Aquatic Toxicology 118–119 (2012) 54–61

Contents lists available at SciVerse ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Effects of a triazole fungicide and a pyrethroid insecticide on the decomposition of leaves in the presence or absence of macroinvertebrate shredders

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ARTICLE INFO

Article history:
Received 21 February 2012
Received in revised form 23 March 2012
Accepted 27 March 2012

Keywords:
Leaf litter decomposition
Pesticides
Macroinvertebrates
Microorganisms
Mixture
Pulse exposure

ABSTRACT

Previously, laboratory experiments have revealed that freely diluted azole fungicides potentiate the direct toxic effect of pyrethroid insecticides on *Daphnia magna*. More ecologically relevant exposure scenarios where pesticides are adsorbed have not been addressed. In this study we exposed beech leaves (*Fagus sylvatica*) to the azole fungicide propiconazole (50 or 500 μg L^{-1}), the pyrethroid insecticide alpha-cypermethrin (0.1 or 1 μg L^{-1}) or any combination of the two for 3 h. Exposed leaves were transferred to aquaria with or without an assemblage of macroinvertebrate shredders, and we studied treatment effects on rates of microbial leaf decomposition, microbial biomass (using C:N ratio as a surrogate measure) and macroinvertebrate shredding activity during 26 days post-exposure. Microbial leaf decomposition rates were significantly reduced in the propiconazole treatments, and the reduction in microbial activity was significantly correlated with loss of microbial biomass (increased C:N ratio). Macroinvertebrate shredding activity was significantly reduced in the alpha-cypermethrin treatments. In addition, the macroinvertebrate assemblage responded to the propiconazole treatments by increasing their consumption of leaf litter with lower microbial biomass, probably to compensate for the reduced nutritional quality of this leaf litter. We found no interaction between the two pesticides on macroinvertebrate shredding activity, using Independent Action as a reference model. In terms of microbial leaf decomposition rates, however, alpha-cypermethrin acted as an antagonist on propiconazole. Based on these results we emphasise the importance of considering indirect effects of pesticides in the risk assessment of surface water ecosystems.

1. Introduction

The conversion of coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM) is a fundamental stream ecosystem process that is primarily mediated by microorganisms and shredding macroinvertebrates. Microorganisms, especially hyphomycetes, are important for the conversion of leaf litter into more palatable food resources for macroinvertebrate shredders and collectors/gatherers as they degrade indigestible polysaccharides and increase the nutritional value of leaves (Bärlocher and Kendrick, 1975b; Gessner et al., 2007). Shredding macroinvertebrates convert CPOM into minor leaf fragments and faecal pellets (Graca, 2001), and several species of macroinvertebrates have been shown to consistently select or reject leaf fragments dependent on the species of aquatic fungi that colonised the leaf material (Arsuffi and Suberkropp, 1989). However, total microbial biomass on leaves is recognised as an overall robust indicator for preferred macroinvertebrate shredding activity (Graca, 2001; Gulis et al., 2006).

Periodic contamination with agricultural pesticides potentially impairs the structure and/or the function of stream biota, and agricultural streams are recognised as some of the most impacted on earth (MEA, 2005). Indeed, the co-occurrence of numerous pesticides in agricultural streams has been reported frequently in scientific studies and monitoring programs (Bøgestrand, 2007; Martin et al., 2003; Rasmussen et al., 2011; Schäfer et al., 2011b).

Research reviews of the interaction between chemical toxicants in mixtures show that the vast majority of effects can be predicted with the model of concentration addition (Belden et al., 2007; Cedergreen et al., 2008; Deneer, 2000). However, in approximately 5% of the studies of binary mixtures the effect was significantly potentiated, and the majority of these studies involved azole fungicides (Cedergreen et al., 2006, 2008; Nørgaard and Cedergreen, 2010). Azole fungicides (including triazoles and imidazoles) are C14α-demethylase inhibitors that obstruct the

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http://dx.doi.org/10.1016/j.aquatox.2012.03.015

biosynthesis of ergosterol (a significant part of fungi cell walls) by inhibiting the activity of an enzyme belonging to the group of P450 monooxygenases (Copping and Hewitt, 1998). Their synergising potential is hypothesised to be due to inhibition of P450 monooxygenases responsible for the oxygenation and thereby degradation of other xenobiotics in target organisms (Walker, 2009). Azole fungicides are often applied to agricultural fields in tank mixtures with pyrethroid insecticides, and recent studies have confirmed that some azole fungicides synergise the effect of pyrethroid insecticides both under laboratory and field conditions (Bjergager et al., 2011; Nørgaard and Cedergreen, 2010). Field screenings of stream water have disclosed concentrations up to 175 $\mu g\,L^{-1}$ and 6.2 $\mu g\,L^{-1}$ of azole fungicides and pyrethroid insecticides, respectively (Elsaesser and Schulz, 2008; Liess et al., 1999). However, due to their physicochemical properties pyrethroids and some azole fungicides probably primarily occur in surface waters as sorption-complexes with organic particles (Ding et al., 2010; Ensminger et al., 2011).

