Science of the Total Environment 996 (2025) 180154

Contents lists available at ScienceDirect

Science of the Total Environment

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

Species Sensitivity Distribution (SSD) profiles of carabid beetle communities exposed to acetamiprid, chlorpyrifos or λ-cyhalothrin

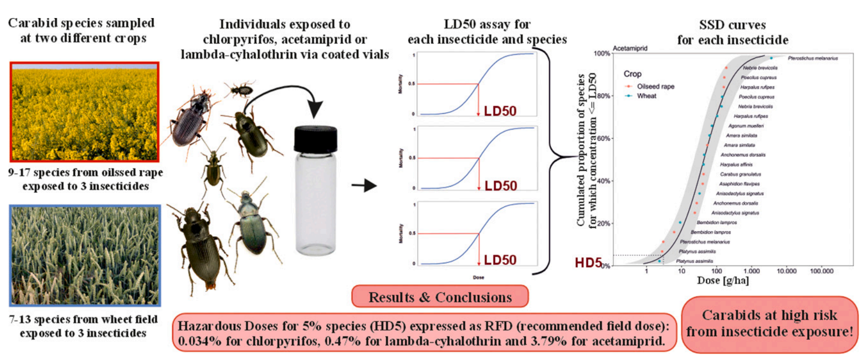
Agnieszka J. Bednarska [a,b,*], Grzegorz Sowa [a], Danuta Frydryszak [a], Renata Śliwińska-Grochot [a], Ryszard Laskowski [a]

[a] Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Gronostajowa 7, 30-378 Kraków, Poland
[b] Institute of Nature Conservation, Polish Academy of Sciences, A. Mickiewicza 33, 31-120 Kraków, Poland

HIGHLIGHTS

- Carabid beetles collected from agricultural landscapes.
- Species Sensitivity Distributions determined for chlorpyrifos, λ-cyhalothrin, and acetamiprid.
- Predicted impacted fractions were between 66 % and 100 % at recommended field doses.
- Chlorpyrifos posed the greatest hazard, and acetamiprid the least.
- Carabids are ecosystem service providers at high risk from insecticide exposure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
ground beetles
neonicotinoids
organophosphates
pyrethroids
HD5
LD_{50}
ecological risk assessment

ABSTRACT

Many carabid species are essential for pest management in agricultural areas, but at the same time can be exposed to pesticides. To understand how pesticides may affect ecosystem services provided by carabids, it is crucial to assess the effects of exposure not only for individual species but for whole communities. The objective of our study was thus to assess the distribution of sensitivity in carabid communities inhabiting agricultural landscapes toward three insecticides representing three major groups: neonicotinoid acetamiprid, organophosphate chlorpyrifos, and pyrethroid λ-cyhalothrin. Using a vial test, in which an active ingredient dissolved in acetone is evenly distributed inside a glass vial, we first assessed the median lethal doses (24-h LD_{50}) for each insecticide to 10–20 species collected in agricultural areas, which were further used to establish Species Sensitivity Distribution (SSD) profiles. Each insecticide tested caused a serious threat to beetle communities at the recommended field doses, with the strongest effect caused by chlorpyrifos and the weakest by acetamiprid. The estimated Potentially Affected Fraction (PAF) of species at doses of the insecticides recommended for field use was 100 % for chlorpyrifos, 99.8 % for λ-cyhalothrin, and ca. 66 % for acetamiprid. The Hazardous Doses for 5 % species (HD5) were 0.034, 0.47, and 3.79 % of their recommended field doses, respectively. Our findings prove that carabids are a group of ecosystem service providers at considerable risk from insecticide exposure.

* Corresponding author at: Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Gronostajowa 7, 30-378 Kraków, Poland.
E-mail addresses: a.bednarska@uj.edu.pl, bednarska@iop.krakow.pl (A.J. Bednarska).

https://doi.org/10.1016/j.scitotenv.2025.180154
Received 7 May 2025; Received in revised form 25 July 2025; Accepted 25 July 2025
Available online 30 July 2025

1. Introduction

The decline in insect biodiversity in the last few decades has been proven in many studies (e.g., Hallmann et al., 2017; Grevé et al., 2024; Sánchez-Bayo and Wyckhuys, 2019; Seibold et al., 2019), with species inhabiting open areas being the most endangered. The reason is that in large parts of the world, such areas are dominated by arable land where pesticides are extensively used to protect crops from weeds, arthropod pests, and diseases. Among the large and chemically diverse group of pesticides, insecticides play a special role in the decline of insect biodiversity (Geiger et al., 2010; Sánchez-Bayo and Wyckhuys, 2019), as most are not specific enough to eliminate only pests and disease-transmitting insects. This leads to detrimental effects on insects living in and around agricultural areas and the loss of important ecosystem services such as pollination, pest control, and nutrient cycling. As ecosystem service providers (ESPs) are essential for human well-being, the threat from pesticide use is becoming a major concern.

From the perspective of ecosystem services and overall ecosystem functioning, assessing the risks of pesticide use at the level of entire communities or ecological guilds is crucial, rather than focusing solely on individual species as current legal frameworks require. The two guilds that are most important economically are pollinators, without whom much of agricultural production would not be possible, and pest control agents, who help suppress agricultural pest populations. Of the latter, the ground beetles (Carabidae) are among the most important (Kromp, 1999) and have been frequently used as a model taxon in agricultural landscapes (Rischen et al., 2021; Deppe and Fischer, 2023).

As species differ in sensitivity to pesticides (and other stressors), this variation can be described by a statistical distribution – Species Sensitivity Distribution (SSD). The SSD became an invaluable tool in estimating concentrations (or doses) hazardous to a certain proportion (x%) of species (HCx, HDx) and the potentially affected fraction of species (PAF) at a given concentration or dose (cf. Posthuma et al., 2002). The U.S. Environmental Protection Agency (EPA) (1980) recommended a multispecies approach (although not called SSD at that time) for the derivation of water quality criteria as early as 1980. In that document, it was assumed that no more than 5 % of a freshwater community should be put at risk, and the final acute value (FAV) has been defined as the 5th percentile of a community represented by freshwater fish and invertebrates. Since then, in ecological risk assessment, the concentration (or dose) that puts at risk no more than 5 % of species in a community has been agreed as a benchmark (Van Straalen and Denneman, 1989). Thus, HC5 (HD5) is the concentration (dose) at which 5 % of species in the community reach the toxicity endpoint used to establish the SSD. The HC5 values derived from SSDs based on acute toxicity measures, such as 50 % effect concentration (EC_{50}) and median lethal concentration (LC_{50}), are primarily used in pesticide risk assessment (EFSA, 2013). To determine predicted concentrations that do not cause significant adverse effects in non-target species communities (PNECs) from SSD, ECHA (2008) recommends dividing HC5 (HD5) by an assessment factor (AF) of one to five, considering the following criteria: quality of the endpoints used (in particular whether the dataset covers chronic studies and different life stages), the diversity and representativeness of tested taxonomic groups, statistical uncertainty around the HC5 (HD5) estimate, etc. (see also Checkai et al., 2014).

