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Schulz, R.; Moore, M.T.; Bennett, E.R.; Milam, C.D.; Bouldin, J.L.; Farris, J.L.; Smith, S;
Cooper, CM. 2003. Acute toxicity of methyl-parathion in wetland mesocosms: Assessing the
influence of aquatic plants using laboratory testing with Hyalella azteca. Archives of
Environmental Contamination and Toxicology 45(3):331-336.
NAL Call Number: TD172.A7

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Matt Moore, 12/9/03
T (662) 232-2955
mtmoore@ars.usda.gov

Acute Toxicity of Methyl-Parathion in Wetland Mesocosms: Assessing the Influence of Aquatic Plants Using Laboratory Testing with *Hyalella azteca*

R. Schulz,[1] M. T. Moore,[1] E. R. Bennett,[2] C. D. Milam,[3] J. L. Bouldin,[3] J. L. Farris,[3] S. Smith Jr.,[1] C. M. Cooper[1]

[1] USDA-Agricultural Research Service, National Sedimentation Laboratory, P.O Box 1157, Oxford, Mississippi 38655, USA

[2] GLIER, University of Windsor, Windsor, Ontario, Canada N9B 3P4

[3] Environmental Science Program, Arkansas State University, P.O. Box 847, State University, Arkansas 72467, USA

Abstract. Methyl-parathion (MeP) was introduced into constructed wetlands for the purpose of assessing the importance of distance from the source of contamination and the role of emergent vegetation on the acute toxicity to *Hyalella azteca* (Crustacea. Amphipoda). A vegetated (90% cover. mainly *Juncus effuses*) and a nonvegetated wetland (each with a water body of 50 × 5.5 × 0.2 m) were each exposed to a simulated MeP storm runoff event. *H. azteca* was exposed for 48 h in the laboratory to water samples taken from the wetlands at a distance of 5, 10, 20, and 40 m from the pesticide inlet 3 h, 24 h, 96 h, and 10 days following application. Methyl-parathion was detected throughout the nonvegetated wetland, whereas the pesticide was only transported halfway through the vegetated wetland. A repeated-measure three-way analysis of variance (ANOVA) using time, location, and vegetation indicated significantly lower toxicity in the vegetated wetland. Furthermore, the mortality decreased significantly with both increasing distance from the inlet and time (48-h $LC_{50} \pm$ 95% CI: 9.0 ± 0.3 μg/L). A significant three-way interaction of time × vegetation × location confirmed higher toxicity at the inlet area of the nonvegetated wetland immediately after contamination. Significant linear regressions of maximum mortality (independent of time) versus distance from the pesticide inlet indicated that 44 m of vegetated and 111 m of nonvegetated wetland would reduce *H. azteca mortality* to ≤5% These results suggest that vegetation contributes to reduced MeP effects in constructed wetlands.

The utility of aquatic plants for removal of insecticides from water has been shown (Hand *et al.* 2001, Karen *et al.* 1998; Wolverton and Harrison 1975) Processes important for removal of nonpoint-source pesticide runoff in vegetated wetlands may include adsorption, decomposition, and microbial

Correspondence to R. Schulz, Syngenta Crop Protection AG, Ecological Sciences, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK; *email* Ralf.Schulz@syngenta.com

metabolism (Rodgers *et al.* 1999). 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 (Hand *et al* 2001; Karen *et al.* 1998; Luckeydoo *et al.* 2002) Furthermore, they may contribute directly to chemical metabolism (Wetzel 1993). It has also been demonstrated that emergent vegetation reduces resuspension of sediments in wetlands (Dieter 1990).

The effects of the organophosphate phorate have been assessed using littoral mesocosms in South Dakota wetlands (Dieter *et al.* 1996). Recent studies have emphasized the ability of constructed wetlands to retain nonpoint-source insecticide pollution, preventing it from entering receiving aquatic habitats (Moore *et al.* 2002; Schulz and Peall 2001; Schulz *et al.* 2001). The implementation of retention ponds in agricultural watersheds was mentioned by Scott *et al.* (1999) as part of an integrated strategy to reduce the amount and toxicity of runoff-related insecticide pollution discharging into estuaries. However, there are few other studies in the published literature dealing with the fate or effects of agricultural insecticide input in constructed wetlands.

