Fate and Effects of Insecticides in Vegetated Agricultural Drainage Ditches and Constructed Wetlands: A Valuable Approach in Risk Mitigation

Verbleib und Wirkung von Insektiziden in landwirtschaftlichen Entwässerungsgräben und künstlichen Feuchtgebieten: Ein wertvoller Ansatz zur Risikominimierung

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ABSTRACT

Studies have shown that runoff and spray-drift are important sources of nonpoint-source pesticide pollution of surface waters. Owing to this, public concern over the presence of pesticides in surface and ground water has resulted in intensive scientific efforts to find economical, yet environmentally sound solutions to the problem. Implementation of best management practices (BMPs), such as vegetated drainage ditches and constructed wetlands, have been proposed to retain/reduce nonpoint-source pollution from entering receiving aquatic habitats. The primary objective of this research was to assess the effectiveness of vegetated aquatic systems in providing buffering between natural aquatic ecosystems and agricultural landscape following insecticide associated runoff and spray-drift events. Multiple studies were designed using drainage ditches and constructed wetlands to test this objective. Prior to the initiation of the field studies, an efficient multi-residue analytical method for insecticides in plants and sediments was developed and validated for the extraction and analysis of the synthetic pyrethroids, bifenthrin, lambda-cyhalothrin, and the organophosphate, methyl parathion (MeP). Extraction methods utilized ultra-sonication, while extracts were analyzed by GC-μECD. This method was later adapted for the extraction and analysis azinphos-methyl (AZP). The first set of studies were implemented using vegetated agricultural ditches, one in Mississippi, USA, using the pyrethroids (bifenthrin, lambda-cyhalothrin) under simulated runoff conditions and the other in the Western Cape, South Africa using the organophosphate insecticide AZP, under natural runoff and spray-drift conditions. The second set of studies were implemented using constructed wetlands, one in the Western Cape using AZP under natural spray-drift conditions and the other in Mississippi, USA using the organophosphate MeP under simulated runoff conditions. The wetland study in Mississippi was the most complex study because it tested the mitigative

effectiveness of both vegetated and non-vegetated wetland systems in reducing loadings of MeP following a simulated runoff event using multiple tests. In addition, macroinvertebate communities were assessed before and after runoff, and in situ bioassays using *Chironomus tentans* (Insecta), and lab based aquatic and sediment toxicity tests using *Ceriodaphnia dubia* (Crustacea) and *Pimephales promelas* (Pisces) were performed.

Results from the Mississippi-ditch study indicated that ditch lengths of less than 300 m would be sufficient to mitigate bifenthrin and lambda-cyhalothrin. In addition, data from mass balance calculations determined that the ditch plants were the major sink (generally > 90%) and/or sorption site for the rapid dissipation of the above pyrethroids from the water column. Similarly, results from the ditch study in South Africa showed that a 180 m vegetated system was effective in mitigating AZP after natural spray drift and low flow runoff events. The system was less effective in mitigating AZP when flow rates were high following a runoff event caused by increased precipitation. Analytical results from the first wetland study show that the vegetated wetland was more effective than the non-vegetated wetland in reducing loadings of MeP. Mass balance calculations indicated approximately 90% of MeP mass was associated with the plant compartment. Moreover, it was calculated that a vegetated wetland length of 18.8 m would have been adequate to reduce methyl parathion concentrations to 0.1 % of the inflow concentration, while a non-vegetated wetland length of 62.9 m would be required for a similar reduction in MeP concentration. Ninety-six hours after the contamination, a significant negative acute effect of contamination on abundances was found in 8 out of the 15 macroinvertebrate species in both wetland systems. Even with these toxic effects, the overall reaction of macroinvertebrates clearly demonstrated that the impact of MeP in the vegetated wetland was considerably lower than in the non-vegetated wetland. Furthermore, results from both the in situ bioassays and lab

based toxicity testing indicated that the majority of toxicity was measured within the first 10 m of the vegetated wetland while it was found throughout the un-vegetated wetland (>40 m). Results from the constructed wetland study in South Africa revealed that concentrations of AZP at the inlet of the 134 m wetland system were reduced by 90% at the outlet. Mass balance calculations showed that 10.5% of the total mass of AZP associated with plants was retained in the wetland. Since plants were only sampled at two sites, and senesced plant matter and other plant species were not collected, the actual AZP associated mass maybe underestimated. Regardless, results indicate that the plant compartment plays an important role in the mitigative capacity of this wetland system either as a sorptive surface or in retaining compound allowing for increased volatilization, photolysis, hydrolysis or metabolic degradation. Overall, results from all of the studies in this thesis indicate that the presence of the plant compartment was essential for the effective mitigation of insecticide contamination introduced after both simulated and natural runoff or spray-drift events. Finally, both types of BMPs, vegetated drainage ditches and vegetated constructed wetlands, studied would be effective in mitigating pesticide loadings introduced from either runoff or spray-drift, in turn lowering or eliminating potential pesticide associated toxic effects in receiving aquatic ecosystems. Data produced in this research thus provide important information to reduce insecticide risk in exposure assessment scenarios. It should be noted that incorporating these BMPs will decrease the risk of acute toxicity, but chronic exposure may still be an apparent overall risk.

Zusammenfassung

Mehrere Untersuchungen haben gezeigt, daß Oberflächen-Runoff und Sprühabdrift wichtige Eintragsquellen diffuser Pestizideinträge in Gewässer darstellen. Darauf basierende Unsicherheiten haben zu intensiven wissenschaftlichen Anstrengungen hinsichtlich ökonomischer und umweltfreundlicher Lösungsstrategien geführt. Verschiedene Varianten "Guter Landwirtschaftlicher Praxis", wie vegetationsreiche Gräben oder künstliche Feuchtgebiete wurden zum Rückhalt diffuser Stoffeinträge in Gewässer vorgeschlagen. Die Hauptzielsetzung dieser Untersuchung war die Beurteilung der Effektivität vegetationsreicher aquatischer Habitate als Puffer zwischen Gewässern und landwirtschaftlichen Flächen während Runoff oder Abdrift von Insektiziden. Entsprechend wurden verschiedene Studien durchgeführt. Zunächst wurde eine effektive Methode zur Extraktion und Analytik von Insektiziden in Pflanzen und Sedimenten entwickelt und validiert, wobei die synthetischen Pyrethroide Bifenthrin und Lambda-Cyhalothrin, und das Organophosphat Methyl-Parathion (MeP) verwendet wurden. Die Extraktion erfolgte mit Ultraschall, während die Extrakte mittels GC-µECD analysiert wurden. Diese Methode wurde später für die Extraktion- und Analyse von Azinphos-Methyl (AZP) angepasst. Die ersten Studien fanden in vegetationsreichen Gräben in Mississippi, USA mit Pyrethroiden (Bifenthrin, Lambda-Cyhalothrin) unter experimentellen Runoffbedingungen und im Western Cape, Südafrika mit dem Organophosphatinsektizid AZP unter natürlichen Runoff- und Abdrift-Situationen statt. Der zweite Teil von Studien wurde in künstlichen Feuchtgebieten, im Western Cape mit AZP unter natürlichen Abdrift-Situationen und in Mississippi mit MeP unter experimentellen Runoffbedingungen durchgeführt. Diese letztgenannte Studie in Mississippi war sehr komplex, da sie vegetationsreiche und vegetationslose Feuchtgebiete vergleichend hinsichtlich des Rückhaltes von MeP mit Hilfe

verschiedener Tests gleichzeitig untersuchte. Zusätzlich wurden die Makroinvertebaten-Gemeinschaften vor und nach dem simulierten Runoff, in-situ exponierte *Chironomus tentans* (Insecta), und Wasser- und Sedimenttests mit *Ceriodaphnia dubia* (Crustacea) und *Pimephales promelas* (Pisces) im Labor durchgeführt.

Die Ergebnisse der Studie in den Gräben in Mississippi zeigten an, dass Längen von weniger als 300 m ausreichen, um die Konzentrationen von Bifenthrin und Lambda-Cyhalothrin deutlich zu verringern. Durch Massenbilanzberechnungen konnte gezeigt werden, dass Pflanzen als wichtigste Senke (im Allgemeinen > 90%) und/oder der wichtigste Sorptionsort für die schnelle Verminderung der oben genannten Pyrethroide aus der Wasserphase verantwortlich waren. In ähnlicher Weise zeigten die Resultate der Studie in Südafrika, dass ein 180 m langer vegetationsreicher Graben wirkungsvoll die AZP-Konzentrationen nach Abdrift und Runoff verringerte. Dieses System war weniger wirkungsvoll, bei höheren Strömungsgeschwindigkeiten infolge stärkerer Niederschläge. Die analytischen Ergebnisse der Studie in Feuchtgebieten zeigten, dass vegetationsreiche Feuchtgebiete wirkungsvoller die MeP-Konzentrationen verringerten als vegetationslose Systeme. Massenbilanzberechnungen zeigten, dass etwa 90% des MeP an den Pflanzen gebunden wurde. Außerdem zeigte sich, dass eine Feuchtgebietslänge von 18,8 m im vegetationsreichen System ausreichend ist, um die MeP-Konzentration auf 0.1% der Ausgangskonzentration zu verringern, während eine Feuchtgebietslänge von 62,9 m im vegetationslosen System für den gleichen Effekt notwendig wäre. Sechsundneunzig Stunden nach der Belastung, wurde ein signifikanter negativer Effekt auf die Abundanzen von 8 der 15 Makroinvertebraten-Taxa in beiden Feuchtgebieten festgestellt. Trotz dieser toxischen Effekte, war die generelle Reaktion der Makroinvertebraten auf MeP im vegetationsreichen System wesentlich schwächer als im vegetationslosen System. Zusätzlich wiesen die Ergebnisse der in

situ und der Laborstudien im vegetationsreichen System eine Toxizität auf den ersten 10 m und im vegetationslosen System auf der Gesamtstrecke von mehr als 40 m nach. Die Ergebnisse der Feuchtgebietsstudie in Südafrika zeigten, dass die Konzentrationen von AZP am Auslauf des 134 m langen Systems um 90% gegenüber denen am Einlauf verringert waren. Massenbilanzberechnungen ergaben, dass 10,5% der Gesamtmasse von AZP im Feuchtgebiet von den Pflanzen zurückgehalten wurden. Da die Pflanzen nur an zwei Stationen beprobt und altes Pflanzenmaterial sowie weitere Arten nicht einbezogen wurden, könnte die tatsächliche AZP-Masse in den Pflanzen möglicherweise unterschätzt worden sein. Nichtsdestotrotz spielen die Pflanzen eine wichtige Rolle für die Risikominimierung einerseits als sorptive Oberfläche und andererseits durch den Rückhalt und das damit verbundene erhöhte Potential für Verflüchtigung, Photolyse, Hydrolyse oder metabolischen Abbau.

Insgesamt zeigen die Ergebnisse aller hier beschriebenen Studien, dass das Pflanzenkompartiment für die wirkungsvolle Risikominimierung von Insektizidbelastungen infolge simulierter und natürlicher Runoff- und Abdrift-Situationen wesentlich war. Außerdem stellten sich beide Risikominimierungsverfahren, vegetationsreiche Gräben und künstliche Feuchtgebiete als geeignet für die Reduktion von Pestizidbelastungen und resultierenden toxischen Effekten heraus. Die Ergebnisse sind somit für Reduktion des Risikos von Insektiziden in der Expositionsabschätzung von Bedeutung. Obgleich die akute Toxizität vermindert wird, könnten jedoch nach wie vor chronische Effekte auftreten.

1 INTRODUCTION

Over the past few decades, pesticide application has become an integral component of modern agricultural practices. Due to their importance in efficient food production, high rates of pesticides are used worldwide. Even though their use is highly beneficial, their effects may be less than desirable when they leave the target compartments of the agricultural ecosystem. Any unintended loss of pesticide is not only wasteful, but also represents a reduced efficiency and incurs increased costs to the user and the nontarget environment (Bowles and Webster, 1995; Falconer, 1998). Nonpoint-source agricultural pollution is generally considered one of the major threats to surface water quality in rural areas (Loague et al., 1998; Gangbazo et al., 1999). Spraydrift and edge-of-field runoff are the most important routes of entry for agricultural nonpoint source insecticide pollution into surface waters (Groenendijk et al., 1994). Spraydrift and edge-of-field runoff may differ considerably (Erstfield, 1999), where spraydrift leads to an input of pesticides dissolved in the water phase and runoff leads to an input of pesticides associated with suspended particles (Mian and Mulla, 1992).

When rivers, streams, and lakes adjacent to agricultural areas receive runoff following rainfall events and pesticide loading from spray-drift events, an increased potential for damage to water resources, such as increased sedimentation or fish kills, may occur. These episodic pollution events can lead to a short-term contamination of aquatic ecosystems with pesticides (Baughmanet al., 1989; Kreuger, 1995; Liess and Schulz, 1999; Schulz et al., 1998; Schulz and Liess, 1999). Sub-lethal concentrations of potential agricultural contaminants may affect growth, reproduction, behavior, physiology, and long-term survival of aquatic flora and fauna (Anderson and Zeeman, 1995; Rice et al., 1997). Moreover, this is especially relevant for insecticides due

to their relatively high toxicity to fauna in the aquatic environment (Siegfried, 1993; Brock et al., 2000).

Owing to this, public concern over the presence of pesticides in surface waters has resulted in intensive scientific efforts to find economical, yet environmentally sound solutions to the problem. Efforts have been made by government agencies and farmers to develop innovative best management practices (BMPs) to decrease the effects of pesticide associated spray-drift events and storm runoff events containing nutrients, bacteria, sediment and pesticides. BMPs, such as, grass filter strips, hooded sprayers, conservation tillage, slotted board risers and winter cover crops have been developed to decrease the amount of potential agricultural contaminants leaving fields (Caruso, 2000; Cooper et al., 2004). Another effective BMP that is currently used is the implementation of constructed wetlands. Wetlands serve as transitional zones between terrestrial and aquatic systems (Mitsch and Gooselink, 1993). They have several physical, chemical, and biological uptake and degradative processes useful for treatment of point and non-point source pollution (Wolverton and Harrison, 1973; Nichols, 1983; Catallo 1993). One reason that wetlands are so effective is due to the presence of aquatic plants. According to Luckeydoo et al. (2002), the vital role of vegetation in processing water passing through wetlands is accomplished through biomass nutrient storage, sedimentation, and by providing unique microhabitats for beneficial microorganisms. Macrophytes serve as filters by allowing contaminants to flow into plants and stems, which are then sorbed to macrophyte biofilms (Headley et al., 1998; Kadlec and Knight, 1996). According to Zablotowicz and Hoagland (1999), whether or not plants are capable of transferring contaminants from environmental matrices depends upon several factors including contaminant chemistry, plant tolerance to the contaminant, and sediment surrounding the plant (e.g. pH, redox, clay content). Several studies

have shown the capacity of vegetated wetlands to mitigate pollutants such as metals, industrial/municipal wastewater, dairy wastes, urban stormwater, road runoff, and petroleum products (Livingston, 1989; Jackson, 1989; Nix et al., 1994; Scholes et al., 1998; Osterkamp et al, 1999; Matsin et al., 2001; Birch et al., 2004). Prior to the work in this thesis, studies were focused on examining the fate and effects of specific herbicide exposures in vegetated constructed wetlands (Rodgers and Dunn, 1992; Lee, 1995; Moore et al., 2000). Moreover, there was little research on the fate and effects of insecticides in these systems except for some research in South Africa that was occurring at that time (Schulz and Peall, 2001a; Schulz et al., 2001b). Constructed vegetated wetlands are sometimes not an option in agricultural row-crop farming mainly due to space limitations, thus vegetated drainage ditches have been suggested as a simple alternate BMP.

Drainage ditches are an essential component of the agricultural production landscape. Most agricultural fields are surrounded by a network of ditches to facilitate field drainage and to reduce flooding on production acreage. In the past, the value and function of such marginal land has been generally ignored; however, due to their crucial role in transfer/transformation of contaminants (nutrients, sediment, pesticides) more research is required to assess the intricacies of drainage ditches for mitigation purposes. Historically, drainage ditches have been dredged to remove built up sediment and aquatic plants that impede efficient drainage. It has been proposed that the colonization of aquatic plants in these ditches may provide a cheap environmentally sound mitigation measure. Few studies have focused on the capacity of drainage ditches as a BMP to decrease the concentration of pesticides entering receiving aquatic ecosystems (Crum et al., 1998; Moore et al., 2001; Cooper et al., 2002). More research has been conducted in the Netherlands, where drainage ditches serve as important habitat and potential transports for

drinking water (Van Strien et al., 1989; Van Strien et al., 1991; Meuleman and Beltman B, 1993; Crum et al., 1998). Many of these studies focused on ditch maintenance and management practices, since the use of ditches is more restrictive in the Netherlands than in the United States. Overall, there has been little research involving vegetated constructed wetlands and vegetated agricultural drainage ditches on their overall capacity as a mitigative tool in reducing pesticide loading following runoff and spraydrift events. Since these systems are transitional zones between agricultural landscape and natural aquatic ecosystems, their effectiveness as a BMP will be assessed by the objectives put forth below.

2 OBJECTIVES

- The primary objective of this research was to assess the effectiveness of vegetated aquatic systems in providing buffering between natural aquatic ecosystems and agricultural landscape following insecticide associated runoff and spray-drift events. In order to accomplish this primary objective, the following sub-objectives were established:

    - To develop an efficient analytical method for the extraction and analysis of multiple classes of pesticides in water, plants and sediments (Appendix 11.1).

    - To assess the effectiveness of agricultural vegetated drainage ditches for insecticide mitigation (Appendix 11.2, 11.3)

    - To assess the effectiveness of agricultural vegetated constructed wetlands for insecticide mitigation (Appendix 11.4, 11.7).

- To assess the effectiveness of these systems in reducing aquatic toxicity using invertebrate sampling, in situ and lab based toxicity testing (Appendix 11.5, 11.6, 11.7).

- To assess toxicity test results for validation of measured analytical results.

3 PHYSICAL AND CHEMICAL PROPERTIES

Before one can consider the development of analytical methods, environmental fate and/or aquatic toxicity of a chemical it is important to take into account the physical/chemical properties of the given chemical(s). In this thesis, two types of current use pesticides were used during the field exposures, two synthetic pyrethroids and two organophosphate insecticides (Figure 3.0). The pyrethroids bifenthrin [(2-methyl-2-methylbiphenyl-3-ylmethyl(Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclo-propane-carboxylate] and lambda-cyhalothrin (a 1:1 mixture of (S)-a-cyano-3-phenoxybenzyl -(Z)-(1R,3R)-3- (2-chloro-3,3,3-trifluoroprop-1-enyl) -2,2- dimethylcyclopropane carboxylate and (R)-a-cyano-3-phenoxybenzyl-(Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropanecarboxylate [(RS)-alpha-cyano-3-phenoxybenzyl3-(2-chloro-3,3,3 trifluror-propenyl) -2,2 dimethylcyclopropane carboxylate] and the organophosphate insecticides methyl parathion (O,O-dimethyl O-(4-nitrophenol) phosphorothioate) and azinphos-methyl (S-(3,4-dihydro-4-oxobenzo[d]-[1,2,3]-triazin-3-ylmethyl) O,O-dimethyl phosphordithioate) were used. The physical and chemical properties of these two types of compounds are quite different (Table 3.0) indicating that their behavior in the environment will vary between each class as well as each other. In understanding the fate of

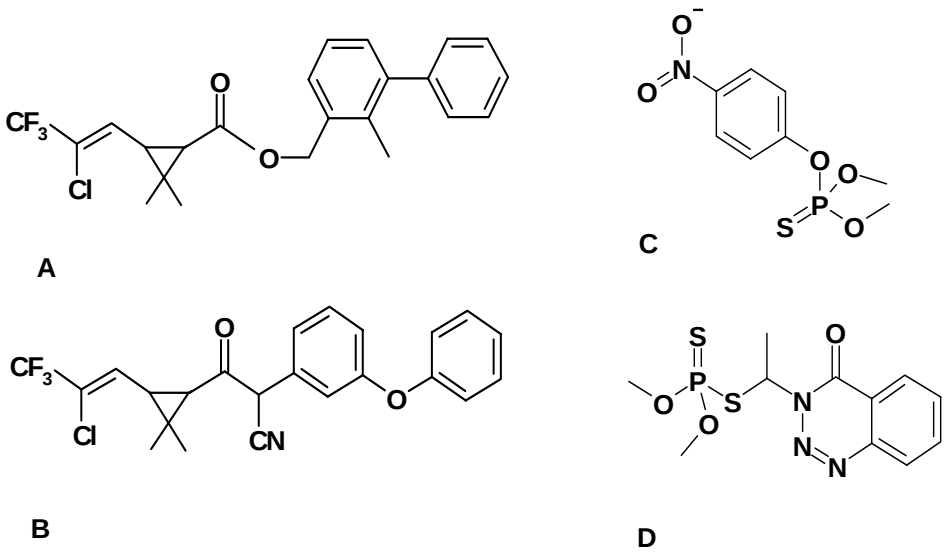

Figure 3.0. Chemical structures of **A**) Bifenthrin, **B**) lambda-Cyhalothrin, **C**) Methyl parathion and **D**) Azinphos-methyl.

Table 3.0. Physical and chemical properties of bifenthrin, lambda-cyhalothrin (adapted from Laskowski [25] and methyl parathion and azinphos-methyl (ATSDR, 2001; PMRA, 2003; EXTOXNET, 1996) (N.A. = not available).

		Bifenthrin	lambda-Cyhalothrin	Methyl-Parathion	Azinphos-Methyl
Molecular weight (g/mol)		422.9	449.9	263.2	317.3
Vapor pressure (mm Hg)		1.8×10^{-7}	1.7×10^{-9}	9.7×10^{-6}	1.0×10^{-5}
Water solubility (mg/L)		1.4×10^{-5}	5.0×10^{-3}	$5.0 \times 10^{+1}$	$3.0 \times 10^{+1}$
Henry's Law Constant (atm m^3/mol)		7.2×10^{-3}	7.9×10^{-7}	6.2×10^{-6}	2.0×10^{-8}
log K_{ow}		6.4	7.0	3.7	3.3
log K_{oc}		5.4	5.5	3.7	3.0
Hydrolysis ½ life (days)	pH 5	Stable	Stable	Stable	Stable (38)
	pH 7	Stable	Stable	Stable	Stable (37)
	pH 9	Stable	8.7	Not Stable	6.9
Photolysis ½ life (days)	Water	408	24.5	N.A.	N.A.
	Soil	96.9	53.7	N.A.	N.A.
Soil ½ life (days)	Aerobic	96.3	42.6	5	21
	Anaerobic	425	N.A.	N.A	68

organic compounds, especially in the aquatic environment, probably the most important physical properties include a compounds octanol-water partition coefficient (Kow), air-water partition coefficient (Henry's law constant) and organic carbon adsorption coefficient (Koc). For example the degradation pathway of a pesticide in the aquatic environment can be quite complex. The proposed degradation pathway for methyl parathion may involve multiple pathways (Figure 3.1) while the proposed aquatic pathway for bifenthrin is less complex (Figure 3.2).

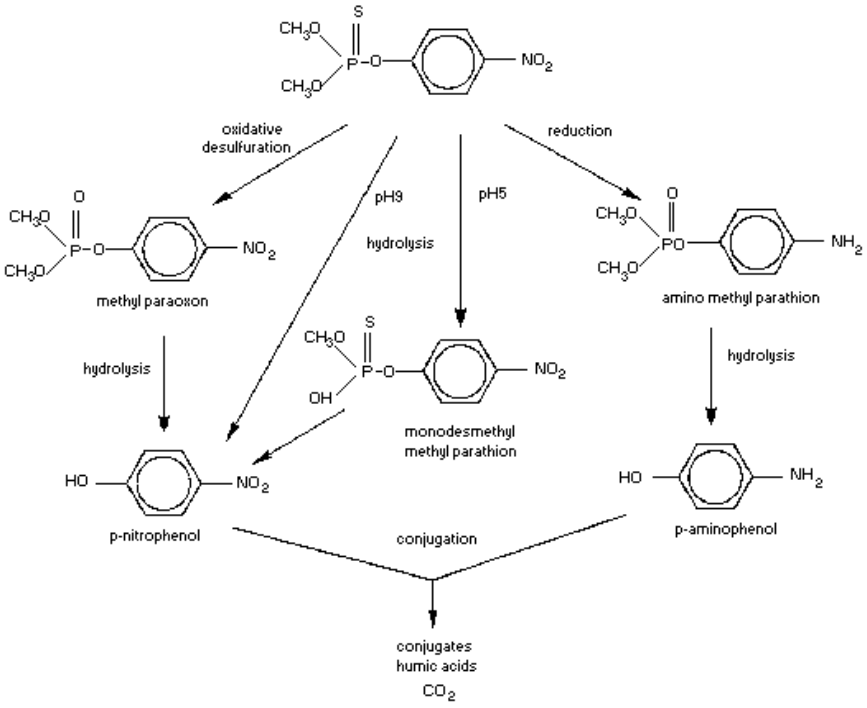

Figure 3.1. Proposed pathway of for the breakdown of methyl parathion in aquatic systems (from IPCS, 1993; modified from Bourquin et al., 1996; Wilmes, 1987).

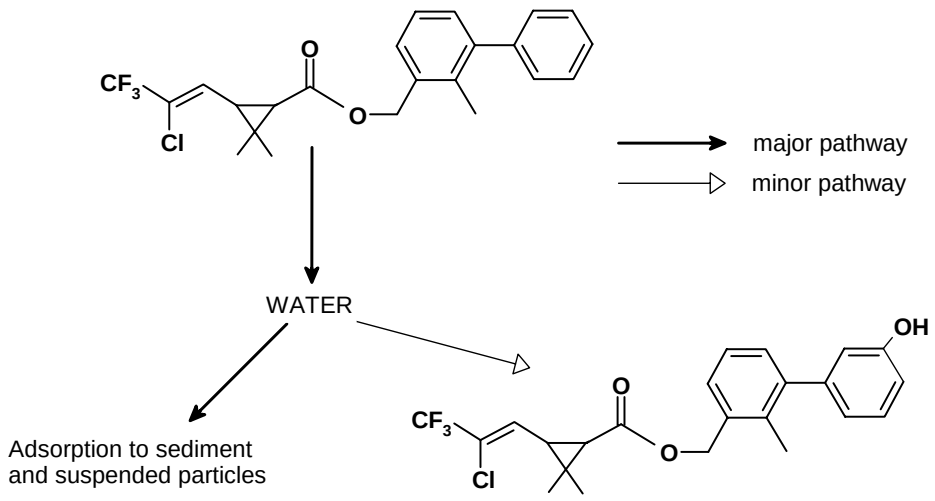

Figure 3.2. Proposed pathway for the breakdown of bifenthrin in water (modified from Fecko, 1999).

Overall, physical and chemical information data for each compound provide information on how a chemical may behave in the aquatic environment, but since aquatic environments are such dynamic systems, a chemicals behavior may be greatly altered by other factors. Biotic factors, such as the presence of microbial and algal communities, or abiotic factors, such as temperature, dissolved oxygen and pH, may drive the ultimate fate of a chemical in the aquatic. For example, a recent study by López et al. (2005) showed that *Bacillus* sp. and *Exiguobacterium aurantiacum* showed high pesticide removal capacities from the aquatic environment, while other studies have shown that elevated pH levels, due to photosynthetic activity in aquatic systems rich with aquatic plants and algae, cause an increase in hydrolysis of some pesticides (Prins et al., 1980; Kersting and van den Brink, 1997; Table 3.0).

4 THESIS LAYOUT AND METHOD OVERVIEW

This thesis focuses on the use of vegetated agricultural drainage ditches and vegetated constructed wetlands to assess the objectives outlined above using numerous methodologies. In order to carry out these studies, multiple laboratories and field sites were used in Mississippi, USA and the Western Cape Province of South Africa (Figure 4.0). To understand how this multi-tier thesis was arranged and how methodologies were carried out, the thesis was broken into three main sections and is described below:

1. The first part of the thesis was to develop an analytical method for the analysis of the pesticides that were chosen for the field experiments. Originally, atrazine and lambda-cyhalothrin were chosen as example compounds to determine an efficient method to be used throughout this thesis (**Appendix 11.1**). This analytical method was developed and validated for the extraction and analysis of these pesticides from water, sediment and aquatic macrophytes. In the end, the pesticides used in this thesis included the pyrethroid insecticides, bifenthrin and lambda-cyhalothrin and the organophosphate insecticides, methyl parathion (MeP) and azinphos-methyl (AZP). The method was later adapted from the original analytical method for the extraction of AZP.

2. The second part of the thesis involved two field studies to determine the pesticide mitigation capacity of a vegetated agricultural drainage ditch in the Greenwood, Mississippi, USA and the capacity of a vegetated agricultural stream in the Western Cape Province of South Africa.

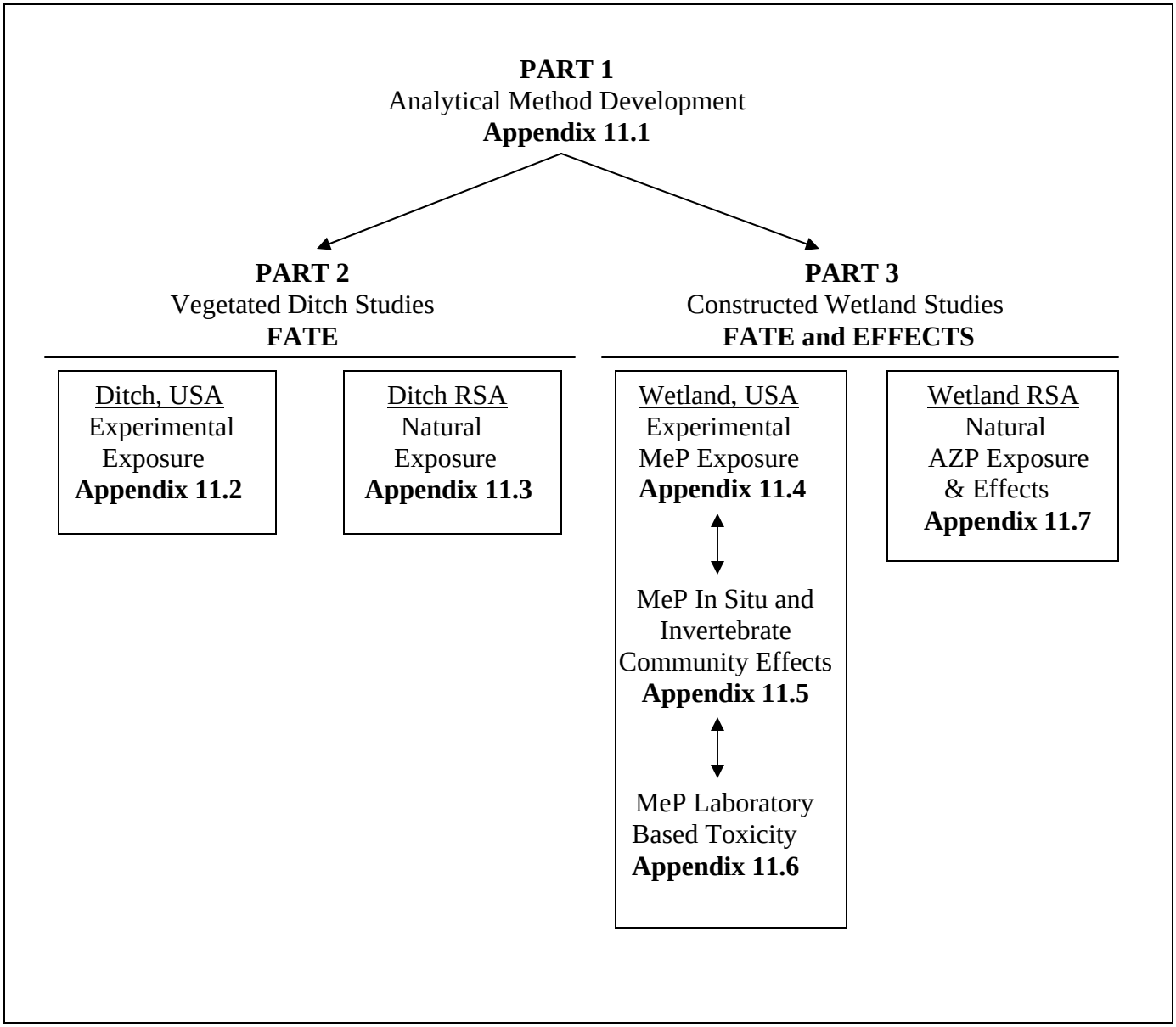

Figure 4.0. Thesis flowchart indicating how each appendix ties the thesis together in 3 steps. In addition, both Part 2 and Part 3 are separated out to show that there were both experimental and natural exposures in both sections of the thesis.

I) In **Appendix 11.2**, a mixture of bifenthrin and lambda-cyhalothrin were amended into a 650 m vegetated agricultural drainage ditch. Water, sediment and plant samples were collected from sampling sites (0 m, 25 m, 75 m, 100 m, 200 m, 400 m and 650 m) along the ditch system throughout the study (99 days). Analytical data from these samples were used to calculate a mass balance to determine the effectiveness of vegetation in the mitigation of these pesticides and to determine pesticide half lives in this system. Furthermore, ditch lengths for effective mitigation of each compound were estimated. II) In **Appendix 11.3**, a vegetated stream (agricultural drainage ditch) located in close proximity to adjacent pear orchards in the Western Cape, South Africa was used. The stream has been shown to regularly receive runoff- and spraydrift-related pesticide input under normal farming practice. This system was used to monitor the movement/mitigation of AZP during two runoff events and a single spraydrift event. Water, plant and sediment samples were collected at 5 m, 25 m, 45 m, and 180 m downstream from the input. Data collected from these trials were used to compare the differences in mitigation capacity of vegetated streams receiving AZP contamination from spray-drift or runoff. In addition, the effect of flowrate was addressed during the two runoff trials.

