

Sensitivity of non-target groups of invertebrates to cypermethrin

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Agrogenic pollution with pyrethroid insecticides has been impacting the structure of populations of terrestrial invertebrates, causing decline in their taxonomic diversity and tolerance to critical values of environmental factors. In a laboratory experiment, we evaluated the sensitivity of 46 non-target invertebrate species to cypermethrin. In most examined species, we observed correlation between the body parameters (length and weight of body) and tolerance to this insecticide. We determined that the greater body size of the invertebrates, the better their tolerance to cypermethrin. Differences in LD₅₀ were the highest for groups of invertebrates with the body weight of 1.0–3.9 mg (1.9 ± 0.5 g/ha) and 16.0–63.9 mg (16.4 ± 3.2 g/ha). We observed a relationship between the trophic specialization and sensitivity to the insecticide in phytophages and zoophages. Average LD₅₀ values for phytophages were 2.1 ± 0.5 g/ha, much lower than for zoophages – 15.6 ± 3.3 g/ha. Among zoophages, the greatest tolerance to cypermethrin was demonstrated by ground beetles *Carabus coriaceus* L., *Pterostichus niger* (Schall.), *P. melanarius* (Ill.), *Pseudoophonus rufipes* (De Geer), and earwigs *Forficula auricularia* L. Analysis of various taxonomic groups of insects revealed the parameter to be 24.00 ± 4.66 for Carabidae, 8.60 ± 2.72 for Formicidae, and 0.23 ± 0.08 for Staphylinidae. Among the taxonomic groups we studied, the most sensitive to cypermethrin (LD₅₀ = 0.002–0.99 g/ha) were *Philonthus decorus* (0.0029), *Ph. rectangulus* (0.0035), *Ophonus rufibarbis* (0.121), *Oxytelus sculptus* (0.124), *Myrmica ruginodis* (0.39), *Aleochara lanuginosa* (0.49), *Carabus granulatus* (0.51), *Oxythyrea funesta* (0.52), *Tachinus signatus* (0.55), Cixiidae sp. (0.56), *Lygus pratensis* (0.56), *Carabus convexus* (0.71), and *C. hortensis* (0.83). Lower sensitivity to cypermethrin (LD₅₀ = 1.00–9.99 g/ha) was seen in *Lasius fuliginosus* (1.05), *Pyrrhocoris apterus* (1.28), *Chortippus* sp. 2 (1.96), *Rhyparochromus phoeniceus* (2.24), *Phosphuga atrata* (2.25), *Chironomus plumosus* (2.58), *Labia minor* (2.86), *Graphosoma italicum* (2.86), *Hister fenestus* (3.39), *Cylindroiulus truncorum* (3.61), *Opilio saxatilis* (3.71), *Chortippus* sp. 1 (3.94), *Epaphius secalis* (4.54), *Lasius niger* (4.77), *Silpha carinata* (4.84), *Aphodius foetens* (4.94), *Porcellio laevis* (5.68), *Coreus marginatus* (6.50), *Leistus ferrugineus* (7.39), and *Lasius alienus* (9.73). The most tolerant to cypermethrin (LD₅₀ = 10.00–108.00 g/ha) were *Calathus fuscipes* (12.14), *Limodromus assimilis* (12.22), *Trochosa terricola* (12.55), *Lithobius forficatus* (13.98), *Calathus ambiguus* (20.85), *Nebria brevicollis* (23.20), *Ponera coarctata* (27.04), *Megaphylum* sp. (29.01), *Pseudoophonus rufipes* (41.75), *Pterostichus melanarius* (45.78), *P. niger* (58.29), *Forficula auricularia* (80.57), and *Carabus coriaceus* (107.71). The differences we found in tolerance to cypermethrin ranged 100,000 times. This evidences the necessity of further research of taxonomic differences in tolerance of invertebrates to cypermethrin.

Keywords: non-target groups of invertebrates; pyrethroids; susceptibility to insecticide; median lethal dose; survivability of species.

Introduction

Intensive methods of the modern arable farming include the use of chemical means of plant protection. Extensive application of agrochemicals has inflicted a chronic global contamination on farm land. Toxic compounds are found in all environments. Every year, over 5 B tons of pesticides are introduced into the environment (Kyrychenko et al., 2022). Insecticides are effective against target species, despite some pests attaining tolerance to it. Pyrethroid compounds are considered perfect insecticides because of their fast toxic effect against pests in minimum doses. An advantage of these compounds is reduction of parasitic infections (malaria, filariasis, dengue fever) transmitted by insects. However, at the same time, insecticides are detrimental to many non-target invertebrates, including pollinators, and also predatory invertebrates – entomophages, which are promising for biological defense of plants (Pandey et al., 2016; Dhananjay & Ravichandran, 2018; Rani et al., 2020; Langraf et al., 2021; Kumar et al., 2023).

First of all, toxic insecticides are dangerous to humans and agricultural animals, because are able to accumulate in plant tissues or remain long on the surface of plant products. Directly or indirectly, they affect wild vertebrates. According to the literature data, the greatest threat posed by pyrethroid insecticides is to aquatic organisms (for example, fishes), while

it is less dangerous to mammals, birds, and amphibians. However, pyrethroids cause a number of generative and toxic effects in vertebrates: hepato-, immune-, neuro-, embryotoxicity, degeneration of the central nervous system, bone marrow, reproductive system, and DNA damages, etc. (Gibbons et al., 2014; Ullah et al., 2019; Nedzvetsky et al., 2020; Farag et al., 2021).

These compounds are no less damaging to taxonomic diversity of invertebrates and functioning of entomocenoses of soil-litter and other terrestrial strata. Little study has been conducted regarding side-effects of agrochemicals and their impacts on non-target groups of arthropods. The most comprehensive research has been performed focusing on sensitivity to insecticides among pollinators. For example, it is known that *Apis mellifera* bees in various natural conditions are constantly exposed to various pesticides and their metabolites. Impact of field doses of insecticides on bees and bumblebees leads to disorientation, decrease in fertility, life span, and behavioural changes. Pyrethroids (cypermethrin, deltamethrin, and permethrin) are highly toxic to *A. mellifera* (Decourtye et al., 2005; Desneux et al., 2007; Liao et al., 2018).