Runoff is the major source of insecticide input to aquatic systems in the intensively cultivated Mississippi delta region (Cooper and Lipe 1992). Constructed wetlands may serve as a suitable risk mitigation strategy for agricultural runoff, given that enough information is made available on their effectiveness with specific reference to the importance of the wetland vegetation.

Biological effects of pesticides in wetlands have been studied under experimental conditions using mesocosms (Detenbeck *et al.* 1996), in littoral enclosures (Dieter *et al.* 1996), or in the field employing organisms in situ (Schulz and Peall 2001; Schulz *et al.* 2001; Scott *et al.* 1999). Unfortunately, no information is available on the design of wetlands with respect to the resulting toxicity reduction. This implies a need to provide quantitative data on the effectiveness of wetlands in reducing insecticide toxicity in relation to characteristics such as the presence of emergent macrophyte vegetation. The following study was undertaken for this purpose.

Methyl-parathion (MeP), 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 (USDA 1997) and it has been detected at high levels in agricultural runoff (Wauchope 1978). Following application to vineyards in southwest Germany, MeP was detected at levels up to 213 μg/L in surface waters (Aufsess et al. 1989). MeP has an organic carbon–water partition coefficient (K_{oc}) of 5100 ml/g O.C. and a water solubility of 55 mg/L at 25°C (Hornsby et al. 1995). A laboratory half-life of 12 h has been reported for MeP in water (Ferrando et al. 1992). Based on a model approach, Lassiter et al. (1986) showed the importance of microorganisms for the fate of MeP in the aquatic environment. Acute toxicity was assessed using *Hyalella azteca* Saussure (Crustacea: Amphipoda), the most widespread species of amphipod in North America (Cooper 1965), as a test organism.

Materials and Methods

Description of the Wetland Mesocosms

Constructed wetlands at the University of Mississippi Field Station were specifically designed to evaluate the fate of pesticides in wetlands (Rodgers and Dunn 1992). Four of those constructed wetlands (in consecutive series) were used for this research Two wetlands (water body $50 \times 55 \times 02$ m) differing in vegetation coverage were chosen as experimental cells. The vegetated wetland had a macrophyte coverage of >90% (*Juncus effusus*, 171 ramets/m², and *Leersia* sp , 12 ramets/m²) and the nonvegetated wetland had a macrophyte coverage of <5%. The two remaining wetlands were used as water sources for the simulated storm event Above-surface platforms at distances of 5, 10, 20, and 40 m from the inlet of the wetlands were used in each wetland to ensure that sampling would not cause unnecessary damage to the macrophytes and/or sediments

Experimental Procedure and Pesticide Analysis

Each of the two wetlands 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 worst-case runoff scenario assumptions of an immediate (postapplication) 6.35-mm rainfall on 50-ha agricultural fields to which commercial-grade MeP (Clean Crop) at a rate of 8 6 kg active ingredient per 20 ha had been applied Based on the assumption of 1% pesticide runoff (Wauchope 1978), a total of 43 g active ingredient of MeP in a volume of 6500 L of water was added to each wetland. Additional inclusion of 2 5 kg sandy loam (84% sand, 16% silt) per wetland was designed to simulate the typical suspended solid load (400 mg/L) in the Mississippi Delta Ecoregion An amount of 50 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 application period. The soil and pesticide mixture was homogenized for a 24-h period prior to the experiment. Concentrations of MeP in the mixing chamber varied from 19 to 23 mg/L in water and from 34 to 58 mg/L in sediments

Water samples for pesticide analysis were taken at 3 h, 6 h, 24 h, 96 h, and 10 days postapplication at the eight sampling stations in the two wetlands 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°C) pending analysis Sample extraction and analysis are outlined by Moore et al. (2001). Sediment and subsurface plant samples were taken at 24 h postapplication and analyzed as documented by Bennett et al. (2000).

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 0.1 μg/L, 0 1 μg/kg dry weight, and 0 1 μg/kg dry weight, respectively. Based on fortified samples, mean extraction efficiencies were >90%.

The mean values ($n = 3$ per wetland) for pH, dissolved oxygen, temperature, and conductivity, measured between 9 and 10 A.M., shortly before contamination, were 6.7 ± 0.1, $2 3 \pm 1 0$ mg/L, $25.2 \pm 1 3$°C, and $116 \pm 6 5$ μS/cm, respectively, in the vegetated and $6.9 \pm 0 2$, $6 6 \pm 0 7$ mg/L, 27.8 ± 0.5°C, and $43 \pm 3 4$ μS/cm, respectively in the nonvegetated wetland.