3. The third part of the thesis involved two field studies to determine the pesticide mitigation capacity of vegetated wetland systems located in Mississippi, USA and in the Western Cape Province of South Africa. In addition to the ditch studies above, the biological effects of runoff and spraydrift events in these wetland systems were addressed. **I)** The study conducted in Mississippi involved the comparison of a vegetated wetland system to a non-vegetated wetland system to determine the differences in the mitigation of MeP following a

simulated runoff event. Two constructed wetlands (width = 11m; length = 50 m; depth = 0.2 m) located at the University of Mississippi Field station (Bay Springs, MS) were divided longitudinally, each into two replicate cells using aluminum flashing. Two of the cells included aquatic vegetation and the other two cells were void of vegetation. Sampling stations, using above-surface platforms, were set up at 2.5 m, 5 m, 10 m, 20 m and 40 m in each wetland cell. The overall MeP study was broken into three separate studies that were run in parallel. **Appendix 11.4** involved the collection of water, sediment and plant samples for analysis to determine concentration of MeP at various sites over time. These data were used to calculate mass balances to estimate the fate of MeP in the vegetated versus the non-vegetated wetlands and to compare the mitigative capacity of each wetland system. In addition, wetland lengths for effective mitigation of MeP in each wetland were estimated. The purpose of **Appendix 11.5** was to assess the macroinvertebrate communities present in the wetland systems two days prior to the MeP exposure and 96 h after the exposure to determine the differences in impact on the communities present in the vegetated and non-vegetated wetlands. In addition to this, in situ exposure bioassays, using midges (*Chironomus tentans*), were set up at various sites in each wetland to assess MeP toxicity with distance. The objective of **Appendix 11.6** was to collect water and sediment samples for acute toxicity bioassays using *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyalella azteca* and *Chironomus tentans* to determine spatial and temporal differences between the wetland systems. Samples were collected at 3 h, 24 h, 96 h, and 10 d post application from the sampling stations listed above. Aqueous toxicity tests were conducted using *C. dubia*, *P. promelas* and *H. azteca* and sediment tests were conducted using solid phase, 10 d acute toxicity assays to determine relative inhibition of survival and growth in exposed *C. tentans*.

II) The study conducted in South Africa (**Appendix 11.7**) involved the determination of the fate and effects of AZP in a flow-through vegetated wetland in the Western Cape of South Africa. The inflow for this wetland was a tributary that flows through a highly agricultural area (fruit orchards) and the outflow enters a nearby river (Lourens River). During a spraydrift event upstream of the wetland, water samples were collected at the inlet and outlet of the wetland and at 17 m, 45 m, 85 m, and 110 m from the inlet over a 7 d period for AZP analysis. Plant and sediment samples were also taken from the 17 m and 110 m sites. In addition to this zooplankton samples were taken at various times 7 d prior to the exposure and 7 d after at the 85 m sampling site.

5 METHOD DEVELOPMENT AND ADAPTATION

Before the latter chapters of this thesis could be developed and carried out, an efficient multi-residue analytical method had to developed and validated. Through this process, an easy and rapid method for the extraction and analysis of two classes of pesticides, pyrethroids and triazines, from water, sediment and aquatic plants using a sonication method was established (Appendix 11.1). Furthermore, this same method was later validated for the extraction and analysis of MeP from the three above matrices (Appendix 11.4). During the development stage of this analytical method, stability storage tests were performed on natural water samples spiked with pesticides. Results from this study (Table 5.0) provided important information for the sampling design for the fate studies performed in this thesis. Data from this study indicated that there could be a considerable loss of pesticide residue if water samples were stored for more than 24 h. This was especially noticeable for lambda-cyhalothrin, a pyrethroid insecticide, where

Table 5.0 Results of the pesticide stability storage test in surface waters fortified with trazine, λ-cyhalothrin and metolachlor in surface waters (n=3).

	Storage Time	% Recovery		
Water Sample	(days)	metolachlor	λ-cyhalothrin	atrazine
Lake Water	0	100 ±4	82 ±4	100 ±1
	1	87 ±6	56 ±3	79 ±7
	2	87 ±10	NA	61 ±14
	4	73 ±6	51 ±2	43 ±12
	8	83 ±4	60 ±3	47 ±7
Ditch Water	0	93 ±13	87 ±12	100 ±2
	1	78 ±3	69 ±1	100 ±7
	2	66 ±14	NA	75 ±30
	4	68 ±5	72 ±0.3	35 ±6
	8	66 ±4	54 ±5	43 ±10

approximately 30% of this residue was lost within a 24 h storage period. Owing to these results, all water samples that were collected in the field were immediately extracted after sampling.

For all of the analytical work that was performed in South Africa, some method adaptations had to be done due to the availability of equipment and solvents. In both studies, Appendix 11.3 and 11.7, the analyte of interest was AZP. A similar sonication method was used for the extraction of AZP from sediment and aquatic plants with the exception that methanol was used as the extracting solvent instead of ethyl acetate. Since the method was altered from the original method, validations studies were performed to determine the effectiveness of the extraction procedure. For example, extraction efficiencies for spiked plant samples were calculated to be 95.5%±8.04 (unpublished data, 2001). In the case of water extractions, water samples were extracted using solid-phase extraction (SPE) instead of the above sonication method. This method proved to be a more appropriate method because samples could not be extracted and analyzed immediately, as in the USA studies, due to laboratory restraints. Thus, water samples were extracted onto SPE (C18) cartridges and stored in the freezer (-10 °C) until they could be extracted and analysed. Extraction efficiencies for AZP spiked water samples were calculated to be between 79 and 106% (Appendix 11.3).

6 AQUEOUS MITIGATION

In general the overall goal of this thesis was met by studying multiple methods for testing the effectiveness of vegetated drainage ditches and vegetated constructed wetlands for the reduction or elimination of pesticide related runoff or spray-drift from entering receiving water bodies, such as rivers and lakes. The following subchapters give an overview of the general results

obtained in each of the studies. The overall results and importance of aquatic vegetation will be discussed in more detail in following chapter.

6.1 Fate in Agricultural Drainage Ditches

The studies in this section were designed to determine the effectiveness of vegetated drainage ditches on the retention of insecticides following a simulated worst-case scenario runoff event (Appendix 11.2) and after natural (not simulated) runoff and spray-drift events (Appendix 11.3). Results from Appendix 10.2 illustrated that measured water concentrations of bifenthrin and lambda-cyhalothrin applied to the 650 m ditch system decreased rapidly with distance and were not detected in water beyond the 400 m sampling site throughout the study (Table 6.0). In fact, only 33.9% and 36.3% of the original dose of bifenthrin and lambda-cyhalothrin, respectively, remained in the water 3 h post application and were further reduced to 3.09% and 1.24% after 1 d. From these data, it was calculated that ditch lengths of 120 m and 280 m were required to reduce bifenthrin and lambda-cyhalothrin to 1.00% and 0.100%, respectively, of the original loading from the simulated runoff event. The estimated flowrate in this test system was 0.05 m^3/sec.

Interestingly, results from the second drainage ditch study (Appendix 11.3) produced a similar relationship to the simulated runoff study where pesticide concentrations declined with distance Figure 6.0). For example, after the spray-drift event, a 180 m section of a vegetated drainage ditch successfully reduced aqueous AZP concentrations by 90%. During a 10 mm rain event, the same ditch section effectively reduced the average concentrations of AZP entering the system via runoff by 61%. However, during a 22 mm rain event, the vegetated ditch system was not as effective in reducing the loadings of AZP along the 180 m stretch indicating that flow rate may

be a limiting factor in a ditches mitigation capacity. Approximated flow rates during the 10 mm and 22 mm rain events were 0.06 m^3/sec and 0.10 m^3/sec, respectively.

Table 6.0. Downstream dissipation of bifenthrin and lambda-cyhalothrin concentrations (µg/L) in water at the 3 h, 12 h and 7 d sampling times (N.D. = Not Detected, below detection limits; 1.00 ng/L) [Appendix 11.2].

	Bifenthrin			**lambda-Cyhalothrin**		
Distance (m)	3 h	12 h	7 d	3 h	12 h	7 d
0	666	10.7	0.887	375	5.29	0.250
25	235	25.9	7.76	115	11.8	2.32
50	77.2	6.33	0.178	39.1	3.44E	5.50E-02
75	33.8	1.03	0.270	20.6	0.745	7.38E-02
100	27.8	1.32	6.38E-02	16.6	0.899	2.38E-02
200	0.724	0.454	5.07E-02	0.309	0.296	2.00E-02
400	N.D.	0.471	N.D.	0.144	9.88E-02	N.D.
650	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

In addition to water samples, both sediment and plant samples were also collected during both of the vegetated drainage ditch studies. Data from these studies showed that pesticide plant associated concentrations were generally more concentrated near the source of contamination and decreased with distance. In contrast bifenthrin, lambda-cyhalothrin and AZP sediment concentrations were generally lower compared to plant concentrations.

Since it is difficult to directly compare insecticide concentrations between water, sediments and plants, mass balance calculations were performed to better understand the distribution and fate between compartments and along the ditch system. Mass balance calculations were performed

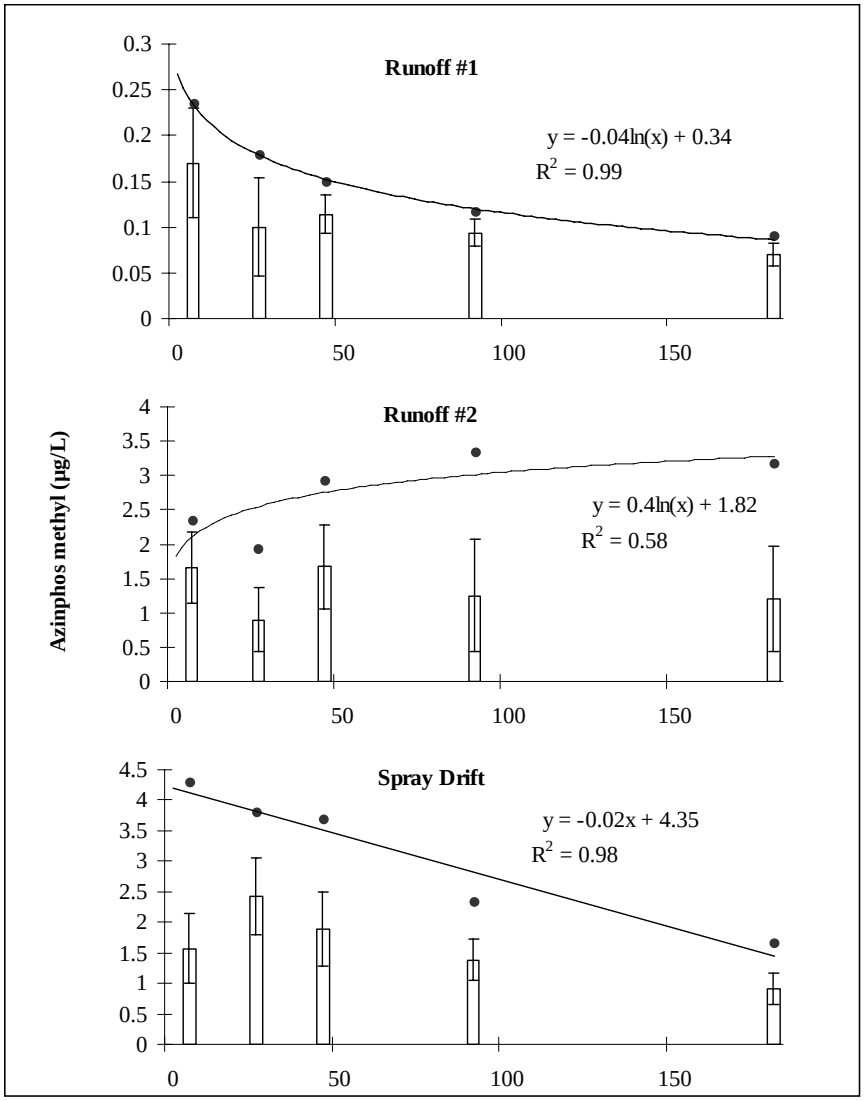

Figure 6.0. Peak (dots) and average (bars; ± standard error; *n* = 4) concentrations of azinphos-methyl (AZP) plotted against distance from point of input for a 10 mm (Runoff #1) and a 22 mm (Runoff #2) rainfall event and a spray-drift event in a vegetated stream in the Lourens River catchment, South Africa. Lines indicate best fitting logarithmic or linear regression relationships between observed peak AZP concentrations and distance downstream from the point of input [Appendix 11.3].

using water, sediment and plant data collected along transects of the 650 m ditch length for each sampling time point (Appendix 11.2.). This enabled quantitative evaluation of chemical partitioning and losses that occurred over the study duration. The mass balance at a given time point was determined as:

$$m_{total(t)} = m_{w(0-650)} + m_{p(0-650)} + m_{s(0-650)} \quad (1)$$

where $m_{w(0-650)}$, $m_{p(0-650)}$ and $m_{s(0-650)}$ reflect the total chemical mass (g) in water, plants and sediments over the 650 m ditch length. Integration of chemical masses in water was performed according to the trapezoidal rule:

$$m_{w(0-650)} = \sum_{i=0}^{n-1} \left(\left(V_{w(i+1)} - V_{w(i)} \right) \cdot \left(\frac{C_{w(i+1)} + C_{w(i)}}{2} \right) \right) \quad (2)$$

where the term $(V_{w(i+1)} - V_{w(i)})$ represents the volume of water (L) bounded by transects i and i+1 (i.e. 0-25m, 25-50m, 200-400m etc.) and $C_{w(i)}$ is the water concentration measured at transect i. Water volumes between transects were estimated as the product of mean water depth, mean water width. The mass calculations for plants and sediments were similar to Equation 2 except that concentrations were measured in units of mg/kg (d.w.) and the volume terms were replaced by bulk sediment mass (kg) or plant biomass (kg).

Results from the mass balance indicated that after 12 h, the majority of pesticide mass was found in the plant compartment giving evidence that this compartment was the most important and effective compartment for the mitigation of these insecticides (Table 6.1). It should be noted that sediment mass stabilized over the 14 d sampling period for both bifenthrin and lambda-cyhalothrin, while bifenthrin decreased further after 44 d, indicating that the sediment compartment acted as a sink during the majority of the study. Regardless, levels in sediments

were relatively low throughout the duration of the study. More importantly, pesticide mass in the plant compartment decreased with time in the ditch system. It is unlikely that either pyrethroid were redistributed into the water column due to the rapid decrease of pyrethroid mass in the water compartment over time.

Table 6.1. Estimated mass (g) of bifenthrin and lambda-cyhalothrin in the water, sediment and plant compartments relative to each sampling time (*total A.I. amended to ditch at time zero) [Appendix 11.2]

	Bifenthrin (g)					Lambda–Cyhalothrin (g)			
Time	Water	Plants	Sediment	Total	Time	Water	Plants	Sediment	Total
0 h	-	-	-	*11.4	0 h	-	-	-	*5.70
3 h	5.78	6.29	3.85E-02	12.1	3 h	3.10	6.13	6.24E-02	9.29
12 h	0.718	7.22	3.30E-02	7.97	12 h	0.353	3.76	1.12E-02	4.13
24 h	0.191	4.03	1.13E-02	4.24	24 h	0.106	1.59	3.68E-03	1.70
7 d	0.134	1.93	6.31E-02	2.13	7 d	4.05E-02	6.74E-02	1.79E-02	0.126
14 d	4.48E-02	3.00	5.25E-02	3.10	14 d	6.90E-03	2.09E-01	1.29E-02	0.229
30 d	4.37E-03	0.199	1.93E-03	0.206	30 d	1.05E-03	3.41E-02	4.24E-02	7.75E-02
44 d	8.82E-04	4.89E-02	8.31E-04	5.07E-02	44 d	6.22E-03	9.10E-02	5.04E-02	0.148

6.2 Fate in Constructed Wetlands

The studies in this section were designed to determine the effectiveness of vegetated constructed wetlands on the retention of insecticides during a simulated worst-case scenario runoff event and a “real time” spray-drift event. As with the drainage ditch studies above, vegetated wetlands were very effective in reducing loadings of insecticide introduced from runoff events (Appendix 11.4) and spray-drift events (Appendix 11.7). Results from the spray-drift trials using the vegetated flow-through wetland system showed that the wetland was capable of an average AZP reduction of 90±1% (Table 6.2). To further compare the efficiency of vegetated wetlands in

mitigating these loadings a non-vegetated wetland was examined in parallel during the runoff study.

Table 6.2. Concentration and mass of AZP during five separate spray drift trials at the inlet and outlet of the constructed wetland. Inlet values are from 3-h composite samples (n = 1), whereas those for the outlet are from 12-h composite samples (n = 1) during spray drift trials [Appendix 11.7].

	AZP concentration			AZP mass		
Trial	inlet (µg/L)	outlet (µg/L)	reduction (%)	inlet (mg)	outlet (mg)	retention (%)
1	0.41	0.04	90	159.4	62.2	61
2	0.36	0.04	89	186.6	82.9	56
3	0.34	0.02	94	143.2	33.7	77
4	0.51	0.04	92	225.8	70.8	69
5	0.27	0.04	85	145.8	86.4	41
average (±SE; n = 5)	0.4±0.03	0.04 ±0.003	90±1	172.2±12.6	67.2±7.7	61±5

Table 6.3. Mean aqueous methyl parathion concentrations (µg/L) in wetland mesocosms during the first 10 d of exposure. (ND = below limits of analytical detection) [Appendix 11.4].

	Vegetated					
Distance (m)	30 min	3 h	6 h	1 d	4 d	10 d
2.5	1400	452	423	253	444	N.S.
5	717	421	298	192	22.0	4.00
10	687	184	118	90.0	14.5	0.600
20	14.0	8.15	24.9	8.00	0.600	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	Non-Vegetated					
Distance (m)	30 min	3 h	6 h	1 d	4 d	10 d
2.5	493	1110	615	247	72.5	3.50
5	337	552	352	185	39.0	3.00
10	54.5	121	189	164	29.5	3.50
20	4.75	44.0	72.5	35.5	22.5	0.600
40	0.150	0.300	0.550	6.00	9.00	0.550

In both wetland systems aqueous MeP concentrations decreased rapidly with distance (Table 6.3). The major difference was that MeP was not detected at the 40 m sampling site throughout the study, whereas it was detected throughout the non-vegetated wetland. Regression formulas predicted that a vegetated wetland length of 18.8 m would have been adequate to reduce MeP concentrations to 0.1 % of the inflow concentration. In contrast, a non-vegetated length of 520 m would be required to reduce inflow concentrations to 0.1% of the inflow. As with the drainage ditch studies, sediments and aquatic vegetation were sampled in both of the wetland systems. Once again, insecticide concentrations in plants collected near the front end of the system were high, while sediment levels remained low in the vegetated systems.

Due to the difficulties in directly comparing water, sediment and plant concentrations, mass balance calculations were performed using similar equations as discussed above (Appendix 11.2). A full mass balance was not performed in Appendix 11.7 as in Appendix 11.4 due to the limited plant data and the fact that AZP was not detected in any of the sediment samples. Regardless, the calculated mass of AZP in the inlet versus the outlet of the wetland during the spray-drift trials reflects the measured concentration data (Table 6.2). Mass balance data from Appendix 11.4 indicated that both the vegetated and non-vegetated wetlands were effective in reducing the aqueous loadings of MeP introduced into each of the systems (Table 6.4). After 1 d the majority of the pesticide mass in the vegetated wetland was found in the plant compartment while the majority of the pesticide was located in the sediment compartment of the non-vegetated wetland. As in the ditch study, pesticide mass in the plant compartment decreased with time while sediment mass generally stabilized in both the vegetated and un-vegetated wetlands. It should be noted that sediment-associated pesticide mass in the vegetated wetland was an order of

magnitude lower that the mass in the non-vegetated wetland. The significance of this will be discussed in the next section.

Table 6.4. Estimated mass (g) of methyl parathion in the water, sediment and plant compartments relative to each sampling time in the vegetated and non-vegetated wetland cells [Appendix 11.4].

	Vegetated - MeP (g)				Non-Vegetated - MeP (g)		
Distance (m)	Plant	Sediment	Water	Total	Sediment	Water	Total
Mix. Chamber	-	-	-	85.6 (± 3.2)	-	-	63.1 (± 4.7)
30 m	7.84	0.136	21.9	29.9	4.63	7.48	12.1
3 h	18.4	0.206	6.42	25.0	0.289	11.0	11.29
1 d	5.22	0.127	3.22	8.57	3.43	3.18	6.61
10 d	1.58	0.329	0.0266	1.94	4.16	0.130	4.29

6.3 Effects in Constructed Wetlands

The studies in this subsection were run in parallel with appendix 11.4 to assess the differences in MeP induced aquatic toxicity in the vegetated and non-vegetated wetlands relative to distance from the inlet. As outlined in Appendix 11.5, a total of 15 macroinvertebrate species were found in the wetlands prior to the simulated runoff event. *Caenis latipennis* (Ephemeroptera) and *Chironomus* sp. (Diptera) were the most dominant species, forming more than 50% of the individuals present in both systems prior to contamination. Six out of the 15 species were odonate species and 13 species belonged to the insect group. Ninety-six hours after the contamination, a significant negative acute effect of contamination on abundances was found in 8 out of the 15 species in both wetland systems. Both the mayfly species and the caddisfly, *Oecetis cinerascens,* were no longer present in either of the systems. Even with these toxic effects, the overall reaction of macroinvertebrates clearly demonstrated that the impact of MeP in

the vegetated wetland was considerably lower than in the non-vegetated wetland. In addition to these findings, results from the in situ exposed *C. tentans* showed that lowest survival occurred

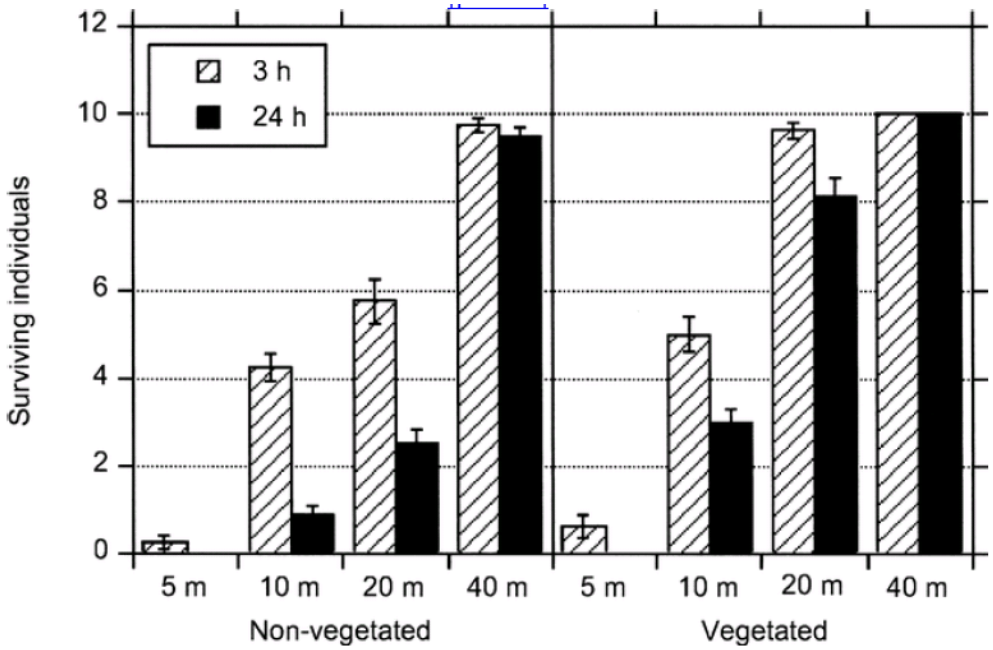

Figure 6.1. Mean (± standard error, n = 8) survival of in situ exposed *Chironomus tentans* in wetland mesocosms at different times after introduction of methyl-parathion. Initial number of individuals was 10 per replicate (Appendix 11.5).

within 10 m of vegetated wetland inlet while the lowest survival occurred within 20 m of the non-vegetated wetland inlet at 3 h and 24 h following the exposure (Figure 6.1). These results revealed the positive effect of vegetation on the spatial extent of MeP toxicity in theses test wetland systems.

Aqueous acute toxicity data collected from Appendix 11.6 and Schulz et al. (2003) further illustrated the importance of the presence of aquatic vegetation in these test systems for efficient mitigation/reduction of MeP. Furthermore, these data validated the concentration and mass balance results collected in Appendix 11.4 (Table 6.5). For example, 48-h toxicity results for *C.*

Table 6.5 Mean methyl parathion concentrations in water samples (µg/L) collected at each sampling site during the 3h, 24h and 96h sampling periods relative to mass balance results [Appendix 11.4] and acute (aqueous) toxicity survival results (*C. dubia* [Appendix 11.6], *H. azteca* [Schulz et al., 2003] and *P. promelas* [Appendix 11.6]) from vegetated and non-vegetated wetland mesocosms. Note: N.D. = below detection limits; NA = No available data.

Concentration of MeP (µg/L)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	421	192	22.0	551	184	39
10 m	184	90.0	14.5	120	164	29.5
20 m	8.15	8.00	0.600	44	35.5	22.5
40 m	N.D.	N.D.	N.D.	0.300	6.00	9.00

Mass of MeP in Water (g)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	2.66	1.24	0.2	3.7	1.92	0.376
10 m	1.69	0.862	0.133	1.81	2.19	0.572
20 m	0.145	0.143	0.012	0.974	0.847	0.693
40 m	0.001	0.001	0.001	0.0033	0.033	0.099

C. dubia (% Survival)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	0%	0%	0%	0%	0%	0%
10 m	0%	0%	0%	0%	0%	0%
20 m	0%	50%	100%	0%	0%	0%
40 m	75%	100%	100%	100%	0%	0%

H. azteca (% Survival)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	0%	0%	23% (±0.6)	0%	0%	1.7% (±0.2)
10 m	1.7% (±0.2)	5% (±0.3)	28% (±0.5)	0%	0%	10% (±0.5)
20 m	30% (±0.3)	37% (±1.0)	92% (±0.5)	3.3% (±0.2)	1.7% (±0.2)	20% (±1.1)
40 m	97% (±0.3)	87% (±0.6)	98% (±0.2)	92% (±0.5)	62% (±1.2)	32% (±0.3)

P. promelas (% Survival)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	98	80	N.A.	90	93	N.A.
10 m	93	100	N.A.	68	90	N.A.
20 m	98	100	N.A.	93	70	N.A.
40 m	88	100	N.A.	90	100	N.A.

dubia exposed to water samples (Appendix 11.6) taken at 3 h indicated 0% survival in both wetlands up to the 20 m sampling sites. By 96 h the water in the vegetated wetland remained toxic to *C. dubia* through the 10 m site while 0% survival was recorded throughout the non-vegetated system at this time point (Table 6.5). Schulz et al (2003) reported similar toxicity results (24 h LC_{50}) for *H. azteca* exposed to water samples collected from both vegetated and unvegetated wetlands at 3 h, 24 h and 96 h (Table 6.5). Interestingly, results for the exposed *P. promelas* indicated low toxicity in each wetland demonstrating that there was minimal effect to this test organism relative to other test organisms in this study.

7 Discussion: Importance of Aquatic Vegetation

In summary, these studies suggest that macrophyte vegetated drainage ditches and wetlands have a strong potential in reducing aqueous loading of insecticides introduced from runoff and spray-drift events. Vegetated ditch lengths of < 200 m were shown to mitigate loadings of bifenthrin, lambda-cyhalothrin and AZP while a 134 m vegetated constructed flow through wetland successfully reduced loadings of AZP by 90%. In addition, a 50 m densely vegetated constructed wetland effectively reduced MeP to below analytical detection limits (<0.1 µg/L) while a similar non-vegetated wetland was not effective in retaining the loading within this length. In this same wetland system, there were minimal effects of the pesticide on macroinvertebrate communities, in situ exposed chironomids or lab based toxicity test organisms detected at 40 m distance from the pesticide inlet in the vegetated wetland. Furthermore, concentration and fate data were confirmed and backed up by the toxicity data produced in this wetland study (Table 6.5). One factor that may reduce the mitigative effectiveness of these vegetated systems is increased flowrate, especially during runoff events.

The one factor that links the success of all of these studies together was the presence of aquatic vegetation in each of the test systems. Data produced from mass balances calculations in Appendix 11.2 and 11.4 further highlighted the importance of the plant compartment for effective overall pesticide mitigation. Interestingly, regardless that different aquatic macrophytes species were present in each test system (Table 7.0), results from these studies show they were all effective in the ditch and wetland exposures. For example, in both Appendix 11.2 and Appendix 11.4 submerged plant coverage were dominated by *Ludwiga sp.* (88% coverage) and *Juncus effuses* (85% coverage), respectively. Further research is required to determine if some specie are more effective in the retention/degradation of individual pesticides. In both the

drainage ditch and wetland studies, the overall dense plant coverage within the sediment water interface played an important role in their mitigative capacity.

Table 7.0. Aquatic macrophyte species present in each of the agricultural and constructed wetland studies.

Study	Dominant Plant Species	Other Species Present
Appendix 11.2	*Ludwiga sp.*	*Lemna sp. and Polygonum sp.*
Appendix 11.3	*Juncus capensis*	*Fuirena hirsuta* and *Pycreus sp.*
Appendix 11.4	*Juncus effusus*	*Leersia oryzoides*
Appendix 11.7	*Typha capensis*	*Juncus kraussii* Hochst and *Cyperus dives* Delile

Each of the vegetated agricultural ditch and vegetated wetland studies in this thesis addressed that the plant compartment is an essential component for the effective mitigation of pesticide exposure in these systems. The processes via which the plants acts in these test systems has not been directly researched, but there are many processes, abiotic and biotic, that could be occurring. In the systems tested in this thesis, results would indicate that aquatic macrophytes play an important role in providing an increased surface area for pesticide sorption. Analytical data from Appendix 11.2, 11.3, 11.4 and 11.7 illustrate this by the high pesticide residue levels detected in collected plant samples. This concept of increased surface area for pesticide sorption on plant material has been described by other researchers (Wetzel 1993; Rodgers et al., 1999; Luckeydoo et al., 2002). Furthermore, Kadlec and Knight (1996) described that macrophytes serve as filters by allowing contaminants to flow into plants and stems, which are then sorbed to macrophyte biofilms. The process of pesticide sorption was especially shown in Appendix 10.2.

It was expected that sediments would have been the major sink for the pyrethroids, bifenthrin and lambda-cyhalothrin, due to their high K_{ow} and K_{oc} values (Table 3.0). In fact sediments were a minor sink due to the dense plant community that limited the movement and/or partitioning of bifenthrin and lambda-cyhalothrin to the sediment compartment. Similar results were found in a microcosm study by Hand et al. (2001) where aquatic plants significantly reduced the amount of lambda-cyhalothrin reaching the sediments. Moreover, in the same study, it was shown that lambda-cyhalothrin plant adsorption was virtually irreversible in turn reducing sediment partitioning.

Once the pesticide is sorbed to the plant surface, multiple biotic and abiotic processes could take place affecting pesticide fate. It has been shown that aquatic plant populations not only contain individual plant species, but may also play host to multiple algal species and Aufwuchs that contain diverse microbial communities (Crossland and Bennett, 1984, Holm et al., 1983; Wetzel, 1993; Headley et al., 1998). The presence of these communities may increase biotransformation or degradation of pesticides. It has been speculated that algal species either provide sites for pesticide sorption or facilitate pesticide degradation (Friesen-Pankratz et al., 2003). For example, *Anabaena sp*, a blue-green algal species, has been shown degrade MeP under aerobic, photosynthetic conditions (Barton et al., 2004) while other studies have shown that green and blue-green algae presence accelerate the photoreaction of MeP (Zepp and Scholtzhauer, 1983). In addition, biofilms and aufwuchs, which contain a wide range of microbial organisms, have been shown to rapidly degrade insecticides (Holm et al., 1983; Crossland and Bennett, 1984; Headley et al., 1998).

Studies have shown that the degradation of insecticides in the aquatic environment occurs more rapidly in alkaline conditions relative to neutral or acidic conditions (USEPA, 1978; Badawy and

El-Dib, 1984; Lartigues and Garrigues, 1995; Kaur et al., 1997; Lakowski, 2002). In systems rich with aquatic plants and algae, pH has been shown to exceed 9 at the water algal/plant interface as a result of photosynthetic activity (Prins et al., 1980; Kersting and van den Brink, 1997). Therefore, if insecticides were present in this interface they would be susceptible to alkaline hydrolysis. Both pyrethroids and organophosphate insecticides have been shown to be unstable under alkaline conditions (Table 3.0)

Mass balance data produced in Appendix 11.4 indirectly highlighted the potential for pesticide degradation in the plant compartment as discussed above. In this study a vegetated wetland system was compared to a non-vegetated system to compare the effectiveness on reducing the loading of MeP during a simulated runoff event. Within the first 3 hours of the study, mass balance data showed that the majority of mass of MeP in the vegetated wetland had partitioned into the plant compartment while the majority of mass was located in the sediment compartment of the un-vegetated wetland (Table 6.4). After 10 d, MeP mass in the plant compartment declined by an order of magnitude while overall sediment mass in the un-vegetated system remained unchanged. There was little change in the sediment associated MeP mass in the vegetated wetland indicating that the plant compartment limited the movement and/or partitioning of MeP to the sediment where little degradation would have taken place (as indicated in the un-vegetated wetland). Since there was no increase in the MeP mass in either the sediment or water compartment, these data indicate that the MeP retained by the plant compartment was more than likely subject to multiple biotic and abiotic degradation processes.