Although the SSD technique dates back to the early, 1980s (U.S. EPA, 1980, but for an extensive review, discussion and examples see Posthuma et al., 2002), it was originally developed for aquatic communities and to date there are surprisingly few studies on the effects of insecticides on terrestrial communities (e.g., Blanco-Moreno et al., 2024; Humann-Guilleminot et al., 2019). This study aimed to fill this gap by estimating SSDs for carabids for three widely used insecticides representing three different chemical classes: acetamiprid, chlorpyrifos, and λ-cyhalothrin. Acetamiprid is currently the only neonicotinoid registered in the European Union. It was shown to have small effects on honey bees and bumble bees at field-realistic concentrations (Camp et al., 2020; EFSA, 2016; Tóth and Kovács, 2024; Varga-Szilay and Tóth, 2022). Still, Varga-Szilay and Tóth (2022) indicate that it is poorly studied, and more research is needed, especially considering that extensive use of acetamiprid may lead to high concentrations in the field. Also, the report by EFSA (2016), generally assessing the risk to soil organisms from exposure to acetamiprid as low, points to the need for further research into its potential negative effects on non-target arthropods. Chlorpyrifos is an organophosphate insecticide, widely used in agriculture and to control disease-transmitting insects. It has, however, well-proven toxic effects on various non-target organisms (Frampton and Van den Brink, 2007; Svobodová et al., 2020), being highly toxic to bees (Stanley et al., 2015), reducing the abundance of carabids (Asteraki et al., 1992; Luff and Rushton, 1989), termites, earthworms and organic matter decomposition rate (De Silva et al., 2010). Due to the accumulated evidence of serious negative effects on non-target organisms and ecosystem processes at recommended application rates, it was banned in 2016 in the UK, in 2020 in the EU and 2021 in the USA (but returned to the market of several states in 2023). In some countries, its use has been restricted to only certain crops and application methods (e.g., China, India, Thailand, Malaysia). However, it is still widely used in many countries, with global use estimated at 50,000 t annually (ECHA, 2022). Λ-cyhalothrin is a quick-acting pyrethroid, commonly used in the EU and elsewhere to control agricultural pests, mosquitoes, flies, and ticks (Depalo et al., 2022; Leprince et al., 1992). In recent years, the use of pyrethroid insecticides increased substantially due to the ban on chlorpyrifos (He et al., 2008; Hites, 2021) but λ-cyhalothrin also has some negative effects on non-target organisms. For example, Liao et al. (2018) found that even short-term exposure to λ-cyhalothrin reduced lifespan, affected learning and memory performance, and reduced the homing ability of worker honey bees. In turn, Blanco-Moreno et al. (2024) proved that this pesticide can cause strong negative effects in whole pest-control communities dominated by carabids and spiders across Europe in the acute contact test (the data in Blanco-Moreno et al. (2024) partly overlap with those presented here for λ-cyhalothrin). On the other hand, Wanner et al. (2005) did not find clear negative effects on the activity density of carabids in general, while the abundance of some species, e.g., *Carabus violaceus*, increased on plots treated with λ-cyhalothrin using foliar spray. The authors hypothesised that this might be caused by the abundance of dead insects on the treated plots which might have attracted necrophagous carabids which are highly mobile and can immigrate quickly after the spray. Given these inconclusive results for individual species, estimating SSDs for whole guilds or communities and the resulting HD5 and PAFs for the three insecticides provide better insight into the actual risks to ESPs and other non-target organisms associated with the use of each insecticide.

2. Materials and methods

Adult carabids were collected in the agricultural landscape of southern Poland using pitfall traps placed along borders of conventionally managed oilseed rape or wheat fields, depending on the crop rotation in the year of the survey. The beetles were collected every 2nd or 3rd day in the following periods: 27.04.2020–10.06.2020, 12.05.2021–30.07.2021, and 09.05.2022–08.07.2022 from three separate locations. After transporting to the laboratory, the beetles were identified to the species level with the use of the key of Hurka (1996), placed in transparent plastic boxes with perforated lids and kept in a climatic chamber at 20 ± 2 °C, relative humidity 70 ± 5 %, light intensity 600–700 lx and 16:8 h light:dark regime. The beetles were not fed either before or during the bioassay. After 24-h acclimation, the beetles were exposed to the tested insecticides using the insecticide-coated glass vial assay based on IRAC (Insecticide Resistance Action Committee) Method 031, described by Zimmer et al. (2014). The insecticides were applied to the vials (5 cm high, 2 cm diameter; internal glass surface area 34.56 cm^2, S Murray and Co, UK) as an acetone solution of the active ingredient, and the vials were rotated on a laboratory roller until the acetone had completely evaporated (~24 rpm for 2 h). Before preparing the insecticide-coated vials, the evenness of distribution of the solution on the vial walls was checked with a tinted acetone solution. Such prepared vials were capped, concentration-labelled, and stored at 4 °C until use. The beetles were exposed to five insecticide doses (plus solvent control), relative to the Recommended Field Dose (RFD), with specific doses for each insecticide determined in a range-finder to allow for precise estimation of 24-h LD_{50} values, which were later used to establish SSD curves. For acetamiprid (PESTANAL analytical standard, Sigma-Aldrich) and λ-cyhalothrin (PESTANAL analytical standard, Sigma-Aldrich), the doses were 0.8 % RFD, 4 % RFD, 20 % RFD, 100 % RFD, and 200 % RFD, and for chlorpyrifos (PESTANAL analytical standard, Sigma-Aldrich) 0.064 % RFD, 0.032 % RFD, 0.16 % RFD, 0.8 % RFD, and 4 % RFD. Because for different crops different field doses are recommended, we assumed the following values as best representing the application in the studied crops: for acetamiprid 1 RFD = 80 g a.i./ha, for chlorpyrifos 1 RFD = 288 g a.i./ha and for λ-cyhalothrin 1 RFD = 7.5 g a.i./ha.

The beetles were placed into vials individually to standardise between species of varied sizes and avoid potential interactions between individuals. In most cases, 60 individuals per species and crop were used, with 10 individuals per insecticide dose. Due to the limited availability of beetles, a smaller number of individuals were used in some assays, the most extreme cases being *Anisodactylus signatus* from oilseed rape in the acetamiprid test (23 individuals), *Carabus granulatus* from oilseed rape in the chlorpyrifos test (30 individuals), and *Asaphidion flavipes* from oilseed rape fields and *Amara aranea* from wheat fields in the λ-cypermethrin test (23 and 24 individuals respectively; see Tables 1–3 for more details). The available individuals were distributed among the treatments with different doses as evenly as possible. The physical condition of the tested individuals was checked 24 h after exposure. Three categories were used: 'mobile'– normally active and responsive, capable of moving in a coordinated way; 'paralysed' – showing signs of paralysis of the legs, incapable of coordinated movement; 'dead' – not responding to mechanical stimuli or showing minimal responses, e.g., antennal movements only. Even though insects exposed to insecticides, especially pyrethroids, often recover from temporary paralysis, in statistical analysis paralysed individuals were treated as dead, because in ecological reality, i.e. under field conditions, these beetles would have a negligible chance of survival, either because they are unlikely to recover or because they would become prey for predators due to their limited mobility.

