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Toxicity of three biocides to springtails and earthworms in a soil multi-species (SMS) test system

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ABSTRACT

Effects of three biocides, esfenvalerate, picoxystrobin and triclosan, were studied in a Soil-Multi-Species (SMS) test system with four springtail- and one earthworm species exposed for eight weeks. All biocides affected springtail population growth negatively, and species- and chemical specific differences had clear impacts on their dominance structure. We suggest that differences in vulnerability may be influenced by activity related to species-specific vertical distribution in the soil. The responses of the springtail *Folsomia fimetaria* differed strongly from previous results from a single-species experiment with the same biocides. Survival of juvenile earthworms was more sensitive to biocide exposure than weight gain, and earthworm performance was significantly correlated with springtail abundance. Feeding activity of soil organisms measured with the bait lamina test system was a sensitive criterion for esfenvalerate and picoxystrobin exposure, but not for triclosan. The results suggest that interspecific interactions such as resource- and interference competition may influence the toxicity of biocides. SMS test systems may hence be a suitable method for investigating direct and indirect effects in combination under standardized laboratory conditions.

1. Introduction

The use of agricultural pest control agents and soil amendments has increased agricultural output worldwide, but it has also increased the risk of affecting non-target organisms (Aktar et al., 2009). The potential of a biocide as an environmental stressor is commonly assessed in single-species tests, i.e. selected species are individually exposed to the respective chemical in the laboratory. Such tests are useful in studying direct effects of chemicals, both singly and in mixture, on the studied organism, and are important for understanding processes determining direct effects. However, they do not include indirect effects due to species interactions (Chapman, 2002), nor do they take differences in species sensitivity into account (unless many tests are performed), which limit their usefulness for extrapolating the risks chemicals pose to natural species assemblages or communities.

In order to meet these challenges, alternative studies on the effects of toxicants under "semi-field conditions" have been performed, i.e. testing field-collected, intact soil cores containing an indigenous pool of soil-dwelling species in the laboratory (Gyldenkaerne et al., 2000; Forster et al., 2004; Knacker et al., 2004). Employing intact field-collected cores inevitably introduces large variations among sampled "replicates", due to the heterogeneity of natural systems (Scott-Fordsmand et al., 2008; Jensen and Scott-Fordsmand, 2012), which reduces the ability to identify between treatment differences. A step between single-species and semi-field studies are Soil-Multi-Species (SMS) test systems (e.g. (Scott-Fordsmand et al., 2008; Jensen and Scott-Fordsmand, 2012)). These are experimentally constructed, simplified and standardized food webs, consisting of homogenous soil with a defined number of selected soil-dwelling invertebrate species, and a defined number of organisms per species. These systems can represent a specific ecosystem, in a simplified way, but lack the full complexity of "the whole soil community". Instead they facilitate the cumulative measurement of direct and indirect toxicant effects on multiple soil invertebrates under standardized conditions. They may be used to compare interspecific variations in sensitivities, taking into account ecological interactions such as

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competition and predation. In combination with single-species experiments, SMS test systems may also serve to detangle chemical and ecological stress impacts. A benefit of the method is that the faunal composition can be adjusted according to the aims of the study, and the experimental setup enables precise repetitions of experiments.

The present study investigated the responses of a simplified soil food web to exposure to esfenvalerate, picoxystrobin and triclosan in an SMS test system. The studied system comprised four springtail and one earthworm species. The springtails are potential competitors for space and/or food resources. Since both, springtails and earthworms are secondary decomposers, they may compete for food, but more likely the earthworm may influence soil arthropods through interference. This may be positive interference by creating hiding space and access to deeper soil layers (Wickenbrock and Heisler, 1997; Salmon et al., 2005), or negative interference, e.g. by disturbing the soil habitat physically. The SMS system was hence driven by resource- and interference competition.

The pyrethroid insecticide esfenvalerate and the strobilurin fungicide picoxystrobin are used in crop production (Unger, 1996; Bartlett et al., 2002), and may hence pose a risk to non-target soil organisms such as springtails and earthworms. The synthetic bactericide triclosan is used in many personal care products, and is therefore commonly found in wastewater, from where it is removed by activated sludge in wastewater treatment plants and hence transferred to the soil (Reiss et al., 2002; Amorim et al., 2010) where it may pose a health risk to soil organisms. All three biocides have been documented to be toxic to soil invertebrates, especially earthworms (ctgb, 2005; Reiss et al., 2009; Amorim et al., 2010; Lin et al., 2010; Wang et al., 2012; EFSA, 2013; Schnug et al., 2013).