8 CONCLUSION

Overall, multiple methodologies were used to test the effectiveness of vegetated agricultural drainage ditches and vegetated wetland systems for mitigating pesticide loadings introduced via runoff and spraydrift from entering downstream receiving water bodies. Both analytical and mass balance results indicate that both of these systems are quite effective in carrying out this task. Furthermore, field sampling, in situ and lab based toxicity testing validated the above analytical and mass balance revealing the importance of utilizing both analytical and toxicological approaches in testing the effectiveness of a study like this one. Both types of BMPs studied in this system would be effective in mitigating pesticide loadings introduced from either runoff or spraydrift, in turn lowering or eliminating potential pesticide associated toxic effects in receiving aquatic ecosystems. Finally, from a risk assessment standpoint, by incorporating these BMPs, the risk of acute toxicity of the studied insecticides will be greatly reduced, but chronic exposure may still be an apparent overall risk.

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11 APPENDICES

Listing of Appendices

APPENDIX 11.1

Method for the simultaneous extraction and analysis of two current use pesticides, atrazine and lambda-cyhalothrin, in sediment and aquatic plants.

Bennett, E.R., M.T. Moore, C.M. Cooper and S. Smith, Jr.

Bull. Environ. Contam. Toxicol., 64(6): 825-833 (2000)

INTRODUCTION

It has been estimated that 375,000 tons of pesticides are used for agriculture annually in the Midwest area of the United States, where approximately 65% of this total is used for production of corn and soybean crops (Clark *et al.*, 1999). Within the Mississippi River Basin, greater than 100,000 tons of herbicides are used annually (Clark *et al.*, 1999). Triazine herbicides (e.g. atrazine) and synthetic pyrethroids insecticides (e.g. λ-cyhalothrin) are two classes of pesticides currently used in this agricultural area. These two types of pesticides are very dissimilar, due to their differences in structural and physical properties. A major difference between these pesticides is polarity, where triazines are relatively more polar than pyrethroids.

With demand for more rapid extractions and multiresidue pesticide analyses, analytical methods for environmental matrices, such as soil and plants, have been developed using new technologies (Kahn, 1995; S<nchez-Brunete *et al.*, 1998). For example, traditional extraction methods, such as soxhlet and sonication, are being replaced with solid-phase extraction (SPE), solid-phase microextraction (SPME) and supercritical fluid extraction (SFE) techniques because they are less time consuming and require lower volumes of organic solvents (Hengel *et al.*, 1997; Miege and Dugay, 1998; Camel, 1998). In some cases, newer analytical equipment is not available, therefore older methods using traditional equipment must be reevaluated.

The following study presents the development of a rapid and sensitive gas chromatographic method using sonication for extraction of atrazine and λ-cyhalothrin in sediment and aquatic macrophytes. This method requires lower solvent volumes and reduced sample weights relative to the traditional EPA sonication method.

In addition to method development, a storage stability test was performed to determine acceptable storage times for natural water samples containing pesticide residues. Natural water samples were fortified with atrazine, λ-cyhalothrin, and metolachlor, a chloroacetamide herbicide. These three pesticides represent three of the major classes of pesticides used extensively in row crop production in the area of the lower Mississippi River Basin known as the Mississippi Delta.

MATERIALS AND METHODS

Atrazine [2-chloro-4-ethylamine-6-isopropylamino-1,3,5-triazine], λ-cyhalothrin [(*RS*)-alpha-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2,-dimethyl-cyclo-propanecarboxylate] and metolachlor [2-chloro-6'-ethyl-*N*-(2-methoxy-1-methylethyl)-acet-*o*-toluidide were obtained from USEPA (Research Triangle Park, NC). Chemical structures and physical properties are presented Figure 1 and Table1, respectively.

Sediment and aquatic plant samples were collected from an agricultural drainage ditch located in one of the Mississippi Delta Management System Evaluation Areas (MDMSEA) (Beasley Lake Watershed, Sunflower County, Mississippi). Samples were wrapped in solvent washed foil, transported to the laboratory on ice and placed in the fumehood to dry.

Individual dried sediment and plant samples were ground using a Wiley-mill fitted with a size 10-mesh screen, mixed and placed in solvent washed erlenmeyer flasks equipped with foil caps. Triplicate samples of 1000 mg of dry ground sediment were placed in separate 50 mL glass centrifuge tubes followed by the addition of 7 mL of ethyl acetate. A similar method was followed for plants with the exception that plant samples were prewetted with 1 mL of ultrapure water prior to the addition of ethyl acetate. The mixture was sonicated (Sonics GE600 sonicator) for 1 min in pulse mode using an 80% duty cycle. Following sonication, the mixture was centrifuged on high (~ 2000-2500 rpm) using an IEC HN-S Centrifuge with a 4 place rotor (4 x 50 mL). The solvent layer was transferred into another 50 mL centrifuge tube. The extraction was repeated and the solvent layer was combined with the previous extract. The extract was concentrated to near dryness under a stream of UHP nitrogen using a nitrogen evaporator (N-EVAP, Organomation) and solvent exchanged into hexane (0.5 mL). These methods were

derived from methods developed by O'Neal *et al.* (1996) for the extraction of pesticides and PCBs from aquatic organisms.

For comparison of extraction methods, triplicate sediment samples were extracted using Soxhlet extraction with ethyl acetate (DIG). Equal amounts of sediment (1000 mg) were ground with sodium sulfate and extracted for 6 hours. The extract was rotary evaporated to approximately 2 mL and further concentrated under a stream of UHP nitrogen to a volume of 1mL.

All extracted sediment and plant samples were subject to silica gel clean-up prior to analysis. A glass micro-column (7 mm i.d.) was fitted with a glass wool plug and 5 cm silica gel (60-100 mesh) activated at 200ºC was poured into the column. Approximately 5 mm of sodium sulfate was added to the top of the silica. The column was pre-wetted with 3 mL of hexane and the eluent was discarded. When the hexane reached the top of the sodium sulfate, the concentrated sample extract(0.5 mL) was loaded onto the column with three rinses of hexane, allowing each rinse to sink onto the column. The column was eluted as follows: A) 5 mL of hexane, and B) 10 mL of 10% acetone in hexane. Fraction A was discarded and the hexane reached the top of the sodium sulfate, the concentrated sample extract (0.5 mL) was loaded onto the column with three rinses of hexane, allowing each rinse to sink onto the column. The column was eluted as follows: A) 5 mL of hexane, and B) 10 mL of 10% acetone in hexane. Fraction A was discarded and Fraction B containing atrazine and λ-cyhalothrin was collected in a 12 mL glass centrifuge tube, concentrated under a stream of UHP nitrogen to a final volume of 1 mL.

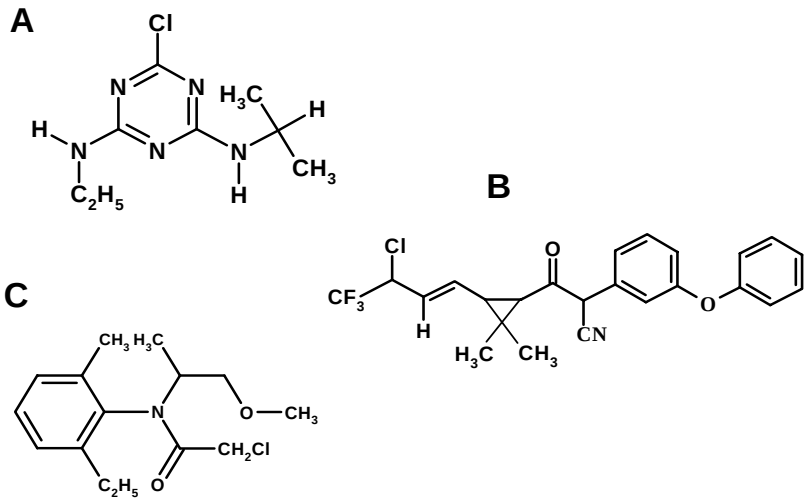

Figure 1. Chemical structures of chlorinated pesticides: A - Atrazine; B - λ-Cyhalothrin; C – Metolachlor.

Table 1. Physical properties of pesticides studied (ARS Pesticide Database, 1995).

Parameter	**Atrazine**	**8-cyhalothrin**	**Metolachlor**
Molecular wt	215.7	449.9	283.8
Water Solubility (mg/L @ 20°C)	30	0.005	500
Vapour Pressure (mPa @ 20°C)	$4.0E^{-02}$	$2.0E^{-03}$	1.7
Henry's Law (Pa m^3/mol @ 25°C)	$2.48E^{-04}$	$1.80E^{-02}$	$2.44E^{-03}$
Log K_{ow} (@ 20°C)	2.6	7.0	3.1
Log K_{oc}	$1.5E^{02}$	$1.8E^{05}$	$2.5E^{02}$

A water sample storage stability study was performed in a manner similar to the methods described previously by Hengel *et al*. (1998). Briefly, water samples collected from Beasley Lake and an agricultural drainage ditch were transferred into separate 125 mL amber glass bottles and fortified with atrazine and metolachlor at the 50 ng/mL level and λ-cyhalothrin at the 100 ng/mL level and then stored at 4°C (n = 18 per sample type). Three samples for each water type were selected randomly and transferred into individual 200 mL glass jars in addition to 500 mg of KCl and 25 mL of ethyl acetate. Each sample was extracted using sonication and transferred into a 250 mL separatory funnel. After discarding the water layer, the organic layer was transferred through sodium sulfate and collected in a 125 mL erlenmeyer flask. The extract was concentrated under a stream of UHP nitrogen to approximately 2 mL, transferred to a 12 mL centrifuge tube and further concentrated to a final volume of 1 mL.

All three analytes were analyzed by gas chromatography-electron capture detection using a Tracor 540 gas chromatograph equipped with a Dynatech Precision GC-411V autosampler and a 15 m x 0.53mm i.d. J&W 30 m DB-5 (1 μm film thickness) Megabore™ column. Column oven, inlet and detector temperatures were 195ºC, 240ºC and 350ºC, respectively. The carrier gas was UHP helium (nexAir, Memphis, TN) at 12.3 cc/min, whereas both the column makeup gas and detector purge gas were UHP nitrogen (nexAir, Memphis, TN) at 60 and 10 cc/min, respectively. Digital data were collected using a PE Nelson 2700 chromatography data system and analyzed using Turbochrom™ 4.11 software. A multi-level calibration procedure was used with standards and was updated every ninth sample. The limits of detection (LOD) for atrazine and cyhalothrin in water were 0.34 ng/mL and 0.05 ng/mL, respectively while the LODs for sediment and plants were 12 ng/mL and 1.4 ng/mL, respectively.

Concentrations of all analytes in sediment and plant samples were calculated in units of μg/g dry weight. Mean, standard deviation and coefficient of variance about the mean for each analyte were calculated from sample replicates (n=3). Data were analyzed using a two-way analysis of variance (ANOVA) with a significance level of $P \leq 0.05$. When significant differences were detected, a Tukey's multiple comparison test was utilized to compare efficiencies of different solvents and extraction procedures.

RESULTS AND DISCUSSION

Traditionally, when methods are being developed or compared, environmental matrices are amended with the compound(s) of interest and extracted using various solvents to determine the most efficient extraction method (Snyder *et al.*, 1992; Hengel *et al.*, 1998; Babić *et al.*, 1998). Initially this method was followed, where sediment samples were fortified with atrazine and λ-cyhalothrin, made up in acetone, and rolled for 24 hrs to homogenize the sediment. Sub-samples were extracted in ethyl acetate by sonication and extraction efficiencies were calculated to be 90% and >99 % for atrazine and λ-cyhalothrin, respectively. One problem with this type of amendment method is that analytes may not mimic the process in which these compounds are associated to matrices in the aquatic environment, therefore actual extraction efficiencies may be lower in environmental samples. Thus, instead of using this traditional method, native sediments known to be recently contaminated with atrazine and λ-cyhalothrin were used for method development.

Results from native sediment and plant samples sonicated using three different solvents indicate, in both cases, that ethyl acetate was the most effective extraction solvent ($p < 0.05$)(Table 2). It

was expected that the mixture of hexane and methylene chloride would be more effective at extracting these pesticides from these matrices. To further validate this method and to ensure that ethyl acetate was the more effective solvent to use, sediment and plant samples were pre-wetted with water prior to extraction. In the case of sediment, extraction efficiencies were increased for both hexane and the hexane/methylene chloride (DCM) mix, but there was no observed significant difference ($p < 0.05$) between ethyl acetate groups (Table 3). Conversely, atrazine recoveries increased significantly ($p < 0.05$) for all three extraction solvents, but there was no observed significant difference ($p < 0.05$) between their recoveries in each pre-wetted group.

Since atrazine is much more polar than λ-cyhalothrin, organic solvents were not as effective in extracting this compound from plant material (Table 2 and 3). By pre-wetting the plant samples with water prior to addition of organic solvent, atrazine extraction efficiencies increased by 6 fold. Similar results were shown by Lino and Noronha da Silveira (1997), where they used a mixture of water and organic solvents to extract a range of organochlorine pesticides. They found that their extraction efficiencies improved for more polar pesticides with the addition of water. It is thought that the addition of water to dried plant samples causes the deactivation of the cellulose active sites where these pesticides are sorbed, therefore increasing extraction efficiencies (Luke and Doose, 1983).

To further validate this method, extraction efficiencies from this procedure were compared to extraction efficiencies using a traditional soxhlet extraction. Sediments containing two levels of atrazine and λ-cyhalothrin contamination were compared using these methods. Results from the

comparison show that the present sonication method, which requires considerably less organic solvent, is as effective as soxhlet extraction (Table 4).

As part of the Mississippi Delta MSEA project, routine water samples are collected from agricultural ditches, monitoring wells and lakes for pesticide analysis. To ensure optimal pesticide recovery from these samples, a study was performed to determine acceptable sample storage time.

Table 2. Mean (n=3) extraction efficiencies of atrazine and λ-cyhalothrin (μg/g) in native sediment and plant samples using various organic solvents.

SEDIMENT				
Solvent	Atrazine	C.V.	λ-Cyhalothrin	C.V.
Ethyl acetate	2.8 ±(0.31)	11	0.064 ±(0.007)	11
Hexane	N.D.	-	N.D.	-
50:50 (Hex:DCM)	0.25 ±(0.04)	16	0.030 ±(0.004)	12
PLANT				
Solvent	Atrazine	C.V.	λ-Cyhalothrin	C.V.
Ethyl acetate	2.4 ±(0.70)	29	0.51 ±(0.13)	25
Hexane	0.47 ±(0.08)	18	0.18 ±(0.03)	19
50:50 (Hex:DCM)	1.3 ±(0.56)	43	0.39 ±(0.15)	38

C.V. = coefficient of variation

Table 3. Mean (n=3) extraction efficiencies of atrazine and λ-cyhalothrin (μg/g) for native sediment and plant samples pre-wetted with water prior to the addition of various organic solvents.

pre-wetted **SEDIMENT**

Solvent	Atrazine	C.V.	λ-Cyhalothrin	C.V.
Ethyl acetate	2.1 ±(0.25)	12	$5.4E^{-02}$ ±($6.5E^{-03}$)	12
Hexane	0.76 ±(0.12)	16	$3.0E^{-03}$ ±($5.1E^{-04}$)	17
50:50 (Hex:DCM)	0.89 ±(0.06)	7.3	$9.0E^{-03}$ ±($2.1E^{-03}$)	23

pre-wetted **PLANT**

Solvent	Atrazine	C.V.	λ-Cyhalothrin	C.V.
Ethyl acetate	13.7 ±(2.3)	17	0.44±(0.11)	25
Hexane	13.3 ±(1.3)	10	0.20 ±(0.03)	16
50:50 (Hex:DCM)	13.1 ±(1.1)	8.7	0.28 ±(0.05)	17

Table 4. Comparison of soxhlet vs sonication extraction methods using ditch sediment contaminated with high and low concentrations of atrazine and λ-cyhalothrin (μg/g).

	Soxhlet		**Sonication**	
Sediment A	Mean (n=3)	C.V.	Mean (n=3)	C.V.
Atrazine	11.1 ±(1.04)	9.4	9.48 ±(0.81)	8.5
λ-Cyhalothrin	$9.4E^{-03}$ ±($7.1E^{-04}$)	7.6	$9.50E^{-02}$ ±($5E^{-03}$)	4.8
Sediment B				
Atrazine	0.23 ±(0.06)	25	0.28 ±(0.04)	14
λ-Cyhalothrin	$2.6E^{-03}$ ±($3.9E^{-04}$)	15	$2.5E^{-03}$ ±($7.3E^{-04}$)	29

C.V. = coefficient of variation

The USEPA recommends a maximum storage time of 7 days for chlorinated pesticides (EPA Method 608). To determine this, water samples taken from an oxbow lake (Beasley Lake) in the Mississippi Delta region and an agricultural ditch that drains into the lake were spiked with

atrazine, λ-cyhalothrin and metolachlor. For both water samples, results show that there was a rapid reduction in recovery of atrazine and λ-cyhalothrin at 1, 2, 4 and 8 days (Table 5). Hengel *et al.* (1998) reported similar data with pyrethroids, esfenvalerate and permethrin (cis and trans), using a similar test method. Results for metolachlor indicate that, over an 8 day period, recoveries were reduced by approximately 20%.

Table 5. Results of the pesticide stability storage test in surface waters fortified with atrazine, λ-cyhalothrin and metolachlor in surface waters.

		% Recovery (n = 3)			Storage Time
Water Sample	(days)	metolachlor	λ-cyhalothrin	atrazine	
Beasley Lake	0	100 ±4	82 ±4	100 ±1	
	1	87 ±6	56 ±3	79 ±7	
	2	87 ±10	NA	61 ±14	
	4	73 ±6	51 ±2	43 ±12	
	8	83 ±4	60 ±3	47 ±7	
Ditch Water	0	93 ±13	87 ±12	100 ±2	
	1	78 ±3	69 ±1	100 ±7	
	2	66 ±14	NA	75 ±30	
	4	68 ±5	72 ±0.3	35 ±6	
	8	66 ±4	54 ±5	43 ±10	

These losses may be attributed to adsorption on glass (Sharom and Solomon, 1981) but it is more likely that these losses were due to adsorption to suspended solids. Both water samples contained a high amount of suspended solids (~ 400 mg/L). Other possibilities for reduction in recovery may include chemical degradation via oxidative free radicals or microbial degradation (Sharom and Solomon, 1981). These data indicate, as also reported by Hengel *et al.* (1998), the importance of immediate extraction of water samples upon returning from the field.

Overall, this research presents an easy and rapid method for the extraction and analysis of two classes of pesticides using a sonication method that requires lower solvent volumes than traditional methods. This method could be used for the extraction of other triazine herbicides and pyrethroid insecticides simultaneously, but it would be suggested that all sediment and plant samples be pre-wetted with water prior to the addition of solvents. Even though there was no difference in recoveries between dry and pre-wetted sediments using ethyl acetate, polar pesticides, such as other triazines, may require this pre-wetting step for optimal recoveries.

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APPENDIX 11.2

Vegetated Agricultural Drainage Ditches for the Mitigation of Pyrethroid Associated Runoff

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ABSTRACT

Drainage ditches are indispensable components of the agricultural production landscape. An environmental benefit of these ditches is mitigation of contaminants associated with agricultural storm water runoff. This study was designed to determine bifenthrin and lambda-cyhalothrin (two pyrethroid insecticides) partitioning and retention in ditch water, sediment, and plant material, as well as estimate necessary ditch length required for effective pesticide mitigation. A controlled-release storm runoff simulation was conducted on a 650 m vegetated drainage ditch in the Mississippi Delta. Bifenthrin and lambda-cyhalothrin were released into the ditch in a slurry with water and sediment. Samples of ditch water, sediment, and plants were collected and analyzed for pesticide concentrations. Three hours following the initiation of the storm runoff simulation, bifenthrin and lambda-cyhalothrin water concentrations ranged from 666 µg/L and 374 µg/L, respectively, at the inlet to 7.24 µg/L and 5.23 µg/L at 200 m downstream. No chemical residues were detected at the 400 m sampling site. A similar trend was observed throughout the first 7 d of the study where water concentrations were elevated at the front end of the ditch (0 – 25 m) and greatly reduced by the 400 m sampling site. Regression formulas predicted that bifenthrin and lambda-cyhalothrin concentrations in ditch water were reduced to 0.1% of the initial value within 280 m. Using mass balance calculations it was determined that the ditch plants were the major sink and/or sorption site for the rapid dissipation of bifenthrin and lambda-cyhalothrin from the water column. By 12-h into the study, the majority of the mass of each pesticide (>93%) was present in the ditch plants. By incorporating vegetated drainage ditches into a watershed management program, agriculture can continue to decrease potential non-point source threats to downstream aquatic receiving systems. Overall results of this study illustrate that aquatic macrophytes play an important role in the retention and distribution of pyrethroids in vegetated agricultural drainage ditches.

Key words: Drainage ditches, Bifenthrin, lambda-Cyhalothrin, Aquatic plants, Mitigation

INTRODUCTION

Available literature on surface drainage ditch research is limited [1,2]. Other than Moore et al. [3] and Cooper et al. [4], few studies in the United States have focused on the capacity of drainage ditches to decrease the concentration of pesticides entering receiving aquatic ecosystems. More research has been conducted in the Netherlands, where drainage ditches serve as important habitat and potential transports for drinking water [5-8]. Many of these studies focused on ditch maintenance and management practices, since the use of ditches is more restrictive in the Netherlands than in the United States. Historically, the value and function of agricultural ditches have been ignored except for their general upkeep, which may include periodic dredging to remove built up sediment and plants impeding efficient drainage. It has been proposed that these marginal lands colonized with aquatic plants could be used as a simple and inexpensive method for the mitigation of agricultural runoff. Natural or constructed vegetated wetlands are sometimes not an option in agricultural row-crop farming mainly due to space limitations, thus vegetated drainage ditches are a simple alternative for this currently suggested best management practice (BMP).

Recently, research by Moore et al. [3] has shown that vegetation in agricultural ditches aids in the trapping of many commonly used pesticides. Other studies on the role of aquatic plants as a mitigative tool for pesticide reduction and retention have been limited to wetland and mesocosm studies using both spraydrift [9-12] and runoff [13-15] scenarios. Schulz et al. [13] recently showed that the aquatic macrophyte, *Typha capensis*, facilitated the reduction of the loading of an organophosphate insecticide, azinphos-methyl, into a nearby waterway following a spray drift event in a South African wetland.

In the Mississippi Delta region there are a wide range of pesticides currently used in production farm acreage. Of these, synthetic pyrethroids are one of the main classes of insecticides used, especially in cotton and corn production. For example, bifenthrin [(2-methyl-2-methylbiphenyl-3-ylmethyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclo-propanecarboxylate] is a fourth generation pyrethroid insecticide. Sold under such trade names as Capture® and Brigade®(FMC, Philadelphia, PA, USA) approximately 52,000 kg active ingredient bifenthrin was applied to US corn (94%), cotton (3%), and blackberry (3%) crops in 2001 (NASS, 2003, http://www.nass.usda.gov). Lambda-cyhalothrin, a 1:1 mixture of (S)-a-cyano-3-phenoxybenzyl -(Z)-(1R,3R)-3- (2-chloro-3,3,3-trifluoroprop-1-enyl) -2,2-dimethylcyclo propane carboxylate and (R)-a-cyano-3-phenoxybenzyl-(Z)-(1S,3S) -3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate [(RS)-alpha-cyano-3-phenoxybenzyl3-(2-chloro-3,3,3-triflurorpropenyl)-2,2 dimethylcyclopropanecarboxylate], another fourth generation pyrethroid, is commonly sold as Karate®(Syngenta, Greensboro, NC, USA) Over 45,000 kg of lambda-cyhalothrin (as active ingredient) was applied to US cotton (54%), corn (41%) and soybean (5%) crops in 2001 (NASS, 2003, http://www.nass.usda.gov). The basic mode of action for synthetic pyrethroids is the disruption of the central and peripheral nervous system of insects causing paralysis [16]. Pyrethroids have been shown to elicit toxic effects at extremely low concentrations in non-target aquatic organisms, such as mayflies (*Heptageniidae*) and damselflies (*Enellagma* and *Ishnura* spp.) [17]. Owing to these toxic effects, there is increased concern about such compounds reaching aquatic environments, especially during agricultural runoff and spray drift events.

The purpose of this present study was two fold. The first objective was to evaluate the retention and partitioning (water, plant, sediment) of two currently used pyrethroids, bifenthrin

and lambda-cyhalothrin, within a vegetated agricultural drainage ditch located in the Mississippi Delta, MS, USA during a simulated, worst case scenario runoff event. From these data, the relative importance of aquatic vegetation in facilitating the removal of insecticide from water was evaluated using mass balance calculations and insecticide physico-chemical properties. The second objective of this study was to estimate drainage ditch lengths for effective mitigation of bifenthrin and lambda-cyhalothrin using pesticide distribution, given recommended field application rates, and other rainfall and runoff variable assumptions.

MATERIALS AND METHODS

Ditch exposure

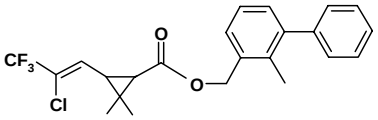
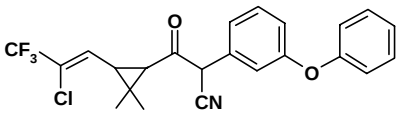
A 650-m segment of an agricultural drainage ditch located within the Mississippi Delta Management Systems Evaluation Area (MDMSEA), near Indianola, MS, was used for this study. The ditch was approximately 4.5 m wide (top width), 0.35 m deep (water depth), and had a water width of approximately 2.8 m. Sampling sites were established within the ditch at the simulated runoff inlet, 10 m upstream, and 25, 50, 75, 100, 200, 400 and 650 m downstream. One week prior to the simulated storm runoff event, ditch vegetative cover was determined to be approximately 88% by sampling multiple 0.69 m^2 quadrats at each sampling site and the dominant plant species were determined to be *Ludwiga* followed by *Lemna* and *Polygonum*. In addition to this survey, basic water quality and centerline velocity measurements were made using a handheld, YSI-85 meter and a Marsh-McBirney electromagnetic current meter, respectively. Mean ditch measurements were as follows: velocity, 0.03±0.02 m/s; dissolved oxygen., 3.3±0.09 mg/L; temperature, 30.1±0.4 °C; conductivity, 559±5 µmhos; pH 7.4±0.2.

A storm runoff event was simulated in the drainage ditch in late July 1999. A mixture of bifenthrin (Capture®) and lambda-cyhalothrin (Karate®) was amended directly into the ditch. Physical and chemical properties of these compounds are presented in Table 1. Pesticide concentrations in simulated runoff (0.89 mg/L bifenthrin and 0.44 mg/L lambda-cyhalothrin) were based on recommended application rates (0.011 kg/ha and 0.0057 kg/ha, respectively) and an assumed 1% runoff from a 0.64-cm storm event across a 20-ha contributing area [18]. Simulated runoff was pumped through a 2.0-m length of 7.6-cm diameter polyvinyl chloride pipe with 16, 1.5-cm holes evenly dispersed along the pipe's length for even diffusion. Four, 3800-L water tanks filled with groundwater were connected (one at a time) to the diffuser and used as a water source for the simulated event. The mixture of bifenthrin and lambda-cyhalothrin was added to water in a 110 L mixing chamber, and then delivered to the top of the PVC diffuser via Tygon™ tubing using an Atwood™ V450 submersible pump at a rate of 0.02 L/s for 90 min. A 5-cm hose delivered water from the 3800-L water tanks directly to the diffuser at a rate of approximately 1 L/s for about 250 min.

Collection of water, sediment, and plant samples

Grab samples of water (n=1) were collected in 1 L amber glass bottles 1 week prior to the application, at 0 h, at 15 minute intervals for 2 h, and at 3 h, 6 h, 12 h, 24 h, 7 d, 14 d, 30 d, 44 d, and 99 d post-application from each site. After samples were collected, they were stored on ice and returned to the laboratory for extraction (within 24 h). Sediment and plant samples were collected 1 week prior to application and at 0 h, 3 h, 12 h, 24 h, 7 d, 14 d, 30 d, 44 d, and 99 d post-application from each site, wrapped in solvent washed foil, stored on ice, and returned to the laboratory to be dried. It should be noted that sediment samples were obtained from the top 1 cm using solvent-rinsed stainless steel spatulas, while plant materials were collected with solvent-

Table 1. Physical and chemical properties of bifenthrin and lambda-cyhalothrin (adapted from Laskowski [25]; N.A. = not available).

		Bifenthrin	Lambda-cyhalothrin
Structure			
Molecular weight (g/mol)		422.9	449.9
Vapor pressure (mm Hg)		1.8×10^{-7}	1.7×10^{-9}
Water solubility (mg/L)		1.4×10^{-5}	5.0×10^{-3}
Henry's Law Constant (atm m^3/mol)		7.2×10^{-3}	7.9×10^{-7}
log K_{ow}		6.4	7.0
log K_{oc}		5.4	5.5
Hydrolysis ½ life (days)	pH 5	Stable	Stable
	pH 7	Stable	Stable
	pH 9	Stable	8.7
Photolysis ½ life (days)	Water	408	24.5
	Soil	96.9	53.7
Soil ½ life (days)	Aerobic	96.3	42.6
	Anaerobic	425	N.A.

rinsed scissors. Only that plant material exposed in the water column (between sediment-water surface) was collected for analysis.

It should be noted that only the top 0.01 m of sediment was collected using a metal spatula. Also, only the portion of plant present in the water column (from sediment surface to water surface) was collected.

Extraction and analysis of water, sediment and plant samples

Water, sediment and plant samples were extracted using previously described methods by Bennett et al. [19] and Moore et al. [3]. Briefly, water samples were extracted by liquid-liquid extraction using ethyl acetate, while both sediment and plant samples were extracted by ultra-sonication using ethyl acetate. Sediment and plant extracts were also subject to silica gel cleanup before analysis. Water samples were partitioned with ethyl acetate in the field and transported in coolers back to the laboratory for final extraction.

Pyrethroids were analyzed by gas chromatography-microelectron capture detection using a HP 6890 gas chromatograph equipped with a 30 m HP-1MS column. The following oven temperature program was used: 75°C (held for 1 min) to 225°C at a rate of 40°C/min. The injector and detector temperatures were set to 250°C and 325°C, respectively. The carrier gas, ultra high purity helium (nexAir, Memphis, TN), was set to a constant flow of 1 mL/min and makeup gas, ultra high purity Nitrogen (nexAir, Memphis, TN), was set at a constant makeup flow of 60.0 mL/min. A multi-level calibration procedure was used with bifenthrin and lambda-cyhalothrin standards (AccuStandard, New Haven, CT, USA) and was updated every ninth sample. Procedural blanks and solvent blanks were analyzed with each batch of standards. Limits of detection for bifenthrin and lambda-cyhalothrin in water, sediment and plants were 1-10 ng/L (ppt). Mean extraction efficiencies, based on fortified samples, were > 90% for water, sediment, and plants.

Data Analysis

Ordinary least-squares linear regression analyses [20] were used to fit curves to log-transformed bifenthrin and lambda-cyhalothrin water concentrations (y) versus the log of the distance down ditch from the inlet (x). For simplicity, only the maximum concentrations

observed at each sampling site during the first 3 h following injection were used in the analyses. Concentrations at the most distant sampling site (x = 650 m) were below detection limits, so regressions were run either without this data point or with the assumption that the concentration at this site equaled the detection limit. The latter set of regressions produced results similar to those without the x = 650 m data, so they will not be presented below. Concentrations at the injection point were also omitted from the regression in order to improve curve fits at greater distances.

Mass balances were performed using data on water, plant and sediments collected along transects of the 650 m ditch length for each sample time point (3 h, 12 h, 24 h, 7 d, 24 d, 30 d and 44 d). This enabled quantitative evaluation of chemical partitioning and losses that occurred over the study duration. The mass balance at a given time point was determined as:

$$m_{total(t)} = m_{w(0-650)} + m_{p(0-650)} + m_{s(0-650)} \qquad (1)$$

where $m_{w(0-650)}$, $m_{p(0-650)}$ and $m_{s(0-650)}$ reflect the total chemical mass (g) in water, plants and sediments over the 650 m ditch length. Integration of chemical masses in water was performed according to the trapezoidal rule:

$$m_{w(0-650)} = \sum_{i=0}^{n-1} \left(\left(V_{w(i+1)} - V_{w(i)} \right) \cdot \left(\frac{C_{w(i+1)} + C_{w(i)}}{2} \right) \right) \qquad (2)$$

where the term $(V_{w(i+1)} - V_{w(i)})$ represents the volume of water (L) bounded by transects i and i+1 (i.e. 0-25m, 25-50m, 200-400m etc.) and $C_{w(i)}$ is the water concentration measured at transect i. Water volumes between transects were estimated as the product of mean water depth, mean water width, and the distance between transects less the water displacement by plants (assuming an estimated 20% of water was displaced by plant biomass in each transect). The mass

calculations for plants and sediments were similar to Equation 2 except that concentrations were measured in units of mg/kg and the volume terms were replaced by bulk sediment mass (kg) or plant biomass (kg). In the former case, the ditch bed surface area (m^2) within a given interval was multiplied by a sediment depth of 0.01 m and converted to sediment mass by assuming a bulk sediment density of 1200 kg/m^3. Plant biomass was calculated at 0, 25, 50, 75, 100, 200, 400 and 600 m transects and ranged from 0 to 4.22 kg/m^2 (d.w.). The average area plant biomass within a transect interval was multiplied by the ditch width and interval distance to arrive at a plant biomass (kg) estimate.

A ditch chemical depuration rate constant (k_2), representative of all measured compartments, was determined by plotting ln m_{total} as a function of time and determining the slope using linear regression analysis. The ditch chemical depuration rate constant represents the summation of individual clearance rate constants occurring within each phase and includes losses to advection (e.g. outflow from the ditch via water), volatilization and abiotic/biotic reactions. Chemical half lives ($t_{1/2}$) were subsequently estimated as $\ln(2)/k_2$. Chemical depuration rate constants and half lives were also derived using mass balances for individual media (water, plants and sediments).