To calculate LD_{50} values, Abbott's formula was used in those cases where control mortality was recorded. All bioassays with a control mortality >20 % were excluded from the analyses. The dose-response curves were fitted to Abbott-corrected mortality using generalised linear models with binomial error distribution and probit link function, using the glm function in base R (R Core Team, 2022). The LD_{50}s were estimated by applying the function dose.p in the package MASS for R (Venables and Ripley, 2002) to the fitted glms. The LD_{50}s are reported in grams of active ingredient per hectare (g a.i./ha) and as a percentage of the recommended field dose (% RFD). The SSDs were estimated with the R package ssdtools (Thorley and Schwarz, 2018). First, log-logistic, log-normal, and gamma distributions were fit to the data, and the SSD was estimated based on three or two distributions which differed only marginally based on Akaike's Information Criterion corrected for sample size (AICc). The average fit, with a 95 % confidence interval, was estimated using the distributions with AICc-based relative weights ≥0.05. The HD5 values were derived as the 5th percentile of the model-averaged SSDs and are reported both in g a.i./ha and relative to the recommended field dose (% RFD) with bootstrap-estimated 95 % confidence intervals. Because SSDs were estimated based on 24-h LD_{50}s, HD5 represents the dose at which populations of no more than 5 % of species would experience 50 % or greater mortality within 24 h after the exposure. Similarly, PAF represents the percentage of species in a community for which the recommended field dose results in 50 % or greater mortality within 24 h.

Table 1
Acetamiprid LD_{50}s for 24 h, reported as percent of the recommended field dose (% RFD) of 80 g a.i./ha and in g a.i./ha, for carabid beetles collected in oilseed rape and wheat crops. The very wide confidence intervals in two cases were due to a sharp increase in mortality between two consecutive doses, leading to a survival curve based on only two or three data points (see data in Supplementary Materials). N-number of individuals used in the assay.

Species	Crop	N	LD_{50} [% RFD]	LD_{50} [g a.i./ha]	95 % confidence interval [g a.i./ha]
Amara similata	Oilseed rape	60	68.0	54.40	22.89–129.30
Anchonemus dorsalis	Oilseed rape	60	33.8	27.06	14.63–50.06
Anisodactylus signatus	Oilseed rape	23	29.9	23.88	7.06–80.79
Asaphidion flavipes	Oilseed rape	56	50.1	40.09	19.30–83.26
Bembidion lampros	Oilseed rape	60	7.7	6.13	2.87–13.08
Carabus granulatus	Oilseed rape	57	53.0	42.43	20.57–87.50
Harpalus rufipes	Oilseed rape	60	200.0	160.00	147.30–173.79
Nebria brevicollis	Oilseed rape	60	236.5	189.20	0–2.26E+163
Platynus assimilis	Oilseed rape	59	3.5	2.79	1.56–5.00
Poecilus cupreus	Oilseed rape	59	207.2	165.79	0–1.37E+27
Pterostichus melanarius	Oilseed rape	48	3.8	3.01	1.54–5.88
Agonum muelleri	Wheat	42	91.5	73.18	41.82–128.08
Amara similata	Wheat	54	78.3	62.65	25.79–152.19
Anchonemus dorsalis	Wheat	60	55.1	44.08	17.19–113.01
Anisodactylus signatus	Wheat	55	40.7	32.55	17.24–61.49
Bembidion lampros	Wheat	60	11.4	9.12	3.66–22.74
Harpalus affinis	Wheat	60	53.1	42.46	19.82–90.95
Harpalus rufipes	Wheat	60	130.0	104.02	60.225–179.65
Nebria brevicollis	Wheat	60	173.0	138.36	54.39–351.96
Platynus assimilis	Wheat	36	2.9	2.34	1.05–5.22
Poecilus cupreus	Wheat	60	180.3	144.20	67.06–310.08
Pterostichus melanarius	Wheat	60	4568.9	3655.12	11.01–1.21E+6

3. Results

Altogether, 20 species were used which represented different breeding types (spring breeders and autumn breeders), food preferences (herbivorous, omnivorous, and carnivorous), wing morphologies (macropterous and brachypterous) and body sizes (small <5 mm), medium-sized (5–10 mm) and large (>10 mm) (Eyre et al., 2013) (see Table S1 in Supplementary Materials). The 24 h LD_{50}s for acetamiprid were estimated for 11 species collected from oilseed rape fields and 11 from wheat fields, for a total of 13 species, as most species occurred in both crops (Table 1). Ten species were tested for sensitivity to chlorpyrifos, including nine from oilseed rape and seven from wheat fields (Table 2). For λ-cyhalothrin, LD_{50}s were estimated for 20 species, including 17 from oilseed rape and 13 from wheat (Table 3). The probit regression mostly fitted the data well, with R^2 0.66–1 for acetamiprid, 0.75–1 for chlorpyrifos and 0.56–1 for λ-cyhalothrin. However, in several cases the regression could only be fitted to a subset of data points, resulting in very wide confidence intervals (Tables 1–3). Despite that, all calculated LD_{50}s were used in estimating SSDs.

As noted already in a rangefinder, chlorpyrifos applied even at a fraction of RFD appeared lethal to carabids, with the lowest LD_{50} of only 0.03 % RFD, and the highest at 3.60 % RFD (Table 3). Next in terms of

Table 2
Chlorpyrifos LD_{50}s for 24 h, reported as per cent of the recommended field dose (% RFD) of 288 g a.i./ha and in g a.i./ha, for carabid beetles collected in oilseed rape and wheat crops. The very wide confidence intervals in several cases were due to a sharp increase in mortality between two consecutive doses, leading to a survival curve based on only two or three data points (see data in Supplementary Materials). N-number of individuals used in the assay.

Species	Crop	N	LD_{50} [% RFD]	LD_{50} [g a.i./ha]	95 % confidence interval [g a.i./ha]
Agonum muelleri	Oilseed rape	60	0.357	1.028	0 – ∞
Amara similata	Oilseed rape	60	1.789	5.153	3.221–8.244
Bembidion lampros	Oilseed rape	60	0.170	0.489	0–3.07E+54
Carabus granulatus	Oilseed rape	30	1.781	5.130	0 - ∞
Harpalus affinis	Oilseed rape	60	0.357	1.028	0 - ∞
Harpalus rufipes	Oilseed rape	60	0.357	1.028	0 - ∞
Nebria brevicollis	Oilseed rape	60	0.549	1.580	0.681–3.664
Poecilus cupreus	Oilseed rape	60	0.666	1.919	0–2.03E+118
Pterostichus melanarius	Oilseed rape	60	0.618	1.779	0–3.48E+240
Anchomenus dorsalis	Wheat	60	0.066	0.189	0.103–0.345
Bembidion lampros	Wheat	60	0.031	0.089	0.039–0.205
Harpalus affinis	Wheat	60	0.066	0.189	0.103–0.345
Harpalus rufipes	Wheat	60	0.054	0.156	0.089–0.274
Nebria brevicollis	Wheat	60	3.602	10.373	3.697–29.104
Poecilus cupreus	Wheat	60	0.357	1.028	0 - ∞
Pterostichus melanarius	Wheat	60	0.358	1.030	0.644–1.64

