 5890's, Hewlett Packard, Avondale, PA, USA) fitted with a standard Hewlett Packard electron-capture, nitrogen-phosphorus detector and confirmed using a flame-photometric detector according to methods outlined in Schulz *et al.* (Schulz *et al.* 2001a). Sediment samples were extracted and analysed according to methods described by Schulz *et al.* (Schulz *et al.* 2001a). The detection limit for water and sediment samples were 0.01 µg/L and 0.1 µg/kg dw, respectively and spiked overall recovery efficiencies were between 79 and 106%.

3.2.7 Evaluation of Yearly Loads

Based on the evaluation of spraying programmes and meteorological data, the annual AZP loading by nonpoint-source pollution events at site LR was determined. The main insecticide application period lasts from early November to the beginning of February, during which time 12 applications are made per plot. Based on ten-year rainfall data, the frequency of rainfall events between 10 and 15 mm/d and above 15 mm/d is 3.4 and 1.7, respectively. A 15-mm event with a frequency of one was used for the first rainfall of the wet season following a non-application period (Schulz *et al.* 2001a). Annual loads were calculated by multiplying predicted loads for peak application and post-application scenarios with the respective frequencies.

An annual load for spray-drift was calculated by multiplying the predicted load for the predominant wind direction by the total number of spraying applications (12). The factors obtained for the differences between predicted and measured loads at site LR (1.89 and 1.34 for runoff and spray-drift respectively) were used for correction of the annual loads.

3.2.8 Data analysis

Regression analyses were used to determine whether AZP loads measured in the tributaries during runoff and spray-drift events could be predicted by the relevant GIS-based calculations. A Mann-Whitney Rank Sum test was used to determine significant differences between runoff and spray-drift in terms of mean measured AZP loads and concentrations at LR.

3.3 RESULTS

3.3.1 Runoff Prediction and Measurement

According to the definitions of the formula, tributaries A, B and I had a WBZ of zero and J a WBZ of 2. Tributaries F, G and H ranged between 5 and 6 meters. Tributaries I and J had 30 and 18 erosion rills respectively, the highest amongst all the tributaries, while the remaining tributaries had between 2 (H) and 12 (F) erosion rills per tributary.

Regression analysis showed a significant positive correlation ($r^2 = 0.95$; $p < 0.0001$; $n = 9$) between predicted and measured average runoff loads in the tributaries of the Lourens River (Fig. 3.2). Predicted loads were between a factor of 0.81 and 1.34 different to measured loads, apart from site G at which only 3 samples during moderate runoff events were analysed, resulting in an average measured value that was considerably lower than the predicted value. Tributaries F, I and J showed high average contamination up to 1 g/event, while tributaries A, B, G and H showed comparatively lower contamination, up to 0.1 g/event. No contamination was measured or predicted for sub-catchments C-E.

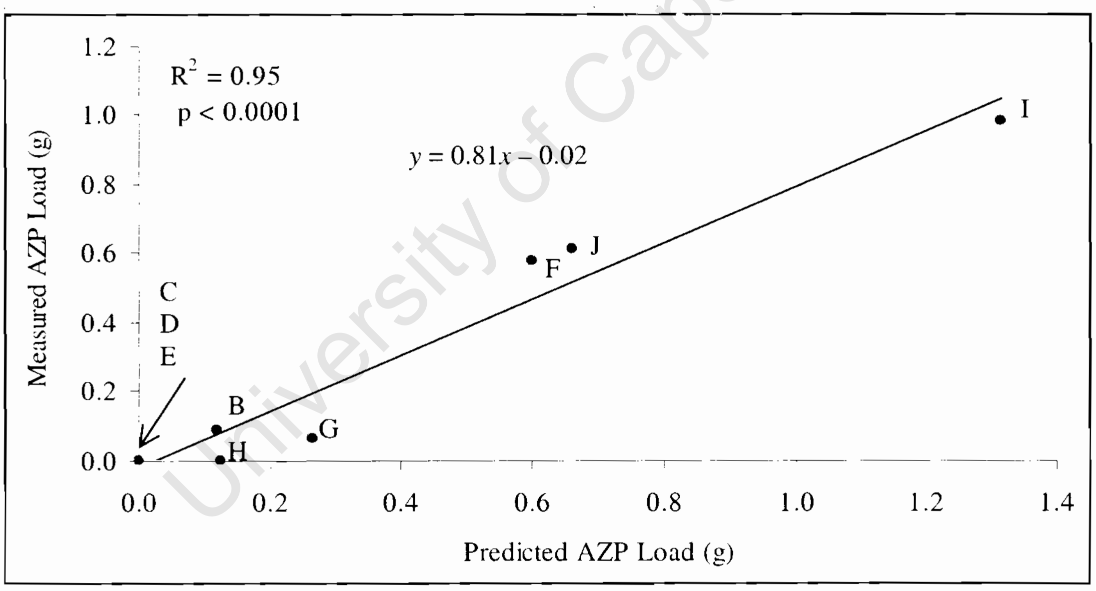

Fig. 3.2: Linear regression of measured versus predicted loads of azinphos-methyl (AZP) in nine tributaries (B-J) of the Lourens River during runoff. The measured values each represent means of 15 runoff events. No contamination was measured or predicted for sub-catchments C-E.

3.3.2 Spray-drift Prediction and Measurement

The basic drift deposition values given by Ganzelmeier *et al.* (1995) (r^2 = 0.96; p = 0.0006; n = 8) predicted in-stream loads which were between a factor of 1.2 and 1.58 higher than loads measured in the tributaries (Fig. 3.3). In-stream concentrations were between 1.5 and 3.6 μg/L and were mainly dependent on the distance of orchards from the tributary and the application rate of AZP.

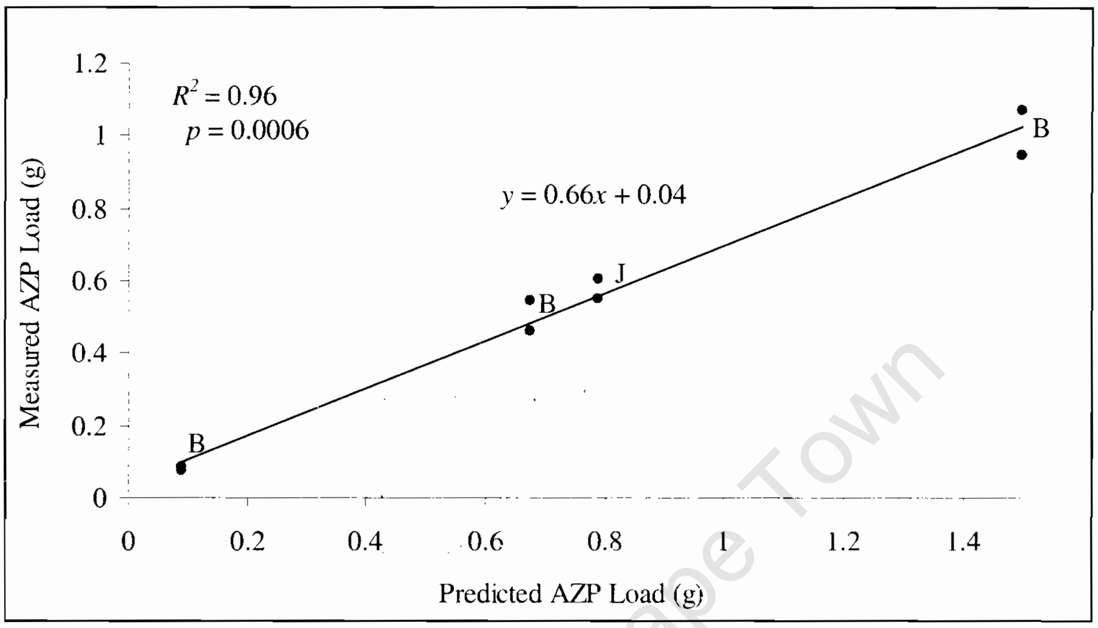

Fig. 3.3: Linear regression of measured versus predicted loads of azinphos-methyl (AZP) in two tributaries (B and J) of the Lourens River during eight spray-drift trials. The measured values each represent means of 6 samples per spray-drift event.

3.3.3 Comparison of Runoff and Spray-drift

Six runoff events were measured at LR; three during intensive spraying (November and December), and three long after the completion of spraying (April and May) (Table 3.1). Rainfall varied from 6 mm to 35 mm and lead to high variations in peak discharges (7.5 to 28 m^3/s). Based on the GIS-integrated runoff formula, predicted loads were similar to measured loads at LR (between a factor of 1.03 and 1.86 lower). Concentrations and loads measured during the November and December events were in all cases higher than those measured during the April and May events. Sediment concentrations of AZP ranged from 8 µg/kg (19.05.01) to 1247 µg/kg (14.12.98).

Table 3.1: Comparison of runoff and spray-drift based on measured and predicted loads in the Lourens River (LR). The measured concentrations (± standard error) are also provided.

Runoff	Date						
	14.12.98	15.4.99	19.11.99	20.12.99	4.5.01	19.5.01	Mean (± SE)
Measured Concentration (μg/L)	1.50	0.15	0.52	0.30	0.03	0.02	0.42 (± 0.23)
Measured Load (g/event)	121.0	5.4	28.2	8.2	3.1	1.0	27.8 (± 19.1)
Predicted Load (g/event)	78.0	2.9	15.3	4.8	1.2	0.6	17.1 (± 12.4)
Spray-drift	Date						
	27.1.99	28.1.99	21.1.01	7.2.01	10.2.01	12.2.01	Mean (± SE)
Measured Concentration (μg/L)	0.04	0.031	0.045	0.069	0.294	0.053	0.09 (± 0.04)
Measured Load (g/event)	0.32	0.25	0.35	0.54	2.25	0.41	0.69 (± 0.32)
Predicted Load (g/event)	0.60	0.60	0.83	0.83	2.42	0.83	1.02 (± 0.28)

Sampling during spray-drift events at LR took place on six occasions with average wind speeds of 1.6 to 3.1 m/s. Discharge did not vary between sampling dates and was 0.27 m^3/s. Based on the GIS-integrated calculations for spray-drift, predicted AZP loading at LR correlated well to measured 8-hr integrated loads (between a factor of 1.1 and 2.4 higher) (Table 3.1).

Mean measured loads were significantly ($p = 0.004$, Mann-Whitney Rank Sum Test) higher for runoff (27.81 ± 19.06 g) per event than for spray-drift (0.69 ± 0.32 g) per event. Only the highest measured load during spray-drift (2.25 g) was higher than the lowest value obtained during runoff (0.97 g). In terms of mean concentrations, runoff (0.42 ± 0.23 µg/L) led to higher values than spray-drift (0.09 ± 0.04 µg/L) however, the differences were not significant. This is most probably due to runoff events during the post-application period, when detected concentrations were in the range of average spray-drift events.

3.3.4 Evaluation of Annual Loads

Based on calculated annual loads, runoff is responsible for considerably higher levels of AZP nonpoint-source pollution than spray-drift (Fig. 3.4a & b).

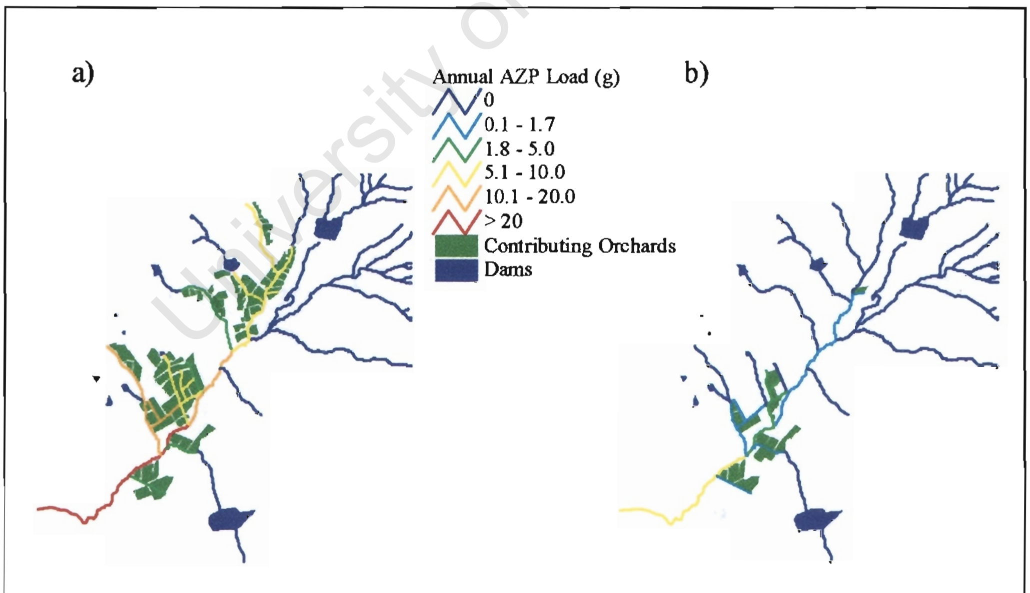

Fig. 3.4: Orchards affected by runoff (a) and spray-drift (b) and the corresponding predicted annual loads of azinphos-methyl (AZP) in the tributaries and mainstream of the Lourens River.

The loads at site LR during runoff and spray-drift were 46.7 g/year (89.6 % of total AZP load) and 5.5 g/year (10.4% of total AZP load), respectively. Tributaries F, J and I are responsible for most of the runoff contamination, and tributary I is responsible for most of the spray-drift pollution.

3.4 DISCUSSION

3.4.1 Runoff Prediction and Measurement

Measured loads in the tributaries were generally well predicted by the GIS-integrated runoff formula, indicating its suitability for use in South African orchard areas. The original use of the formula is intended to predict the loss of insecticide during a specific event at a specific site and not along the entire length of a tributary. Many assumptions had to therefore be made, which may have resulted in the systematic over-prediction of measured in-stream loads. The assumption which could have had the greatest influence on the accuracy of the predictions was that surface runoff from the entire area of an orchard adjacent to a tributary eventually enters the tributary. This may not always be the case, specifically if the orchard is very wide and flat, which might mean that runoff has to travel a distance of up to 200 m to reach the tributary in order to satisfy the assumptions of the model. The maximum distance over which runoff travels for a given slope is not incorporated into the formula and is a difficult parameter to quantify. According to the regression equation (Fig. 3.2) measured loads were approximately 81% of the value of the predicted loads. This value could be incorporated into the formula as an empirical correction factor to calibrate the model and improve the accuracy of the predictions.

Other runoff models, such as AGNPS, GLEAMS, and PRZM have been validated in the field with varying degrees of success (Donigian Jr. & Huber 1991; Grunwald & Norton 2000), but normally require a large number of input variables that may not be generally available (Adriaanse *et al.* 1997a). The main advantage of the approach used in this study is that it is very simple in its application, has minimal data requirements and combines field based measurements with a modelling approach, which is very useful for the assessment of water quality (Adriaanse *et al.* 1997a). High AZP input in sub-catchments F, I and J was most probably as a result of a high number of erosion rills in some cases associated with narrow buffer strips and steep slopes, all of which increase surface runoff (Wilcox & Wood 1989; Castelle *et al.* 1994).

3.4.2 Spray-drift Prediction and Measurement

The basic drift values (Ganzelmeier *et al.* 1995) could accurately predict loads measured in the tributaries and thus seem to be suitable for use in South African orchard areas. In a previous study in the same catchment, predictions based on the drift values of Ganzelmeier and the SDTF (USEPA 1999) correlated to measured concentrations in a similar way (Schulz *et al.* 2001b), indicating that the values proposed by the SDTF may also be applicable to South African conditions. Short-term peak concentrations detected in the tributaries were generally very high which is in accordance with previous studies (Gilbert & Bell 1988). Predictions were generally higher than measured loads. While the drift values utilized in this study have been rigorously validated in European countries (Ganzelmeier *et al.* 1995), very few studies have used these values in South African orchards (Schulz *et al.* 2001b) and further validation of the drift values is required in order to determine how applicable they are to South African conditions.

3.4.3 Comparison of Runoff and Spray-drift

This is perhaps the first study that has used and integrated GIS, modelling and pesticide monitoring to directly compare spray-drift and runoff. The result is an efficient, time saving method to pre-determine an area's vulnerability in terms of runoff- and spray-drift-induced pesticide contamination of surface waters and thus provides an integrated approach to pesticide management of agricultural catchments.

Runoff related AZP loading in the Lourens River (LR) is clearly a far more important nonpoint-source than spray-drift-related input (Table 3.1). This has rarely been shown so far in a direct comparison in the same catchment. Furthermore, this result is unexpected, since specific pesticide application in orchards results in a large amount of spray-drift due to small droplet size, the crop morphology and the resulting trajectory of release (Groenendijk *et al.* 1994).

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. GIS analysis clearly shows that each tributary in the catchment is influenced simultaneously during a runoff event, resulting in high potential input of pesticides (Fig. 3.4b). While buffer strips can reduce pesticide loss (Patty *et al.* 1997), the presence of erosion rills may jeopardize their positive effect and can thus result in rapid pesticide input (Castelle *et al.* 1994). Furthermore, rainfall is non-directional and does not restrict the number of orchards prone to runoff. Spray-drift on the other hand is dependent on downwind orientation to the point of application (Longley & Sotherton 1997) and can be prevented by windbreaks (Adriaanse *et al.* 1997b). Thus, the land use and the meteorological conditions restrict the number and length of tributaries that can possibly be affected by spray-drift.

Results from this and other studies (Williams *et al.* 1995; Kreuger 1998) show that runoff-induced pesticide loading can occur long after the previous application, indicating that runoff integrates chemical input over a large time span. In comparison, spray-drift is instantaneous and contamination can only occur during application, in combination with specific meteorological requirements, which further restricts the potential for contamination.

AZP concentrations detected during peak pesticide application periods (November and December) were higher than any other concentrations detected during spray-drift events and are potentially toxic to macroinvertebrates (Dortland 1980; Tanner *et al.* 1995). Furthermore, Water Quality Criteria defined by the U.S. Environmental Protection Agency (EPA) for AZP (0.01 μg/L) were greatly exceeded (USEPA 1988).

Although there was not a significant difference in the concentrations of pesticides during runoff and spray-drift events at LR, the fact that all tributaries in the catchment are simultaneously subjected to pesticide contamination during runoff may be of ecotoxicological significance. The fact that runoff is associated with higher loads but not significantly higher concentrations is most likely as a result of the increased discharges associated with runoff, which dilutes pesticide concentrations. Further research should compare the duration of exposure of typical runoff and spray-drift events, which could give more insight into the ecotoxicological implications of the two exposure scenarios. The comparison of different flow velocities characteristic of runoff and spray-drift conditions in combination with pesticide contamination is also worthy of further investigation as toxicity of pollutants has been shown to be dependant on flow velocities (Lowell *et al.* 1995).

Sediment associated loads of AZP were of minor importance during most of the measured runoff events (less than 0.18 g), which is most probably as a result of the relatively low K_{OC} value and high water solubility of AZP (Hornsby *et al.* 1995). In extreme cases however, contamination of sediments by AZP has been known to occur (Wan *et al.* 1995), and on 14.12.98 loads as high as 52.3 g of AZP were measured (Schulz 2001a), indicating the added risk of sediment associated input of pesticides during runoff conditions.

It is important to note that the different routes of entry could be heavily influenced by the physicochemical characteristics of the substance under consideration. For example, it has been shown that the chemistry of atrazine plays a far more important role than land use in determining its loss in surface runoff (Blanchard & Lerch 2000; Capel & Larson 2001). In the case of less water soluble pesticides, such as organochlorines and pyrethroids, sediment loadings and the resulting chronic effects may become more important (Schulz & Liess 2001a).

Both modelling approaches predict accurate loads of AZP at the catchment level, highlighting the importance of the contribution of tributaries to the nonpoint-source pollution of the Lourens River. Similar conclusions have been implicated by other workers (Pereira *et al.* 1996). In contrast to the predictions made in the tributaries the runoff predictions at the catchment level were under-predicted. Given the size of the catchment, it is most probable that a number of smaller intermittent streams (which only flow during heavy rainfall conditions) were not considered in the catchment-based predictions.

The predictions of spray-drift loads at LR were consistent with the predictions in the tributaries and were also over-predicted by the model.

3.4.4 Evaluation of Annual Loads

Annually, runoff is responsible for greater nonpoint AZP pollution than spray-drift (Fig. 3.4a & b). This has major implications on the implementation and focus of mitigation strategies designed to improve surface water quality. Pesticide loss via runoff is assumed to be more important than via leaching (Flury 1996), necessitating the need to reduce this route of contamination. Based on the results of this study, it is now possible to plan mitigation strategies, firstly in relation to the most important route of pollution of surface waters and secondly, in relation to problem areas (sub-catchments) responsible for large proportions of the contamination. It must be noted however, that the findings of this study may be specific to the land use of the area and the fact that there are a high number of windbreaks in the catchment. The physicochemical properties of pesticides also play an important role, and other more insoluble pesticides with higher adsorption co-efficients may lead to spray-drift being a more important route of aqueous dissolved pesticide exposure, and runoff contributing mainly to particle associated contamination.

Several options are available for agricultural best management practice or the implementation of buffer strips in order to mitigate the risk of nonpoint-source pollution (Humenik *et al.* 1987). Furthermore, constructed wetlands are effective in mitigating agricultural runoff and have been shown to significantly reduce aqueous and suspended particle associated pesticide input from tributaries into the Lourens River mainstream (Schulz & Peall 2001).

3.5 CONCLUSION

The GIS-based model successfully identified areas of concern associated with runoff and spray-drift contamination within the catchment. The validated model clearly showed that runoff is a more important source of nonpoint pesticide pollution in the studied catchment, leading to greater loads and concentrations of dissolved azinphos-methyl at the catchment scale. This is most likely because of the fact that runoff integrates contamination over a much larger spatial (contamination occurs over a wider area during a single rainfall event) and temporal (contamination can occur long after the most recent pesticide application) scale. Azinphos-methyl is however relatively water soluble, and the same study approach using a pesticide with a lower water solubility may show runoff to be less of an important source.

CHAPTER 4

COMBINED EFFECTS OF DISCHARGE, TURBIDITY AND CYPERMETHRIN ON MAYFLY BEHAVIOUR: EXPERIMENTAL EVALUATION OF RUNOFF AND SPRAY-DRIFT SCENARIOS

4.1 INTRODUCTION

Spray-drift and edge-of-field runoff are regarded as two major nonpoint-sources of pesticides in surface waters and typically result in short-term exposure, as insecticides are present in peak concentrations for only a few hours at most (Schulz & Liess 2001a). Both may however differ considerably in their resulting exposure scenarios (Groenendijk *et al.* 1994). Spray-drift leads to input of pesticides dissolved directly in the water phase, which is the only additional factor influencing aquatic fauna. Runoff-related input however, usually leads to increased discharge, flow velocity as well as increased levels of total suspended solids and pesticides, which may enter the surface water either as water-dissolved or particle-associated chemicals (Wauchope 1978). Despite reduced bioavailability through adsorption of pesticides to sediment (Hill 1989), contaminated suspended particles have been shown to cause toxicological (Schulz & Liess 2001b) as well as physical (Newcombe & MacDonald 1991) effects on aquatic macroinvertebrates. For aquatic organisms, flow velocity is also a particularly relevant abiotic factor and has been shown to influence the toxicity of contaminants towards macroinvertebrates (Lowell *et al.* 1995).

Hence, a multitude of factors can influence the aquatic environment under runoff conditions. Studies of these environmental factors have however usually been single-factor studies and little attention has focussed on their interactive impacts on aquatic organisms. Few studies have compared the ecotoxicological effects of typical runoff and spray-drift exposure scenarios on macroinvertebrates, and those that have, have only compared water-dissolved to particle-associated contamination (Chandler *et al.* 1994; Schulz & Liess 2001a), without incorporating the inherent differences in flow velocity in the experimental design. A comparative study of the sub-lethal effects of runoff and spray-drift related pesticide contamination that combines the various single factors is very relevant, particularly with regards to higher tier risk assessments of particular pesticides.

Increased activity and downstream drift of aquatic macroinvetebrates has been shown to increase as a result of exposure to low, field relevant concentrations of pesticides, and have been documented in both field (Schulz & Liess 1999a) and microcosm studies (Crossland *et al.* 1992; Breneman & Pontasch 1994; Schulz &

Dabrowski 2001). Increased activity and downstream drift can be regarded as a behavioural adaptation, enabling aquatic macroinvertebrates to escape from temporary stressful situations to unaffected regions during transient unfavourable conditions (Brittain & Eikeland 1988). Thus, drift response and changes in benthic activity in response to abiotic stresses can have a major influence on the distribution and activity of aquatic organisms.

