Applied Soil Ecology 81 (2014) 22–29

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Interactive effects of pyrimethanil, soil moisture and temperature on *Folsomia candida* and *Sinella curviseta* (Collembola)

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ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form 25 April 2014
Accepted 27 April 2014
Available online 14 May 2014

Keywords:
Pesticide
Fungicide
Springtail
Environmental factor
Full factorial design
Climate change

ABSTRACT

The aim of the presented investigation was to study effects resulting from specific aspects of climate change and chemical stress (individually or in interaction) on soil organisms. In detail, the interaction of different temperatures (20 °C and 26 °C) and soil moisture levels (30%, 50% and 70% of the water holding capacity) were examined in combination with the fungicide pyrimethanil on the reproduction of two Collembola species (*Folsomia candida* and *Sinella curviseta*). Testing was based on the standard collembolan reproduction test (OECD-Guideline 232), following an EC_X design. Low soil moisture led to a significant reduction of the juveniles in the control groups in contrast to medium or high soil moisture. Furthermore, the results showed a significant influence of both climatic factors on the toxicity of the fungicide. In general, both species reacted more sensitive when exposure was conducted in dry soil or at enhanced temperature.

1. Introduction

With reference to the fourth assessment report of the Intergovernmental Panel on Climate Change (IPCC) we are faced with increasing global temperatures within the next decades. Depending on human population development and exploitation of fossil resources, IPCC modeled different scenarios. The worst case scenario, A1FI, forecasts a temperature increase of 6.4 °C by the year 2100 (Meehl et al., 2007). In connection with global climate change, IPCC also predict a shift of precipitation. A significant decline of summer precipitation (up to 30–45%) and an increase in winter precipitation (up to 15–30%) in central Europe is anticipated (Alcamo et al., 2007). Changes in precipitation may be linked to changes in soil moisture. However, a detailed prediction could not be given since such measurements are typically not integrated in soil monitoring programs due to technical reasons (e.g. its rapid changes within short distances or over time, depending on soil characteristics). A proliferation of intense rain events may cause higher soil moisture contents while a decrease of mean precipitation may lead to drought stress for soil organisms.

Soil organisms stressed by changes in soil moisture or temperature may respond differently to chemicals. This is particularly significant for plant protection products, since these chemicals are intentionally released to ecosystems targeting pest organisms, e.g. insects, pest plants or fungi. To exclude a potential risk for non-target organisms, e.g. earthworms, bees or collembolans, their environmental risk has to be assessed prior to market registration and laboratory tests, conducted in accordance to guidelines (e.g. OECD, ISO, etc.), are already requested. However, further potential stressors as they may occur due to climate change are not considered in the environmental risk assessment of pesticides to date.

As part of the research center BiK-F (Biodiversity and Climate Research Center, Frankfurt, Germany), one project aims to assess the effects of pesticides in combination with different climatic scenarios in several experiments. The main objective is to research one subset issue of global climate change on soil ecosystems by evaluating the combined effects of a pesticide and different soil moisture contents and temperatures on soil organisms, e.g. collembolans.

Collembolans (springtails) are one of the most prevalent and copious microarthropods of soil biocoenoses (Choi et al., 2006; Hopkin, 1997). Temperature and soil water content are ecological key factors that influence collembolan populations and their distribution (Choi et al., 2006). They live mainly on bacteria, dead organic material, fungal hyphae and fine roots. Hence,

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http://dx.doi.org/10.1016/j.apsoil.2014.04.010
0929-1393/

collembolans participate in essential soil functions, especially decomposition of organic material (Hopkin, 1997).

Since Collembola represent an important class of soil fauna, a couple of guidelines for chemical testing have been introduced using them. Firstly, *Folsomia candida* WILLEM, 1902 (Isotomidae) (ISO, 1999, 2011; OECD, 2009) is proposed as a "standard" test species based on its easy handling in the laboratory, cultivability, data availability and ecological relevance. The handling of this candidate is simple due to its' parthenogenetically reproduction strategy and hence, sex ratio is not relevant in a reproduction test. Many tests with different chemicals have already been performed with *F. candida*, for instance metals (e.g. Nakamori et al., 2008), (veterinary) pharmaceuticals (e.g. Noël et al., 2006) and pesticides (e.g. Campiche et al., 2007; Chernova et al., 1995).

As an alternative to *F. candida*, the species *Sinella curviseta* BROOK, 1882(Entomobryidae) is proposed in OECD (2009) among further species. It is sexually reproducing and occurs in comparable habitats as *F. candida* (e.g. in the Zhejiang province (China); Xu et al., 2008). *S. curviseta* prefers higher temperatures in contrast to *F. candida* (Gist et al., 1974; Jänsch et al., 2005; Fountain and Hopkin, 2005). If temperature rises due to global climate change, it could be assumed that thermophilic collembolans (as e.g. *S. curviseta*) may prevail over collembolans that prefer cooler temperatures (as e.g. *F. candida*). Additional factors, both climatic and chemical, may affect this coexistence.

In order to examine a potential interaction of these factors, a multifactorial reproduction experiment with collembolan was performed. The fungicide pyrimethanil was selected as a model substance. Even though effects through pyrimethanil were investigated on arthropods, ecotoxicological data on Collembola are not available. However, data regarding for instance, degradation and fate in soil, mode of action, etc. are published (European Commission, 2005), which may be helpful to discuss the outcome of these experiments. Furthermore, with a half-life of 56 days, a near-constant exposure can be assumed during the 28 days lasting reproduction test. Finally, pyrimethanil is a broad spectrum fungicide with global use in agriculture.