Laboratory Toxicity Tests

Testing procedures followed general U S EPA guidelines (USEPA 1993) Water samples (1.5 L) were taken in amber glass bottles from the eight sampling sites at 3 h, 24 h, 96 h, and 10 days following treatment of the wetlands and were transported to the USDA. Following temperature equilibration using a water bath, the tests were performed at a standard temperature of 21 ± 2°C and under an 8-h dark 16-h light regime *H azteca* from an established culture at the USDA were used as the test organism Culturing procedures followed the methods of de March (1981) Ten individuals were kept in 200 ml of test water and six replicates were performed per sampling site and sampling time The number of surviving animals was counted after 48 h and data are expressed as the mean mortality of the six replicates.

Data Analysis

A 48-h LC_{50} ($\pm 95\%$ CI) was calculated based on the observed mortalities in relation to the measured MeP concentrations using the probit method (USEPA 1994). Effects of vegetation (vegetated versus nonvegetated), location (5, 10, 20, and 40 m from the inlet), and time after application of the simulated runoff (3 h, 24 h, 96 h, and 10 days following MeP introduction; both repeated-measure variables) on the toxicity to *H. azteca* were analyzed using a three-way analysis of variance (ANOVA) Unfortunately, pseudoreplication is unavoidable in the study type undertaken here; hence, it is difficult to assess or exclude the effect of unmeasured or unknown covariables (Hurlbert 1984) However, with regard to the size of the wetland mesocosms, the samples taken at one site are regarded as sufficiently independent to justify an analysis of variance 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 $\alpha = 0.012$. Linear regression analysis was used to fit curves to mortality of *H. azteca* (y) versus distance downstream of the pesticide inlet (x). The maximum mortality observed at each sampling distance (regardless of time) was used in the analysis.

Results

Methyl-Parathion Concentrations

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 from the inlet, while the respective values were 20 and <0.1 μg/L in the vegetated wetland (Table 1) At 5 and 10 m from the inlet, the contamination was generally higher but did not differ greatly between the nonvegetated (550 and 190 μg/L,

Table 1. Methyl-parathion concentrations in water samples (μg/L) taken at different distances from the inlet between 3 h and 10 days postapplication[a]

Distance from inlet	Time after application				
	3 h	6 h	24 h	96 h	10 days
Nonvegetated					
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

[a] Each value represents the mean of analysis of two separate samples. nd—not detectable, i e., 0.1 μg/L

respectively) and the vegetated wetland (420 and 180 μg/L, respectively). Apart from the 20- and 40-m station in the nonvegetated wetlands, all MeP levels were at least five times higher between 3 and 24 h postapplication than at 96 h and at 10 days.

On the basis of the pesticide added and the water volume of the wetland, a theoretical concentration of 700 μg/L would be obtained in the treated wetlands, assuming that all the MeP remained in the water and no degradation occurred. As expected, the measured concentrations were generally lower than this target value. Sediment and plant samples taken at 24 h postapplication contained MeP concentrations up to 2000 μg/kg dry weight and 10,000 μg/kg dry weight, respectively. For all sampling locations and times within the vegetated wetland, mean MeP concentrations were 170 $\pm$ 50 μg/L, 140 $\pm$ 40 μg/kg, and 2020 $\pm$ 890 μg/kg for water, sediment, and plants, respectively, therefore indicating that 87% of measured MeP was associated with vegetation.

Methyl-Parathion Toxicity

The 48-h LC_{50} ($\pm$95% CI) calculated based on our toxicity data for *H. azteca* in relation to the measured MeP concentrations is 9.0 $\pm$ 0.3 μg/L. Three-way ANOVA using time, location (both repeated-measure variables), and vegetation indicated a significant ($p < 0\ 001$) reduction in toxicity of water from the vegetated wetland (Fig. 1, Table 2). Furthermore, the observed toxicity decreased significantly ($p < 0.001$) with both increasing distance from the inlet and time. However, survival rates were not significantly (ANOVA-Bonferroni with a critical p value $= 0\ 0042$) different in water from the 5- and 10-m stations and they were also not significantly different between the 3- and 24-h sampling times. Survival increased generally over time and survival was higher in water from the 20- and 40-m station

