

# **Chemical sensitivity of spiders and earthworms: effects of ecology, phylogeny and habitat**

by

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# Abstract

Biodiversity has declined by approximately 70% in the last 50 years for vertebrate and invertebrate species. This loss in biodiversity is strongly connected with anthropogenic activities, such as agricultural intensification and pollution. Currently, pesticides are needed to secure the growing global food demand, although they are recognized as one of the main drivers of biodiversity loss, mainly in agricultural areas.

In the European Union, pesticides are regulated within the risk assessment framework, which aims to protect both the environment and human health from undesirable effects. The effects on non-target organisms are mostly assessed following a “one-size-fits-all” approach, focused on sensitive species tests. However, it has been recognized that the current methodology can be improved in order to minimize undesirable effects. Aiming to provide valuable data to inform future risk assessment, this thesis focused on two terrestrial organism groups that play beneficial roles, especially in agroecosystems: earthworms and spiders.

Although the earthworm *Eisenia fetida* is included in pesticide regulation, its use as the only earthworm representative may lead to uncertainties for the risk assessment. Therefore, we collected ecotoxicological data on field-captured earthworm species via acute exposure to imidacloprid and copper. In addition, we investigated the relationships between earthworm chemical sensitivity, biological traits and habitat preferences, and potential links with their ecosystem services (Chapter 2). We found that earthworms sampled from extremely acidic soils were less sensitive to copper than earthworms from neutral soils. Moreover, anecic and endogeic earthworms were more sensitive to imidacloprid than epigeic earthworms.

Spiders have, thus far, been understudied in regulatory risk assessment in comparison to other non-target arthropods. Thus, we aimed to collect ecotoxicological data of spider species sampled in different European climates via acute exposure to lambda-cyhalothrin. Moreover, we explored relationships between spider chemical sensitivity, phylogeny, biological traits and habitat preferences, as well as potential links with their ecosystem services (Chapter 3). Spiders showed a high sensitivity to lambda-cyhalothrin. Furthermore, our results showed that spider sensitivity varies depending on climate. We confirmed this relationship by incorporating different rearing and test temperatures into the toxicity testing protocol (Chapter 4).

The outcomes of this thesis contribute to informing pesticide regulatory practices, allowing for an improved protection and conservation of terrestrial organism groups and the ecosystem services they provide. The consideration of ecological traits, habitat variability and related plasticity, key species, and ecological network structure could improve the risk assessment framework and minimize the effects of pesticides and other stressors on an ecosystem-level.

# Chapter 1

General introduction

*Tomás Duque*

## **Biodiversity loss**

On a global scale, the relative abundance of terrestrial, freshwater and marine vertebrate populations has declined by 69% on average between 1970 and 2018 (WWF, 2022). Similar rates of decline have been observed for entomofauna (e.g., Hallmann et al., 2017), which may lead to a global extinction rate of around 1% per year of terrestrial insects species in the next decades (van Klink et al., 2020a; van Klink et al., 2020b). The high rates of biodiversity loss have been directly linked with anthropogenic activities. Researchers have identified five major drivers responsible of biodiversity decline: habitat loss, pollution, invasive species, overexploitation and climate change (Díaz et al., 2019). In addition, the world's human population is continuously growing and is predicted to reach 9.7 billion in 2050 (UN, 2022). As a consequence, agricultural intensification is also expected to increase in the next years, to meet the growing food demand.

Agricultural intensification will require larger arable areas and use of agrochemicals, such as pesticides, resulting in a major threat to biodiversity (Abudulai et al., 2022; Emmerson et al., 2016). Pesticide application is recognized as the main driver of biodiversity loss in agricultural areas, as they can also affect several non-target organisms (Beaumelle et al., 2023; Geiger et al., 2010). Moreover, pesticides can unintentionally reach areas outside agroecosystems and may also affect other aquatic and terrestrial non-target organisms (Kaur & Garg, 2014). Hence, to mitigate adverse effects of pesticides on non-target organisms and ensure safe use, pesticides must undergo rigorous testing before they can be approved to sell on the market.

## **Regulatory risk assessment in the European Union**

The European Food Safety Authority (EFSA) is the responsible authority for pesticide registration in the European Union (EU) under the Regulation (EC) No. 1107/2009. The main goal of the pesticide regulatory process is to achieve a high level of protection of the environment, human and animal health, as well as to safeguard agricultural production (EU, 2009). In the context of environmental protections, the EFSA has developed and published several scientific opinions and guidance documents describing the requirements for assessing the risk of pesticides for different aquatic and terrestrial organism groups (e.g., EFSA, 2010). If needed, guidance

documents are revised, improved and updated based on scientific evidence identifying gaps in the risk assessment process. For example, the guidance document on the risk assessment of pesticides on bees was first published in 2013, revised in 2019 and updated in 2023 (EFSA, 2023).

Nevertheless, it has been recognized that the current risk assessment methodology can be improved by a more responsive regulatory framework to minimize undesirable effects (Brühl & Zaller, 2019; Storck et al., 2017). As a response, the EFSA is aiming to shift the focus of the pesticide risk assessment towards incorporating ecosystem services, multiple stressors and environmental compartments (Devos et al., 2019; EFSA, 2016b). Hence, ecotoxicological data need to be linked with ecosystem functions and services to identify sensitive organism communities and protect their ecological roles (Faber et al., 2019; Maltby et al., 2017). Additionally, the development of a new regulatory risk assessment framework has been suggested to consider the recovery of terrestrial organism groups at relevant spatial and temporal scales (EFSA, 2016a). However, ecotoxicological information of different taxa, except for a few standard test species, is lacking, especially for terrestrial organisms. Hence, this thesis is focused on taxa of two terrestrial organism groups that provide beneficial ecosystem services, especially in agroecosystems: earthworms and spiders, to provide valuable data and inform future risk assessment.

## **Earthworms**

Considered ecosystem engineers (Jouquet et al., 2006), earthworms play key roles in soil formation (Edwards, 2004), soil structuring (Kavdir & İlay, 2011) and soil fertility (Edwards & Bohlen, 1996). Moreover, they are suitable bioindicators of soil contamination with toxic substances, e.g., heavy metals (Suthar et al., 2008) and pesticides (Pelosi et al., 2014). The compost earthworm *Eisenia fetida* (SAVIGNI, 1826) has been used as the standard organism for the risk assessment of pesticides for in-soil organisms (EU, 2011). *E. fetida* meets the basic requirements for being a standard organism because it is easy to rear and reproduce under laboratory conditions (Paradise, 2001). Several standardized guidelines have been developed to assess the acute (ISO, 2012; OECD, 1984), chronic and sublethal effects (ISO, 2008, 2023; OECD, 2016) of pesticides and other pollutants on *E. fetida* under laboratory conditions.

Although pesticide testing on *E. fetida* is well-established for regulatory purposes, its use as the only earthworm representative has often been criticized. *E. fetida* is not a typical soil species (Krück, 2018) and there is a lack of realism when its chemical responses are extrapolated to other earthworm species with different functional roles in the field (Edwards, 2002). Nevertheless, earthworm ecotoxicological data is mainly based on *E. fetida* tests, representing almost 80% of soil acute earthworm toxicity studies (EPA, 2022). Moreover, there is a lack of information on more sensitive endpoints, as only 16% of available toxicity data have assessed sublethal effects of pesticides on earthworms (EPA, 2022). Therefore, the current risk assessment leads to uncertainties when *E. fetida* responses are extrapolated to different ecosystems inhabited by earthworms with variable chemical sensitivities, and fails to establish links with their ecosystem services (Forbes et al., 2021). Consequently, the inclusion of more ecologically-relevant earthworm species would be required in an improved future risk assessment.

## Spiders

Distributed in most terrestrial ecosystems, spiders are among the most abundant and diverse predators (Wise, 1993). They also play a key role in biocontrol as they consume a variety of insects, including pest species (Nyffeler, 1999). This function is especially relevant for agroecosystems (Michalko et al., 2019). However, their beneficial role can be disrupted by negative effects from the application of pesticides (Reiff et al., 2023; Theiling & Croft, 1988). Previous research has identified that acaricides and synthetic insecticides are among the most toxic compounds to spiders, affecting survival and spider performance (Pekár, 2012). As a result, spider abundance and their services may be reduced in field scenarios (Rodrigues et al., 2013).

Despite the known adverse effects of pesticides, spiders have received little attention in risk assessment compared with other non-target arthropod taxa (EFSA, 2015). In addition, spiders are not routinely included as test organisms for the risk assessment of pesticides in Europe (European Commission, 2013). Although some methods for testing pesticides on spiders from the genus *Pardosa* (Wehling et al., 1998) and the family Linyphiidae (Aukema et al., 1990) have been proposed, so far no standard protocol applicable to all spider taxa has been accepted for regulatory risk

assessment. In addition, spider pesticide sensitivity varies between different laboratory test designs. This variation has been mainly attributed to abiotic parameters of the test design, such as temperature (Jagers Op Akkerhuis et al., 1997). Apart from test temperature, their climatic origin and rearing conditions may influence the pesticide sensitivity of spiders (Duque et al., 2023), but information about potential interactions of test temperature, rearing temperature and climatic origin on spider sensitivity is lacking. In summary, spider ecotoxicological data is scarce compared to other predators (Pekár, 2012) and future risk assessment should consider the inclusion of these organisms to protect their ecosystem services, e.g., biocontrol (Michalko et al., 2019).

## Research questions

Earthworms and spiders provide beneficial functions in soil processes and pest control, respectively. These key roles are especially relevant in agroecosystems, making them suitable test organisms to study the link between chemical sensitivity and ecosystem services. Hence, the following questions were assessed in this thesis: (1) What is the relationship between earthworm chemical sensitivity, their biological traits and ecosystem characteristics and how can this relationship be connected to their ecosystem services? (2) What is the relationship between spider chemical sensitivity, phylogenetic signal, traits and habitat preferences and how can this relationship be connected to their ecosystem services? (3) Can the interactions between climatic origin, rearing and test temperature influence the chemical sensitivity of spiders?

*(1) What is the relationship between earthworm chemical sensitivity, their biological traits and ecosystem characteristics, and how can this relationship be connected to their ecosystem services?*

Most of the available earthworm ecotoxicological data has been derived using the species *Eisenia fetida* (Pelosi et al., 2014). However, *E. fetida* is not relevant for agroecosystems, and it is generally less sensitive than other earthworm species (Short et al., 2021). Thus, the extrapolation of its responses to pesticides to earthworm species found in the field may lead to uncertainties in risk assessment (Edwards, 2002). We collected information on the sensitivity of five earthworm field species (*Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea longa*,



*Aporrectodea rosea*, and *Lumbricus rubellus*), as well as *E. fetida*, to imidacloprid and copper in single-species acute toxicity tests. We derived species sensitivity distributions (SSD) for both pesticides and examined the relationship between earthworm traits (ecotype and weight), habitat characteristics (ecosystem type and soil pH), and chemical sensitivity. Moreover, we aimed to establish a link between earthworm chemical sensitivity and their ecosystem services (see Chapter 2).

(2) *What is the relationship between spider chemical sensitivity, phylogenetic signal, traits and habitat preferences, and how can this relationship be connected to their ecosystem services?*

Spiders may be adversely affected by the application of pesticides (Reiff et al., 2023; Theiling & Croft, 1988), yet they have received little attention in ecotoxicology compared to other non-arthropod taxa (Pekár, 2012). We collected information on the sensitivity of 28 spider species in single-species acute toxicity tests with lambda-cyhalothrin. We derived SSDs to identify sensitive species, and examined the relationship between spider chemical sensitivity, phylogenetic signal, traits (foraging mode and weight) and habitat preferences. In addition, we established possible implications of pesticide exposure on their ecosystem services (see Chapter 3).

(3) *Is there a relationship of climatic origin, rearing and test temperature with the chemical sensitivity of spiders?*

In the previous chapter, climatic origin had a strong influence on spider chemical sensitivity. Spiders originating from boreal and polar climates were more sensitive than spiders collected in cool temperate and warm temperate regions. However, other factors under laboratory conditions, such as the rearing or test temperature, may also influence their sensitivity (Everts et al., 1991; Jagers Op Akkerhuis et al., 1997). We examined the influence of climatic origin, and rearing and test temperature on the chemical sensitivity of *Pardosa amentata*. This species showed different sensitivities depending on their origin (Chapter 3). *P. amentata* was collected in boreal and cool temperate climates, reared in the laboratory at three different temperatures (15, 20 and 25°C) and tested with the insecticide lambda-cyhalothrin in a crossed treatment design at the test temperatures of 15, 20 and 25°C (see Chapter 4).