Mayflies in particular, commonly exhibit drift behaviour (Corkum 1978). In addition to being among the most common benthic invertebrates in lotic environments, mayflies form an important part in the aquatic food web and the productivity of stream environments. They affect the composition and abundance of algal communities and are also a significant prey item for vertebrate and invertebrate predators (Forrester *et al.* 1999). Mayflies belonging to the genus *Baetis* are widely distributed and abundant in the Western Cape of South Africa (Dallas *et al.* 1999) and are thus at potential risk of pesticide exposure via spray-drift or runoff. The dynamics of mayfly populations are influenced by numerous environmental factors and are therefore used in many studies to investigate ecological effects caused by a variety of different factors such as pesticide input (Schulz & Dabrowski 2001) or increase in hydraulic stress (Ciborowski 1982).

The aim of this study is to compare the impact of simulated spray-drift and runoff scenarios on the drift behaviour of a Baetid mayfly species (*Baetis harrisoni*) in experimental microcosms. The pyrethroid insecticide cypermethrin was used in experimental trials, as pyrethroids are known to elicit sensitive drift responses (Breneman & Pontasch 1994; Schulz & Liess 2001a). Cypermethrin [(R,S)-alpha-cyano-3-phenoxybenzyl(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcycloprpane-carboxylate] has a water solubility of 0.01 mg/L at 20°C (Kidd & James 1989) and a high adsorption coefficient (K_{OC}: 100 000) and thus readily sorbs to sediment, suspended particles and plants (Wauchope *et al.* 1992). It has a moderate persistence in soils with a half-life of four days to eight weeks (Kidd & James 1989) and is highly toxic towards aquatic invertebrates with a 96-hour median lethal concentration (LC_{50}) of 0.2 μg/L for *Daphnia magna* (Bradbury & Coats 1989) and 0.01 μg/L for the mayfly *Baetis rhodani* (USEPA 2000). The independent and combined effects of cypermethrin, sediment input and increased flow speed on mayfly activity and drift behaviour were evaluated. Pesticide and sediment concentrations and flow speeds used in the microcosms were typical of exposure scenarios likely to occur during runoff and spray-drift events.

4.2 MATERIALS & METHODS

4.2.1 Field collection and maintenance of mayflies

The studies were carried out with mayfly nymphs captured from the upper Liesbeek River near Table Mountain, Cape Town, Western Cape, South Africa (S33°47′; E18°58′) and were performed during December 2002 and January 2003, a period of high pesticide application in the Western Cape. Nymphs were gently washed from the surface of stones into 20 L buckets and then transferred to climate-controlled holding facilities at the University of Cape Town. The nymphs were kept in continuously aerated aquariums, which contained water collected from the river and were kept at a constant temperature of 18°C. Mayfly nymphs were late-instar *Baetis harrisoni* without black wing pads, ranging in size from 4 to 8 mm. Animals of different sizes were randomly taken for each trial to reduce differences in results caused by their developmental stage. The animals were used from 1 h up to 24 h after capture.

4.2.2 Artificial stream microcosms

The experiments were conducted in a stainless steel microcosm (1.5 x 0.2 x 0.2 m) filled with 30 L of water resulting in a water depth of 10 cm (Fig. 4.1). Water current was produced by a paddle wheel (diameter: 30 cm; width: 8 cm) attached to an axis driven by a 100-W electric motor. A glass plate (9 x 15 x 0.5 cm) could be inserted into brackets mounted permanently in a position approximately 30 cm downstream from the paddle wheel. With the glass plate inserted the current velocity was 0.07 ± 0.01 m/s and represents a normal flow case. High flow (0.2 ± 0.02 m/s) was achieved by simply removing the glass plate. Current velocities are within the range of those reported for natural streams (Ciborowski 1982). A drift net with a mesh width of 1 x 1 mm was placed at the end of the paddle wheel channel. Six rocks (~ 5 x 5 x 3 cm) were collected from the Liesbeek River and were placed in the experimental channel to provide the mayflies with a substrate representative of natural habitats (no sand or gravel was added). The rocks were cleaned of any algae, preventing any potential interaction with the pesticide.

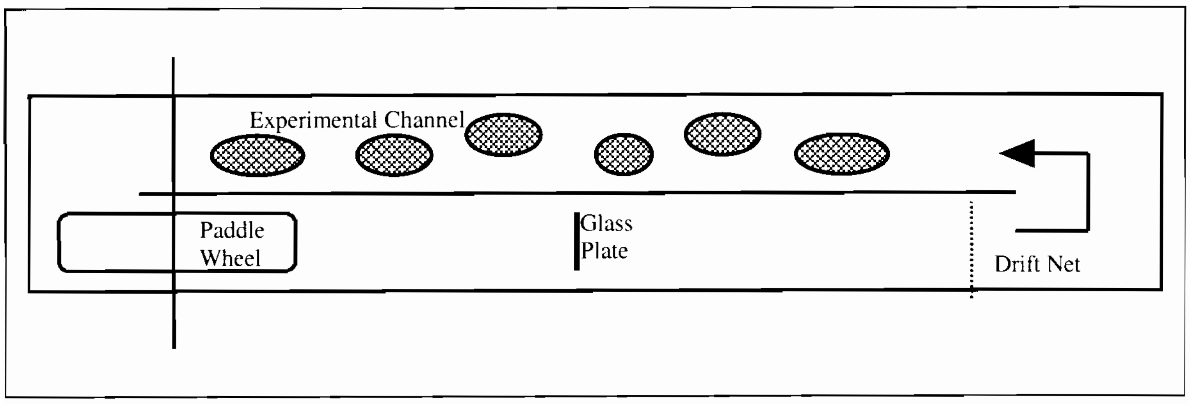

Fig. 4.1: Schematic view, from above, of the stainless steel microcosm (1,5 x 0,2 m) showing the channel with paddle wheel, removable glass plate and drift net and the experimental channel with rocks. The arrow indicates the flow direction.

Pilot microcosm trials revealed no difference in the behaviour of mayflies when tap water or stream water (collected from the stream from which the mayflies were captured) was used. Therefore, tap water was used for logistical reasons. Before experiments were undertaken, tap water was aerated in 25 L containers for 48 h in darkness to allow the oxidation of chlorine.

4.2.3 Exposure set-ups

Pesticide-water and pesticide-sediment stock solutions containing the desired concentration of cypermethrin and contaminated sediment for simulation of the spray-drift and runoff-induced pesticide exposure scenarios were prepared before the experiments. The emulsifiable concentrate, 200 g/L active ingredient (a.i.) was used rather than the pure substance (technical grade) in order to approximate conditions in the field. All concentrations given here refer to the active ingredient.

Field relevant concentrations of 1 μg/L for water-dissolved (Crossland *et al.* 1982) and 2000 μg/kg (Schulz & Liess 1999a) for particle associated cypermethrin were chosen in order to simulate spray-drift- and runoff-related exposure concentrations which also ensured that experiments were directly comparable in terms of mass of pesticide per volume of water (1 μg/L aqueous dissolved cypermethrin in 30 L water and 2000 μg/kg particle associated cypermethrin at a total suspended solid (TSS) concentration of 500 mg/L). It should be noted, that a direct, quantitative comparison of water and sediment-water exposure could only be made under the assumption that all cypermethrin was in the water during the sediment-water exposure, which may not be the case. For spray-drift exposures a 10000 μg a.i./L stock solution was prepared daily (0.01 μg a.i. cypermethrin in 1 liter distilled water) and used for all treatments within six hours. The sediment for the particle-associated trials study was collected from the upper stretch of a tributary of the Lourens River, Somerset West, (34°06′S, 18°48′E), where no pesticide input occurs owing to the lack of any farming activity (5% Silt; 95% Sand, 27% total organic carbon). No contamination of the sediment with current-use pesticides was measurable. The contamination of the sediment was done following the method of Schulz and Liess (2001b). The fine-fraction of sediment for the contamination trials was collected by washing it through a 90 μm sieve. The sediment was then homogenised after which it was allowed to settle for 48 hours.

After separation of the aqueous phase, a small amount of the sediment was weighed and dried in order to determine the water content (43.2%). The remaining wet sediment was stored in closed glass jars to minimise any risk of evaporation and changes in the water content. Two 2.25 L stock sediment-water solutions with a sediment load of 100 g/L were prepared by adding 396 g of the wet sediment to 2.078 L water. One stock solution served for trials with contaminated sediment, the other one for trails with uncontaminated sediment. Both solutions were aerated in darkness for 8 days to allow oxidation of ammonia and nitrite. For the preparation of the contaminated and the control sediment samples, a sample of 100 mL (containing 10 g sediment) was taken from each stock solution during continuous homogenisation and then dried at 60 °C. An amount of 10 mL of acetone containing 450 μg cypermethrin was added to the dry sediment for the contaminated sample and pure acetone for the control sediment. The sediment was then stored for 1 hour to allow evaporation of the acetone, confirmed by a gravimetric control.

The sediment samples were refilled with water to 100 mL, combined with their stock solution and allowed to equilibrate with constant aeration for 48 hours in darkness.

4.2.4 Experimental design

Eight treatments were carried out (Table 4.1), each of which was replicated five times. All trials were conducted in one microcosm and were run in random order. After each trial, the microcosm was cleaned with acetone and rinsed with tap water. For each trial stones and water were replaced. The experiments took place during December 2002 and January 2003 and were performed in a walk-in, temperature-controlled climate room of the University of Cape Town, South Africa. The temperature was 18 ± 1 °C.

Table 4.1: Experimental exposure scenario's used to determine the individual and combined effects of cypermethrin (CYP), suspended particles (PART) and increased flow speed (FLOW) on the drift and activity of mayflies (*Baetis harrisoni*) in experimental microcosms.

Treatment	Aqueous- dissolved Cypermethrin (μg/L)	Particle-associated Cypermethrin (μg/kg)	Suspended Particles (mg/L)	Flow (m/s)
Control	None	None	None	0.07
CYP	1.0	None	None	0.07
PART	None	None	500	0.07
FLOW	None	None	None	0.2
CYP x PART	None	2000	500	0.07
CYP x FLOW	1.0	None	None	0.2
PART x FLOW	None	None	500	0.2
CYP x PART x FLOW	None	2000	500	0.2

Prior to the start of each trial 20 mayfly nymphs were gently placed into the upper region of the experimental channel under either normal or high flow conditions depending on the ensuing trial. Only externally undamaged and active individuals were used. After their release into the microcosm the mayflies were given approximately 10 minutes to settle down beneath the rocks. Mayflies that had not settled on or underneath the rocks during this period were replaced by new ones, which were also given 10 minutes in which to settle on the rocks. Prior to the start of each experiment all mayflies had settled on the rocks, with more than 70% of the mayflies settling in positions underneath the rocks. After the 10 minute settling period the appropriate contaminants were added depending on the trial to be executed. For the CYP and CYP x FLOW treatments 3 mL of the 10 000 μg/L stock solution of cypermethrin was added to the microcosm in front of the paddle wheel, ensuring rapid distribution in the water column. For the treatments with sediment exposure a volume of 150 mL of the contaminated or uncontaminated sediment stock solution was added in the same way. Observations were made from a position next to the middle region of the microcosm and opposite to the experimental channel in order to avoid any influence of the observation process on the mayfly behaviour. All experiments were performed between 10 AM and 2 PM in light conditions.

4.2.5 Monitoring of activity and drift

Once contaminants had been added the experiment started and involved monitoring the activity and drift of the mayflies via visual scans that were performed every minute for a period of 30 minutes. Activity was determined by counting the number of individuals that actively crawled from underneath the stones to a position on top of the stones. As a result of the high turbidity, activity could not be observed during the suspended sediment trials. Drift was determined by counting individuals in the drift net after every minute. Each individual caught in the drift net was removed after counting. Most of the mayflies entered the drift from the top of the rocks and only a few individuals drifted directly from their position under a rock.

4.2.6 Water quality and toxicant analysis

Physico-chemical parameters of the microcosm water were measured with electronic meters under low flow as well as high flow conditions (Table 4.2). The slightly acidic pH is typical of rivers in the Western Cape of South Africa (Dallas *et al.* 1999). Cypermethrin exposure concentrations were measured at the Forensic Chemistry Laboratory (Department of Health, Cape Town, South Africa). Water and sediment samples (after extraction) were solid phase extracted with Chromabond® C18 columns (Macherey-Nagel, Düren, Germany). The measurements were made with gas chromatographs (Hewlett-Packard 5890, Avondala, PA, USA) fitted with standard Hewlett-Packard electron-capture, nitrogen-phosphorous, and flame-photometric detectors, with a quantification limit of 0.1 μg/L, and spiked recovery efficiencies were between 79 and 106%. A more detailed explanation of the extraction and analytical procedure can be viewed in (Dabrowski *et al.* 2002). Average (± standard error) concentrations of cypermethrin in samples taken from separate trials containing aqueous-dissolved (n = 3) and particle-associated (n = 3) pesticide were 0.89 ± 0.3 μg/L and 1875 ± 301 μg/kg, respectively.

Table 4.2: Means (± standard error, n = 3) of pH, conductivity and oxygen saturation for the microcosm water under low and high flow conditions.

	low flow	high flow
pH	5.2 ± 0.01	5.3 ± 0.01
Conductivity (μS/cm)	150 ± 2.52	150 ± 0.15
Oxygen saturation (%)	85.3 ± 1.15	87.0 ± 1.00
Oxygen (mg/L)	9.1 ± 0.06	9.1 ± 0.01

4.2.7 Data analysis

Drift behaviour was expressed either as drift rate or as drift density. The drift rate is the total number of individuals drifting downstream per observation period, which was 30 minutes. The drift density is the number of individuals drifting per volume of water, which was 1260 L over the total period of this experiment at normal flow rate and 3600 L for high flow treatments. The use of drift density is recommended for comparative studies that take into account the effect of flow changes on the drift of organisms (Brittain & Eikeland 1988). Thus the drift rate was used for most comparisons, while the drift density was used for the assessment of the effects of flow changes. The drift density was calculated by dividing the drift rate measured during high flow (0.2 m/s) trials by 2.86, which was the factor increase of volume flow in comparison to the normal flow (0.07 m/s) trials. Drift rate and drift density were expressed as the average absolute number of mayflies that drifted in 30 minutes and the number of mayflies drifting in 1260 L of water, respectively.

Differences in the two endpoints (drift rate and drift density) between any of the treatments were analysed using a three factorial analysis of variance (ANOVA) with cypermethrin, suspended sediment and increased speed as factors. The data were transformed using ln (x+1) to satisfy the assumptions of ANOVA. As activity could only be registered visually in the non-turbid treatments without suspended particles, a separate two-factorial ANOVA was performed using cypermethrin and increased flow speed as factors. The average number of active mayflies was expressed as a percentage relative to the total number of mayflies present in the microcosm after every minute. Activity data was thus arcsin transformed before performing the ANOVA. A Kolmogorov-Smirnov-Test was applied (p = 0.55, 0.41 and 0.96 for drift rate, drift density and activity) to confirm that the data were normally distributed.

In order to interpret potential synergistic or antagonistic combined effects, an expected value was calculated for each endpoint in the treatments showing significant interactions (e.g., CYP x PART). The factor increase or decrease in drift rate/density between the control and one variable (e.g., CYP) was calculated and then multiplied with the measured drift rate/density of the other variable (e.g., PART). The results are expressed as the absolute number of mayflies drifting.

4.3 RESULTS

4.3.1 Single factor effects

Results of the three-factorial ANOVA show that cypermethrin, suspended sediment and increased flow speed all had a significant influence on the drift rate and density, respectively (Table 4.3). For single factor effects, only the addition of pesticide resulted in an increase in drift rate, from 3 in the CONTROL to 11.6 individuals in the CYP treatment (Fig. 4.2). In contrast the PART and FLOW trials resulted in a reduced drift rate (from 3 to 1.8 individuals) and density (from 3 to 1.4 individuals), respectively. The two-factorial ANOVA, indicated that only increased flow speed had a significant impact on activity (Table 4.4, $p < 0.05$), which decreased from 41.4% to 28.2% individuals (Fig. 4.3).

Table 4.3: Three-factorial analysis of variance of the effect of pesticide (Pest.) suspended sediment (Sed.) and increased flow (Flow) on *Baetis harrisoni* drift (ln(x+1) transformed).[a]

	Source	*df*	MS	*F*	*p*
Drift rate	Pest.	1	8.519	148.874	<0.001
	Sed.	1	2.938	51.335	<0.001
	Flow	1	0.350	6.111	0.019
	Pest. x Sed.	1	0.331	5.788	0.022
	Pest. x Flow	1	0.037	0.650	0.426
	Sed. x Flow	1	0.001	0.011	0.916
	Pest. x Sed. x Flow	1	0.000	0.000	1.000
Drift density	Pest.	1	6.749	152.434	<0.001
	Sed.	1	2.328	52.585	<0.001
	Flow	1	3.764	85.015	<0.001
	Pest. x Sed.	1	0.329	7.441	0.010
	Pest. x Flow	1	0.264	5.964	0.020
	Sed. x Flow	1	0.046	1.029	0.318
	Pest. x Sed. x Flow	1	0.000	0.000	0.994

[a] *df* = degrees of freedom; MS = mean square; *F* = likelihood ratio; *p* = probability

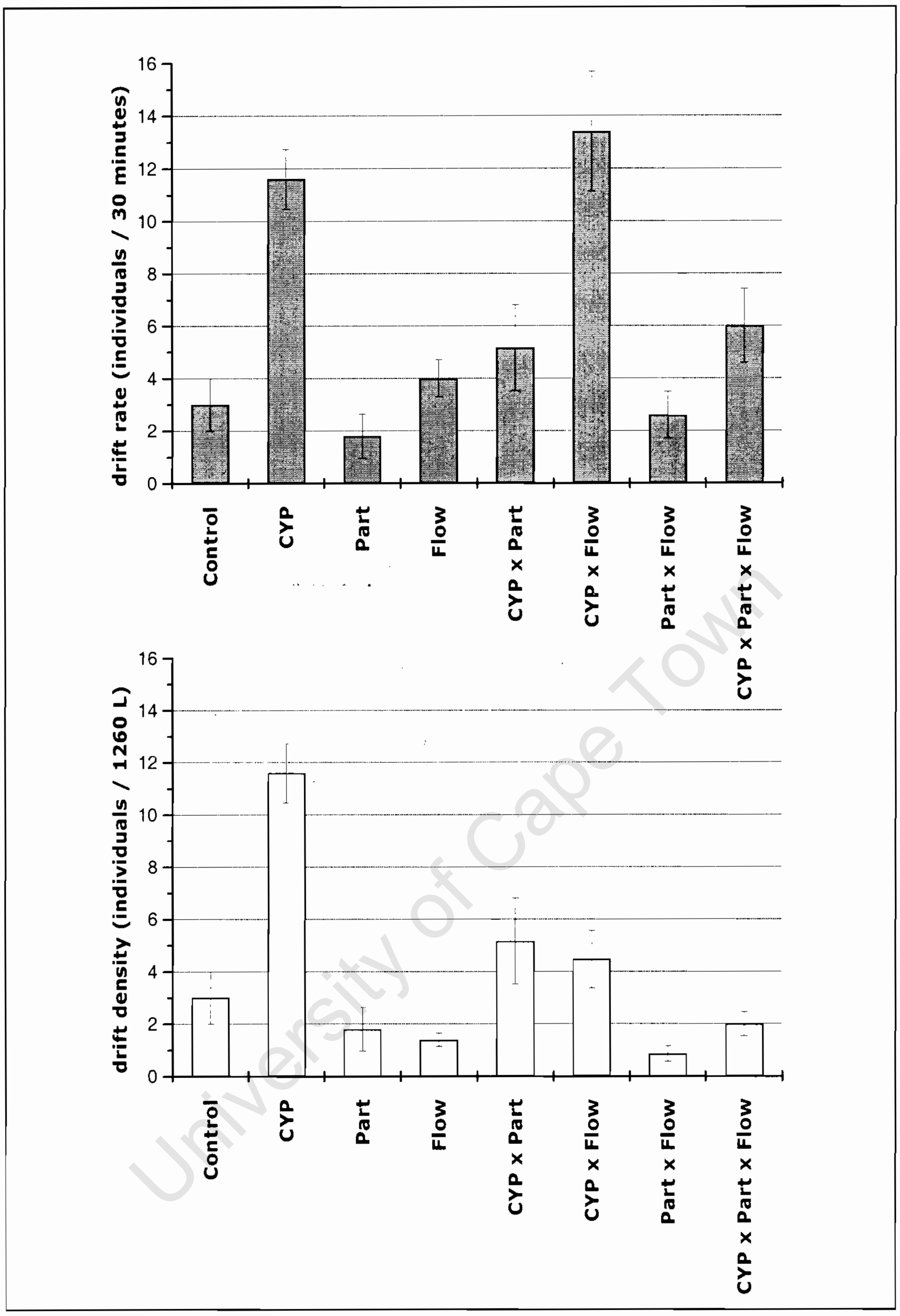

Fig. 4.2: Mean (± standard error; n = 5) of drifting mayflies (*Baetis harrisoni*) expressed as drift rate (*top*) and as drift density (*bottom*) in 30-min treatments either with pesticide (CYP), suspended particles (Part) or flow increase (Flow) and their combinations.

Table 4.4: Two-by-two factorial analysis of variance of the effect of pesticide (Pest) and increased flow rate (Flow) on *Baetis harrisoni* activity (arcsin transformed).[a]

	Source	*df*	MS	*F*	*p*
Activity	Pest.	1	0.008	0.665	0.427
	Flow	1	0.055	4.820	0.043
	Pest. x Flow	1	0.007	0.598	0.450

[a] *df* = degrees of freedom; MS = mean square; *F* = likelihood ratio;*p* = probability

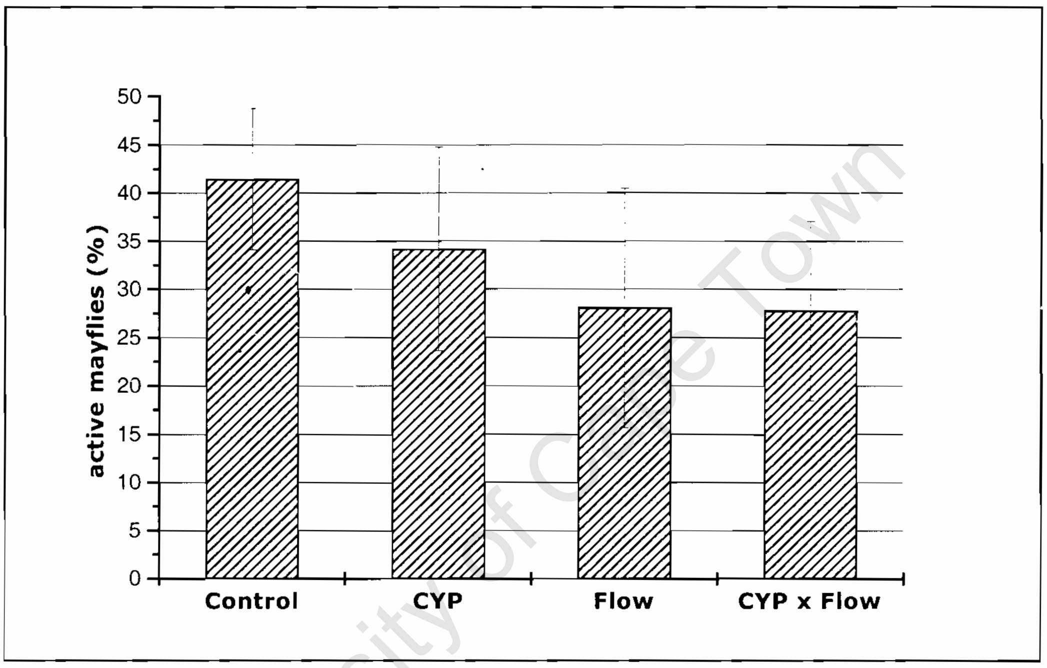

Fig. 4.3: Mean (± standard error; n = 5) of percentage of active mayflies (*Baetis harrisoni*) 30-min treatments either with pesticide (CYP), flow increase (Flow) or their combination (CYP x Flow).