A number of studies have attempted to identify how toxic pyrethroids are to non-target freshwater species of invertebrates, namely crustaceans, insects (nymphs of mayflies and stoneflies, larvae of mosquitoes), and mollusks. Especially vulnerable to pesticide contamination are freshwater

bodies that are in close proximity to agricultural lands. Having been largely spread, pyrethroid insecticides have accumulated in bental deposits, causing toxic impacts on aquatic invertebrates (Mugni et al., 2013; Li et al., 2017; Crowley et al., 2021). *Daphnia*, copepods, and larvae of insects (Hasenbein et al., 2016) have been mentioned as the most sensitive to pyrethroids. Some freshwater crustaceans (*Hyalella azteca*) and mollusks (*Chilina parhappii*) are tolerant to cypermethrin. Among species subject to continuous impact of insecticides, tolerance arises during detoxication of the body (increase in the activity of some enzymes) or as a result of mutations that reduce the sensitivity to toxins (Weston et al., 2005; Major et al., 2018; Fernandez San Juan et al., 2020).

The literature contains analysis of possible ecological implications of neurotoxins for non-target organisms. Despite the fact that most toxicological studies focused on acute toxic effects of insecticides, ecologically significant chronic (long) effects of those compounds on communities of terrestrial invertebrates have been studied poorly. There has been a comparative assessment of influence of various groups of insecticides on non-target species of arthropods living in winter-wheat fields. It found that organic-phosphorus compounds exerted the highest toxicity towards invertebrates compared with pyrethroid and carbamate compounds. Studies have rarely concentrated on the simultaneous overall effect of various groups of insecticides on non-target species. There is also a paucity of data about cumulative properties of pyrethroids (Moreby et al., 2001; Diao et al., 2011; Kumar et al., 2023). In natural conditions, living organisms are always subject to many factors at the same time. Complex effects of insecticides and other stress factors was studied on pollinators. Scientific data about influence of pyrethroids on soil-litter fauna are limited by the small number of species. Cypermethrin is known to cause metabolic disorders in earthworms, and has effects on the behavior, lifespan, reproduction of soil springtails (Hartnik et al., 2008; Ch et al., 2015; Zortéa et al., 2015). Non-target groups of terrestrial invertebrates in this aspect have been studied insufficiently.

Cypermethrin (C₂₂H₁₉Cl₂NO₃) is a second-generation synthetic pyrethroid with a strong insecticide activity and a broad range of action. Currently, this insecticide is broadly used in agriculture, although it emerged on the global market back in the 1970s. Cypermethrin is a neurotoxin, which can easily penetrate the coatings of arthropods. In invertebrates, it causes oxidative stress, neuro-, geno-, and immunotoxicity, reduces fertility, periods of development of pre-imago phases, and causes unnatural models of behavior. It has no systemic action and is resilient to abiotic environmental factors. In agrocenoses, it is utilized against a wide range of pests of cereal crops, sunflowers, potatoes, tomatoes, grapes, cotton, etc. Also, cypermethrin is used to deal with synanthropic insects and ectoparasites of animals (Maund et al., 2011; Kumar et al., 2023).

The objective of the study was to identify the extent of action of cypermethrin, a popular insecticide in arable farming, on various groups of non-target species of invertebrates by establishing LD₅₀ for each studied invertebrate species in laboratory conditions.

Material and methods

The laboratory experiments were performed on 46 common species of invertebrates, common in Lithuania. They mostly consisted of epigeal species and a large number of grass-stand dwellers. The species belonged to different systematic groups. We collected the field material in the territory of the Kamša Botanical and Zoological Reserve (near the city of Kaunas) and the dendrarium of the Vytautas Magnus University Agriculture Academy (Akademija, west of Kaunas) between June and August 2023. To capture the invertebrates, we used the generally accepted entomological methods: Barber pit-fall traps, manual collection from the litter and soil, exhausters, butterfly nets, and luring using light in the night (Lamarre et al., 2018).

The studies were performed in a well-ventilated laboratory at 21.5–23.5 °C and relative air humidity of 43–56%. To study the sensitivity of the mesofauna to cypermethrin, the invertebrates were put in small closable 10 × 6 × 4 cm containers (with microholes in the lids), made of edible plastic. Onto the bottom of the containers, we put cotton disks to absorb surplus moisture. During the experiment, the containers were kept in fume hoods. Plastic pulverizers were filled with aqueous solution of cypermethrin (Cyperkill 500 EC, UPL, Poland) in various concentrations. We used 8 concentrations of the insecticide (Table 1). The manufacturer's instruction indicated that the recommended dose for most types of agrocenoses is 0.05 L/ha. Because the drug contains 500 g/L of the active agent (cypermethrin), the dose recommended by the manufacturer corresponds to 25 g of active agent per hectare, or 2.5 mg/m² (Table 2).

In each container, we put 8 specimens of one species and using a pulverizer, we introduced the dose of 0.37 mL of the solution in a certain concentration (microscopic drops of aqueous solution of cypermethrin with the same likelihood reached the cuticle of invertebrates, filter paper on the bottom of the container and wall of the plastic container). We analyzed the live invertebrates 24 h after the beginning of experiment (Table 1). Only adult specimens of invertebrates were used in the experiment. Dead arthropods were fixated in 70% ethanol for further study of changes that had occurred in their bodies.

The statistical analysis of the results was performed using a set of Statistica 8.0 (StatSoft Inc., USA). The results were analyzed using probit analysis. The tables present mean value (x) ± standard error (SE).

Results

The LD₅₀, calculated according to the results of the laboratory experiments, was not always related to species, body sizes, and trophic specialization. Drastically different reactions to the insecticide were observed even in related species of the same genus, which have similar biological and ecological specifics and body parameters. Bright examples are species of the *Lasius* genus and ground beetles of the *Carabus* genus (Table 2).

