Applied Soil Ecology 115 (2017) 27–30

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Short communication

Ecotoxicological assessment of propiconazole using soil bacterial and fungal growth assays

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ARTICLE INFO

Article history:
Received 29 November 2016
Received in revised form 21 February 2017
Accepted 7 March 2017
Available online xxx

Keywords:
Biocides
Ecotoxicology
Fungicides
Microbial activity
Soil microorganisms

ABSTRACT

Effects of the fungicide propiconazole on soil microorganisms were tested using [^{3}H] leucine incorporation and [^{14}C] acetate in ergosterol incorporation to measure bacterial and fungal growth inhibition, respectively. Growth was compared to basal respiration (BR) and substrate-induced respiration (SIR) in soil microcosms established according to the OECD 217 guideline. Fungal growth was most sensitive with IC_{50} values remaining around 300 mg kg^{-1} during 40 days of incubation. SIR was initially less sensitive (IC_{50} 1300 mg kg^{-1}), but IC_{50} values progressively decreased over time to reach 380 mg kg^{-1} after 40 days. Bacterial growth was affected at concentrations $\geq$200 mg kg^{-1}, but exhibited more complex dose-response relationships possibly due to a combination of direct toxicity, bacterial community adaptation, and competitive release from the more severely affected fungi. BR was either stimulated or not affected by propiconazole. Our results indicate that group-specific endpoints targeting microbial growth will improve ecotoxicological assessment of toxicants for environmental risk assessment.

1. Introduction

Microbial community-based approaches have potential for ecotoxicological assessment of toxicants, but only a few standardized methods are included in current guidelines for environmental risk assessment (ERA) (Brandt et al., 2015). Recommended standard methods specifically targeting soil microorganisms currently include OECD 216 and 217, which use potential nitrification and potential soil respiration, respectively (Brandt et al., 2015). Collectively, these tests quite broadly cover soil microorganisms including archaea (nitrifiers), bacteria (nitrifiers and organotrophs) and eukaryotic microorganisms (organotrophic fungi). However, these standard tests may be rather insensitive as respiration may be less affected by toxicants than microbial growth (Brandt et al., 2009). Furthermore, many toxicants such as specific antibiotics and fungicides will primarily affect one of these broad taxonomic groups causing a risk that toxic effects will be overlooked due to functional redundancy in C mineralization (Rousk et al., 2009). Thus, there is a need for more sensitive, group-specific endpoints targeting microbial growth for improved ecotoxicological assessment of toxicants in soil. Bacterial growth rate, estimated using the leucine or thymidine incorporation methods, and fungal growth rate, estimated using the acetate in ergosterol incorporation method, offer such endpoints (Rousk and Bååth, 2011), but surprisingly direct inhibition of *in situ* fungal growth has not been determined in studies of organic fungicides.

Propiconazole is a systemic triazole fungicide efficient against a wide range of fungal pathogens (Schwinn, 1983; Komárek et al., 2010). Propiconazole is also used as a biocide for preservation of wood and other materials (US EPA, 2006) and can be found in urban storm- and wastewater (Bollmann et al., 2014a,b). The aim of this work was to compare toxic effects of propiconazole on bacterial and fungal growth with effects on substrate-induced respiration (SIR) based on the OECD 217 guideline routinely used for ERA. Propiconazole acts as an ergosterol biosynthesis inhibitor (Schwinn, 1983) and we therefore hypothesized that fungal growth

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http://dx.doi.org/10.1016/j.apsoil.2017.03.009
0929-1393/

would confer a more sensitive endpoint than the ecotoxicological endpoints currently in use for ERA. We also hypothesized that propiconazole levels inhibiting fungal growth would stimulate bacterial growth due to competitive release.

2. Material and methods

An agricultural soil (sandy loam, 1.6% organic C, pH_{water} 6.4) used for cultivation of barley was collected from the University of Copenhagen experimental farms in Taastrup (Field 26) during November 2013. The field soil was fertilized exclusively with inorganic fertilizers (NPKS) and had not been amended with propiconazole during the 7 years preceding soil sampling, but the related triazole fungicide tebuconazole was applied at recommended rates during the previous 6 years as a single summer application. Sampling was performed according to the OECD 217 guideline (OECD, 2000). In brief, soil (0–20 cm depth) was sampled by multiple coring (10 randomly positioned cores) and stored at 4 °C until analysis (3 months). After sieving (2 mm mesh) the soil was first pre-incubated (10 days, 22 °C) and then spiked with different concentrations of propiconazole (CAS 60207-90-1; $n = 3$) using quartz sand as a carrier (0.05 g g^{-1} soil). The sand was spiked with propiconazole dissolved in acetone, or acetone without propiconazole as a control, added to soil after the acetone had evaporated, and was mixed into the soil using a spatula. The final propiconazole concentrations in the soil were 0, 12.5, 25, 50, 100, 200, 400 and 800 mg kg^{-1}. These concentrations were selected in order to obtain dose-response curves to estimate toxicity using different methods. The soil microcosms (20 g) were performed in triplicate in 50 mL glass bottles (resulting in a total of 24 microcosms) and incubated at 22 °C in the dark. Fungal and bacterial growth, SIR and basal respiration (BR) assays (see