Interactions were significant ($p < 0.001$) for time $\times$ vegetation and for location $\times$ vegetation highlighting greater toxicity in the nonvegetated wetland with respect to both spatial and temporal patterns (Fig. 1, Table 2) In addition, the time $\times$

location interaction was significant ($p < 0.001$) due to the fact that survival increased with time at the 5- and 10-m stations, while at the 20- and 40-m stations, the survival decreased from 3 to 24 and 96 h before it increased again at 10 days A significant ($p = 0.005$) three-way interaction of time $\times$ vegetation $\times$ location confirmed greater toxicity at the inlet area of the nonvegetated wetland This was specifically pronounced for the 96-h sampling period after simulated runoff application and with reduced survival at the 40-m station in the 24- and 96-h samples (Fig 1, Table 2).

Significant ($p < 0.001$) linear regressions of maximum mortality (independent of time) versus distance from the pesticide inlet were obtained for both the nonvegetated and the vegetated wetland (Fig. 2). It is evident that differences in the mortality are most prominent at the 20-m and even more at the 40-m station. Based on the regression model it is possible to calculate wetland lengths that should contribute to reduced MeP toxicity. In the case of a target mortality $\leq$5%, the necessary wetland length is 44 m for the vegetated and 111 m for the nonvegetated wetland. However, the geometry of the wetlands should be taken into account if the data are applied to another situation.

Discussion

Methyl-Parathion Concentrations

The pesticide analysis revealed differences in the transport of MeP between the two wetlands, presumably due to the presence or absence of vegetation. This is of specific interest as the MeP contamination applied represented a worst-case runoff exposure scenario. It is likely that the dense vegetation coverage in the vegetated wetland, with more than 180 ramets/m², leads to a reduced hydraulic conductivity (Madsen *et al* 2001) and thus reduces the pesticide transport. Similar processes are well known for nutrients (Uusi-Kämppä *et al*. 2000) and suspended particles (Sand-Jensen 1998) but have rarely been 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 (Hand *et al*. 2001; Karen *et al*. 1998; Moore *et al*. 2002). The importance of sorption and partitioning of MeP in river biofilms has been demonstrated under experimental conditions by Headley *et al*. (1998). Our results concerning MeP concentrations associated with the plants and sediments 24 h postapplication confirm the importance of sorption in reducing pesticide levels in the water.

Unfortunately there are no field data available for MeP half-life. A half-life of 12 h has been reported in water at initial levels of 200 μg/L using 20-L glass aquaria in the laboratory (Ferrando *et al*. 1992). Based on this half-life, an initial concentration of 550 μg/L at the 5-m station in the nonvegetated wetland would be reduced to values below the detection limit (<0.1 μg/L) after about 7 days. However, levels between 0.6 and 4 μg/L measured after 10 days suggest that the degradation of MeP was slower under the high-temperature and low-oxygen conditions in the wetlands. A rather rapid degradation rate would be assumed from the high temperatures measured in the wetlands, but it is questionable to what extent laboratory and field degradation may be directly comparable.
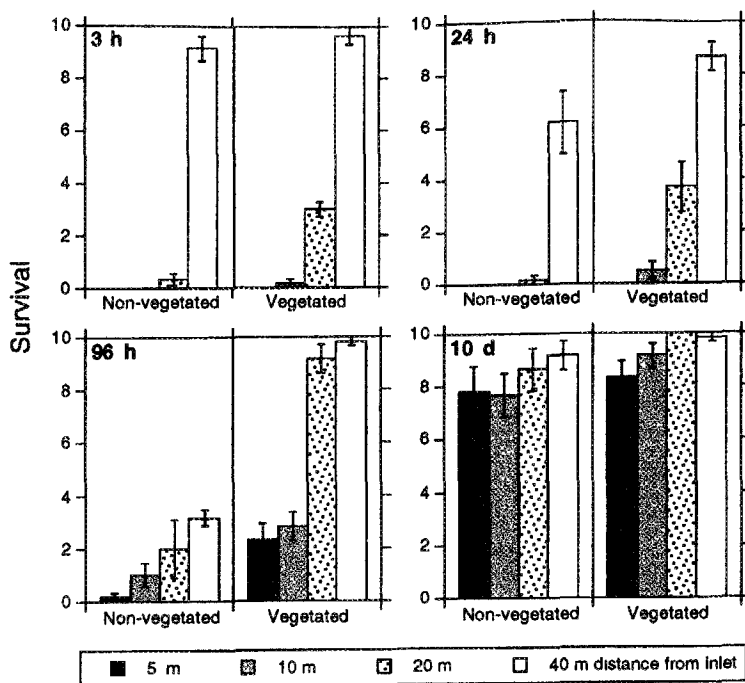
Fig. 1. Mean (± SE, $n = 6$) survival of *Hyalella azteca* exposed for 48 h to water samples taken in wetland mesocosms at different times after introduction of methyl-parathion and at different distances from the pesticide inlet station. Initial number of individuals was 10 per replicate