lethal effects expressed in RFD was λ-cyhalothrin, with LD_{50}s ranging from 0.64 % RFD to 57.47 % RFD (Table 3). Acetamiprid treatment was the least lethal and the only one among the tested pesticides with LD_{50}s of several species above RFD, including one extreme case, ***Pterostichus melanarius***, with $LD_{50} = 4568$ % RFD. Apart from this special case, the LD_{50}s for acetamiprid were in the range of 2.92–236.5 % RFD (Table 1), with four species with LD_{50}s > 100 % RFD: *Harpalus rufipes*, *Nebria brevicollis*, *Poecilus cupreus* and *P. melanarius*. The particularly interesting case was the abovementioned *P. melanarius* whose population from wheat fields appeared extremely resistant to acetamiprid while in oilseed rape fields it was one of the most sensitive species to this insecticide and moderately sensitive to chlorpyrifos and λ-cyhalothrin.

When the dose is expressed in units of active ingredient per hectare, the toxicity ranking of the insecticides differs from the risk ranking based on recommended field doses. In that case, λ-cyhalothrin appeared most toxic (LD_{50}s 0.05–4.31 g a.i./ha), closely followed by chlorpyrifos (LD_{50}s 0.09–10.37 g a.i./ha). Acetamiprid was the least toxic, with LD_{50}s between 3.34 and 189.2 g a.i./ha, plus the extreme value of 3655 g a.i./ha for *P. melanarius* (Tables 1–3).

As could be assumed from the LD_{50}s alone, the SSD-estimated HD5 (Fig. 1, Table 4) expressed in RFD units was extremely low for chlorpyrifos: 0.034 % RFD (95 % CI, 0.012–0.115 % RFD). For λ-cyhalothrin, the HD5 was also below 1 % RFD, namely 0.47 % RFD (0.27–0.90 % RFD), and even for the least toxic acetamiprid the HD5 was only a fraction of the recommended field dose: 3.79 % RFD (95 % CI, 1.47–12.09 % RFD). The estimated PAFs at the recommended field doses were: 100 % (95 % CI, 99.8–100 %) for chlorpyrifos, 99.8 % (99–100 %) for λ-cyhalothrin, and 65.7 % (48.1–82.1 %) for acetamiprid.

Table 3
Λ-cyhalothrin LD_{50}s for 24 h, reported as per cent of the recommended field dose (% RFD) of 7.5 g a.i./ha and in g a.i./ha, for carabid beetles collected in oilseed rape and wheat crops. N-number of individuals used in the assay.

Species	Crop	N	LD_{50} [% RFD]	LD_{50} [g a.i./ha]	95 % confidence interval [g a.i./ha]
Abax parallelepipedus	Oilseed rape	60	1.04	0.078	0.039–0.157
Amara aenea	Oilseed rape	60	0.81	0.061	0.025–0.149
Amara similata	Oilseed rape	60	1.48	0.111	0.052–0.239
Anchomenus dorsalis	Oilseed rape	60	0.76	0.057	0.017–0.192
Anisodactylus sp.	Oilseed rape	60	0.90	0.070	0.042–0.116
Asaphidion flavipes	Oilseed rape	23	1.0	0.073	0.009–0.559
Bembidion lampros	Oilseed rape	60	3.17	0.238	0.105–0.539
Carabus granulatus	Oilseed rape	60	0.89	0.067	0.024–0.186
Harpalus affinis	Oilseed rape	60	0.69	0.052	0.010–0.281
Harpalus rufipes	Oilseed rape	60	57.47	4.310	2.002–9.278
Harpalus tardus	Oilseed rape	48	1.13	0.085	0.039–0.184
Nebria brevicollis	Oilseed rape	60	12.43	0.932	0.447–1.946
Notiophilus palustris	Oilseed rape	30	8.90	0.671	0.304–1.478
Platynus assimilis	Oilseed rape	42	12.44	0.933	0.369–2.360
Poecilus cupreus	Oilseed rape	60	3.41	0.256	0.132–0.495
Pterostichus melanarius	Oilseed rape	60	7.87	0.590	0.286–1.221
Zabrus tenebrioides	Oilseed rape	36	2.60	0.197	0.110–0.355
Agonum muelleri	Wheat	60	4.56	0.342	0.179–0.654
Amara aenea	Wheat	24	1.80	0.135	0.057–0.321
Amara similata	Wheat	60	1.00	0.077	0.048–0.126
Anchomenus dorsalis	Wheat	60	2.77	0.208	0.098–0.440
Anisodactylus signatus	Wheat	56	1.13	0.085	0.025–0.284
Bembidion lampros	Wheat	60	5.01	0.376	0.195–0.723
Calathus fuscipes	Wheat	36	15.70	1.174	0.645–2.140
Carabus granulatus	Wheat	48	2.80	0.212	0.127–0.353
Harpalus affinis	Wheat	60	1.79	0.134	0.084–0.215
Harpalus rufipes	Wheat	60	5.10	0.382	0.240–0.606
Platynus assimilis	Wheat	60	10.39	0.779	0.437–1.386
Poecilus cupreus	Wheat	60	10.85	0.814	0.392–1.688
Pterostichus melanarius	Wheat	60	5.28	0.396	0.209–0.748

4. Discussion

The coated glass tube test – a method that represents a worst-case scenario, with test individuals constantly exposed to the insecticide-covered glass surface – revealed significant adverse effects of all three insecticides on carabid communities. At the assumed recommended field dose of chlorpyrifos of 288 g a.i./ha, not only is the estimated PAF 100 %, meaning that LD_{50} is reached for 100 % species, but all LD_{50}s are 2–3 orders of magnitude lower than the RFD. While this may suggest almost complete eradication of carabids from chlorpyrifos-treated crops, toxicity testing under more realistic exposure would provide more information on the actual effects. In most field application scenarios, interception by leaves/crops occurs, which significantly reduces exposure at the soil surface. Nevertheless, the results obtained for chlorpyrifos are particularly worrying, especially considering that in some crops doses considerably higher than those used in our experiment are

