Environmental Pollution 213 (2016) 84–89

Contents lists available at ScienceDirect

Environmental Pollution

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

Sublethal effect of agronomical surfactants on the spider *Pardosa agrestis*☆

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ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form
29 January 2016
Accepted 1 February 2016
Available online xxx

Keywords:
Spider
Pardosa
Surfactant
Agrochemicals
Foraging behavior

ABSTRACT

In addition to their active ingredients, pesticides contain also additives — surfactants. Use of surfactants has been increasing over the past decade, but their effects on non-target organisms, especially natural enemies of pests, have been studied only very rarely. The effect of three common agrochemical surfactants on the foraging behavior of the wolf spider *Pardosa agrestis* was studied in the laboratory. Differences in short-term, long-term, and overall cumulative predatory activities were investigated. We found that surfactant treatment significantly affected short-term predatory activity but had no effect on long-term predatory activity. The surfactants also significantly influenced the cumulative number of killed prey. We also found the sex-specific increase in cumulative kills after surfactants treatment. This is the first study showing that pesticide additives have a sublethal effect that can weaken the predatory activity of a potential biological control agent. More studies on the effects of surfactants are needed to understand how they affect beneficial organisms in agroecosystems.

1. Introduction

Surfactants are a common component of pesticides (Krogh et al., 2003). They are also conventionally mixed with liquid pesticides, which modifies the medium's properties at the surface or interface and increases the diffusion rates of agrochemical compounds through plant cuticles (American Society for Testing and Materials, 1999; Holloway and Stock, 1989; Leaper and Holloway, 2000). They also reduce the surface tension of insect exoskeletons (Goodwin and McBrydie, 2000).

Despite the fact that surfactants are widely used in agroecosystems and their global consumption is growing at a rate of 5.2% annually (MarketsAndMarkets, 2015), the effects of pure surfactants are studied far more rarely than are those of pesticides. The limited studies indicate that some surfactants can have similar or even higher lethal effects on pests than the pesticides themselves, and so the surfactants alone can act as pesticides (Cowles et al., 2000; Druart et al., 2010; Imai et al., 1994; Sims and Appel, 2007). However, surfactants can be also toxic to such beneficial fauna in agroecosystems as pollinators. Goodwin and McBrydie (2000) found that 4 of the 11 surfactants they tested were highly toxic to bees. Moreover, surfactants have been also found to have strong negative effects on water ecosystems (Dorn et al., 1993; Gillespie et al., 1996; Mann and Bidwell, 2001; Mottier et al., 2014). They can reduce the total clutch size and larval survival of the fathead minnow (*Pimephales promelas*) (Dorn et al., 1996) and are toxic to *Daphnia magna* (Wong et al., 1997). Surfactants can also inhibit the oxidative functions of the submitochondrial particles and intact mitochondria of rat liver (Oakes and Pollak, 1999). In addition to their potential toxicity to many non-target organisms, surfactants have very low degradation rates in the environment (Cirelli et al., 2008; Lara-Martin et al., 2008).

There has been only very few studies dealing with the effect of surfactants on natural enemies of pests. Evans et al. (2009) found that a combination of herbicide and surfactant resulted in increased mortality for the spider *Steatoda capensis*, Ray and Hoy (2014) found a lethal effect of surfactant Silwet L-77 in combination with horticulture oil on two predatory mites *Typhlodromus occidentals* (Nesbitt, 1951) and *Hemicheyletia wellsina* (De Leon, 1967)

☆ This paper has been recommended for acceptance by Sarah Michele Harmon.

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http://dx.doi.org/10.1016/j.envpol.2016.02.005
0269-7491/

and Cocco and Hoy (2008) found toxic effect of the surfactant Kinetic and Silwet L-77 in combination with imidacloprid on the parasitoid *Tamarixinia radiata* (Waterston, 1922). The almost complete lack of studies of surfactants' effects on natural enemies is rather surprising given that it is estimated that biological pest control saves from 4.5 to 17 billion dollars in the United States alone (Losey and Vaughan, 2006; Pimentel et al., 1997; Wyckhuys et al., 2013). Spiders are among the most abundant predators in many agroecosystems and have been found to reduce pest populations significantly (e.g., Birkhofer et al., 2008; Marc and Canard, 1997; Pekár et al., 2015). However, spiders are highly susceptible to non-specific agrochemicals and to an even greater extent than are some insect pests (Pekár, 2012). Spiders therefore represent ideal model organisms for testing the effects of surfactants on beneficial arthropods.

In addition to lethal effects, agrochemicals can have various sublethal effects (Benamú et al., 2010; Desneux et al., 2007; Evans et al., 2010; Pekár, 2012). Sublethal effects can be connected to such changes in behavior as mobility, orientation, feeding, oviposition, and learning performance (Desneux et al., 2007). In the case of biological controls, sublethal effects are sometimes comparable to lethal effects because biocontrol agents completely cease attacking prey (e.g., Michalko and Košulič, 2015).