4.3.2 Combined factor effects

The three-factorial ANOVA reveals a significant two-way interaction of contaminated sediment for drift rate (Table 4.3), which increased from 3 (CONTROL) to 5.2 individuals in the CYP x PART trials (Fig. 4.2). The combination of cypermethrin and increased flow speed also showed a significant interactive effect for drift density, which increased from 3 to 4.7 individuals in the CYP x FLOW trials. The combination of suspended sediment and increased flow speed as well as contaminated sediment and increased flow speed did not have any significant interactive effects on drift density (Table 4.3) and resulted in lower drift densities than the control.

4.3.3 Antagonistic effects of CYP x PART and CYP x FLOW exposure

For a visualisation of the interactive effects of CYP, PART and FLOW, it is useful to compare the expected and measured values for those combined treatments that show a significant reaction in drift behaviour. These are CYP x PART for the drift rate and CYP x FLOW for the drift density (Fig. 4.4). In both cases the measured values (5.2 and 4.7 individuals for CYP x PART and CYP x FLOW, respectively) are lower than the expected values (7 and 5.4 individuals for CYP x PART and CYP x FLOW, respectively), suggesting the variables have an antagonistic effect on the drift behaviour of the mayflies.

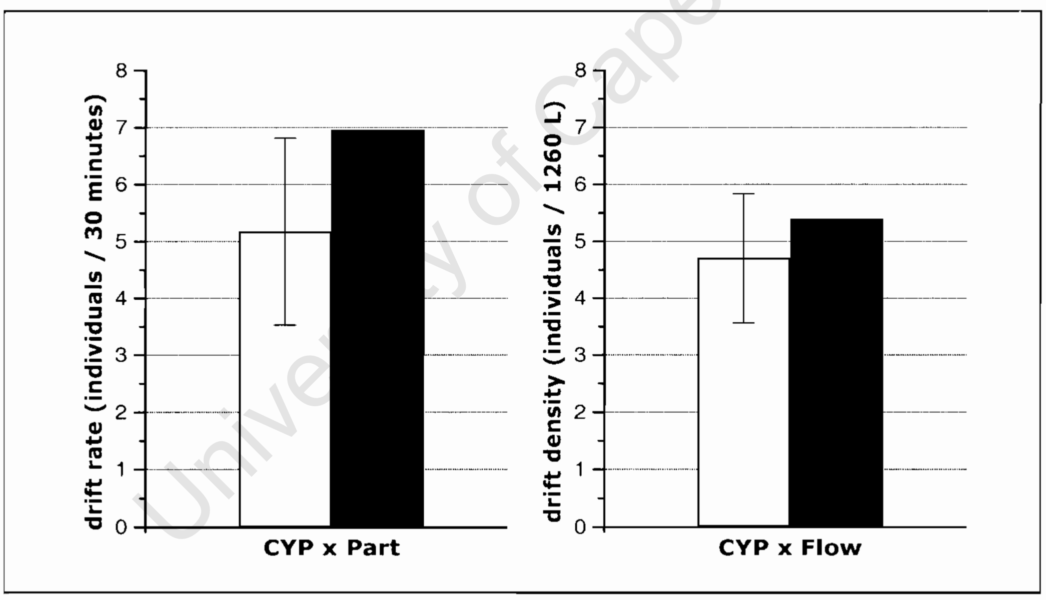

Fig. 4.4: Mean measured (white bars ± standard error; n = 5) and expected (black bars) drift for the significant interaction CYP x Part (significant for drift rate) and CYP x Flow (significant for drift density).

4.4 DISCUSSION

4.4.1 Single factor effects on drift and activity

Results show that in comparison to suspended sediment and increased flow speed, cypermethrin exposure led to the greatest increase in drift response by the mayflies (Fig. 4.2). Most importantly this increased drift rate took place under field relevant exposure conditions, in terms of length and concentration of pesticide exposure (Crossland *et al.* 1992; Schulz & Liess 1999a). This finding is in agreement with many other studies that have reported increased invertebrate drift as a response to insecticide exposure. Breneman & Pontasch (1994) found that the drift of Baetid mayflies increased due to exposure of 1 μg/L of the pyrethroid fenvalerate in experimental stream microcosms. Schulz & Dabrowski (2001) reported a significant increase in drift of Baetis sp. following a 0.2 μg/L exposure by the pyrethroid insecticide fenvalerate in microcosms. Mayflies and Baetis in particular are highly sensitive to pesticide pollution and frequently respond to lower concentrations of toxicants than other species included in the same multi-species tests (Siegfried 1993; Breneman & Pontasch 1994). Increased drift as a result of repeated exposure to pesticides can lead to changes in benthic community structure (Wallace *et al.* 1989) and thus poses a serious risk to natural communities in agricultural catchments.

Although studies have shown increased drift behaviour in response to sediment exposure, no studies have shown a reduction in drift as found in this study (Fig. 4.2). Macroinvertebrate drift rate has been shown to increase with more fine sediments present (Doeg & Milledge 1991), which has been attributed to factors such as physical disturbance by moving sediment (Culp *et al.* 1986), a response to light reduction (Gammon 1970) and settling of silt. Yet studies have also shown no change in macroinvertebrate drift behaviour in response to sediment contamination (Hose *et al.* 2002). Cooper (1993) found that invertebrates have the ability to withstand high concentrations of suspended sediments (> 1 g/L) for brief periods but that their mortality rates increase with time. *Baetis harrisoni* is a relatively tolerant mayfly species (Palmer & Scherman 2002) and is most probably relatively well adapted to the periodical return of suspended particles during heavy rainfall events in the Western Cape. *Baetis harrisoni* is also found in a wide range of aquatic habitats and sediment that settled on the bottom of the microcosm may have increased habitat availability, thereby reducing the drift. The composition of the sediment may also influence the drift reaction of mayflies. It is possible that the sediment used in this study may have been perceived as a potential source of nutrients due to its organic carbon content, thus reducing drift, while coarser grained sand or very fine clay particles may be more stressful due to physical abrasion or smothering of respiratory surfaces respectively (Culp *et al.* 1986; Fairchild *et al.* 1987).

Mayfly drift has also been shown to increase during daylight in order to avoid predation by visually feeding fish (Poff *et al.* 1991) Drift may thus decrease in the presence of sediment as result of a perceived decrease in predation risk. The individual and combined effects of particle characteristics, light intensity, predation risk and sediment concentration on macroinvertebrate drift are complex and provides opportunity for further research in this area.

The reduced drift density in the FLOW treatment may be as a result of changes in the activity of the mayflies. Increased current velocity increases the likelihood of dislodgement from the substrate and mayflies therefore decrease their activity at high flow levels and maintain a position beneath the rocks so as to avoid hydraulic stress and thus reduce drift (Williams 1990). This assumption is supported by the decreased activity observed in the FLOW treatments (Fig. 4.3). Decreased activity during high flow conditions has been observed for Baetid mayflies and other invertebrates, such as *Gammarus* (Elliott 1967; Brittain & Eikland 1988).

4.4.2 Combined effects on drift

4.4.2.1 CYP x PART

Due to their high K_{oc} and low water solubility, pyrethroids often enter surface waters via runoff attached to sediment (Muir *et al.* 1985) and thus potentially pose a risk towards natural macroinvertebrate communities. The CYP x PART trials resulted in an increase in drift (Fig. 4.2) thus supporting results from similar microcosm experiments (Schulz & Liess 2001a; Hose *et al.* 2002). The significant interaction of cypermethrin and sediment (Table 4.3) however led to a drift rate that was considerably lower than in the

CYP treatment and measured rates were significantly lower than the expected rate (Fig. 4.4).

This is most likely as a result of the pesticide binding strongly to sediment particles, thereby reducing its bioavailability (Brittain & Eikland 1988; Hill 1989). The degree of sorption of pesticides to sediment is however strongly related to the physicochemical properties of the pesticide and other pesticides with higher water solubilities, such as the organophosphate azinphos-methyl, are less likely to sorb to sediment particles (Dabrowski *et al.* 2002) and may thus be more bioavailable. As in the case of the PART trials, factors such as increased habitat availability, perceived food source and light interactions may also have influenced the amount of drift in the CYP x PART trials.

4.4.2.2 CYP x FLOW

The combination of cypermethrin and increased flow speed resulted in a significant effect on drift density (Table 4.3). The increased drift density in the CYP x FLOW treatments was however comparatively lower than that observed in the CYP treatments (Fig. 4.2). This suggests that the increased current velocity has an antagonistic effect on the pesticide exposure and is supported by the fact that the drift density in the CYP x FLOW treatments was lower than expected (Fig. 4.4). The influence of current velocity on the toxicity of pollutants has rarely been studied.

A comparison of the toxicity of contaminants in flow-through and static systems showed that macroinvertebrates reacted more sensitively in the flow-through systems (Crossland *et al.* 1992), thus indicating that the presence of flow may increase the toxicity of pollutants. Lowell *et al.* (1995) however compared the toxicity of NaCl to *Baetis tricaudatus* in three different current velocities, 0, 0.06 and 0.12 m/s, and found that the absence of flow in combination with NaCl exposure significantly decreased the median effective concentration (EC_{50}) of the species. They therefore concluded that at least some flow was required in toxicity testing so as to obtain realistic toxicological effects. They found no difference however, in the EC_{50} or lowest observed effect concentration (LOEC) between the 0.06 and 0.12 m/s trials and suggested a velocity threshold between 0 and

0.06 m/s above which the effect of current velocity on toxicity was no longer measurable. Although the current velocities in this present study (0.07 and 0.2 m/s for normal and high flow trials, respectively) were comparable to those used in the Lowell *et al.* (1995) study, both the CYP and CYP x FLOW trials resulted in an increased drift rate. The drift in the CYP x FLOW (0.2 m/s) treatment was however far lower than that measured in the CYP (0.07 m/s) treatment, which supports the findings of Lowell *et al.* (1995) and suggests that an increased flow rate may reduce the toxic effect of contaminants.

Insufficient oxygen delivery to the gills was cited as the most likely explanation for the significant effect of the NaCl exposure on the EC_{50} of *B. tricaudatus* during the 0 m/s trials in the study by Lowell *et al.* (1995). It is possible that the presence of cypermethrin may place the mayflies under respiratory stress, necessitating improved oxygen exchange. Although oxygen levels in the CYP experiments were identical to those in the CYP x FLOW experiments (Table 4.2), it is possible that the rate of delivery of oxygen to the gills was higher in the high flow experiment, rendering the mayflies less susceptible to the transient increase in pesticide concentrations.

This argument is further justified when taking the activity into consideration. The activity of the mayflies in the CYP experiment was higher than during the CYP x FLOW experiment, suggesting that the mayflies may have been actively seeking a position to expose themselves to faster current velocities and more efficient oxygen exchange on top of the stones, which could have made them more susceptible to drift. Wiley & Kohler (1980) showed that mayflies increase their activity by crawling to the top of stones when exposed to low oxygen concentrations.

4.4.3 Active/passive drift behaviour

Multiple factors can influence drift, ranging from environmental conditions to species-specific attributes such as behaviour, morphology and mode of locomotion (Poff *et al.* 1991; Breneman & Pontasch 1992) or activity and exposure to current velocity (Elliott 1967). Studies have investigated whether the entry of drifting individuals into the water is passive due to physiological impairment or an active purposeful form of behaviour (Allan *et al.* 1986).

Baetids are good swimmers and have often been reported to actively enter and remain in the drift under conditions of low velocities (Corkum 1978). Poff *et al.* (1991) hypothesised that mayfly nymphs can actively control their entry into the drift as a result of predator avoidance. Under high velocity conditions with risk of erosion passive entry into the water column has been observed by Elliot (1967). Schulz & Dabrowski (2001) reported that the active movement of mayflies to the upper surface of the rocks is a prerequisite for entering the drift. This positive relationship between activity on stone top surfaces and drift is the underlying basis of the active drift model (Allan *et al.* 1986). It should be noted however that both concordance (Schulz & Dabrowski 2001) and nonconcordance (Allan *et al.* 1986) between drift and positioning on the substrate surface have been reported.

In the present study, in terms of pesticide exposure, nonconcordance behaviour was observed. Assuming that increased activity is a preliminary stage of active drift suggests that the low activity in combination with high drift, observed during both the CYP and the CYP x FLOW experiment represents a passive drift process. Furthermore, the fact that the exposure concentration was two orders of magnitude higher than the LC_{50} for *Baetis rhodani* (USEPA 2000) suggests that the mayflies were most likely to have been physiologically affected by the pesticide. In terms of current velocity however, the results suggest concordance with the active drift model. The fact that increased current velocity has been shown to result in passive drift due to accidental dislodgement of mayflies from the substrate (Williams 1990; Kidd & James 1991) indicates that the increased current velocity in the FLOW treatment was not high enough to cause hydraulic stress, which is justified by the fact that both activity and drift density were significantly reduced during this treatment. Therefore the higher drift in the control treatment must have been an active process. Thus it is possible that mayflies may exhibit both active and passive drift, depending on the nature of the stressor to which they are exposed.

4.4.4 Comparison of runoff and spray-drift

Previous studies have compared the toxicological effects of runoff and spray-drift related pesticide exposure by exposing test organisms to aqueous dissolved (spray-drift) and particle associated (runoff) pesticide concentrations, without considering increased current velocity in the runoff experiments. The results of this study however, still support those observed in previous studies, and show a reduced response in trials simulating runoff related pesticide exposure (CYP x PART x FLOW). A major contributor to the decreased drift reaction is the reduced bioavailability of the pesticide as a result of its adsorption to sediment and the antagonistic effect of increased flow velocity on the effectiveness of the pesticide (Fig. 4.4). Accordingly the drift density in the CYP x PART x FLOW trials was not higher than that measured in the control trials. In contrast the spray-drift exposure (CYP) resulted in a large significant increase in drift rate. The results thus suggest that in terms of immediate short-term effects, spray-drift poses more of a serious ecotoxicological threat to aquatic macroinvertebrates than runoff related pesticide exposure.

It should be noted however, that in the case of a more water-soluble pesticide such as azinphos-methyl, the antagonistic effect of the sediment might not be as pronounced resulting in more of the pesticide being available. The relative risk of spray-drift versus runoff may thus be highly dependent on the water solubility or the K_{OC} of the chemical. Furthermore in comparison to spray-drift, runoff has resulted in higher concentrations of dissolved pesticide at the catchment scale (Dabrowski & Schulz 2003), which may result in runoff posing more of a significant ecotoxicological risk.

4.5 CONCLUSION

Low, field relevant cypermethrin concentrations (1 μg/L) have the potential to cause large increases in macroinvertebrate drift after a short period of exposure (30 mins). Suspended sediment and increased flow speed both had an antagonistic effect on the toxicity of cypermethrin, suggesting that typical runoff exposures (high flow and increased sediment input) pose less of a risk towards macroinvertebrates than those associated with spray-drift (low flow and aqueous-dissolved pesticide concentrations).

The antagonistic effect of sediment may however not be as pronounced for a more water soluble pesticide, possibly resulting in more of the pesticide being available during runoff conditions.

CHAPTER 5

MITIGATION OF AZINPHOS-METHYL IN A VEGETATED AND A NON-VEGETATED STREAM: COMPARISON OF RUNOFF AND SPRAY-DRIFT

5.1 INTRODUCTION

Amidst a global movement towards sustainable development, increased attention has focused on developing solutions to reduce nonpoint-source pesticide pollution in surface waters. In this respect, aquatic macrophytes have been shown to play an essential role in reducing water-dissolved pesticide concentrations through physical adsorption (Karen *et al.* 1998; Erstfeld 1999; Hand *et al.* 2001). As a result there is a growing interest amongst regulators, the agrochemical industry and researchers in characterizing the potential of different water bodies to mitigate against pesticide exposure through the presence or absence of aquatic macrophytes (Adriaanse 1997; Mackay *et al.* 2002).

Field studies have shown that vegetated drainage ditches (Moore *et al.* 2001) and constructed wetlands (Schulz & Peall 2001) are highly effective in reducing concentration, loads and toxicity of non-point-derived pesticides and have been able to correlate decreased water dissolved pesticide concentrations with increased plant associated concentrations of pesticide (Schulz *et al.* 2003a). As a result, vegetated agricultural water bodies have been proposed as efficient buffer zones for the protection of more sensitive receiving waters (Moore *et al.* 2000; Borin *et al.* 2001).

Runoff and spray-drift are important sources of nonpoint-source pesticide pollution of surface waters (Groenendijk *et al.* 1994; Dabrowski & Schulz 2003). Vegetated ditches (Brock *et al.* 1992a; Moore *et al.* 2001) and wetlands (Schulz & Peall 2001; Moore *et al.* 2002) have been shown to be effective in mitigating against both of these inputs. Studies have however been performed as isolated approaches and no direct comparisons have been made of the effectiveness of aquatic macrophytes in mitigating against runoff- and spray-drift-related pesticide contamination in a single type of water body.

Such comparisons are important, both for regulatory and management decisions. In the Western Cape of South Africa, application of pesticides in fruit orchards takes place during the hot, dry summer months, resulting in spray-drift related pesticide input into tributaries associated with low flow conditions (Schulz *et al.* 2001b). Occasional heavy rainfall events occur during this period and result in runoff-induced pesticide inputs associated with short-term increases in discharge (Schulz 2001a). Variation in discharge levels may have a significant impact in terms of the mitigation potential of a vegetated water body, particularly those such as small streams or ditches that are subjected to significant changes in flow speed and water depth during heavy rainfall events. It is thus possible that under high-flow runoff conditions the potential of a vegetated water body to mitigate against pesticide transport may be less effective than during low-flow spray-drift conditions. Additionally, the intensity of a rainfall event and the subsequent relative changes in hydrology may also influence mitigation potential.

Accordingly the fate of runoff- and spray-drift-related pesticide input was studied in a vegetated and non-vegetated tributary of the Lourens River, Western Cape, South Africa so as to test two hypotheses. The first hypothesis is that a vegetated stream is more effective in mitigating against both runoff- and spray-drift-related pesticide input than a non-vegetated stream. The second hypothesis is that the effectiveness of reduction of aqueous-dissolved pesticide concentrations is dependent on prevailing flow conditions, with high flow conditions resulting in less effective reduction of aqueous-dissolved pesticide concentrations. Consequently, pesticide concentrations associated with larger runoff events and higher discharge and flow levels will be less effectively mitigated than spray-drift events and smaller runoff events.

5.2 MATERIALS & METHODS

5.2.1 Study Area

The streams investigated in this study are tributaries of the Lourens River catchment in the Western Cape, South Africa. About 87% of the river's 35×10^6 m^3 mean annual discharge occurs during the winter months between April and October (Tharme *et al.* 1997), as is characteristic of the region's Mediterranean climate. Heavy rainfall events do however occur at frequencies of 3.4 and 1.7 per spraying season (October to February) for events > 10 and > 15 mm/d respectively (Schulz 2001b).

The streams were selected, as they were similar in all morphological and hydrological features, apart from macrophyte coverage. Both streams had similar average discharges. The non-vegetated stream was faster flowing and had a shallower average depth and narrower average width. The vegetated stream had a coverage of emergent macrophyte of almost 80 % (*Juncus capensis* – 47 %, *Fuirena hirsuta* – 39 % and *Pycreus* sp. – 14 %) which resulted in the formation of a central unvegetated channel (0.56 ± 0.04; n = 19) flanked by wide vegetated zones either side of the channel (left zone, 1.15 m ± 0.12; n = 19; right zone, 0.98 m ± 0.07; n = 19) (Fig. 5.1). All species occur throughout the Western Cape and are common in damp lowland systems (Goldblatt & Manning 2000). *J. capensis* has a relatively broader, filiform leaf shape, while *F. hirsuta* as well as the *Pycreus* species have comparatively narrower, pubescent leaves and stems. Estimated densities were 125, 170 and 151 ramets/m^2 for *J.capensis*, *F.hirsuta* and *Pycreus* sp. respectively.

Both streams are in close proximity to adjacent pear orchards (5 m) and previous studies have shown that the streams regularly receive runoff- and spray-drift-related pesticide input under normal farming practice (Schulz 2001b; Dabrowski *et al.* 2002). Insecticides regularly applied to pear orchards, include azinphos-methyl, phosmet, chlorpyrifos, endosulfan, cypermethrin and fenvalerate (Rijk Louw, Imbila Orchards, Somerset West, pers. comm.). Azinphos-methyl (AZP) is the most commonly applied insecticide and is used on pears between October and February at up to one application every two weeks on each plot (Schulz 2001b). It has a relatively low K_{OC} of 1000 L/kg and a high water solubility of 29 mg/L at 25 C° (Hornsby *et al.* 1995). Its frequent use and relatively high water solubility thus make it suitable for comparison of runoff- and spray-drift-associated pesticide concentrations. Thus, all experiments were based on measurement of AZP concentrations. During each application AZP was applied at 0.15 kg a.i./ha on orchards bordering the vegetated stream and at 0.525 kg a.i./ha on orchards bordering the non-vegetated stream.

5.2.2 Experimental Design

Five sampling sites (Fig. 5.1) were selected in the vegetated stream and were positioned at 5, 25, 45, 90 and 180 m downstream from the point of runoff and spray-drift input. The runoff entry point was a deep erosion rill (width 0.2 m, slope 45°) leading from an adjacent pear orchard (11.35 ha in area) directly into the stream. Additional runoff input along the 180 m stretch of stream was prevented by a berm (width 0.5 m, height 0.4 m) running along both sides of the stream for the entire 180 m downstream section.

Three sites were selected at 5, 45 and 180 m downstream from the point of input in the non-vegetated stream. Additional runoff input along the 180 m stretch of stream was again prevented by the presence of a berm (width 0.5 m, maximum height, 0.3 m) on the right bank and the absence of orchards (and thus of pesticide application) on the left bank.

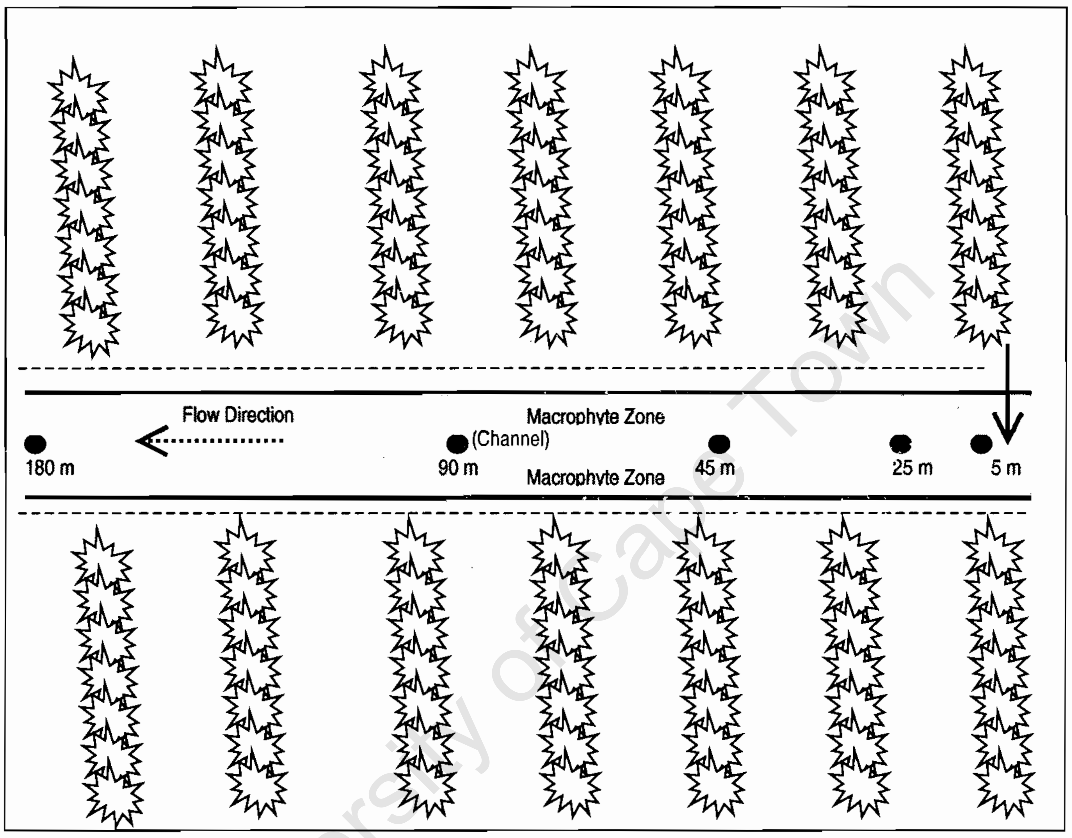

Fig. 5.1: Diagram (not to scale) of the position of sampling sites (black dots) at 5, 25, 45, 90 and 180 m after the point of runoff and spray-drift input (bold black arrow) in a vegetated stream

5.2.3 Runoff Sampling

Water samples (n = 4) (Schulz *et al.* 2001a) and suspended sediment samples (n = 1) (Liess *et al.* 1996) were collected at each site within each stream during two heavy rainfall events; a 10 mm (Runoff #1) and a 22 mm (Runoff #2) event (Table 5.1). Plant and sediment samples were taken at 5, 45 and 180 m in the vegetated stream before the 10 mm runoff event (so as to determine background pesticide levels) and immediately after both runoff events. Plant sampling was restricted to *Juncus capensis* as this was the dominant species in the stream. Only the submerged section of the plant was sampled (from sediment surface to water surface). Samples were immediately wrapped in foil and placed on ice until they were placed in a freezer pending analysis. Sediment samples were collected from the top 5 cm of the streambed using sterilized stainless steel scoops. Turbidity was measured at each site in both streams during both runoff events using a turbidity meter (Dr. Lange, Duesseldorf, Germany).