RESULTS

Concentrations in water

Both bifenthrin and lambda-cyhalothrin concentrations measured in water decreased rapidly with distance (Table 2). For example, 3 h following the initiation of the storm runoff simulation, bifenthrin water concentrations ranged from 666 µg/L at the inlet to 7.24 µg/L at 200 m downstream, while concentrations were below detection limits at the 400 m sampling site. A similar trend was observed throughout the first 14 d of the study where water concentrations

were elevated at the front end of the ditch (0 – 25 m) and greatly reduced by the 400 m sampling site. By day 30, the distribution of bifenthrin water concentrations showed slightly greater dispersion, exhibiting a peak concentration at 50 m, elevated levels at 100 m and non-detectable levels after 200 m (Fig. 1)

Table 2. Downstream dissipation of bifenthrin and lambda-cyhalothrin concentrations (µg/L) in water at the 3 h, 12 h and 7 d sampling times (N.D. = Not Detected, below detection limits; 1.00 ng/L).

Distance	Bifenthrin			lambda-Cyhalothrin		
(m)	3 h	12 h	7 d	3 h	12 h	7 d
0	666	10.7	0.887	375	5.29	0.250
25	235	25.9	7.76	115	11.8	2.32
50	77.2	6.33	0.178	39.1	3.44E	5.50E-02
75	33.8	1.03	0.270	20.6	0.745	7.38E-02
100	27.8	1.32	6.38E-02	16.6	0.899	2.38E-02
200	0.724	0.454	5.07E-02	0.309	0.296	2.00E-02
400	N.D.	0.471	N.D.	0.144	9.88E-02	N.D.
650	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

This pattern was also observed for lambda-cyhalothrin (Table 2 and Fig. 1). During and after day 14 of sampling, bifenthrin and lambda-cyhalothrin water concentrations were approximately 0.05 µg/L and 0.02 µg/L, respectively, when detected. By day 99, all water samples collected were below detection limits. When these data are expressed on a percentage basis, pesticide concentrations at the 200 m sampling site were >99% lower than concentrations detected at the 25 m sampling throughout the study.

Concentrations in/on plants

Ditch plant samples collected one week prior to experiment initiation indicated bifenthrin and lambda-cyhalothrin concentrations that were below detection. The highest concentrations for both bifenthrin and lambda-cyhalothrin in/on plants were detected in the ditch within 50 m of

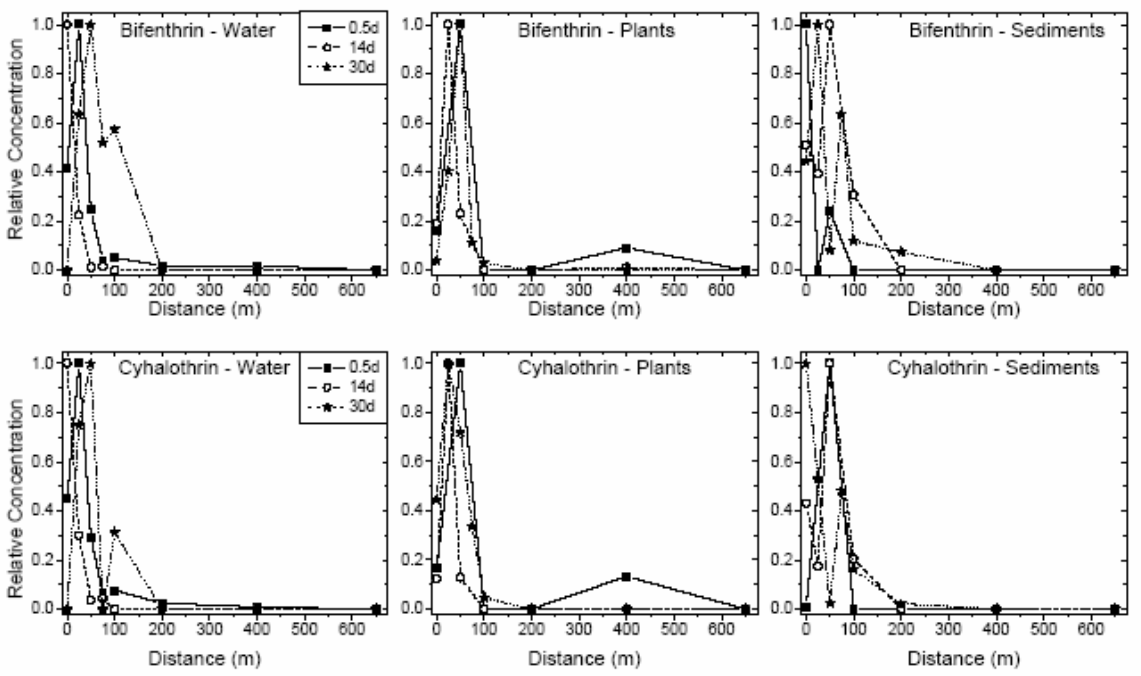


Figure 1. Comparison of relative bifenthrin and lambda-cyhalothrin concentrations measured at 0.5d, 14d and 30d for each compartment (water, plants, sediment) to sampling sites downstream of the injection point.

the inlet throughout the study (Fig. 1). Maximum measured concentrations for bifenthrin and lambda-cyhalothrin were 10.8 mg/kg (d.w) and 8.79 mg/kg (d.w.), respectively. By 12 h after runoff initiation, both pesticides were detected at all of the sampling sites between the inlet and the 400 m sampling site. Unlike water, bifenthrin and lambda-cyhalothrin showed little evidence of dispersion from the initial distribution along the ditch length over time (Fig. 1).

Pesticide concentrations associated with plants stabilized after 12 h throughout the ditch for approximately 30 d post application. Bifenthrin concentrations were between 7.26 and 10.8 mg/kg at the 50 m sampling site within the first day of the study with concentrations stabilizing (~ 3 mg/kg) after 7 d until they dropped considerably after 30 d (Fig. 2). A similar trend was observed for lambda-cyhalothrin with the exception that there was not a considerable decline in concentration after 30 d.

Concentrations in sediment

Background sediment samples collected one week prior to experiment initiation were below detection limits for concentrations of the two insecticides. Bifenthrin and lambda-cyhalothrin in sediment reached maximum concentrations of 0.0917 mg/kg and 0.0538 mg/kg, respectively, within the first 25 m of the ditch system. Generally, sediment pesticide concentrations were two orders of magnitude lower at sites beyond the 50 m sampling site. However, after 14 d, both bifenthrin and lambda-cyhalothrin showed some evidence of downstream dispersion, with low to moderate concentrations being measured at 100 m and 200m (bifenthrin only; Fig. 1).

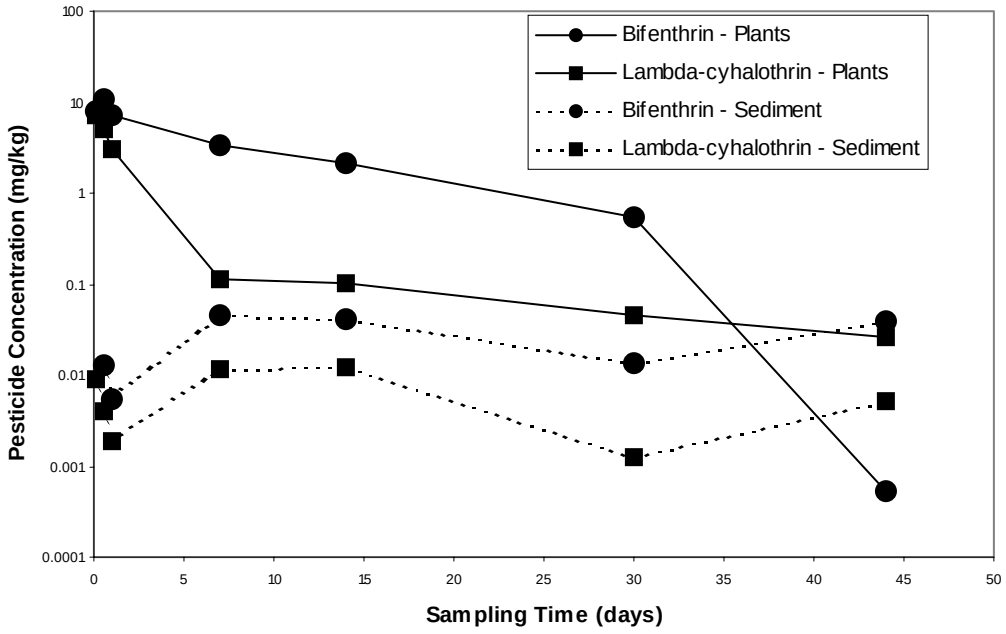


Figure 2. Measured bifenthrin and lambda-cyhalothrin concentrations (mg/kg) in plants and sediments collected from the 50 m sampling site at the 3 h, 12 h, 24 h, 7 d, 14 d, 30 d and 44 d sampling periods.

Overall, bifenthrin and lambda-cyhalothrin sediment concentrations stabilized in the ditch system after 24 h. Bifenthrin sediment concentrations at the 50 m sampling site stabilized to approximately 0.0400 mg/kg 24 h post application and this concentration was maintained for the remainder of the study (Fig. 2). A similar trend was also observed for lambda-cyhalothrin.

Ditch length predictions

Maximum observed pesticide concentrations (determined during 0-3h) were inversely proportional to distance downstream from the inlet, producing regression coefficients that were significant at $p < 0.004$ (Table 3 and Fig. 3). The fit standard errors were about ±0.01, but 95% confidence intervals were rather broad due to the relatively small number of data points. The formulas predict that bifenthrin and lambda-cyhalothrin concentrations in ditch water were reduced to 1% of the initial value within the first 120 m of ditch downstream from the inlet, and fall to only 0.1% of the initial value within 280 m.

Mass balance and half-lives

The total mass of bifenthrin and lambda-cyhalothrin at 3 h ($m_{total(3h)}$) was consistent with the nominal mass of chemical added to the system (Table 3). The $m_{total(3h)}$ for bifenthrin was 12.1 g or 106% of the nominal mass added to the system, while the $m_{total(3h)}$ for lambda-cyhalothrin was 163% of the nominal mass. Mass balance calculations for different sampling times showed that there were changes in phase distribution occurring over the study duration. At the 3 h sampling time point, the majority of mass for both bifenthrin and lambda-cyhalothrin were concentrated in the water and plant compartments (Table 3). By 12 h into the study, almost all the mass of both pesticides had shifted to the plant compartment. This trend continued throughout the study. For both water and plant compartments, there was a steady reduction of

Table 3. Summary of linear regression formulas produced using maximum pesticide concentrations versus distance downstream from inlet. Data were log-transformed. Relations are power functions of the form $y = a\ x^{b}$ where y is the maximum concentration in mg/L (water) observed during the first 3 h following injection, and x is the distance in meters. Alternatively, $y' = a'x^{b}$ where y' is concentration in mg/L (water) divided by the maximum observed concentration at the inlet. Data for the injection point (x = 0) were not used in the regression in order to improve curve fits for larger x values (Fig. 3).

Compound	No. of observations	a	a'	b	r^2	p
Bifenthrin	**6**	**20,460**	**30,770**	**-3.19**	**0.937**	**0.0015**
λ-Cyhalothrin	**6**	**1,537**	**4,099**	**-2.69**	**0.902**	**0.0037**

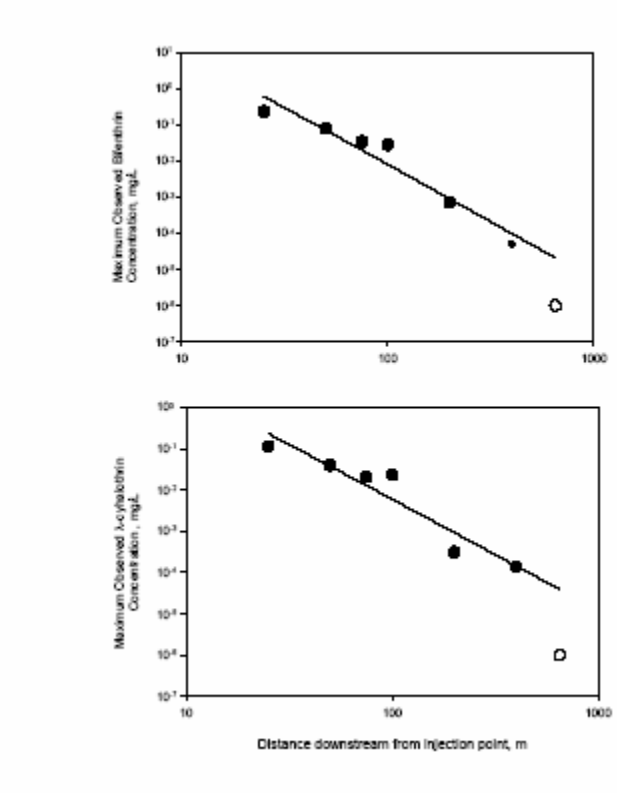


Figure 3. Least-squares regression relationships fit to log-transformed maximum observed pesticide concentration in water versus distance downstream from injection point. Concentrations at x = 650 m (open circles) are detection limits.

pesticide mass, while there was enrichment and/or retention of lambda-cyhalothrin in the sediment compartment over time. However, the total mass in sediment was relatively low.

Combined changes in $m_{w(0\text{-}650)}$, $m_{s(0\text{-}650)}$ and $m_{p(0\text{-}150)}$ with time were used to estimate ditch chemical half-lives. Bifenthrin and lambda-cyhalothrin half-lives were calculated to be 6.12 and 1.35 d, respectively. Bifenthrin data used in this calculation followed first order kinetics while initial lambda-cyhalothrin half-life calculations were based on assumed first order kinetics. In fact, lambda-cyhalothrin data followed a bi-phasic pattern of elimination with was occurring with an initial rapid drop in mass during the first 7 d followed by a much slower elimination rate over the remaining 36 d (Table 4). Alternately, half-lives for lambda-cyhalothrin were calculated to be 1.24 d within the 7 d sampling period and >100 d after the 7 d sampling period.

Table 4. Estimated mass (g) of bifenthrin and lambda-cyhalothrin in the water, sediment and plant compartments relative to each sampling time (*total A.I. amended to ditch at time zero).

Bifenthrin (g)					Lambda–Cyhalothrin (g)				
Time	Water	Plants	Sediment	Total	Time	Water	Plants	Sediment	Total
0 h	-	-	-	*11.4	0 h	-	-	-	*5.70
3 h	5.78	6.29	3.85E-02	12.1	3 h	3.10	6.13	6.24E-02	9.29
12 h	0.718	7.22	3.30E-02	7.97	12 h	0.353	3.76	1.12E-02	4.13
24 h	0.191	4.03	1.13E-02	4.24	24 h	0.106	1.59	3.68E-03	1.70
7 d	0.134	1.93	6.31E-02	2.13	7 d	4.05E-02	6.74E-02	1.79E-02	0.126
14 d	4.48E-02	3.00	5.25E-02	3.10	14 d	6.90E-03	2.09E-01	1.29E-02	0.229
30 d	4.37E-03	0.199	1.93E-03	0.206	30 d	1.05E-03	3.41E-02	4.24E-02	7.75E-02
44 d	8.82E-04	4.89E-02	8.31E-04	5.07E-02	44 d	6.22E-03	9.10E-02	5.04E-02	0.148

DISCUSSION

This study was designed to determine the effectiveness of a vegetated drainage ditch on the retention and partitioning of pyrethroids during a simulated runoff event. Results showed that vegetated drainage ditches are effective tools for lowering/removing pyrethroid associated runoff from the ditch water column, consequently reducing pesticide loadings into receiving water bodies. In this study, 33.9% and 36.3% of the original dose of bifenthrin and lambda-cyhalothrin, respectively, remained in the water 3 h post application and were further reduced to 3.09% and 1.24% after 1 d. Similar results by Leistra et al. [11] showed that 1.8-6.5% of the original dose of lambda-cyhalothrin applied into vegetated ditch enclosures remained after 3 d. These results indicate that there was a rapid reduction of both pesticides within the first day of application. This rapid decrease in water concentrations is of great importance since pyrethroids have been shown to elicit toxic effects at extremely low concentrations in non-target aquatic organisms [17,21,22]. To reduce loadings and toxic effects in receiving water bodies after a runoff event, an effective vegetated drainage ditch length is required. Ditch lengths of 120 m and 280 m were estimated from this study to reduce bifenthrin and lambda-cyhalothrin to 1.00% and 0.100% of initial values, respectively. These values are based on a worst-case scenario indicating that shorter ditch lengths may be effective, but depending on space limitations more conservative distances would likely be more effective.

Water, plant and sediment samples were collected throughout the study to determine the relative importance of each compartment. Since it is difficult to directly compare concentrations between compartments, mass balance calculations were performed to better understand the distribution and fate of bifenthrin and lambda-cyhalothrin in this system. After 12 h the majority of pesticide mass was found in the plant compartment giving evidence that this compartment was

the most important and effective compartment in the mitigation of these insecticides. Recent reports [3,10-12] have also shown the importance of aquatic vegetation in pesticide mitigation. It would be expected that sediments would also play an important role in this mitigation process since pyrethroids have a relatively high K_{oc} (Table 1).

In this study, sediments were a minor sink due to the dense plant community that limited the movement and/or partitioning of bifenthrin and lambda-cyhalothrin to the sediment compartment. Similar results were found in a microcosm study by Hand et al. [10] where aquatic plants significantly reduced the amount of lambda-cyhalothrin reaching the sediments. Moreover, in the same study, it was shown that lambda-cyhalothrin plant adsorption was virtually irreversible in turn reducing sediment partitioning. The results of the present study suggest that a small amount of bifenthrin and lambda-cyhalothrin was mobilized from the more concentrated upstream portion of the ditch as evidenced by an increase in dispersion distance of relative water and sediment contamination along ditch length at the 30 d (Figure 2) and 44 d sampling intervals. An increase in pesticide dispersion distance was not evident for plants at later time points.

Differences in stability between bifenthrin and lambda-cyhalothrin in this test system was evident from the half-lives calculated for each pesticide. Bifenthrin exhibited a half-life of 6.12 d, while the half-life for lambda-cyhalothrin was only 1.35 d. Hand et al. [10] reported similar results in a study investigating the route of metabolism of [^{14}C]lambda-cyhalothrin following adsorption to aquatic plants where lambda-cyhalothrin quickly bound to the plant surface and was readily degraded by ester cleavage. This was evident due to the rapid increase in the cyclopropane acid metabolite and lack of parent compound present in the water of their test system. Alternatively, this shorter half-life may have been attributed to alkaline hydrolysis.

Studies have shown that the pH in surface waters can exceed 9 due to the photosynthesis by plants and algae [23,24]. Lambda-cyhalothrin has been shown to be unstable under these basic conditions while bifenthrin has been shown to be stable [25].

The fate of bifenthrin and lambda-cyhalothrin reflects a complex combination of processes, and there are other important factors also affecting their fate in drainage ditches, especially in the Mississippi delta region. In this region, water temperatures can be greater that than 30°C, while air temperatures near the water surface can reach 40°C. Even though pyrethroids have low volatility (Table 1), these higher temperatures may play a role in their degradation and volatilization from the ditch system. Other factors that are related to this process include plant growth and water level fluctuation which expose plant material to ambient air and direct sunlight leading to possible volatilization and photolysis. Rudel [26] reported pesticide volatilization from plant surfaces were higher as compared to soil surfaces for a range of pesticides, including lambda-cyhalothrin, due to increased air flow and exposure to these surfaces. In relation to water and submerged plant surfaces, these air exposed plant surfaces previously submerged may be an important vector for pesticide loss from the ditch system. Furthermore, many of the drainage ditches in the Mississippi Delta are ephemeral, and by late summer/early fall are often void of water. This may play an important role in the cycling or degradation of compounds that remain sorbed to remaining plant material and sediments. With the loss of water, light and air have direct contact to these surfaces possibly increasing the photo- and aerobic degradation of agrichemical compounds. This annual cycling may act as a self-cleaning mechanism for these ditch systems and reduce pesticide loadings into receiving water bodies during the winter and spring when water returns to these systems.

CONCLUSION

Ditch lengths of less than 300 m are required to reduce loadings into receiving water bodies during worst-case scenario runoff events. This demonstrates the importance and effectiveness of vegetated drainage ditches as a BMP for the mitigation of pyrethroid runoff. For this tool to be optimally effective, it would need to work in parallel with other existing BMPs and farming practices to facilitate single entry points into surrounding ditch systems. Since most fields are surrounded by these ditch systems in the Mississippi Delta, multiple entry points may be required, especially in larger acreage fields. Buffer strips on the fringes of agricultural fields would also help in the funneling of runoff into these ditch entry points. In many cases where other BMPs are not available, simple vegetated drainage ditches would still be an effective tool.

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APPENDIX 11.3

Mitigation of Azinphos-Methyl in a Vegetated Stream: Comparison of Runoff and Spray-Drift

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Abstract: - The effectiveness of aquatic macrophytes in reducing runoff- and spray-drift-induced azinphos-methyl (AZP) input was compared in a vegetated stream. Water, sediment and plant samples were taken at increasing distances from a point of input during a spray-drift event and two runoff (10 and 22 mm/day) events. Peak concentrations of AZP decreased significantly (R^2 = 0.99; p < 0.0001; n = 5) from 0.24 µg/l to 0.11 µg/l during the 10 mm runoff event. No reduction took place during the 22 mm event. AZP concentrations were reduced by 90 % following spray-drift input, with peak concentrations decreasing significantly (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. Plant samples taken after the spray-drift event showed increased AZP concentrations in comparison to before the event indicating sorption of the pesticide to the macrophytes. Although peak concentrations of AZP were as effectively mitigated during the 10 mm runoff event as during the spray-drift event, 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.

Keywords: azinphos-methyl, runoff, spray-drift, mitigation, vegetation.

Introduction

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 and 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).

Runoff and spray-drift are important sources of nonpoint-source pesticide pollution of surface waters (Dabrowski and Schulz 2003). Vegetated ditches (Brock et al. 1992; Moore et al. 2001) and wetlands (Schulz and 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. 2001a).

Occasional heavy rainfall events occur during this period and result in runoff-induced pesticide inputs associated with short-term increases in discharge (Schulz 2001b). 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 tributary of the Lourens River, Western Cape, South Africa, so as to assess the potential of aquatic macrophytes in reducing pesticide concentrations under different hydrological conditions.

Materials and Methods

Study Area

The stream investigated in this study is a tributary of the Lourens River catchment in the Western Cape, South Africa. About 87% of the river's $\underline{3{,}5x10^7}$ 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 occur at frequencies of 3.4 and 1.7 per spraying season (October to February) for events > 10 and > 15 mm/d, respectively (Schulz et al. 2001a).

The stream had a coverage of emergent aquatic macrophytes of almost 80 % (*Juncus capens*is – 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 on either side of the channel (left zone, 1.15 m ± 0.12; *n* = 19; right zone, 0.98 m ± 0.07; *n* = 19) (Fig. 1). Estimated densities were 125, 170 and 151 ramets/m^2 for *J. capensis*, *F. hirsuta* and *Pycreus* sp. respectively.

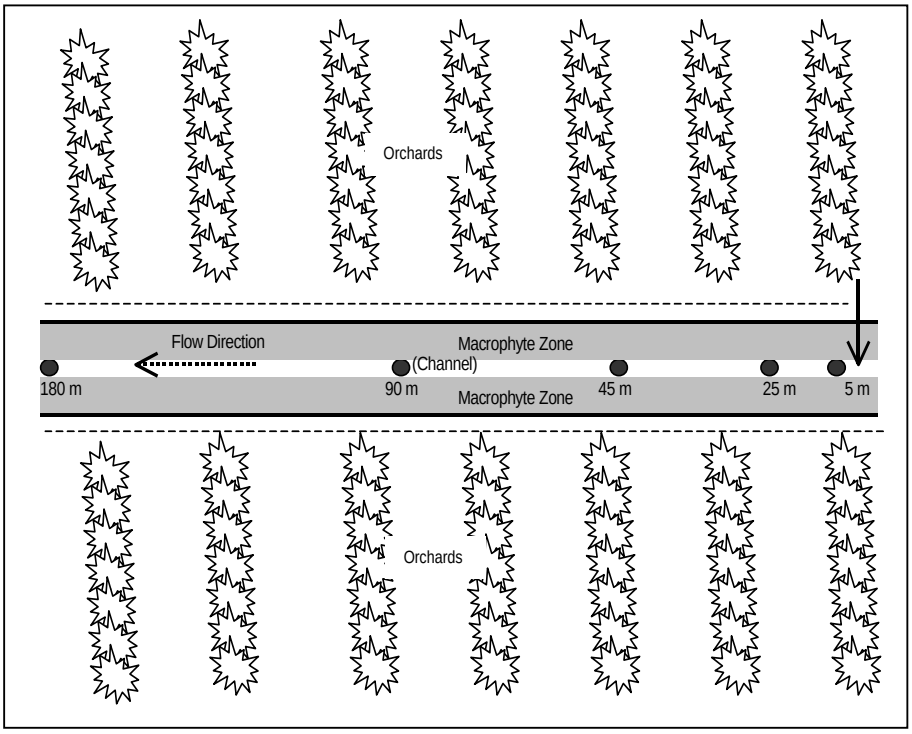

Fig. 1**:** Diagram (not to scale) of the position of sampling sites (black dots) at 5, 25, 45, 90 and 180 m after the point of runoff and spray-drift input (bold black arrow) in a vegetated stream. Dotted lines represent runoff barriers on either side of the stream.

The stream was in close proximity to adjacent pear orchards (5 m) and previous studies have shown that the stream regularly receives runoff- and spray-drift-related pesticide input under normal farming practice (Dabrowski and Schulz, 2003). 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 et al. 2001a). 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 make it suitable for comparison of runoff- and spray-drift-associated pesticide concentrations. All experiments were based on measurement

of AZP concentrations. AZP was applied at 0.15 kg a.i./ha on orchards bordering the stream.

Experimental Design

Five sampling sites (Fig. 1) were selected 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 runoff barrier (width 0.5 m, height 0.4 m) running along both sides of the stream for the entire 180 m downstream section.

Runoff Sampling

Water samples (n = 4) (Schulz et al. 2001b) and suspended sediment samples (n = 1) (Liess et al. 1996) were collected at each sampling site within the stream during two heavy rainfall events; a 10 mm (Runoff #1) and a 22 mm (Runoff #2) event (Table 1).

Table 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

Plant samples were taken at 5, 45 and 180 m 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. Turbidity was measured at each site in both streams during both runoff events using a turbidity meter (Dr. Lange, Duesseldorf, Germany).

Spray-drift Sampling

One spray-drift event was monitored in detail during an application of AZP on adjacent pear orchards (Table 1) so as to make a comparison with runoff-related pesticide transport in the same stream. 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 within the stream. 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. 2001a). Plant and sediment samples were also collected before and after the event at the 5, 45 and 180 m sampling sites. Sediment samples were collected from the top 5 cm of the streambed using sterilized stainless steel scoops.

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 matrices 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.

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 expected during each of the different events. This was accomplished by using basic drift values (Rautmann et al. 2001) for spray-drift predictions and a runoff formula by Reus et al. (1999). Both formulae have previously been applied and successfully validated in the Lourens River catchment (Dabrowski and Schulz 2003). 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:

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

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).

Data Analysis

Reductions in peak and average concentrations of pesticide 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.

Results

Runoff trials

Both runoff events resulted in increased discharge, flow and water depth (Table 2). All water-dissolved concentrations of AZP measured at each site 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 for the 10 mm event; however, no consistent reduction was apparent during the 22 mm event (Fig. 2 and Table 3). No obvious decrease in particle-associated AZP concentrations took place during either runoff events (Table 3). A slight decline in turbidity occurred during the 10 mm runoff event.

Spray-drift trials

Discharge, flow and depth were comparable to normal background levels measured during the spraying season (Table 2). A significant 90 % reduction in AZP concentrations was observed from top (2.4 ± 1.3 µg/L) to bottom (0.23 ± 0.02 µg/L) sites ($p = 0.02$, $n = 6$) after spray-drift input.

Table 2: Hydrological measurements in a 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 during November and December 2002 and January 2003.

	Discharge (m^3/s)	Current Velocity (m/s)	Water Depth (m)	Width (m)
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

Comparison of runoff and spray-drift

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 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. 2). Peak concentrations were equally reduced (61% reduction from the 5m to the 180 m sampling point) during both events. Average concentrations 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

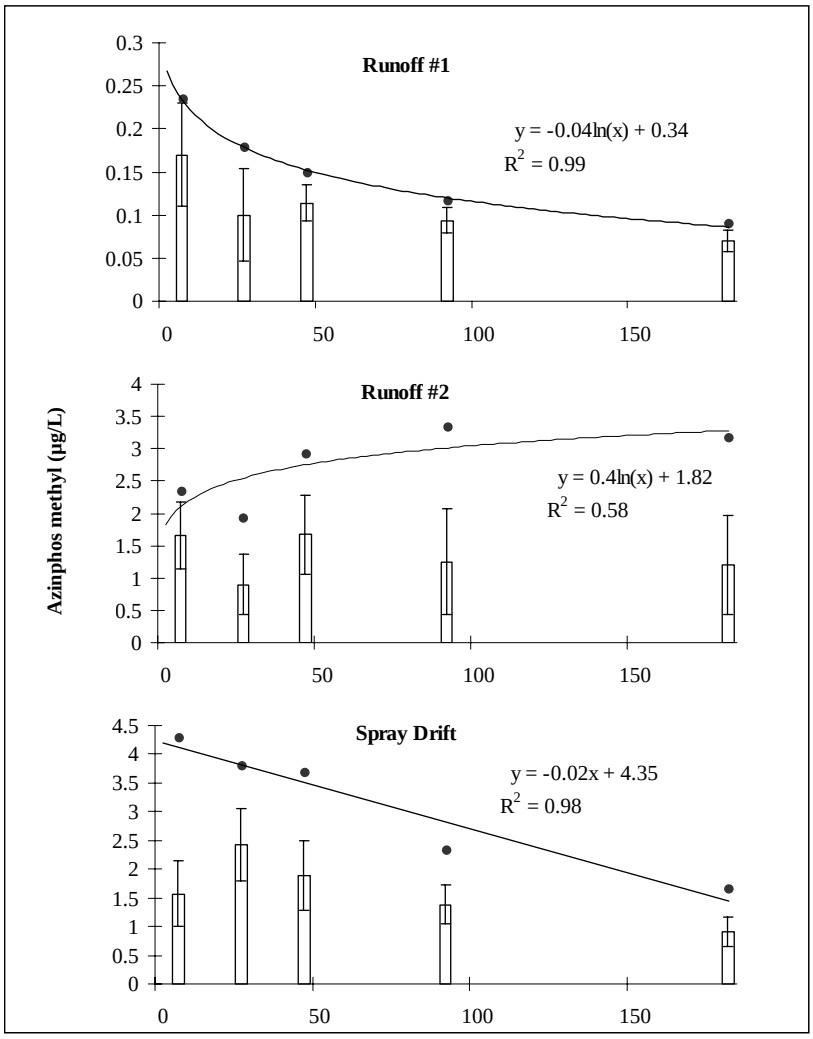

Fig. 2: Peak (dots) and average (bars; ± standard error; *n* = 4) concentrations of azinphos-methyl (AZP) plotted against distance from point of input for a 10 mm (Runoff #1) and a 22 mm (Runoff #2) rainfall event and a spray-drift event in a vegetated stream in the Lourens River catchment, South Africa.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

Table 3: Total suspended sediment and average water (± standard error) and sediment concentrations of azinphos-methyl (AZP) measured in a 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
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

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

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 4). 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.

Table 4: 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

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.

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. Predictions were therefore 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).

Table 5: 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

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.

Discussion

Effect of macrophytes

The results clearly indicate that pesticide concentrations decreased along the length of the stream during the spray-drift and 10 mm runoff events. Reduction in runoff- and spray-drift-derived pesticide concentrations as a result of sorption to aquatic macrophytes have been reported in constructed wetlands and ditches (Brock et al. 1992; Schulz and Peall 2001; Schulz et al. 2003a). 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 4 and Fig. 2), clearly indicating that the AZP sorbs to aquatic macrophytes. Sorption of the organophosphate, chlorpyrifos to *Juncus effusus* (Lytle and 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 4). 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 may have played an important role in reducing pesticide concentrations during the 10 mm runoff event.

It is important to note that sediment may also be an important sink for dissolved pesticides associated with runoff and spray-drift events. An increase in AZP concentrations in sediments taken from the 45 m sampling point 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 et al. 2001a) 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.

Effect of Flow Velocity

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.

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 3, Fig. 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 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 and Peall 2001; Moore et al. 2002) or have been performed in static mesocosms (Hand et al. 2001; Lytle and 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. 1992; Karen et al. 1998). AZP is a relatively water soluble pesticide, 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 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. Further studies with more replicates may help to further validate the results of this study.

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.

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

Apart from previously verified mitigation strategies, such as increased buffer zone width (De Snoo and De Wit 1998), vegetated buffer strips (Patty et al. 1997) and wind-breaks (Ucar and 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. 1992). 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), which are an order of magnitude lower for a 10 mm runoff event than for a spray-drift event. Predicted environmental concentrations 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 and 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 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 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.