Table 1
Results of the laboratory experiments on invertebrates subject to cypermethrin

| Species | Dose, grams of cypermethrin per hectare | 1600 | 400 | 100 | 25 | 6.25 | 1.56 | 0.39 | 0.10 |
|--|--|------|-----|-----|----|------|------|------|------|
| | | | | | | | | | |
| <i>Porcellio laevis</i> Latreille, 1804 | Live | 0 | 0 | 0 | 7 | 7 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 8 | 1 | 1 | 0 | 0 | 0 |
| <i>Lithobius forficatus</i> (Linnaeus, 1758) | Live | 0 | 2 | 7 | 7 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 6 | 1 | 1 | 0 | 0 | 0 | 0 |
| <i>Cylindroiulus truncorum</i> (Silvestri, 1896) | Live | 0 | 0 | 2 | 2 | 4 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 6 | 6 | 4 | 0 | 0 | 0 |
| <i>Megaphyllum</i> sp. | Live | 0 | 2 | 4 | 8 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 6 | 4 | 0 | 0 | 0 | 0 | 0 |
| <i>Forficula auricularia</i> Linnaeus, 1758 | Live | 0 | 2 | 8 | 8 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Labia minor</i> (Linnaeus, 1758) | Live | 0 | 0 | 0 | 2 | 5 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 8 | 6 | 3 | 0 | 0 | 0 |
| <i>Chortippus</i> sp. 1 | Live | 0 | 0 | 2 | 2 | 5 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 6 | 6 | 3 | 0 | 0 | 0 |
| <i>Chortippus</i> sp. 2 | Live | 0 | 0 | 0 | 1 | 2 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 8 | 7 | 6 | 0 | 0 | 0 |
| <i>Cixiidae</i> sp. | Live | 0 | 0 | 0 | 0 | 1 | 4 | 7 | 8 |
| | Dead | 8 | 8 | 8 | 8 | 7 | 4 | 1 | 0 |

| Species | Dose, grams of cypermethrin per hectare | Dose, grams | | | | | | | |
|--|--|-------------|-----|-----|----|------|------|------|------|
| | | 1600 | 400 | 100 | 25 | 6.25 | 1.56 | 0.39 | 0.10 |
| <i>Rhyparochromus phoeniceus</i> (Rossi, 1794) | Live | 0 | 0 | 0 | 3 | 6 | 5 | 8 | 8 |
| | Dead | 8 | 8 | 8 | 5 | 2 | 3 | 0 | 0 |
| <i>Coreus marginatus</i> (Linnaeus, 1758) | Live | 0 | 0 | 2 | 3 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 6 | 5 | 0 | 0 | 0 | 0 |
| <i>Pyrrhocoris apterus</i> (Linnaeus, 1758) | Live | 0 | 0 | 0 | 1 | 4 | 5 | 8 | 8 |
| | Dead | 8 | 8 | 8 | 7 | 4 | 3 | 0 | 0 |
| <i>Lygus pratensis</i> (Linnaeus, 1758) | Live | 0 | 0 | 0 | 0 | 1 | 4 | 7 | 8 |
| | Dead | 8 | 8 | 8 | 8 | 7 | 4 | 1 | 0 |
| <i>Graphosoma italicum</i> (Muller, 1766) | Live | 0 | 0 | 0 | 2 | 5 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 8 | 6 | 3 | 0 | 0 | 0 |
| <i>Carabus convexus</i> Fabricius, 1775 | Live | 0 | 0 | 0 | 0 | 6 | 4 | 5 | 8 |
| | Dead | 8 | 8 | 8 | 8 | 2 | 4 | 3 | 0 |
| <i>Carabus coriaceus</i> Linnaeus, 1758 | Live | 0 | 3 | 8 | 8 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Carabus granulatus</i> Linnaeus, 1758 | Live | 0 | 0 | 0 | 1 | 3 | 4 | 4 | 8 |
| | Dead | 8 | 8 | 8 | 7 | 5 | 4 | 4 | 0 |
| <i>Carabus hortensis</i> Linnaeus, 1758 | Live | 0 | 0 | 0 | 1 | 3 | 5 | 6 | 8 |
| | Dead | 8 | 8 | 8 | 7 | 5 | 3 | 2 | 0 |
| <i>Nebria brevicollis</i> (Fabricius, 1792) | Live | 0 | 1 | 6 | 7 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 7 | 2 | 1 | 0 | 0 | 0 | 0 |
| <i>Leistus ferrugineus</i> (Linnaeus, 1758) | Live | 0 | 0 | 1 | 6 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 7 | 2 | 0 | 0 | 0 | 0 |
| <i>Epaphius secalis</i> (Paykull, 1790) | Live | 0 | 0 | 3 | 2 | 7 | 7 | 8 | 8 |
| | Dead | 8 | 8 | 5 | 6 | 1 | 1 | 0 | 0 |