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# Chapter 2

Variation in the Chemical Sensitivity of Earthworms from Field Populations to  
Imidacloprid and Copper

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## Hazard/Risk Assessment

## Variation in the Chemical Sensitivity of Earthworms from Field Populations to Imidacloprid and Copper

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**Abstract:** The chemical risk of pesticides for nontarget soil macroorganisms has mainly been assessed using the compost earthworm *Eisenia fetida*. However, *E. fetida* does not occur in agroecosystems, and it is generally less sensitive than other earthworm species. Thus, the extrapolation of its response to pesticides to other earthworm species may lead to uncertainties in risk assessment. Because toxicity data for other earthworms are scarce, we assessed the chemical sensitivity of five species (*Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea longa*, *Aporrectodea rosea*, and *Lumbricus rubellus*) from different habitats (forests, wetlands, and grasslands), as well as *E. fetida*, to imidacloprid and copper in single-species acute toxicity tests. In addition, we examined the relationship between earthworm traits (ecotype and weight), habitat characteristics (ecosystem type and soil pH), and chemical sensitivity. The lower limits of the hazardous concentration affecting 5% (HC5) of species were 178.99 and 0.32 mg active ingredient/kg dry weight for copper and imidacloprid, respectively. Some concentrations that have been measured in European agroecosystems for both pesticides were above the HC5s, indicating toxic risks for these organisms. Furthermore, soil pH from the sampling habitat played a significant role, with earthworms sampled from extremely acidic soils being less sensitive to copper than earthworms from neutral soils. In addition, endogeic earthworms were more sensitive to imidacloprid than epigeic earthworms. This may translate to changes in soil functions such as bioturbation, which is mainly carried out by endogeic earthworms. Our results suggest that risk assessment should include a wider range of earthworms covering different habitats and ecosystem functions to achieve a better protection of the biological functions carried out by these key soil organisms. *Environ Toxicol Chem* 2023;42:939–947. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

**Keywords:** Earthworms; Risk assessment; Species sensitivity distribution

## INTRODUCTION

Earthworms are ecosystem engineers (Jones et al., 1994; Jouquet et al., 2006), playing key roles in pedogenesis (Edwards, 2004; Lee & Foster, 1991), soil structure (Bernier, 1998; Kavdir & İlay, 2011), and soil fertility (Edwards & Bohlen, 1996). In addition to their ecological relevance, earthworms are bio-indicators of soil pollution caused by toxic substances such as heavy metals (Suthar et al., 2008) and pesticides (Pelosi et al., 2014). The compost earthworm *Eisenia fetida* (SAVIGNI, 1826) has been used as a standard organism for the risk

assessment of pesticides on nontarget soil macroorganisms in the European Union, regulated by the European Commission (EC) under legislation 1107/2009 (European Union, 2011) and guidance document SANCO/10329/2002 (Santé et Consommateurs Directorate General Health and Consumers, 2002). *Eisenia fetida* is considered to be a species complex (Römbke et al., 2016) consisting of at least *E. fetida* and *Eisenia andrei* (BOUCHÉ, 1972). This species meets the basic requirements for being a standard test organism because it is easy to rear under laboratory conditions (Paradise, 2001). Consequently, several standardized guidelines have been developed to assess the acute (International Organization for Standardization [ISO], 2012; Organisation for Economic Co-operation and Development [OECD], 1984), as well as chronic, and sublethal effects (ISO, 2008; OECD, 2016) of pesticides and other chemicals on *E. fetida* under laboratory conditions.

Pesticide testing on *E. fetida* is well established for regulatory purposes (Edwards, 2004). However, its use as the

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only earthworm representative has often been criticized because of the lack of realism in terms of extrapolating its responses to chemicals to other species with different functional roles found in the field (Edwards, 2002). Therefore, current risk assessment leads to uncertainties when *E. fetida* responses are extrapolated to different ecosystems inhabited by earthworms with variable chemical sensitivity (Forbes et al., 2021). In addition, the European Food Safety Authority (EFSA) is aiming to shift the focus of the pesticide risk-assessment protection goals on biodiversity and ecosystem services by integrating multiple stressors, scales, and environmental compartments (Devos et al., 2019; EFSA Scientific Committee, 2016). Hence, ecotoxicological data need to be linked with ecosystem functions and services to identify sensitive communities and protect their ecological roles (Faber et al., 2019; Maltby et al., 2017). However, toxicity information for soil organisms is generally scarce (Frampton et al., 2006; Weyers et al., 2004) and, specifically for earthworms, mainly comes from tests with *E. fetida* (Forbes et al., 2021; Pelosi et al., 2014), making up almost 80% of soil acute earthworm toxicity studies (US Environmental Protection Agency [USEPA], 2022). Therefore, a data gap to future risk assessment has been recognized where toxicity data derived from multiple field earthworm species is required to establish links with their ecosystem functions (Forbes et al., 2021).

The present study aimed to provide information on the sensitivity of six earthworm species toward acute chemical exposure. For this purpose, we conducted single-species tests based on the soil test described in OECD guideline 207 (OECD, 1984) using earthworms sampled from different habitats, such as grasslands, forests, and wetlands, as well as *E. fetida*. Insecticides and fungicides are typically the most toxic pesticides for earthworms (Pelosi et al., 2014). Hence, we chose the insecticide imidacloprid and a copper-based fungicide (copper II sulfate pentahydrate) for the present study. Also, both substances have been tested on other earthworm species in addition to *E. fetida* and, therefore, provide further data which may be compared and used to complement our results (see Capowicz et al., 2005; Haque & Ebing, 1983). Imidacloprid acts on the nervous system, blocking nicotinic acetylcholine receptors (Talcott, 2013), and is known to be toxic for earthworms at low soil concentrations (i.e., 0.2 mg/kg; Zang et al., 2000). Copper acts by denaturing proteins and enzymes in an organism's cells (Dalecki et al., 2017), and long-term use causes its accumulation in soil (Fagnano et al., 2020). Thus, copper can be present at high concentrations, >200 mg/kg, in soils treated with this fungicide, especially in vineyards (Komárek et al., 2010; Steinmetz et al., 2017), which may pose a risk for earthworms (Streit, 1984). Thus, we aimed to derive species sensitivity distributions (SSDs) of earthworms for both copper and imidacloprid and the hazardous concentrations which affect 5% of the species (HC5), together with their lower 95% confidence limit (Newman et al., 2000; Posthuma et al., 2001). In addition, we explored relationships between earthworm chemical sensitivity, their biological traits, and ecosystem characteristics.

## MATERIALS AND METHODS

### Source of earthworms and ecosystem characterization

Adult earthworms from the species *Allolobophora chlorotica* (SAVIGNY, 1826), *Aporrectodea caliginosa* (SAVIGNY, 1826), *Aporrectodea longa* (UDE, 1885), *Aporrectodea rosea* (SAVIGNY, 1826), and *Lumbricus rubellus* (HOFFMEISTER, 1843) were collected by hand in winter and fall of 2020 around Landau in der Pfalz, Germany (Supporting Information, Table S1). Sampling sites, located outside of agricultural areas, were selected to cover the major ecosystems of the region, that is, grasslands, forests, and wetlands (Supporting Information, Table S1), including both acidic and neutral soils. The same ecosystems were sampled for imidacloprid and copper tests, and dominant species at the sampling time were collected (Supporting Information, Table S1). Live organisms were first identified to species in situ, which was confirmed prior to the test following the identification keys of Bährmann & Müller (2015) and Krück (2018). Earthworms were then stored in 1-L polypropylene containers (18 cm length × 13.2 cm width × 6.8 cm height) with approximately 700 g of natural soil (i.e., soil collected from their ecosystem of origin; Supporting Information, Table S1), which had been transported to the laboratory and stored for acclimatization in a climate chamber ( $16 \pm 1^\circ\text{C}$ ,  $65 \pm 10\%$  humidity,  $600 \pm 200$  lux, and 16:8-h light: dark cycle) for 1 week prior to the ecotoxicological assessment. In addition, the pH of the natural soil (Supporting Information, Table S1) was measured at the laboratory in 0.01 M  $\text{CaCl}_2$  following ISO (2005). Natural soil pH was classified according to the *Soil Survey Manual* (Soil Science Division Staff, 2017) as follows: extremely acidic (3.5–4.4), slightly acidic (6.1–6.5), and neutral (6.6–7.3). Because *E. fetida* is not a typical soil species (Krück, 2018), this species was obtained from a domestic compost pile (Supporting Information, Table S1) as well as from a laboratory culture (ECT Oekotoxikologie, Flörsheim/Main, Germany).

### Pesticides

The insecticide imidacloprid (Kohinor<sup>®</sup> 70 WG; Leu+Gygax, Birmenstorf, Switzerland; 70% active ingredient [a.i.]) and the fungicide copper II sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; Centrum Metal Odczynnik Chemiczne, Falenty, Polen; 25% a.i.) were used in the present study. For the ecotoxicological assessment, the substances were weighed to the nearest 0.01 mg (AT261 DeltaRange<sup>®</sup> 205 g/0.01 mg; Metler Toledo) and diluted in ultrapure water, and stock solutions were created using serial dilutions.

### Ecotoxicological assessment

The mortality tests were based on OECD guideline 207 (OECD, 1984), with the following adaptations. Instead of artificial soil, we used the standard soil LUFA 2.2 (Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany; Supporting Information, Table S2) as the test

substrate, which is widely used as a standard soil for the ecotoxicological assessment of soil invertebrates (Løkke & van Gestel, 1998). In addition, the test temperature was decreased from 20 °C to 16 °C, which is a more typical temperature for field situations (Lowe & Butt, 2005). Range-finding tests for both substances were done based on previously reported *E. fetida* median lethal concentrations (LC50s): 2.26 mg/kg for imidacloprid (Wang et al., 2019) and 643 mg/kg for copper (Neuhauser et al., 1985). For the tests, geometric series with seven concentrations of imidacloprid (ranging from 0 to 5.41 mg a.i./kg; Supporting Information, Table S3) or copper (ranging from 0 to 1075.6 mg a.i./kg; Supporting Information, Table S3) were tested on earthworms from the same species and ecosystem, with one experimental unit per concentration. An experimental unit consisted of approximately 690 g of moist LUFA soil (Supporting Information, Table S2), spiked with 20 ml (to achieve a final soil moisture of ~20%; Supporting Information, Table S3) of the desired test concentration together with 10 earthworms. Ultrapure water was used for the control. After spiking, the soil was thoroughly mixed, homogenized, and transferred to 1-L polypropylene containers (18 cm length × 13.2 cm width × 6.8 cm height). At the beginning of the test, the earthworms were weighed (Supporting Information, Table S1) to the nearest mg (PA214<sup>®</sup> 210 g/0.0001 g; OHAUS) and introduced to the soil immediately after spiking. Test boxes were closed with perforated lids to allow gas exchange and stored in randomized positions in a climate chamber under the same conditions as for the acclimatization period. The LUFA soil pH (ISO, 2005) and moisture (ISO, 1993) were measured at the beginning and end of the test (Supporting Information, Table S3). Survival was assessed by testing the organism's reaction to a gentle mechanical stimulus on Days 7 and 14 after the chemical application. In addition, approximately 10 g of soil were sampled on Days 0, 7, and 14, and stored at –20 °C to analyze pesticide concentrations.

Imidacloprid concentrations were quantified by Eurofins Umwelt Südwest (Speyer, Germany). Briefly, 20 ml of acetone was added to a 5-g dried soil sample, shaken for 60 min, and centrifuged. Then an aliquot of 200 µl was taken, evaporated to dryness, and reconstituted with 500 µl methanol and 500 µl water. The sample was filtrated, and the imidacloprid concentration was quantified via high-performance liquid chromatography–tandem mass spectrometry using a recovery standard. Copper contents were extracted at the iES Landau as follows: 10 ml of aqua regia (HNO<sub>3</sub> + 3 HCl, 65% and 32% suprapure assay, respectively; Carl Roth, Germany) was added to a 5-g dried soil sample. Samples were digested using microwave-induced (Mars Xpress; CEM, Germany) aqua regia at 800 watts and 60 min of digestion phase at 175 °C. Then, samples were diluted 1:10 with Milli-Q water and quantified with inductively coupled plasma atomic emission spectroscopy (700 Series; Agilent, Germany). Measured copper concentrations were consistently up to 30% lower than nominal concentrations (Supporting Information, Table S4), indicating incomplete recovery from the soil matrix. Imidacloprid measured concentrations (Supporting Information, Table S4) varied around nominal concentrations,

which were always included within the confidence intervals of measurements. Following the majority of existing studies (USEPA, 2022), nominal concentrations of the pesticides at the beginning of the test were measured and are reported throughout our study.

## Data analysis

Following Ritz et al. (2019), LC50s were calculated (Supporting Information, Table S3) after 7 (when possible) and 14 days of exposure for all tested species by fitting binomial dose–response models to the data. Model fits were compared using the Akaike information criterion, and the best-fit model was selected (Supporting Information, Figures S1–S28). The intraspecific differences in LC50s (Supporting Information, Table S5) were assessed via pairwise comparisons of multiple binomial dose–response curves (Ritz et al., 2019). Furthermore, SSDs (Posthuma et al., 2001) were fitted for both pesticides using the 14-day LC50 values for all examined species and literature data from comparable studies (i.e., soils with similar organic matter content; Supporting Information, Table S6) because 7-day LC50s were not available for all tests (Supporting Information, Table S3). If multiple LC50 values from the same species were available, the geometric mean LC50 was calculated (Supporting Information, Table S6). Values of HC5 were derived from these distributions, and parametric bootstrap 95% confidence intervals (CIs), from 1000 iterations, were calculated to obtain the lower limits of the HC5. In addition, potential associations between earthworm chemical sensitivity in terms of LC50, habitat (grassland, wetland, forest), natural soil pH, fresh weight, and ecotype (endogeic, epigeic, anecic) were analyzed via analysis of covariance (ANCOVA). *Eisenia fetida*, an epigeic compost earthworm rarely found in nature (Krück, 2018), was excluded from these calculations. Because *Aporrectodea longa* was the only anecic species tested for both pesticides, anecic and endogeic earthworms were merged into one category, “nonepigeic,” for analysis. In addition, soil pH classes “slightly acidic” and “neutral” (Soil Science Division Staff, 2017) were combined into one category for the analysis because pH values were close to 6.5, which is the limit between these classes. All statistical analyses and figures were created with R Ver 4.2.1 for Windows together with the add-on packages “drc” (Ritz et al., 2015), “multcomp” (Hothorn et al., 2008), “plotrix” (Lemon, 2006) for dose–response modeling, “fitdistrplus” (Delignette-Muller & Dutang, 2015), “reshape2” (Wickham, 2007), “ggplot2” (Wickham, 2016), “ggpubr” (Kassambara, 2020) for the SSD, and “car” (Fox & Weisberg, 2019) for the ANCOVA.