In this study, we examined the sublethal effects of three commonly used nonionic surfactants: Wetcit®, Šaman®, and Trend 90® on prey capture efficiency and its changes over time in the agrobiont lycosid spider *Pardosa agrestis* (Westring 1861), because prey capture efficiency is related to important pest regulation services in agroecosystems (Carter and Rypstra, 1995).

2. Materials and methods

2.1. Studied species

We studied the effects of surfactants on the wolf spider *P. agrestis*. This is widely distributed Palearctic spider species (Nentwig et al., 2016). Wolf spiders (Lycosidae) are ground-dwelling spiders common within agroecosystems in central and north-western Europe (Clough et al., 2005; Öberg et al., 2007). These spiders could be exposed to different agrochemicals including surfactants, because surfactants are a common part of pesticides that are widely used for plant protection in agroecosystems (Krogh et al., 2003). The genus *Pardosa* is one of the most abundant ground-dwelling predatory arthropod groups in temperate-zone agroecosystems (Lang, 2003), and it is very likely that this genus comes into contact with herbicides containing surfactants. *Pardosa* species are widely used as model organisms for studying the effects of herbicides (Evans et al., 2010; Michalková and Pekár, 2009; Wehling et al., 1998; Wrinn et al., 2012).

2.2. Spider collection and maintenance

Subadult specimens of *P. agrestis* of both sexes were collected in plots untreated by agrochemicals (Arboretum of Mendel University) in Brno, Czech Republic. Spiders ($n = 80$) were collected by hand to the plastic tubes on 28 February 2015. Spiders were kept singly in plastic tubes (15 mm in diameter and 120 mm long) with a periodically moistened half of a facial wipe at the bottom. Spiders were acclimated to the laboratory for 2 weeks. Laboratory conditions were 22 ± 1 °C with a natural photoperiod.

2.3. Laboratory testing

Spiders were held in the laboratory to acclimate for three weeks after capture. They were fed *ad libitum* with laboratory-reared fruit flies (*Drosophyla hydei* Sturtevant 1921) to standardize their hunger levels for two weeks. Seven days prior to the experiments, spiders were left to starve. Starvation time can be from 4 days (Michalková and Pekár, 2009) to three weeks (Řezáč et al., 2010). Experiments were performed in laboratory conditions. First, we sorted individuals according to their sex and assigned to the experimental groups randomly. Consequently, each experimental group of spiders consisted of 20 (9 males, 11 females) randomly selected individuals (total $N = 80$, 36 males, 44 females). Each individual was used only once. Each experimental group was exposed to solutions of one of three different nonionic surfactants that are commonly used in agroecosystems as herbicide additives: Wetcit®, Šaman®, and Trend 90® (Table 1). The control group was exposed to distilled water, which is used as a solvent for surfactant dilution. The surfactants were diluted with distilled water. The concentrations of the surfactant solutions were: 0.2% for Šaman®, 0.1% for Trend 90®, and 0.15% for Wetcit®. Surfactants and water were applied through direct spraying. The amount of surfactant solution sprayed on the Petri dish with a spider was recalculated from field rates to the Petri dish area. A pharmaceutical pump sprayer with a precisely measured aerosol dose of 0.05 ml was used to spray the bottom of Petri dishes (50 mm in diameter) covered with filter paper and a spider. The filter paper was removed from the Petri dishes after spraying to prevent residual effects, because we studied the effects of surfactants of spiders getting exposed directly to spraying. The prey was untreated to prevent the effect of contaminated prey.

We studied short- and long-term predatory activity after treatment because surfactants persist in the environment for a relatively long time (some surfactants from 30 days to eight months) and they are found in sludge, in freshwaters, in seas and oceans (Cirelli et al., 2008; Lara-Martin et al., 2008; Scott and Jones, 2000). Fruit flies (*D. hydei*) were provided as prey. All spiders were offered three flies. In the short-term horizon, killed flies were replaced by live flies (in each Petri dish, there were always constant density of prey (three flies)) and counted each hour during the 4-h period immediately following exposure to the surfactants or water. The experiments on the long-term predatory activity followed over the next 4 days with the same set of spiders. In the long-term horizon, spiders were provided with three fruit flies every 24 h over 4 days and the number of consumed or killed fruit flies was recorded each 24 h.

Spiders that did not accept prey and molted within 24 h (altogether 9 spiders) were excluded from further analyses in order to avoid the effect of feeding cessation (Foelix, 1996). Sex was also recorded to study sex-specific effects.

2.4. Data assessment

All analyses were performed within the R environment (R Development Core Team, 2013). We investigated the differences in short-term, long-term, and overall cumulative predatory activities. To assess short- and long-term differences in predatory activity among treatments, the number of killed flies was compared using negative binomial generalized linear models with log link (GLM-nb) as the data were counts and were overdispersed (Pekár and Brabec, 2009). Treatment and sex and their interaction acted as explanatory variables. To compare the cumulative number of killed prey among treatments, we used a linear mixed effects model (LME) as the data were autocorrelated. Treatment, count number, and sex as well as all their two- and three-fold interactions acted as fixed effects. Time and the individual acted as random effects. We used the “varIdent” variance function as the data were heteroscedastic (Pekár and Brabec, 2012).