To summarize, the main aim of this investigation was to answer, whether and how environmental factors impact the toxicity of the fungicide against the two selected collembolan species.

2. Material and methods

2.1. Test organisms

For the reproduction tests two species were selected as test organisms: *Folsomia candida* (the "standard test species") and *Sinella curviseta* (the "alternative collembolan species"). The parthenogenetic *F. candida* prefers an optimum temperature of 15–21 °C. Its clones are distributed worldwide in soils rich in humus and litter (Jänsch et al., 2005; Fountain and Hopkin, 2005). The second test species *S. curviseta*, is an obligate sexual reproducing organism (Waldorf, 1971). It colonizes similar habitats as *F. candida* and is found in Southeast Asia, North and Central America as well as parts of Europe (www.collembola.org). Its temperature optimum is 30 °C (Gist et al., 1974). The two species were cultured separately in plastic containers (ca. 200 mL) onto a 1 cm layer of plaster of Paris. The cultures were kept in the dark and fed twice a week with dry baker yeast and a few drops of deionized water. In order to acclimatize the collembolans to the two test temperatures, two cultures of each species were established at 20 ± 2 and 26 ± 2 °C, respectively.

2.2. Model substance pyrimethanil

The model substance pyrimethanil (CAS: 53112-28-0; IUPAC: N-(4,6-dimethylpyrimidin-2-yl) aniline) is a broad spectrum fungicide that belongs to the group of anilino-pyrimidines (mode-of-action: methionine biosynthesis inhibition). It restrains the secretion of hydrolytic enzymes of the fungi (FAO/WHO, 2007). Under aerobic laboratory conditions (20 °C) half life in soil was determined between 27 and 82 days with a mean of 56 days (European Commission, 2005). The water solubility (121 $mg/L_{25\,°C,\ pH\ 6.1}$) is moderate as well as log K_{OW} ($2.84_{25\,°C,\ pH\ 6.1}$) and hence, accumulation neither in biota nor in soil is expected (European Commission, 2005). In this study, pyrimethanil was applied as formulation "SCALA", distributed by BASF SE, Germany. This suspension concentrate contains 404.6 g/L active substance according to the certificate of analysis. Besides pyrimethanil, no further hazardous substances are ingredients of "SCALA" (BASF, 2005). All concentrations declared in this study refer to the active substance [a.s.] pyrimethanil and dry weight [dw] of soil. In Germany, the maximum single application rate for "Scala" is 2.5 L/ha in strawberry cultures (Federal Office of Consumer Protection and Food safety, 2009), i.e. 1011.5 g pyrimethanil per hectare. To allow a comparison of the effects from laboratory tests, this application rate has to be transformed into mg a.s./kg dw soil. To simplify the calculation, a soil density of 1.5 g/cm^3 and an average soil depth of 5 cm is assumed in EU regulatory procedures (European Economic Community, 2007). Thus, the maximum application rate complies with 1.35 mg a.s./kg dw soil. The reproduction tests were conducted with one negative control in untreated soil and 11 concentrations of pyrimethanil, starting from 0.98 up to 1000 mg a.s./kg dw soil (nominal values) with a spacing factor of two. The concentration range was chosen based on prior range finding tests (data not shown).

For chemical analysis, one extra vessel containing the highest concentration (1000 mg a.s./kg dw soil) was arranged for each experiment. At day 0 and day 28, two sub samples of this vessel were stored deep frozen for chemical analysis via HPLC/DAD. The chemical analyses were conducted at TZW (Technologie Zentrum Wasser) in Karlsruhe, Germany. The limit of quantification was assessed to be 0.5 mg a.s./kg dw soil.

2.3. Experimental testing

All tests were conducted as described in OECD (2009) with a 16/8 h light/dark regime. Ten female *F. candida* with an age between 9 and 12 days were used in the experiments. Due to the findings of Bandow et al. (2014) 20 individuals of *S. curviseta* were used with an age of 20–23 days. As it is not possible to recognize the sexes of the living individual of *S. curviseta* morphologically, 20 individuals were inserted into each test vessel, supposing an even distribution of both sexes due to randomization (i.e. achieving a comparable amount of females per test vessel).

The experiments with the two different species were performed independently at two different temperatures, 20 ± 2 and 26 ± 2 °C, resulting in four reproduction tests all in all.

The OECD soil contained 5% peat, 20% kaolin, 74–74.9% quartz sand and 0.1–1% calcium carbonate to regulate the pH value to 6.5 ± 0.5. The maximum water holding capacity (WHC) was determined according to Annex 5 of OECD (2009). In the tests with *S. curviseta* as well as in the test with *F. candida* conducted at 20 °C, the WHC was 49.2%. In the test with *F. candida* (26 °C), the WHC was 45.6%. To attain the desired soil moisture content (in % w/w of WHC), the soil was pre-moistened with the respective volume of deionized water less than the application volume of 10 mL, which was blended into the soil at the beginning of the test. In order to identify interactive effects between chemical and climatic factors, the soil was finally moistened to 30%, 50% and 70% of the water holding capacity, respectively. These three moisture levels were combined with the above mentioned 11 concentrations of pyrimethanil plus the negative control. Two replicates were used