## RESULTS

### Acute toxicity

In total, 14 tests were run for each pesticide, with six species of earthworms from the genera *Allolobophora*, *Aporrectodea*, *Eisenia*, and *Lumbricus* (Supporting Information, Table S3). Earthworm 14-day LC50s (Supporting Information, Table S3) for

imidacloprid ranged between 0.72 and 3.53 mg a.i./kg dry weight, and values for copper ranged from 199.99 to 433.09 mg a.i./kg dry weight. Intraspecific differences (Supporting Information, Table S5) showed that *Aporrectodea caliginosa* collected from an extremely acidic grassland (4.24 pH; Supporting Information, Table S1), for imidacloprid (Figure 1A), and an extremely acidic forest (4.16 pH; Supporting Information, Table S1), for copper (Figure 1B), were significantly less sensitive than *Aporrectodea caliginosa* sampled from the other ecosystems. Furthermore, laboratory-raised *E. fetida* were significantly less sensitive than *E. fetida* collected in the field for both chemicals (Figure 1C,D).

## SSDs

Additional LC50 values were included from the literature to fit the SSDs (Supporting Information, Table S6). *Allobophora chlorotica* tested in the present study was the most sensitive species to acute imidacloprid and copper exposure. The HC5 (95% CI) derived from the SSDs for imidacloprid (Figure 2A) and copper (Figure 2B) were 0.70 (0.32–1.47) and 201.51 (178.49–234.07) mg a.i./kg dry weight, respectively.

## Earthworm sensitivity and habitat characteristics

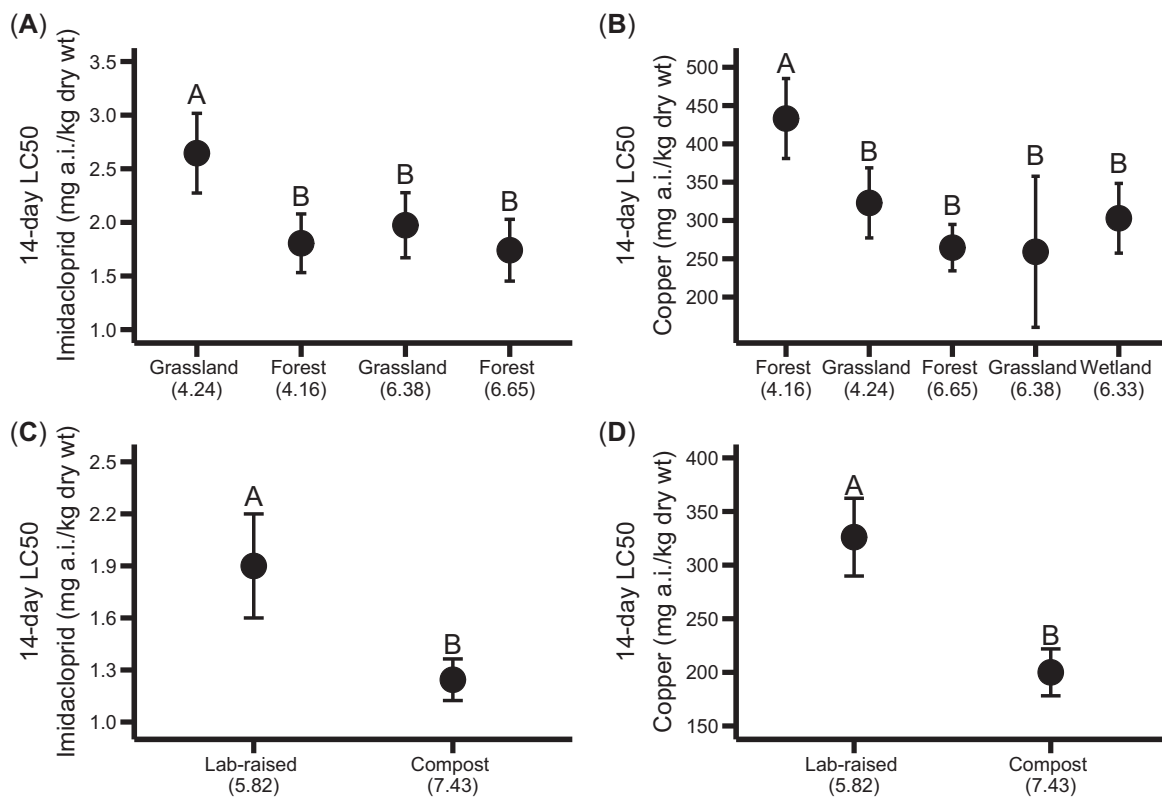
Chemical sensitivity to imidacloprid differed significantly between epigeic and nonepigeic earthworms ( $F_{(1,6)} = 17.45$ ,

$p < 0.01$ ; Table 1). The LC50s of nonepigeic earthworms were generally twice as low as those from epigeic earthworms (Figure 3). Earthworm sensitivity to copper increased significantly with increasing soil pH of their ecosystem of origin ( $F_{(1,6)} = 11.66$ ,  $p = 0.01$ ; Table 1). Thus, earthworms obtained from extremely acidic soils were approximately twice as resistant to copper than those sampled in neutral soils (Figure 4).

## DISCUSSION

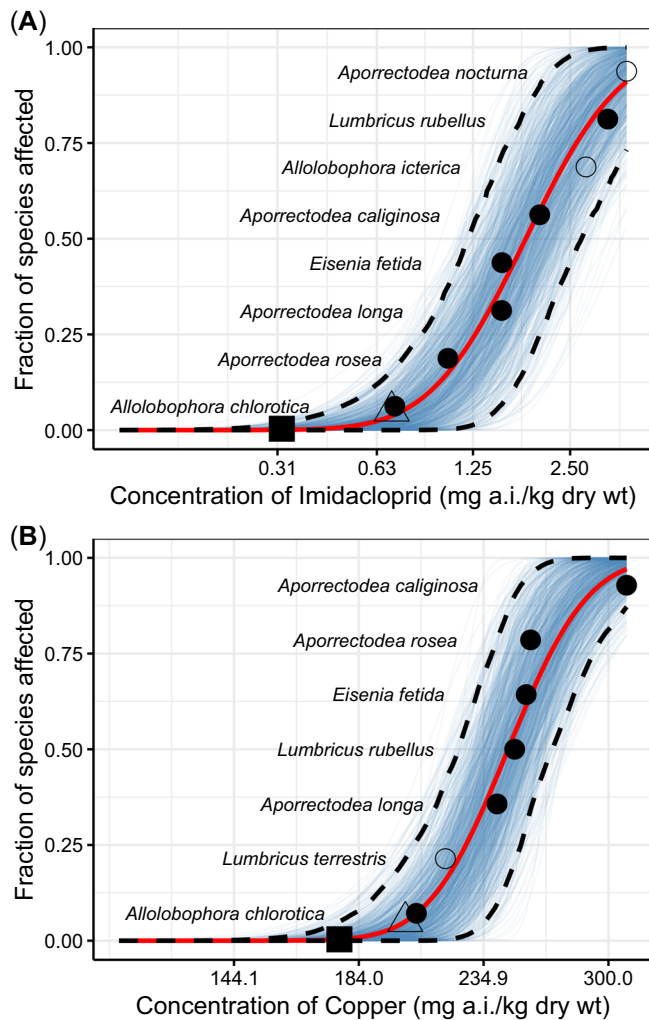
### SSDs and implications for risk assessment

The European Union (EU) pesticide risk assessment for soil organisms defines a safety factor of 5 (EFSA Panel on Plant Protection Products and Their Residues et al., 2017) to assess the acceptable risk of a substance (Regulation EU 546/2011 [EU, 2011]). For an acceptable risk, the differences in the sensitivity of the standard test organism *E. fetida* and other earthworm species (i.e.,  $LC50_{E. fetida}/LC50_{other\ species}$ ) should be lower than the safety factor of 5 (Frampton et al., 2006). Although *E. fetida*, tested in the present study, was not the most sensitive species to imidacloprid (Figure 2A) and copper (Figure 2B), the ratio of the  $LC50_{E. fetida}$  to the most sensitive species in our study, *Allobophora chlorotica*, was less than the safety factor for both substances (Supporting Information, Table S3; Figure 2A,B). Still, the species tested in our study, including *E. fetida*, were very sensitive to copper and imidacloprid, which are known to be toxic compounds for



**FIGURE 1:** Intraspecific variation of earthworm chemical sensitivity for *Aporrectodea caliginosa* exposed to imidacloprid (A) and copper (B) and *Eisenia fetida* exposed to imidacloprid (C) and copper (D). Black points represent the 14-day median lethal concentration and whiskers their respective 95% confidence interval. For *Aporrectodea caliginosa* the x-axis shows the habitat of origin (soil pH). Different letters show significant differences ( $p < 0.05$ ). LC50 = median lethal concentration; a.i. = active ingredient.





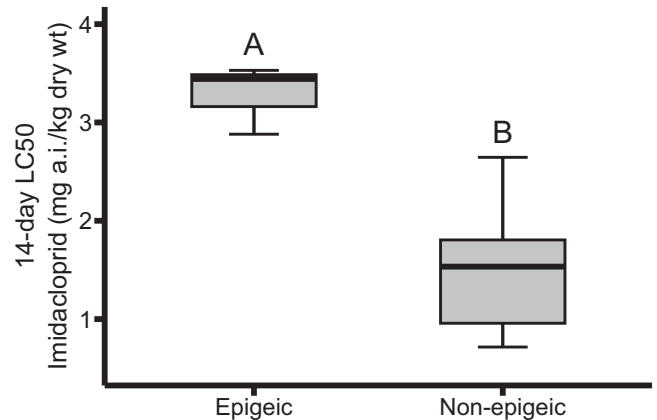
**FIGURE 2:** Species sensitivity distributions for imidacloprid (A) and copper (B) calculated from earthworm species sensitivity (red line). Black points (data from the present study) and open points (literature) represent the 14-day median lethal concentration values of earthworm species. Species names are aligned by sensitivity in ascending order from bottom to top on the y-axes, with the most sensitive at the bottom. Dashed lines enclose the parametric bootstrap (95% confidence interval; 1000 iterations). Blue transparent lines display all parametric bootstrap samples. The open triangle marks the hazardous concentration affecting 5% and the black square its lower limit. a.i. = active ingredient.

earthworms (see Streit, 1984; Wang et al., 2012). Short et al. (2021) exposed different earthworm species to imidacloprid and found that the safety factor proposed by the EFSA did not cover the most sensitive species tested, *Amyntas gracilis*

**TABLE 1:** Comparison between earthworm traits, habitat characteristics, and chemical sensitivity for imidacloprid and copper

Covariate	LC50 imidacloprid			LC50 copper		
	df	F	p	df	F	p
Ecotype	1	17.45	<b>0.005</b>	1	0.01	0.91
Weight	1	0.23	0.65	1	0.04	0.85
Habitat	2	1.54	0.29	2	0.31	0.74
pH	1	1.1	0.34	1	11.66	<b>0.01</b>

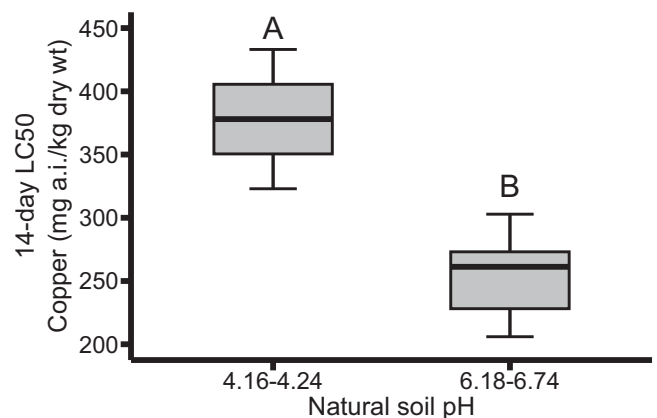
Statistically significant differences ( $p < 0.05$ ) are printed in bold. LC50 = median lethal concentration.



**FIGURE 3:** Comparison between epigeic and nonepigeic earthworm imidacloprid median lethal concentrations. Different letters show significant differences ( $p < 0.05$ ). LC50 = median lethal concentration; a.i. = active ingredient.

(KINBERG, 1866). In addition, Frampton et al. (2006) performed a pesticide analysis using SSDs and soil invertebrates. In addition to oligochaetes, mainly arthropods reacted very sensitively to insecticides, as expected. They also concluded that *E. fetida* was not the most sensitive soil organism, and in most cases, the safety factor did not cover the range of chemical acute sensitivities of all species analyzed. These findings question the strong reliance of the current risk-assessment framework on *E. fetida* and underline the need to test pesticides on more ecologically relevant and sensitive soil organisms (Forbes et al., 2021).

The HC5s derived for imidacloprid (Figure 2A) and copper (Figure 2B) are useful as a proxy for potential mortality risk for earthworms under field conditions when compared with measured and recommended field concentrations. To be more conservative, the lower limit of the CI of the HC5 is often considered to achieve a higher level of protection. In European vineyards, concentrations of copper in topsoil and subsoil were



**FIGURE 4:** Comparison between copper median lethal concentrations of earthworms sampled in extremely acidic (4.16–4.24), slightly acidic (6.18–6.38), and neutral (6.65–6.74) soils. Different letters show significant differences ( $p < 0.05$ ). LC50 = median lethal concentration; a.i. = active ingredient.

reported up to 600 mg/kg (Komárek et al., 2010) and even 1600 mg/kg in the study area, around Landau in der Pfalz, Germany (Steinmetz et al., 2017), which is characterized by long-term intensive viticulture. This shows that the soils in some areas that are heavily contaminated with copper, especially vineyards, may exert acute toxicity to earthworms.