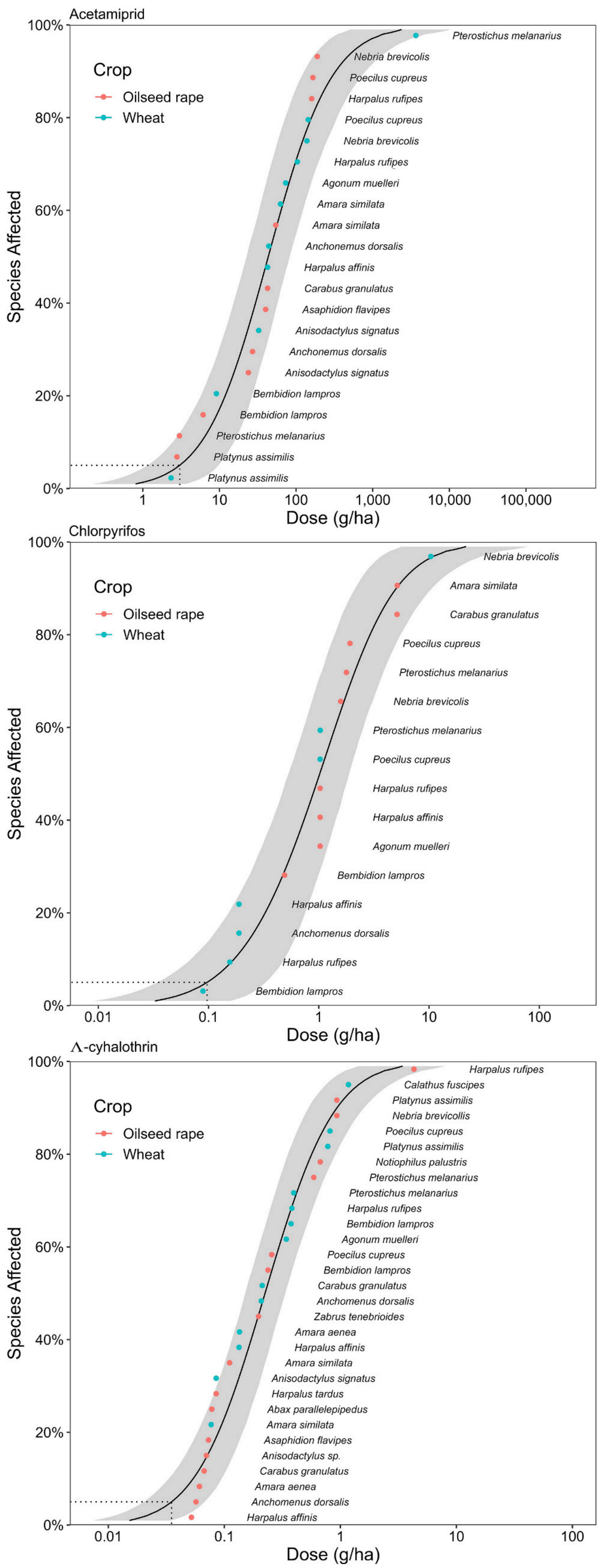


Fig. 1. Species sensitivity distributions (SSDs) for adult carabid beetles collected in 2020–2022 in southern Poland from oilseed rape (red) and wheat (blue) fields toward acetamiprid, chlorpyrifos, and λ-cyhalothrin.

Table 4
Predicted Affected Fraction (PAF) of carabid beetle communities at assumed recommended field doses (RFD) and hazardous doses for 5 % species (HD5) collected from oilseed rape and wheat crops in Poland and exposed for 24 h in glass vials coated with acetamiprid, chlorpyrifos and λ-cyhalothrin; S – number of species tested; n – number of data points per SSD (can be larger than the number of species if tested in more than one crop).

Pesticide (RFD)	S	n	PAF [%] (95 % CI)	HD5 [g a.i./ha] (95 % CI)	HD5 as% RFD (95 % CI)
Acetamiprid (80 g/ha)	13	22	65.7 (48.4–82.1)	3.03 (1.17–9.67)	3.79 (1.46–12.09)
Chlorpyrifos (288 g/ha)	10	16	100 (99.8–100)	0.097 (0.035–0.330)	0.034 (0.012–0.115)
λ-cyhalothrin (7.5 g/ha)	20	30	99.8 (99.0–100)	0.035 (0.020–0.068)	0.47 (0.27–0.90)

applied.

The results obtained for λ-cyhalothrin were slightly better, although the estimated PAF = 99.8 % RFD means that also in this case the recommended dose is higher than LD_{50}s of almost 100 % of species. However, in this case, LD_{50}s for a few species were higher than 10 % RFD, and for the least sensitive *Harpalus rufipes* from the oilseed rape field reached 57.5 % RFD. Still, as all LD_{50}s are much lower than the recommended field dose, the carabid beetle communities are expected to be significantly affected in λ-cyhalothrin-treated crops. However, as mentioned above, these tests represent the worst-case scenario, hence, actual effects in the field would probably be more acceptable than in the case of chlorpyrifos.

The only insecticide among the three tested that may not affect carabid communities to that great extent is acetamiprid. Although the recommended dose of acetamiprid resulted in reaching or exceeding LD_{50} in more than 66 % of species, several species showed high tolerance to the insecticide, with $LD_{50} > 100$ % RFD, and in only four cases (i.e., species/crop combinations) LD_{50}s were below 10 % RFD. This is in line with studies on acetamiprid toxicity to a range of species, including solitary bees (Mokkapati et al., 2021), honey bees (Badawy et al., 2015; Uhl et al., 2019), and bumble bees (Varga-Szilay and Tóth, 2022). Although risk assessment results indicate that field-realistic concentrations of acetamiprid may have minor acute lethal effects, further research is necessary to evaluate its potential sublethal impacts on non-target organisms. This is particularly important given the anticipated increase in acetamiprid use across Europe in the coming years, following the ban on other neonicotinoids, which could result in elevated field concentrations.

Research on insecticides has been skewed, with a recent emphasis on neonicotinoids stimulated largely by social and political debate (Godfray et al., 2014). There is a lack of data on the toxicity of older-generation insecticides (e.g., organophosphates), despite their continued large-scale use in many developing regions (Basu et al., 2024). In that context, it is worth noting that, similarly to our studies on carabids, chlorpyrifos has been shown highly toxic to the red mason bee, *Osmia bicornis*, whereas the pyrethroid cypermethrin and acetamiprid did not show acute toxicity to the bees when exposed topically at recommended field concentrations (Mokkapati et al., 2021).

It should be stressed, however, that from an exposure point of view, the method used in this study represents a worst-case scenario. The beetles were constantly exposed for 24 h to the insecticide at the tested doses, without a possibility of hiding or leaving the contaminated area. In nature, carabids are at least partly protected from the direct spray by plants and may spend part of the day hidden in soil. Also, carabid communities are not isolated from the surrounding habitats, so if non-sprayed areas are nearby, they can serve as refugia for beetles from the treated crops and as a source of healthy individuals in case of decreased densities of populations within the crop (Holland et al., 2016). Finally, it was assumed in this experiment that paralysed beetles had no chance of surviving and recovering and were treated as dead in the statistical analysis, which may not always be the case in the natural environment. Nevertheless, our assumption is in line with the EPA's guidelines on the protection of aquatic organisms (Stephan et al., 1985), which recommend using the percentage of immobilised individuals plus the percentage killed rather than mortality alone when calculating the final acute value. It is also important to remember that the idea behind applying the SSD approach to risk assessment is not to develop more realistic bioassays, but to draw conclusions for entire communities using data from standardised acute bioassays (here – the coated glass tube test, but any other acute test driving LC_{50} or LD_{50} could be used). On the other hand, the test duration was short (24 h), the beetles were exposed to a single insecticide at a time, and no sublethal effects of chronic exposure were investigated. If the carabids had been exposed to the insecticides for a longer time, the estimated LD_{50}s would likely have been lower than the 24-h LD_{50}s. Unfortunately, longer exposure resulted in elevated control mortality in several species and did not allow proper determination of SSD. Also, including sublethal effects, such as decreased fecundity or impaired mobility, would exacerbate the adverse effects on the populations. Finally, in conventional agriculture, several agrochemicals are usually used per crop in a season, which may result in difficult-to-predict synergistic effects. All this may weaken the 'worst-case scenario' argument outlined above.