| <i>Pterostichus niger</i> (Schaller, 1783) | Live | 0 | 2 | 8 | 7 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 6 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Pterostichus melanarius</i> (Illiger, 1798) | Live | 0 | 1 | 8 | 8 | 7 | 8 | 8 | 8 |
| | Dead | 8 | 7 | 0 | 0 | 1 | 0 | 0 | 0 |
| <i>Limodromus assimilis</i> (Paykull, 1790) | Live | 0 | 1 | 5 | 5 | 7 | 8 | 8 | 8 |
| | Dead | 8 | 7 | 3 | 3 | 1 | 0 | 0 | 0 |
| <i>Calathus ambiguus</i> (Paykull, 1790) | Live | 0 | 0 | 7 | 8 | 7 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 1 | 0 | 1 | 0 | 0 | 0 |
| <i>Calathus fuscipes</i> (Goeze, 1777) | Live | 0 | 0 | 5 | 6 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 3 | 2 | 0 | 0 | 0 | 0 |
| <i>Pseudoophonus rufipes</i> (De Geer, 1774) | Live | 0 | 1 | 8 | 7 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 7 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Ophonus rufibarbis</i> (Fabricius, 1792) | Live | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 8 |
| | Dead | 8 | 8 | 8 | 8 | 8 | 8 | 2 | 0 |
| <i>Aleochara lanuginose</i> Gravenhorst, 1802 | Live | 0 | 0 | 0 | 1 | 2 | 1 | 7 | 8 |
| | Dead | 8 | 8 | 8 | 7 | 6 | 7 | 1 | 0 |
| <i>Philonthus decorus</i> (Gravenhorst, 1802) | Live | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 6 |
| | Dead | 8 | 8 | 8 | 7 | 7 | 8 | 7 | 2 |
| <i>Philonthus rectangulus</i> Sharp, 1874 | Live | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 6 |
| | Dead | 8 | 8 | 8 | 8 | 8 | 7 | 7 | 2 |
| <i>Oxytelus sculptus</i> Gravenhorst, 1806 | Live | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 8 |
| | Dead | 8 | 8 | 8 | 8 | 7 | 6 | 6 | 0 |
| <i>Tachinus signatus</i> Gravenhorst, 1802 | Live | 0 | 0 | 0 | 0 | 0 | 3 | 8 | 8 |
| | Dead | 8 | 8 | 8 | 8 | 8 | 5 | 0 | 0 |
| <i>Silpha carinata</i> Herbst, 1783 | Live | 0 | 0 | 3 | 4 | 6 | 7 | 8 | 8 |
| | Dead | 8 | 8 | 5 | 4 | 2 | 1 | 0 | 0 |
| <i>Phosphuga atrata</i> (Linnaeus, 1758) | Live | 0 | 0 | 1 | 2 | 4 | 6 | 8 | 8 |
| | Dead | 8 | 8 | 7 | 6 | 4 | 2 | 0 | 0 |
| <i>Hister fenestus</i> Erichson, 1834 | Live | 0 | 0 | 0 | 4 | 5 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 8 | 4 | 3 | 0 | 0 | 0 |
| <i>Aphodius foetens</i> (Fabricius, 1787) | Live | 0 | 0 | 1 | 5 | 6 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 7 | 3 | 2 | 0 | 0 | 0 |
| <i>Oxythyrea funesta</i> (Poda, 1761) | Live | 0 | 0 | 0 | 0 | 2 | 4 | 6 | 8 |
| | Dead | 8 | 8 | 8 | 8 | 6 | 4 | 2 | 0 |
| <i>Ponera coarctata</i> (Latreille, 1802) | Live | 0 | 0 | 8 | 7 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Myrmica ruginodis</i> Nylander, 1846 | Live | 0 | 0 | 0 | 1 | 1 | 2 | 6 | 8 |
| | Dead | 8 | 8 | 8 | 7 | 7 | 6 | 2 | 0 |
| <i>Lasius alienus</i> (Foerster, 1850) | Live | 0 | 0 | 4 | 5 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 4 | 3 | 0 | 0 | 0 | 0 |
| <i>Lasius fuliginosus</i> (Latreille, 1798) | Live | 0 | 0 | 0 | 0 | 4 | 6 | 7 | 8 |
| | Dead | 8 | 8 | 8 | 8 | 4 | 2 | 1 | 0 |
| <i>Lasius niger</i> (Linnaeus, 1758) | Live | 0 | 0 | 2 | 5 | 6 | 7 | 8 | 8 |
| | Dead | 8 | 8 | 6 | 3 | 2 | 1 | 0 | 0 |
| <i>Chironomus plumosus</i> (Linnaeus, 1758) | Live | 0 | 0 | 3 | 2 | 3 | 6 | 8 | 8 |
| | Dead | 8 | 8 | 5 | 6 | 5 | 2 | 0 | 0 |
| <i>Trochosa terricola</i> Thorell, 1856 | Live | 0 | 0 | 4 | 7 | 8 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 4 | 1 | 0 | 0 | 0 | 0 |
| <i>Opilio saxatilis</i> C. L. Kokh, 1839 | Live | 0 | 0 | 0 | 4 | 6 | 8 | 8 | 8 |
| | Dead | 8 | 8 | 8 | 4 | 2 | 0 | 0 | 0 |