Regarding imidacloprid, concentrations in agricultural soils were reported up to 0.65 mg/kg after 1 month of application in crops (Donnarumma et al., 2011). The most sensitive species, that is, *Allobophora chlorotica* and *Aporrectodea rosea* (Figure 2A; Supporting Information, Table S3), may have a survival risk in such soils. Nevertheless, because imidacloprid use is currently restricted to greenhouses in the EU (Regulation EU 2018/783 [EC, 2018]), a low risk for earthworms populations can be expected. In a recent monitoring study, the maximum concentration of imidacloprid found was 0.06 mg/kg (Silva et al., 2019). However, sublethal endpoints, such as reproduction, are more relevant for risk assessment than acute endpoints because they are typically affected at much lower concentrations than the observed LC50 (Neuhauser & Callahan, 1990). For example, *E. fetida* reproduction was negatively affected at a concentration of 0.87 mg imidacloprid/kg soil, while its acute LC50 was 2.26 mg imidacloprid/kg soil (Wang et al., 2019). Nevertheless, information on sublethal effects for other substances and earthworm species is scarce compared with acute data; only approximately 16% of earthworm toxicity studies addressed sublethal endpoints (USEPA, 2022). Although laboratory culturing of field earthworm species may be challenging, the mineral dweller *Aporrectodea caliginosa* promises to be a good candidate for evaluating the chronic effects of pesticides (Bart et al., 2018). Moreover, the update of the ISO 11268-2 (ISO, 2023) incorporates environmentally relevant species, for example, *Aporrectodea caliginosa* and *Dendrodrilus rubidus* (SAVIGNY, 1826), for testing pollutant effects on earthworm reproduction.

### Intraspecific variation in chemical sensitivity

The differences in chemical sensitivity among populations of *Aporrectodea caliginosa* could be partially related to the organisms' ecosystem of origin (Figure 1A,B; Supporting Information, Table S5). *Aporrectodea caliginosa* is a species complex, often divided into different species (see Sims & Gerard, 1985) or subspecies (see Briones, 1996). Although differences in this classification are rather phenotypic than taxonomic (Bart et al., 2018), organisms used in the present study were identified morphologically (cf., Krück, 2018). Thus, the sensitivity differences obtained (Figure 1A,B; Supporting Information, Table S5) may be between different species that could not be morphologically separated. In this context, DNA barcoding probably will reveal *Aporrectodea caliginosa* cryptic species, which should be considered in future studies (Römbke et al., 2016). Furthermore, the chemical sensitivity of *E. fetida* differed by origin, with individuals from a laboratory culture being less sensitive than those from compost (Figure 1C,D; Supporting Information, Table S5). Laboratory-raised organisms fulfilled the standardization recommendations of the acute

OECD guideline (OECD, 1984), for example, adult, weight, age, whereas only adult earthworms of unknown age were considered from compost. Moreover, laboratory test organisms were cultured in a moderately acid substrate (pH 5.82; Supporting Information, Table S1), whereas compost earthworms were raised in a slightly alkaline substrate (pH 7.43; Supporting Information, Table S1). The different substrates may have influenced their chemical response because pH appears to affect earthworm sensitivity to pesticides (see next section). Including additional earthworm species in risk assessment would be confronted with the challenge of standardization, with field organisms potentially failing to live and reproduce under laboratory conditions (Fründ et al., 2010). Nevertheless, the inclusion of field earthworms in standardized guidelines, such as the ISO 11268-2 (ISO, 2023), will help to improve risk assessment of soil organisms.

### Earthworm sensitivity and habitat characteristics

Abiotic soil characteristics, such as soil type, pH, and moisture, influence earthworm biodiversity (Edwards & Bohlen, 1996). Furthermore, our results show that soil pH appears to affect earthworm sensitivity to pesticides (Figure 4 and Table 1). We are not aware of other studies that investigated the relationship between earthworm pesticide sensitivity and habitat characteristics and can only speculate on the reasons for our results. Ontogenetic traits acquired during earthworm development may explain the observed differences with soil pH (see Briones & Álvarez-Otero, 2018). For example, a reduced sensitivity to copper in earthworms from highly acidic soil may be an adaptation to low pH values (<5.5), in which toxic metals such as copper are mobilized (Fernández-Calviño et al., 2008). Moreover, recent studies have used toxicogenomic analysis to investigate and explain why some species are more sensitive to a certain compound (cf., Short et al., 2021). Pesticide uptake in earthworms is mainly through direct contact and oral ingestion (see Short et al., 2021; Streit, 1984). Uptake varies among different species, as do their toxicokinetic and toxicodynamic traits; and these dynamics mainly determine organism sensitivity to pesticides (Ashauer & Jager, 2018). Thus, toxicogenomic experiments combined with earthworm populations from different habitats could clarify the differences in earthworm sensitivity and habitat relationships observed in the present study.

The variation in earthworm sensitivity seen in the present study may affect soil functions such as bioturbation, that is, reworking of soil performed by soil organisms (Meysman et al., 2006). Nonepigeic earthworms contribute considerably to this process (Lee & Foster, 1991). Furthermore, anecic and endogeic earthworms are more sensitive than epigeic earthworms (Figure 3), especially to insecticides (Pelosi et al., 2014), which may result in a reduction in populations and cast production (Lal et al., 2001) and affect ecosystem functioning. Identifying sensitive traits and thresholds for safeguarding ecological functions would require further studies considering earthworm ecological groups and species within these groups (Forbes et al., 2021).

## CONCLUSION

Our results confirm that the standard test organism *E. fetida* is not the most sensitive earthworm species (Frampton et al., 2006; Pelosi et al., 2013). Protecting the ecosystem services and functions provided by these soil invertebrates would require the inclusion of more ecologically relevant and sensitive earthworms in risk assessment (Forbes et al., 2021; ISO, 2023). While the sensitivities of earthworms showed no clear differences between ecosystem types, they varied with soil pH. The protection of a region-specific soil community and its ecological roles would require considering the soil characteristics of their habitat of origin. Furthermore, the higher sensitivity to imidacloprid shown by soil-inhabiting compared with epigeic earthworms could affect ecosystem services, such as bioturbation, if sensitive species are lost.

**Supporting Information**—The Supporting Information is available on the Wiley Online Library at <https://doi.org/10.1002/etc.5589>.

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**Data Availability Statement**—R codes for toxicity data calculations are available from Figshare: <https://doi.org/10.6084/m9.figshare.21119884>. Data, associated metadata, and calculation tools are available from the corresponding author ([duque@uni-landau.de](mailto:duque@uni-landau.de)).

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## Supporting Information

**Table S1** Collected earthworm species for imidacloprid and copper experiments with their respective habitat characterization (mean values with  $\pm$  standard deviation).

Pesticide	ID	Species	Ecological group	Fresh weight (g)	Source	EUNIS habitat type classification	Coordinates (WGS84)	Elevation (m.a.s.l)	Soil pH
Imidacloprid	1	<i>A. longa</i>	Anecic	1.96 $\pm$ 0.49	Grassland	E2.2	49.193975, 8.096792	152	6.74 $\pm$ 0.13
	2	<i>A. caliginosa</i>	Endogeic	0.60 $\pm$ 0.22	Forest	G1.6	49.223472, 8.187417	127	6.65 $\pm$ 0.07
	3	<i>A. rosea</i>	Endogeic	0.31 $\pm$ 0.08	Forest	G1.6	49.223472, 8.187417	127	6.65 $\pm$ 0.07
	4	<i>A. caliginosa</i>	Endogeic	1.58 $\pm$ 0.37	Forest	G1.A	49.211944, 8.188722	140	4.16 $\pm$ 0.11
	5	<i>A. caliginosa</i>	Endogeic	0.78 $\pm$ 0.25	Grassland	E2.2	49.228772, 8.003750	223	6.38 $\pm$ 0.04
	6	<i>E. fetida</i>	Epigeic	0.32 $\pm$ 0.08	Compost	-	-	-	7.43 $\pm$ 0.15
	7	<i>A. rosea</i>	Endogeic	0.31 $\pm$ 0.11	Forest	G1.2	49.200283, 8.092964	150	6.28 $\pm$ 0.09
	8	<i>A. rosea</i>	Endogeic	0.37 $\pm$ 0.12	Wetland	E3.4	49.199667, 8.096469	153	6.33 $\pm$ 0.05
	9	<i>A. chlorotica</i>	Endogeic	0.28 $\pm$ 0.08	Forest	G1.2	49.200547, 8.092903	153	6.18 $\pm$ 0.21
	10	<i>L. rubellus</i>	Epigeic	1.41 $\pm$ 0.39	Forest	G1.2	49.200283, 8.092964	150	6.28 $\pm$ 0.09
	11	<i>L. rubellus</i>	Epigeic	0.83 $\pm$ 0.19	Forest	G1.A	49.211944, 8.188722	140	4.16 $\pm$ 0.11
	12	<i>L. rubellus</i>	Epigeic	0.88 $\pm$ 0.29	Grassland	E2.2	49.284167, 7.914136	518	4.24 $\pm$ 0.09
	13	<i>A. caliginosa</i>	Endogeic	0.69 $\pm$ 0.18	Grassland	E2.2	49.284167, 7.914136	518	4.24 $\pm$ 0.09
	14	<i>E. fetida</i>	Epigeic	0.47 $\pm$ 0.08	Lab-raised	-	-	-	5.82 $\pm$ 0.01
Copper	1	<i>A. rosea</i>	Endogeic	0.28 $\pm$ 0.10	Forest	G1.6	49.223472, 8.187417	127	6.65 $\pm$ 0.07
	2	<i>E. fetida</i>	Epigeic	0.37 $\pm$ 0.09	Compost	-	-	-	7.43 $\pm$ 0.15
	3	<i>A. caliginosa</i>	Endogeic	1.21 $\pm$ 0.41	Forest	G1.A	49.211944, 8.188722	140	4.16 $\pm$ 0.11
	4	<i>A. chlorotica</i>	Endogeic	0.26 $\pm$ 0.06	Forest	G1.2	49.200547, 8.092903	153	6.18 $\pm$ 0.21
	5	<i>A. rosea</i>	Endogeic	0.25 $\pm$ 0.07	Forest	G1.2	49.200283, 8.092964	150	6.28 $\pm$ 0.09
	6	<i>A. caliginosa</i>	Endogeic	1.07 $\pm$ 0.33	Grassland	E2.2	49.228772, 8.003750	223	6.38 $\pm$ 0.04
	7	<i>A. caliginosa</i>	Endogeic	0.90 $\pm$ 0.27	Grassland	E2.2	49.284167, 7.914136	518	4.24 $\pm$ 0.09
	8	<i>A. caliginosa</i>	Endogeic	0.64 $\pm$ 0.17	Forest	G1.6	49.223472, 8.187417	127	6.65 $\pm$ 0.07
	9	<i>A. caliginosa</i>	Endogeic	0.83 $\pm$ 0.35	Wetland	E3.4	49.199667, 8.096469	153	6.33 $\pm$ 0.05
	10	<i>A. longa</i>	Anecic	2.29 $\pm$ 0.58	Grassland	E2.2	49.193975, 8.096792	152	6.74 $\pm$ 0.13
	11	<i>A. longa</i>	Anecic	2.18 $\pm$ 0.65	Wetland	E3.4	49.199667, 8.096469	153	6.33 $\pm$ 0.05
	12	<i>A. rosea</i>	Endogeic	0.39 $\pm$ 0.14	Wetland	E3.4	49.199667, 8.096469	153	6.33 $\pm$ 0.05
	13	<i>L. rubellus</i>	Epigeic	0.94 $\pm$ 0.34	Forest	G1.2	49.200283, 8.092964	150	6.28 $\pm$ 0.09
	14	<i>E. fetida</i>	Epigeic	0.47 $\pm$ 0.07	Lab-raised	-	-	-	5.82 $\pm$ 0.01

**Table S2** Chemical and physical characteristics of the standard soil LUFA 2.2 at the moment of packing (mean values with  $\pm$  standard deviation).

Soil parameter	Value
Organic carbon (%C)	1.61 $\pm$ 0.44
Nitrogen (%N)	0.18 $\pm$ 0.04
pH (0.01 M CaCl <sub>2</sub> )	5.6 $\pm$ 0.4
Cation exchange capacity (meq/100g)	8.5 $\pm$ 12.1
Soil type	sandy loam
Dry matter (%)	87.14 $\pm$ 0.87
Water content (g water/100g soil)	14.76 $\pm$ 1.14
Maximum water holding capacity (g/100g)	30.37 $\pm$ 2.33
Weight per volume (g/1000mL)	1232 $\pm$ 93.8

**Table S3** Additional information on ecotoxicological assessment. LUFA 2.2 pH and moisture at the beginning and the end of the tests (mean values with  $\pm$  standard deviation) and LC<sub>50</sub>s ( $\pm$  95% confidence intervals) for day 7 and day 14.