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APPENDIX 11.4

Influence of Vegetation in Mitigation of Methyl Parathion Runoff

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ABSTRACT

Wetlands have been characterized as "nature's kidneys" due to their effective contaminant filtering capacity. Unfortunately, many wetlands in the United States and elsewhere were drained in the early 20th century (with heavy losses in the 1960s and 1970s) to allow for increased agricultural production acreage. With this loss of wetlands came a concomitant loss of water quality enhancement by these former wetland areas. The result was an increase in downstream contamination from agricultural storm runoff. In the last two decades, researchers have focused on the capacity of constructed wetlands, or wetlands placed where natural wetlands once stood, for reducing the concentrations of both point and non-point source pollutants. Constructed wetlands play an important role as an agricultural best management practice for water quality improvement of storm runoff. In this study, a pesticide runoff event was simulated using two 10 m x 50 m constructed wetlands (one non-vegetated; one vegetated with Juncus effusus) to evaluate the fate of methyl parathion. A partition wall divided each wetland longitudinally into replicate cells. Water, sediment, and plant samples were collected at five sites downstream of the inflow for a period of approximately 120 days. Additionally, semi-permeable membrane devices (SPMDs) were deployed at the outflow of each wetland cell to determine the total load of pesticide reaching that distance. Results from the non-vegetated wetland cells indicated that methyl parathion was detected in water throughout the entire system (50 m) 30 minutes post-exposure and was still detected after 10 days. In the vegetated wetland, methyl parathion was detected 20 m from the inflow 30 minutes post-exposure, while after 10 days it was detected only at 10 m. Methyl parathion was detected only in SPMDs deployed in the non-vegetated wetland cells, which further suggests that detectable levels were not present near the vegetated wetland outflow. These results demonstrate the importance of vegetation as sorption sites for pesticide mitigation in constructed wetlands.

KEYWORDS: constructed wetlands, methyl parathion, plants, remediation

INTRODUCTION

Wetlands serve as transitional zones or "ecotones" between terrestrial and aquatic systems (Mitsch and Gosselink, 1993). They have several physical, chemical, and biological uptake and degradative processes useful for treatment of point and non-point source pollution. Since the early 20th century, wetland losses attributed to agriculture have been dramatic. Extensive wetland draining in the 1960s and 1970s led to increased agricultural production acreage but decreased water quality enhancement capabilities of these former wetland areas. When rivers, streams, and lakes adjacent to these new agricultural areas receive runoff following rainfall events, an increased potential for damage to water resources, such as increased sedimentation or fish kills, may occur. Sub-lethal concentrations of potential agricultural contaminants may affect growth, reproduction, behavior, physiology, and long-term survival of aquatic flora and fauna (Anderson and Zeeman, 1995; Rice et al., 1997). In response to historic wetland losses, the U.S. Department of Agriculture Natural Resource Conservation Service (USDA NRCS) has established four conservation practice standards (Codes 656, 657, 658, and 659) relating to constructed wetlands (USDA NRCS, 2002). By establishing these standards, farmers and other agricultural landowners are given instructions on how to develop and use constructed wetlands as a best management practice (BMP) to minimize non-point source pollution of water bodies.

Methyl parathion (O,O-dimethyl O-4-nitrophenol phosphorothioate) was applied to US agricultural land in an amount in excess of 270,000 kg active ingredient in 2002 (NASS, 2005). In Mississippi, methyl parathion is the most intensively used pesticide (Coupe et al., 2000). Although not approved for urban use, methyl parathion was detected in rain and air samples from both agricultural and urban sites (Foreman et al., 2000). Majewski et al. (2000) consistently detected methyl parathion in air samples along the Mississippi River from mid May-June

through September. Methyl parathion has a water solubility of 55 mg/L, log K_{ow} of 3.5, and K_{oc} of 5.1×10^3.

The purpose of this study was to evaluate effectiveness of vegetated and non-vegetated wetlands in retaining methyl parathion during a simulated, worst case scenario runoff event. From these data, the relative importance of aquatic vegetation versus no vegetation in facilitating the removal and/or retention of methyl parathion was evaluated using mass balance calculations.

MATERIALS AND METHODS

Wetland exposure

Two constructed wetlands (W=11 m; L=50 m; D=0.2 m) located at the University of Mississippi Field Station (Bay Springs, Mississippi, USA) were divided longitudinally, each into two replicate cells (5.5 m x 50 m x 0.2 m) using aluminum flashing. Two cells contained two types of vegetation, Juncus effusus (256 plants/m^2) and Leersia oryzoides (43 plants/m^2). The remaining two cells were non-vegetated. Sampling sites for the study were established within each cell at 2.5, 5, 10, 20 m, and outflow (50 m). Background water, sediment, and plant samples were collected one month prior to test initiation to determine initial methyl parathion concentrations. All samples were non-detectable for methyl parathion.

A storm runoff event was simulated within the wetland systems in July 2000. Prior to amendment, basic water quality data were obtained for the vegetated and non-vegetated wetlands (Table 1). In addition, StowAway Tidbit temperature loggers (Onset Computers, Bourne, MA) were deployed in the water of each wetland during initial 10 d of the study. Initial methyl

Table 1. Mean (± standard error) water quality parameters for vegetated and non-vegetated wetland cells at the University of Mississippi, Abbeville, MS, USA (n=3) prior to initiation of study and median, maximum and minimum water temperatures recorded over first 10 d of the study.

Parameter	Vegetated	Non-vegetated
pH (standard units)	6.7 ± 0.058	6.9 ± 0.11
Dissolved oxygen (mg/L)	2.3 ± 0.57	6.6 ± 0.23
Temperature (^{o}C)	25.2 ± 0.75	27.8 ± 0.29
Conductivity (µS/cm)	116 ± 3.8	42.6 ± 2.0

Parameter		Vegetated	Non-vegetated
Temperature (^{o}C)	Median:	25.5	29.5
	Maximum:	29.0	36.5
	Minimum:	21.5	22.5

parathion concentrations were based on recommended application rates (0.42 kg active ingredient per ha). Suspended sediment, water, and methyl parathion were mixed in a 110 L polyethylene mixing chamber prior to entering a 7.6 cm diameter PVC pipe for delivery. A mixture of methyl parathion (0.24 kg active ingredient per liter), water, and suspended sediment (400 mg/L) was pumped into each cell simulating a 1% pesticide and water runoff from a 0.64 cm storm event across a 20 ha contributing area. The simulated runoff event took a total of 30 minutes to deliver at a rate of 530 L / minute.

Wetland sampling

One liter amber glass bottles were used to collect grab samples of water at 30 min, 3 h, 6 h, 24 h, 96 h, 10 d, 27 d, 41 d, 64 d, and 182 d. Following collection, water samples were iced and transported to the USDA-ARS National Sedimentation Laboratory (USDA-ARS-NSL) Oxford, MS, USA, for immediate extraction. Additionally, semi-permeable membrane devices (SPMDs supplied by EST, Kansas City, MO) were deployed (n = 2 per cell) for the first 96 h at the outflow (50 m) sites of each wetland cell. Sediment and plant samples were collected with all water samples, with the exception of the 6 h sampling period (water only), wrapped in aluminum foil, placed on ice for transport, and then immediately stored in a freezer (-10^{o}C) upon arrival at USDA-ARS NSL until being dried for analysis. Using solvent-rinsed stainless steel spatulas, sediment samples were obtained from the top 1 cm, while plant materials were collected with solvent-rinsed scissors. Only that plant material exposed in the water column (between sediment-water surface) was collected for analysis.

Sample extraction and analysis

Water, plant and sediment samples were extracted using methods described by Bennett et al. (2000) and Moore et al. (2001). Briefly, aqueous samples were extracted with ethyl acetate using a liquid-liquid extraction method, while sediment and plants were extracted using ultrasonication with ethyl acetate. Silica gel cleanup was performed on water, sediment and plant extracts prior to final analysis. SPMDs were extracted using methods described by Bennett et al. (1996). Each SPMD was dialysed in a beaker containing 400 mL of hexane, covered with foil and placed in the dark at approximately 18°C for 48 h. Subsequently, SPMDs were removed

and the dialysates were passed through anhydrous sodium sulfate and rotary evaporated to a volume of about 1 mL. Extracts were subject to gel permeation chromatography to remove co-extracted triolein and polyethylene constituents followed by silica gel clean up, as above, prior to analysis.

Methyl parathion was analyzed via gas chromatography-microelectron capture detection with a HP 6890 gas chromatograph equipped with a DB5 MS column. The oven temperature program was as follows: 75^{o}C held for one minute, to 225^{o} C at a rate of 40 ^{o}C / min. Injector and detector temperatures were set to 250 ^{o}C and 325 ^{o}C, respectively. Ultra high purity helium, the carrier gas (nexAir, Memphis, TN, USA), was set to a constant flow of 1 mL/min and ultra high purity nitrogen, the makeup gas (Whatman Nitrogen Generator), was set at a constant makeup flow of 60 mL/min. A multilevel calibration procedure was implemented with standards updated every ninth sample. Limits of detection (LOD) for methyl parathion in water, sediments, and plants were 0.1 ng/mL, 0.5 ng/g, and 0.5 ng/g, respectively. Based on fortified samples, mean extraction efficiencies were >90% for water, sediments, and plants.

Data analysis

Mass balances were performed using data on water, plant, and sediments collected along transects of the 50 m vegetated wetland cells and water and sediment data collected from the non-vegetated wetland cells for each sample time point (30 min, 3 h, 24 h, and 10 d). This enabled quantitative evaluation of chemical partitioning and losses that occurred over the study duration. The mass balance at a given time point was determined as:

$$m_{total(t)} = m_{w(0-50)} + m_{p(0-50)} + m_{s(0-50)}$$

(1)

where $m_{w(0-50)}$, $m_{p(0-50)}$ and $m_{s(0-50)}$ reflect the total chemical mass (g) in water, plants and sediments over the 50 m wetland length. Integration of chemical masses in water was performed according to the trapezoidal rule:

$$m_{w(0-50)} = \sum_{i=0}^{n-1} \left(\left(V_{w(i+1)} - V_{w(i)} \right) \cdot \left(\frac{C_{w(i+1)} + C_{w(i)}}{2} \right) \right)$$

(2)

where the term $(V_{w(i+1)} - V_{w(i)})$ represents the volume of water (L) bounded by a given transect interval (i.e. 0-2.5m, 2.5-5.0m, 5.0-10m etc.) and is multiplied by the average water concentration (C_w) measured at the interval boundaries. Water volumes between transects were estimated using the mean water depth (0.20 m) multiplied by the surface area of the transect interval and by accounting for water displacement by plants (assuming 20% of water was displaced by plant biomass in each transect). The mass calculations for plants and sediments were similar to Eq. 2 except that concentrations were measured in units of mg/kg and the volume terms were replaced by bulk sediment mass (kg) or plant biomass (kg). In the former case, the wetland surface area (m^2) within a given interval was multiplied by a sediment depth of 0.01 m and converted to sediment mass by assuming a bulk sediment density of 1200 kg/m^3. The average area plant biomass (10.8 kg/m^2) within a transect interval was multiplied by the wetland width and interval distance to arrive at a plant biomass (kg) estimate. Aqueous methyl parathion chemical half lives ($t_{1/2}$) were estimated as $\ln(2)/k_2$ using chemical depuration rate constants and

half lives were also derived using mass balances for individual media (water, plants and sediments).

Ordinary least-square linear regression analyses were used to fit curves to base 10 log-transformed methyl parathion water concentrations (y) versus log-transformed distance downstream from the injection point (x) (Sokal and Rohlf, 1981) to determine wetland lengths required to decrease initial loadings by 0.1%. Semilog functions were also fit to the data, but produced inferior fits. For simplicity, only the maximum concentrations observed at each sampling distance (within 3 h) were used in the analyses. For regression, data from the two vegetated cells were pooled and the two unvegetated cells were pooled.

RESULTS

Methyl parathion concentrations in water

In vegetated wetlands, aqueous methyl parathion concentrations decreased rapidly with distance (Table 2). For example, 3 h following the initiation of the runoff simulation, mean water concentrations ranged from 452 µg/L at the 2.5 m sampling site to 8.15 µg/L at 20 m downstream, while concentrations were below detection limits at the 40 m sampling site. A similar trend was observed throughout the study's first 4 d. By 10 d, methyl parathion was only detected within the first 10 m of the wetland. In non-vegetated wetlands, methyl parathion concentrations generally decreased with distance within the initial 6 h of the study, while concentrations between sites began to stabilize to similar concentrations after 1 d of the study (Table 2). Methyl parathion was detected at the 40 m sampling site throughout the study in non-vegetated wetlands. Overall, water concentrations measured in both vegetated and non-vegetated wetlands on 10 d were approximately 1% of methyl parathion concentrations detected at the beginning in the study. Results from deployed SPMDs indicated that methyl parathion was not detected at the outflow (50 m) of the vegetated wetland within 96 h of the study, while a mean SPMD/methyl parathion (n=2) concentration of 8.83 µg/g was measured at the outflow of the non-vegetated wetland.

Fate of methyl parathion

The total nominal mass of methyl parathion added to each wetland system was 86 g (43 g into each replicate wetland mesocosm). Measured masses applied to the wetland cells were consistent with the nominal mass, where the mass of methyl parathion added to the vegetated system was calculated to be 85.6 g (±3.2) and 63.1 g (±4.7) for the non-vegetated wetland

Table 2. Mean aqueous methyl parathion concentrations in wetland mesocosms during the first 10 d of exposure. NS indicates no sample. ND indicates below limits of analytical detection.

	Vegetated					
Distance (m)	**30 min**	**3 h**	**6 h**	**1 d**	**4 d**	**10 d**
2.5	1400	452	423	253	444	N.S.
5	717	421	298	192	22.0	4.00
10	687	184	118	90.0	14.5	0.600
20	14.0	8.15	24.9	8.00	0.600	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Non-Vegetated					
Distance (m)	**30 min**	**3 h**	**6 h**	**1 d**	**4 d**	**10 d**
2.5	493	1110	615	247	72.5	3.50
5	337	552	352	185	39.0	3.00
10	54.5	121	189	164	29.5	3.50
20	4.75	44.0	72.5	35.5	22.5	0.600
40	0.150	0.300	0.550	6.00	9.00	0.550

(Table 3) The ($m_{total(30min)}$) for methyl parathion in vegetated wetlands was 29.9 g or 34.9% of the measured mass added to the system, while the $m_{total(30min)}$ for methyl parathion in the non-vegetated wetlands was 12.1 g or 19.2% of the measured mass (Table 3). Mass balance calculations for different sampling times showed there were changes in phase distribution occurring over the study duration (Table 3). Thirty minutes following the initiation of the simulated storm event, 73.3% of methyl parathion mass in vegetated cells was in the aqueous phase, while only 26.2% of the pesticide mass was associated with plant material. Interestingly, after 3 h, the earlier mass pattern had shifted to where 73.5% of the calculated mass was present in plant material. This trend continued throughout the study, but overall mass values where decreased in the water and plant compartments after the 3 h sampling period. Throughout the study, sediment methyl parathion mass was minimal in the vegetated wetland system with slight enrichment over time. Results from the non-vegetated wetland showed that 30 min following the

Table 3. Mean methyl parathion mass located in water, sediment, and plant phases of vegetated and non-vegetated wetland cells.

	Vegetated - MeP (g)					Non-Vegetated - MeP (g)			
Distance (m)	**Plant**	**Sediment**	**Water**	**Total**	**Distance (m)**	**Sediment**	**Water**	**Total**
Mix. Chamber	-	-	-	85.6 (± 3.2)	**Mix. Chamber**	-	-	63.1 (± 4.7)
30 m	7.84	0.136	21.9	29.9	**30 m**	4.63	7.48	12.1
3 h	18.4	0.206	6.42	25.0	**3 h**	0.289	11.0	11.29
1 d	5.22	0.127	3.22	8.57	**1 d**	3.43	6.18	9.61
10 d	1.58	0.329	0.0266	1.94	**10 d**	4.16	0.130	4.29

initiation of the study that 61.7% of methyl parathion mass was in the aqueous phase. As with the vegetated wetland, a phase shift occurred over time where by 1 d the mass in water relative to sediment were relatively the same. By 10 d, the majority of the methyl parathion mass in the non-vegetated wetland system was associated with the sediment compartment

After 10 d a total mass of 1.94 g remained (mostly associated with plants) in the vegetated wetland, while 4.29 g remained in the non-vegetated wetland. In both the vegetated and non-vegetated wetlands, less than 5% of methyl parathion mass was associated with the water column after 10 d.

Combined changes in $m_{w(0\text{-}50)}$, $m_{s(0\text{-}50)}$ and $m_{p(0\text{-}50)}$ with time were used to estimate wetland chemical half-lives. Methyl parathion half-lives were calculated to be 2.84 and 7.80 d for the vegetated and non-vegetated wetlands, respectively, using all three compartments combined and half lives in water were calculated to be 1.27 and 1.69 d.

Wetland length calculations

Maximum observed methyl parathion concentrations were inversely proportional to distance downstream from the point of injection, producing regression coefficients that were significant at $p < 0.05$ (Figure 1). Standard errors for the exponents (b) were between 0.12 and 0.59, but 95%

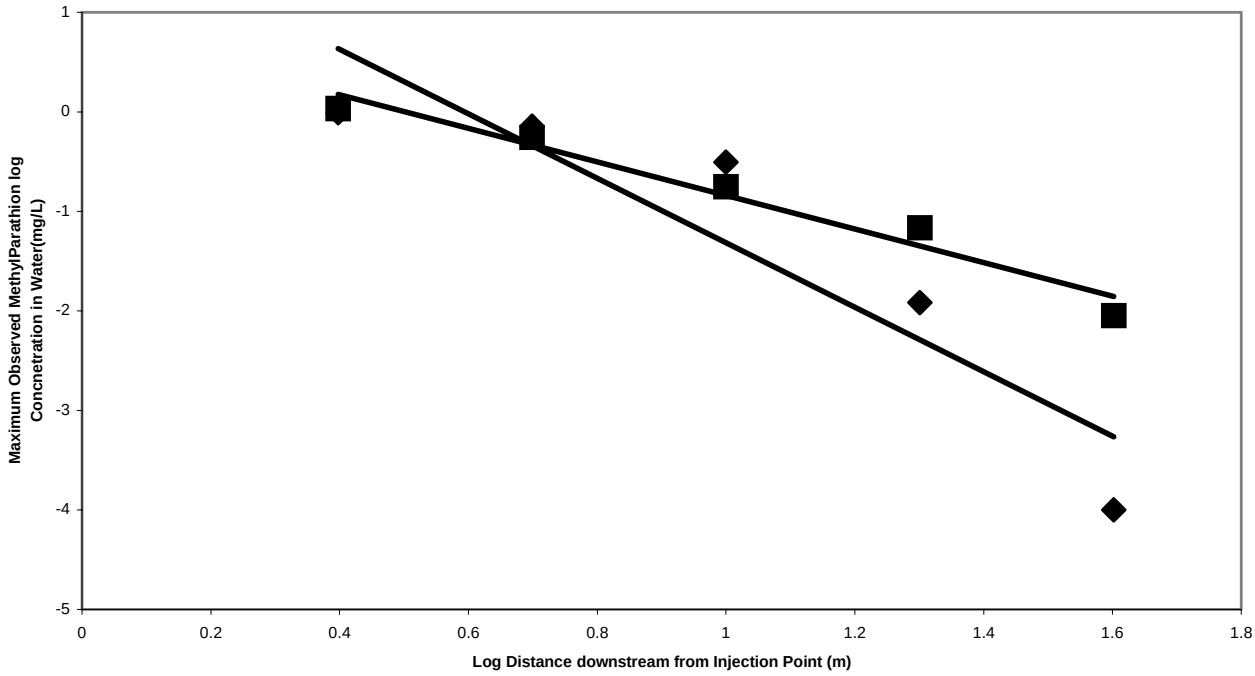

Figure 1. Least-squares regression relationships fit to log-transformed mean maximum observed pesticide concentration in water collected from the vegetated wetland (♦) and the un-vegetated wetland (■) versus distance downstream from injection point.

confidence intervals spanned several orders of magnitude in terms of untransformed concentration due to the relatively small number of data points. Formulas predicted that a vegetated wetland length of 18.8 m would have been adequate to reduce methyl parathion concentrations to 0.1 % of the inflow concentration of 8.01 mg/L. In contrast, regression formulas indicated that a length of 62.9 m would reduce methyl parathion concentrations to 0.1 % of the inflow concentration in a non-vegetated wetland.

DISCUSSION

This study was designed to evaluate the effectiveness and compare the differences of vegetated and non-vegetated wetlands on the retention and partitioning of methyl parathion during a simulated runoff event. Results indicate the importance of a multi compartment system that includes vegetation for efficient mitigation and degradation of methyl parathion. The concept of vegetation as a tool for contaminant mitigation is not new. Many studies have evaluated the use of wetland plants to mitigate pollutants such as acid mine drainage, metals, dairy wastes, and even municipal wastes. According to Luckeydoo et al. (2002), the vital role of vegetation in processing water passing through wetlands is accomplished through biomass nutrient storage, sedimentation, and providing unique microhabitats for beneficial microbial organisms. Macrophytes serve as filters by allowing contaminants to flow into plants and stems, which are then sorbed to macrophyte biofilms (Kadlec and Knight, 1996). According to Zablotowicz and Hoagland (1999), whether or not plants are capable of transferring contaminants from environmental matrices depends upon several factors including contaminant chemistry, plant tolerance to the contaminant, and sediment surrounding the plant (e.g. pH, redox, clay content). Recent studies have shown the importance of aquatic vegetation for mitigation of pesticides in flow through wetlands and agricultural drainage ditches (Moore et al., 2001; Schulz et al., 2003a; Bennett et al., 2005). The current study has shown that aquatic vegetation plays an important role for pesticide mitigation and retention in constructed detention wetlands. The wetlands used in this study differ from that of a flow through wetland, as in Schulz et al. (2003b), by allowing only the displaced volume to exit the wetland cell. For this type of wetland system, stand pipes are used to control the wetland volume. Thus, during a runoff event wetland water is replaced/displaced until the inflowing volume has stopped. Data from this study illustrated that

vegetated wetlands were capable of retaining the pulse of methyl parathion introduced into the system during the 30 min simulated runoff. Throughout the study, methyl parathion was not detected in water at the 40 m sampling site of the vegetated wetlands, whereas the pesticide was detected at all sites in the non-vegetated wetlands throughout the 10 d study (Table 2).

To further understand the fate of methyl parathion in the vegetated versus the non-vegetated wetlands, mass balance calculations were performed to estimate the partitioning between the water, plant and sediment compartments. Results from the mass balance model used in this study showed that only 34.9% and 19.2% of the methyl parathion added to the vegetated and non-vegetated wetland systems, respectively, were accounted for. In an earlier study where pyrethoids were applied to a vegetated agricultural drainage ditch, the same mass balance model was utilized and pesticide recoveries were much greater (Bennett et al., 2005). It is expected that methyl parathion mass was underestimated in this study because samples were not taken at the inlet of the wetland (point were methyl parathion water/sediment mixture was added to the wetland systems) as in the above ditch study, and sampling was initiated at 30 min instead of at time zero (as in ditch study). Owing to this, it is possible that methyl parathion mass may have been underestimated at the front end of the system. Furthermore, methyl parathion was detected at the outflow of the non-vegetated wetland at the 30 min sampling period indicating loss from the system. Due to the high air and water temperatures (Table 1), sunny and windy conditions (personal observations) during the study's first day, other factors for methyl parathion loss from the system may include volatilization, photodegradation, hydrolysis and biotransformation. These abiotic and biotic processes will be discussed in the following section. Regardless of the

low recovery, data produced from the mass balance calculations still reveal important information on the fate of methyl parathion in the two wetland systems.

Mass balance data indicated that both the vegetated and non-vegetated wetlands were effective in reducing the aqueous loadings of methyl parathion introduced into each of the systems. After 10 d the majority of pesticide mass in the vegetated wetland was found in the plant compartment while the majority of pesticide was located in the sediment compartment in the non-vegetated wetland. It would be expected that sediments would play an important role in the wetland systems since methyl parathion has a moderately high K_{oc} (5.1×10^3) (USDA ARS, 2005). Mass balance data from this study indicate that methyl parathion mass in sediments stabilized over time in both wetland systems, but levels were an order of magnitude higher in the non-vegetated wetland (Table 3). These data illustrate that sediment partitioning was a major process for the loss of methyl parathion from the non-vegetated wetland system. Similarly, Crossland et al. (1986) showed that sediments were a sink for methyl parathion in the aquatic environment and that sorption of methyl parathion onto sediment was one of the dominant processes for loss from the water compartment. This process did not occur to the same extent in the vegetated wetland due to the dense plant community which limited the movement and/or partitioning of methyl parathion to the sediment compartment. Similar results were found in a microcosm study by Hand et al. (2001) where aquatic plants significantly reduced the amount of insecticide reaching the sediment. By limiting the movement into the sediment (sink), the plant compartment played an important role in the overall reduction of methyl parathion in the system. By 10 d, the loading of methyl parathion in the vegetated wetland was less than half of the loading predicted in the non-vegetated wetland even though the measured mass introduced into the system (85.6±3.2g) was much greater than what was introduced into the non-vegetated

Table 4. Mean methyl parathion concentrations in water samples (µg/L) collected at each sampling site during the 3h, 24h and 96h sampling periods relative to acute (aqueous) toxicity survival results (*C. dubia* [Milam et al., 2004], *H. azteca* [Schulz et al., 2003a] and *C. tentans* [Schulz et al., 2003b]) from vegetated and non-vegetated wetland mesocosms. Note: N.D. = below detection limits; NA = No available data.

Concentration of MeP (µg/L)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	421	192	22.0	551	184	39
10 m	184	90.0	14.5	120	164	29.5
20 m	8.15	8.00	0.600	44	35.5	22.5
40 m	N.D.	N.D.	N.D.	0.300	6.00	9.00

Mass of MeP in Water (g)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	2.66	1.24	0.2	3.7	1.92	0.376
10 m	1.69	0.862	0.133	1.81	2.19	0.572
20 m	0.145	0.143	0.012	0.974	0.847	0.693
40 m	0.001	0.001	0.001	0.0033	0.033	0.099

C. dubia (% Survival)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	0%	0%	0%	0%	0%	0%
10 m	0%	0%	0%	0%	0%	0%
20 m	0%	50%	100%	0%	0%	0%
40 m	75%	100%	100%	100%	0%	0%

H. azteca (% Survival)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	0%	0%	23% (±0.6)	0%	0%	1.7% (±0.2)
10 m	1.7% (±0.2)	5% (±0.3)	28% (±0.5)	0%	0%	10% (±0.5)
20 m	30% (±0.3)	37% (±1.0)	92% (±0.5)	3.3% (±0.2)	1.7% (±0.2)	20% (±1.1)
40 m	97% (±0.3)	87% (±0.6)	98% (±0.2)	92% (±0.5)	62% (±1.2)	32% (±0.3)

C. tentans (% Survival)

	Vegetated			Non-vegetated		
Site	3-h	24-h	96-h	3-h	24-h	96-h
5 m	5% (±0.3)	0%	-	3% (±0.2)	0%	-
10 m	53% (±0.4)	28% (±0.3)	-	43% (±0.3)	0%	-
20 m	95% (±0.2)	80% (±0.4)	-	58% (±0.5)	28% (±0.3)	-
40 m	100%	100%	-	98% (±0.2)	93% (±0.2)	-

wetland (63.1±4.7g; Table 3). This reduction/degradation in the vegetated wetland may have been due to many abiotic and biotic processes. Studies have shown that the degradation of methyl parathion occurs more rapidly in alkaline conditions relative to neutral or acidic conditions (USEPA, 1978; Badawy and El-Dib, 1984; Lartigues and Garrigues, 1995; Kaur et al., 1997). In systems rich with aquatic plants and algae, pH has been shown to exceed 9 at the water algal/plant interface as a result of photosynthetic activity (Prins et al., 1980; Kersting and van den Brink, 1997). Furthermore, *Anabaena sp*, a blue-green algal species, has been shown degrade methyl parathion under aerobic, photosysnthetic conditions (Barton et al., 2004) while other studies have shown that green and blue-green algae presence accelerate the photoreaction of methyl parathion (Zepp and Scholtzhauer, 1983). These dense aquatic plant systems are generally laden with aufwuchs that contain bacteria that have been shown to rapidly degrade methyl parathion (Holm et al., 1983; Crossland and Bennett, 1984). Other factors such as increased water temperature (Kaur et al., 1997) (Table 1) and photolysis have also been shown to cause degradation of methyl parathion (USEPA, 1978).

Overall, these data illustrate the importance of the plant compartment in wetland system for the mitigation of methyl parathion. Without this compartment, methyl parathion may be readily transferred to the sediment compartment. This process is less effective, especially from a toxicity standpoint, since the sediment becomes a sink for high levels of methyl parathion. Data from this study show that methyl parathion would degrade at a slower rate in the sediment than in the water and plant compartments. Furthermore the effectiveness of the vegetated wetland can further be illustrated by the fact that methyl parathion was not detected in SPMDs deployed at the outlet of the wetland and that data from acute toxicity testing run in parallel with this study reflect the concentration and mass balance data (Table 4). There were no recorded rain events

during the first 10 days of the study; however, if an event had occurred during or after the initial 10 days, it is projected that the vegetated wetland would continue to efficiently mitigate any pesticide which may be resuspended. Conversely, a lack of mitigation would likely occur in the non-vegetated wetland, due to resuspension and potential transport from the wetland system.

CONCLUSIONS

The presence of vegetation in stormwater treatment areas has both positive and negative effects. From an observational standpoint, excessive vegetation may slow down drainage. While this may be seen as a negative impact by some, others would view it as a positive effect, allowing increased contact time for contaminant binding to plant organic material. In fact, vegetation is often ignored in contaminant fate and effects modeling for several reasons (Cousins and Mackay, 2001). Kinetics are not well defined, because of the lack of data from vegetation uptake experiments. Vegetation is sometimes thought to contribute little to the mitigation process as compared to sediment or other matrices. However, this research reaffirms the importance of vegetation in the mitigation of pesticide-associated runoff. If contaminants (e.g. pesticides) can be bound or retained by wetland or ditch vegetation, then they will have less impact upon downstream aquatic systems.

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APPENDIX 11.5

Methyl Parathion Toxicity in Vegetated and Unvegetated Wetland Mesocosms

Schulz, R., M.T. Moore, E.R. Bennett, J.L. Farris, S. Smith Jr., and C.M. Cooper

Env. Tox. Chem., 22(6): 1262-1268 (2003)

Abstract—Methyl-parathion (MeP) was introduced into constructed wetlands for the purpose of assessing the influence of emergent vegetation on transport and toxicity of the pesticide. Two vegetated (90% cover, mainly *Juncus effusus*) and two non-vegetated wetland cells (each with a water body of 50 m × 5.5 m × 0.2 m) were each dosed with 6.5 m^3 of water containing 6.6 mg/L a.i. MeP associated with 400 mg/L suspended soil to simulate a storm runoff event. Acute toxicity was assessed by sampling benthic macroinvertebrates at 5, 10, 20 and 40 m distance from the inlet before and 96 h after contamination and by in situ exposure of *Chironomus tentans* (Diptera) up to 24 h after contamination. Methyl-parathion was detected throughout the non-vegetated wetland cells (70 µg/L at 20 m, 8 µg/L at 40 m), whereas the pesticide was not transported through the vegetated wetland cells (20 µg/L at 20 m, <0.1 µg/L at 40 m). A three-way analysis of variance (ANOVA) using contamination (repeated measure variable), location and vegetation indicated significant negative effects of contamination on various insect taxa, such as mayfly and caddisfly larvae. Seven out of the total of 15 species revealed a significant contamination × vegetation effect, with individuals in the vegetated wetlands being less affected. Four species showed a significant contamination × location effect confirming a higher toxicity in the inlet area of the wetlands. A significant three-way interaction of contamination × vegetation × location was detected in *Chironomus* sp. which was most strongly affected at the inlet area of the non-vegetated wetland cells. The in situ bioassay employing *C. tentans* confirmed the positive effect of wetland vegetation on MeP toxicity. These results demonstrate the importance of vegetation for pesticide mitigation in constructed wetlands.

Keywords—Insecticides Risk mitigation Nonpoint-source pollution Vegetation Wetland communities

INTRODUCTION

It has been recently shown that constructed wetlands have the ability to retain nonpoint-source insecticide pollution, preventing it from entering receiving aquatic habitats [1-3]. The implementation of retention ponds in agricultural watersheds was mentioend by Scott et al. [4] as one strategy to reduce the amount and toxicity of runoff-related insecticide pollution discharging into estuaries. The usefulness of aquatic plants for removal of insecticides from water has been shown [5] and the effects of the organophosphate phorate have been assessed using littoral mesocosms in South Dakota wetlands [6]. However, there are almost no other studies in the open literature dealing with the fate or effects of agricultural insecticide input in constructed wetlands.

Processes important for removal of nonpoint-source pesticide runoff in wetlands may include adsorption, decomposition and microbial metabolism [7]. The macrophytes present in the wetland may play an important role in providing an increased surface area for sorption as well as for microbial activity [8,9]. Furthermore, they may contribute directly to chemical metabolism [10]. It was demonstrated that emergent vegetation reduces the resuspension of sediments in wetlands [11].

Spraydrift and runoff are important routes for nonpoint-source pesticide pollution of aquatic habitats, and it has been shown that runoff may contribute to greater concentrations and loads of insecticides than spraydrift [12]. Runoff is the major source of aquatic insecticide input in the intensively cultivated Mississippi delta region [13]. Constructed wetlands could serve as a suitable risk mitigation strategy for agricultural runoff, given that enough information on their effectiveness with specific reference to the importance of the wetland-vegetation is available.

Biological effects of pesticides in wetlands have been studied under experimental conditions using mesocosms [14], in littoral enclosures [6] or in the field employing organisms in

situ [1,2,4]. There is now a need to link wetland characteristics such as the presence of emergent macrophyte vegetation with the transport of nonpoint-source pesticide contamination and the resulting biological effects. The following study was undertaken for this purpose. Methyl-parathion, an organophosphate insecticide primarily applied to cotton, was chosen as the test substance for a simulated runoff event. Its use in the lower Mississippi delta averages approximately 400,000 kg of active ingredient per year [15] and it has been detected at high levels in agricultural runoff [16]. Methyl-parathion has an organic carbon-water partition coefficient (K_{OC}) of 5,100 and a water solubility of 55 mg/L.