Table 2
Use of cypermethrin in the laboratory experiment

| Order | Family | Species | LD ₅₀ (mean ± standard error), gram of cypermethrin per hectare | Trophic group | Mean body weight (dry weight), mg | Body length, mm |
|----------------|----------------|--|--|---------------|-----------------------------------|-----------------|
| Isopoda | Porcellionidae | <i>Porcellio laevis</i> Latreille, 1804 | 5.68 ± 2.47 | s | 29 | 10–20 |
| Lithobiomorpha | Lithobiidae | <i>Lithobius forficatus</i> (Linnaeus, 1758) | 13.98 ± 9.90 | z | 21 | 18–30 |
| Julida | Julidae | <i>Cylindroiulus truncorum</i> (Silvestri, 1896) | 3.61 ± 2.17 | s | 42 | 15–20 |
| Julida | Julidae | <i>Megaphyllum</i> sp. | 29.01 ± 8.10 | s | 47 | 17–22 |
| Dermaptera | Forficulidae | <i>Forficula auricularia</i> Linnaeus, 1758 | 80.57 ± 10.89 | p | 29 | 12–14 |
| Dermaptera | Spongiphoridae | <i>Labia minor</i> (Linnaeus, 1758) | 2.86 ± 1.69 | p | 3 | 4–7 |
| Orthoptera | Acrididae | <i>Chortippus</i> sp. 1 | 3.94 ± 2.41 | f | 72 | 20–28 |
| Orthoptera | Acrididae | <i>Chortippus</i> sp. 2 | 1.96 ± 1.09 | f | 63 | 18–25 |
| Hemiptera | Cixiidae | <i>Cixiidae</i> sp. | 0.56 ± 0.36 | f | 0.2 | 3.0–4.0 |
| Hemiptera | Lygaeidae | <i>Rhyparochromus phoeniceus</i> (Rossi, 1794) | 2.24 ± 1.40 | f | 4 | 7.5–9.0 |
| Hemiptera | Coreidae | <i>Coreus marginatus</i> (Linnaeus, 1758) | 6.50 ± 3.13 | f | 23 | 13–15 |
| Hemiptera | Pyrrhocoridae | <i>Pyrrhocoris apterus</i> (Linnaeus, 1758) | 1.28 ± 0.91 | p | 32 | 9–11 |
| Hemiptera | Miridae | <i>Lygus pratensis</i> (Linnaeus, 1758) | 0.56 ± 0.36 | f | 2 | 5.5–7.0 |
| Hemiptera | Pentatomidae | <i>Graphosoma italicum</i> (Muller, 1766) | 2.86 ± 1.69 | f | 45 | 8–12 |
| Coleoptera | Carabidae | <i>Carabus convexus</i> Fabricius, 1775 | 0.71 ± 0.42 | z | 120 | 15–18 |
| Coleoptera | Carabidae | <i>Carabus coriaceus</i> Linnaeus, 1758 | 107.71 ± 13.10 | z | 1043 | 30–42 |
| Coleoptera | Carabidae | <i>Carabus granulatus</i> Linnaeus, 1758 | 0.51 ± 0.36 | z | 106 | 17–23 |
| Coleoptera | Carabidae | <i>Carabus hortensis</i> Linnaeus, 1758 | 0.83 ± 0.60 | z | 237 | 23–30 |
| Coleoptera | Carabidae | <i>Nebria brevicollis</i> (Fabricius, 1792) | 23.20 ± 7.49 | z | 29 | 10–14 |
| Coleoptera | Carabidae | <i>Leistus ferrugineus</i> (Linnaeus, 1758) | 7.39 ± 3.32 | z | 7 | 6.5–8.0 |
| Coleoptera | Carabidae | <i>Epaphius secalis</i> (Paykull, 1790) | 4.54 ± 2.65 | z | 0.5 | 3.5–4.5 |
| Coleoptera | Carabidae | <i>Pterostichus niger</i> (Schaller, 1783) | 58.29 ± 9.88 | z | 74 | 15–21 |
| Coleoptera | Carabidae | <i>Pterostichus melanarius</i> (Illiger, 1798) | 45.78 ± 7.33 | z | 71 | 12–18 |
| Coleoptera | Carabidae | <i>Limodromus assimilis</i> (Paykull, 1790) | 12.22 ± 6.07 | z | 15 | 10–13 |
| Coleoptera | Carabidae | <i>Calathus ambiguus</i> (Paykull, 1790) | 20.85 ± 5.00 | z | 25 | 8–12 |
| Coleoptera | Carabidae | <i>Calathus fuscipes</i> (Goeze, 1777) | 12.14 ± 4.70 | z | 25 | 10–14 |
| Coleoptera | Carabidae | <i>Pseudoophonus rufipes</i> (De Geer, 1774) | 41.75 ± 7.71 | p | 47 | 11–16 |
| Coleoptera | Carabidae | <i>Ophonus rufibarbis</i> (Fabricius, 1792) | 0.121 ± 0.087 | f | 11 | 7.5–10.0 |
| Coleoptera | Staphylinidae | <i>Aleochara lanuginosa</i> Gravenhorst, 1802 | 0.49 ± 0.31 | z | 0.3 | 3.0–5.5 |
| Coleoptera | Staphylinidae | <i>Philonthus decorus</i> (Gravenhorst, 1802) | 0.0029 ± 0.0058 | z | 12 | 11–13 |
| Coleoptera | Staphylinidae | <i>Philonthus rectangularis</i> Sharp, 1874 | 0.0035 ± 0.0062 | z | 2 | 6.5–9.0 |
| Coleoptera | Staphylinidae | <i>Oxytelus sculptus</i> Gravenhorst, 1806 | 0.124 ± 0.094 | z | 0.6 | 3.5–4.0 |
| Coleoptera | Staphylinidae | <i>Tachinus signatus</i> Gravenhorst, 1802 | 0.55 ± 0.31 | z | 0.8 | 5–6 |
| Coleoptera | Silphidae | <i>Silpha carinata</i> Herbst, 1783 | 4.84 ± 3.21 | p | 74 | 12–23 |
| Coleoptera | Silphidae | <i>Phosphuga atrata</i> (Linnaeus, 1758) | 2.25 ± 1.67 | z | 42 | 10–16 |
| Coleoptera | Histeridae | <i>Hister fenestus</i> Erichson, 1834 | 3.39 ± 1.95 | z | 6 | 4–6 |
| Coleoptera | Aphodiidae | <i>Aphodius foetens</i> (Fabricius, 1787) | 4.94 ± 2.93 | s | 9 | 6.0–8.5 |
| Coleoptera | Cetoniidae | <i>Oxythyrea funesta</i> (Poda, 1761) | 0.52 ± 0.36 | f | 45 | 8–12 |
| Hymenoptera | Formicidae | <i>Ponera coarctata</i> (Latreille, 1802) | 27.04 ± 5.32 | p | 0.3 | 2.0–4.0 |
| Hymenoptera | Formicidae | <i>Myrmica ruginodis</i> Nylander, 1846 | 0.39 ± 0.27 | p | 0.7 | 3.0–4.5 |
| Hymenoptera | Formicidae | <i>Lasius alienus</i> (Foerster, 1850) | 9.73 ± 4.22 | p | 0.3 | 2.5–4.0 |
| Hymenoptera | Formicidae | <i>Lasius fuliginosus</i> (Latreille, 1798) | 1.05 ± 0.67 | p | 0.5 | 3.5–5.5 |
| Hymenoptera | Formicidae | <i>Lasius niger</i> (Linnaeus, 1758) | 4.77 ± 3.17 | p | 0.4 | 3.0–4.5 |
| Diptera | Chironomidae | <i>Chironomus plumosus</i> (Linnaeus, 1758) | 2.58 ± 1.62 | s | 1 | 6–8 |
| Araneae | Lycosidae | <i>Trochosa terricola</i> Thorell, 1856 | 12.55 ± 4.71 | z | 4 | 4–6 |
| Opiliones | Phalangidae | <i>Opilio saxatilis</i> C. L. Kokh, 1839 | 3.71 ± 2.10 | p | 1 | 3.0–4.5 |

Note: manufacture-recommended dose of cypermethrin in the conditions of agroecosystems is 25 g/ha.

The ascending straight line in Figure 1 represents relationship between the body length of the studied arthropods and their sensitivity to cypermethrin. Median lethal dose for most small species of up to 6 mm body length did not exceed 12.55 ± 4.71 g/ha. An exception was the ant *Ponera coarctata* (Latr.). Median lethal dose for this species with 2–4 mm body lengths equaled 27.04 ± 5.32 g/ha. For species with body length of 6 to 14 mm, values of LD₅₀ varied more significantly. It is worth noting the very high LD₅₀ for *Forficula auricularia* L. (80.57 ± 10.89 g/ha). Also, as the most tolerant species in this size group, we should name the ground beetles *Pseudoophonus rufipes* (De Geer), *Nebria brevicollis* (F.), *Calathus ambiguus* (Payk.), *C. fuscipes* (Goeze), and *Limodromus assimilis* (Payk.) (Table 2). High LD₅₀ values for the mentioned species much higher than those for the small species.