Pesticide	ID	Species	Test date	Soil pH (0.01 M CaCl <sub>2</sub> )		Soil moisture (%)		LC <sub>50</sub> $\pm$ C.I. (mg a.i./kg d.w.)		Concentrations tested (mg a.i./kg d.w.)
				Day 0	Day 14	Day 0	Day 14	Day 7	Day 14	
Imidacloprid	1	<i>A. longa</i>	19 Feb 2020	5.90 $\pm$ 0.05	5.46 $\pm$ 0.04	21.43 $\pm$ 0.64	13.14 $\pm$ 0.43	-	1.53 $\pm$ 0.16	0, 0.86, 1.12, 1.46, 1.89, 2.46, 3.2
	2	<i>A. caliginosa</i>	24 Mar 2020	5.71 $\pm$ 0.14	5.53 $\pm$ 0.01	22.03 $\pm$ 0.27	12.59 $\pm$ 0.56	-	1.74 $\pm$ 0.29	0, 0.86, 1.12, 1.46, 1.89, 2.46, 3.2
	3	<i>A. rosea</i>	24 Mar 2020	5.71 $\pm$ 0.14	5.57 $\pm$ 0.01	22.03 $\pm$ 0.27	14.88 $\pm$ 1.02	2.01 $\pm$ 0.31	1.32 $\pm$ 0.29	0, 0.86, 1.12, 1.46, 1.89, 2.46, 3.2
	4	<i>A. caliginosa</i>	25 Mar 2020	5.53 $\pm$ 0.06	5.51 $\pm$ 0.01	20.14 $\pm$ 0.39	13.66 $\pm$ 0.59	-	1.81 $\pm$ 0.27	0, 0.86, 1.12, 1.46, 1.89, 2.46, 3.2
	5	<i>A. caliginosa</i>	10 Apr 2020	5.53 $\pm$ 0.06	5.40 $\pm$ 0.05	20.57 $\pm$ 0.12	13.42 $\pm$ 0.26	2.46 $\pm$ 0.34	1.97 $\pm$ 0.30	0, 1.12, 1.46, 1.89, 2.46, 3.2, 4.16
	6	<i>E. fetida</i>	10 Apr 2020	5.66 $\pm$ 0.11	5.40 $\pm$ 0.05	22.53 $\pm$ 0.07	15.24 $\pm$ 0.89	-	1.24 $\pm$ 0.12	0, 0.86, 1.12, 1.46, 1.89, 2.46, 3.2
	7	<i>A. rosea</i>	14 May 2020	5.66 $\pm$ 0.11	5.94 $\pm$ 0.02	21.05 $\pm$ 0.16	15.67 $\pm$ 0.08	-	0.96 $\pm$ 0.32	0, 0.86, 1.12, 1.46, 1.89, 2.46, 3.2
	8	<i>A. rosea</i>	14 May 2020	5.66 $\pm$ 0.11	5.83 $\pm$ 0.03	21.05 $\pm$ 0.16	15.44 $\pm$ 0.07	1.61 $\pm$ 0.24	0.91 $\pm$ 0.27	0, 0.86, 1.12, 1.46, 1.89, 2.46, 3.2
	9	<i>A. chlorotica</i>	14 May 2020	5.79 $\pm$ 0.02	5.90 $\pm$ 0.02	21.05 $\pm$ 0.16	15.32 $\pm$ 0.22	2.05 $\pm$ 0.37	0.72 $\pm$ 0.28	0, 0.86, 1.12, 1.46, 1.89, 2.46, 3.2
	10	<i>L. rubellus</i>	15 May 2020	5.79 $\pm$ 0.02	5.97 $\pm$ 0.03	20.36 $\pm$ 1.15	15.80 $\pm$ 0.44	-	3.44 $\pm$ 0.44	0, 1.46, 1.89, 2.46, 3.2, 4.16, 5.41
	11	<i>L. rubellus</i>	15 May 2020	5.79 $\pm$ 0.02	5.96 $\pm$ 0.03	20.36 $\pm$ 1.15	16.85 $\pm$ 0.26	3.19 $\pm$ 0.45	2.88 $\pm$ 0.44	0, 1.46, 1.89, 2.46, 3.2, 4.16, 5.41
	12	<i>L. rubellus</i>	15 May 2020	5.79 $\pm$ 0.02	5.92 $\pm$ 0.07	20.36 $\pm$ 1.15	17.15 $\pm$ 0.49	-	3.53 $\pm$ 0.46	0, 1.46, 1.89, 2.46, 3.2, 4.16, 5.41
	13	<i>A. caliginosa</i>	15 May 2020	5.79 $\pm$ 0.02	5.98 $\pm$ 0.01	20.36 $\pm$ 1.15	16.83 $\pm$ 0.41	2.97 $\pm$ 0.34	2.65 $\pm$ 0.37	0, 1.12, 1.46, 1.89, 2.46, 3.2, 4.16
	14	<i>E. fetida</i>	16 Jun. 2021	5.82 $\pm$ 0.02	5.81 $\pm$ 0.02	18.67 $\pm$ 0.12	16.88 $\pm$ 0.19	-	1.90 $\pm$ 0.30	0, 0.86, 1.12, 1.46, 1.89, 2.46, 3.2
Copper	1	<i>A. rosea</i>	2 Apr 2020	5.53 $\pm$ 0.06	5.57 $\pm$ 0.06	20.14 $\pm$ 0.39	14.14 $\pm$ 0.34	296.44 $\pm$ 38.84	220.29 $\pm$ 97.34	0, 200, 280, 392, 548.8, 768.3, 1075.6
	2	<i>E. fetida</i>	2 Apr 2020	5.53 $\pm$ 0.06	5.47 $\pm$ 0.09	20.14 $\pm$ 0.39	13.24 $\pm$ 0.81	387.37 $\pm$ 54.46	199.99 $\pm$ 21.87	0, 200, 280, 392, 548.8, 768.3, 1075.6
	3	<i>A. caliginosa</i>	20 May 2020	5.81 $\pm$ 0.08	6.00 $\pm$ 0.13	21.08 $\pm$ 0.09	15.74 $\pm$ 0.04	663.12 $\pm$ 69.76	433.09 $\pm$ 52.21	0, 148.7, 208.2, 291.5, 408.2, 571.4, 800
	4	<i>A. chlorotica</i>	3 Jun 2020	5.95 $\pm$ 0.03	5.89 $\pm$ 0.01	19.21 $\pm$ 0.08	14.18 $\pm$ 0.25	219.70 $\pm$ 45.50	205.95 $\pm$ 80.44	0, 215.5, 280.1, 364.1, 473.4, 615.4, 800
	5	<i>A. rosea</i>	3 Jun 2020	5.95 $\pm$ 0.03	5.91 $\pm$ 0.03	19.21 $\pm$ 0.08	13.86 $\pm$ 0.30	432.87 $\pm$ 57.41	275.95 $\pm$ 32.67	0, 215.5, 280.1, 364.1, 473.4, 615.4, 800
	6	<i>A. caliginosa</i>	26 Nov 2020	5.83 $\pm$ 0.04	5.70 $\pm$ 0.06	17.33 $\pm$ 0.38	13.47 $\pm$ 0.32	284.26 $\pm$ 85.82	259.09 $\pm$ 98.62	0, 296, 361.1, 440.7, 537.5, 655.7, 800
	7	<i>A. caliginosa</i>	26 Nov 2020	5.83 $\pm$ 0.04	5.72 $\pm$ 0.03	19.47 $\pm$ 0.06	13.51 $\pm$ 0.23	330.91 $\pm$ 68.75	322.95 $\pm$ 45.75	0, 296, 361.1, 440.7, 537.5, 655.7, 800
	8	<i>A. caliginosa</i>	1 Dec 2020	5.75 $\pm$ 0.03	5.83 $\pm$ 0.04	18.83 $\pm$ 0.03	14.37 $\pm$ 0.25	288.11 $\pm$ 40.50	264.52 $\pm$ 30.28	0, 148.7, 208.2, 291.5, 408.2, 571.4, 800
	9	<i>A. caliginosa</i>	1 Dec 2020	5.75 $\pm$ 0.03	5.87 $\pm$ 0.02	18.83 $\pm$ 0.03	15.21 $\pm$ 0.17	331.26 $\pm$ 47.70	302.84 $\pm$ 45.53	0, 148.7, 208.2, 291.5, 408.2, 571.4, 800
	10	<i>A. longa</i>	2 Dec 2020	5.75 $\pm$ 0.03	5.87 $\pm$ 0.03	18.83 $\pm$ 0.03	15.77 $\pm$ 0.44	280.18 $\pm$ 35.59	263.97 $\pm$ 34.79	0, 148.7, 208.2, 291.5, 408.2, 571.4, 800
	11	<i>A. longa</i>	2 Dec 2020	5.75 $\pm$ 0.03	5.80 $\pm$ 0.05	18.83 $\pm$ 0.03	15.75 $\pm$ 0.18	271.01 $\pm$ 26.25	220.94 $\pm$ 41.19	0, 148.7, 208.2, 291.5, 408.2, 571.4, 800
	12	<i>A. rosea</i>	4 Dec 2020	5.75 $\pm$ 0.03	5.88 $\pm$ 0.01	18.83 $\pm$ 0.03	15.61 $\pm$ 0.46	313.61 $\pm$ 26.01	281.09 $\pm$ 19.63	0, 196.6, 245.8, 307.2, 384, 480, 600
	13	<i>L. rubellus</i>	24 Apr 2021	5.85 $\pm$ 0.05	5.79 $\pm$ 0.02	18.25 $\pm$ 0.12	16.18 $\pm$ 0.26	281.56 $\pm$ 23.77	249.66 $\pm$ 20.03	0, 196.6, 245.8, 307.2, 384, 480, 600
	14	<i>E. fetida</i>	16 Jun 2021	5.82 $\pm$ 0.02	5.84 $\pm$ 0.06	18.67 $\pm$ 0.12	14.74 $\pm$ 0.26	-	326.07 $\pm$ 36.26	0, 163.8, 204.8, 256, 320, 400, 500

**Table S4** Nominal and measured (mean with  $\pm$  95% confidence intervals,  $n = 3$ ) concentrations of imidacloprid or copper used in the earthworm ecotoxicological assessments.

Pesticide	Concentrations in mg a.i./kg d.w.			
	Nominal	Fresh	7-day old	14-day old
Imidacloprid	0	<LOQ*	-	-
	0.86	0.57 $\pm$ 0.24	-	-
	1.12	1.19 $\pm$ 0.57	-	-
	1.46	2.10 $\pm$ 1.05	1.10 $\pm$ 0.52	0.95 $\pm$ 0.48
	1.89	2.43 $\pm$ 1.24	-	-
	2.46	2.57 $\pm$ 1.29	-	-
	3.2	4.00 $\pm$ 2.00	2.95 $\pm$ 1.48	1.05 $\pm$ 0.52
	4.16	4.19 $\pm$ 2.10	-	-
	5.41	4.76 $\pm$ 2.48	-	-
Copper	0	5.15 $\pm$ 0.76	-	-
	148.75	139.89 $\pm$ 21.56	-	-
	163.84	119.08 $\pm$ 57.77	-	-
	196.60	137.69 $\pm$ 5.47	-	-
	200.00	139.72 $\pm$ 36.19	145.20 $\pm$ 8.80	156.18 $\pm$ 3.94
	204.80	134.66 $\pm$ 17.12	-	-
	208.20	157.90 $\pm$ 8.29	-	-
	215.50	172.25 $\pm$ 15.93	-	-
	245.80	249.57 $\pm$ 37.30	-	-
	256.00	163.77 $\pm$ 16.42	-	-
	280.00	181.32 $\pm$ 6.70	-	-
	280.10	238.74 $\pm$ 71.63	-	-
	291.50	189.69 $\pm$ 52.31	-	-
	296.00	252.04 $\pm$ 54.16	-	-
	307.20	285.54 $\pm$ 52.96	-	-
	320.00	208.90 $\pm$ 30.45	-	-
	361.12	293.89 $\pm$ 24.11	-	-
	364.10	299.30 $\pm$ 13.22	-	-
	384.00	331.93 $\pm$ 25.10	-	-
	392.00	320.01 $\pm$ 20.71	-	-
	400.00	326.86 $\pm$ 13.78	-	-
	408.20	366.52 $\pm$ 8.76	355.37 $\pm$ 16.67	335.44 $\pm$ 11.02
	440.67	471.12 $\pm$ 46.70	-	-
	473.40	434.22 $\pm$ 35.02	-	-
	480.00	495.92 $\pm$ 37.17	-	-
	500.00	506.67 $\pm$ 14.59	-	-
	537.49	599.72 $\pm$ 29.43	-	-
548.80	537.36 $\pm$ 40.18	-	-	
571.40	593.40 $\pm$ 12.07	-	-	
600.00	538.64 $\pm$ 12.90	-	-	
615.40	551.58 $\pm$ 9.12	-	-	
655.74	657.79 $\pm$ 38.85	-	-	
768.30	689.52 $\pm$ 65.67	-	-	
800.00	783.58 $\pm$ 12.29	781.22 $\pm$ 25.72	639.77 $\pm$ 8.12	
1075.60	1280.41 $\pm$ 19.82	-	-	

\*LOQ (Limit of quantification) = 0.05 mg a.i./kg d.w.

**Table S5** Intra-specific pairwise comparisons of LC<sub>50</sub>s values via analysis of multiple binomial dose-response curves. Soil pH classes are according to Soil Science Division Staff, 2017. All *p*-values <0.05 are printed in bold.