5.2.4 Spray-drift Sampling

One spray-drift event was monitored in each of the streams. By arrangement with the farmer, in each case only one row of trees was sprayed, which ensured that no additional pesticide input took place during the sampling programme. Immediately after deposition, water samples (n = 4) were taken at a position approximately 2 m from the point of deposition, so as to determine an average peak deposition concentration. Samples were then taken at a position 180 m downstream (n = 6) from the point of deposition so as to determine peak concentrations and the reduction in pesticide transport in each stream.

A more detailed sampling design was applied in the vegetated stream so as to gain accurate insight into the fate of AZP in the stream and to make a comparison with runoff-related pesticide transport in the same stream. Accordingly, one spray-drift event was monitored during an application of AZP on adjacent pear orchards (Table 5.1). Application was arranged with the farmer such that only one row of trees (approx 10 m upstream from the 5 m sampling point) was sprayed, ensuring that no additional input of pesticide would take place during the sampling programme. The remainder of the rows were sprayed the following day. Based on the discharge of the stream, the sampling programme was coordinated so that peak concentrations of AZP could be detected at each site. Samples were collected in pre-washed acetone and distilled water rinsed 700 ml glass jars by dipping closed sampling jars into the water column and opening the jars approx. 10 cm below the water surface to avoid contamination with surface film (Schulz *et al.* 2001b).

Table 5.1: Timing of runoff and spray-drift events in relation to the application of azinphos-methyl to pear orchards bordering a vegetated stream in the Lourens River catchment, South Africa.

Event	Date	No. of days after most recent application (days)	Rainfall (mm)	Minimum application distance from stream (m)
Runoff #1	18.10.2002	3	10	5
Runoff #2	17.12.2002	1	22	5
Spray-drift	26.11.2002	0	0	5

5.2.5 Pesticide Extraction and Analysis

Plant, water and sediment samples were analysed as documented in (Bennett *et al.* 2000) and (Dabrowski *et al.* 2002). Measurements were done using gas chromatography (HP 5890's) fitted with standard HP electron-capture, nitrogen- phosphorus, and flame-photometric detectors. Concentrations for sediments and plants were expressed as micrograms per kilograms dry weight (μg/kg dw). Identity of AZP was confirmed by matching retention times on three different stationary phases. Method validation employed water matrixes that were found to have no detectable levels of the investigated pesticides and consisted of spiking water at eight spiking levels over the range of concentrations found in the actual samples. Overall mean recoveries were between 79 and 106%. For quality control, a matrix blank was analysed with each extraction set. The investigated pesticides were never detected in matrix blanks.

5.2.6 Prediction of worst-case scenarios

For those events where pesticide concentrations were reduced, worst-case scenario predictions of pesticide concentrations were made so as to determine the maximum concentrations expected for those events. This could provide an indication of the relative efficiency of the macrophytes in reducing runoff- and spray-drift-related AZP concentrations, in terms of the magnitude of the concentrations present during each of the different events. This was accomplished by using basic drift values for spray-drift predictions (Ganzelmeier *et al.* 1999) and a runoff formula by (Reus *et al.* 1999). Both formulae have previously been applied and successfully validated in the Lourens River catchment (Dabrowski & Schulz 2003) and in other catchments (Verro *et al.* 2002). The formulae were first validated by incorporating real-time input variables as measured during the sampling events and comparing the resulting predicted concentrations to measured concentrations. Worst-case scenarios were predicted by adjusting the input variable for the number of days since the last pesticide application for runoff, and the distance of a water body from the point of application for spray-drift. The runoff formula is:

$$L\%_{\mathrm{runoff}} = \frac{Q}{P} \cdot f \cdot e^{-t \cdot \frac{\ln 2}{DT_{50soil}}} \cdot \frac{100}{1+K_{\mathrm{d}}}$$

where; $L\%_{runoff}$ = percentage of application dose being available in run-off water as a dissolved substance; Q = runoff amount (mm) calculated according to hydrological models (Lutz 1984; Maniak 1992); P = precipitation amount (mm); DT_{50soil} = half-life of active ingredient in soil (10 d for AZP) (Hornsby *et al.* 1995); f = f1 x f2 x f3; correction factor reflecting the influence of slope (f1 = 0.02153 x slope + 0.001423 x slope^2), plant interception (PI), the percentage of applied pesticide intercepted by trees in the orchards (f2 = 1-PI/100) and buffer width (f3 = 0.83^{WBZ} and WBZ is the width of buffer zone (meters); if the buffer zone is not densely covered with plants, the width is set to zero); t = time between application and rainfall in days; K_{d} = (K_{OC} x OC); factor reflecting the tendency of the pesticide to bind to organic carbon in the soil, where K_{OC} is the sorption coefficient of the active ingredient to organic carbon (1000 L/kg for AZP) and OC% is the organic carbon content of the soil (0.75%, F. Ellis, Department of Soil Science, University of Stellenbosch, South Africa, personal communication).

5.2.7 Data Analysis

Reductions in peak and average concentrations of pesticide in the vegetated and non-vegetated streams during the spray-drift and two runoff and events were compared using an ANOVA and Fischers PSLD analysis. Values for spray-drift reduction were determined by comparing the difference between the average measured deposition concentration and the peak concentration measured at the sampling site 180 m downstream.

The relative efficiency of the vegetated stream in reducing runoff and spray-drift related AZP concentrations was compared by performing regression analyses and plotting peak and average concentrations detected at each site against the distance from the point of input. Formulas generated by the analyses were used to predict pesticide concentrations at various distances within the stream.

5.3 RESULTS

5.3.1 Runoff trials

Both runoff events resulted in increased discharge, flow and water depth in both the vegetated and the non-vegetated streams (Table 5.2). All water-dissolved concentrations of AZP measured at each site in the vegetated stream were significantly higher during the 22 mm runoff event than the 10 mm runoff event ($p = 0.006$, $n = 20$). Average concentrations of AZP were significantly reduced (by 61%; $p = 0.05$, $n = 3$) from the 5 m to 180 m sampling point in the vegetated stream for the 10 mm event, however no consistent reduction was apparent in the vegetated stream during the 22 mm event (Fig. 5.2 & Table 5.3). No reduction in pesticide concentrations took place during either of the events in the non-vegetated stream. No obvious decrease in particle-associated AZP concentrations took place in either of the streams during either runoff events (Table 5.3). A slight decline in turbidity occurred in the vegetated stream during the 10 mm runoff event

5.3.2 Spray-drift trials

Discharge, flow and depth in both streams were comparable to normal background levels measured during the spraying season (Table 5.2). A significant reduction in AZP concentrations was observed from top to bottom sites in the vegetated stream ($p = 0.02$, $n = 6$) after spray-drift input (Table 5.4). Reduction in pesticide concentrations also took place in the non-vegetated stream, although changes were not significant.

Table 5.2: Hydrological measurements in a vegetated and non-vegetated stream in the Lourens River catchment, South Africa. Measurements during a 10 mm (Runoff #1) and a 22 mm (Runoff #2) rainfall event and a spray-drift event are compared to average measurements taken November, December 2002 and January 2003.

		Discharge (m^3/s)	Current Velocity (m/s)	Water Depth (m)	Width (m)
Vegetated	Average (± s.e.; n = 5)	0.04 ± 0.01	0.08 ± 0.01	0.28 ± 0.05	2.4 ± 0.2
	Runoff #1	0.06	0.096	0.33	2.7
	Runoff #2	0.1	1.23	0.41	2.7
	Spray-drift	0.031	0.073	0.2	1.7
Non-vegetated	Average (± s.e.; n = 5)	0.04 ± 0.004	0.15 ± 0.02	0.17 ± 0.03	1.4 ± 0.06
	Runoff #1	0.045	0.2	0.16	1.4
	Runoff #2	0.052	0.32	0.2	1.7
	Spray-drift	0.035	0.16	0.1	1.3

5.3.3 Comparison of runoff and spray-drift in a vegetated stream

Discharge, depth and water velocity were higher during both runoff events in comparison to those measured during spray-drift event, with the highest values being observed during the 22 mm event (Table 5.2). A significant logarithmic ($R^2 = 0.99$; $p < 0.0001$, $n = 5$) decline in peak AZP concentrations was observed for the 10 mm runoff event, while a significant linear decline ($R^2 = 0.93$; $p = 0.0084$, $n = 5$) was observed for the spray-drift event (Fig. 5.2). Peak concentrations were equally reduced (61% reduction from the 5m to the 180 m sampling point) during both events. Average concentrations

Table 5.3: Total suspended sediment and average water (± standard error) and sediment concentrations of azinphos-methyl (AZP) measured in a vegetated and a non-vegetated stream in the Lourens River catchment South Africa, during a 10 mm (Runoff #1, $n = 3$) and a 22 m (Runoff #2, $n = 4$) rainfall event.

	AZP – Water (μg/L)			AZP – Suspended Sediment (μg/kg)			Total Suspended Solids (mg/L)		
	5 m	45 m	180 m	5 m	45 m	180 m	5 m	45 m	180 m
Vegetated									
Runoff # 1	0.2 ± 0.06	0.1 ± 0.02	0.07 ± 0.01*	N.D.	18.9	13.1	180	168	154
Runoff # 2	1.7 ± 0.52	1.7 ± 0.6	1.2 ± 0.77	86.4	158	148	190	189	190
Non-vegetated									
Runoff # 1	0.05 ± 0.01	0.05 ± 0.01	0.4 ± 0.5	N.D.	N.D.	N.D.	60	71	130
Runoff # 2	0.2 ± 0.1	0.4 ± 0.23	0.4 ± 0.1	N.D.	1.8	252	141	144	145

*Significant reduction in comparison to concentrations measured at the 5 m sampling point ($p < 0.05$); N.D. = not detected

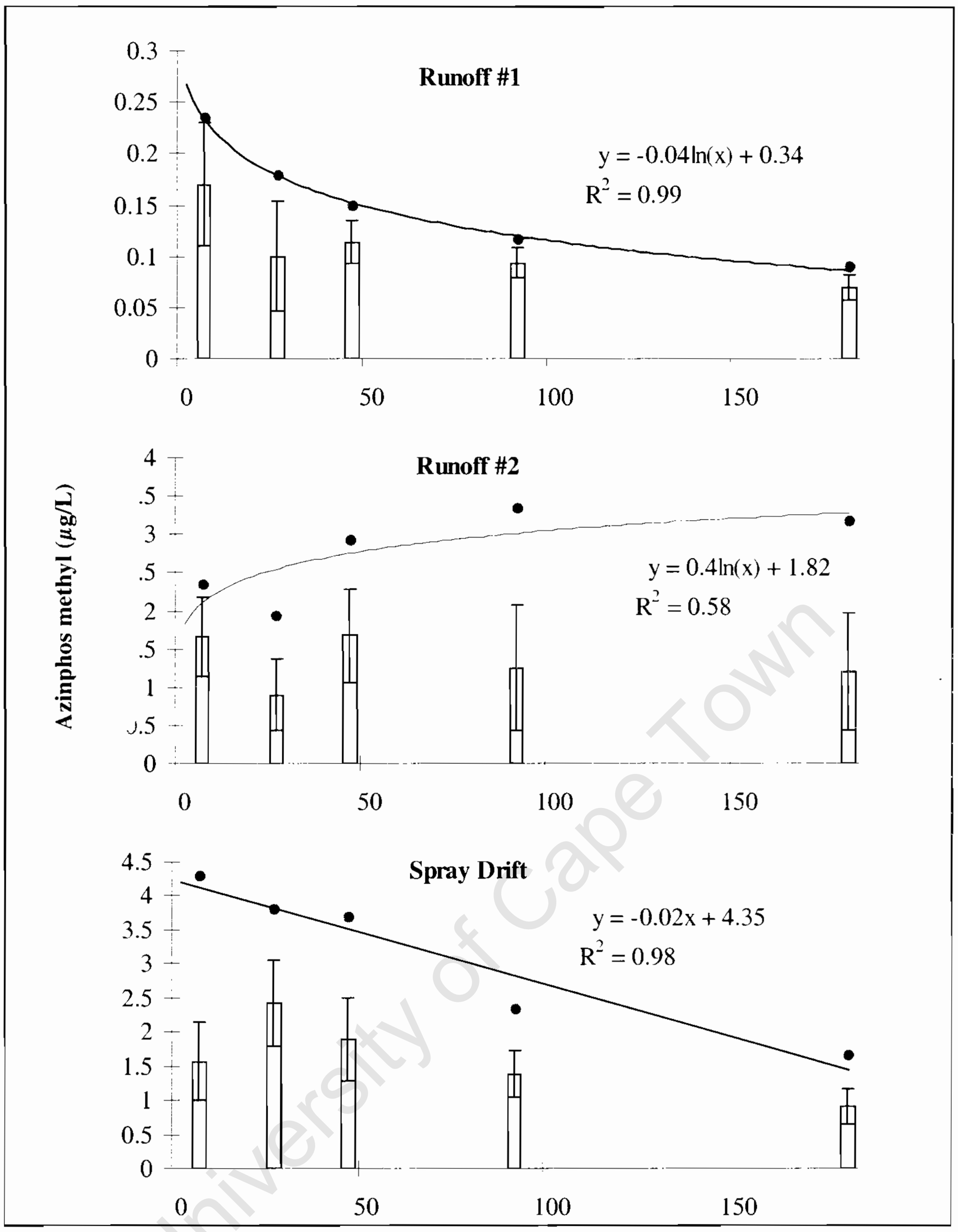

Fig. 5.2: Peak (dots) and average (bars; ± standard error; n = 4) concentrations of azinphos-methyl (AZP) plotted against distance from point of input for a 10 mm (Runoff #1) and a 22 mm (Runoff #2) rainfall event and a spray-drift event in a vegetated stream in the Lourens River catchment, South Africa. Lines indicate best fitting logarithmic or linear regression relationships between observed peak AZP concentrations and distance downstream from the point of input.

Table 5.4: Concentrations of azinphos-methyl (AZP) (± standard error) measured immediately after deposition of spray-drift (5 m below the point of deposition; $n = 4$) and at 180 m ($n = 6$) below the point of deposition in a vegetated and a non-vegetated stream in the Lourens River catchment, South Africa.

	Vegetated (μg/L)	Non-vegetated (μg/L)
5 m	2.4 ± 1.3	1.4 ± 0.35
180 m	0.23 ± 0.02	0.66 ± 0.33
% Reduction	90.3	52.1

also showed a general step-wise decline in AZP concentration over distance for both events. No significant trend in average or peak AZP concentrations was observed during the 22 mm runoff event. Concentrations of AZP were significantly higher during the spray-drift event than in the 10 mm runoff event ($p < 0.0001$, $n = 68$), whilst no significant differences in AZP concentrations were observed between the spray-drift event and the 22 mm runoff event. Concentrations of plant-associated AZP measured 90 minutes after the beginning of the spray-drift event increased in comparison to pre-spraying levels and showed a consistent decline with increasing distance (from 5 to 45, to 180 m) from the point of deposition (Table 5.5). No increase in plant-associated AZP was detected after the 10 mm runoff event and no step-wise decline in AZP concentrations along a longitudinal gradient was observed after either runoff event. The highest concentrations of plant associated AZP for all three pesticide input events were measured at the 5 m sampling point.

Analysis of sediment samples taken prior to the spray-drift event showed no detectable levels of AZP at the 5 m and 90 m sampling points and 218 μg/kg AZP at the 180 m point. Samples taken after spraying contained 212 μg/kg AZP at the 90 m sampling point, while no change in AZP levels were detected at the 5 m (no detectable levels) and 180 m (202 μg/kg) sampling points.

Table 5.5: Concentrations of azinphos-methyl (AZP) associated with an aquatic macrophyte (*Juncus capensis*) from a vegetated stream (Lourens River catchment, South Africa) before and after a 10 mm (Runoff #1) and 22 mm (Runoff #2) runoff event and a spray-drift event.

	Event	Date	AZP (µg/kg dw)		
			5m	45m	180m
Runoff	Pre-Runoff #1	17.10.2002	40.3	37.5	82.4
	Runoff #1	18.10.2002	47.4	22.6	41.9
	Runoff #2	17.12.2002	132.3	20.7	91.9
Spray-drift	T - 10 mins	26.11.2002	52.4	40.6	40.3
	T + 90 mins	26.11.2002	145.2	119.3	62.6

5.3.4 Prediction of worst-case runoff and spray-drift scenario

Only the 10 mm runoff event and the spray-drift event resulted in decreased AZP concentrations along a longitudinal gradient. Thus predictions were only made for these two events. The concentration measured during the 10 mm runoff event, which took place 3 days after the most recent application, compared more favourably to the 0 day predicted concentration (worst-case scenario), but was only a factor of 1.2 higher than the predicted concentration for 3 days after application (Table 5.6).

The measured spray-drift concentration was slightly higher (by a factor of 1.14) than the predicted concentration of 3.75 µg/l and was representative of a worst-case scenario event (assuming a minimum buffer strip width of 5 m). According to predicted values, drift deposition resulting from a worst-case scenario 5 m buffer strip width would result in a peak concentration a factor of 6.7 higher than for a 20 m buffer strip width. Worst-case scenario predictions were an order of magnitude higher for spray-drift than for a 10 mm runoff event.

Table 5.6: Table showing the effect of timing of event after most recent application (for a 10 mm rainfall event) and the distance from the point of application (for a spray-drift event) on the predicted peak concentrations of azinphos-methyl (AZP) in a vegetated stream in the Lourens River catchment, South Africa.

	Measured	Predicted			
Runoff (10 mm)					
No. of days after application	3	0	1	2	3
Peak AZP conc. (μg/l)	0.235	0.238	0.223	0.207	0.194
Spray-drift					
Distance (m) from point of application	5	5	10	15	20
Peak AZP conc. (μg/l)	4.28	3.75	1.69	0.94	0.56

5.4 DISCUSSION

5.4.1 Vegetated vs. Non-vegetated stream

The results clearly indicate that, in comparison to a non-vegetated stream, a vegetated stream is more effective in reducing both runoff and spray-drift associated AZP concentrations (Table 5.3, Fig. 5.2).

A few studies have illustrated the effectiveness of vegetated water bodies in reducing runoff induced aqueous dissolved pesticide concentrations along a longitudinal gradient (Moore *et al.* 2000; Moore *et al.* 2001; Moore *et al.* 2002; Schulz *et al.* 2003b), however, these studies have been performed under simulated conditions where pesticides have been experimentally added to the water body. This is the first study that has measured a reduction in pesticides during real-time runoff conditions and thus incorporated the transient increase in discharge and flow levels into the experimental design.

Although a reduction in spray-drift induced AZP concentrations was measured in both the vegetated and the non-vegetated stream, the vegetated stream was almost twice as effective (Table 5.4). Reduction in spray-drift-derived pesticide concentrations as a result of sorption to aquatic macrophytes have been reported in constructed wetlands and ditches (Brock *et al.* 1992a; Schulz *et al.* 2003a). Potential factors resulting in decreased AZP concentrations in the non-vegetated stream are adsorption to sediment (Brock *et al.* 1992a) and degradation as a result of volatilisation, hydro- and phytolysis (Rodgers Jr. & Dunn 1992, Maguire, 1992).

A comparison of plant associated AZP concentrations before and after the intensive spray-drift study show an increase in levels at each sampling site after spraying, which consistently decrease from the 5 m sampling point to the 180 m sampling point in correlation to decreased average and peak (from 4.28 to 1.66 μg/L) aqueous dissolved AZP concentrations (Table 5.5 & Fig. 5.2), clearly indicating that the AZP sorbs to aquatic macrophytes. Sorption of the organophosphate, chlorpyrifos to *Juncus effesus* (Lytle & Lytle 2002) and *Elodea densa* (Karen *et al.* 1998) in mesocosms and AZP to *Typha capensis* (Schulz *et al.* 2003a) in a constructed wetland have been previously documented following spray-drift trials. The consistent decrease in water dissolved AZP concentrations along the length of the stream during the 10 mm runoff event was not reflected by increased plant associated AZP concentrations, with higher concentrations being measured in pre-runoff samples than in post-runoff samples (Table 5.5). The high pre-runoff and spray-drift levels associated with the plants are most likely as a result of spray deposition occurring during the previous application (3 and 10 days prior to the runoff and spray-drift event, respectively) and could thus account for the poor correlation between water dissolved and plant associated concentrations of AZP. Previous studies have reported an increase in plant associated concentrations of the organophosphate insecticide methyl parathion, atrazine and lambda-cyhalothrin (pyrethroid) corresponding to decreased water concentrations following simulated runoff events (Moore *et al.* 2001; Schulz *et al.* 2003a), which, along with the fact that the highest measured concentrations during both runoff events were immediately below the input point, suggests that the macrophytes played an important role in reducing pesticide concentrations during the 10 mm runoff event.

An increase in AZP concentrations in sediments taken from the 45 m sampling point in the vegetated stream suggests that adsorption to sediment may be a relatively important process during a spray-drift event. Previous studies have reported low levels of AZP in sediments following spray-drift in an agricultural stream (Schulz 2001b) and chemigation of cranberry bogs (Wan *et al.* 1995). Partitioning of pesticides between water, sediment and plant phases is however highly dependent on the physicochemical properties of individual pesticides. (Wan *et al.* 1995)

Most studies investigating the fate of runoff associated pesticides in ditches and wetlands have been performed under simulated conditions (i.e. pesticide is added manually) (Moore *et al.* 2000; Runes *et al.* 2001; Moore *et al.* 2002). Depending on the catchment size, streams drain water from a wide area during a runoff event, resulting in significant changes in stream hydrology, an aspect that may not be accurately represented under simulated conditions.

5.4.2 Effect of Flow Velocity

While AZP concentrations associated with both the 10 mm runoff event and the spray-drift event were effectively mitigated in the vegetated stream, no reduction took place during the 22 mm runoff event (Table 5.3, Fig. 5.2). A potential reason for the lack of reduction of pesticide concentrations during this event may be related to the elevated discharge levels during the event. The aquatic macrophytes in the vegetated stream decrease the velocity (Table 5.1) of the water flow, which presumably facilitates the sorption of AZP to the plants. Based on this assumption, increased discharge levels associated with heavy rainfall events may in turn diminish the effectiveness of the vegetation in reducing water dissolved pesticide concentrations. This seems to be the case in the 22 mm runoff event, where discharge, depth and flow velocity increased in comparison to the 10 mm event and the spray-drift event.