However, not all Carabidae species were tolerant to cypermethrin. High sensitivity to the insecticide was seen in *Ophonus rufibarbis* (F.) (0.121 ± 0.087 h/ha), with an average body size (7.5–10.0 mm). Abnormally high sensitivity was found in staphylinids of the *Philonthus* genus (*Ph. decorus* (Grav.) – 0.0029 ± 0.0058 g/ha). In this case, no relationship between body length and increase in tolerance to the insecticide was observed. However, such a strong reaction of *Philonthus* to cypermethrin should, perhaps, be identified to taxonomic specifics. It would be promi-

sing to conduct expanded laboratory studies using a large number of *Philonthus* species. The latter size group included species with the body length of 15 mm. The highest LD₅₀ values were seen in *Carabus coriaceus* L., *Pterostichus niger* (Schall.), and *P. melanarius* (Ill.) (107.71 ± 13.10 , 58.29 ± 9.88 , and 45.78 ± 7.33 g/ha, respectively).

We found significant correlation between trophic specialization and sensitivity to cypermethrin in phytophages and zoophages (Fig. 2). In our research, phytophages were mainly represented by species of the Coleoptera order, families Coreidae, Miridae, Pentatomidae, Lygaeidae, and also the Orthoptera order – the Acrididae family. The trophic group of zoophages was taxonomically more diverse: centipedes Lithobiidae, spiders Lycosidae, coleopterans of families Carabidae, Staphylinidae, Silphidae, and Histeridae. Mean LD₅₀ values for phytophages accounted for 2.1 ± 0.5 g/ha, which are much lower than for zoophages – 15.6 ± 3.3 g/ha. The dominant group of zoophages in the environment by species diversity is Carabidae. Among the studied species of ground beetles, the highest tolerance to cypermethrin were exhibited by *Carabus coriaceus* L., *Pterostichus niger* (Schall.), *P. melanarius* (Ill.). *Carabus coriaceus* L. was the largest of all the examined invertebrates. Its body length varies 30 to 42 mm. Perhaps, the very high median lethal dose of cypermethrin for this species (107.71 ± 13.10 g/ha) could be attributable to the body size.

We did see such a relationship in invertebrates used in the experiment (Fig. 1, 3). Other species of *Carabus*, despite large sizes, were very sensitive to the insecticide, their LD₅₀ not exceeding 0.83 ± 0.60 g/ha (Table. 2).

We observed a tendency for the median lethal dose of cypermethrin to be higher for the invertebrates with greater body weight (Fig. 3). Differences were significant for weight groups of 1.0–3.9 mg (LD₅₀ mean ± standard error: 1.9 ± 0.5 g/ha) and 16.0–63.9 mg (16.4 ± 3.2 g/ha).

The first group was represented by ants, small species (body length up to 6 mm) of staphylinids, ground beetles, and hemipterans. The second group contains woodlice, millipedes Lithobiidae, Julidae, average-sized species of groundbeetles of genera *Calathus*, *Pseudoophonus*, *Nebria* (up to 15 mm), large species of hemipterans – *Graphosoma italicum* (Muller), *Pyrhocoris apterus* (L.), and *Coreus marginatus* (L.) (up to 15 mm).

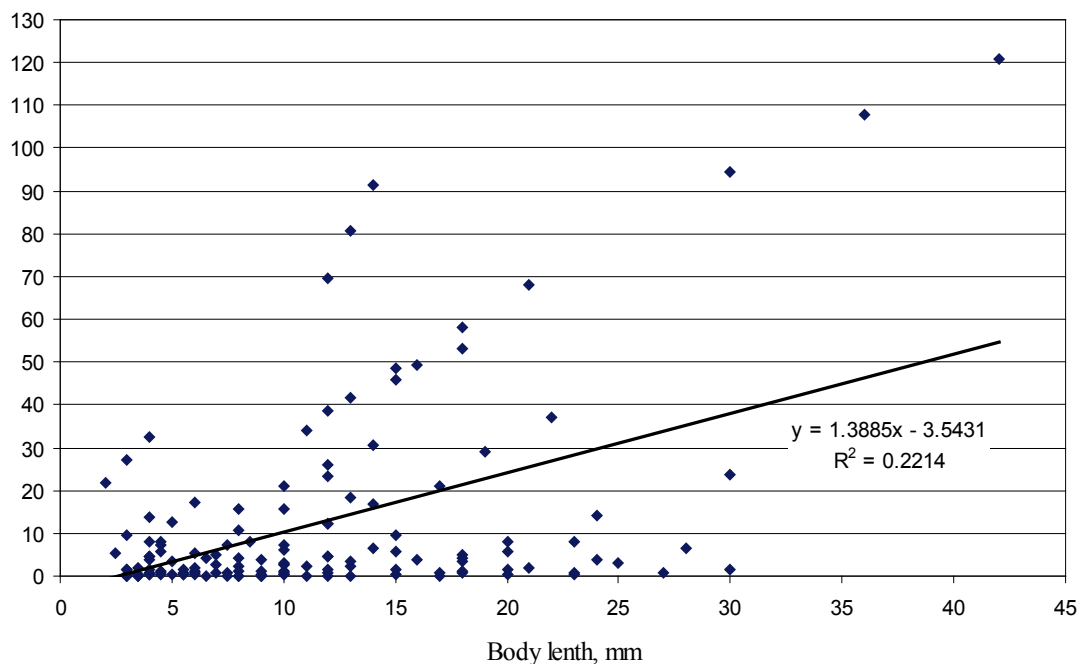


Fig. 1. Correlation between sensitivity (ordinate axis – LD₅₀, grams of active agent per hectare) of the studied invertebrates and their body length (abscissa axis)

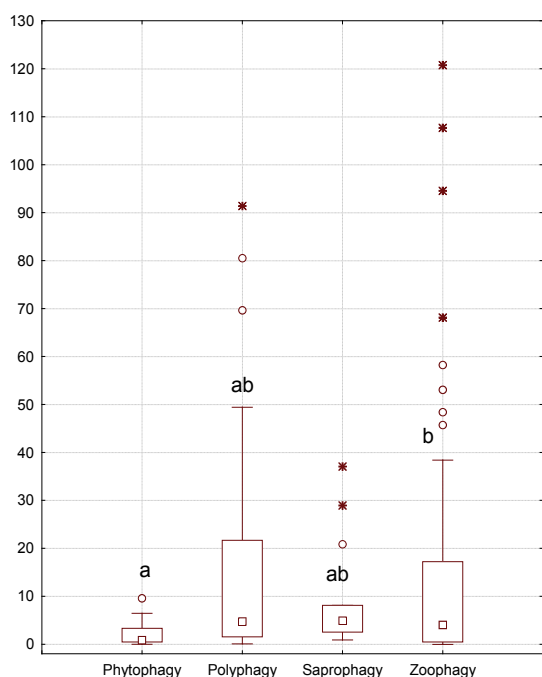


Fig. 2. Relationship between sensitivity to cypermethrin (ordinate axis, LD₅₀, grams of active agent per hectare) and trophic specialization (abscissa axis): phytophages – 2.1 ± 0.5 (mean ± standard error), polyphages – 16.2 ± 4.3, saprophages – 9.2 ± 2.8, zoophages – 15.6 ± 3.3 gram of active agent per hectare