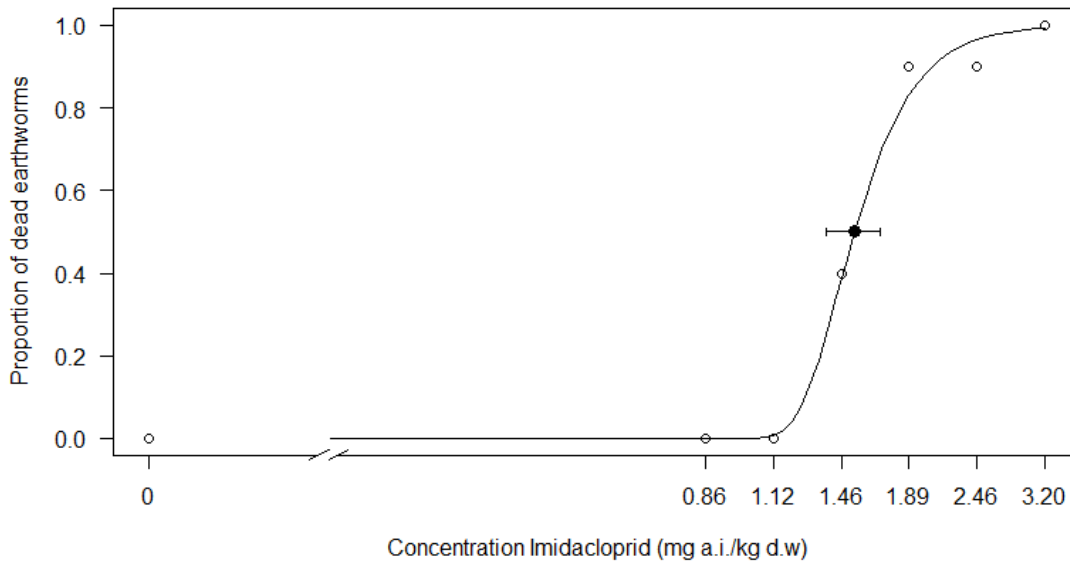
Pesticide	Species	Compared habitats (soil pH class)		LC <sub>50</sub> difference (mg a.i./kg d.w.)	<i>p</i> -value
		Habitat 1	Habitat 2		
Imidacloprid	<i>A. caliginosa</i>	Forest (Extremely acidic)	Forest (Neutral)	0.07	0.989
		Grassland (Slightly acidic)	Forest (Neutral)	0.23	0.694
		Grassland (Extremely acidic)	Forest (Neutral)	0.91	<b>&lt;0.001</b>
		Grassland (Slightly acidic)	Forest (Extremely acidic)	0.16	0.850
		Grassland (Extremely acidic)	Forest (Extremely acidic)	0.84	<b>0.002</b>
		Grassland (Slightly acidic)	Grassland (Extremely acidic)	-0.68	<b>0.031</b>
	<i>A. rosea</i>	Forest (Slightly acidic)	Forest (Neutral)	-0.36	0.225
		Wetland (Slightly acidic)	Forest (Neutral)	-0.41	0.116
		Wetland (Slightly acidic)	Forest (Slightly acidic)	-0.05	0.882
	<i>E. fetida</i>	Lab-raised	Compost	0.66	<b>&lt;0.001</b>
	<i>L. rubellus</i>	Forest (Slightly acidic)	Forest (Extremely acidic)	0.56	0.185
		Grassland (Extremely acidic)	Forest (Extremely acidic)	0.65	0.114
		Grassland (Extremely acidic)	Forest (Slightly acidic)	0.09	0.961
	Copper	<i>A. caliginosa</i>	Grassland (Slightly acidic)	Forest (Extremely acidic)	-174
Grassland (Extremely acidic)			Forest (Extremely acidic)	-110.14	<b>0.015</b>
Forest (Neutral)			Forest (Extremely acidic)	-168.57	<b>&lt;0.001</b>
Wetland (Slightly acidic)			Forest (Extremely acidic)	-130.25	<b>0.018</b>
Grassland (Extremely acidic)			Grassland (Slightly acidic)	63.86	0.765
Forest (Neutral)			Grassland (Slightly acidic)	5.43	0.999
Wetland (Slightly acidic)			Grassland (Slightly acidic)	43.75	0.798
Forest (Neutral)			Grassland (Extremely acidic)	-58.43	0.284
Wetland (Slightly acidic)			Grassland (Extremely acidic)	-20.11	1.000
Wetland (Slightly acidic)			Forest (Neutral)	38.32	0.400
<i>A. longa</i>		Wetland (Slightly acidic)	Grassland (Neutral)	-43.03	0.167
<i>A. rosea</i>		Forest (Slightly acidic)	Forest (Neutral)	55.66	0.154
		Wetland (Slightly acidic)	Forest (Neutral)	60.8	0.125
		Wetland (Slightly acidic)	Forest (Slightly acidic)	5.14	0.972
<i>E. fetida</i>	Lab-raised	Compost	126.08	<b>&lt;0.001</b>	

**Table S6** Earthworms 14-day LC<sub>50</sub> (mg a.i./kg d.w.) values used for the species sensitivity distributions for imidacloprid and copper.

Pesticide	Species	Ecological group	14-day LC <sub>50</sub>	Soil type (Organic matter %)	Source		
Imidacloprid	<i>A. longa</i>	Anecic	1.53	LUFA 2.2 (2.77)	Present study		
	<i>A. caliginosa</i>	Endogeic	2.01				
	<i>A. chlorotica</i>	Endogeic	0.72				
	<i>A. rosea</i>	Endogeic	1.05				
	<i>E. fetida</i>	Epigeic	1.54				
	<i>L. rubellus</i>	Epigeic	3.27				
	<i>A. nocturna</i>	Anecic	3.74			Natural soil (2.8)	Capowiez et al. 2005
	<i>A. icterica</i>	Endogeic	2.80			Natural soil (2.8)	Capowiez et al. 2005
Copper	<i>A. longa</i>	Anecic	241.22	LUFA 2.2 (2.77)	Present study		
	<i>A. caliginosa</i>	Endogeic	310.86				
	<i>A. chlorotica</i>	Endogeic	205.95				
	<i>A. rosea</i>	Endogeic	257.57				
	<i>E. fetida</i>	Epigeic	255.37				
	<i>L. rubellus</i>	Epigeic	249.66				
	<i>L. terrestris</i>	Anecic	218.00			Natural soil (1.7)	Haque & Ebing, 1983

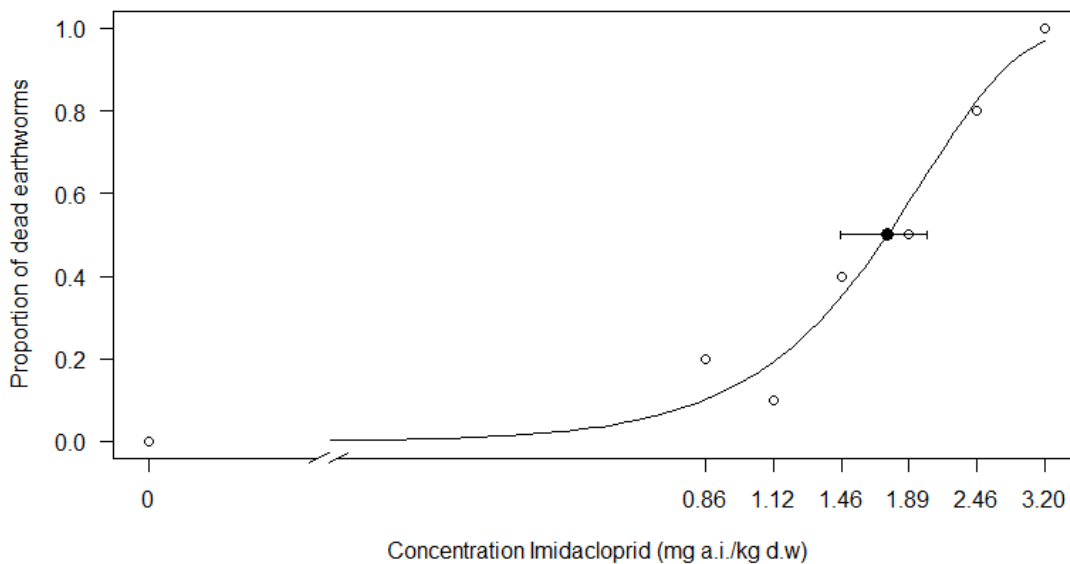


1. *Aporrectodea longa*



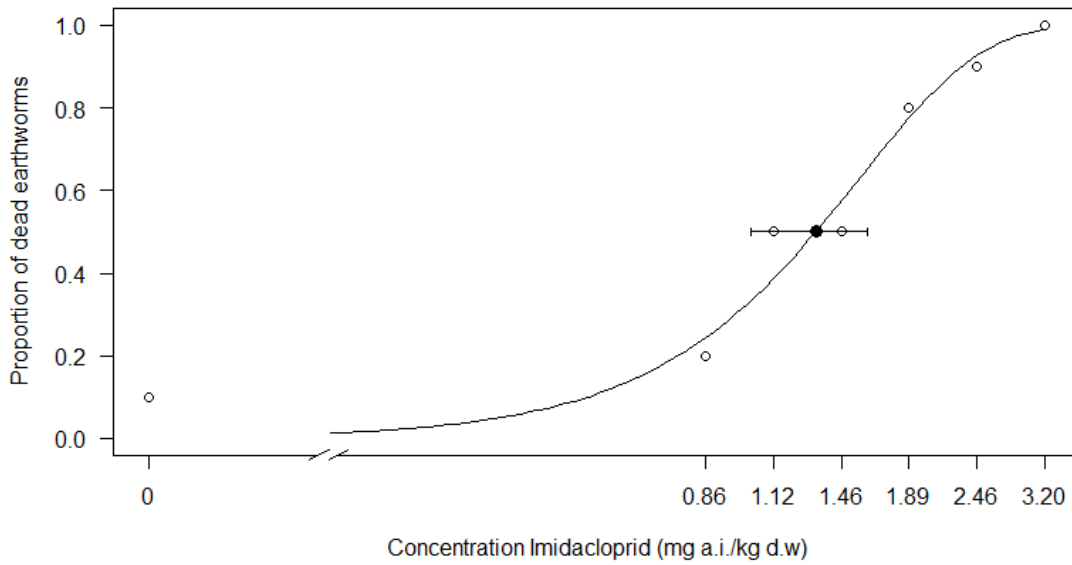
**Figure S1** Dose-response curve from *A. longa* (ID 1; Table S3) 14-day toxicity test with imidacloprid.

2. *Aporrectodea caliginosa*



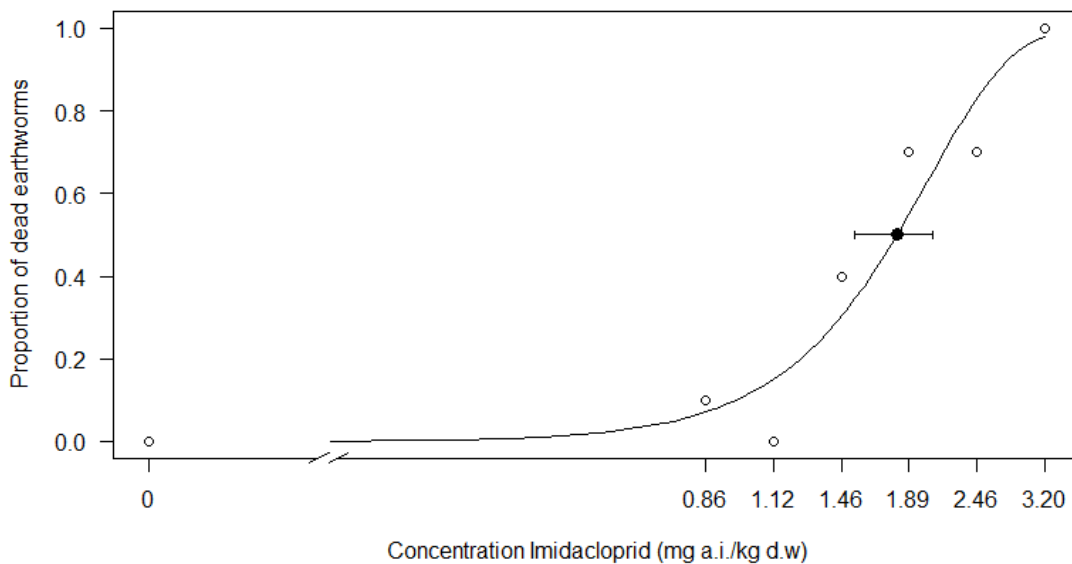
**Figure S2** Dose-response curve from *A. caliginosa* (ID 2; Table S3) 14-day toxicity test with imidacloprid.

### 3. *Aporrectodea rosea*



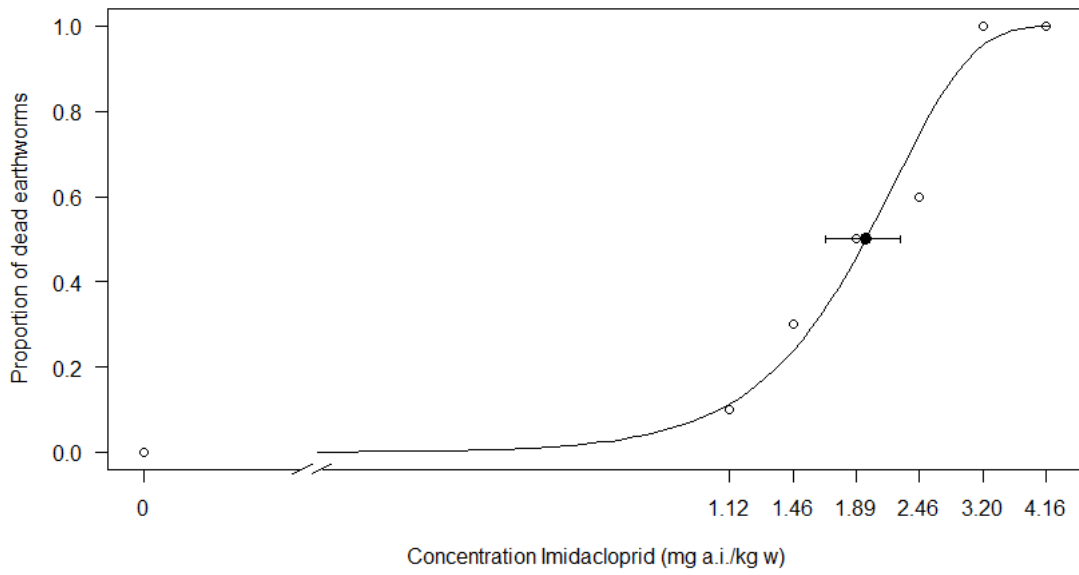
**Figure S3** Dose-response curve from *A. rosea* (ID 3; Table S3) 14-day toxicity test with imidacloprid.

