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Biochemical and microbial soil functioning after application of the insecticide imidacloprid

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ABSTRACT

Imidacloprid is one of the most commonly used insecticides in agricultural practice, and its application poses a potential risk for soil microorganisms. The objective of this study was to assess whether changes in the structure of the soil microbial community after imidacloprid application at the field rate (FR, 1 mg/kg soil) and 10 times the FR (10× FR, 10 mg/kg soil) may also have an impact on biochemical and microbial soil functioning. The obtained data showed a negative effect by imidacloprid applied at the FR dosage for substrate-induced respiration (SIR), the number of total bacteria, dehydrogenase (DHA), both phosphatases (PHOS-H and PHOS-OH), and urease (URE) at the beginning of the experiment. In 10× FR treated soil, decreased activity of SIR, DHA, PHOS-OH and PHOS-H was observed over the experimental period. Nitrifying and N_2-fixing bacteria were the most sensitive to imidacloprid. The concentration of NO_3^- decreased in both imidacloprid-treated soils, whereas the concentration of NH_4^+ in soil with 10× FR was higher than in the control. Analysis of the bacterial growth strategy revealed that imidacloprid affected the r- or K-type bacterial classes as indicated also by the decreased eco-physiological (EP) index. Imidacloprid affected the physiological state of culturable bacteria and caused a reduction in the rate of colony formation as well as a prolonged time for growth. Principal component analysis showed that imidacloprid application significantly shifted the measured parameters, and the application of imidacloprid may pose a potential risk to the biochemical and microbial activity of soils.

Introduction

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-*N*-nitro-imidazolidin-2-ylideneamine) is a systemic insecticide used to control a number of agricultural insect pests and animal parasites (Dryden et al., 1999; Tomizawa and Casida, 2005). Imidacloprid acts as a neurotoxin, with a highly specific affinity to the nicotinic acetylcholine receptor (nAChR) of insects (Stygar et al., 2013). Recent research suggests that widespread agricultural use of imidacloprid and other neonicotinoids may be contributing to honey bee colony collapse disorder, and the decline of honey bee colonies in many countries (Whitehorn et al., 2012). The European Food Safety Authority stated that neonicotinoids pose an unacceptably high risk to bees, and that the industry-sponsored research findings upon which regulatory

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agencies' claims of safety have relied may be flawed, or even deceptive.

Imidacloprid enters the soil during plant treatment and exhibits high persistence in soil, with half-life ($t_{1/2}$) of up to 229 days (Miles Inc., 1993). The behavior of imidacloprid in soils depends on the physicochemical parameters of the soil, such as the type and content of the organic matter, pH, temperature, insecticide concentrations, presence of cover crops, and time (Oi, 1999; Flores-Céspedes et al., 2002; Wu et al., 2012). The presence of imidacloprid in soils may affect non-targeted microorganisms. Since they play key roles in the functioning of soil ecosystems, the changes in their activity and biodiversity in response to imidacloprid contamination is of great interest.

In our previous study we used the phospholipid fatty acid (PLFA), denaturing gradient gel electrophoresis (DGGE), and community level physiological profile (CLPP) approaches to evaluate the genetic, structural and functional biodiversity of the soil microbial community after imidacloprid application at field rate (FR, 1 mg/kg soil) and 10 times the FR (10× FR, 10 mg/kg soil) (Cycoń et al., 2013a). PLFA profiles showed that imidacloprid significantly shifted the microbial community structure and decreased the biomass of the total, bacterial and fungal PLFAs. The alterations in DGGE patterns caused by imidacloprid application confirmed the occurrence of considerable changes in the overall richness and diversity of dominant bacteria. As a result of imidacloprid application, the metabolic activity of microbial communities was generally lower. Moreover, imidacloprid degradation and the appearance of some new bands in DGGE profiles suggest the evolution of bacteria capable of degrading imidacloprid among indigenous microflora (Cycoń et al., 2013a). Due to a lack of comprehensive information, the aim of this study was to assess whether the changes in the structure of the soil microbial community after imidacloprid application may also have an impact on the soil microbial activity. To ascertain this effect, the substrate-induced respiration (SIR), soil enzyme activities, and numbers of total heterotrophic bacteria as well as the numbers of specific groups of bacteria involved in soil nitrogen transformation were determined. Changes in the concentrations of ammonium and nitrate ions, indicating the rates of ammonification and nitrification processes, were also ascertained. In addition, the r/K-strategy approach was used to evaluate the effect of this insecticide on the community structure of the culturable soil bacteria.

1. Materials and methods

1.1. Soil

A loamy sand soil that had not been previously treated with imidacloprid or other pesticides was collected from the top layer (0–20 cm) of a grass-covered field that was located within the Pszczyna area of Upper Silesia in southern Poland (49°59′48″ N, 18°55′14″E). According to the FAO Soil Classification, the soil is classified as Orthic Luvisol, and its physico-chemical properties are shown in Table 1. Determination of soil parameters was performed according to the methods described in our previous studies (Cycoń et al., 2010a, 2010b).

1.2. Experimental design

Analytical standard grade imidacloprid (99.8% purity) purchased from Sigma-Aldrich (Germany) was used in this study. The experiment had a completely randomized block design with three replications and the following treatments: control and two insecticide dosages (1 and 10 mg/kg soil). The lower dosage is the recommended field rate (FR) of imidacloprid, assuming a homogeneous distribution of the insecticide to a depth of 5 cm and a soil density of 1.5 g/cm^3. The higher dosage of imidacloprid corresponded to 10 times the FR. The 10× FR dosage was used to evaluate the potential hazards of imidacloprid on soil microorganisms during undesirable events such as insecticide spills in high amounts into soil, which can occur during damage of devices used for its application, and due to uncontrolled disposal in soil of waste and water used to wash equipment, as well as *via* transport or industrial accidents.

The soil was divided into three portions of equal weight (3000 g) that were placed into plastic pots. Two portions were treated with the above-mentioned dosages of the insecticide while the third portion of soil was used as the control. Finally,

Table 1 – General characteristics of the soil used in the experiment.

Parameter	Value	Method of determination	Reference
Origin	Pszczyna, Poland	Randomized method	ISO 10381-2:2002
Sand (2000–50 m) (%)	86.0 ± 2.7	Sedimentation and sieving method	ISO 11277:2009
Silt (<50–2 m) (%)	11.0 ± 2.4	Sedimentation and sieving method	ISO 11277:2009
Clay (<2 m) (%)	3.0 ± 0.5	Sedimentation and sieving method	ISO 11277:2009
Density (g /cm^3)	1.2 ± 0.2	Core method	ISO 11272:1998
$pH_{(in\ water)}$ (1:5)	6.6 ± 0.3	Measurement with glass electrode	ISO 10390:2005
Cation exchange capacity (CEC) (cmol+/kg)	12.9 ± 1.7	Modified Gillman method	ISO 11260:1994
Water holding capacity (WHC) (%)	32.4 ± 2.8	Gravimetric method	ISO 14239:1997
C_{org} (%)	1.0 ± 0.2	Oxidation in the presence of H_2SO_4	ISO 14235:1998
N_{tot} (%)	0.09 ± 0.03	Modified Kjeldahl method	ISO 11261:1995
Microbial biomass (mg/kg dry weight)	668.0 ± 34.2	Substrate-induced respiration (SIR)	ISO 14240-1:1997

The values are the means of three replicates with the standard deviation, which was within 5% of the mean.