Most studies dealing with the impact of aquatic macrophytes on pesticide fate have either not reported on current velocity (Moore *et al.* 2000; Schulz & Peall 2001; Moore *et al.* 2002) or have been performed in static mesocosms (Hand *et al.* 2001; Lytle & Lytle 2002). Schulz *et al.* (2003b) and Moore *et al.* (2001) reported flow velocities of < 0.05 m/s and 100 % and 88 % reduction of methyl-parathion and lambda-cyhalothrin, and atrazine, respectively. Lower overall reductions were measured in this study (69 %), which again suggests that the reduction efficiency may decrease with increasing flow speed (0.07 and 0.1 m/s for the spray-drift and 10 mm runoff event, respectively). It must be noted however that the tendency of a pesticide to sorb to aquatic macrophytes will also be highly dependent on the physicochemical properties of the pesticides. High degrees of sorption to aquatic macrophytes have been reported for relatively insoluble pesticides, such as pyrethroids (Erstfeld 1999; Hand *et al.* 2001) and organophosphates such as chlorpyrifos (Brock *et al.* 1992a; Karen *et al.* 1998; Crum *et al.* 1999). AZP is a relatively water soluble pesticide (Hornsby *et al.* 1995), which could also possibly account for its lower retention in comparison to previous studies. A 90 % reduction in AZP concentrations was however measured in a flow through wetland in South Africa (Schulz *et al.* 2003a), which again suggests that flow velocity is an important factor influencing the sorption of pesticides to macrophytes.

A direct link between flow velocity and pesticide adsorption to plants has not yet been shown. Previous studies have indicated the loss of the pyrethroid, permethrin, from the water column during transport, with the greatest losses occurring during low discharge conditions (House *et al.* 2000), such as those measured during the 10 mm runoff and spray-drift events in this study. (Mitsch *et al.* 1995) showed that riparian wetlands were more effective in reducing phosphate concentrations during low flow conditions than during high flow conditions. Aquatic macrophytes have also been shown to promote sedimentation through decreasing flow velocities (Sand-Jensen 1998). Turbidity measurements consistently decreased along the length of the vegetated stream during the 10 mm runoff event (Table 5.3). No reduction in turbidity was measured during the 22 mm event however, suggesting that discharge levels and flow velocities were too high for effective sedimentation by the macrophytes. This provides further support to the hypothesis that pesticide adsorption to macrophytes may be heavily dependent on flow velocity and thus decrease under high flow conditions.

The influence of flow velocity on pesticide sorption to macrophytes suggests that different water bodies will vary in their effectiveness in pesticide mitigation, depending on their hydrological characteristics. Thus larger, slower flowing water bodies, such as wetlands may be more effective in mitigating pesticide transport than smaller faster flowing water bodies that are subjected to large changes in hydrological conditions during runoff events. In this respect, (Uusi-Kämppä *et al.* 2000) showed that vegetated constructed wetlands (shallower than 0.5 m) were more efficient than retention ponds (deeper than 0.5 m) in retention of nutrients. The potential of aquatic macrophytes to mitigate non-point pesticide pollution may also be dependent on the type of vegetation growing in the stream. The vegetated stream used in this study had well defined zones; a narrow central channel free of aquatic macrophytes, flanked on either side by wide zones of emergent aquatic macrophytes. Macrophytes that inhabit streams along the entire width of the stream (e.g. *Typha* sp.) will not lead to the formation of a channel and may be more effective in mitigating runoff associated pesticide levels under high flow conditions.

5.4.3 Comparison of runoff and spray-drift in a vegetated stream.

Apart from previously verified mitigation strategies, such as increased buffer zone width (De Snoo & De Wit 1998), vegetated buffer strips (Patty *et al.* 1997) and wind-breaks (Ucar & Hall 2001), vegetated ditches and wetlands have been proposed as effective mitigation tools for reducing both runoff and spray-drift-derived pesticide concentrations (Rodgers Jr. *et al.* 1999). Whilst the results from this present study support those from previous ones, new perspective is added on the issue, in that the effectiveness of reduction would appear to be dependent on prevailing discharge conditions during the nonpoint-source event.

Based on the premise that increased flow velocity reduces the effectiveness of aquatic macrophytes in adsorbing pesticides, it follows that runoff-related pesticide concentrations in combination with high discharge levels, are less likely to be effectively reduced by the presence of vegetation than spray-drift related pesticide concentrations. Despite this assumption, peak AZP concentrations during a 10 mm rainfall event associated with elevated discharge levels were reduced as effectively as spray-drift-related concentrations. The fact that spray-drift is most commonly not associated with increased discharge thus indicates that a consistent decrease in pesticide concentrations can be expected and will thus be generally more effectively reduced by plants than runoff-associated pesticide concentrations. This is further indicated by the magnitude of predicted worst-case concentrations (Table 5.6), which are an order of magnitude lower for a 10 mm runoff event than for a spray-drift event. PECs for the 10 mm runoff event and the spray-drift event corresponded well to measured concentrations, and both formulae have previously been successfully validated through field-based sampling in the

Lourens River catchment (Dabrowski & Schulz 2003). Based on a comparison of predicted peak AZP levels resulting from 10 mm runoff events 0 and 3 days after the most recent application (0.24 and 0.19 μg/l respectively), it is clear that, in comparison to a spray-drift event, low concentrations (< 0.5 μg/l) of AZP can be expected during a worst-case scenario 10 mm runoff event (a factor of 1.2 higher than what was measured) (0 days after application). In contrast, higher concentrations of AZP, comparable to those measured during the spray-drift event, can be expected only during larger runoff events, as in the case of the 22 mm runoff event (peak 3.4 μg/l), when no reduction is possible. Thus, in terms of runoff events, based on the fact that only concentrations associated with a 10 mm runoff event can be potentially reduced, the studied stream is only capable of reducing only relatively low pesticide concentrations, even during a worst case scenario event (0 days between application and event). In contrast, spray-drift events result in peak concentrations of pesticides comparable to those measured during the 22 mm runoff event. Due to the low flow conditions during the pesticide application period, the high concentrations can however be effectively reduced by vegetation in the stream. Thus, the comparison of maximum expected concentrations for each event suggest that the aquatic macrophytes are more effective in mitigating against pesticide concentrations resulting from spray-drift than those resulting from runoff. It must be noted, however, that under different geological conditions, such as loamy soils or increased slope, concentrations in the stream resulting from a 10 mm event might be higher than those predicted for the studied stream. Under such conditions, aquatic macrophytes could potentially reduce relatively high pesticide concentrations during a 10 mm event.

5.5 CONLCUSION

Vegetated streams are more effective in mitigating against runoff and spray-drift-induced pesticide concentrations than non-vegetated streams. As a result of increased flow velocity during heavy rainfall conditions the potential for vegetated streams to mitigate against high runoff-induced pesticide concentrations is however far lower than their ability to mitigate against high spray-drift-induced pesticide concentrations.

CHAPTER 6

INTERCEPTION OF AZINPHOS-METHYL BY EMERGENT AQUATIC MACROPHYTES: POTENTIAL TO MITIGATE SPRAY-DRIFT RELATED PESTICIDE INPUT IN SURFACE WATERS.

6.1 INTRODUCTION

Spray-drift is one of the most important sources of nonpoint-source pesticide pollution in edge-of-field surface waters, such as ditches, streams and ponds (Groenendijk *et al.* 1994). Pesticide concentrations associated with spray-drift are often high due to the direct nature of input of pesticides (Gilbert & Bell 1988; AEDG 1992) and thus pose significant risk for aquatic fauna. Amidst heightened awareness for sustainable development and increasingly stringent regulatory measures, increased emphasis has been placed on mitigatory measures in an attempt to reduce nonpoint-source pesticide pollution in agricultural surface waters (Streloke & Brown 2003). In particular, higher-tier risk assessments play an important role in characterizing the impact of mitigatory measures on the fate and toxicity of pesticides in surface waters (Mackay *et al.* 2002).

With respect to spray-drift, higher tier risk assessments have focused on the potential of submerged aquatic macrophytes in reducing drift-related pesticide exposure in surface waters. Aquatic macrophytes in ditches and wetlands have been shown to be effective in reducing spray-drift related concentrations of water-dissolved pesticides (Brock *et al.* 1992a; Schulz *et al.* 2003a) through providing large surface areas for adsorption of water dissolved pesticides (Hand *et al.* 2001), a factor which has recently been incorporated into fate models such as TOXSWA (Adriaanse 1997).

Foliar interception of pesticides by sprayed crops results in a decrease in deposition rate on the soil surface, the extent of which is dependent on factors such as the growth stage and physical characteristics of the crop. Interception factors for different crop types have been established and are currently used in EU and US risk assessment procedures (Linders *et al.* 2000, Pfleeger *et al.* 1996). Many aquatic macrophyte species found in agricultural ditches are emergent (e.g. *Juncus* spec., *Typha* sp.), with their leaves protruding above the water surface. In addition to adsorbing water-dissolved pesticide concentrations, emergent macrophytes may thus provide a mitigatory function in reducing spray-drift-related pesticide input in surface waters through foliar interception of spray droplets before they land on the water surface. This feature has been alluded to in a previous study (Schulz *et al.* 2001b), although no detailed study has investigated this potentially efficient mitigatory factor.

Azinphos-methyl (AZP) is commonly applied to pear orchards at approximately one application every two weeks from October to January (Dabrowski & Schulz 2003) in the Lourens River catchment in the Western Cape of South Africa. Numerous studies performed in the catchment have shown that the pesticide enters tributaries as a result of spray-drift (Schulz *et al.* 2001b; Dabrowski & Schulz 2003) at concentrations capable of eliciting ecotoxicological community responses (Schulz *et al.* 2002). Most of the tributaries of the river are essentially man-made drainage ditches, many of which are intensively vegetated with emergent aquatic macrophytes.

The main aim of this study was to determine the potential of emergent aquatic macrophytes in reducing spray-drift related AZP deposition in a heavily vegetated tributary bordering an intensively sprayed pear orchard. Measured AZP deposition rates were compared to predicted rates made using basic 90^{th} percentile drift values (Rautmann *et al.* 2001). Results were used to develop a basic predictive formula that was used to forecast deposition rates based on different macrophyte coverage percentages.

6.2 MATERIALS & METHODS

6.2.1 Study Site

The study took place in the Kleinvlei tributary of the Lourens River, which has a catchment area of $92km^2$ of which 400 ha are cultivated under fruit orchards (apples, pears and plums) and vineyards. The tributary is separated from an adjacent pear orchard by a 5-15 m buffer zone and is dominated by dense emergent aquatic vegetation, which covers almost 80% of the surface area of the stream (Table 6.1). The vegetation grows to form a distinct, narrow, unvegetated central channel, flanked on either side by dense, wide marginal aquatic emergent vegetation zones that grow from within the water body (left and right vegetated zone). The macropyhte community comprises three species, *Juncus capensis*, *Fuirena hirsuta* and *Pycreus* sp., of which *J. capensis* and *F. hirsuta* are the most dominant (Table 6.1). Both species occur throughout the Western Cape and are common in damp lowland systems (Goldblatt & Manning, 2000). *F. hirsuta* and

Table 6.1: Coverage and species compostion of emergent aquatic macrophytes (± standard error; *n* = 20) in a 200 m stretch of tributary of the Lourens River, South Africa

	Width (m)	Area (m^2)	Area (%)	Species Composition (%)		
				Juncus capensis	*Fuirena hirsuta*	*Pycreus* sp.
Unvegetated Channel	0.56 (± 0.04)	101	21.8	0	0	0
Veg. Left Zone	1.15 (± 0.12)	208	42.7	56 (± 8.2)	36.2 (± 9.8)	7.8 (± 9.8)
Veg. Right Zone	0.98 (± 0.07)	178	36.5	37.4 (± 6.9)	41.8 (± 8.9)	20.9 (± 6)
Total Veg. Coverage	-	386	79.2	37.5	31.1	11.3

Pycreus sp. has narrow, needle-like pubescent leaves and stems, while *J. capensis* has a comparatively broader filiform leaf shape (Table 6.2).

Table 6.2: Physical characteristics (± standard error; n = 15) of three emergent macrophyte species in a vegetated tributary of the Lourens River, South Africa.

Species	Density (ramets/m^2)	Leaf Area cm^2	Height cm
Juncus capensis	125	12.1 (± 2.4)	18.1 (± 1.2)
Fuirena hirsuta	170	6.3 (± 1.5)	23.6 (± 3.3)
Pycreus spec.	151	5.7 (± 2.0)	21.4 (± 4.1)

6.2.2 Experimental Design

Drift deposition was measured in the tributary during an application of AZP to the adjacent pear orchards on 13 November 2002. AZP was applied to the orchards by Jacto Airbus (Sao Paulo, Brazil) air-assisted mist blowers, which delivered AZP at a rate of 0.15 kg a.i./ha in 1000 L of water at a pressure of approximately 1200 kPa. The distance of the tributary from the edge of the treated area was 5 m. Spray deposition was measured by drift collectors (n = 6 for each species) that consisted of acetone- and distilled-water-rinsed flat straight-sided glass Petri-dishes (diameter: 15 cm; surface area: 0.02 m^2) containing distilled water. Drift deposition rates were measured in four treatment areas (Fig. 6.1): the surface of the vegetation canopy (S), where deposition was expected to be 100 %; in the mid-stream on the surface of the non-vegetated channel (M) so as to measure any possible shielding effect caused by adjacent emergent vegetation; and beneath the coverage of *J. capensis* (J.c) and *F. hirsuta* (F.h) so as to determine the degree of interception by the two plant species. Wooden beams laid across the stream supported dishes collecting drift deposition in the channel. Dishes placed beneath emergent vegetation (F.h. and J.c) were placed on the water surface and were small enough so as not to disturb the density and structure of the vegetation above the dishes. Surface vegetation (S) collectors were placed on top of the vegetation and the dense aggregation of the plants prevented the dish from falling through the canopy.

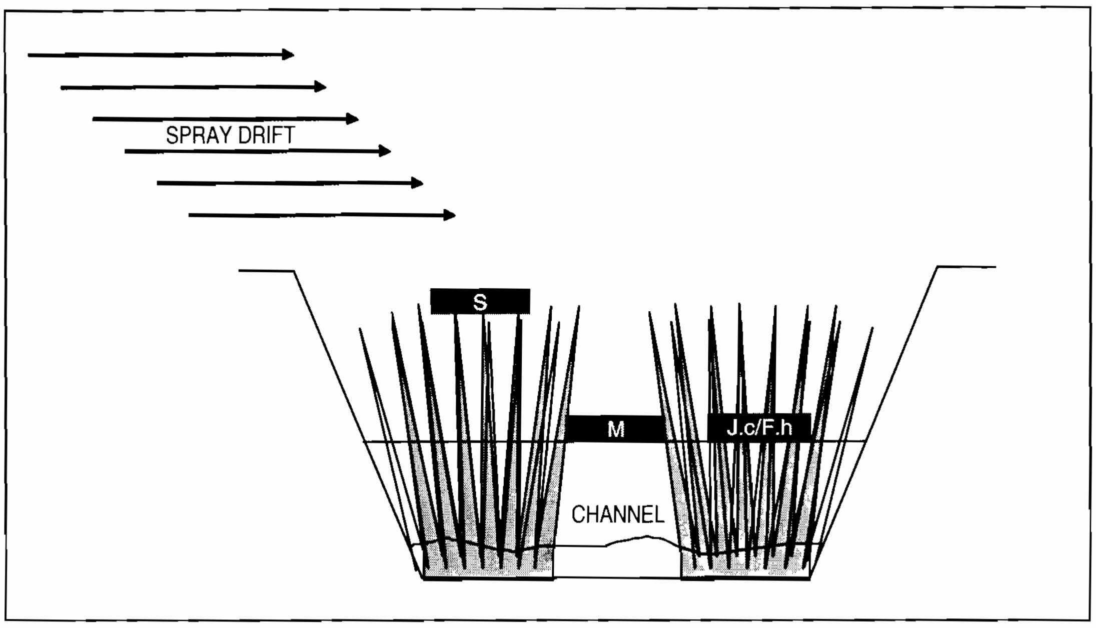

Fig. 6.1: Diagram (not to scale) of the placement of the drift collectors (black bars) deployed for the measurement of deposition of azinphos-methyl (AZP) in a vegetated tributary of the Lourens River, South Africa. At each sampling point deposition of AZP was measured on the surface of the vegetation canopy (S), on the surface of the unvegetated channel (M) and at the base of two emergent aquatic macrophytes; *Juncus capensis* (J.c.) and *Fuirena hirsuta* (F.h.).

Three sampling zones (SZ1 – SZ3, each 10 m long) were selected along the stretch of the stream (Fig. 6.2). Each zone accommodated two driving rows (gaps in between orchard rows along which the spraying tractors moved) along which pesticide was applied to adjacent tree rows. All four treatments (S, M, F.h and J.c) were sampled twice within each zone such that mid-stream samples were taken at six sites along the center of the stream in the exposed channel, and surface vegetation samples and samples beneath the two vegetation types were collected at three sites in the right and left zone respectively. Thus in total six replicate samples were collected for each treatment. As the orchard tree rows were orientated perpendicular to the tributary a well-defined cloud of spray-drift moved from the orchard in the direction of the tributary. Prior to the start of the application collectors were placed in SZ1. After spraying was completed in the two

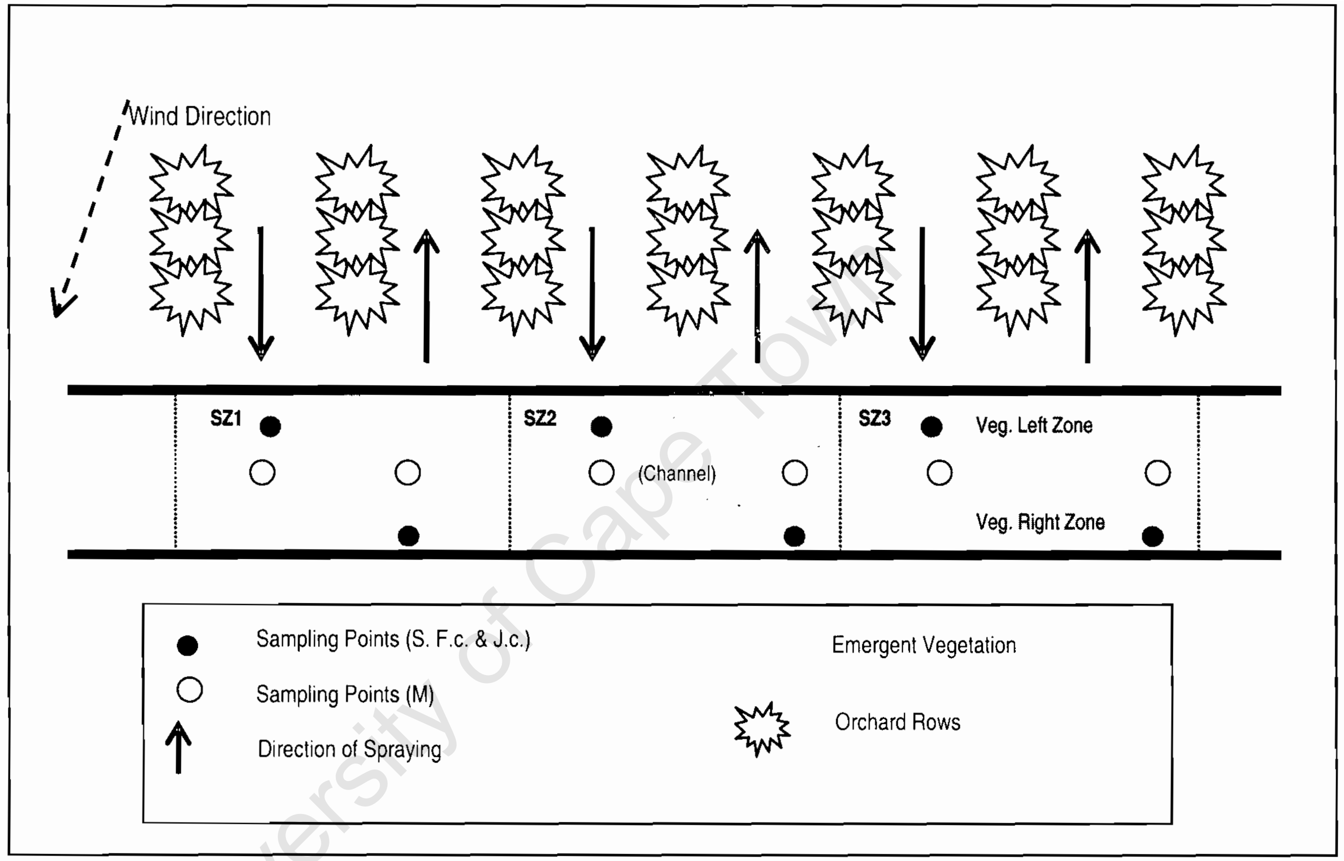

Fig. 6.2: Diagram (not to scale) of the position of sampling points within sampling zones (SZ1, SZ2 and SZ3) for measurement of deposition of azinphos-methyl on the surface of the emergent vegetation canopy (S) at the base of two emergent aquatic macrophytes, *Juncus capensis* (J.c) and *Fuirena hirsuta* (F.h) and on the surface of the unvegetated channel (M).

rows within SZ1, the collectors were immediately removed and new collectors were then placed in SZ2. The same procedure was repeated for SZ3. The purpose of this was to prevent cross contamination of collectors between the zones so that each collector represents one single deposition event. Once the collectors were removed from the stream, the water was immediately poured from the dishes into acetone- and distilled water-rinsed glass jars and kept on ice until solid phase extraction was carried out.

6.2.3 Pesticide Analysis

Samples were solid-phase extracted within 5 h after sampling using Chromabond® C18 columns (Macherey-Nagel, Düren, Germany). The columns were air-dried for 30 minutes and kept at -18 °C until analysis. Analysis was performed according to methods described in (Dabrowski *et al.* 2002). Measurements were done using gas chromatography (HP 5890) fitted with standard HP electron-capture, nitrogen-phosphorus, and flame-photometric detectors. Identity of AZP was confirmed by matching retention times on three different stationary phases. Method validation employed water matrixes that were found to have no detectable levels of the investigated pesticides and consisted of spiking water at eight spiking levels over the range of concentrations found in the actual samples. Overall mean recoveries were between 79 and 106%. For quality control, a matrix blank was analysed with each extraction set. The investigated pesticides were never detected in matrix blanks. The detection limits were 0.01 μg/L.

6.2.4 Data Analysis

Based on concentrations measured in the drift collectors the deposition rate in each treatment (mg/m^2) was ascertained. Significant differences between treatments were tested by means of a one-way ANOVA and a Fischer's PSLD post-hoc test. Measured deposition rates were compared to predicted rates in order to validate the sampling technique. Deposition rates in the stream were predicted using basic 90th percentile drift values (Rautmann *et al.* 2001) using a value of 8.41% of applied pesticide landing on the stream surface (based on a 5 m buffer zone in between the orchards and the tributary). Thus, based on an application rate of 0.15 Kg a.i/ha (or 0.015g/m^2), predicted deposition rates on the stream surface were calculated to be 6.75 x $10^{-4}/m^2$. Using the surface area of the dish, the total expected load of azinphos-methyl landing in the dish was estimated and compared to measured loads.

6.3 RESULTS & DISCUSSION

6.3.1 Interception

Predicted deposition rates ($1.3 mg/m^2$) compared well with average measured deposition rates ($1.5 mg/m^2 \pm 0.5$; $n = 6$) on the vegetation surface. The basic drift values (Rautmann *et al.* 2001) used for predicting values in the stream are widely used in aquatic pesticide exposure assessments for surface waters (FOCUS 2001) and have been shown to be valid in previous studies in the Lourens River catchment (Schulz *et al.* 2001b; Dabrowski & Schulz 2003). Pesticide deposition was highest in the surface vegetation drift collectors, with lower rates being measured on the surface of the unvegetated channel (Fig. 6.3).

The close correlation between measured and predicted deposition values indicates that the sampling method utilized in the study was appropriate and further suggests that the 33.6 % reduction of spray deposition measured on the surface of the exposed channel is lower than normally would be expected. It would thus appear that the emergent vegetation shields the channel to a degree and intercepts drift particles before they land on the channel surface. The fact that vegetation restricts the channel to a narrow width presumably facilitates this reduction. Reduction in macrophyte coverage and the resulting increase in channel width would thus potentially reduce this shielding effect. Vegetation in buffer zones (Hall *et al.* 1996) and edge-of-field vegetative wind-breaks (Ucar & Hall 2001) and hedgerows (Longley & Sotherton 1997; Longley *et al.* 1997) have been shown to be highly effective in intercepting drift from orchards to adjacent water bodies, although the present study is the first that has demonstrated the potential of in-stream marginal emergent aquatic vegetation in reducing pesticide deposition on the non-vegetated water surface. Physical characteristics of the plants would presumably influence the shielding effect to a large degree. In particular, vegetation height would play an important role by reducing the angle of incidence of the exposed channel to the direction of the on-coming drift.