Short term effects of pyrethroid insecticides on benthic macroinvertebrates have been reported to include reduced feeding rate of some shredders and grazers (Lauridsen et al., 2006; Rasmussen et al., 2008) and altered locomotor behaviour (Nørum et al., 2010), whereas long term effects include reduced growth, fecundity and emergence success (Liess and Schulz, 1996; Schulz and Liess, 2001a,b). Recently, research on effects of fungicides on non-target microorganisms has received increasing attention, and changes in structure or function of microbial communities have been documented in laboratory studies and in the field (Dijksterhuis et al., 2011; Rasmussen et al., 2012; Schäfer et al., 2011a). Moreover, Bundschuh et al. (2011) showed that *Gammarus fossarum* preferred unexposed leaves over those that were previously exposed to an azole fungicide, and this selective behaviour was ascribed to pesticide-induced changes in the microbial community structure associated with the leaf fragments. In the field, however, these effects may be counteracted by functional redundancy and high abundances of shredders such as Gammaridae (Piscart et al., 2009; Rasmussen et al., 2012). Moreover, in agricultural streams shredding macroinvertebrates may not have the option to select between exposed and unexposed sources of food.

In the present study we applied a classic cross-factorial design to study the decomposition rates of leaf litter that was pulse-exposed to the triazole fungicide propiconazole and/or the pyrethroid insecticide alpha-cypermethrin. The decomposition rates were studied in the presence or absence of an assemblage of macroinvertebrate shredders. In addition, we used the C:N ratio of leaves as a proxy for microbial biomass and nutritional quality for macroinvertebrate shredders (as C:N ratio decreases with increasing biomass of fungi). More specifically we tested the hypotheses that (1) propiconazole exposure reduces the biomass of leaf-associated fungi (increasing the C:N ratio) which is additionally reflected by reduced rates of microbial leaf decomposition, (2) alpha-cypermethrin exposure decreases the rate of macroinvertebrate shredding due to either direct toxic effects or a repelling effect, (3) the toxicity of alpha-cypermethrin to macroinvertebrates is potentiated by the presence of propiconazole, and (4) the average macroinvertebrate shredding activity will increase with decreased nutritional quality of leaves (higher C:N ratio), as the shredding macroinvertebrates must compensate for low nutritional quality of ingested food by increasing the feeding rate in order to maintain basic body functions. This picture may, however, be obscured by the direct toxic effect of alpha-cypermethrin on macroinvertebrates in the insecticide and combination treatments. The joint effects of alpha-cypermethrin and propiconazole were predicted using the model of independent action (IA) (Bliss, 1939). This model was chosen as the two pesticides are expected to have different modes of action on the two measured endpoints, microbial and shredder mediated leaf decomposition, with propiconazole having a direct fungicidal effect on the fungal community colonising the leaves, while alpha-cypermethrin will most likely only exhibit a narcotic toxicity towards microorganisms which lack the nervous system of higher organisms. The shredders are, however, likely to be directly affected by alpha-cypermethrin through its effect on the nervous system (Copping and Hewitt, 1998), whereas propiconazole should not have a direct toxic effect apart from the mentioned potential effects on the P450 mediated metabolism of the macroinvertebrates (Walker, 2009).

Table 1
Chemical parameters for stream water used in the laboratory experiment.

Chemical parameters	Concentration ($mg\,L^{-1}$)
NH_4-N	0.006
(NO_2 + NO_3)-N	2.57
Total N	2.79
PO_4-P	0.002
Total P	0.003
Total Fe	0.004
pH	7.0

2. Materials and methods

2.1. Leaf packs

Beech leaves (*Fagus sylvatica*) were collected in April, 2009 from Søndervinge Brook – an unpolluted first order stream in a catchment that is dominated by old beech forest. Thus, the collected leaves were supposed to be uncontaminated by pesticides prior to the experiment and were further assumed to be colonised with microorganisms. The leaves were stored in lightly aerated stream water at 10 °C for three weeks prior to the experiment to allow for the microorganism community to adapt to laboratory conditions using a diurnal light/darkness cycle of 14 h/10 h, respectively. Water chemistry of the stream water used at all stages of the experiment is presented in Table 1.

Leaves used in the experiment were carefully selected with highest possible similarity in terms of texture and colour. Based on 50 randomly selected leaves the relation between fresh weight (FW) and dry weight (DW) for the leaves was established prior to the experiment. The leaves were blotted and the petiole was removed from all leaves before establishing the relationship between FW and DW. The DW was obtained by drying the leaves to constant mass at 60 °C. The leaves were subsequently weighed using a Mettler Toledo XP-204 (10 μg accuracy).

Leaf packs for the experiment were produced the day before exposure by stacking 4–6 leaf discs (2 cm diameter, petioles removed if present) with a total weight of 0.30 ± 0.01 g FW. The introduced leaf packs function as substrate for microbial organisms and as food source for macroinvertebrate shredders. The leaf discs were threaded in a bundle using a polyester string.

2.2. Macroinvertebrates

The macroinvertebrates that were used in this study included two species: the amphipod *Gammarus pulex* (L.) and the caddisfly *Halesus radiatus* (Curtis). They were collected in early May 2009, in uncontaminated streams (Hagenstrup Millbrook, eastern Jutland, for *G. pulex* and Silke Stream, South Funen, for *H. radiatus*). All macroinvertebrates were stored in lightly aerated stream water at 10 °C with sufficient leaf material to avoid biased rates of leaf decomposition in the early phase of the experiment due to starved individuals. To harmonise the size of animals for the study, *G. pulex* smaller than 10 mm in length were discarded, whereas only instars IV and V of *H. radiatus* were selected. However, in order to relate