Since crop type and its corresponding cultivation technology have an impact on the structure of local carabid communities (Eyre et al., 2013; Maksimovich et al., 2023), to increase the taxonomic and lifestyle diversity of the carabids used to estimate SSDs, we surveyed two crop types. Of the 10 species included in the SSD curves for all three insecticides, the small carnivorous *Bembidion lampros* was the most sensitive species to acetamiprid and chlorpyrifos, while the much larger herbivorous *Harpalus affinis* was the most sensitive to λ-cyhalothrin. Among the species most resistant to the insecticides were both the large autumn-breeding carnivorous *Nebria brevicolis* (in the case of acetamiprid and chlorpyrifos) and the large spring-breeding omnivorous *Harpalus rufipes* (in the case of λ-cyhalothrin). The special case of the autumn breeding carnivorous *Pterostichus melanarius*, which was the species most resistant to acetamiprid in wheat crop (LD_{50} = 3655 g a.i./ha) and the third most susceptible in oilseed rape crop (3.01 mg a.i./ha) deserves a separate discussion. One possible explanation could be that the result obtained for the wheat population was an experimental error of unknown origin. If this is the case, it should not be used in SSD estimation. However, considering that this test was based on 60 individuals and the mortality rate did not exceed 20 % even at the two highest doses, the more plausible explanation is that the wheat-originating population evolved a high resistance to acetamiprid due to repeated sprays with this insecticide or other neonicotinoids. While the three orders of magnitude difference in the sensitivity to a pesticide of a single species may seem unlikely, Blanco-Moreno et al. (2024) found an over 250-fold difference in sensitivity to λ-cyhalothrin among different populations of ladybird *Coccinella septempunctata* and parasitoid wasp *Diaeretiella rapae*. Among pest species, such large differences in sensitivity are not uncommon. For example, Ijaz et al. (2016) found 10,631-fold increased resistance to acetamiprid in populations of the cotton mealybug (*Phenacoccus solenopsis*) after 24 generations of selection. Basit et al. (2012) showed that only 10 generations of laboratory selection are sufficient to increase the resistance of the whitefly (*Bemisia tabaci*) to buprofezin 7000-fold, and Katundu and Aliniazee (1990) reported for the filbert aphid (*Myzocallis coryli*) up to 4090-fold resistance toward carbaryl and 49,096-fold against phosalone. Without knowing the actual reason for that large difference in sensitivity of *P. melanarius* between the two crop types, we decided to include it in SSD, especially since excluding this extreme LD_{50} value from SSD did not change the SSD-estimated endpoints significantly: the HD5 estimated from the complete dataset was 3.03 g a.i./ha (95 % CI, 1.17–9.67 g a.i./ha) while excluding this single data point decreased it to 2.89 g a.i./ha (95 % CI, 0.76–11.00 g a.i./ha). At the same time, after eliminating the extreme value, PAF increased from 65.7 g a.i./ha (95 % CI, 48.4–82.1 %) to 71.2 g a.i./ha (95 % CI, 55.1–86.4 %). This outcome indicates that SSDs based on >10 species and > 20 data points are robust to individual outliers which is important and good news for ecological risk assessment. Since it has been repeatedly shown that individual populations of a species can differ in their sensitivity to toxic chemicals by orders of magnitude, ecological risk assessment based on single-species tests is much more prone to error than if the SSD approach is used. In this context, it is also worth mentioning that *P. cupreus*, which is one of the species recommended by the International Organisation for Biological and Integrated Control for evaluating the side-effects of plant protection products on the non-target arthropods (Candolfi et al., 2000), belonged to the least sensitive carabids tested in our study.

We have shown that all tested insecticides at the assumed recommended field doses (RFDs) have serious negative effects on beetle communities, and thus can negatively affect the dynamics of food webs and the potential of beetles to suppress agricultural pest populations (Toft and Bilde, 2002; Kotze et al., 2011). Therefore, it is important to broaden our knowledge of the species sensitivity profiles for different taxonomic and functional groups of ESPs. A deeper knowledge based on the effects of pesticides representing different modes of action at the community level can then be used to develop a more robust ecological risk assessment framework.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180154.

CRediT authorship contribution statement

Agnieszka J. Bednarska: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Grzegorz Sowa:** Writing – review & editing, Investigation. **Danuta Frydryszak:** Investigation. **Renata Śliwińska-Grochot:** Project administration, Investigation. **Ryszard Laskowski:** Writing – original draft, Project administration, Methodology, Formal analysis, Conceptualization.

Funding

This research was funded by the European Union Horizon 2020 Research and Innovation Program "EcoStack" (grant agreement no. 773554) and supported by the statutory funds of the Institute of Nature Conservation, Polish Academy of Sciences.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Ryszard Laskowski reports financial support was provided by European Union. Financial support was provided by EU Framework Programme for Research and Innovation EcoStack (grant agreement no. 773554). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Sara Bąk, Karina Kapela, Ligia Kuriańska-Piątek for their assistance in laboratory and fieldwork. We also thank the University of Agriculture in Kraków for making the oilseed rape and wheat research fields available. The open-access publication of this article was funded by the programme "Excellence Initiative – Research University" at the Faculty of Biology of the Jagiellonian University in Kraków, Poland.

Data availability

Original survival data used to calculate LD_{50}s and then estimate SSDs are available in the Supplementary Materials.

References

Asteraki, E.J., Hanks, C.B., Clements, R.O., 1992. The impact of two insecticides on predatory ground beetles (Carabidae) in newly-sown grass. Ann. Appl. Biol. 120 (1), 25–39. https://doi.org/10.1111/j.1744-7348.1992.tb03400.x.

Badawy, M.E.I., Nasr, H.M., Rabea, E.I., 2015. Toxicity and biochemical changes in the honey bee *Apis mellifera* exposed to four insecticides under laboratory conditions. Apidologie 46 (2), 177. https://doi.org/10.1007/s13592-014-0315-0.

Basit, M., Saleem, M.A., Saeed, S., Sayyed, A.H., 2012. Cross resistance, genetic analysis and stability of resistance to buprofezin in cotton whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae). Crop Prot. 40, 16–21. https://doi.org/10.1016/j.cropro.2012.03.024.

Basu, P., Ngo, H.T., Aizen, M.A., Garibaldi, L.A., Gemmill-Herren, B., Imperatriz-Fonseca, V., Klein, A.M., Potts, S.G., Seymour, C.L., Vanbergen, A.J., 2024. Pesticide impacts on insect pollinators: current knowledge and future research challenges. Sci. Total Environ. 954, 176656. https://doi.org/10.1016/j.scitotenv.2024.176656.