Table 2. Three-way analysis of variance of the effect of vegetation (vegetated versus nonvegetated), location (5, 10, 20, and 40 m from the inlet; repeated-measure variable), and contamination (3 h, 24 h, 96 h, and 10 days after methyl-parathion introduction, repeated-measure variable) on the survival [$\ln(x + 1)$ transformed] of *Hyalella azteca* (denominator degrees of freedom = n − residual df − 1)

Source	df	Mean square	Likelihood ratio	Wilks' λ	p
Vegetation	1	11.95	137.09	137.1	<0 001
Time	3	23.86	179.47	538.4	<0.001
Location	3	21 03	318 12	954.4	<0 001
Time × vegetation	3	1 89	14 28	42.9	<0 001
Location × vegetation	3	1.51	22 77	68.3	<0 001
Time × location	9	2 66	33.34	300.0	<0 001
Time × location × vegetation	9	0.23	2.87	25.8	0.005

Methyl-Parathion Toxicity

Acute toxicity in *H. azteca* clearly increased with increasing MeP concentrations, leading to an estimated 48-h LC_{50} of 9.0 ± 0.3 μg/L based on measured pesticide concentrations. There are no published acute toxicity data for MeP and *H. azteca*. However, Borthwick (1988) documented a 24-h mortality of 80% for *H. azteca* exposed in situ in an 0.8-ha prairie wetland during aerial application of 0.56 g/ha MeP. Generally, the measured MeP concentrations and respective high mortalities observed in the present study are in agreement with levels known to be acutely toxic to other aquatic invertebrates. The 24-h EC_{50} for the cladoceran *Ceriodaphnia dubia* is 5.5 μg/L (Norberg-King *et al.* 1991), the 96-h LC_{50} for the damselfly *Ischnura verticalis* is 33 μg/L (Johnson and Finley 1980), and sublethal effects on cholinesterase activities were documented at 1–3 μg/L in the amphipod *Gammarus pulex* (Streit and Kuhn 1994).

Most importantly, the survival data of *H. azteca* clearly demonstrated that the impact of MeP in the vegetated wetland was lower than in the nonvegetated wetland. This result is in accordance with the observed differences in transport of MeP

through the wetland and the resulting differences in exposure levels. The levels of MeP detected in the nonvegetated wetland were in a range of concentrations that would induce mortality in *H. azteca* for more than 4 days. In contrast, levels in the vegetated wetland were reduced more quickly and the nonaffected areas of this wetland may serve as a refuge for invertebrate species. Such a link of vegetation coverage in wetlands, pesticide transport, and toxicity has not yet been described in the published literature. However, input–output studies of constructed wetlands and retention ponds in agricultural watersheds recently demonstrated reductions of insecticide-associated toxicity (Moore *et al.* 2002; Schulz and Peall 2001, Schulz *et al.* 2001). 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 (Hand *et al.* 2001; Karen *et al.* 1998). It should be noted that mortality was used as an endpoint in this study and it is thus possible that sublethal effects would likely occur even at lower toxicant levels.