The positive effect of the vegetation on pesticide drift reduction is further apparent when comparing deposition rates beneath the two vegetation types to expected and measured values on the vegetation surface. Respective deposition rates measured beneath *J. capensis* and *F. hirsuta* were 88 % (significant reduction; $p = 0.005$; $n = 6$) and 65 % (not significant) lower than those measured on the surface of the vegetation. Deposition rates measured below *J. capensis* were also significantly lower than those measured in the mid-stream channel ($p = 0.048$; $n = 6$); rates beneath *F. hirsuta* were lower but not significant (Fig. 6.3).

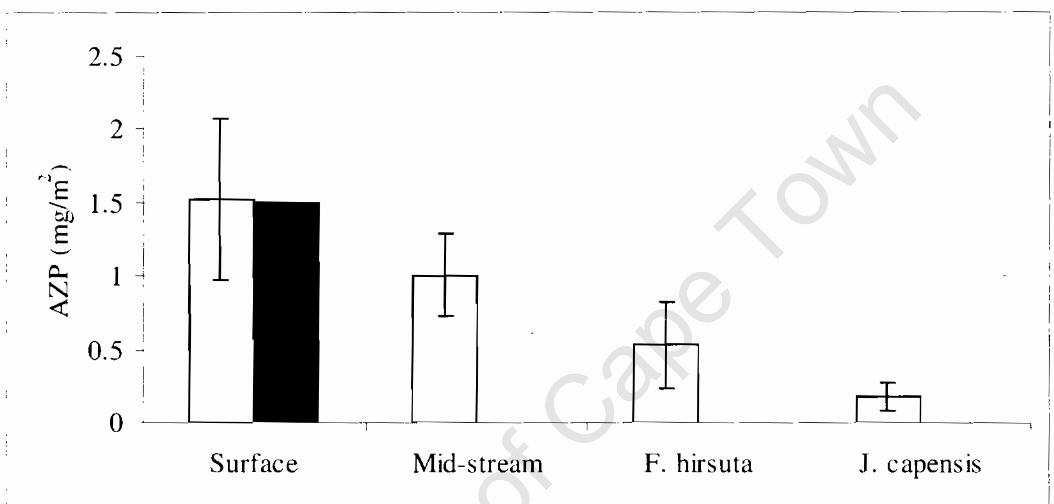

Fig. 6.3: Graph showing the measured deposition of azinphos-methyl (AZP; white bars; ± standard error; $n = 6$) on the surface of the canopy of marginal emergent vegetation (Surface), on the surface of the unvegetated channel (Mid-stream) and at the base of two species of emergent aquatic macrophytes; F.h (*Fuirena hirsuta*) and J.c (*Juncus capensis*); in a vegetated tributary of the Lourens River, South Africa. The black bar represents the predicted deposition on the surface of the canopy of marginal emergent vegetation

Two studies performed on alfalfa have shown that the plants intercept applications of AZP and thus reduce pesticide concentrations on the soil surface by up to 84% (Bennett *et al.* 1994; Schauber *et al.* 1995). Values of interception factors for emergent aquatic macrophytes have not been reported in the literature. Interception factors have however been studied in greater detail for agricultural crops, and recently a proposal for determining interception factors based on the growth phase or leaf area index of the crop has been derived (Linders *et al.* 2000). Interception factors reported in this study are within the upper range of these values. Additional variables influencing interception factors include the surface area of the leaves (Pfleeger *et al.* 1996), vegetation height (Schauber *et al.* 1995), canopy structure and plant densities (Linders *et al.* 2000). The highly efficient interception by *J. capensis* is most likely as a result of the broad leaf shape occurring in high densities which presents a larger surface area than leaves of *F. hirsuta*, which are in the form of long thin spikes (Table 6.2). The high degree of variance in treatments could be explained by the variation in distance of drift collectors from the point of application. Collectors placed in the left vegetated zone were up to 1 m further away from those in the right zone and generally received less drift, although no statistical differences were apparent. Previous studies measuring interception of AZP also found a high degree of variance which was attributed to the distance of treatment plots from the spraying boom (Bennett *et al.* 1994). Although not studied in detail, the leaves of *J. capensis* were orientated relatively uniformly (upright position), whilst those of *F. hirsuta* were orientated at a variety of angles, which may also account for the high degree of variance of percentage interception by this species (Koch & Weisser 2001).

The half-life of AZP on plant surfaces is reported to be from 1.7 – 5.1 days (Bennett *et al.* 1994) and thus degrades fairly rapidly after interception. Interception data from this present study and half-life data thus indicate that emergent aquatic macrophytes are highly effective in reducing spray-drift-related AZP loads to the water column of the stream. Aquatic macrophytes are often removed from agricultural ditches to promote rapid drainage from fields during heavy rainfall events (Beltman 1984). The benefit of submerged sections of aquatic macrophytes in improving water quality via assimilation and adsorption of pollutants has been well documented however (Meulemann *et al.* 1990; Schulz *et al.* 2003a).

The findings of this present study provide additional support for the overall positive effect of aquatic macrophytes in reducing nonpoint-source pesticide input. Most importantly, aquatic macrophytes need to be effectively managed in agricultural surface waters so as to realize to their full benefits in improving water quality. The Lourens River has a high conservation status (Tharme *et al.* 1997) and effective management of the catchment is required to minimize potential pesticide-related risks. As the tributaries serve as the main source of pesticide contamination in the mainstream (Dabrowski *et al.* 2002), aquatic macrophytes can have a significant impact in reducing drift deposition in the tributaries. Furthermore, as pesticide application takes place during the hot, dry summer months the risk of intercepted pesticides entering the water body via rainfall is relatively low. Thus, in the context of the Lourens River, clearing of vegetation could took place in winter and thus facilitate rapid drainage of fields, without compromising the benefits that the macrophytes provide in terms of interception and adsorption of spray-drift derived pesticide concentrations during the summer spraying season.

6.3.2 *Extrapolated predictions*

Based on the 33.6% reduction of spray-drift deposition in the unvegetated channel as a result of the shielding effect of the adjacent vegetation, a negative exponential relationship could be derived between drift reduction in the unvegetated area of the stream and the percentage of exposed area. This relationship assumes 75% drift reduction when 0% of the channel is unvegetated (based on the mean of the reduction for *J. capensis* and *F. hirusta*) and 0% drift reduction when 100% of the stream is unvegetated (based on the expected deposition derived from 90th percentile basic drift values).

The resulting equation can thus be used to calculate the reduction of spray deposition in the non-vegetated channel based on the percentage non-vegetated channel area of the stream. The model thus assumes that the unvegetated area of the stream will always be confined to the center of the stream and will be flanked on either side by the emergent aquatic vegetation. Based on this model an interception factor of 0.65 and 0.88 for *F. hirsuta* and *J. capensis* respectively, and percentage coverage of each plant type with respect to the total area of the stream, a predictive formula was generated:

$$\textit{Total Load (mg)} = \text{SA.ED}\ (f(\text{M}) + 0.65(\text{F.h}) + 0.88(\text{J.c}) + 0.65(\text{Pyc}))$$

Where:

SA = Total surface area of stream adjacent to an orchard (m^2)

ED = Expected deposition (mg/m^2) based on basic 90th Percentile drift values (Rautmann *et al.* 2001)

$f = 77.215e^{-0.0434M}$ (variable describing the exponential relationship between non-vegetated channel area and the shielding effect of adjacent emergent vegetation, where M = % non-vegetated channel area).

F.h = % Area covered by *F. hirsuta*

J.c = % Area covered by *J. capensis*

Pyc. = % Area covered by *Pycreus* spec. (an interception factor of 0.65 was assumed as the plant was similar in morphological characteristics to *F. hirsuta*)

Using the formula, predictions were generated for different total percentages of area of vegetation coverage in order to estimate total drift reductions with respect to application of AZP at 5, 10 and 15 m distance from the stream. The proportion of area covered by each of the three plant species was assumed to be the same for each prediction (37.5% *J. capensis*; 31.1% *F. hirsuta*; 11.3% *Pycreus* spec.).

Predicted drift deposition reductions were calculated relative to expected deposition with a buffer strip of 5 m (as in the case of the studied stream) with no vegetative coverage. Predicted drift reductions provide additional support for the efficiency of emergent aquatic vegetation in reducing drift related pesticide loads in water bodies, with 67% of drift deposition being potentially reduced along the entire stretch of the stream at 80% macrophyte coverage (Table 6.3). Thus the high interception factors of the two plant species in combination with the fact that the extensive macrophyte coverage reduces the area of the exposed non-vegetated channel to such a degree, results in a large reduction in drift deposition of AZP along the entire stretch of the stream.

Table 6.3: Prediction of spray-drift derived loads of azinphos-methyl (AZP) in a vegetated tributary of the Lourens River, South Africa, based on different percentage area coverages of emergent macrophytes and buffer zone distances of 5, 10 and 15 meters.

	Vegetation Coverage			
	0%	25%	50%	80%
mg AZP (5m)	740.8	539.8	382.7	242.1
% Reduction	0	27.1	48.3	67.3
mg AZP (10m)	326.82	182.54	148.10	106.79
% Reduction	44.1	75.4	80.0	85.6
mg AZP (15m)	136.58	76.29	61.89	44.63
% Reduction	81.6	89.7	91.7	94.0

Interception factors for different crop types are incorporated into models (e.g. PRZM) used in regulatory exposure assessments in order to estimate the amount of applied pesticide that reaches the soil and is thus available for processes such as leaching and surface runoff (Linders *et al.* 2000). In the USA, the Kenaga nomogram is used in terrestrial exposure assessments according to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and can be used to calculate plant interception factors based on the structure of the plant and the application rate of the applied pesticide (Pfleeger *et al.* 1996). The existence of these models coupled with the results of the study therefore present a strong argument for the development of interception factors related to emergent aquatic vegetation, which could then be incorporated into fate models. Existing fate models designed to predict PECs in surface waters as a result of drift deposition (e.g. TOXSWA) do not incorporate the effect of interception by emergent aquatic plants (Adriaanse 1997) and could thus over-estimate PECs in ditches where emergent macrophytes are present. Further development of the model and interception factors would rely on trials investigating the potential of different emergent aquatic plant species in reducing drift deposition based on characteristics such as density, height, surface area and orientation of emergent leaves.

Increasing the width of the buffer zone has been proposed as one of the most effective mitigation strategies for reducing drift of pesticides from the edge-of-field to adjacent water bodies (De Snoo & De Wit 1998). This option may often not be agronomically feasible, as it means that a large surface area is lost to farmers for production of crops (Hewitt 2000). In the present study, in the absence of macrophyte coverage an increase in buffer zone width from 5 to 10 m results in a 44% reduction in spray deposition which is comparable to reductions with 50% in-stream vegetation coverage and lower than 80% coverage in combination with a buffer zone width of 5 m. The results thus indicate that emergent macrophytes may be as effective as increasing buffer zone width in terms of reducing drift deposition in aquatic water bodies and may therefore be considered as a highly effective and viable risk mitigation strategy. It is also clear that overall positive effect of the emergent vegetation in terms of percentage reduction of drift deposition reduces with increasing buffer strip distance. In the case of a 5 m buffer strip, 80% coverage results in a 67.3% increase in reduction of drift deposition in comparison to 0% coverage. In the case of a 10 and 15 m buffer strip, reduction is only increased by 41.5 and 12.4% respectively. Thus, in terms of a management scenario, the possibility for compromise exists between maintaining a dense coverage of emergent aquatic vegetation adjacent to fields with narrow buffer strips (< 5 m), or by establishing a wide buffer strip, with relatively little emergent coverage in an adjacent stream or ditch. It is essential however, to establish the potential ecological consequences of pesticide adsorbed on plant surfaces on terrestrial species (e.g., birds and insects) that may utilise the vegetation for habitat or nesting, before interception can be recommended as a viable mitigation strategy.

Risk mitigation is a relatively new field and is coming under ever increasing scrutiny, particularly with respect to pesticide registration. Different mitigatory strategies are generally poorly understood (FOCUS 2001) which has resulted in the formation of bodies such as the FOCUS group on Landscape and Mitigation Factors in Ecological Risk Assessment, which aim to harmonize and develop mitigation options (Streloke & Brown 2003). In this respect the results of the present paper provide additional insight into the role of aquatic macrophytes in effective pesticide risk mitigation and suggest that the effective management of aquatic macrophytes in agricultural ditches may be one of the most important mitigation options with respect to spray-drift-derived pesticide input into agricultural surface waters.

6.4 CONCLUSION

Interception of spray-drift by emergent aquatic vegetation is potentially a highly efficient mitigation tool, with pesticide deposition being reduced by up to 67% in the studied stream. Predictive modelling using 90^{th} percentile drift values indicates that emergent aquatic vegetation may be as effective in reducing spray deposition in surface waters as increasing buffer zone width. The potential ecological consequences of pesticide deposition on plant surfaces needs to be considered however, before interception by emergent aquatic macrophytes can be regarded as a viable mitigation strategy.

CHAPTER 7

GENERAL DISCUSSION

Nonpoint-source pollution poses one of the greatest threats to the water quality of surface waters (Loague *et al.* 1998). Pesticides in particular pose a high risk due their high toxicity towards non-target organisms (Cooper 1993). Of the major nonpoint-sources, runoff and spray-drift have been identified as routes, which lead to relatively high concentrations of pesticide entering surface waters. Due to the direct nature of contamination spray-drift has often been presumed to pose the greatest risk to surface waters, however very few studies have compared these two routes of exposure in a risk assessment scenario. Central to this thesis is the question of which of runoff and spray-drift potentially poses the greatest risk towards aquatic systems in terms of pesticide contamination and what are the key processes that distinguish their respective risks towards aquatic environments. Chapters 3 to 6 have each focused on integral components of a risk assessment scenario in an attempt to answer this question: exposure (Chapter 3), effect (Chapter 4) and mitigation (Chapters 5 and 6). In all chapters every effort was made to perform experiments under field-relevant conditions and field experiments and sampling exercises were performed during real-time spray-drift and runoff events. Thus contamination of study sites was not simulated and sampling exercises are representative of typical nonpoint-source pollution events and integrate aspects such as changes in discharge in the experimental design.

Exposure

Exposure assessments and modelling of nonpoint-source pesticide events have generally been performed at the edge-of-field and thus cover a relatively small spatial scale (FOCUS 2001; Brown *et al.* 2003). The use of predictive models in particular, forms an integral component of exposure assessments, particularly in developed (FOCUS 1997; 2001) countries. In fact, predicted environmental concentrations (PECs) derived from the models are used as benchmark concentrations against which toxicity data is compared so as to determine whether the use of a plant protection product may be authorized. Landscape-based exposure assessments cover larger areas and help refine the risk assessment process by placing preliminary exposure and effect assumptions into context within the agricultural landscape (Brown *et al.* 2003). A comparison of the predicted loads in the initial validation of the runoff and spray-drift models in the tributaries (Figs 3.2 and 3.3) indicates no clear difference between runoff- and spray-drift-derived azinphos-methyl loads. In fact, if concentrations were compared, those measured during the spray-drift (high concentration, low discharge) validation where higher than those measured during the runoff events (low concentrations high discharge). The spatial scale incorporated by these measurements is quite small however, and is almost representative of an edge-of-field scenario. On a larger scale, runoff leads to higher contamination as a result of the fact that many orchards over a large area are simultaneously influenced by a rainfall event. The broader landscape-based assessment is thus advantageous in that it takes measurements made at a small spatial scale and integrates them within the context of the entire agricultural catchment.

It is also however important to take physico-chemical properties of pesticides into consideration. As is apparent from the effect study in Chapter 5, water-dissolved pesticide concentrations potentially pose the greatest risk towards aquatic macroinvertebrates. Thus, in an exposure assessment, it is important to focus on water-dissolved pesticide concentrations. Azinphos-methyl is a relatively water soluble pesticide and was thus detected in high concentrations in the water-phase during runoff conditions. An additional study, employing identical methods, but focusing on a pesticide with low water solubility could show that runoff may pose less of a risk in terms of water-dissolved pesticide concentrations, with spray-drift becoming a more important route of entry at the catchment level. A study that predicted runoff concentrations of 3 different pesticides; azinphos-methyl, chlorpyrifos and endosulfan, (the latter two of which have low water solubilities) predicted higher concentrations of azinphos-methyl in comparison to chlorpyrifos and endosulfan (Dabrowski *et al.* 2002). Thus spray-drift could potentially become a more important route of entry for the latter two pesticides. Schulz (2001b) however showed that even for endosulfan, runoff resulted in higher concentrations in the Lourens River mainstream than those measured after spray-drift events.

The larger temporal scale associated with runoff is however also important to consider. Even though azinphos-methyl has a relatively short half-life (10 days) it was still detected in relatively high concentrations in the Lourens River mainstream up to 3 months after the last application. The potential for runoff-associated pesticide input thus exists long after the pesticide has been applied, whereas spray-drift-related contamination can only occur during the application process. It is also important to note that each catchment will have its own unique land use and geographical features, which may also have a large influence on the outcome of measured and predicted pesticide levels resulting from runoff and spray-drift.

Thus, considering different land-use features and application of pesticides with different pyhsico-chemical properties, it is possible that spray-drift may become more important at the catchment scale. The spatial and temporal scales associated with runoff-induced pesticide contamination will however mean that runoff is most likely to present the highest exposure risk towards aquatic environments on the catchment scale. The predictive modelling used in this study provides the opportunity to determine which of runoff and spray-drift poses a more important exposure risk for a particular pesticide, based on the physico-chemical properties of the pesticide in question. It is also important to note that the modelling approach is able to identify areas where pesticide contamination may be particularly high, for both runoff and spray-drift events, and could thus potentially serve as a useful tool in the management of pesticide pollution in agricultural catchments.

Although the exposure assessment in this study focused on one pesticide only, it is also important to consider that runoff generally leads to more than one pesticide entering a system simultaneously (Dabrowski *et al.* 2001), resulting in organisms being exposed to a mixture of pesticides. Studies have reported additive and synergistic effects as a result of exposure to a combination of pesticides (Hoagland *et al.* 1993; Thompson 1996; Pape-Lindstrom & Lydy 1997; Kungolos *et al.* 1999). Spraying, and thus spray-drift, of combinations of mixed pesticide is possible due to intentional mixing or lack of washing of spraying apparatus after application. This can however be prevented through efficient cleaning or changes in the spraying programme if possible. Furthermore, in addition to water-dissolved pesticide concentrations, runoff also leads to input of particle-associated pesticide, which although is less bioavailable, still has potential to elicit negative toxicological effects (Schulz & Liess 2001c).

EFFECT

Ecotoxicological effects are obviously directly influenced by exposure. Thus, the results of the exposure study would suggest that, at the edge-of-field, spray-drift could potentially pose the highest risk towards aquatic fauna, while at a larger catchment scale, runoff is likely to lead to potentially higher risk as a result of higher concentrations. The results of the microcosm study however indicate that the ecotoxicological effect of a specific pesticide concentration may vary according to the flow velocity. The findings of the present study show that increased flow velocity decreases the magnitude of the toxic effect, suggesting that for any given concentration of pesticide, runoff potentially poses less of a toxic threat than spray-drift (assuming that flow velocity is lower during spray-drift events as is typical of the Western Cape, when pesticides are applied during the hot, dry summer months).

Exposure scenarios used in the microcosm experiment in Chapter 4 were specifically chosen to be as representative of field conditions as possible and to determine individual and combined effects of pesticide, flow increase and turbidity on mayfly behaviour. Drift behaviour has been shown to be a highly sensitive endpoint with regards to pesticide contamination and has been documented for many different pesticides, including organophosphates, organochlorines and pyrethroids (Breneman & Pontasch 1994; Schulz & Dabrowski 2001; Hose *et al.* 2002). Once again, differences in physico-chemical properties of different pesticides could lead to differences in expected exposure scenarios, which could in turn influence the observed effect with respect to runoff and spray-drift. Cypermethrin has a low water solubility and a high K_{OC} value and would thus be expected to be highly associated with particles during runoff as has already been documented (Hadfield *et al.* 1993). In this case bioavailability of the pesticide is reduced (Erstfeld 1999), resulting in a decrease in effect as was observed by the decreased drift rate during the CYP x PART trials in the present microcosm study. Chapters 3 and 5 however both showed that for a relatively water soluble pesticide such as azinphos-methyl, water-dissolved concentrations of pesticide are as high or even higher during runoff events when compared to spray-drift events. It is thus important to consider that whilst most simulated runoff studies in microcosms assume sorption to sediments and thus low concentrations of water-dissolved pesticide, it is possible that concentrations may be comparable to those observed during spray-drift conditions.

The results of the present study suggest that increased flow velocity has an antagonistic effect on the toxicity of cypermethrin, resulting in a drift density that was lower than expected. This can clearly be seen by comparing the CYP and CYP x PART experiments with the CYP x FLOW and CYP x PART x FLOW experiments respectively. Both the CYP and CYP x PART treatment led to increases in drift rate. The addition of increased flow velocity in the design of these exposure scenarios still had an antagonistic effect on the toxicity of cypermethrin and thus decreased the drift density. The decrease was most pronounced in the CYP x PART x FLOW experiment where drift density was comparable to the CONTROL experiment. Based on these results, it can be concluded that due to an increase in flow velocity, runoff potentially poses less of an ecotoxicological risk than spray-drift, even if water-dissolved concentrations resulting from each type of event were comparable. Further toxicity testing under a variety of flow velocities is necessary in order to gain a broader understanding of the influence of flow velocity on toxicity of chemicals. These tests also need to be conducted on different test organisms, as different macroinvertebrate species are adapted to different flow regimes and may thus react differently towards changes in flow velocity. These results also suggest that water bodies or different sections of a river (mountain stream, foothill and lowland sections) may vary in their vulnerability towards pesticides based on their respective flow velocities. Further research focusing on the impact of pesticides on water bodies with different flow velocities and their respective macroinvertebrate communities may provide further insight on the ecological effects of pesticides in surface waters.

Other studies have shown an increase in drift density as a result of increased flow velocity (in the absence of pesticide) (Ciborowski *et al.* 1977; Ciborowski 1982). It is thus likely that under very high discharge conditions drift may be a passive process, whereby macroinvertebrates are literally swept away as a result of the increased current velocity. In the case of the microcosm experiment conducted for this thesis it is possible that the current velocity in the CONTROL experiments was too low, a situation that presented the mayflies with a degree of stress. This assumption is justified by the fact that drift density was reduced below that of the CONTROL during the FLOW experiment.

The findings of the microcosm experiments highlight the importance of incorporating changes in current velocity when investigating impacts of pesticides on macroinvertebrates.

MITIGATION

The results of this study indicate that emergent aquatic macrophytes are potentially a useful feature in agricultural ditches and help to mitigate against the transport and input of spray-drift-derived pesticide concentrations. Whilst increased buffer zones have been shown to be effective in minimizing deposition of pesticides into water bodies, the results presented in this thesis indicate that emergent aquatic macrophytes may be as effective via foliar interception of pesticide drift. Based on the fact that increased discharge and flow conditions decrease the sorption potential of aquatic macrophytes (Chapter 5) it follows that at least during heavy rainfall events, vegetated streams or ditches that show a marked increase in discharge during the runoff event may not be suitable to reduce the transport of pesticides. Thus in assessing the suitability of vegetated water bodies to mitigate against the transport of runoff-derived pesticides, careful consideration of the hydrological characteristics of the water body is necessary.

Larger water bodies, such as wetlands, where changes in discharge and flow velocity are not as pronounced during heavy rainfall events, are most likely better suited to mitigate against runoff-induced pesticide transport. Schulz & Peall (2001) showed a high reduction in pesticide concentrations at the outlet of a vegetated wetland following an 18 mm runoff event. Based on the literature review and the results of Chapters 5 and 6, it is apparent that there are generally more options available for mitigating against spray-drift in comparison to runoff. In addition to increased buffer zone width, wind-breaks and advances in spraying technology, the present study has shown vegetated streams to be effective in reducing water-dissolved pesticide concentrations through sorption to plant surfaces and via interception of drift deposition on the water surface. Runoff poses more of a problem in that water naturally leaves fields following rainfall events, which means that there is always potential for transport of pesticides. In contrast to spray-drift, it would seem that the most efficient method of reducing runoff-related pesticide concentrations in surface waters is through preventing runoff entering the water body in the first place or via use of constructed wetlands that do not show a marked increase in current velocity following a rainfall event. In this respect erosion control, conservation tillage and vegetated buffer strips have been shown to be effective in reducing pesticide concentrations in runoff.