Blanco-Moreno, J.M., Caballero-López, B., Cook, S.M., Foster, S.P., Frydryszak, D., Laskowski, R., Ortega-Ramos, P., Rasko, M., Reichardt, P., Sousa, J.P., Sowa, G., Śliwińska-Grochot, R., 2024. Species sensitivity distribution (SSD) profiles towards λ-cyhalothrin for key ecosystem service provider (ESP) species across five European countries representing different pedoclimatic zones. Sci. Total Environ. 954, 176412. https://doi.org/10.1016/j.scitotenv.2024.176412.

Camp, A.A., Williams, W.C., Eitzer, B.D., Koethe, R.W., Lehmann, D.M., 2020. Effects of the neonicotinoid acetamiprid in syrup on *Bombus impatiens* (Hymenoptera: Apidae) microcolony development. PloS One 15 (10), e0241111. https://doi.org/10.1371/journal.pone.0241111.

Candolfi, M.P., Blümel, S., Forster, R., Bakker, F.M., Grimm, C., Hassan, S.A., Heimbach, U., Mead-Briggs, M.A., Reber, B., Schmuck, R., 2000. In: Vogt, H. (Ed.), Guidelines to Evaluate Side-Effects of Plant Protection Products to Non-Target Arthropods. International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC/WPRS), 158 pp.

Checkai, R., Van Genderen, E., Sousa, J.P., Stephenson, G., Smolders, E., 2014. Deriving site-specific clean-up criteria to protect ecological receptors (plants and soil Invertebrates) exposed to metal or metalloid soil contaminants via the direct contact exposure pathway. Integr. Environ. Assess. Manag. 10, 346–357. https://doi.org/10.1002/ieam.1528.

De Silva, P.M.C.S., Pathiratne, A., Van Straalen, N.M., Van Gestel, C.A.M., 2010. Chlorpyrifos causes decreased organic matter decomposition by suppressing earthworm and termite communities in tropical soil. Environ. Pollut. 158, 3041–3047. https://doi.org/10.1016/j.envpol.2010.06.032.

Depalo, L., Pasqualini, E., Jan, E., Slater, R., Daum, E., Zimmer, C.T., Masetti, A., 2022. Evaluation of the susceptibility to emamectin benzoate and lambda cyhalothrin in European populations of Cydia pomonella (L.) (Lepidoptera: Tortricidae). Crop Prot. 157, 105968. https://doi.org/10.1016/j.cropro.2022.105968.

Deppe, F., Fischer, K., 2023. Landscape type affects the functional diversity of carabid beetles in agricultural landscapes. Insect Conserv. Divers. 16, 441–450. https://doi.org/10.1111/icad.12634.

ECHA, 2008. Guidance on information requirements and chemical safety assessment Chapter R.10: Characterisation of dose [concentration]-response for environment. European Chemicals Agency (ECHA), Helsinki, Finland.

ECHA, 2022. Chlorpyrifos. Draft risk profile. https://echa.europa.eu/documents/10162/8a51d7d9-e9a4-2513-e975-492fb70f825c accessed 17.09.2024.

EFSA (European Food Safety Authority), 2013. Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA J. 11, 3295. https://doi.org/10.2903/j.efsa.2013.3295.

EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment of the active substance acetamiprid. EFSA J. 14 (11), 4610, 26 pp. https://doi.org/10.2903/j.efsa.2016.4610.

Eyre, M.D., Luff, M.L., Leifert, C., 2013. Crop, field boundary, productivity and disturbance influences on ground beetles (Coleoptera, Carabidae) in the agroecosystem. Agric. Ecosyst. Environ. 165, 60–67. https://doi.org/10.1016/j.agee.2012.12.009.

Frampton, G.K., Van den Brink, P.J., 2007. Collembola and macroarthropod community responses to carbamate, organophosphate and synthetic pyrethroid insecticides: Direct and indirect effects. Environ. Pollut. 147, 14–25. https://doi.org/10.1016/j.envpol.2006.08.038.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Pärt, T., Bretagnolle, V., Plantegenest, M., Clement, L.W., Dennis, C., Palmer, C., Oñate, J.J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hänke, S., Fischer, C., Goedhart, P.W., Inchausti, P., 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic Appl. Ecol. 11, 97–105. https://doi.org/10.1016/j.baae.2009.12.001.

Godfray, H.C.J., Blacquière, T., Field, L.M., Hails, R.S., Petrokofsky, G., Potts, S.G., Raine, N.E., Vanbergen, A.J., McLean, A.R., 2014. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proc. R. Soc. B 281, 20140558. https://doi.org/10.1098/rspb.2014.0558.

Grevé, M.E., Marx, M.T., Eilmus, S., Ernst, M., Herrmann, J.D., Baden, C.U., Maus, C., 2024. Insect decline—evaluation of potential drivers of a complex phenomenon. Insects 15 (12), 1021. https://doi.org/10.3390/insects15121021.

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., De Kroon, H., 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PloS One 12 (10), e0185809. https://doi.org/10.1371/journal.pone.0185809.

He, L.M., Troiano, J., Wang, A., Goh, K., 2008. Environmental Chemistry, Ecotoxicity, and Fate of Lambda-Cyhalothrin. In: Whitacre, D.M. (Ed.), Reviews of Environmental Contamination and Toxicology, 195. Springer, New York, NY. https://doi.org/10.1007/978-0-387-77030-7_3. Rev. Environ. Contam. Toxicol.

Hites, R.A., 2021. The rise and fall of chlorpyrifos in the United States. Environ. Sci. Technol. 55 (3), 1354–1358. https://doi.org/10.1021/acs.est.0c06579.

Holland, J.M., Bianchi, F.J., Entling, M.H., Moonen, A.C., Smith, B.M., Jeanneret, P., 2016. Structure, function and management of semi-natural habitats for conservation biological control: a review of European studies. Pest Manag. Sci. 72 (9), 1638–1651. https://doi.org/10.1002/ps.4318.

Humann-Guilleminot, S., Binkowski, Ł., Jenni, L., Hilke, G., Glauser, G., Helfenstein, F., 2019. A nation-wide survey of neonicotinoid insecticides in agricultural land with implications for agri-environment schemes. J. Appl. Ecol. 56, 1502–1514. https://doi.org/10.1111/1365-2664.13392.

Hurka, K., 1996. Carabidae of the Czech and Slovak republics. Zlin, Czech Republic, Edit by Vit Kabourek.

Ijaz, M., Afzal, M.B.S., Shad, S.A., 2016. Resistance risk analysis to acetamiprid and other insecticides in acetamiprid-selected population of *Phenacoccus solenopsis*. Phytoparasitica 44, 177–186. https://doi.org/10.1007/s12600-016-0519-0.

Katundu, J.M., Aliniazee, M.T., 1990. Variable resistance of filbert aphid (Homoptera: Aphididae) to insecticides in the Willamette Valley, Oregon. J. Econ. Entomol. 83, 41–47. https://doi.org/10.1093/jee/83.1.41.