MATERIALS AND METHODS

Description of the wetland mesocosms

Constructed wetlands (water body: 50 m × 11 m × 0.2 m) at the University of Mississippi Field Station were specifically designed to evaluate the fate of pesticides in wetlands [3]. Four of these constructed wetlands, orientated in parallel and independent from each other in terms of water supply, were used for this research. Two wetlands differing in vegetation coverage were chosen as experimental cells. The vegetated wetland had a macrophyte coverage of > 90% (*Juncus effusus*: 171 ramets/m^2 and *Leersia* sp.: 12 ramets/m^2) and the non-vegetated wetland had a macrophyte coverage of < 5%. Both wetlands were divided longitudinally 5 days prior to contamination, so that each comprised two wetland cells (water body: 50 m × 5.5 m × 0.2 m); the divider consisted of metal flashing (50 m × 0.5 m × 1 mm) that was pressed 10 cm into the wetland sediment. The two remaining wetlands were used as water sources for the simulated storm event. Above-surface platforms at 5 m, 10 m, 20 m and 40 m distance from the inlet were

used in each wetland cell to ensure that sampling could be done without the necessity to walk into the wetlands, which may destroy the macrophytes and/or sediments. The total number of sampling sites was 16 (two non-vegetated and two vegetated wetland cells with four stations each).

Experimental procedure and pesticide analysis

Each of the four wetland cells was treated at the inlet with MeP in a soil-water mixture to simulate agricultural runoff on August 11, 2000. The amount of MeP applied as simulated runoff was based on assumptions of an immediate (post-application) 6.35-mm rainfall on 50-hectare agricultural fields to which commercial grade MeP (Clean Crop®, United Agri Products, Greely, CO, USA) at a rate of 8.6 kg active ingredient per 20 hectares had been applied. Based on the assumption of 1% pesticide runoff [16], a total of 43 g active ingredient of MeP in a volume of 6500 L of water was added to each of the four wetland cells. Additional inclusion of 2.5 kg sandy loam (84% sand; 16% silt) per wetland cell was designed to simulate the typical suspended solid load (400 mg/L) in the Mississippi Delta Ecoregion. An amount of 100 L of water per wetland was mixed with soil and pesticide in a mixing chamber and was introduced into runoff water during the 30-min contamination period. The soil and pesticide mixture was homogenized for a 24-h period prior to the experiment. The surface velocity through the wetlands during runoff simulation was below 0.05 m/s.

Water samples for pesticide analysis were taken at 3 h, 6 h, 24 h, 96 h and 10 days post-application at the 16 sampling stations. Solvent-washed 1000-ml amber glass bottles were used to collect aqueous samples. Following collection, samples were placed on ice (< 2 h) until transported to a walk-in cooler (4 ^{o}C) pending analysis. Sample extraction and analysis are outlined in Moore et al. [17]. Sediment and subsurface plant samples were taken at 24 h post-

application and analysed as documented in Bennett et al. [18]. All samples were analysed via gas chromatography-microelectron capture detection (GC-uECD) with a Hewlett-Packard (Avondale, PA, USA) 6890 gas chromatograph equipped with a DB5 MS column. The limit of detection for MeP in water, sediments and plants was 0.001 µg/L, 0.02 µg/kg dry weight and 0.02 µg/kg dry weight, respectively. Based on fortified samples, mean extraction efficiencies were >90%.

The mean values (n = 3) for pH, dissolved oxygen, temperature and conductivity in the vegetated and non-vegetated wetland were 6.7±0.1; 6.9±0.2; 2.3±1.0 mg/L; 6.6±0.7 mg/L; 25.2±1.3 °C; 27.8±0.5 °C; 116±6.5 µS/cm; 43±3.4 µS/cm, respectively.

Macroinvertebrate sampling and in situ exposure bioassays

Sampling of macroinvertebrates was performed two days prior to contamination and 96 h after the contamination. Four samples were taken at each of the 16 sites and each of the two dates using an Ekman sampler (area: 15 × 15 cm). Samples were transported within 1 h to the lab, sorted out in white plastic tubs, preserved in 70 % ethanol and determined to species or genus level using dissecting microscopes.

Midges (*Chironomus tentans*) were used as a test organism. Animals were obtained from a culture at the Ecotoxicology Research Facility at Arkansas State University. At each of the 16 sites, four replicate exposure beakers containing 10 fourth instar larvae were installed 1 h prior to contamination. The number of surviving larvae was counted at 3 h and 24 h post-exposure. The in situ exposure methodology is outlined in detail in Schulz et al. [19].

Data analysis

Linear regression analyses were used to fit curves to base 10 log-transformed MeP water concentrations (y) versus distance downstream of the pesticide inlet (x). Only the maximum

concentrations (of the means from $n = 2$ samples) observed at each sampling distance (regardless of time) were used in the analysis. Biological data obtained for the two non-vegetated and vegetated wetland cells correlated well ($r^2 = 0.94$; $p < 0.0001$; $df = 255$) and were thus combined. Unfortunately, pseudoreplication is unavoidable in studies of the type undertaken here; hence, it is difficult to assess or exclude the effect of unmeasured or unknown covariables [20]. However, with regard to the size of the wetland mesocosms, the invertebrate samples taken at one site are regarded as sufficiently independent to justify an analysis of variance. Effects of vegetation (vegetated versus non-vegetated, factorial), location (5, 10, 20 and 40 m distance from the inlet, factorial) and contamination (before versus 96 h after MeP introduction, repeated measure variable) on the abundances of macroinvertebrates were analysed using a three-way ANOVA. Similarly, the survival of in situ exposed *C. tentans* was analysed using time (3 h and 24 h after MeP introduction) as a repeated measure variable instead of contamination as with the community data. Abundance and survival data were transformed using $\ln(x + 1)$ to satisfy the assumptions of ANOVA. We applied a Bonferroni correction to control for type I statistical errors and assessed statistical significance with $\alpha = 0.012$.

RESULTS AND DISCUSSION

Methyl-parathion concentrations

Maximum observed MeP concentrations in water were inversely proportional to the distance from the inlet (Fig. 1). However, the transport of MeP through the wetlands differed greatly depending on the vegetation coverage. Peak levels in the non-vegetated wetland were as high as 70 µg/L at 20 m and 8 µg/L at 40 m distance from the inlet, while the respective values

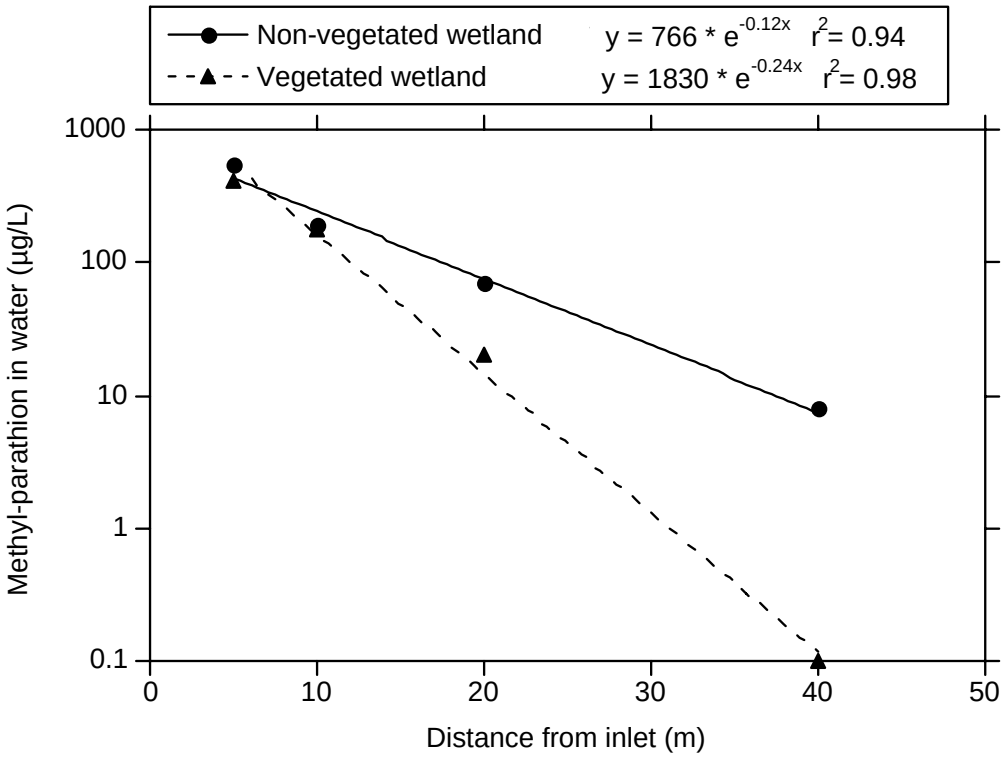

Fig. 1. Linear regression relationship between log-transformed maximum observed methyl-parathion concentrations (detection limit: 0.1 µg/L) and distance downstream from the pesticide inlet.

were 20 µg/L and <0.1 µg/L in the vegetated wetland (Table 1). At 5 m and 10 m distance from the inlet, the contamination was generally higher, but did not differ greatly between the non-vegetated (550 and 120 µg/L, respectively) and the vegetated wetland (420 and 180 µg/L, respectively). Apart from the 20- and 40-m station in the non-vegetated wetlands, all MeP levels were at least five times higher between 3 h and 24 h post-application than at 96 h and at 10 d. On the basis of the pesticide added and the water volume of the wetland, a theoretical target concentration of 700 µg/L would result, assuming all the MeP to be in the water, immediate and equal mixing, and no degradation. As expected, the measured concentrations were generally lower than this target value. Sediment and plant samples taken at 24 h post-application contained MeP concentrations up to 2,000 µg/kg dry weight and 10,000 µg/kg dry weight, respectively.

Table 1. Methyl-parathion concentrations in water samples (µg/L) taken at different distances from the inlet between 3 h and 10 d post-application. Each value represents the mean of analysis of two separate samples taken from each wetland cell replicate (nd = not detectable, i.e. 0.1 µg/L).

	Distance	Time after application				
	from inlet	3 h	6 h	24 h	96 h	10 d
Non-vegetated	5 m	550	350	180	40	3
	10 m	120	190	160	30	4
	20 m	40	70	40	20	0.6
	40 m	0.3	1	6	8	0.6
Vegetated	5 m	420	300	190	20	4
	10 m	180	120	90	10	1
	20 m	10	20	10	1	nd
	40 m	nd	nd	nd	nd	nd

The pesticide analysis clearly revealed differences in the transport of MeP depending on the vegetation coverage of the wetland. It is likely that the dense vegetation coverage in the vegetated wetland, with more than 180 ramets/m^2, leads to a reduced flow rate of the water through the wetland and their rhizomes may very well reduce the seepage velocity through the sediments [21] and thus reduces the pesticide transport. Similar processes are well known for nutrients [22] and suspended particles [23] but have been rarely demonstrated for insecticides. However, a role for aquatic plants in facilitating the removal of pesticides from the water via adsorption has been implicated by a number of workers [8,9]. The importance of sorption and partitioning of MeP in river biofilms has been demonstrated under experimental conditions by Headley et al. [24]. Our results concerning high MeP contents in the plants and sediments 24 h

post-application confirm the importance of sorption in reducing pesticide levels in the water.

A half-life time of 12 h has been reported for MeP in water at initial levels of 200 µg/L using 20-L glass aquaria in the laboratory [25]. Based on this half-life time, an initial concentration of 550 µg/L at the 5-m station in the non-vegetated wetland would be reduced to values below the detection limit (<0.1 µg/L) after about 7 days. The fact that levels between 0.6 and 4 µg/L were measured even after 10 days indicate that the degradation of MeP under high-temperature and low-oxygen conditions in the wetlands was much slower than would have been expected from the reported laboratory half-life times.

Macroinvertebrate community responses

A total of 15 species were found in the wetlands (Table 2). *Caenis latipennis* (Ephemeroptera) and *Chironomus* sp. (Diptera) were the most dominant species, forming more than 50% of the individuals before contamination. Six out of the 15 species were odonate species and 13 species belonged to the insect group. The macroinvertebrate composition is typical for static mud bottom ponds with low oxygen concentrations [26].

The abundances before and 96 h after the contamination are compared for the non-vegetated and vegetated wetland in Figure 2. A significant negative acute effect of contamination on abundances was found in 8 out of the 15 species (Fig. 2; Table 2), resulting also in a significant negative effect of contamination on the total numbers of individuals (Fig. 2 p; Table 2). Both of the two mayfly species and the caddisfly *Oecetis cinerascens* were not found any more after contamination (Fig. 2 b, c & k). The strong negative effects of MeP on the abundance of macroinvertebrates are in accordance with the fact that the measured concentrations were well above levels that are reported to be acutely toxic to aquatic invertebrates. The 24-h EC50 (effective concentration) for the cladoceran *Ceriodaphnia dubia* is 5.5 µg/L [27] and the 96-h

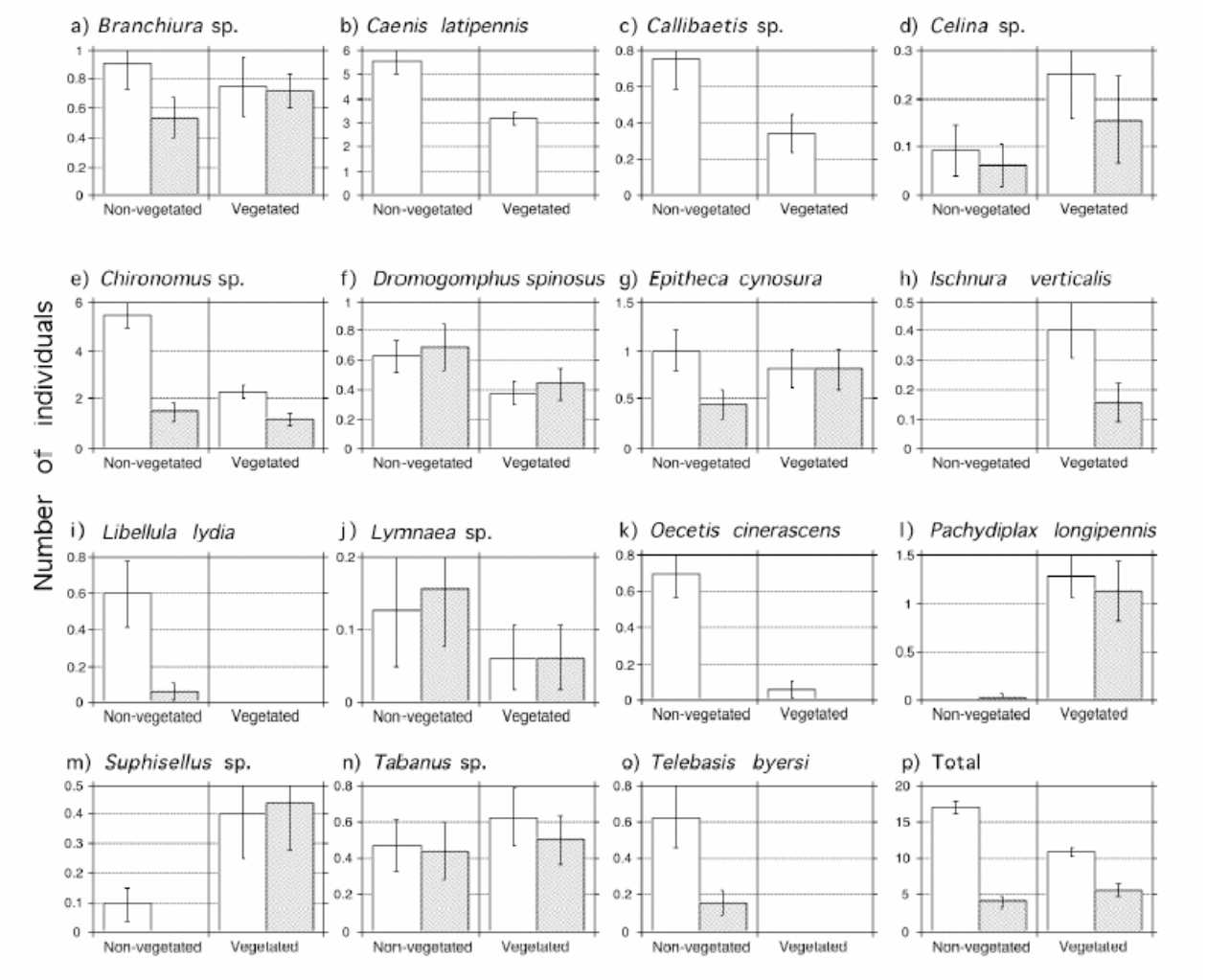

Fig. 2. Mean (± standard error, n = 32) abundance (individuals per sampler) of macroinvertebrate species in non-vegetated and vegetated wetland mesocosms before (white bars) and 96 h after (black bars) introduction of methyl-parathion.

LC50 (lethal concentration) for the damselfly *Ischnura verticalis* is 33 µg/L [28]. A high susceptibility of mayfly and caddisfly species to insecticide components has been reported in other studies [29]. A significant decline of mayfly abundance was also reported for experimental outdoor ponds treated with 100 µg/L of MeP [30].

Seven of the 8 species that were affected by the contamination showed a stronger negative response in the unvegetated than in the vegetated wetland (Fig. 2), as indicated by the significant

contamination × vegetation interaction in the ANOVA (Table 2). This interaction is also significant for the total number of individuals (Table 2). As 4 of these 8 species were not present in either the vegetated or the non-vegetated wetland prior to contamination, a direct comparison of the effects of contamination in relation to vegetation coverage is not possible for these species. However, *Chironomus* sp. was found in both wetlands and was affected to a significantly higher extent by contamination in the non-vegetated wetland (Fig. 2 e; Table 2). *C. latipennis*, *Callibaetis* sp., and *O. cinerascens* were 100% eliminated from both wetlands after treatment (Fig. 2 b, c & k), however, these three species occured at higher densities in the non-vegetated wetland than in the vegetated wetland prior to contamination.

The reaction of macroinvertebrates clearly demonstrated that the impact of MeP in the vegetated wetland was considerably lower than in the non-vegetated wetland. This result is in accordance with the observed differences in transport of MeP through the wetland and the resulting differences in exposure levels. Such a link of vegetation coverage in wetlands, pesticide transport and toxicity to the inhabiting fauna has not been described yet in the open literature. However, input-output studies of constructed wetlands and retention ponds in agricultural watersheds recently demonstrated reductions of insecticide-associated toxicity [1,3,4]. The decreased toxicity in the vegetated wetland may result from a combination of reduced transport and increased sorption of the pesticide to the aquatic plants [8,9]. It is unlikely that differences in other water quality parameters would have contributed to the observed stronger effects in the non-vegetated wetland, since the oxygen concentrations were even lower, the temperature was only 2.6°C higher in the vegetated wetland and the hardness was low in both wetlands.

Table 2. Summary of the statistical significance from three-way analysis of variance (ANOVA) of the effect of vegetation (vegetated versus non-vegetated), location (5, 10, 20 and 40 m distance from the inlet) and contamination (before versus 96 h after methyl-parathion introduction, repeated measure variable) on the abundances (ln(x + 1) transformed) of various macro-invertebrate species (Significance levels are: NS (not significant) $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Species[a]		veg.[b]	loc.[b]	veg. × loc.	cont.[b]	cont. × veg.	cont. × loc.	cont. × veg. × loc.
Branchiura sp.	C	NS	NS	NS	NS	NS	NS	NS
Caenis latipennis	E	***	NS	NS	***	***	NS	NS
Callibaetis sp.	E	*	NS	NS	***	*	NS	NS
Celina sp.	C	NS	NS	NS	NS	NS	NS	NS
Chironomus sp.	D	***	***	NS	***	***	***	*
Dromogomphus spinosus	O	*	***	NS	NS	NS	***	NS
Epitheca cynosura	O	NS	NS	NS	*	NS	**	NS
Ischnura verticalis	O	***	NS	NS	*	*	NS	NS
Libellula lydia	O	***	NS	NS	**	**	NS	NS
Lymnaea sp.	G	NS	NS	NS	NS	NS	NS	NS
Oecetis cinerascens	T	***	NS	NS	***	***	NS	NS
Pachydiplax longipennis	O	***	*	NS	NS	NS	NS	NS
Suphisellus sp.	C	**	NS	NS	NS	NS	NS	NS
Tabanus sp.	D	NS	NS	NS	NS	NS	NS	NS
Telebasis byersi	O	**	NS	NS	**	**	*	*
Total number of individuals		NS	***	NS	***	***	***	NS

[a] A = Annelida, C = Coleoptera, D = Diptera, E = Ephemeroptera, G = Gastropoda, O = Odonata, T = Trichoptera.
[b] veg. = vegetation, loc. = location, cont. = contamination.

Within both wetlands, there was a clear spatial gradient in the abundances of various species. The abundances of 4 out of the 15 species were reduced at a significantly higher rate near to the inlet (5 and 10 m station) than further away (20 and 40 m station, Fig. 3 a, b, c & e), which is indicated by the signficant interaction for contamination × location from ANOVA (Table 2).

The same contamination × location effect is present if the total number of individuals is taken into consideration (Fig. 3 f, Table 2). The contamination × location effect is not significant for *Pachydiplax longipennis*, although there was a trend for this species to be more affected near to the inlet as well (Fig. 3 d). The spatial differences in the biological reactions are again in accordance with the observed MeP distribution and thus further reinforce the inference that the pesticide is the cause of the changes in invertebrate abundances.

Four odonate species were present in even greater numbers at the 20- and 40-m station following contamination (Fig 3 b, c, d & e), suggesting that they may have migrated within the wetland as a reaction to the MeP exposure. A spatial response of odonate larvae to environmental factors has been implicated by other workers [31,32]. *Chironomus* sp. showed a significant three-way interaction of contamination × vegetation × location (Table 2), confirming that this species was affected at a higher level in the inlet area of the non-vegetated wetland than in the inlet area of the vegetated wetland (Fig. 3 a), which again highlights the positive effect of vegetation coverage on survival. A similar trend was also present in *Epitheca cynosura* and *Dromgomphus spinosus*, but the abundances of these species were too low to indicate significant differences.

In situ toxicity to Chironomus tentans

Analysis of variance revealed a significant ($p < 0.001$) decrease in survival of in situ exposed *C. tentans* at 3 h and 24 h following exposure (Fig. 4). Furthermore, there was a significant ($p < 0.001$) effect of vegetation, with survival being higher in the vegetated wetland cells. This effect is mainly based on differences at the 10 m and 20 m sampling station, where survival was more than three times higher in the vegetated wetland at 24 h. Survival was generally below 10% at the 5 m station at 3 h and zero at 24 h, while the respective values for the 40 m station were all ≥ 95%, demonstrating that there was also a significant location effect ($p < 0.001$).

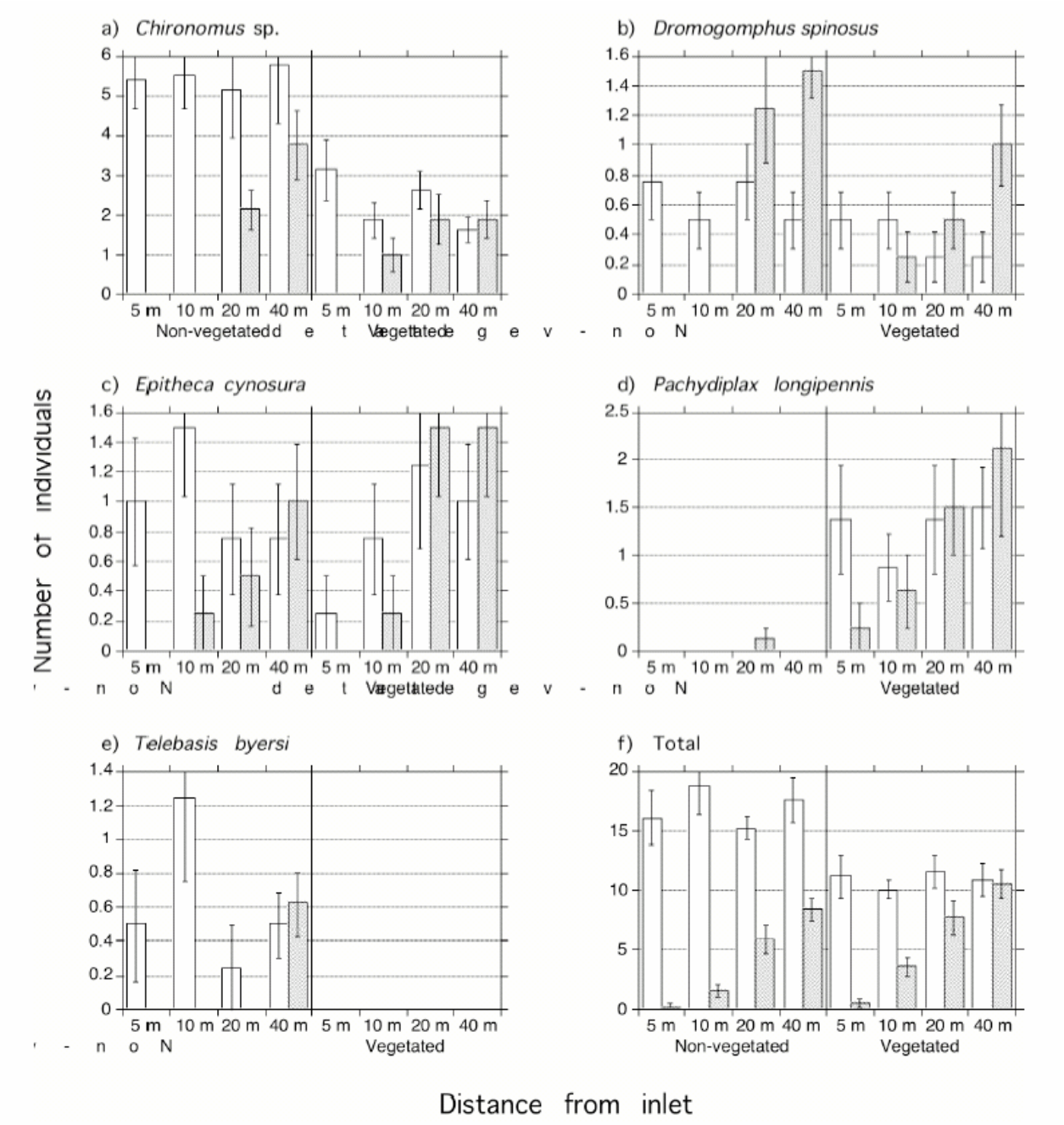

Fig. 3. Mean (± standard error, $n = 8$) abundance (individuals per sampler) of macroinvertebrate species in wetland mesocosms at different distances from the pesticide inlet station before (white bars) and 96 h after (black bars) introduction of methyl-parathion.

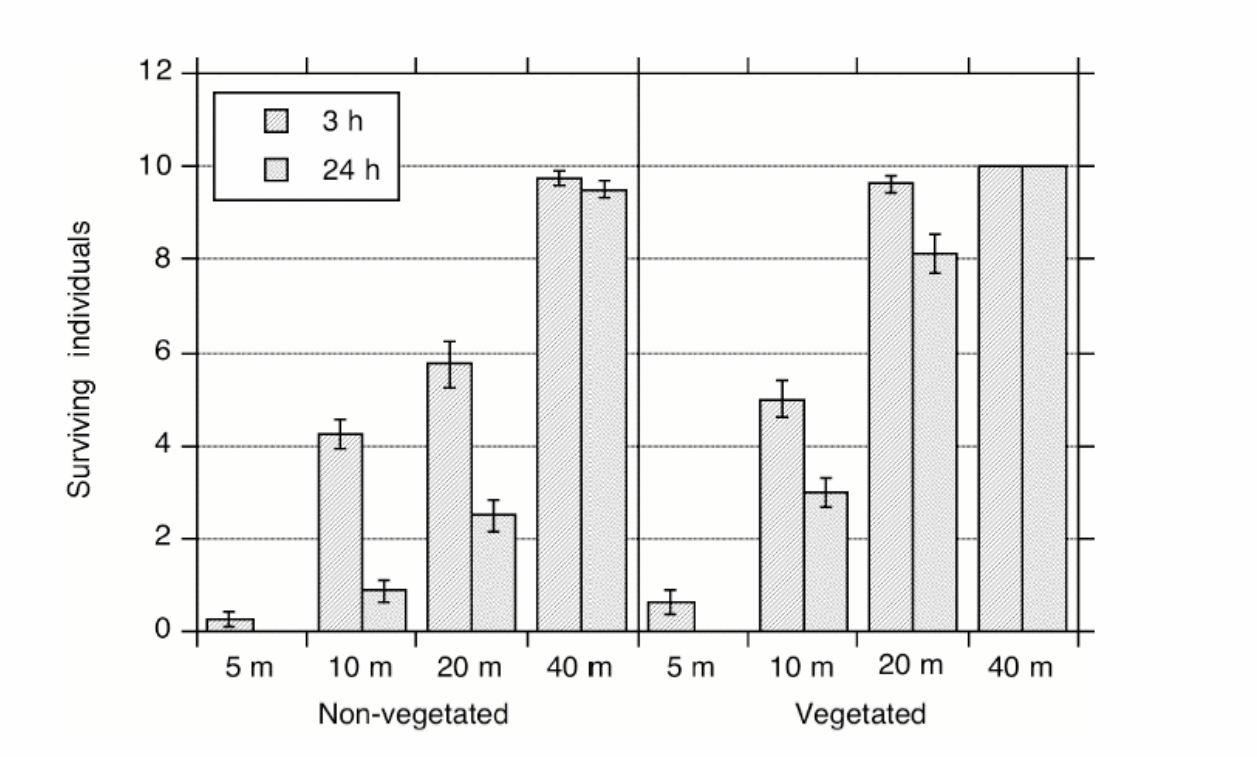

Fig. 4. Mean (± standard error, n = 8) survival of in situ exposed *Chironomus tentans* in wetland mesocosms at different times after introduction of methyl-parathion. Initial number of individuals was 10 per replicate.

The in situ bioassay results confirmed the invertebrate community results, in indicating a positive effect of vegetation on the spatial extent of MeP toxicity in the wetlands. Approximately 30% of the exposed larvae survived concentration levels between 40 µg/L at the 20-m station in the non-vegetated wetland and 90 µg/L at the 10-m station in the vegetated wetland during the 24-h exposure. This is in accordance with the 24-h LC50 of 58 µg/L reported for *Chironomus* sp. [33]. A 96-h EC50 of 32.3 µg/L was reported for *C. tentans* [34]. In situ exposure with chironomids have been used already for detection of insecticide toxicity in constructed wetland studies [1,2].

In summary, this study suggests that macrophyte vegetated weltands have a strong potential to contribute to aquatic pesticide risk mitigation. A 40-m stretch of dense vegetation cover effectively reduced a target MeP concentration of about 700 µg/L to below detection limit

(<0.1 µg/L). Furthermore, there were no effects of the pesticide on macoinvertebrate communities or in situ exposed chironomids detected at 40 m distance from the pesticide inlet in the vegetated wetland. These results confirm the importance of vegetated buffer zones represented either as wetland areas within streams or ditches or as vegetation coverage in the streams or ditches. It can be concluded that the conservation and management of vegetation in small drainage channels may be an effective tool to avoid agricultural pesticide contamination of receiving larger water bodies.

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APPENDIX 11.6

Evaluating Acute Toxicity of Methyl Parathion Application in Constructed Wetland Mesocosms

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Abstract

Wetland ecosystems have served to reduce ambient levels of various organic and metallic compounds, although their effectiveness on agricultural pesticides is not well documented. Five stations within each of four 10 x 50m constructed wetlands (two vegetated; two non-vegetated) were selected to measure fate and effects of methyl parathion (MeP). Following a simulated storm event (0.64 cm rainfall), aqueous, sediment, and plant samples were collected and analyzed spatially (5, 10, 20, and 40m from inlet) and temporally (3h – 10d) for MeP concentrations and impact to the aquatic fauna. Aqueous toxicity to fish decreased spatially and temporally in the vegetated mesocosm. *Pimephales promelas* survival was significantly reduced to 68% in non-vegetated mesocosms at the 10-m station (3h post application) with pesticide concentrations averaging 9.6 μg MeP/L. *Ceriodaphnia* in both vegetated and non-vegetated mesocosm, were sensitive (i.e. significant acute response to MeP) to pesticide exposures through 10-d post application. Mean MeP concentrations in water ranged from 0.5 to 15.4 μg/L and 0.1 to 27.0 μg/L in vegetated and non-vegetated wetlands, respectively. *Hyalella azteca* aqueous tests resulted in significant mortality at the 5m vegetated segment following 10-d post exposure of MeP (2.2 μg/L). Solid-phase (10-d) sediment toxicity tests showed no significant reduction in *Chironomus tentans* survival or growth with the exception of the sediments sampled 3 hours post application in the mesocosm without vegetation (65% survival). Thereafter, midge survival averaged >87% in sediments sampled from both treated mesocosms. These data suggest that wetlands play a significant role in mitigating MeP exposures to sensitive aquatic biota.

Key Words: agriculture, insecticides, BMPs, fate and effects

Introduction

Wetland research has been thoroughly documented for describing the efficiency of aquatic plants in removing toxic chemicals from waters, as well as the effective role that wetlands play in reducing suspended sediments and nutrients (Catallo 1993, Nichols 1983, Wolverton and Harrison 1973). Structure and function of wetlands have been reported by various researchers over the last two decades to improve water quality and provide specific mitigating properties for metals, industrial/municipal wastewater, urban stormwater, and petroleum products (Mastin et al. 2001, Gillespie et al. 2000, Hawkins et al. 1997, Detenbeck et al. 1996, Skousen et al. 1994, Nix et al. 1994, Jackson 1989, Livingston 1989). Fewer studies, however, have examined the fate and effects of specific pesticide exposures (i.e. organophosphate insecticides) in constructed wetlands (Schulz et al. 2003, Moore et al. 2002, Rodgers and Dunn 1992). The beneficial role of wetland ecosystems for improving water quality has most often been established through investigations examining their potential for trapping or transforming contaminants (Cooper et al. 1994). Investigations associated with agricultural runoff should provide landowners guidance for reducing pesticides prior to their reaching critical receiving streams.