It is unlikely that differences in other water quality parameters would have contributed to the more intense effects observed in the nonvegetated wetland, since the oxygen concen-
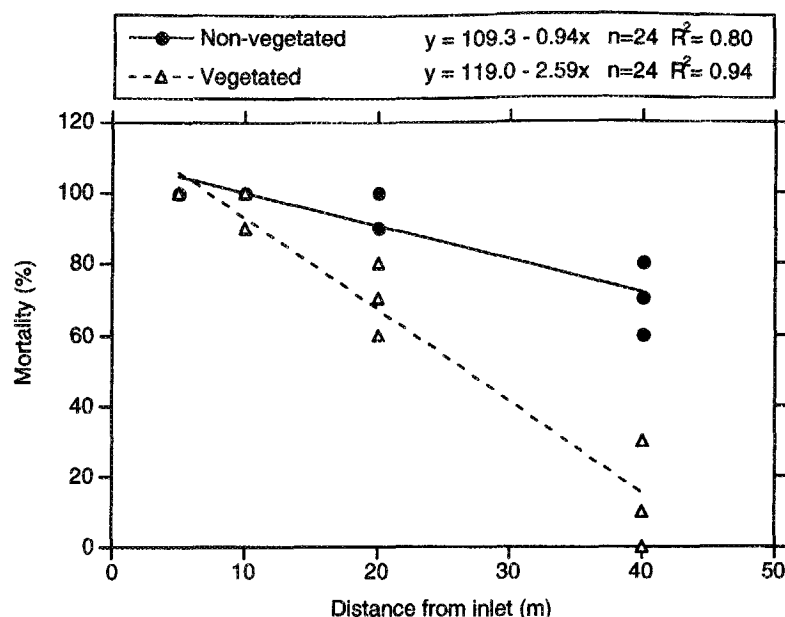
Fig. 2. Linear regression relationships ($p < 0.001$) between maximum mortality of *Hyalella azteca* (independent of time) and distance downstream from the pesticide inlet

trations were even lower in the vegetated wetland, the temperature was equilibrated in all samples, and the hardness was low in both wetlands. *H. azteca* has been found at high densities in a small wetland in Alabama with temperatures up to 28°C and dissolved oxygen concentrations as low as 2 mg/L (Pickard and Benke 1996). The spatial and temporal differences in wetland water samples and their effect upon the survival of *H. azteca* 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. The observed significant three-way interaction of time × vegetation × location confirmed the stronger effect in the inlet area of the nonvegetated wetland than in the inlet area of the vegetated wetland, which again highlights the positive effect of vegetation coverage on survival. Similar positive effects of aquatic vegetation have been shown for other organophophate insecticides with similar water solubilities, such as azinphos-methyl (Schulz and Peall 2001), and, moreover, for organophosphate (Moore *et al.* 2002) or pyrethroid insecticides with considerably lower water solubilities (Moore *et al.* 2001).

Vegetated buffer zones in surface waters have been suggested as a contribution to mitigation of agricultural nonpoint source pollution by various workers (Rodgers *et al.* 1999; Uusi-Kämppä *et al.* 2000). However, specific guidelines for wetland construction are quite rare with respect to the mitigation of pesticide levels (Moore *et al.* 2000, 2002) and they are not at all available with respect to the resulting toxic effects. As a result of this study, we reported a vegetated wetland length of 44 m to be sufficient for a MeP-toxicity reduction ≤5%, while a 111-m nonvegetated wetland would be necessary to obtain the same effectivity. However, when buffer zones of 44 m are needed, some habitats, such as ephemeral floodplain regions, may still be vulnerable On the other hand, the present study dealt with a worst-case simulated runoff scenario and lower buffer width might be protective to mitigate average runoff scenarios. A 134-m vegetated wetland in South Africa effec-

tively reduced runoff- and spraydrift-related organophosphate toxicity in in situ exposed midges, *Chironomus* sp. (Schulz and Peall 2001; Schulz *et al.* 2001). Scott *et al.* (1999) reported a reduction of insecticide- toxicity below an extensive retention pond system as part of an integrated risk reduction approach in an estuarine environment.

Conclusions

In conclusion, this study suggests that vegetated wetlands have a strong potential to contribute to aquatic pesticide risk mitigation. A 40-m stretch of dense vegetation cover effectively reduced a MeP concentration in simulated runoff of about 700 µg/L to below the detection limit (< 0.1 µg/L). Furthermore, the mortality to *H. azteca* detected in samples taken at a 40-m distance from the pesticide inlet in the vegetated wetland was always below 15%, in comparison to up to 68% in the non-vegetated wetland. These results confirm the importance of vegetated buffer zones as filters preceeding field drainage to streams or ditches or as vegetation coverage within 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. However, further studies are needed on the potential toxic effects of contaminated detritus, sediments and vegetation in these systems.

tance with the field operations The paper benefited from reviews by B. Helling.

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