The results of our studies indicate that the taxa did correlate with the median lethal dose (Fig. 4). Most of the ground beetles were tolerant to cypermethrin (LD₅₀ mean ± standard error: 24.00 ± 4.66 g/ha). Among the analyzed taxa, Carabidae had the highest LD₅₀ values. Despite the fact that there were exceptions, large species of the *Carabus* genus (body

length of 15–30 mm) turned out to be very sensitive to the insecticide. The ants could be characterized as an average-tolerant group (8.60 ± 2.72 g/ha). The data we obtained are conflicting. Even within one genus *Lasius*, species with only slight differences in body length and weight varied quite broadly in sensitivity to the neurotoxin. Therefore, LD₅₀ for *Lasius alienus* (F.) it was 9.73 ± 4.22 g/ha, and for *L. fuliginosus* (L.) it was much lower, measuring 1.05 ± 0.67 g/ha. Staphylinids was the most vulnerable family of Coleoptera of all the examined (0.23 ± 0.08 g/ha). Especially sensitive to cypermethrin were species of the *Philonthus* genus.

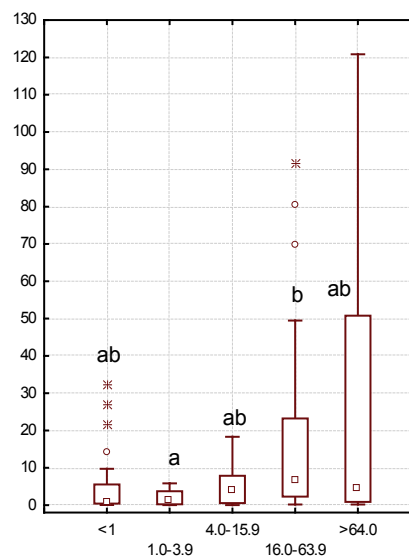


Fig. 3. Correlation between LD₅₀ (ordinate axis, grams of active compound per hectare) and body weight (abscissa axis, mg, weight of dried invertebrates): <1 mg (mean ± standard error: 4.9 ± 1.5 g/ha), 1.0–3.9 mg (1.9 ± 0.5 g/ha), 4.0–15.9 mg (5.4 ± 1.1 g/ha), 16.0–63.9 mg (16.4 ± 3.2 g/ha), >64.0 mg (27.8 ± 7.8 g/ha)

The median lethal dose for forest litter dweller *Ph. decorus* (Grav.) was only 0.0029 ± 0.0058 g/ha, even though it has quite a long 11–13 mm body and 12 mg dry weight. Representatives of other taxonomic groups of Staphylinidae were more tolerant to the insecticide. For example, LD₅₀ for coprophilous *Tachinus signatus* Grav. and *Aleochara lanuginose* Grav. was higher (0.55 ± 0.31 and 0.49 ± 0.31 g/ha, respectively). It would be promising to study the relationship between median lethal dose and confinement to type of biocenose. According to our studies, coprozoic and coprophilous species of insects – inhabitants of feces of bovine cattle – were more tolerant to cypermethrin than the litter species with the same body sizes. Besides the said species, an example can be the coprozoic species *Hister fenestus* Erich. and *Aphodius foetens* (F.). However, the exception was again the *Philonthus* staphylinids. For coprophilous *Ph. rectangulus* Sharp (life cycle occurs in swine and bovine feces), LD₅₀ was only 0.0035 ± 0.0062 g/ha.

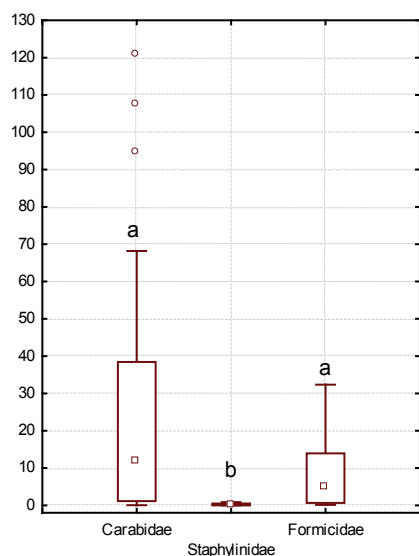


Fig. 4. Correlation between LD₅₀ (ordinate axis, grams of active agent per hectare) and taxa: Carabidae (mean \pm standard error: 24.00 ± 4.66 g/ha), Staphylinidae (0.23 ± 0.08 g/ha), Formicidae (8.60 ± 2.72 g/ha)

Discussion

Our large-scale research into the sensitivity of invertebrates to cypermethrin, involving a large number of taxa, revealed conflicting results. We identified a broad range of median lethal doses. High sensitivity to cypermethrin, which was observed in staphylinids, most species of hemipterans, some species of ground beetles, ants, and others, suggest that in the natural conditions those species disappear from the invertebrate communities living in the treated fields and adjacent areas. Those species belong to large, broadly common taxa and perform important functional roles in terrestrial ecosystems. Staphylinidae is one of the dominant group of epigeal coleopterans, both by composition and number. According to the same characteristics, they are inferior only to Carabidae. The practical importance of ground beetles is that they regulate the number of invertebrates, including agricultural pests. At the same time, some species of Carabidae are phytophages and mixophytophages and cause great damage to agricultural and forest plants (Avtaeva et al., 2019, 2021a; Puchkov et al., 2020; Brygadyrenko et al., 2021).

Pterostichus melanarius is a broadly common species in Europe and is more ecologically adaptable to unfavourable environmental factors (Korolev & Brygadyrenko, 2014; Avtaeva et al., 2021b). Being highly resistant to pyrethroid insecticides, in Lithuania, it is numerous in both forest ecosystems and agroecosystems.