Conventional wetland attributes (e.g. plant species, soil, and hydrologic regime) can also be found in agricultural drainage ditches. Very often the residence time in the drainage ditch, aquatic plant species inhabiting the ditch, and the soils that often include some organic and fine particles can facilitate the transformation of some pesticides that pose risk to sensitive aquatic communities.

Recent recognition that drainage ditches have an inherent ability to transform pesticide-associated agricultural runoff supports their use as a tool to transfer and transform contaminants

and subsequently decrease the potential for effects on downstream receiving systems due to non-point source runoff (Cooper et al. 2003, Cooper et al. 2002). Agricultural ditches receive drainage water that may result from both irrigation and stormwater runoff and therefore may contain elevated sediment, nutrient, and pesticide loads often in pulsed doses (Reinert et al. 2002, Morton et al. 2000). These ditches not only become an efficient means to promote drainage and reduce flooding on production acreage, but also provide a buffer between areas of intensive chemical use and aquatic ecosystems at potential risk. Concern over the presence of various pesticides in surface and groundwater has resulted in recent consideration for higher-tier aquatic risk assessments (Giddings et al. 2001, Solomon et al. 2001, Morton et al. 2000, US EPA 2000, Campbell et al. 1999, Solomon et al. 1996).

Agricultural drainage ditches are integral components of the Mississippi Delta production landscape and have been proposed as comparable substitutes for edge-of-field constructed wetlands (Moore et al. 2001). Research is now needed to quantify their attributes (i.e. determine runoff residence time, plant communities, water quality, and geomorphic characteristics of ditches) that support agricultural ditch use in best management practices for runoff-related issues.

This project evaluates aquatic system attributes that support the use of drainage ditches as best management practices for runoff-related issues, and measures the chemical exposure as changed by partitioning, movement, and transformation that occurs in actual field settings. It presents a specific approach for assessing the fate and effects of methyl-parathion (MeP) [O,O-dimethyl O-4-nitrophenyl phosphorothioate] by way of constructed wetland mesocosms (vegetated and non-vegetated) to reduce insecticide concentrations associated with agricultural runoff . Finally, it evaluates the various attributes of agricultural drainage ditches often

associated with wetland ecosystems for support in determining the potential impact of stormwater or irrigation runoff to sensitive aquatic organisms.

Materials & Methods

Study Site

Experiments were conducted in four outdoor wetland mesocosms located at the University of Mississippi Field Station near Oxford, Mississippi. Mesocosms were provided with water from a spring-fed source and rainfall collection. The project objectives included vegetated and non-vegetated wetlands to determine the fate and effects of MeP concentration exposures. Mesocosms were constructed in 1988 (average dimensions, 50 x 10 x 0.32 m; average bed slope = 0.006%) and had been previously planted with wild rice (*Zizania aquatica*), cattail (*Typha latifolia*), and bulrush (*Scirpus cyperinus*); however, natural succession had since dominated the mesocosms with 86% rush (*Juncus effusus*) and 14% cut grass (*Leersia oryzoides*). The latter two plant species were identified and quantified (256 and 43 plants / m^2, respectively) for the vegetated mesocosm exposure. Platforms were built at each station (5, 10, 20, and 40m from inlet) to collect water, plants, and sediment to reduce the possibility of disturbing the wetland mesocosm during sampling events.

Exposure Regime

Methyl parathion is a highly toxic insecticide in EPA toxicity class I. Some or all formulations of MeP may be classified as restricted-use pesticides, and it is primarily used as an organophosphate insecticide on cotton. Its use in the lower Mississippi delta averages

approximately 402,000 kg of active ingredient per year. The water solubility of MeP ranges from 55 - 60 mg/L @ 25^{o}C and has a partition coefficient range of 3.5 to 3.8 (Kidd and James 1991).

Two types of wetland mesocosms were used and included vegetated (VW) and non-vegetated (NVW) habitats. Each mesocosm was divided lengthwise using metal flashing to provide replication within each wetland cell. Each side was treated with commercial grade MeP (43.8% active ingredient) at a rate of 0.43 kg active ingredient / hectares. Treated mesocosms were exposed using a stock solution of wetland water and 6.6 mg MeP/L. Approximately 6,435 L of pesticide mixture was added to each side of the mesocosms at a rate of 9.5 L/second. Pesticide application included a simulated storm event of 0.64 cm rainfall on a 50-ha agricultural field; additional inclusion of 5 kg dry sediment was added to the pesticide mixture to simulate the typical suspended sediment load (400 mg/L total suspended solids) in the Mississippi Delta ecoregion (Smith et al. 2002).

Analytical Methods

Water quality was measured in wetland mesocosms, vegetated (VW) and non-vegetated (NVW) following amendments with methyl-parathion and included pH, dissolved oxygen, temperature, and conductivity (APHA 1995). Temperature in VW and NVW ranged from 23.7 – 27.8 and 27.0 – 28.6^{o}C, respectively. Conductivity was higher in VW, which ranged from 103-125 µs/cm (VW), while values measured in NVW ranged from 37-48 µs/cm. Values for pH were relatively neutral for both mesocosms ranging from 6.5-7.1. Dissolved oxygen indicated some differences for VW, where measured values ranged from 1.1 – 4.2 mg/L, and in NVW, where measured values ranged from 5.8 – 7.2 mg/L.

Plants, sediments, and surface water samples were analyzed throughout the 10-d exposure period for MeP uptake at pre-determined locations (stations) in the wetland mesocosms (Bennett et al. 2000, Moore et al. 2001, Cooper et al. 2002). Samples were collected at 0.5h, 3h, 24h, 96h, and 10d for water and sediment, and at 0.5h, 3h, 24h, and 10d for plants. All samples were collected and immediately placed on ice for shipment to the laboratory and subsequent analysis. Methyl parathion was analyzed by gas chromatograph (GC) equipped with a Hewlett-Packard 6890 GC equipped with a DB5 MS column and followed methods described by Moore et al. (2001) and Bennett et al. (2000). A multi-level calibration procedure was used with standards and updated every ninth sample. Limits of detection (LOD) for MeP in water, sediments, and plants were 0.1 μg/L, 0.02 μg/kg, and 0.02 μg/kg, receptively. Mean extraction efficiencies were >90%.

Acute Toxicity

Water and sediments were collected at 3h, 24h, 96h, and 10d post application for acute toxicity bioassays using *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyalella azteca*, and *Chironomus tentans*. All samples were collected spatially to identify MeP movement through each mesocosm. Surface water samples were collected at 5, 10, 20, and 40 m from the inlet point (0 m). Sediments were collected at 5, 10, and 20 m based on preliminary sediment analyses of MeP, which indicated that the pesticide had not bound to sediment particles beyond 20 m. All samples were transported on ice to Arkansas State University's Ecotoxicology Research Facility within 6 hours of collection and test organisms were immediately exposed to water and sediments.

Aqueous, 48-h toxicity tests were conducted using *C. dubia*, *P. promelas*, and *H. azteca* following methods outlined by U.S. EPA (1993). Comparison of organism survival with control exposures (moderately hard synthetic water; hardness = 80-100 mg$CaCO_3$/L) was the measured endpoint for all aqueous tests.

Sediment tests were conducted using solid-phase, 10-day acute toxicity assays to determine relative inhibition of survival and growth in exposed *C. tentans* (U.S. EPA 1994). A static flow-through system was used to renew overlying water in test chambers three times daily. Additionally, each test replicate chamber (six per site) was fed 1 ml of a Tetramin® solution (4g/L) daily. Test controls for sediment toxicity bioassays used to determine statistical significance included the use of sediments collected from the same ecoregion that have shown to support survival and growth in *C. tentans* (Moore et al. 1996).

Statistical Analysis

Results from the toxicity bioassays were statistically analyzed using Toxcalc® (version 5.0.25) (α = 0.05). Sediment data were tested for normality using Kolmogorov D test and homogeneity of variance using Bartlett's test. Wilcoxon Rank Sum test with a Bonferroni T adjustment was used to determine significance among stations. Aqueous data were tested for normality using Shapiro-Wilk's test and Steel's Many-One Rank test to determine significance in survival.

Results & Discussion

In this study, constructed wetlands have proven beneficial in mitigating the toxic effects of MeP in simulated storm events. Analytical data indicate that partitioning of MeP was

predominantly measured in plant tissues. Others have supported the significant role of plant species to limit the bioavailability of organophosphate pesticides in field and laboratory exposures (Moore et al. 2002, Schulz et al. 2003). In this study, the uptake of MeP from the water column by plants subsequently reduced the insecticide concentrations and reduced the magnitude of acute toxicity in exposed laboratory organisms. The water solubility of this chemical and its subsequent partitioning into the soil allows for the uptake by aquatic plants as was seen by levels of MeP in plant material extracted at the selected segment locations. Our research supports the importance of assessing temporal and spatial movement of MeP through the wetlands and the importance of these systems to efficiently transform MeP from water column to plant tissues or sediments. Using these mesocosms, we were able to measure the effects of the insecticide on aquatic and sediment test organisms and the fate of the insecticide for designing constructed wetlands or utilizing existing drainage ditches in agricultural landscapes for best management practices (BMPs).

Methyl Parathion Partitioning

Methyl parathion was analyzed from sediment and plant tissues prior to application for this study to determine background concentrations of MeP in wetland mesocosms. Sediment and plant tissue concentrations in vegetated mesocosms ranged from 21 to 230 μg/Kg and 67 to 212 μg MeP/Kg, respectively. Sediment concentrations in non-vegetated mesocoms ranged from 31 to 115 μg MeP/Kg.

Measured aqueous concentrations of MeP in vegetated (VW) and non-vegetated (NVW) mesocosms generally decreased as runoff progressed through the wetland (Table 1). Sediment concentrations in vegetated mesocosms were as high as 688 μg MeP/L (10d; 2.5m station).

Table 1. Methyl parathion concentrations measured in water, sediment, and plants from vegetated and non-vegetated wetlands. Values (±1SD are presented as averages (n=2, unless otherwise noted).

	VEGETATED			NON-VEGETATED	
Station	**H_2O (μg/L)**	**Sediment (μg/kg)**	**Plant (μg/kg)**	**H_2O (μg/L)**	**Sediment (μg/kg)**
0-h					
2.5 m	---	82.7 (n=1)	---	---	---
5 m	---	---	211.6	---	30.8 (n=1)
10 m	---	---	---	---	---
20 m	---	20.9 (n=1)	66.9	---	89.2 (n=1)
40 m	---	230.6 (n=1)	148.5	---	115 (n=1)
0.5 h					
2.5 m	5.14 (1.88)	229.8 (325)	8,155 (10,984)	7.89 (3.33)	9,270 (4,949)
5 m	11.47 (0.89)	15.2 (21.4)	2,533 (2,161)	27.01 (14.12)	1,444 (1,934)
10 m	6.67 (5.73)	0.8 (1.1)	1,275 (1,671)	4.36 (5.29)	581 (793)
20 m	11.16 (12.92)	0.0 (0.0)	2.1 (2.9)	3.64 (4.54)	20.7 (26.7)
40 m	ND	24.6 (30.7)	425.3 (128.4)	0.11 (0.03)	2.8 (4.0)
3 h					
2.5 m	7.22 (4.36)	53.9 (68.3)	21,523 (28,454)	17.68 (4.90)	585 (327)
5 m	6.74* (2.24)	108.4 (142.2)	4,650 (5,638)	8.82* (0.67)	131.0 (160.4)
10 m	4.09* (2.44)	77.2 (109.2)	4,519 (5,891)	9.62* (0.50)	0.5 (0.6)*
20 m	6.60* (8.95)	7.7 (6.4)	26.2 (18.1)	3.52* (0.92)	0.0 (0.0)
40 m	ND	12.8 (0.2)	249.7 (145.0)	0.24 (0.12)	4.2 (6.0)

[a]Biological test organisms elicited significant toxicity at these concentrations.

[b]Stations represent distance from inlet point (0m).

--- Not sampled for toxicity testing or chemical analysis.

ND: Non detect (LOD = 0.1μg/L)

Table 1 contd. Methyl parathion concentrations measured in water, sediment, and plants from vegetated and non-vegetated wetlands. Values (±1SD are presented as averages (n=2, unless otherwise noted).

	VEGETATED			NON-VEGETATED	
Station	**H_2O (µg/L)**	**Sediment (µg/kg)**	**Plant (µg/kg)**	**H_2O (µg/L)**	**Sediment (µg/kg)**
6 h					
2.5 m	6.76 (4.11)	---	---	9.84 (0.61)	---
5 m	4.77 (1.58)	---	---	5.62 (3.22)	---
10 m	9.41 (11.31)	---	---	15.16 (7.89)	---
20 m	2.29 (2.27)	---	---	5.78 (2.76)	---
40 m	ND	---	---	0.47 (0.15)	---
24 h					
2.5 m	4.05 (0.33)	214.8 (293.6)	2,481 (2,993)	3.94 (0.39)	10,189 (9,432)
5 m	15.38[a] (17.21)	40.3 (57)	713.1 (16.9)	14.75[a] (9.61)	75.6 (3.2)
10 m	7.22[a] (9.75)	5.4 (7.7)	2,674 (3,688)	13.11[a] (7.03)	0.7 (0.9)
20 m	6.57 (8.12)	0.0 (0.0)	73.5 (60.6)	2.86[a] (2.01)	0.0 (0.0)
40 m	ND	12.3 (4.0)	120.6 (30.4)	5.13[a] (n=1)	7.2 (10.2)
96 h					
2.5 m	7.42 (1.66)	---	---	5.82 (3.67)	---
5 m	1.76[a] (1.96)	---	---	3.09[a] (0.34)	---
10 m	11.51[a] (11.79)	---	---	13.60[a] (15.86)	---
20 m	0.53 (0.55)	---	---	18.07[a] (2.62)	---
40 m	ND	---	---	6.93[a] (1.29)	---
10 d					
2.5 m	---	687.8 (752.1)	133.5 (n=1)	2.99 (0.59)	687 (752)
5 m	3.45[a] (0.02)	18.1 (8.4)	2,186 (n=1)	2.18[a] (0.90)	18.1 (8.4)
10 m	0.48 (n=1)	18.6 (14.0)	96.0 (n=1)	2.79 (0.03)	18.6 (14.0)
20 m	ND	12.9 (1.0)	75.7 (30.9)	0.51 (0.01)	12.9 (1.0)
40 m	ND	23.3 (5.0)	180 (13.4)	0.44 (0.04)	23.3 (5.0)

Likewise, in unvegetated mesocosms, sediment concentrations measured over 10,000 μg MeP/L (24h; 2.5m station). Neither of these concentrations elicited a toxic response from exposed test organisms.

Measured sediment and plant tissue concentrations of MeP in vegetated and non-vegetated mesocosms generally decreased as runoff progressed through 20 m with a latent increase of plant-bound pesticide at 40 m (Table 1). Methyl parathion partitioning in sediments occurred as early as 0.5h (concentrations in vegetated and non-vegetated mesocosms ranged from 0.0 to 230 and 2.8 to 9,270 μg/L, respectively) and decreased in concentration as it moved through the wetland. A peak of sediment-bound MeP in non-vegetated mesocosms occurred at the 2.5 m station following 24-h post application (average = 10,190 μg MeP/L). Within the vegetated wetlands, a greater proportion of measured MeP was bound to plant tissues (average = 2,600 μg MeP/L) than on sediment particles (average = 78 μg MeP/L) post application. While wetland vegetation uptake has been quantified, specific research for the type and number of plant species that most efficiently assimilate MeP has not been determined.

The reported half-life of MeP in surface waters is eight days during summer months (Howard 1989). Using these results, the highest aqueous concentrations following application for vegetated (15.4 μg MeP/L) and non-vegetated wetlands (27.0 μg MeP/L) would be reduced to non-detectable limits (0.1 μg MeP/L) following 56 and 64 days, respectively. Our analytical data indicate that methyl parathion measured *in situ* from vegetated mesocosms is reduced to non-detectable limits within 96h. However, analyses of water in non-vegetated wetlands show that it would take longer than 10d for aqueous concentrations to reach non-detectable limits.

The reported half-life of MeP in water is 12h using laboratory exposures (Ferrando et al. 1992). Using these results, the highest aqueous concentrations following application for

vegetated (15.4 μg MeP/L) and non-vegetated wetlands (27.0 μg MeP/L) would be reduced to non-detectable limits (0.1 μg MeP/L) following four and five days, respectively. Our data indicate that methyl parathion measured *in situ* from vegetated mesocosms would support Ferrando's laboratory study, in that, MeP concentrations measured in water are reduced to non-detectable limits following 96h. However, analyses of water in non-vegetated wetlands show that it would take longer than 10d for aqueous concentrations to reach non-detectable limits.

Toxicity Responses

In this study, constructed wetlands reduced toxicological effects of methyl-parathion to sensitive aquatic biota. Our research has utilized water and sediment quality along with toxicity methods in wetland mesocosms to 1) determine which temporal and spatial measurements that would be needed to sufficiently (>80% survival) mitigate methyl-parathion exposure, and 2) relate this information to similar vegetated agricultural ditch systems.

3-h post application

Significant mortality was measured in *C. dubia* at 5, 10, and 20 m stations in vegetated and non-vegetated mesocosms with average aqueous concentrations of 6.7, 4.1, 6.6 and 8.8. 9.6, 3.5 μg MeP/L, respectively (Table 2). Survival (67.5%) at the non-vegetated, 10-m station for *P. promelas* was significantly lower than all other stations during this collection. Toxicity tests conducted for pesticide registration support this finding and indicate that effects for fathead minnow do not elicit an acute response until average MeP concentrations reach 8.5 ±1.2 mg/L (US EPA 2000).

Table 2. Results of acute (aqueous) toxicity survival (±1SD) results from vegetated and non-vegetated wetland mesocosms. Stations represent distance from inlet point (0m)([a]Significantly different from control exposures).

3-hr Collection

	C. dubia % Survival		*P. promelas* % Survival	
Station	Vegetated	Non-vegetated	Vegetated	Non-vegetated
5 m	0[a]	0[a]	97.5	90
10 m	0[a]	0[a]	92.5	67.5[a]
20 m	0[a]	0[a]	97.5	92.5
40 m	75	100	87.5	90

24-hr Collection

	C. dubia % Survival		*P. promelas* % Survival	
Station	Vegetated	Non-vegetated	Vegetated	Non-vegetated
5 m	0[a]	0[a]	80	92.5
10 m	0[a]	0[a]	100	90
20 m	50[a]	0[a]	100	70
40 m	100	0[a]	100	100

96-hr Collection

	C. dubia % Survival	
Station	Vegetated	Non-vegetated
5 m	0[a]	0[a]
10 m	0[a]	0[a]
20 m	100	0[a]
40 m	100	0[a]

10-d Collection

	C. dubia % Survival		*H. azteca* % Survival	
Station	Vegetated	Non-vegetated	Vegetated	Non-vegetated
5 m	70	40[a]	30[a]	62.5
10 m	100	70	92.5	67.5
20 m	90	100	92.5	100
40 m	100	100	97.5	100

24-h post application

Significant mortality was measured in *C. dubia* at 5, 10, and 20 m stations in the vegetated and non-vegetated wetlands, where MeP concentrations averaged 15.4, 7.2, 6.6, and 14.8, 13.1, 2.9 μg/L, respectively. Similar studies using *C. dubia* support these results, with 48-h LC50 values ranging from 2.6 to 5.5 μg MeP/L (Norberg-King et al. 1991). Reported EC50 values for *Daphnia magna* (average 12.3 ± 14.4μg/L) and LC50 values for *Gammarus fasciatus* (3.8 μg/L) indicate similar sensitivities to toxicity tests conducted in this study where MeP concentrations ranged from 0.3 to 27.6 μg/L (US EPA 2000).

96-h post application

Significant mortality occurred in exposed *C. dubia* from all stations (5, 10, 20, and 40 m) in non-vegetated wetlands, with pesticide concentrations averaging 3.1, 13.6, 18.1, 6.9 μg MeP/L, respectively. *Ceriodaphnia* mortality in vegetated wetlands occurred only at 5 and 10-m stations (average concentrations were 1.8 and 11.5 μg MeP/L, respectively).

10-d post application

In this study, an attempt was made to determine whether exposed organisms to MeP would be affected nearly two weeks following application to the mesocosms. Significant mortality was measured in *C. dubia* at 5m (40% survival) in non-vegetated wetlands (2.2 μg MeP/L) only. This may suggest that by 10 days, MeP was no longer bioavailable in surface waters at concentrations that would elicit an acute response.

Measured impacts to *H. azteca* (30% survival) occurred in vegetated wetlands at 5m; associated MeP concentrations at the same station averaged 3.5 μg/L. Studies conducted by Anderson and Lydy (2002) support the sensitivity of *Hyalella* exposures to MeP, where their 96-h LC50 values were as low as 2.1 μg/L. Their study also indicated that methyl-parathion is more

toxic to amphipods than other organophosphate pesticides (i.e. chlorpyrifos and diazinon). As shown in our study, MeP is still detectable in water for both vegetated and non-vegetation wetlands, with concentrations of 3.5 μg/L and 2.8 μg/L, respectively.

Schulz et al. (2003b) conducted similar studies using *H. azteca*. MeP detections in non-vegetated wetland extended the entire length (40m) of the mesocosm, with concentrations ranging from 0.3 to 550 μg/L. Aqueous MeP concentrations in the vegetated wetlands indicated that the insecticide was only detected through 20m, suggesting that the vegetation contributed to the reduced bioavailability and toxicity to exposed *H. azteca*. Toxicity in these wetlands was significantly lower as time and distance from the inlet increased. The calculated, 48-h LC50 for *H. azteca* was 9.0 ug MeP/L.

Other toxicity studies have included using *Chironomus tentans* in situ for aqueous exposures. Significant acute toxicity was calculated following three hours post application at 5, 10, and 20-m stations in non-vegetated wetland exposures of MeP to *C. tentans* (Schulz et al. 2003a). While results from vegetated wetlands indicated that significant toxicity was only measured through 10m.

Sediment Toxicity

Sediment-bound MeP reduced *C. tentans* survival (65%) at 10m in non-vegetated wetlands during 3-hour, post-application. Methyl-parathion concentrations for this station averaged 0.5 μg/Kg. Average sediment concentrations of MeP for all other stations ranged from 7.7 to 131 μg/Kg. No significant mortality or reduction in growth was measured in any of the other mesocosm stations through 24 hours post application (Table 3). Toxicity at station 10m may be a result of MeP photolysis to other toxic by-products (e.g. 4-nitrophenol) not analytically

Table 3. *Chironomus tentans* acute toxicity (sediment) survival and growth (±1SD) results from vegetated and non-vegetated wetland mesocosms. Stations represent distance from inlet point (0m) ([a]Significantly different from control exposures).

3-hr Collection

	% Survival		Growth (mg)	
Station	Vegetated	Non-vegetated	Vegetated	Non-vegetated
5 m	73.8	93.8	2.3	2.7
10 m	83.8	65[a]	2.9	2.5
20 m	77.5	91.3	2.7	2.4

24-hr Collection

	% Survival		Growth (mg)	
Station	Vegetated	Non-vegetated	Vegetated	Non-vegetated
5 m	87.5	86.3	2.6	2.0
10 m	95	92.5	2.6	2.2
20 m	86.3	92.5	2.5	2.3

measured in this study. Toxicity assays with *C. tentans* in other studies have determined that midges are less sensitive to MeP than other test organisms (Pape-Lindstrom and Lydy 1997). Their research indicated that acute toxicity of *C. tentans* in single-chemical aqueous tests resulted in EC50 values of 32.3 μg MeP/L.

Best Management Practices

Data included in this study may be used to select those parameters that relate to the structure and function of agricultural ditches specific to field application and drainage basin. While the ultimate goal of utilizing wetlands is to achieve no observable effects in organisms once the runoff enters a downstream receiving system, the goal of this research is to utilize acute aqueous and sediment toxicology to measure temporal and spatial reduction of pesticide effects.

Results of these toxicity bioassays indicate while there is similar toxicity at the 5m segment, different toxicity results are shown as the distance from source and time following application increases (Figure 1). Toxicity results support that spatial and temporal scales of vegetated wetlands should be at least 10m long where MeP should be retained in the wetland for at least 96 h to protect *C. dubia.* The same can be applied to the protection of *P. promelas*, where

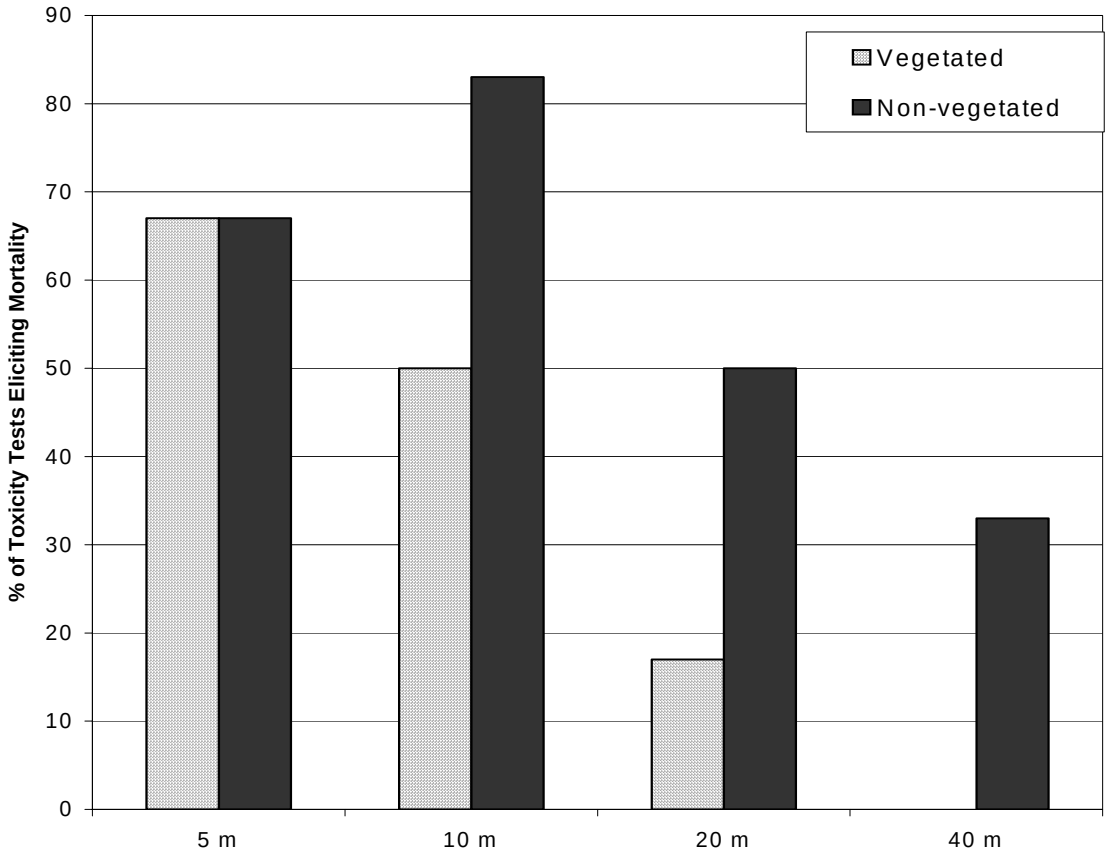

Figure 1. Percentage of all toxicity tests (sediment and water) conducted during this study that elicited significant mortality to exposed organisms. Vegetated wetlands provided more protection for aquatic organisms where partitioning of MeP into sediments and plant material had occurred.

the constructed wetland should be at least 5m long and retain MeP for at least three hours to mitigate the insecticide beyond its toxic effects and consequently protect sensitive vertebrates. *Hyalella azteca* were significantly reduced at 5m during the 10-d collection (MeP concentrations ranged from 12.1 – 24.1 μg/L), consequently, for the protection of this epibenthic species, the wetland would need to be at least 10m in length and be able to retain MeP for 10 days prior to release into receiving streams.

For unvegetated mesocosms, the data suggest that to protect *C. dubia*, the wetland needs to be at least 20 m and be able to retain MeP for 10 days. For the protection of *P. promelas*, the same length (20m) can be applied, however MeP only needs to be retained for three hours. *Hyalella* were not significantly affected at the shortest distance (5m) from the influent for the 10-day collection, more data would be needed to provide specific evidence for effects design using this species.

These data can be utilized by the agriculture industry to manage ditch systems that receive stormwater runoff from fields in order to determine the most efficient use of land (e.g. ditch length) necessary to mitigate pesticides and optimize crop production by minimizing land loss due to other BMPs.

Agricultural ditches approximate realistic pesticide exposure regimes more closely than ponds or with standard laboratory single-species toxicity tests. Since most studies upon pesticides depend on results from ponds, nutrient results from lakes, and sedimentation results from large rivers, ecosystem characteristics at work in ditches often go unnoticed. For this reason, several researchers are now examining the fate and effects of insecticides in mesocosms resembling drainage ditches. Schulz et al. (2003) described similar fate and transport of MeP

through constructed wetland, but assessed the impacts to test organisms *in situ.* That study also supported the effectiveness of wetlands to mitigate certain pesticides.

Our research has utilized water quality and toxicological methods to determine what temporal and spatial measurements would be needed to sufficiently (> 80% survival) mitigate pesticide and sedimentation effects. Utilizing chemical and biological measurements that can relate management in the field to reduction of contaminants in receiving systems will be of greater importance in the future. This study can eventually be used to select those parameters that relate to the structure and function of ditches specific to field application and drainage basin. While the ultimate goal of ditch "buffers" is for no observable effects in organismal response once the runoff enters a downstream receiving system, the goal of this research is to eventually determine wetland efficiency in buffering the effects of contaminants by employing methods of acute aqueous and sediment toxicology. Wetland buffering efficiency could then be predicted according to the results of these (and others) water quality surveys and toxicity assessments.

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APPENDIX 11.7

Fate and Effects of Azinphos-Methyl in a Flow-Through Wetland in South Africa

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Environ. Sci. Tech., 37: 2139-2144 (2003)

Abstract

Our knowledge about the effectiveness of constructed wetlands in retaining agricultural nonpoint-source pesticide pollution is limited. A 0.44-ha vegetated wetland built along a tributary of the Lourens River, Western Cape, South Africa was studied to ascertain the retention, fate and effects of spray drift-borne azinphos-methyl (AZP). Composite water samples taken at the inlet and outlet during five spray drift trials in summer 2000 and 2001 revealed an overall reduction of AZP levels by 90±1% and a retention of AZP mass by 61±5%. Samples were collected at the inlet, outlet and four platforms within the wetland to determine the fate and effect of AZP in the wetland after direct spray drift deposition in the tributary 200 m upstream of the inlet. Peak concentrations of AZP decreased and duration of exposure increased from inlet (0.73 µg/L; 9 h), via platforms 1 and 4 to outlet (0.08 µg/L; 16 h). AZP sorbed to plants or plant surfaces leading to a peak concentration of 6.8 µg/kg dw. The living plant biomass accounted for 10.5% of the AZP mass initially retained in the wetland indicating processes such as volatilization, photolysis, hydrolysis or metabolic degradation being very important. AZP was not detected in sediments. Water samples taken along two 10-m transects situated perpendicular to the shore indicated a homogeneous horizontal distribution of the pesticide: 0.23±0.02 µg/L and 0.14±0.04 µg/L (n = 5), respectively. Both Copepoda ($p = 0.019$) and Cladocera ($p = 0.027$) decreased significantly 6 h post-deposition and remained at reduced densities for at least 7 days. In parallel, the chlorophyll *a* concentration showed an increase, although not significant, within 6 h of spray deposition. The study highlights the potential of constructed wetlands as a risk-mitigation strategy for spray drift-related pesticide pollution.

Introduction

It has recently been shown that constructed wetlands have the potential to retain runoff-related insecticide pollution, preventing it from entering downstream aquatic habitats (*1, 2*). The implementation of retention ponds in agricultural watersheds was mentioned by Scott et al. (*3*) as one strategy to reduce the amount and toxicity of runoff-related insecticide pollution discharging into estuaries. The usefulness of aquatic plants for removal of insecticides from water has been shown in an indoor microcosm study (*4*) and the effects of the organophosphate phorate have been assessed using littoral mesocosms in South Dakota wetlands (*5*). However, information about the fate or effects of spray drift-borne insecticide input in constructed wetlands is limited.

Processes important for removal of nonpoint-source pesticide pollution in wetlands may include adsorption, decomposition, hydrolysis, microbial metabolism, photolysis and volatilisation (*6*). The macrophytes present in the wetland may play an important role in providing an increased surface area for sorption as well as for microbial activity (*7*). Furthermore, they may contribute directly to metabolism (*8*).

Spray drift is an important route for nonpoint-source pesticide pollution of aquatic habitats (*9, 10*). Specifically, orchard applications result in a large amount of drift due to small droplet size and the trajectory of release (*11*). Spray drift has been shown to be a significant route of insecticide entry into tributaries of the Lourens River in South Africa (*12*).

The organophosphate pesticide azinphos-methyl (AZP) [*O,O*-dimethyl-*s*-[(4-oxo-1,2,3-benzotriazine-3(4H)-yl)methyl)]phosphorodithioate], in comparison to other insecticides, has a relatively low K_{OC} of 1000 L/kg, a high water solubility of 29 mg/L at 25 °C (*13*) and a Henry´s law constant of 9.5 x 10^{-11} atm m^3 mol^{-1} (*14*). It has been shown to persist in pond water with a

half-life of about 2.4 days (*15*). Azinphos-methyl 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 Lourens River and its tributaries (*12, 16*). The estimated total application in fruit orchards of the Western Cape is 52,000 kg active ingredient per year. It is also one of the most heavily applied pesticides in the United States, and in 1997 almost 950,000 kg active ingredient were applied throughout the entire country (*17*).