CHAPTER 8

SUMMARY OF CONCLUSIONS

Central to this thesis was the question of which of runoff and spray-drift poses the greatest risk towards aquatic systems in terms of pesticide contamination and what are the essential mechanisms that determine their relative threats to the environment.

The importance of differences in physico-chemical properties of different pesticides has been stressed and the potential implications thereof discussed. All of the studies presented in this thesis would have benefited by focusing on more than one pesticide, each with different physico-chemical properties. Analysis of pesticides at low concentrations is however highly expensive and thus imposes strict limitations on a study of this nature. In fact the expense of analysis coupled with the lack of experts in the field of chemical analysis is one of the main reasons why the environmental fate and effects of pesticides in developing countries is largely unknown. South Africa is the largest producer of pesticides in South Africa (London *et al.* 2000), yet the registration and regulation of pesticides is controlled by the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act of 1947. In contrast to legislation in the EU, where the results of predictive modelling techniques form an integral part of the risk assessment process, no estimation of PECs is performed in the South African registration procedure.

Currently the extent and magnitude of pesticide contamination in South African water bodies is largely unknown as a result of a lack of any continuous monitoring programme (Coetzee & Cooper 1991), although poisoning of fish and birds by pesticides has been frequently documented (Grobler 1994; Roux *et al.* 1994). In spite of the fact that a multitude of pesticides are used throughout the country, water guidelines currently exist for only two pesticides; endosulfan (an organochlorine insecticide) and atrazine (a herbicide) (DWAF 1996). In studies that have documented the occurrence of pesticides in surface waters only a few have attempted to establish a source of contamination. The modelling technique used in this current thesis could thus play a useful role in identifying potential problem areas in catchments and identifying important sources of pesticide contamination and could thus decrease the expense of an expansive monitoring programme.

In summary, the results of this study indicate that, at the catchment scale, runoff leads to higher concentrations of water-dissolved and particle-associated pesticides than spray-drift. This is as a result of the larger spatial and temporal scale associated with runoff contamination. The suitability of vegetated water bodies in reducing the transport of water-dissolved pesticide concentrations is not as great for runoff as it is for spray-drift as a result of decreased sorption to plants during high discharge conditions. This could lead to higher concentrations of pesticide being measured during runoff events in catchments where vegetated ditches are prominent. Furthermore, emergent aquatic macrophytes afford additional protection against spray-drift by effectively intercepting drift before it lands on the water surface, thereby significantly reducing water-dissolved pesticide concentrations.

Thus, in terms of exposure, runoff potentially poses the greatest threat to aquatic systems. The effect study however indicates that increases in flow velocity may reduce the toxic potential of pesticides during runoff events. Thus, even if pesticide concentrations in a given catchment were higher during runoff events than during spray-drift events, the ecotoxicological effect may not be as high as would be predicted by the concentrations present.

Based on these results and the influence of physico-chemical properties on the fate of different pesticides, it is not possible to definitively identify which of runoff and spray-drift poses the greatest risk towards aquatic environments. The thesis has however identified fundamental mechanisms driving exposure, effects and mitigation related to runoff and spray-drift-related pesticide contamination in surface waters. Most importantly these are the impact of different spatial scales, from the edge-of-field to an entire catchment on exposure and the effect of increased flow velocity on toxicity and sorption of pesticides to aquatic macrophytes. Elaboration of these mechanisms in further studies could greatly contribute to an improved understanding of the occurrence and effects of pesticides in aquatic systems and the most effective methods to reduce their presence in non-target environments.

REFERENCES

LITERATURE CITED IN ALL CHAPTERS

ADRIAANSE P.I. (1997). Exposure Assessment of Pesticides in Field Ditches: The Toxswa Model. *Pesticide Science.* **49** (2): 210-212.

ADRIAANSE P., ALLEN R., GOUY V., HOSANG J., JARVIS T., KLEIN M., LAYTON R., LINDERS J., SCHÄFER L., SMEETS L. & YON D. (1997a). Surface Water Models and EU Registration of Plant Protection Products - Dok. 6476/Vi/96. In: Final report of the work of the Regulatory Modelling Working Group of Surface Water - Models of FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe),

ADRIAANSE P.I., BELTMAN W.H.J., WESTEIN E., BROUWER W.W.M. & VAN NIEROP S. (1997b). A Proposed Policy for Differentiated Hazard Evaluation of Pesticides in Surface Waters. In: DLO Winand Staring Centre, Report 141, Wageningen, Netherlands.

AEDG (1992). Improving Aquatic Risk Assessment under FIFRA. Report of the Aquatic Effects Dialogue Group. In: RESOLVE, Washington DC.

ALLAN J.D., FLECKER A.S. & MCCLINTOCK N.L. (1986). Diel Epibenthic Activity of Mayfly Nymphs, and its Nonconcordance with Behavioral Drift. *Limnology and Oceanography.* **31** (5): 1057-1065.

ANKLEY G.T., CALL D.J., COX J.S., KAHL M.D., HOKE R.A. & KOSIAN P.A. (1994). Organic Carbon Partitioning as a Basis for Predicting the Toxicity of Chlorpyrifos in Sediments. *Environmental Toxicology and Chemistry.* **13** (4): 621-626.

BAUGHMAN D.S., MOORE D.W. & SCOTT G.I. (1989). A Comparison and Evaluation of Field and Laboratory Toxicity Tests with Fenvalerate on an Estuarine Crustacean. *Environmental Toxicology and Chemistry.* **8**: 417-429.

BELTMAN B. (1984). Management of Ditches. The Effect of Cleaning of Ditches on the Water Coenoses. *Verhandlungen der Internationalen Vereinigung für Limnologie.* **22**: 2022-2028.

BENNETT E.R., MOORE M.T., COOPER C.M. & SMITH JR. S. (2000). Method for the Simultaneous Extraction and Analysis of Two Current Use Pesticides, Atrazine and Lambda-Cyhalothrin, in Sediment and Aquatic Plants. *Bulletin of Environmental Contamination and Toxicology.* **64**: 825-833.

BENNETT R.S., EDGE W.D., GRIFFIS W.L., MATZ A.C., WOLFF J.O. & GANIO L.M. (1994). Temporal and Spatial Distribution of Azinphos-Methyl Applied to Alfalfa. *Archives of Environmental Contamination and Toxicology.* **27**: 534-540.

BIRD S.L., ESTERLY D.M. & PERRY S.G. (1996). Off-Target Deposition of Pesticides from Agricultural Aerial Spray Applications. *Journal of Environmental Quality.* **25** (5): 1095-1104.

BLACK R.W., HAGGLAND A.L. & VOSS F.D. (2000). Predicting the Probability of Detecting Organochlorine Pesticides and Polychlorinated Biphenyls in Stream Systems on the Basis of Land Use in the Pacific Northwest, USA. *Environmental Toxicology and Chemistry.* **19** (4): 1044-1054.

BLANCHARD P.E. & LERCH R.N. (2000). Watershed Vulnerability to Losses of Agricultural Chemicals: Interactions of Chemistry, Hydrology, and Land-Use. *Environmental Science and Technology.* **34** (16): 3315-3322.

BORIN M., BONAITI G. & GIARDINI L. (2001). Controlled Drainage and Wetlands to Reduce Agricultural Pollution: A Lysimeter Study. *Journal of Environmental Quality.* **30**: 1330-1340.

BRADBURY S.P. & COATS J.R. (1989). Comparative Toxicology of the Pyrethroid Insecticides. *Environmental Toxicology and Chemistry.* **108**: 143-177.

BRASKERUD B.C. (2001). The Influence of Vegetation on Sedimentation and Re-suspension of Soil Particles in Small Constructed Wetlands. *Journal of Environmental Quality.* **30** (4): 1447-1457.

BRENEMAN D.H. & PONTASCH K.W. (1994). Stream Microcosm Toxicity Tests: Predicting the Effects of Fenvalerate on Riffle Insect Communities. *Environmental Toxicology and Chemistry.* **13**: 381-387.

BRENT R.N. & HERRICKS E.E. (1999). A Method for the Toxicity Assessment of Wet Weather Events. *Water Research.* **33** (10): 2255-2264.

BRITTAIN J.E. & EIKELAND T.J. (1988). Invertebrate Drift - a Review. *Hydrobiologia.* **166**: 77-93.

BROCK T.C.M., CRUM S.J.H., VAN WIJNGAARDEN R., BUDDE B.J., TIJINK J., ZUPPELLI A. & LEEUWANGH P. (1992a). Fate and Effects of the Insecticide Dursban 4E in Indoor Elodea-Dominated and Macrophyte-Free Freshwater Model

Ecosytems: I. Fate and Primary Effects of the Active Ingredient Chlorpyrifos. *Archives of Environmental Contamination and Toxicology.* **23**: 69-84.

BROCK T.C.M., VAN DEN BOGAERT M., BOS A.R., VAN BREUKELEN S.W.F., REICHE R., TERWOERT J., SUYKERBUYK R.E.M. & ROIJACKERS R.M.M. (1992b). Fate and Effects of the Insecticide Dursban 4E in Indoor Elodea-Dominated and Macrophyte-Free Freshwater Model Ecosystems: II Secondary Effects on Community Structure. *Archives of Environmental Contamination and Toxicology.* **23**: 391-409.

BROCK T.C.M., VET J.J.R.M., KERKHOFS M.J.J., LIJZEN J., VAN ZUILEKOM W.J. & GIJLSTRA R. (1993). Fate and Effects of the Insecticide Dursban 4E in Indoor Elodea-Dominated and Macrophyte-Free Freshwater Model Ecosystems: III Aspects of Ecosystem Functioning. *Archives of Environmental Contamination and Toxicology.* **25**: 160-169.

BROWN C.D., MAUND S.J., HOLMES C., DUBUS I.G., TURNER N.L., HENDLEY P. & SWEENEY P.J.J. (2003). Landscape-Level Approaches to Risk Assessment for Pesticides. In: *Proceedings of the XII Symposium Pesticide Chemistry.* Eds. A.A M. Del Re, E. Capri, L. Padovani and M. Trevisan, June 4-6, 2003, Piacenza, Italy.

CAMERON K., MADRAMOOTOO C., CROLLA A. & KINSLEY C. (2003). Pollutant Removal from Municipal Sewage Lagoon Effluents with a Free-Surface Wetland. *Water Research.* **37** (12): 2803-2812.

CAPEL P.D. & LARSON S.J. (2001). Effect of Scale on the Behaviour of Atrazine in Surface Waters. *Environmental Science and Technology.* **35** (4): 648-657.

CASTELLE A.J., JOHNSON A.W. & CONOLLY C. (1994). Wetland and Stream Buffer Size Requirements-a Review. *Journal of Environmental Quality.* **23**: 878-882.

CHANDLER G.T., COULL B.C. & DAVIS J.C. (1994). Sediment- and Aqueous-Phase Fenvalerate Effects on Meiobenthos: Implications for Sediment Quality Criteria Development. *Marine Environmental Research.* **37** (3): 313-327.

CIBOROWSKI J.J.H. (1982). Influence of Current Velocity, Density, and Detritus on Drift of Two Mayfly Species (Ephemeroptera). *Canadian Journal of Zoology.* **61**: 119-125.

CIBOROWSKI J.J.H., POINTING P.J. & CORKUM L.D. (1977). The Effect of Current Velocity and Sediment on the Drift of the Mayfly *Ephemerella Subvaria* McDunnough. *Freshwater Biology.* **7**: 567-572.

COETZEE H. & COOPER D. (1991) *Wasting Water. Squandering a Precious Resource.* In: Going Green. People, Politics and the Environment in South Africa. Eds. Cock J and Kock E. pp 129-138. Oxford University Press, Cape Town, South Africa.

COLE J.T., BAIRD J.H., BASTA N.T., HUHNKE R.L., STORM D.E., JOHNSON G.V., PAYTON M.E., SMOLEN M.D., MARTIN D.L. & COLE J.C. (1997). Influence of Buffers on Pesticide and Nutrient Runoff from Bermudagrass Turf. *Journal of Environmental Quality.* **26**: 1589-1598.

COOPER C.M. (1993). Biological Effects of Agriculturally Derived Surface-Water Pollutants on Aquatic Systems - a Review. *Journal of Environmental Quality.* **22**: 402-408.

CORKUM L.D. (1978). The Influence of Density and Behavioural Type on the Active Entry of Two Mayfly Species (Ephemeroptera) into the Water Column. *Canadian Journal of Zoology.* **56**: 1200-1206.

CROSSLAND N.O., MITCHELL G.C. & DORN P.B. (1992). Use of Outdoor Artificial Streams to Determine Threshold Toxicity Concentrations for a Petrochemical Effluent. *Environmental Toxicology and Chemistry.* **11**: 49-59.

CROSSLAND N.O., SHIRES S.W. & BENNETT D. (1982). Aquatic Toxicology of Cypermethrin. 3. Fate and Biological Effects of Spray Drift Deposits in Fresh Water Adjacent to Agricultural Land. *Aquatic Toxicology.* **2**: 253-270.

CRUM S.J.H., VAN KAMMEN-POLMAN A.M. & LEISTRA M. (1999). Sorption of Nine Pesticides to Three Aquatic Macrophytes. *Archives of Environmental Contamination and Toxicology.* **37** (3): 310-316.

CULP J.M., WRONA F.J. & DAVIES R.W. (1986). Response of Stream Benthos and Drift to Fine Sediment Deposition Versus Transport. *Canadian Journal of Zoology.* **64**: 1345-1351.

DABROWSKI J.M., PEALL S.K.C., REINECKE A.J., LIESS M. & SCHULZ R. (2001). Runoff-Related Pesticide Input into the Lourens River, South Africa:

Basic Data for Exposure Assessment and Risk Mitigation at the Catchment Scale. *Water, Air and Soil Pollution.* **135**: 265-283.

DABROWSKI J.M., PEALL S.K.C, VAN NIEKERK A., REINECKE A.J., DAY J.A. AND SCHULZ R. (2002). Predicting Runoff-Induced Pesticide Input in Agricultural Sub-Catchment Surface Waters: Linking Catchment Variables and Contamination. *Water Research.* **36** (20) 4975-4984.

DABROWSKI J.M. & SCHULZ R. (2003). Predicted and Measured Levels of Azinphos-methyl in the Lourens River, South Africa: Comparison of Runoff and Spray Drift. *Environmental Toxicology and Chemistry.* **22**: 494-500.

DALLAS H.F., JANSSENS M.P. & DAY J.A. (1999). An Aquatic Macroinvertebrate and Chemical Database for Riverine Ecosystems. *Water S.A.,Pretoria.* **25** (1): 1-8.

DAY K. (1989). Acute, Chronic and Sub-lethal Effects of Synthetic Pyrethroids on Freshwater Zooplankton. *Environmental Toxicology and Chemistry.* **8**: 411-416.

DE SNOO G.R. & DE WIT P.J. (1998). Buffer Zones for Reducing Pesticide Drift to Ditches and Risks to Aquatic Organisms. *Ecotoxicology and Environmental Safety.* **41** (1): 112-118.

DOEG T.J. & MILLEDGE G.A. (1991). Effect of Experimentally Increasing Concentrations of Suspended Sediment on Macroinvertebrate Drift - Short Commuication. *Australian Journal of Marine and Freshwater Research.* **42** (5): 519-526.

DOMAGALSKI J.L. & DUBROVSKY N.M. (1992). Pesticide Residues in Ground Water of the San Joaquin Valley, California. *Journal of Hydrology.* **130** (1-4): 299-338.

DOMAGALSKI J.L., DUBROVSKY N.M. & KRATZER C.R. (1997). Pesticides in the San Joaquin River, California: Inputs from the Dormant Sprayed Orchards. *Journal of Environmental Quality.* **26** (2): 454-465.

DONIGIAN JR. A.S. & HUBER W.C. (1991). Modelling of Nonpoint-source Water Quality in Urban and Non-Urban Areas. EPA/600/3-91/039. U.S. Environmental Protection Agency, Athens, GA, USA.

DORTLAND R.J. (1980). Toxicological Evaluation of Parathion and Azinphos-Methyl in Freshwater Model Ecosystems. In: Agricultural Research Report 898, Wageningen, The Netherlands.

DWAF (1996). *Draft of South African Water Quality Guidelines. Volume 7: Aquatic Ecosystems.* Department of Water Affairs and Forestry, Pretoria, South Africa.

ECOBICHON D.J. (2001). Pesticide Use in Developing Countries. *Toxicology.* **160** (1-3): 27-33.

ELLIOTT J.M. (1967). The Life Histories and Drifting of the Plecoptera and Ephemeroptera in a Dartmoor Stream. *Journal of Animal Ecology.* **36**: 343-362.

ERSTFELD K.M. (1999). Environmental Fate of Synthetic Pyrethroids During Spray Drift and Field Runoff Treatments in Aquatic Microcosms. *Chemosphere.* **39** (10): 1737-1769.

FAIRCHILD J.F., BOYLE T., ENGLISH W.R. & RABENI C. (1987) Effects of Sediment and Contaminated Sediment on Structural and Functional Components of Experimental Stream Ecosystems. *Water, Air and Soil Pollution.* **36**: 271-293.

FARMER D., HILL I.R. & MAUND S.J. (1995). A Comparison of the Fate and Effects of Two Pyrethroid Insecticides (Lambda-Cyhalothrin and Cypermethrin) in Pond Mesocosms. *Ecotoxicology.* **4** (4): 219-244.

FELSOT A.L., MITCHELL J.K. & KENIMER A.L. (1990). Assessment of Management Practices for Reducing Pesticide Runoff from Sloping Cropland in Illinois. *Journal of Environmental Quality.* **19**: 539-545.

FLURY M. (1996). Experimental Evidence of Transport of Pesticides through Field Soils - a Review. *Journal of Environmental Quality.* **25**: 25-45.

FOCUS (1997). *Surface Water Models and EU Registration of Plant Protection Products.* Document 6476/VI/96. European Commission.

FOCUS (2001). *Focus Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC.* Report of the Focus Working Group on Surface Water Scenarios. SANCO/4802/2001. European Commission.

FORRESTER G.E., DUDLEY T.L. & GRIMM N.B. (1999). Trophic Interactions in Open Systems: Effects of Predators and Nutrients on Stream Food Chains. *Limnology and Oceanography.* **44** (5): 1187-1197.

FRANK R., RIPLEY B.D., LAMPMAN W., MORROW D., COLLINS H., GAMMOND G.R. & MCCUBBIN P. (1994). Comparative Spray Drift Studies of Aerial and Ground Applications 1983-1985. *Environmental Monitoring and Assessment.* **29** (2): 167- 181.

GAMMON J.R. (1970). The Effect of Inorganic Sediment on Stream Biota. In: U.S. Water Pollution Control Administration, Cincinnati, OH, USA.

GANZELMEIER H., RAUTMANN D., SPANGENBERG R., STRELOKE M., HERRMANN M., WENZELBURGER H.-J. & WALTER H.-F. (1995). *Studies of the Spray Drift of Plant Protection Products.* In: Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Blackwell Scientific Publisher, Berlin.

GIDDINGS J.M., SOLOMON K.R. & MAUND S.J. (2001). Probabilistic Risk Assessment of Cotton Pyrethroids: II. Aquatic Mesocosm and Field Studies. *Environmental Toxicology and Chemistry.* **20** (3): 660-668.

GILBERT A.J. & BELL G.J. (1988). Evaluation of the Drift Hazards Arising from Pesticide Spray Application. *Aspects of Applied Biology.* **17**: 363-376.

GIPPEL C.J. (1995). Potential of Turbidity Monitoring for Measuring the Transport of Suspended Solids in Streams. *Hydrological Processes.* **9**: 83-97.

GOLDBLATT P. & MANNING J. (2000). *Cape Plants: A Conspectus of the Cape Flora of South Africa.* MBG Press, Missouri, USA & National Botanical Institute of South Africa, Pretoria, South Africa.

GORDON N.D., MCMAHAN T.A. & FINLAYSON B.L. (1992). *Stream Hydrology: An Introduction for Ecologists.* John Wiley & Sons, Chichester, UK. pp. 526.

GROBLER D.F. (1994) A Note on PCBs and Chlorinated Hydrocarbon Pesticide Residues in Water, Fish and Sediment from the Olifants River, Eastern Transvaal, South Africa. *Water SA, Pretoria.* **20** (3): 187-194.

GROENENDIJK P, VAN DER KOLK JWH & TRAVIS KZ. 1994. Prediction of exposure concentration in surface waters. In I. R. Hill, F. Heimbach, P. Leeuwangh and P. Matthiessen, eds, *Freshwater field tests for hazard assessment of chemicals.* Lewis Publisher, Boca Raton, pp 105-125.

GRUNWALD S. & NORTON L.D. (2000). Calibration and Validation of a Nonpoint-Source Pollution Model. *Agricultural Water Management.* **45**: 17-39.

HADFIELD S.T., SADLER J.K., BOLYGO E., HILL S. & HILL I.R. (1993) Pyrethroid Residues in Sediment and Water Samples from Mesocosm and Farm Pond Studies of Simulated Accidental Aquatic Exposure. *Pesticide Science.* **38** (4): 283-294.

HALL F.R., COOPER J., KIRCHNER L., DOWNER R. & THACKER R. (1996). Assessment of Off-Target Movement of Orchard Pesticides: Capture Efficiencies of Synthetic and Biological Biomarkers. *Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes.* **31** (4): 815-830.

HAND L.H., KUET S.F., LANE M.C.G., MAUND S.J., WARINTON J.S. & HILL I.R. (2001). Influences of Aquatic Plants on the Fate of the Pyrethroid Insecticide Lambda-Cyhalothrin in Aquatic Environments. *Environmental Toxicology and Chemistry.* **20** (8): 1740-1745.

HEWITT A.J. (2000). Spray Drift: Impact of Requirements to Protect the Environment. *Crop Protection.* **19**: 623-627.

HILL R. (1989). Aquatic Organisms and Pyrethroids. *Pesticide Science.* **27**: 429-465.

HOAGLAND K.D, DRENNER R.W., SMITH J.D. & CROSS D.R. (1993). Freshwater Responses to Mixtures of Agricultural Pesticides: Effects of Bifenthrin and Atrazine. *Environmental Toxicology and Chemistry.* **12:** 627-637.

HORNSBY A.G., WAUCHOPE R.D. & HERNER A.E. (1995). *Pesticide Properties in the Environment.* Springer Verlag, New York, NY, USA.

HOSE G.C., LIM R.P., HYNE R.V. & PABLO F. (2002). A Pulse of Endosulfan-Contaminated Sediment Affects Macroinvertebrates in Artifical Streams. *Ecotoxicology and Environmental Safety.* **51**: 44-52.

HOSMER A.J., WARREN L.W. & WARD T.J. (1998). Chronic Toxicity of Pulse-Dosed Fenoxycard to *Daphnia magna* Exposed to Environmentally Realistic Concentrations. *Environmental Toxicology and Chemistry.* **17** (9): 1860-1866.

HOUSE W.A., LONG J.L.A., RAE J., PARKER A. & ORR D.R. (2000). Occurrence and Mobility of the Insecticide Permethrin in Rivers in the Southern Humber Catchment, UK. *Pest Management Science.* **56** (7): 597-606.

HUMENIK F.J., SMOLEN M.D. & DRESSING S.A. (1987). Pollution from Nonpoint-sources. *Environmental Science and Technology.* **21** (8): 737-742.

JONES R.L. & MANGELS G. (2002). Review of the Validation of Models Used in Federal Insecticide, Fungicide, and Rodenticide Act Environmental Exposure Assessments. *Environmental Toxicology and Chemistry.* **21** (8): 1535-1544.

KAREN D.J., JOAB B.M., WALLIN J.M. & JOHNSON K.A. (1998). Partitioning of Chlorpyrifos between Water and an Aquatic Macrophyte (*Elodea Densa*). *Chemosphere.* **37** (8): 1579-1586.

KIDD H. & JAMES D.R. (1991) *The Agrochemical Handbook* (3rd Edition). Royal Society of Chemistry Information Services, Cambridge, UK.