In the conditions of the steppe zone of Ukraine, many Julidae millipedes (*Rossius kessleri*, *Megaphyllum kievense*) are subject to such anthropogenic factors as technogenic and agrogenic contaminations. In a laboratory experiment, heavy metals and pesticides altered their body weight, rates of nutrition, intensity of defecation, and led to death in high concen-

trations (Brygadyrenko & Ivanyshyn, 2015; Kozak & Brygadyrenko, 2018; Kozak et al., 2020). Anthropogenic impact destroys the structure of soil-litter macrofauna through impoverishment of species diversity and degradation of trophic and size structure of invertebrate communities (Brygadyrenko, 2015; Faly et al., 2017).

Pyrethroids are effective in controlling the number of insects harmful to humans, but at the same time are detrimental to species that are beneficial to humans. This leads to dysbalance in the ecosystems. The goal of modern agriculture is to find a balance between protecting crops from pests and maintaining diversity of beneficial species. The concept of sustainable arable farming includes analysis of how insecticides impact the agroecosystems and search for alternative methods to protect the plants. For rare and protected invertebrates, long term toxic poisoning with insecticides, against the background of chemical pollution with other toxic compounds, can lead to decline in their populations and their complete disappearance in the future. Intensive use of insecticides in agroecosystems limits the application of biological methods of combating pests. It is promising to introduce ecological systems of arable farming, completely excluding the use of chemical agents (Brygadyrenko & Nazimov, 2015; Rani et al., 2020; Puchkov & Brygadyrenko, 2022; Langraf et al., 2022).

The cuticle of invertebrates is the first barrier for toxins. Insecticide-resistant invertebrates (insects, Acari) have a thickened cuticle. This is related to heightened expression of certain genes, coding enzymes, involved in cuticle sclerotization. Solid, thickened outer coatings of arthropods restrict the entry of insecticides. Many insecticide-tolerant arthropods were observed to have decreased rates of ingress of toxic compounds through the cuticle. There are some mechanisms by which invertebrates adapt to insecticides: namely decrease in sensitivity of protein – target of toxins, intensification of detoxication, and behavioural changes (choise of living location, food, etc.) that minimize contact with poison (Ottea et al., 2000; Soderlund & Knipple, 2003; Zhu et al., 2013). According to the literature data, insecticide-resistant species belong to different taxonomic groups. The best studied insects in this aspect are species of the Lepidoptera order (Noctuidae family) which are harmful agricultural pests. High resistance to cypermethrin was shown by a species of Noctuidae – *Spodoptera littoralis* (Boisduval, 1833), widely distributed in Africa, the Mediterranean region, and countries of the Near East. This pest of many cultivated plants is a quarantine pest in many countries. High tolerance to pyrethroid insecticides was found in many synanthropic species of the Diptera order (Drosophilidae, Muscidae, Culicidae) (Liu & Shen, 2003; Ahmad et al., 2006; Pan et al., 2009; El-Hassawy et al., 2014).

Conclusion

In a laboratory experiment, we researched sensitivity of 46 invertebrate species to a broadly used agricultural insecticide – cypermethrin. We observed a correlation between the length of arthropods and tolerance to insecticide. Small species with up to 6 mm body length were moderately tolerant (mean LD₅₀ value was 12.55 ± 4.71 g/ha). Median lethal doses for invertebrate species with longer, 6–14 mm, body were much higher. The most resistant species in this size group were earwigs (*Forficula auricularia* L.) and ground beetles (*Pseudoophonus rufipes* (De Geer), *Nebria brevicollis* (F.), *Calathus ambiguus* (Payk.), *C. fuscipes* (Goeze), and *Liodromus assimilis* (Payk.)). The highest median lethal doses were seen for species with over 15 mm body length: *Carabus coriaceus* L., *Pterostichus niger* (Schall.), and *P. melanarius* (Ill.). The results revealed a wide spectrum of LD₅₀ values, not always related to body sizes. Against the background of relatively high tolerance to cypermethrin in some species of Carabidae, there were cases of high sensitivity to the insecticide (*Ophonus rufibarbis* (F.)) which did not correlate with body length (7.5–10.0 mm). High sensitivity was observed in staphylinids. In species of this family which we studied, there were fluctuations in sensitivity to the neurotoxin. The most sensitive species were those of genus *Philonthus* (*Ph. decorus* (Grav.) – 0.0029 ± 0.0058 g/ha). Coprophilous staphylinids were more tolerant to cypermethrin.

The greater was weight of invertebrates, the more tolerant they were to cypermethrin. This correlation was seen in weight groups of 1.0–3.9 mg (1.9 ± 0.5 g/ha) and 16.0–63.9 mg (16.4 ± 3.2 g/ha). Difference between mean values of median lethal doses was significant. We determined corre-

lation between taxa and sensitivity to cypermethrin on the example of the most numerous taxa. Analysis of three taxonomic groups of insects (Carabidae, Formicidae, Staphylinidae) revealed that ground beetles had high resistance to the insecticide (mean LD₅₀ values ranged 24.00 ± 4.66 g/ha). Ants were averagely tolerant (8.60 ± 2.72 g/ha). Staphylinids were the least tolerant to cypermethrin (0.23 ± 0.08 g/ha). Relationship between trophic specialization of invertebrates and their tolerance to cypermethrin was found for phytophages and zoophages. Phytophages were represented by Hemiptera (Coreidae, Miridae, Pentatomidae, Lygaeidae) and Orthoptera (Acrididae), whereas zoophages were represented by centipedes (Lithobiidae), spiders (Lycosidae), and coleopterans (Carabidae, Staphylinidae, Silphidae, Histeridae). Mean values of median lethal doses for phytophages were 2.1 ± 0.5 g/ha, for zoophages these were much higher – 15.6 ± 3.3 g/ha.

The results of the laboratory studies revealed that the invertebrates significantly varied in sensitivity to cypermethrin. However, most species were observed to have a statistically significant relationship between tolerance to the insecticide and body sizes, taxa, and trophic specialization.

Any insecticide can have unpredictable effects on an invertebrate community. Therefore, exhaustive laboratory studies are the only means to accurately assess how toxic an insecticide is to a certain species. Further, it would be practical to conduct in-detail studies of sensitivity of non-target species of invertebrates (with a broadened range of taxa) to different groups of broadly used insecticides.

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