In order to minimize the input of sediment into the Lourens River a 0.44-ha vegetated siltation pond was constructed in 1991 along one of the tributaries. Its effectiveness in retaining runoff-related insecticide input was the subject of an earlier study based on inlet and outlet measurements (*1*). The present study describes the retention, the fate and the biological effects of spray drift-borne aqueous-phase AZP in this flow-through wetland.

Materials and Methods

Study Region and Study Period. The study area is located in the catchment of the Lourens River, which originates at an altitude of 1080 m in fynbos, a naturally sclerophyllous vegetation, and flows in a southwesterly direction for approx. 20 kilometres before discharging into False Bay at the Strand (S34°06′; E18°48′). The Lourens River has a total catchment area of 92 km^2 and receives an annual mean rainfall of 915 mm. Approximately 87% of its $35x10^6$ m^3 mean annual discharge occurs during the winter months between April and October (*18*), as is characteristic of the region's Mediterranean climate.

The 400-ha orchard area in the middle reaches of the Lourens River is mainly covered by pears, plums and apples. The pesticide application period in the study area′s orchards begins in

early August and continues until the end of March. Organophosphorous (OP) insecticides, such as AZP and chlorpyrifos, are applied quite frequently to pears and plums between October and February. Endosulfan is applied mainly in apple orchards (*19*).

The constructed wetland studied in the present investigation is located along one of the tributaries 15 m before its entry into the Lourens River (*1*). This tributary has an average width and depth of 0.89 m × 0.30 m, a current velocity of approximately 0.1 m/s in summer, and approximately 50% of its surface is covered with emergent vegetation. It has a total length of approximately 1.8 km and flows from a dam through a forest area for 800 m and then into pasture land for 400 m before flowing through the orchard area for a further 600 m. Average discharge in the tributary is 0.03 m^3/s in January and 0.32 m^3/s in July. The discharge during the study period was 0.043±0.002 m^3/s; all studies were done during the dry summer season.

The retention of spray drift-borne AZP in the wetland was derived from measurements undertaken in January and February 2000 and 2001 during the dry summer periods with average monthly rainfall <25 mm. A detailed study of the fate and transport of AZP was done on February 5, 2001, during the last pesticide application of the spraying season 2000/2001, and the resulting biological effects were monitored until May 17, 2001. All studies were done while AZP was being applied in the conventional manner to fruit orchards bordering the tributary, which flows through the wetland. Such application resulted in spray deposition into the tributary about 200 m upstream of the wetland, as described in an earlier study (*12*).

Description of the Wetland. The wetland was built in 1991 to prevent nonpoint-source input of suspended sediment into the Lourens River (*1*). The catchment area of the wetland comprises 15 ha of pear and plum orchards, 10 ha of pasture land and 18 ha of forest. The wetland has a length of 134 m and a width of 36 m, giving a total area of 0.44 ha (Figure 1). The water depth varied between 0.3 and 1 m in different parts of the wetland during the study period; this remarkable

shallowness, in contrast to the initial depth of up to 1.5 m at time of construction, indicates the extent of particle sedimentation within the wetland. Using the summer flow rates of the tributary, the theoretical water-renewal time of the wetland is 27 hours. The first 15 m of the wetland are free of vegetation and the remaining area is covered mainly with *Typha capensis* Rohrb. (80% coverage) *Juncus kraussii* Hochst (15% coverage) and *Cyperus dives* Delile (5% coverage).

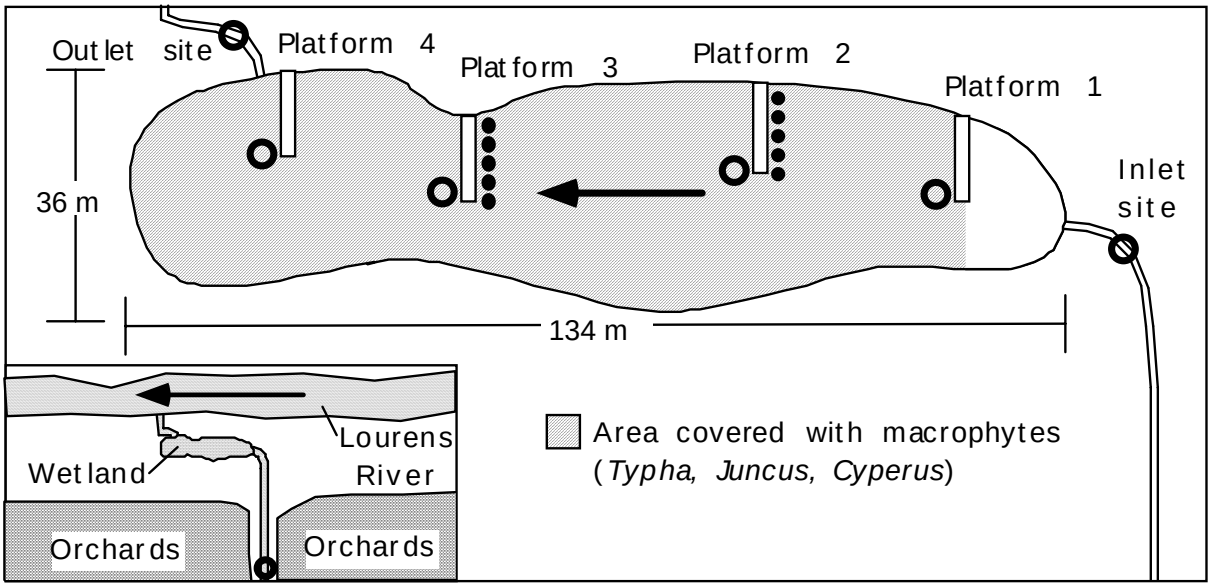

FIGURE 1. Schematic view of the wetland showing size, vegetation coverage and six main sampling sites (large circles) as well as sites for transect sampling at platforms 2 and 3 (small circles). The inset (not to scale) shows the orientation of the wetland to the Lourens River and to the orchard areas as well as the additional site (spray deposition site, circle) about 200 m upstream of the wetland. The arrow indicates the flow direction.

Sampling Procedure and Analysis. Six main sampling stations were used. The inlet and outlet sampling was carried out in the tributary above and below the wetland. Four platforms raised above the water surface, at distances of 17, 45, 85 and 110 m from the inlet, were used as sampling stations in the wetland to ensure that sampling would not cause unnecessary damage to the macrophytes and/or sediments (Figure 1). An additional seventh site, representing the spray

drift deposition site, was located in the tributary about 200 m upstream of the wetland inlet.

Discharge of the tributary was calculated, on the basis of standard formulas (*20*), from velocity measurements along cross-sectional profiles. Total suspended solids (TSS; ±0.1 mg/L) were measured using a turbidity meter (Dr. Lange, Duesseldorf, Germany). Turbidity readings were calibrated as described by Gippel (*22*). Temperature (±0.1 °C), dissolved oxygen (±0.1 mg/L) and pH (±0.1) were measured with portable electronic meters (WTW, Weilheim, Germany). Orthophosphate (molybdenum blue; ±0.01 mg/L) and nitrate (dimethylphenol; ±0.1 mg/L) levels were measured with photometric test kits from Merck, Ingelheim, Germany. The mean values (n = 14 between January and May) of various parameters are summarized in Table 1. Details of the retention of nutrients and suspended particles under different hydrological conditions were described in an earlier study (*1*).

TABLE 1. Mean (±SE; n = 14) of various parameters measured at the wetland inlet between January and May

parameter	mean (±SE)
discharge (m^3/s)	0.041±0.004
total suspended solids (mg/L)	43.7±5.2
temperature (°C)	19.3±0.8
dissolved oxygen (mg/L)	8.6±0.4
pH	7.1±0.1
orthophosphate (mg/L)	0.16±0.02
nitrate (mg/L)	3.6±0.4

In order to describe the overall AZP retention in the wetland, composite samples were taken in 3-litre glass jars at the inlet and the outlet station during five spray drift trials on the following dates: January 28, February 2 and 14, 2000 as well as on January 12, and February 5, 2001. Average discharge through the wetland was 0.043±0.002 m^3/s during these five trials. A previous experiment with the introduction of a tracer dye was undertaken during similar hydrological conditions (discharge: 0.042 m^3/s) to determine the starting point and duration of sampling intervals at the inlet and outlet station of the wetland used for the pesticide sampling. The tracer Rhodamin B was introduced, at a rate of 15 g dissolved in 200 ml of water, into the tributary at a site 200 m above the wetland where spray drift deposition occurs during application to the adjacent pears. Water samples were taken at various time intervals at the inlet and outlet station. Samples were stored at 4°C in the darkness. They were analysed within 24 h using a fluorescence photometer (TD700; GAT Bremerhaven, Germany) with a detection limit of 2.5 ng/L. The concentrations of the tracer are expressed in relative terms, because degradation in the presence of sunlight decreases the absolute concentrations of Rhodamin B with a field half-life of approximately 3 h (*21*). In accordance with the results of this tracer experiment (Figure 2), 3-h composite sampling commenced at the inlet station 30 min after introduction of spray drift 200 m above the wetland, while 12-h composite sampling commenced at the outlet station 6 h after introduction of spray drift. This sampling design potentially enabled collection of the same parcel of water with >95% of the pesticide mass at both stations via composite samples. Each composite sample (1 L) was combined from ten sub-samples (each 100 mL) taken every 20 minutes at the wetland inlet and every 80 minutes at the wetland outlet. As the discharge did not vary over time and showed only very little variation between the five spray drift trials, the concentrations obtained from the composite samples were used for comparisons between inlet and outlet and (along with the discharge data) for calculating AZP mass. However, it should be noted that these

concentrations do not represent peak levels, but average levels based on the ten sub-samples from the composite sampling. As no time-dependent hydraulic residence time distributions were defined, the direct comparison of concentrations between inlet and outled should be interpreted with care.

A detailed description of AZP distribution and fate over time was enabled during the spray drift trial on February 5, 2001 by taking discrete (separate) water samples at the inlet and outlet station and water, plant (rinsed subsurface sections of living *T. capensis* shoots without roots including the attached biofilm) and sediment (upper 2 cm) samples at platform 1 and 4 between time zero at the commencement of spray deposition and day 7 following spray drift.

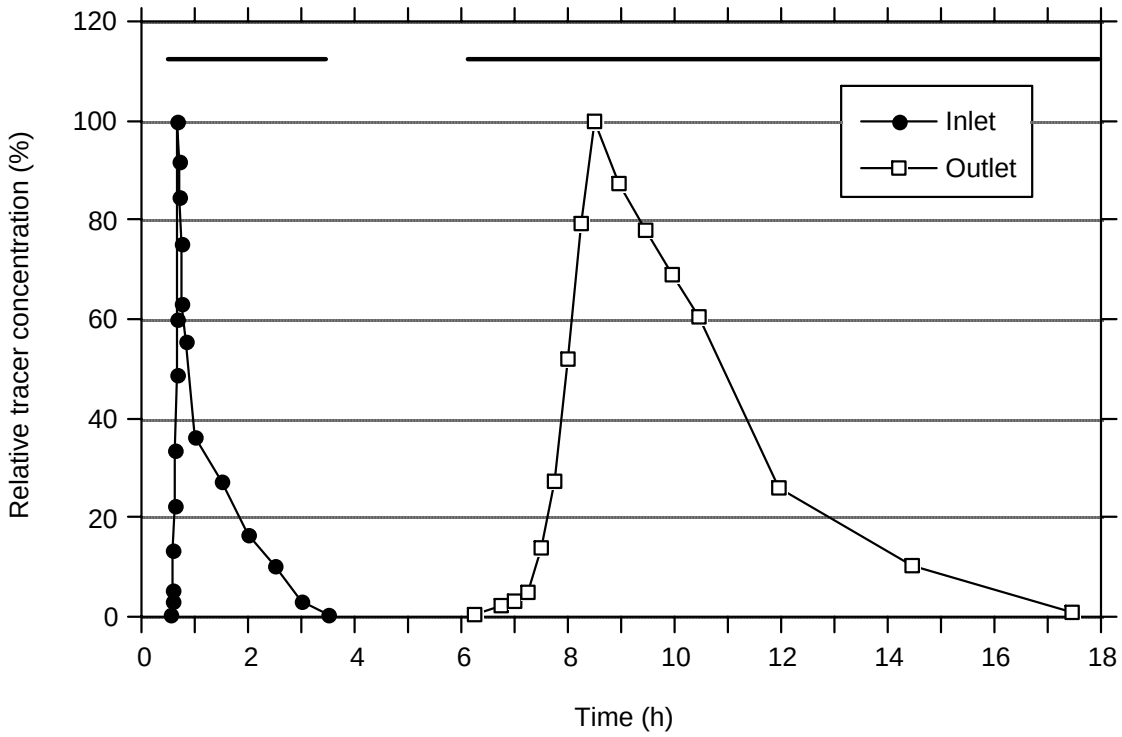

FIGURE 2. Time course of relative concentration of a tracer dye (Rhodamin B) at the two sampling stations at the inlet and outlet of the constructed wetland. The maximum concentration at both stations was set at 100%.

Timing of sampling depended on the expected duration of exposure, i.e. shorter time intervals were used at the inlet station in comparison to the outlet station. In addition, four replicate water samples were taken at the spray deposition site about 200 m upstream of the wetland inlet, according to methods described in Schulz et al. (*12*). For a description of the horizontal distribution of the pesticide in the vegetated wetland area, five water samples were taken at each of the platforms 2 and 3, at 2-m intervals along 10-m transects perpendicular to the wetland shore (Figure 1). The transect used at platform 3 covered more than half of the width of the wetland at this site.

A mass balance was established to estimate the total amount of AZP sorbed to plants or plant surfaces based on the analytical data obtained from the plant samples related to the estimated average inlet mass. The mean (±SE) dry biomass of subsurface shoots sections of living *T. capensis* excluding root material was 1086±57 g/m^2 and the moisture content was 92±0.5% (n = 6). Only 50% of the total area covered by plants was considered in the mass balance as AZP was detected in plants taken at platform 1 but not in those from platform 4. The resulting total biomass was 2280 kg dw.

Water samples (500-900 ml) were solid-phase extracted (SPE) within 10 h after sampling, using C18 columns (Chromabond). The columns were air-dried for 30 minutes and kept at –18°C until analysis. Analyses were performed at the Forensic Chemistry Laboratory of the Department of National Health, Cape Town. Sediment, plant and water samples were analysed as documented in Bennett et al. (*23*) and Schulz et al. (*19*). 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 µg/kg dry weight (dw). Identity of AZP was confirmed by matching retention times on 3 different stationary phases. Method validation employed water matrices that were found to have no detectable levels

of the investigated pesticides, and 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 0.02 µg/L for water, 0.2 µg/kg for sediments and 0.2 µg/kg for plants.

Pesticide Effects. Four replicate zooplankton samples were taken at platform 3 at various times between day -7 and day 7 of the spray drift trial on February 5, 2001. Each water sample (9 L) was filtered through an 80-µm mesh and the zooplankton was preserved in 70% EtOH. Numbers of copepods and cladocerans were counted. Chlorophyll *a* was measured in four replicate samples taken at platform 3 at various times in conjunction with the zooplankton sampling. Volumes of 750 mL each were filtered in the darkness through glass fibre filters immediately following sampling using a vacuum pump. Filters were stored on ice until acetone extraction and analysed according to methods described in Wetzel and Likens (*24*).

Results and Discussion

Overall Pesticide Retention. Concentrations of AZP derived from composite samples taken during five independent spray deposition trials averaged 0.40±0.03 µg/L at the inlet and 0.04±0.003 µg/L at the outlet station (Table 2). Average reduction was 90±1%. The same calculations were undertaken with the loading rates, giving a retention of AZP mass of 61±5% from 172.2±12.6 mg at the inlet to 67.2±7.7 mg at the outlet (Table 2).

The retention of insecticides in constructed wetlands has so far been described in a few studies dealing with runoff scenarios. According to previous results obtained during a runoff

TABLE 2. Concentration and mass of AZP during spray drift at the inlet and outlet of the constructed wetland. Inlet values are from 3-h composite samples (n = 1), whereas those for the outlet are from 12-h composite samples (n = 1) during spray drift trials performed in January and February 2000 and 2001

	AZP concentration			AZP mass		
trial	inlet (μg/L)	outlet (μg/L)	reduction (%)	inlet (mg)	outlet (mg)	retention (%)
1	0.41	0.04	90	159.4	62.2	61
2	0.36	0.04	89	186.6	82.9	56
3	0.34	0.02	94	143.2	33.7	77
4	0.51	0.04	92	225.8	70.8	69
5	0.27	0.04	85	145.8	86.4	41
average (±SE; n = 5)	0.4±0.03	0.04 ±0.003	90±1	172.2±12.6	67.2±7.7	61±5

event in the same wetland, AZP, chlorpyrifos and endosulfan at inlet peak levels of 0.85, 0.02 and 0.2 μg/L, repectively were retained at rates >77% (*1*). Approximately 47-65% by mass of chlorpyrifos was retained in plants and sediments within the first 30-36 m of wetland mesocosms in Mississippi (*2*). A decrease of lambda-cyhalothrin from 460 to <0.02 μg/L within a 50-m stretch was predicted from studies undertaken in a slow-flowing vegetated agricultural drainage ditch (*25*). For the herbicide atrazine, a removal rate between 25 and 95% was demonstrated in the Des Plaines Wetland cells (*26*), while reduction of atrazine concentration in water was 11-14% in 230-m flow-through wetland mesocosms in Minnesota (*27*).

In summary, the wetland studied in the present investigation has very positive effects in reducing the concentration and mass of aqueous-phase AZP entering the surface water via spray drift. Spray deposition resulting in short-term peak AZP levels of 2.5±2.6 μg/L at the deposition site 200 m upstream of the wetland inlet were comparable with results (1.7±0.2 μg/L) obtained from previous spray drift studies undertaken in the same catchment in 1999 (12).

On the other hand, the removal of water-borne toxicants by wetlands could potentially

lead to unwanted long-term accumulation of chemicals, as documented for natural wetland areas (*28*). The effectiveness of pesticide retention in wetlands may differ with season due to fluctuations in water temperature and flow as well as wetland abiotic and biotic conditions (*29*). There is still a need for further studies to demonstrate the long-term fate of insecticides in constructed wetlands.

Pesticide Fate and Distribution. During the spray drift trial on February 5, 2001, AZP was present in the water at the inlet between 40 min and 9 h and at the outlet between 8 h and 1 d post-deposition, and similar durations of detection applied to platforms 1 and 4 (Figure 3). Measured peak concentrations decreased from the inlet (0.73 µg/L) via platforms 1 (0.65 µg/L) and 4 (0.12 µg/L) to the outlet (0.08 µg/L). Plants taken at platform 1 contained about 2 µg/kg AZP at 0 h, 3 h, 12 h, and 7 d and peaked at 6.8 µg/kg at 6 h after commencement of spray deposition (Table 3). AZP was not detectable in plants at platform 4. None of the sediment samples taken at platforms 1 and 4 between 0 h and 7 d, contained any detectable AZP. At both platforms 2 and 3, AZP was distributed virtually uniformly along the transects situated perpendicular to the wetland shore. Average (±SE; n = 5) levels were 0.23±0.02 µg/L and 0.14±0.04 µg/L, with coefficients of variance of 22% and 55% (Table 4).

Detailed measurements of AZP transport through the wetland during the spray drift trial on February 5, 2001, confirmed the positive effect of the wetland in reducing aqueous-phase concentrations. Macrophyte samples contained up to 6.8 µg/kg AZP in comparison to pre-event levels ≤2 µg/kg, which are presumably due to AZP applications earlier in the season. AZP concentrations in plants decreased rapidly and were in the range of 2.2 µg/kg 12 h and 7 d post spray deposition, while the pesticide was not detected in any sediment samples. As studies with chlorpyrifos, which has a much higher K_{OC} value, showed much longer residence times in plants and sediments (*2*), it is assumed that the relatively high water solubility of AZP, along with the

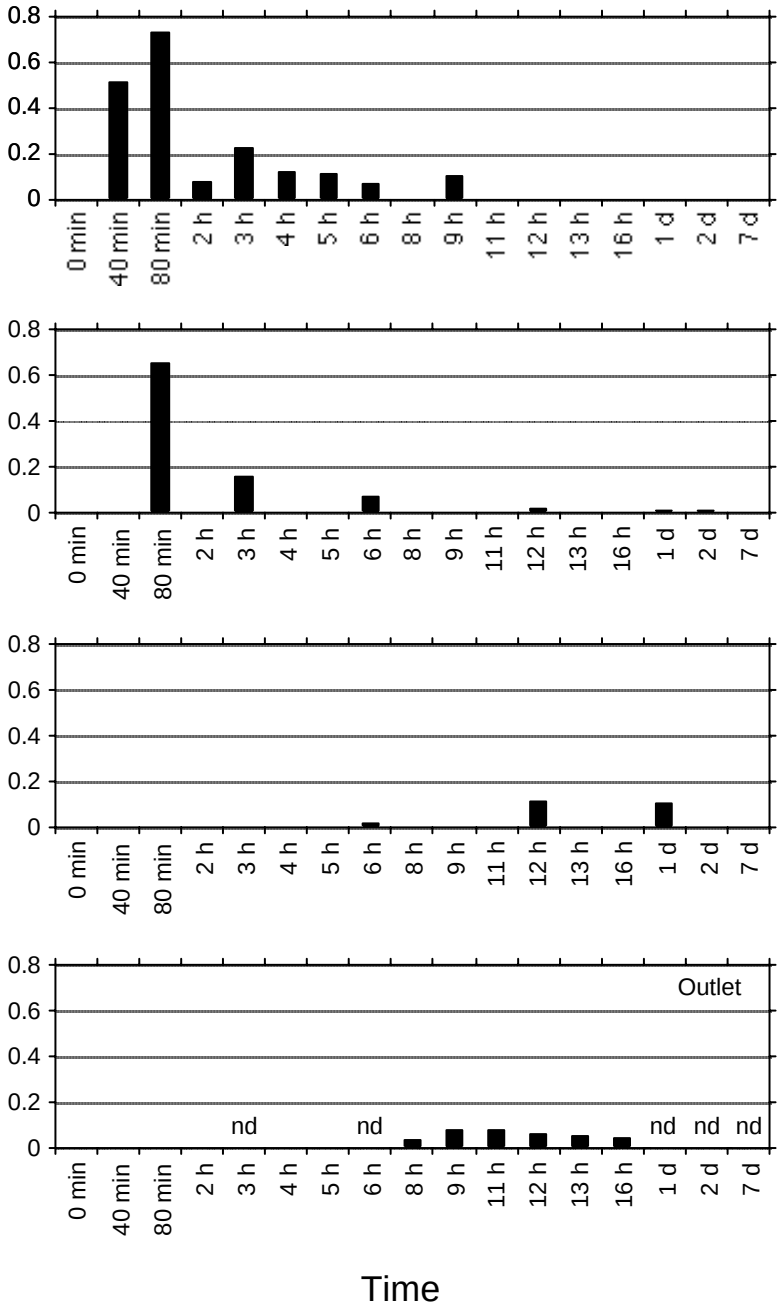

FIGURE 3. Concentrations of azinphos-methyl (nd = not detectable) at various sites during a spray drift event on February 5, 2001. Time zero refers to the commencement of spray deposition about 200 m upstream of the wetland inlet, where peak azinphos-methyl levels were 2.5±2.6 µg/L.

TABLE 3. AZP concentrations in plant samples (µg/kg dw; n =1) taken at platforms 1 and 4 of the wetland (nd = not detectable). Time zero refers to the commencement of spray deposition about 200 m upstream of the wetland inlet on February 5, 2001

time	platform 1	platform 4
0 h	2.0	nd
3 h	1.6	nd
6 h	6.8	nd
12 h	2.2	nd
7 d	2.2	nd

TABLE 4. AZP concentrations in water (µg/L; n =1) along transects across platform 2 at 3.5 h and platform 3 at 6 h following commencement of spray deposition about 200 m upstream of the wetland inlet on February 5, 2001. Five samples (a to e) were taken at each platform at 2-m intervals along 10-m transects perpendicular to the wetland shore

sample	platform 2	platform 3
a	0.17	0.15
b	0.22	0.21
c	0.20	0.16
d	0.29	0.19
e	0.27	0.01

fact that a flow-through wetland with a theoretical water renewal time of 27 h was used in this study, are of importance. Based on the present data, the water-plant (surface) distribution coefficient would be 17 for AZP. Plant samples taken at platform 4 next to the outlet contained no detactable AZP levels, indicating that aqueous-phase AZP concentrations are generally much lower at this site. A mass balance revealed that in total about 11 mg of AZP were sorbed to the

living water-exposed parts of *T. capensis* in the wetland. This equals 10.5% of the total mass of AZP initially retained in the wetland. It should be noted that senesced plant matter and other plant species are not included in this mass balance, which might increase the relative contribution of the aquatic vegetation. However, the majority of the initial loss of AZP in the wetland may be due to processes such as volatilization, photolysis, hydrolysis or metabolic degradation (*6, 7*). On the other hand, the results highlight the importance of aquatic plants for the sorption of insecticides, even if they have relatively low K_{OC}'s and high water solubilities. On the basis of the present data, it is not possible to decide whether the vegetation itself or microbial communities attached to the plant surface are relevant for the AZP sorption. The latter process seems more likely, particularly with respect to the short time periods involved. However, the role of plants including attached algal and microbial communities for insecticide sorption has already been demonstrated by various other workers (*2, 4, 25, 30, 31*).

Another important effect of the plant coverage is related to the flow conditions in the wetland. As was shown by the results from transects along platforms 2 and 3, measured AZP was relatively evenly distributed between sites, indicating that it did not flow in a single main channel through the wetland. It is likely that the dense vegetation coverage in the wetland influences the hydraulic conductivity (*32*) and thus homogenizes the horizontal pesticide distribution along trans-sectional profiles. Since both transects covered only up to half of the wetland width, however, it is possible that concentrations differed in the other half.

Pesticide Effects. Both Copepoda and Cladocera decreased significantly (t-test) 6 h following the spray deposition, from approximately 180 to 100 animals/L ($p = 0.019$; $n = 4$) and from approximately 50 to 20 animals/L ($p = 0.027$; $n = 4$), respectively. They remained at these levels until the end of the sampling period on day 7 (Figure 4). Chlorophyll *a* concentration showed an increase from 1.5 µg/L prior to AZP contamination to >2.5 µg/L between 6 h and day 7, which

was not significant. These generally low concentrations indicate a low phytoplankton density, presumably due to the shading effect and nutrient consumption of the emergent macrophyte vegetation.

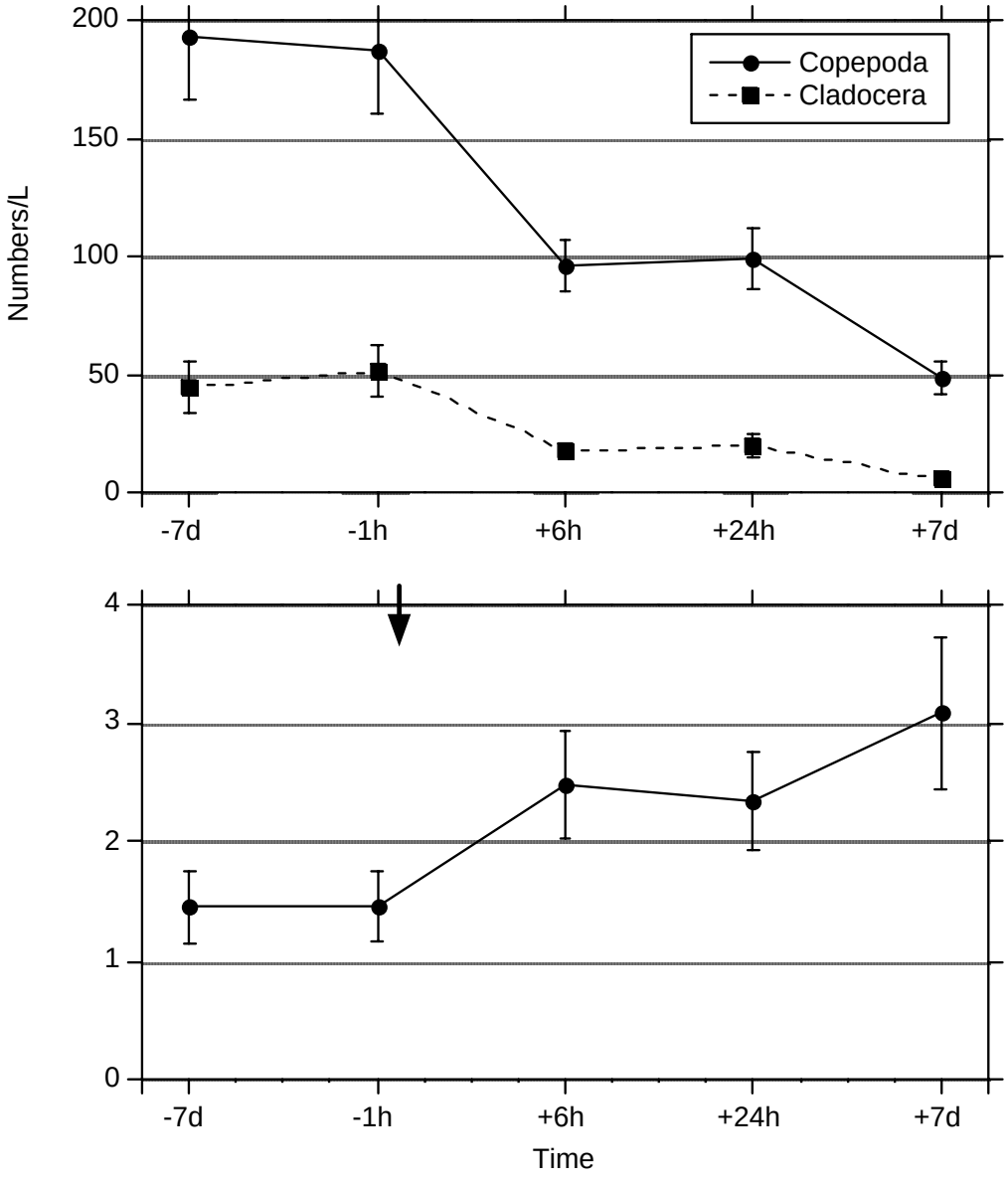

FIGURE 4. Mean (±SE; n = 4) densities of zooplankton and chlorophyll *a* concentrations in the wetland at platform 3. The arrows indicate the spray drift related input of azinphos-methyl on February 5, 2001 at time zero, causing a significant decrease in density of copepods and cladocerans.

Cladocerans and copepods were significantly affected at platform 3, where average AZP concentrations were 0.14±0.04 µg/L at 6 h following spray deposition. It has been reported from littoral enclosures that AZP levels as low as 1 µg/L exhibit short-term effects on zooplankton communities (*15, 33*), with cladocerans being the most sensitive group (*33*). The increased chlorophyll *a* concentrations, although not significant, may indicate an indirect positive effect of the insecticide on the phytoplankton communities. As the grazing pressure may decrease with reduced zooplankton densities, the algae density, expressed as chlorophyll *a*, increases. A similar effect was assumed to be responsible for an increase of chlorophyll *a* concentration in ponds exposed to the organophosphate insecticides temephos and chlorpyrifos (*34*).

Azinphos-methyl concentrations of 0.73 µg/L detected in the inlet water were higher than acute toxic concentrations for various species of crustaceans such as *Gammarus fasciatus* Say (*35*) and *Hyalella azteca* Saussure (*36*). They also exceeded the 96-h LC_{50} of *Chironomus tentans* Fabricius, which is 0.37 µg/L (*36*). In addition, in situ exposure of chironomids during runoff events with 0.85 µg/L and 0.06 µg/L AZP at the inlet and outlet station, respectively, revealed an 89% reduction in toxicity below the same wetland in an earlier study (*1*).

In summary, this study suggests that macrophyte vegetated wetlands have the potential to contribute to aqueous-phase pesticide risk mitigation. However, further studies are needed focussing on the long-term processes in constructed wetlands. These results confirm the importance of vegetated buffer zones, in the form of either wetland areas supplied by streams or ditches, or vegetation coverage in the streams or ditches. It can be concluded that the conservation and management of vegetation in small drainage channels may be an effective tool to avoid agricultural pesticide contamination of larger receiving water bodies.

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APPENDIX 11.8

Thesis Curriculum Vitae

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Education

1990-1993 Technologist Diploma (Environmental Biology) - Canadore College, North Bay, Ontario, Canada.

1993-1996 B.Sc. (Environmental Science) - Trent University, Peterborough, Ontario, Canada.

- Included a 4th year Honours Thesis

1996-1998 M.Sc. (Environmental Chemistry) - Trent University, Peterborough, Ontario, Canada.

- Thesis Title - Distribution of the degradation products of alkylphenol ethoxylates in the aquatic environment. Research supervised by Dr. Chris Metcalfe.

Work Experience

1998-1999 Research Associate – University of Mississippi, Mississippi, USA

- The two main objectives of this position were to provide technical support for investigating the environmental fate of agricultural chemicals in the Mississippi Delta and the screening of organic compounds for potential endocrine system disruption in Japanese medaka.

1999-2000 Sessional Lecturer – Canadore College, North Bay, Ontario, Canada

- Taught courses in Hydrology, Earth Sciences, Inorganic Chemistry, Scientific Writing, Environmental Ethics, and supervised third year projects.

2000-present Ph.D. (Natural Sciences) – University of Koblenz-Landau, Germany

- Proposed Title – Fate, effect and mitigation of pesticides in vegetated agricultural drainage ditches and constructed wetlands. Mentor/supervisor Prof. Dr. Ralf Schulz.
- Research supported by ENVIROMAP, USDA-ARS, the University of Mississippi, the University of Arkansas and the Great Lakes Institute for Environmental Research.