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ScienceDirect
Ecotoxicology and Environmental Safety 68 (2007) 263–271
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Effects of dinoseb on energy reserves in the soil arthropod *Folsomia candida*

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Received 11 January 2006; received in revised form 16 October 2006; accepted 13 November 2006
Available online 26 January 2007

Abstract

This study evaluated the usefulness of energy reserves as an early warning system in *Folsomia candida* when exposed to the pesticide dinoseb. After different exposure times, survival, reproduction, growth (weight and length), lipid and protein content of the organisms were determined. After six days of exposure at 15–30 μg of dinoseb/g dry soil, the weight, lipid, and protein content of the exposed organisms were higher than the controls. This stimulation seems to indicate that Collembola adopt a strategy of increasing their growth in order to improve their reproduction. This hypothesis was confirmed by the number of eggs laid which was greater in exposed organisms. After 21 days, all measured parameters decreased. The results show that after having produced an effort to increase growth and reproduction, lethality increases. The selected energy reserves are not more sensitive than the classically measured parameters such as reproduction, but can be more predictive for a pollutant stress encountered by the organisms.

Keywords: Energy reserves; Early warning; Life-cycle parameters; Growth; Dinitrophenol; *Folsomia candida*; Collembola; Dinoseb

1. Introduction

There is an increasing need for appropriate methods to determine soil quality and the effects of xenobiotics on soil organisms (Tarradellas and Bitton, 1997). The first and most standard methods for risk assessment of chemicals on soil organisms are the use of laboratory toxicity tests. These tests are used to estimate critical threshold concentrations from concentration-response relationships for single life cycle variables such as survival, growth and reproduction.

The development of more sensitive and predictive test methods to characterize the risk associated with exposure to xenobiotics is an area of intense research. One of these approaches is based on the use of biomarkers. By combining this approach with toxicity tests, as done in the present study, it is possible to determine if biomarkers are more sensitive or predictive in detecting the effect of pollutants. The results will represent a step towards a better extrapolation from the cell to the population and from acute to chronic toxicity.

Energy reserve variations are often considered as sensitive indicators for environmental pollution (Donker, 1992). When an organism is exposed to a chemical stress, it can resist in many ways: avoiding or escaping, neutralizing, excreting or repairing damage (Calow, 1991). All these responses are metabolically costly in terms of energy and may reduce the energy left to invest in storage, growth, reproduction and survival. The energy allocation strategy of an organism depends on its physiology. The usefulness of energy reserve macromolecules (lipids and proteins) as biomarkers was studied in various invertebrates (Donker et al., 1993; Van Brummelen and Stuijfzand, 1993; Vink et al., 1995; Khalil et al., 1995; Knigge and Köhler, 2000; De Coen and Janssen, 2003), but was generally not followed over time. Only a few studies provide data on biomarkers in Collembola (Staempfli et al., 2002; Hensbergen et al., 2000; Köhler et al., 1999). Recently the use of fatty acid composition as a biomarker for Collembola was investigated in the context of trophic interactions (Ruess et al., 2005; Chamberlain et al., 2005). To our knowledge,

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0147-6513/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2006.11.007

this approach has not yet been applied to characterize an effect of chemical compounds on Collembola.

In this paper, the effect of dinoseb on the energy reserves (lipid and protein content) of the Collembola *Folsomia candida* was investigated to test the hypothesis that dinoseb exposure reduces energy reserves before an effect can be detected on life cycle parameters (growth, reproduction and survival).

Folsomia candida were chosen as test organisms because, as members of the soil mesofauna, they influence chemical and physical properties of the soil (Bachelier, 1978) and are therefore ecologically important. Their small size and short reproductive cycle make them ideal for conducting laboratory experiments, and their biology and ecology is quite well known (Hopkin, 1997; Van Straalen and Van Gestel, 1993). *Folsomia candida* is a parthenogenic species easily bred in the laboratory. Toxicity of different pollutants has been evaluated on life cycle parameters such as survival, reproduction and growth (Campiche et al., 2006; Fountain and Hopkin, 2005, 2001; Petersen et al., 1997; Crommentuijn et al., 1993; Sandifer and Hopkin, 1997), but little attention has been paid to other physiological responses.

Dinoseb, the pesticide used in this study, is a herbicide and insecticide belonging to the dinitrophenol family. As it was shown to have adverse effects on fertility and teratogenic effects in mammals, it has been banned in most countries of the world (UNEP, 2006). In Switzerland, it was used until 2002 for the defoliation of potatoes (Station fédérale de recherches en arboriculture, viticulture et horticulture, 2002).