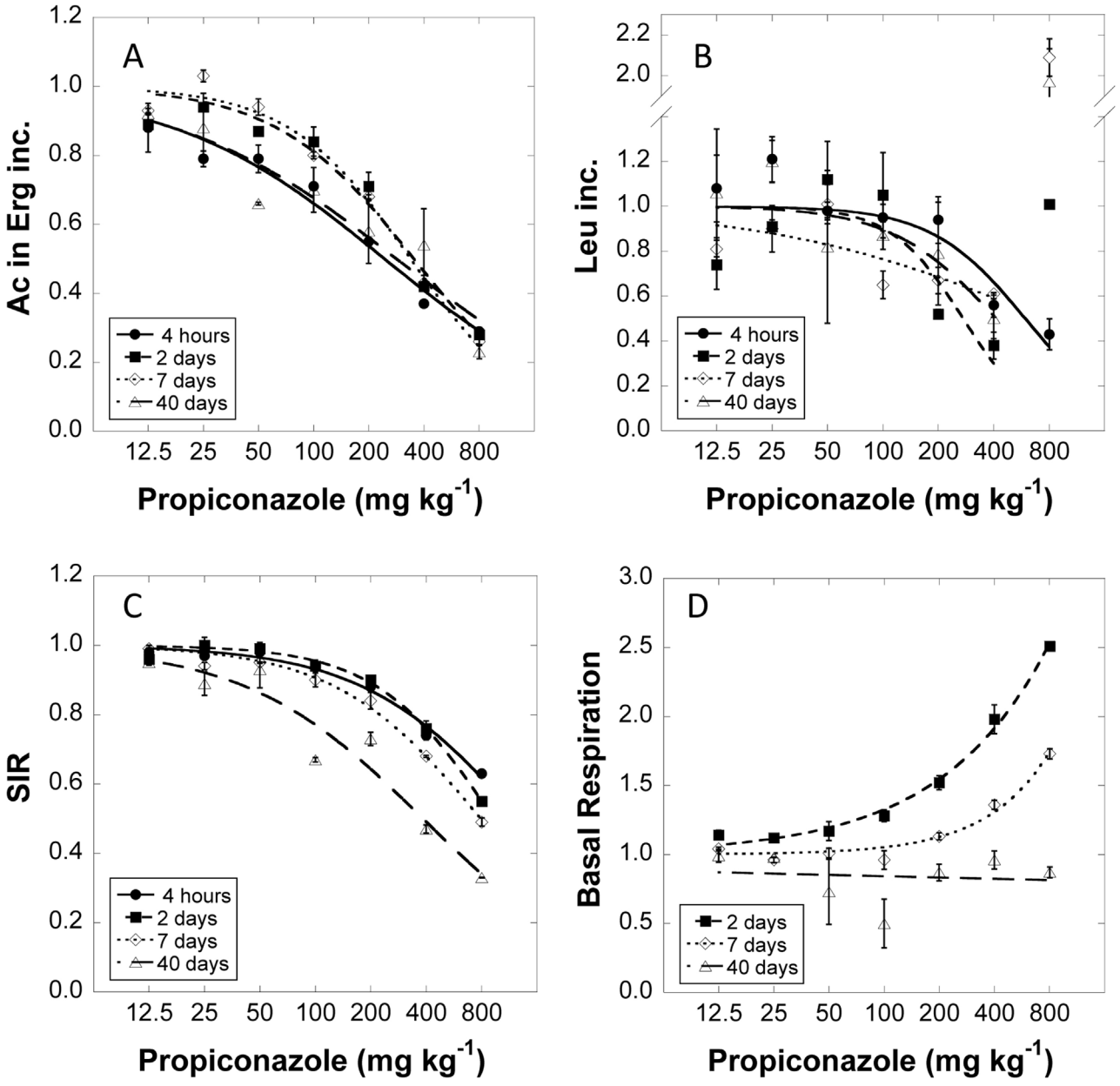

Fig. 1. Propiconazole dose-response curves observed for four microbial activity endpoints measured at different time points after propiconazole addition to soil. Fungal growth measured as acetate in ergosterol incorporation (A); Bacterial growth measured as leucine incorporation (B); Substrate-induced respiration (SIR) (C); Basal soil respiration (D). Microbial activities are normalized relative to the corresponding control with no propiconazole. Means ± standard errors (n = 3) are shown. Lines represent logistic model curve fits.