The aim of the present study was to test the influence of three biocides with different modes of action on the composition of a selected soil community, and to compare species sensitivities between treatments. We hypothesized that the three biocides would influence the multispecies system in different ways, as they primarily target different organisms. The insecticide acts as a sodium channel antagonist disrupting the nervous system of arthropods (Tomlin, 1994), and was therefore expected to affect springtails directly. Direct effects may also occur in earthworms given that they share a similar nervous system with arthropods. Picoxystrobin may act directly on the respiratory system of soil invertebrates by inhibiting the complex III cytochrome bc1 at the Qo site (FRAC, 2013). Triclosan has been shown to affect genotoxicity (Lin et al., 2010) and reproduction (Schnug et al., 2013) of *Eisenia fetida* and may therefore act directly on this species. In addition, picoxystrobin as well as triclosan are likely to have distinct different indirect effects, e.g. by reducing different food resources such as soil fungi and bacteria (Stenrød et al., 2013).

The standardized SMS test system was supplemented with an additional measure of feeding activity of the soil invertebrates (van Gestel et al., 2003), using the bait lamina test system (Kratz, 1998). Finally, the results of the SMS test system were compared with previous single-species studies on the effects of the same biocides on the springtail *Folsomia fimetaria* (Schnug et al., 2014) and the earthworm *E. fetida* (Schnug et al., 2013) in order to investigate differences in sensitivities of these species in the presence and absence of potentially interacting species.

2. Materials and methods

2.1. Experimental design

A selected set of soil invertebrates was exposed to spiked soil (1 kg dry weight, dw) in test containers (polyethylene tubes, $h = 33$ cm, $ø = 9.3$ cm) for eight weeks at 15 ± 1 °C in a 12/12 h light–dark regime. The number of springtail individuals, and survival and weight of earthworms were assessed at test termination.

2.2. Test species

The five soil-dwelling species used in the experiment included both macro- and mesofauna, and consisted of two components: (1) the epigeic and hermaphroditic compost worm *E. fetida* (Annelida: Lumbricidae, Savigny 1826), and (2) two hemi- and two euedaphic springtail (Arthropoda: Collembola) species. The hemiedaphic springtails comprise the mycophagous *Proisotoma minuta* (Tullberg 1871) and the food generalist *Heteromurus nitidus* (Templeton 1835). Also the euedaphic springtail species differ in food preference, with *Folsomia fimetaria* (Linné 1758) being omnivorous (primarily mycophagous) and *Protaphorura fimata* (Gisin 1952) both herbivorous (roots) and mycophagous. *Folsomia fimetaria* reproduces sexually, while the other springtail species are mainly parthenogeneic.

Initially, 10 mature females of the mite *Hypoaspis aculeifer* (Gamasida: Laelapidae, Canestrini 1884) were included per test container as a predator to springtails. Yet, only very few individuals were counted at test termination, both in the controls and in the biocide exposed containers. The average number of individuals per test container was 1.0 ± 1.2 (esfenvalerate), 0.8 ± 0.8 (picoxystrobin), 1.3 ± 1.4 (triclosan), and 1.3 ± 1.1 (control). No concentration-dependent differences were observed. It is most likely that condition of the mites was reduced already before test start, as it was noted that they moved rather slowly when they were introduced to the test containers. Moreover, it may be assumed that remaining mites were weakened with low forage success. Consequently, owing to the very low survival of mites independent of concentration, and the patchy distribution over treatments, any possible effect of their presence in the experiment is regarded to be very small and only in the form of treatment-independent noise. This may to some extent have weakened the power of the analysis, but will not affect conclusions drawn from analysis. Hence, mites will not be regarded in the analyses of the results. The successful component of the studied test system consequently comprised only species of similar trophic levels. Indirect effects on measured responses were hence derived from resource- or interference competition.

The earthworms were purchased in compost from Shop74, Støvring, Denmark (http://www.Shop74.dk), and transferred to soil (same soil as used in the experiments) one week prior to test start. The springtails were taken from cultures routinely maintained at Aarhus University, Institute of Bioscience, Silkeborg, Denmark, where they were kept at 20 ± 1 °C in a 12/12 h light–dark regime, in cultures with substrate consisting of plaster of Paris-charcoal (8:1) and fed on dried baker's yeast.

2.3. Test soil

The topsoil (loam) of an agricultural field located at Voll, Ås, Akershus in south-east Norway (N 59° 65′ E 10° 75′) was used for the experiments. The organic carbon (OC) content of the soil is 1.5 g 100 g^{-1} soil (dw), the pH (H_2O) 5.8 and the water holding capacity 29.7% of dw (Schnug et al., 2014). Prior to test start the air-dried soil was sieved (<2 mm) and dried at 80 °C over night in order to exclude intrinsic soil animals.

2.4. Chemicals

The commercial formulations of esfenvalerate (CAS 66230-04-4) and picoxystrobin (CAS 117428-22-5), Sumi Alpha® (DuPont®, 50 g esfenvalerate L^{-1} Sumi Alpha) and Acanto® 250 SC (DuPont®,