To understand if energy reserves are more sensitive and predictive tools than the life cycle parameters usually measured, *Folsomia candida* was exposed to dinoseb in artificial soil, while survival, reproduction, weight, lipid and protein content were determined at different time intervals in order to determine the relative sensitivity of those parameters. The observed effects at different levels of biological organization were analyzed and compared over time (temporal analysis) as well as after certain time intervals (fixed time analysis).

2. Materials and methods

2.1. Test animals

Folsomia candida were mass reared on a moistened substrate of plaster of Paris and activated charcoal (120 and 15 g, respectively) contained in small plastic boxes ($180 * 135 * 60$ mm with transparent lids closing tightly). Laboratory conditions were constant (20 ± 2°C, 600 lux with a light:dark cycle of 16:8 h, humidity between 70% and 80%). Every 3–4 days the containers were aerated by opening the lids and some drops of bi-distilled water and food (granulated dry yeast, Dr. Oetker, Germany) were added.

To obtain a synchronized culture, adults were transferred to fresh substrate to stimulate their egg production. After 9 days, eggs were transferred with a moist paintbrush to a fresh substrate. Two to three days later, unhatched eggs were removed from the containers. The juveniles were used for the experiments at the age of 10–12 days.

2.2. Preparation of substrate and pesticide application

Artificial soil (OECD, 1984) composed of 70% quartz sand (50% 40–100 mesh, Fluka 84 878 and 50% ⩾230 mesh, Fluka 83 340), 20% of kaolinite clay (Fluka N° 60 609) and 10% of sphagnum peat, was used for testing. The peat (Weißmoortorf, Shagnum extra, ESG, Rastede, Germany) was air dried, ground and sieved to 1 mm. Sufficient $CaCO_3$ was added to reach a pH–KCl of 6 ± 0.5 (ISO, 1994). A quantity of bi-distilled water corresponding to 50% of the water holding capacity determined according to ISO 11267 (ISO, 1999) was added to the various soil constituents which were mixed thoroughly (24 mL of water for 30 g dry soil).

Because the water solubility of dinoseb is low (0.05 mg/mL), a stock solution of 0.5 mg/mL of dinoseb (99.7%, Promochem, code IPO 147) was prepared in acetone and kept at 4 °C.

Animals were exposed to dinoseb via soil. For the preparation of the contaminated artificial soil, the required quantity of the stock solution was added to the quartz sand. The acetone (20 mL for 21.5 g of quartz) was evaporated in a rotary evaporator and the container was then placed under a fume hood for at least 30 min to allow all acetone residues to evaporate. Finally, the contaminated quartz sand was mixed thoroughly with the other soil constituents.

2.3. Determination of dinoseb effect concentrations

In order to determine dinoseb effect concentrations on survival and reproduction of *Folsomia candida*, a reproduction test was conducted according to the ISO 11267 standard (ISO, 1999). For this test, ten 10–12 day old juveniles were introduced into glass containers containing 30 g wet weight of artificial soil. Three replicates were used for each tested concentration (5, 10 and 20 μg/g dry soil) and five replicates for the controls (with and without acetone). Five mg of yeast were added at day 0 and 10 mg at day 14. The containers were aerated once per week and soil moisture content was monitored by weight (no loss was observed). After 28 days, adults and juveniles were recovered by water flotation and counted on a photo taken by a digital camera. As this test does not allow continuous monitoring, the other experiments were conducted on compressed soil (see below).

2.4. Reproduction and survival of adults

The tests were conducted in mini Petri dishes (25 mm diameter × 25 mm height) half-filled with 2 g wet weight of contaminated artificial soil. The soil was compressed in order to prevent Collembola burying into the soil.

Ten to twelve day old Collembola were exposed via substrate to sub-lethal dinoseb concentrations of 5, 10, 15 and 20 μg/g dry soil during 28 days. Thirty Collembola were randomly introduced per box in order to avoid a size difference between concentrations. Two types of control were used: one with and one without acetone. Each concentration was replicated five times and maintained in the same conditions as the breeding culture. Each week, the Petri dishes were aerated, one drop of bi-distilled water was added with a Pasteur pipette and three small sticks (~0.25 mg) of yeast were added. After each oviposition, the adults were counted and transferred to a new contaminated substrate (all contaminations were done on day 0 and the containers were kept in the same experimental conditions until use). After each transfer, the number of eggs on the soil surface was counted under a binocular microscope. After hatching the number of offspring was counted by adding water to each Petri dish. The water–soil slurry was carefully stirred and transferred to a beaker (50 mL). After few minutes the Collembola floating on the water surface could be counted by eye. To increase the visibility, some drops of bromophenol blue were added.

2.5. Energy reserves

The aim of the experiment was to follow over time the impact of dinoseb on the evolution of the weight, the protein content and the lipid content of *Folsomia candida*.