Kotze, D.J., Brandmayr, P., Casale, A., Dauffy-Richard, E., Dekoninck, W., Koivula, M.J., Lövei, G.L., Mossakowski, D., Noordijk, J., Paarmann, W., Pizzolotto, R., Saska, P., Schwerk, A., Serrano, J., Szyszko, J., Taboada, A., Turin, H., Venn, S., Vermeulen, R., Zetto, T., 2011. Forty years of carabid beetle research in Europe–from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation. ZooKeys 100, 55–148. https://doi.org/10.3897/zookeys.100.1523.

Kromp, B., 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agric. Ecosyst. Environ. 74, 187–228. https://doi.org/10.1016/S0167-8809(99)00037-7.

Leprince, D.J., Hribar, L.J., Foil, L.D., 1992. Evaluation of the toxicity and sublethal effects of lambda-cyhalothrin against horse flies (Diptera: Tabanidae) via bioassays and exposure to treated hosts. Bull. Entomol. Res. 82 (4), 493–497. https://doi.org/10.1017/S0007485300042565.

Liao, C., He, X., Wang, Z., Barron, A.B., Zhang, B., Zeng, Z., Wu, X., 2018. Short-term exposure to lambda-cyhalothrin negatively affects the survival and memory-related characteristics of worker bees *Apis mellifera*. Arch. Environ. Contam. Toxicol. 75, 59–65. https://doi.org/10.1007/s00244-018-0514-1.

Luff, M.L., Rushton, S.P., 1989. The ground beetle and spider fauna of managed and unimproved upland pasture. Agric. Ecosyst. Environ. 25, 195–205. https://doi.org/10.1016/0167-8809(89)90051-0.

Maksimovich, K.Y., Dudko, R.Y., Shatalova, E.I., Tsakalof, A.K., Tsatsakis, A.M., Golokhvast, K.S., Novikov, E.A., 2023. Species composition and ecological structure of ground beetles (Coleoptera, Carabidae) communities as biological indicators of the agro-environmental sustainability. Environ. Res. 234, 116030. https://doi.org/10.1016/j.envres.2023.116030.

Mokkapati, J.S., Wnęk, A., Laskowski, R., Bednarska, A., 2021. Acute oral and contact toxicity of three plant protection products to adult solitary bees *Osmia bicornis*. Pol. J. Environ. Stud. 30, 4105–4113. https://doi.org/10.15244/pjoes/130516.

Posthuma, L., Traas, T.P., Suter, G.W., 2002. General introduction to species sensitivity distributions. In: Posthuma, L., Suter, G.W., Traas, T.P. (Eds.), Species Sensitivity Distributions in Ecotoxicology, 2002. Lewis Publishers, Boca Raton, pp. 3–10. https://doi.org/10.1201/9781420032314.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

Rischen, T., Frenzel, T., Fischer, K., 2021. Biodiversity in agricultural landscapes: different non-crop habitats increase diversity of ground-dwelling beetles (Coleoptera) but support different communities. Biodivers. Conserv. 30 (13), 3965–3981. https://doi.org/10.1007/s10531-021-02284-7.

Sánchez-Bayo, F., Wyckhuys, K.A., 2019. Worldwide decline of the entomofauna: a review of its drivers. Biol. Conserv. 232, 8–27. https://doi.org/10.1016/j.biocon.2019.01.020.

Seibold, S., Gossner, M.M., Simons, N.K., Blüthgen, N., Müller, J., Ambarli, D., Ammer, C., Bauhus, J., Fischer, M., Habel, J.C., Linsenmair, K.E., Nauss, T., Penone, C., Prati, D., Schall, P., Schulze, E.-D., Vogt, J., Wöllauer, S., Weisser, W.W., 2019. Arthropod decline in grasslands and forests is associated with landscape-level drivers. Nature 574 (7780), 671–674. https://doi.org/10.1038/s41586-019-1684-3.

Stanley, J., Sah, K., Jain, S.K., Bhatt, J.C., Sushil, S.N., 2015. Evaluation of pesticide toxicity at their field recommended doses to honeybees, *Apis cerana* and *A. mellifera* through laboratory, semi-field and field studies. Chemosphere 119, 668–674. https://doi.org/10.1016/j.chemosphere.2014.07.039.

Stephan, C.E., Mount, D.I., Hansen, D.J., Gentile, J.H., Chapman, G.A., Brungs, W.A., 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. US Environmental Protection Agency Report, Office of Research and Development, Washington, DC.

Svobodová, Z., Skoková Habuštová, O., Spitzer, L., Sehnal, F., 2020. Importance of functional classification in the use of carabids for the environmental risk assessment of the GE crops and other agricultural practices. Insect Sci. 27, 375–388. https://doi.org/10.1111/1744-7917.12643.

Thorley, J., Schwarz, C., 2018. ssdtools: an R package to fit species sensitivity distributions. J. Open Source Softw. 3 (31), 1082. https://doi.org/10.21105/joss.01082.

Toft, S., Bilde, T., 2002. Carabid diets and food value. In: Holland, J.M. (Ed.), The Agroecology of Carabid Beetles. Intercept, Andover, United Kingdom, pp. 81–110.

Tóth, Z., Kovács, Z., 2024. Chronic acetamiprid exposure moderately affects the foraging behaviour of buff-tailed bumblebees (*Bombus terrestris*). Ethology 130, e13425. https://doi.org/10.1111/eth.13425.
U.S. EPA, 1980. Fed. Regist. 45 (231), November 28: 79318–79379.
Uhl, P., Awanbor, O., Schulz, R.S., Brühl, C.A., 2019. Is *Osmia bicornis* an adequate regulatory surrogate? Comparing its acute contact sensitivity to *Apis mellifera*. PloS One 14 (8), e0201081. https://doi.org/10.1371/journal.pone.0201081.
Van Straalen, N.M., Denneman, C.A.J., 1989. Ecotoxicological evaluation of soil quality criteria. Ecotoxicol. Environ. Saf. 18, 241–251. https://doi.org/10.1016/0147-6513(89)90018-3.
Varga-Szilay, Z., Tóth, Z., 2022. Is acetamiprid really not that harmful to bumblebees (Apidae: *Bombus* spp.)? Apidologie 53, 2. https://doi.org/10.1007/s13592-022-00909-6.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics With S, Fourth edition. Springer, New York. 0–387–95457-0.
Wanner, M., Wiesener, C., Otto, L., Xylander, W.E., 2005. Short-term effects of a nun moth suppression programme (*Lymantria monacha*), (Lepidoptera: Lymantriidae) on epigeic non-target arthropods. J. Pest. Sci. 78 (1), 7–11. https://doi.org/10.1007/s10340-004-0060-7.
Zimmer, C.T., Müller, A., Heimbach, U., Nauen, R., 2014. Target-site resistance to pyrethroid insecticides in German populations of the cabbage stem flea beetle, *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae). Pestic. Biochem. Physiol. 108, 1–7. https://doi.org/10.1016/j.pestbp.2013.11.005.