### 4. *Aporrectodea caliginosa*



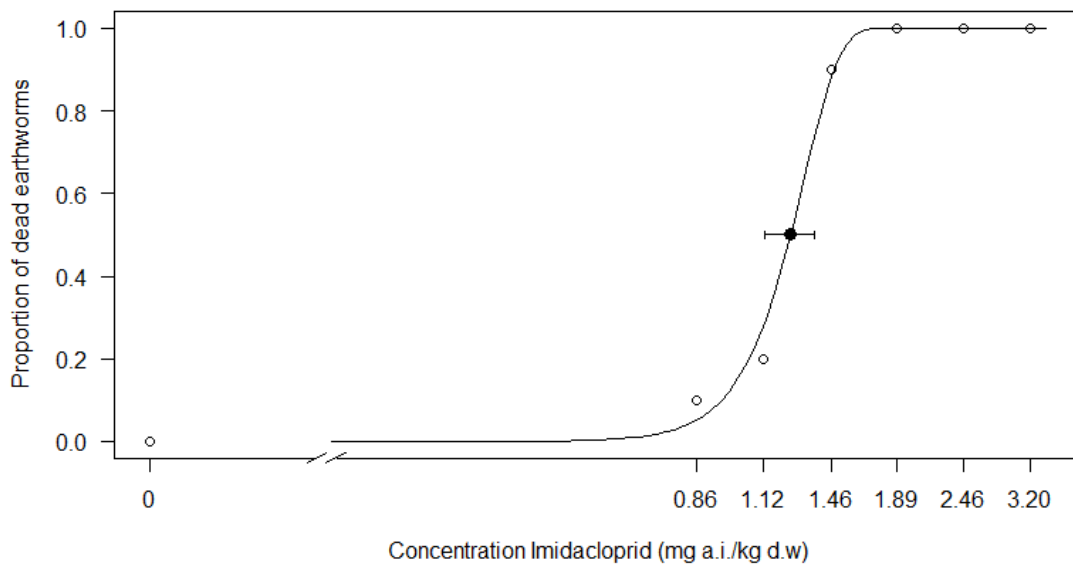
**Figure S4** Dose-response curve from *A. caliginosa* (ID 4; Table S3) 14-day toxicity test with imidacloprid.

5. *Aporrectodea caliginosa*



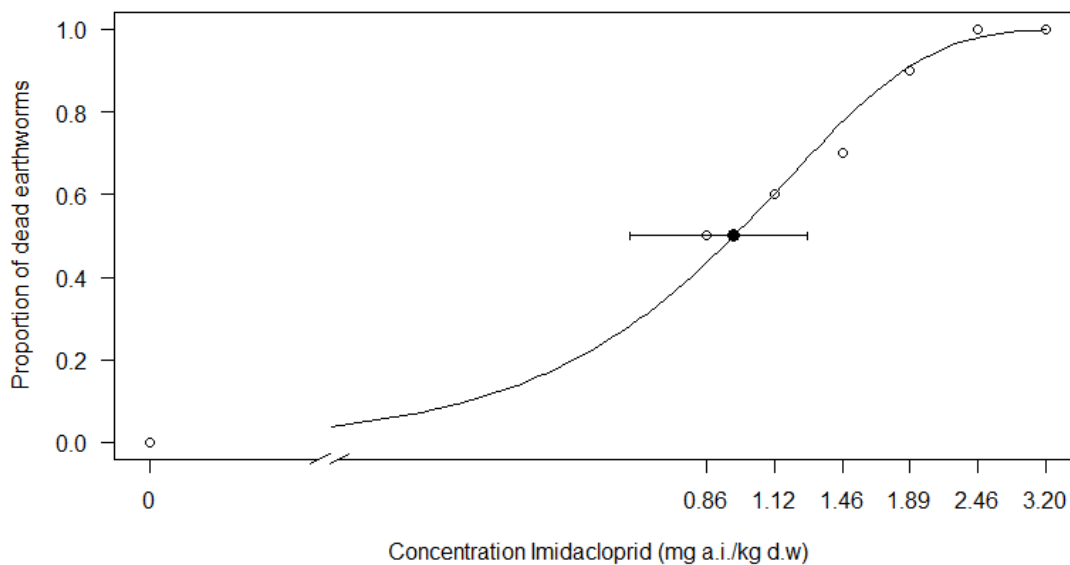
**Figure S5** Dose-response curve from *A. caliginosa* (ID 5; Table S3) 14-day toxicity test with imidacloprid.

6. *Eisenia fetida*



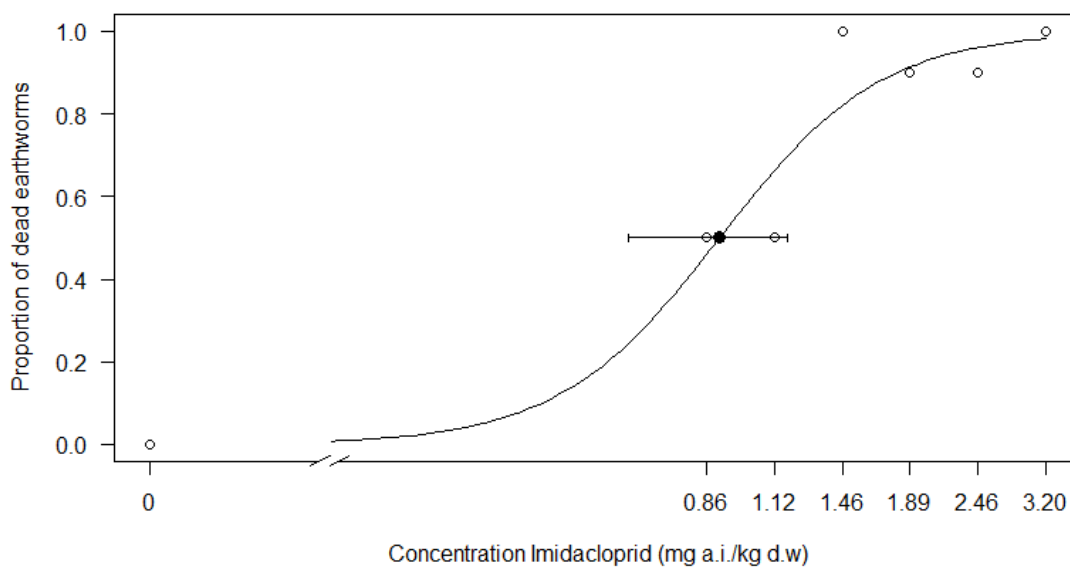
**Figure S6** Dose-response curve from *E. fetida* (ID 6; Table S3) 14-day toxicity test with imidacloprid.

7. *Aporrectodea rosea*



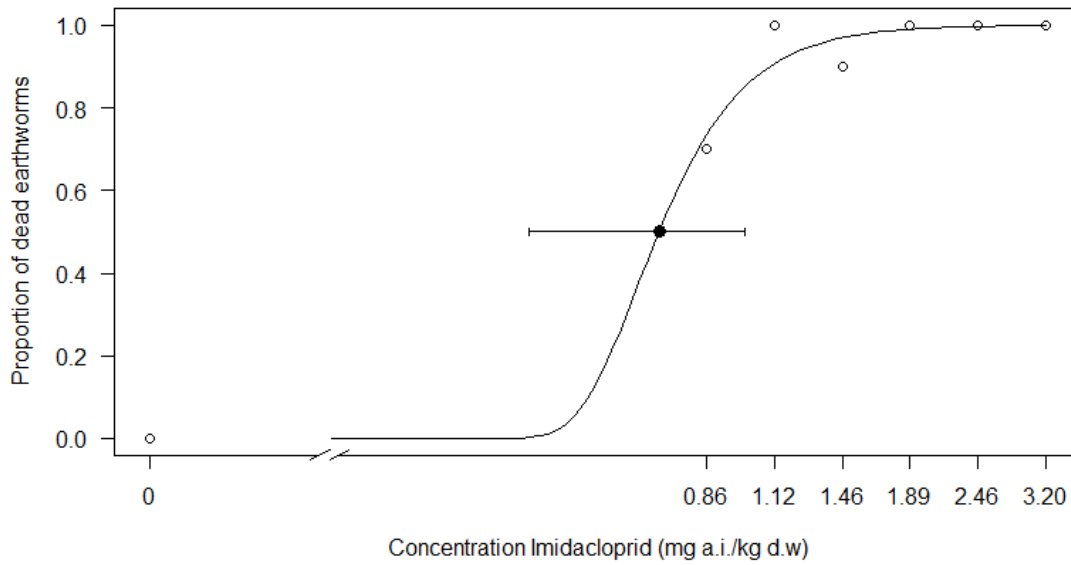
**Figure S7** Dose-response curve from *A. rosea* (ID 7; Table S3) 14-day toxicity test with imidacloprid.

8. *Aporrectodea rosea*



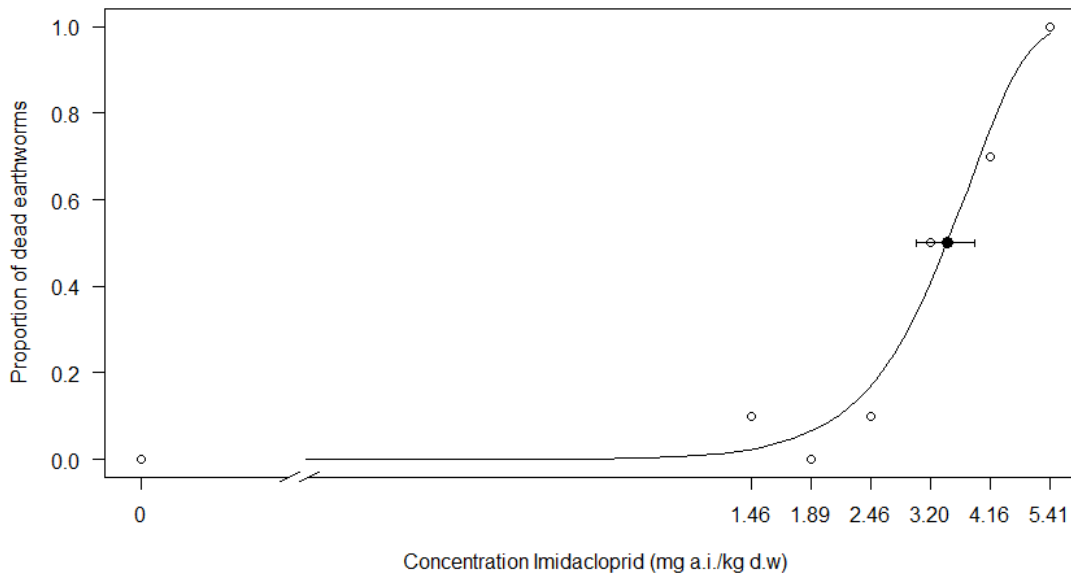
**Figure S8** Dose-response curve from *A. rosea* (ID 8; Table S3) 14-day toxicity test with imidacloprid.

9. *Allolobophora chlorotica*



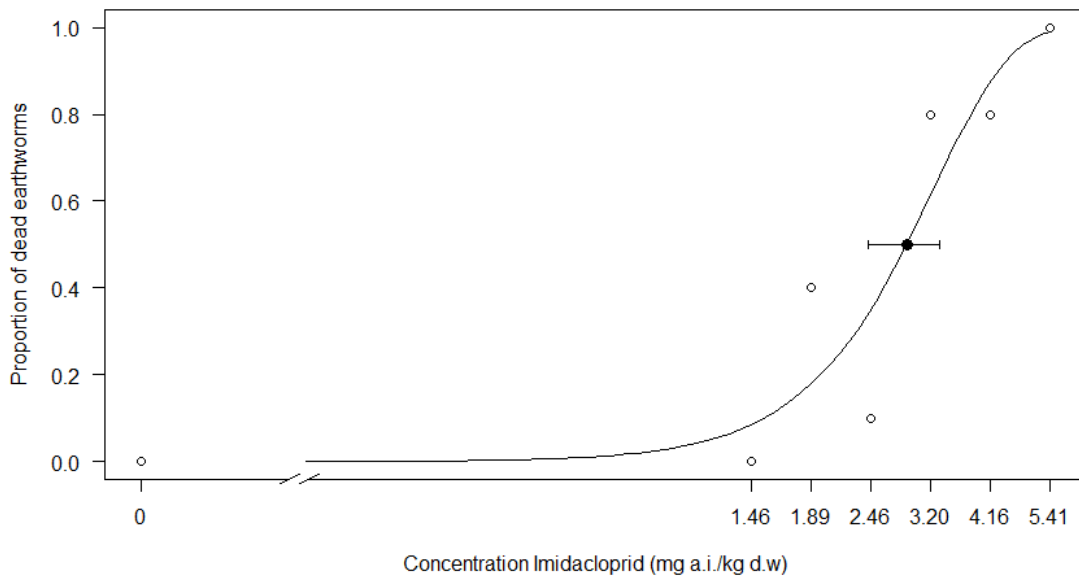
**Figure S9** Dose-response curve from *A. chlorotica* (ID 9; Table S3) 14-day toxicity test with imidacloprid.

10. *Lumbricus rubellus*



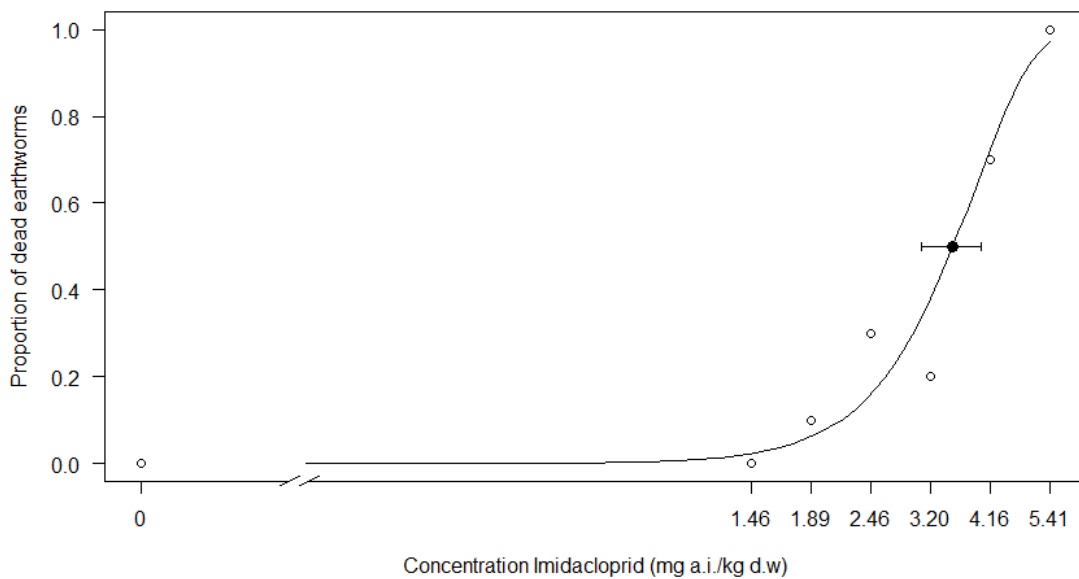
**Figure S10** Dose-response curve from *L. rubellus* (ID 10; Table S3) 14-day toxicity test with imidacloprid.