KOCH H. & WEISSER P. (2001). Spray Deposits of Crop Protection Products on Plants - the Potential Exposure of Non-Target Arthropods. *Chemosphere.* **44**: 307-312.

KREUGER J. (1998). Pesticides in Stream Water within an Agricultural Catchment in Southern Sweden, 1990-1996. *The Science of the Total Environment.* **216**: 227-251.

KREUTZWEISER D.P. & SIBLEY P.K. (1991). Invertebrate Drift in a Headwater Stream Treated with Permethrin. *Archives of Environmental Contamination and Toxicology.* **20**: 330-336.

KUNGOLOS A., SAMARAS P, KIPOPOULOU A.M., ZOUMBOULIS A., & SAKELLAROPOULOS G.P. (1999) Interactive Toxic Effects of Agrochemicals on Aquatic Organisms. *Water, Science and Technology.* **40** (1): 357-364.

LAHR J., GADJI B. & DIA D. (2000). Predicted Buffer Zones to Protect Temporary Pond Invertebrates from Ground-Based Insecticide Applications against Desert Locusts. *Crop Protection.* **19** (7): 489-500.

LEONARD A.W., HYNE R.V., LIM R.P. & CHAPMAN J.C. (1999). Effect of Endosulfan Runoff from Cotton Fields on Macroinvertebrates in the Naomi River. *Ecotoxicology and Environmental Safety.* **42**: 125-134.

LEONARD A.W., HYNE R.V., LIM R.P., PABLO F. & VAN DEN BRINK P.J. (2000). Riverine Endosulfan Concentrations in the Namoi River, Australia: Link to Cotton Field Runoff and Macroinvertebrate Population Densities. *Environmental Toxicology and Chemistry.* **19** (6): 1540-1551.

LIESS M. & SCHULZ R. (1999). Linking Insecticide Contamination and Population Response in an Agricultural Stream. *Environmental Toxicology and Chemistry.* **18**: 1948-1955.

LIESS M., SCHULZ R. & NEUMANN M. (1996). A Method for Monitoring Pesticides Bound to Suspended Particles in Small Streams. *Chemosphere.* **32** (10): 1963-1969.

LINDERS J., MENSINK H., STEPHENSON G., WAUCHOPE D. & RACKE K. (2000). Foliar Interception and Retention Values after Pesticide Application. A Proposal for Standardized Values for Environmental Risk Assessment. *Pure and Applied Chemistry.* **72**: 2199-2218.

LOAGUE K., CORWIN D.L. & ELLSWORTH T.R. (1998). The Challenge of Predicting Nonpoint-source Pollution. *Environmental Science and Technology.* **32**: 130-133.

LONDON L., DALVIE M.A., CAIRNCROSS E. & SOLOMONS A. (2000). *The Quality of Surface and Groundwater in the Rural Western Cape with Regard to Pesticides.* Water Research Commission Report No. 795/1/00. Water Research Commission, Pretoria, South Africa.

LONGLEY M., CILGI T., JEPSON P.C. & SOTHERTON N.W. (1997). Measurements of Pesticide Spray Drift Deposition into Field Boundaries and Hedgerows: 1. Summer Applications. *Environmental Toxicology and Chemistry.* **16** (2): 165-172.

LONGLEY M. & SOTHERTON N.W. (1997). Measurements of Pesticide Spray Drift Deposition into Field Boundaries and Hedgerows: 2. Autumn Applications. *Environmental Toxicology and Chemistry.* **16** (2): 173-178.

LOWELL R.B., CULP J.M. & WRONA F.J. (1995). Toxicity Testing with Artificial Streams: Effects of Differences in Current Velocity. *Environmental Toxicology and Chemistry.* **14** (7): 1209-1217.

LUTZ W. (1984). Calculation of Stormwater Discharge Using Catchment Variables (in German). 24. Technical Report. Institute of Hydrology and Water Research, University of Karlsruhe, Karslruhe, Germany.

LYTLE J.S. & LYTLE T.F. (2002). Uptake and Loss of Chlorpyrifos and Atrazine by *Juncus Effusus* L. in a Mesocosm Study with a Mixture of Pesticides. *Environmental Toxicology and Chemistry.* **21** (9): 1817-1825.

MACKAY N., TERRY A., ARNOLD D. & PEPPER T. (2002). *Approaches and Tools for Higher Tier Assessment of Environmental Fate in the UK.* DEFRA Contract PL0546. Department of Environment, Food and Rural Affairs, UK.

MAGUIRE R.J. (1992) Aquatic Fate of Deltamethrin. *Water, Science and Technology.* **25**: 99-102.

MANDER Ü. (1989). Kompensationsstreifen Entlang Der Ufer Und Gewässerschutz. Landesamt für Wasserhaushalt und Küsten Schleswig-Holstein, Kiel, Germany.

MANIAK U. (1992). Regionalisation of Parameters for Stormwater Flow Curves. In: *Regionalization in Hydrolog, Vol 11 (in German)* (Kleeberg, H.B. ed.). Commission for Water, German Society for the Advancement of Sciences, Bonn, Germany.

MATTHEWS G.A. (1994). Pesticide Application in Relation to Integrated Pest Management. *Insect Science and its Application.* **15** (6): 599-604.

MEULEMANN A.F.M., BELTMAN B. & DE BRUIN H. (1990) The Use of Vegetated Ditches for Water Quality Improvement; A Tool for Nature Conservation in Wetland Areas. In: Constructed Wetlands in Water Pollution Control. Eds. Cooper P.F & and Findlater B.C. pp 599-602. Pergamon Press, Oxford, UK.

MITCHELL G.C., BENNETT D. & PEARSON N. (1993). Effects of Lindane on Macroinvertebrates and Periphyton in Outdoor Artificial Streams. *Ecotoxicology and Environmental Safety.* **25**: 90-102.

MITSCH W.J., CRONK J.K., WU X., NAIRN R.W. & HEY D.I. (1995). Phosphorus Retention in Constructed Freshwater Riparian Marshes. *Ecological Applications.* **5** (3): 830-845.

MOORE M.T., HUGGETT D.B., GILLESPIE JR. W.B., RODGERS JR. J.H. & COOPER C.M. (1998). Comparative Toxicity of Chlordane, Chlorpyrifos, and

Aldicarb to Four Aquatic Testing Organisms. *Archives of Environmental Contamination and Toxicology.* **34** (2): 152-157.

MOORE M.T., RODGERS JR. J.H., COOPER C.M. & SMITH S. (2000). Constructed Wetlands for Mitigation of Atrazine-Associated Agricultural Runoff. *Environmental Pollution.* **110** (3): 393-399.

MOORE M.T., BENNETT E.R., COOPER C.M., SMITH JR. S., SHIELDS JR. F.D., MILAM C.D. & FARRIS J.L. (2001). Transport and Fate of Atrazine and Lambda-Cyhalothrin in Agricultural Drainage Ditches: A Case Study for Mitigation. *Agriculture Ecosystems and Environment.* **87**: 309-314.

MOORE M.T., SCHULZ R., COOPER C.M., SMITH JR. S. & RODGERS JR. J.H. (2002). Mitigation of Chlorpyrifos Runoff Using Constructed Wetlands. *Chemosphere.* **46**: 827-835.

MUIR D.C.G., RAWN G.P., TOWNSEND B.E., LOCKHART W.L. & GREENHALGH R. (1985). Bioconcentration of Cypermethrin, Deltamethrin, Fenvalerate and Permethrin by *Chironomus Tentans* Larvae in Sediment and Water. *Environmental Toxicology and Chemistry.* **4**: 51-61.

NCAPF (1997). *National Pesticide Use Database.* National Center for Food and Agricultural Policy, Washington, D.C., USA.

NEWCOMBE C.P. & MACDONALD D.D. (1991). Effects of Suspended Sediments on Aquatic Ecosystems. *North American Journal of Management.* **11**: 72-82.

OECD (1999). *Report of the OECD Project Pesticide Aquatic Risk Indicators -Report of Phase 1. - 2nd OECD Workshop on Pesticide Risk Indicators.* Technical Report. Braunschweig, Germany.

PALMER G.C. & SCHERMAN P.A. (2000). *Application of an Artificial Stream System to Investigate the Water Quality Tolerances of Indigenous, South African, Riverine Macroinvertebrates.* Water Research Comission Report No 686/1/00. Water Research Commission, Pretoria, South Africa.

PATTY L., REAL B. & GRIL J.J. (1997). The Use of Grassed Buffer Strips to Remove Pesticides, Nitrate and Soluble Phosphorus Compounds from Runoff Water. *Pesticide Science.* **49**: 243-251.

PEREIRA W., DOMAGALSKI J.L., HOSTETTLER F.D., BROWN L.R. & RAPP J.B. (1996). Occurrence and Accumulation of Pesticides and Organic Contaminants in River Sediment, Water and Clam Tissues from the San Joaquin River and Tributaries, California. *Environmental Toxicology and Chemistry.* **15** (2): 172-180.

PFLEEGER T.G., FONG A., HAYES R., RATSCH H. & WICKLIFF C. (1996). Field Evaluation of the EPA (Kenaga) Nomogram, a Method for Estimating Wildlife Exposure to Pesticide Residues on Plants. *Environmental Toxicology and Chemistry.* **15** (4): 535-543.

POFF N.L., DECINO R.D. & WARD J.V. (1991). Size-Dependent Drift Responses of Mayflies to Experimental Hydrologic Variation: Active Predator Avoidance or Passive Hydrodynamic Displacement? *Oecologia.* **88** (4): 577-586.

PONTASCH K.W. & CAIRNS J. (1991). Multispecies Toxicity Tests Using Indigenous Organisms - Predicting the Effect of Complex Effluents in Streams. *Archives of Environmental Contamination and Toxicology.* **20** (1): 103-112.

RAUTMANN D., STRELOKE M. & WINKLER R (2001). New basic drift values in the authorization procedure for plant protection products. *Mitt. Biol. Bundesanst.* Land- Forstwirtsch. No. 383. Berlin

REINERT K.H., GIDDINGS J.M. & JUDD L. (2002). Effects Analysis of Time-Varying or Repeated Exposures in Aquatic Ecological Risk Assessment of Agrochemicals. *Environmental Toxicology and Chemistry.* **21** (9): 1977-1992.

REUS J., LENNDERTSE C., BOCKSTALLER C., FOMSGAARD I., GUTSCHE V., LEWIS K., NILSSON C., PUSSEMIER L., TREVISAN M., VAN DER WERF H., ALFARROBA F., BLUEMEL S., ISART J., MC GRATH D. & SEPPAELAE T. (1999). Comparing Environmental Risk Indicators for Pesticides. Results of the European CAPER Project, CLM 426. Centre for Agriculture and Environment, Utrecht, The Netherlands.

RODGERS JR. J.H. & DUNN A. (1992). Developing Design Guidelines for Constructed Wetlands to Remove Pesticides from Agricultural Runoff. *Ecological Engineering.* **1**: 83-95.

ROUX D.J., BADENHORST J.E., DU PREEZ H.H. & STEYN G.J. (1994). Note on the Occurrence of Selected Trace Metals and Organic Compounds in Water, Sediment and Biota of the Crocodile River, Eastern Transvaal, South Africa. *Water SA, Pretoria.* **20** (4): 333-340.

RUNES H.B., BOTTOMLEY P.J., LERCH R.N. & JENKINS J.J. (2001). Atrazine Remediation in Wetland Microcosms. *Environmental Toxicology and Chemistry.* **20** (5): 1059-1066.

SAND-JENSEN K. (1998). Influence of Submerged Macrophytes on Sediment Composition and near-Bed Flow in Lowland Streams. *Freshwater Biology.* **39** (4): 663-679.

SCHAUBER E.M., EDGE W.D. & WOLFF J.O. (1995). Influence of Vegetation Height on the Distribution and Persistence of Insecticide Residues on Alfalfa and Soil. *Archives of Environmental Contamination and Toxicology.* **29** (4): 449-454.

SCHIAVON M., PERRIN-GANIER C. & PORTAL J.M. (1995). The Pollution of Water by Pesticides: State and Origin. *Agronomy.* **15**: 157-170.

SCHULZ R. (2001a). Rainfall-Induced Sediment and Pesticide Input from Orchards into the Lourens River, Western Cape, South Africa: Importance of a Single Event. *Water Research.* **35**: 1869-1876.

SCHULZ R. (2001b). Comparison of Spraydrift- and Runoff-Related Input of Azinphos-Methyl and Endosulfan from Fruit Orchards into the Lourens River, South Africa. *Chemosphere.* **45**: 543-551.

SCHULZ R. (2003). Using a Freshwater Amphipod In-Situ Bioassay as a Sensitive Tool to Detect Pesticide Effects in the Field. *Environmental Toxicology and Chemistry.* **22** (5): 1172-1176.

SCHULZ R., HAUSCHILD M., EBELING M., NANKO-DREES J., WOGRAM J. & LIESS M. (1998). A Qualitative Method for Monitoring Pesticides in the Edge-of-field Runoff. *Chemosphere.* **36** (15): 3071-3082.

SCHULZ R. & LIESS M. (1999a). A Field Study of the Effects of Agriculturally Derived Insecticide Input on Stream Macroinvertebrate Dynamics. *Aquatic Toxicology.* **46**: 155-176.

SCHULZ R. & LIESS M. (1999b). Validity and Ecological Relevance of an Active In-situ Bioassay Using *Gammarus pulex* and *Limnephilus lunatus*. *Environmental Toxicology and Chemistry*. **18**: 2243-2250.

SCHULZ R. & DABROWSKI J.M. (2001). Combined Effects of Predatory Fish and Sub-lethal Pesticide Contamination on the Behaviour and Mortality of Mayfly Nymphs. *Environmental Toxicology and Chemistry*. **20**: 2537-2543.

SCHULZ R. & LIESS M. (2001a). Toxicity of Aqueous-Phase and Suspended Particle-Associated Fenvalerate: Chronic Effects Following Pulse-Dosed Exposure of *Limnephilus lunatus* (Trichoptera). *Environmental Toxicology and Chemistry*. **20** (1): 185-190.

SCHULZ R. & LIESS M. (2001b). Runoff Simulation with Particle-Bound Fenvalerate in Multispecies Stream Microcosms: Importance of Biological Interactions. *Environmental Toxicology and Chemistry*. **20**: 757-762.

SCHULZ R. & LIESS M. (2001c). Acute and Chronic Effects of Particle-Associated Fenvalerate on Stream Macroinvertebrates: A Runoff Simulation Study Using Outdoor Microcosms. *Archives of Environmental Contamination and Toxicology*. **40**: 481-488.

SCHULZ R. & PEALL S.K.C. (2001). Effectiveness of a Constructed Wetland for Retention of Nonpoint-Source Pesticide Pollution in the Lourens River Catchment, South Africa. *Environmental Science and Technology*. **35** (2): 422-426.

SCHULZ R., PEALL S.K.C., DABROWSKI J.M. & REINECKE A.J. (2001a). Current-Use Insecticides, Phosphates and Suspended Solids in the Lourens River, Western Cape, During the First Rainfall Event of the Wet Season. *Water South Africa*. **27** (1): 65-70.

SCHULZ R., PEALL S.K.C., DABROWSKI J.M. & REINECKE A.J. (2001b). Spray Deposition of Two Insecticides into Surface Waters in a South African Orchard Area. *Journal of Environmental Quality*. **30** (3): 814-822.

SCHULZ R., PEALL S.K.C., HUGO C. & KRAUSE V. (2001c). Concentration, Load and Toxicity of Spraydrift-Borne Azinphos-Methyl at the Inlet and Outlet of a Constructed Wetland. *Ecological Engineering*. **18**: 239-245.

SCHULZ R., THIERE G. & DABROWSKI J.M. (2002). A Combined Microcosm and Field Approach to Evaluate the Aquatic Toxicity of Azinphos-Methyl to Stream Communities. *Environmental Toxicology and Chemistry.* **21** (10): 2172-2178.

SCHULZ R., HAHN C., BENNETT E.R., DABROWSKI J.M., THIERE G. & PEALL S.K.C. (2003a). Fate and Effects of Azinphos-Methyl in a Flow-Through Wetland in South Africa. *Environmental Science and Technology.* **37**: 2139-2144.

SCHULZ R., MOORE M.T., BENNETT E.R., FARRIS J.L., SMITH JR. S. & COOPER C.M. (2003b). Methyl Parathion Toxicity in Vegetated and Non-vegetated Wetland Microcosms. *Environmental Toxicology and Chemistry.* **22** (6): 1262-1268.

SHERRARD R.M., BEARR J.S., MURRAY-GULDE C.L., RODGERS JR., J.H. & SHAH Y.T. (2004). Feasibility of Constructed Wetlands for Removing Chlorothalonil and Chlorpyrifos from Aqueous Mixtures. *Environmental Pollution.* **127** (3): 385-394.

SIEBERS J., BINNER R. & WITTICH K.-P. (2003). Investigation on Downwind Short-Range Transport of Pesticides after Application in Agricultural Crops. *Chemosphere.* **51** (5): 397-407.

SIEGFRIED B.D. (1993). Comparative Toxicity of Pyrethroid Insecticides to Terrestrial and Aquatic Insects. *Environmental Toxicology and Chemistry.* **12**: 1683-1689.

SOLOMON K.R., BAKER D.B., RICHARDS R.P., DIXON K.R., KLAINE S.J., LA-POINT T.W., KENDALL R.J., WEISSKOPF C.P., GIDDINGS J.M., GIESY J.P., HALL L.W.J. & WILLIAMS W.M. (1996). Ecological Risk Assessment of Atrazine in North American Surface Waters. *Environmental Toxicology and Chemistry.* **15** (1): 31-76.

SPATZ R., WALKER F. & HURLE K. (1997). Effect of Grass Buffer Strips on Pesticide Runoff under Simulated Rainfall. *Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent.* **62** (3A): 799-806.

SQUILLACE P.J., CALDWELL J.P., SCHULMEYER P.M. & HARVEY C.A. (1996). Movement of Agricultural Chemicals between Surface Water and Ground Water, Lower Cedar River Basin, Iowa. In: *United States Geological Survey Water-Supply Paper 2448.* United States Government Printing Office, Washington.

STOTTMEISTER U., WIESSNER A., KUSCHK P., KAPPELMEYER U., KASTNER M., BEDERSKI O., MULLER R.A. & MOORMANN H. (2003). Effects of Plants and Microorganisms in Constructed Wetlands for Wastewater Treatment. *Biotechnology Advances.* **22** (1-2 SU -): 93-117.

STRELOKE M. & BROWN C. (2003). Risk Mitigation Measures to Protect Non-Target Life in the Frame of the Authorization of Pesticides. In: *Proceedings of the XII Symposium Pesticide Chemistry.* Eds. A.A M. Del Re, E. Capri, L. Padovani and M. Trevisan, June 4-6, 2003, Piacenza, Italy.

SUNDERAM R.I.M., THOMPSON G.B., CHAPMAN J.C. & CHENG D.M.H. (1994). Acute and Chronic Toxicity of Endosulfan to Two Australian Cladocerans and Their Applicability in Deriving Water Quality Criteria. *Archives of Environmental Contamination and Toxicology.* **27** (4): 541-545.

TANNER C.C., CLAYTON J.S. & UPSDELL M.P. (1995). Effect of Loading Rate and Planting on Treatment of Dairy Farm Wastewaters in Constructed Wetlands .1. Removal of Oxygen Demand, Suspended Solids and Faecal Coliforms. *Water Research.* **29** (1): 17-26.

TANNER D.K. & KNUTH M.L. (1995). Effects of Azinphos-Methyl on the Reproductive Success of the Bluegill Sunfish, *Lepomis macrochirus*, in Littoral Enclosures. *Ecotoxicology and Environmental Safety.* **32**: 184-193.

THARME R., RATCLIFFE G. & DAY E. (1997). *An Assessment of the Present Ecological Condition of the Lourens River, Western Cape, with Particular Reference to Proposals for Stormwater Management.* Freshwater Research Unit, University of Cape Town, Cape Town, South Africa.

THOMPSON H.M. (1996). Interactions between Pesticides; A Review of Reported Effects and their Implications for Wildlife Risk Assessment. *Ecotoxicology.* **5:** 59-81.

TOURAT L.W. & SLIMAK M. (1989). Mesocosm Approach for Assessing the Ecological Risk of Pesticides. *Miscellaneous Publications of the Entomological Society of America.* **75** (0): 33-40.

TYLER C.R., BERESFORD N., WONING M.V.D., SUMPTER J.P. & THORPE K. (2000). Metabolism and Environmental Degradation of Pyrethroid Insecticides

Produce Compounds with Endocrine Activities. *Environmental Toxicology and Chemistry.* **19** (4): 801-809.

UCAR T. & HALL F.R. (2001). Windbreaks as a Pesticide Drift Mitigation Strategy: A Review. *Pest Management Science.* **57** (8): 663-675.

USEPA (1988). Pesticides: Water Quality Standards Criteria Summaries: A Compilation of State/Federal Criteria. United States Environmental Protection Agency No. 440/5-88/021, Duluth, MN, USA.

USEPA (1999). Background Document for the Scientific Advisory Panel on Orchard Airblast: Downwind Deposition Tolerance Bounds for Orchards. Technical Report. United States Environmental Protection Agency, Washington, DC, USA.

UUSI-KÄMPPÄ J., BRASKERUD B., JANSSON H., SYVERSEN N. & UUSITALO R. (2000). Buffer Zones and Constructed Wetlands as Filters for Agricultural Phosphorus. *Journal of Environmental Quality.* **29** (1): 151-158.

VAN DEN BRINK P.J., DONK E.V., GYLSTRA R., CRUM S.J.H. & BROCK T.C.M. (1995). Effects of Chronic Low Concentrations of the Pesticides Chlorpyrifos and Atrazine in Indoor Freshwater Microcosms. *Chemosphere.* **31** (5): 3181-3200.

VERRO R., CALLIERA M., MAFFIOLI G., AUTERI D., SALA S., FINIZIO A. & VIGHI M. (2002). GIS-Based System for Surface Water Risk Assessment of Agricultural Chemicals. 1. Methodological Approach. *Environmental Science and Technology.* **36**: 1532-1538.

WALLACE J.B., LUGTHART G.J., CUFFNEY T.F. & SCHURR G.A. (1989). The Impact of Repeated Insecticidal Treatments on Drift and Benthos of a Headwater Stream. *Hydrobiologia.* **179**: 135-147.

WAN M.T., SZETO S.Y. & PRICE P. (1995). Distribution and Persistence of Azinphos-Methyl and Parathion in Chemigated Cranberry Bogs. *Journal of Environmental Quality.* **24** (4): 589-596.

WAUCHOPE R.D. (1978). The Pesticide Content of Surface Water Draining from Agricultural Fields - a Review. *Journal of Environmental Quality.* **7**: 459-472.

WERNER R.A. & HILGERT J.W. (1992). Effects of Permethrin on Aquatic Organisms in a Freshwater Stream in South-Central Alaska. *Journal of Economic Entomology.* **85** (3): 860-864.

WILCOX B.P. & WOOD M.K. (1989). Factors Influencing Interrill Erosion from Semiarid Slopes in New Mexico (USA). *Journal of Range Management.* **42** (1): 66-70.

WILEY M.J. & KOHLER S.L. (1980). Positioning Changes of Mayfly Nymphs Due to Behavioural Regulation of Oxygen Consumption. *Canadian Journal of Zoology.* **58**: 618-622.

WILLIAMS D.D. (1990). A Field Study of the Effects of Water Temperature, Discharge and Trout Odour on the Drift of Stream Invertebrates. *Archiv für Hydrobiologie.* **119** (2): 167-181.

WILLIAMS P., WHITFIELD M., BIGGS J., BRAY S., FOX G., NICOLET P. & SEAR D. (2004). Comparative Biodiversity of Rivers, Streams, Ditches and Ponds in an Agricultural Landscape in Southern England. *Biological Conservation.* **115** (2): 329-341.

WILLIAMS R.J., BROOKE D., MATTHIESEN P., MILLS M., TURNBULL A. & HARRISON R.M. (1995). Pesticide Transport to Surface Waters within an Agricultural Catchment. *Journal of the Institution of Water and Environmental Management.* **9**: 72-81.

WOIN P. (1998). Short- and Long-Term Effects of the Pyrethroid Insecticide Fenvalerate on an Invertebrate Pond Community. *Ecotoxicology and Environmental Safety.* **41** (2): 137-156.