11. *Lumbricus rubellus*



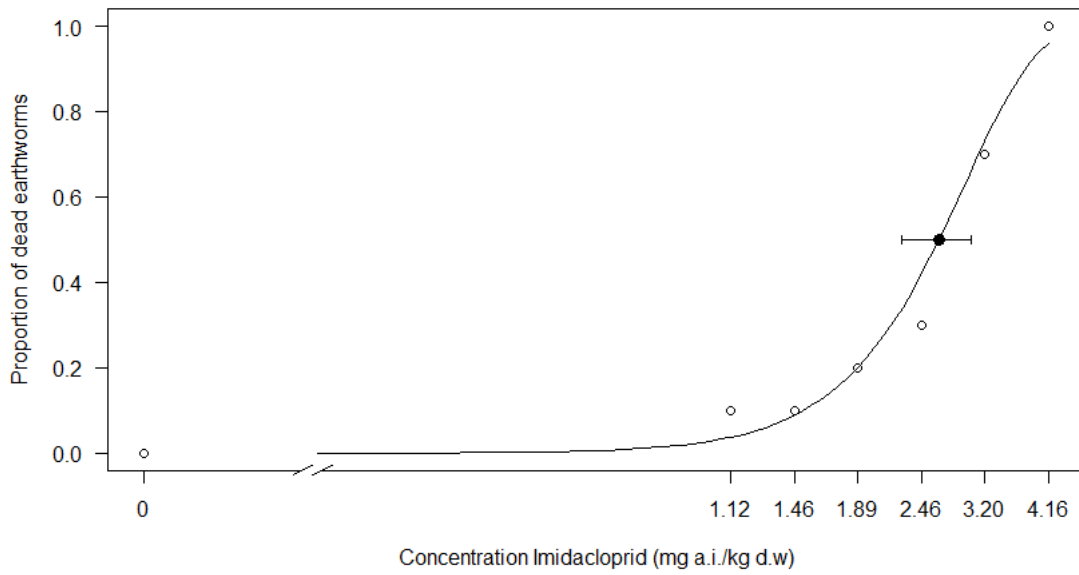
**Figure S11** Dose-response curve from *L. rubellus* (ID 11; Table S3) 14-day toxicity test with imidacloprid.

12. *Lumbricus rubellus*



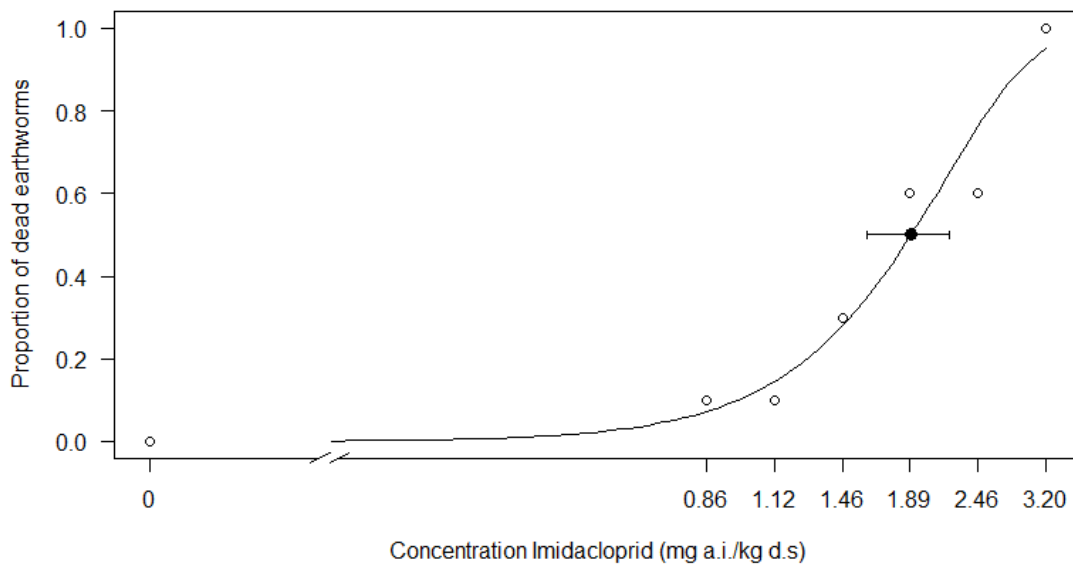
**Figure S12** Dose-response curve from *L. rubellus* (ID 12; Table S3) 14-day toxicity test with imidacloprid.

13. *Aporrectodea caliginosa*



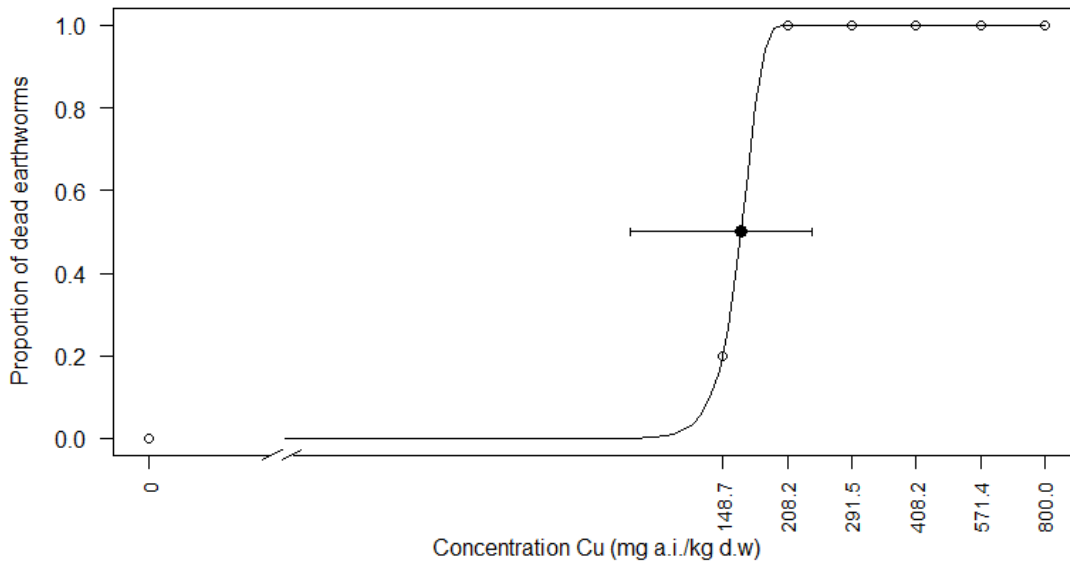
**Figure S13** Dose-response curve from *A. caliginosa* (ID 13; Table S3) 14-day toxicity test with imidacloprid.

14. *Eisenia fetida*



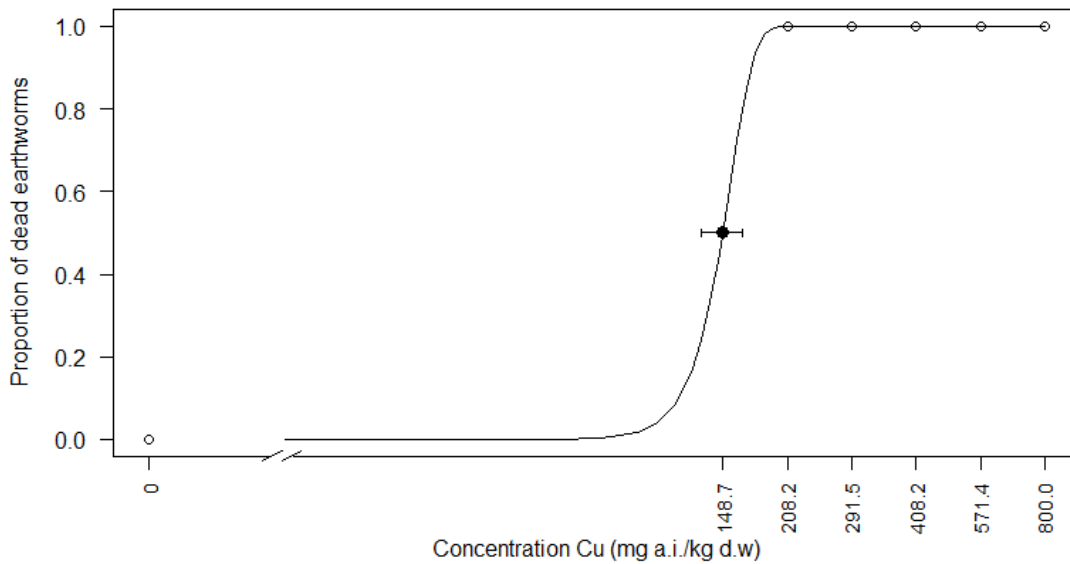
**Figure S14** Dose-response curve from *E. fetida* (ID 14; Table S3) 14-day toxicity test with imidacloprid.

1. *Aporrectodea rosea*



**Figure S15** Dose-response curve from *A. rosea* (ID 1; Table S3) 14-day toxicity test with Copper.

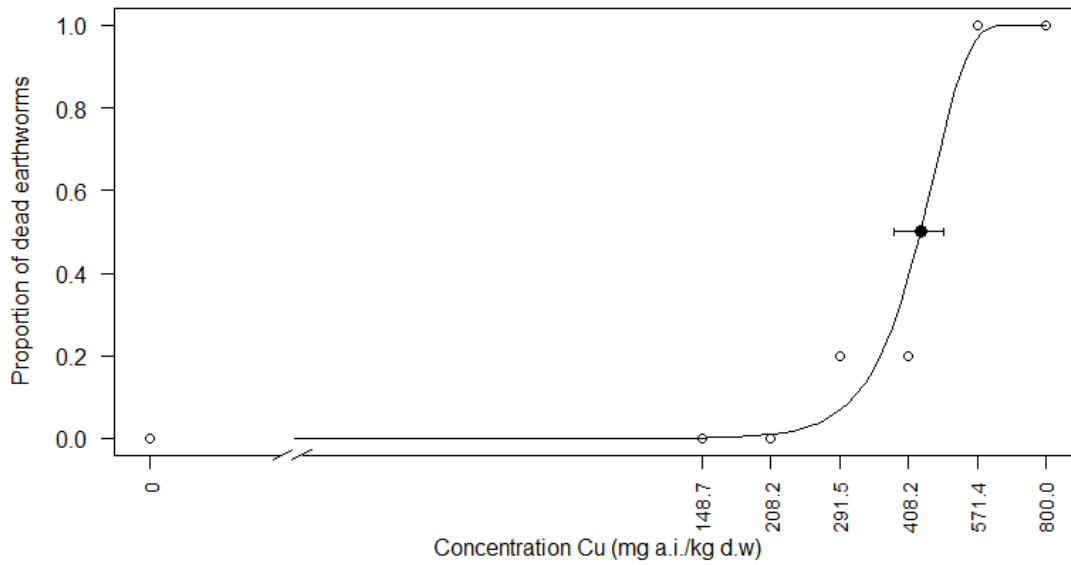
2. *Eisenia fetida*



**Figure S16** Dose-response curve from *E. fetida* (ID 2; Table S3) 14-day toxicity test with Copper.

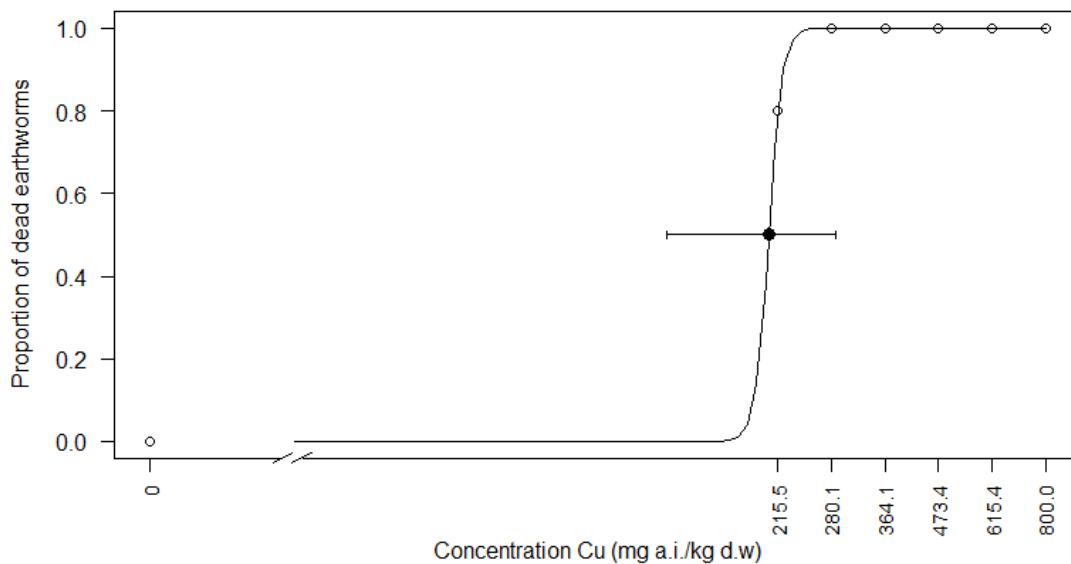


### 3. *Aporrectodea caliginosa*



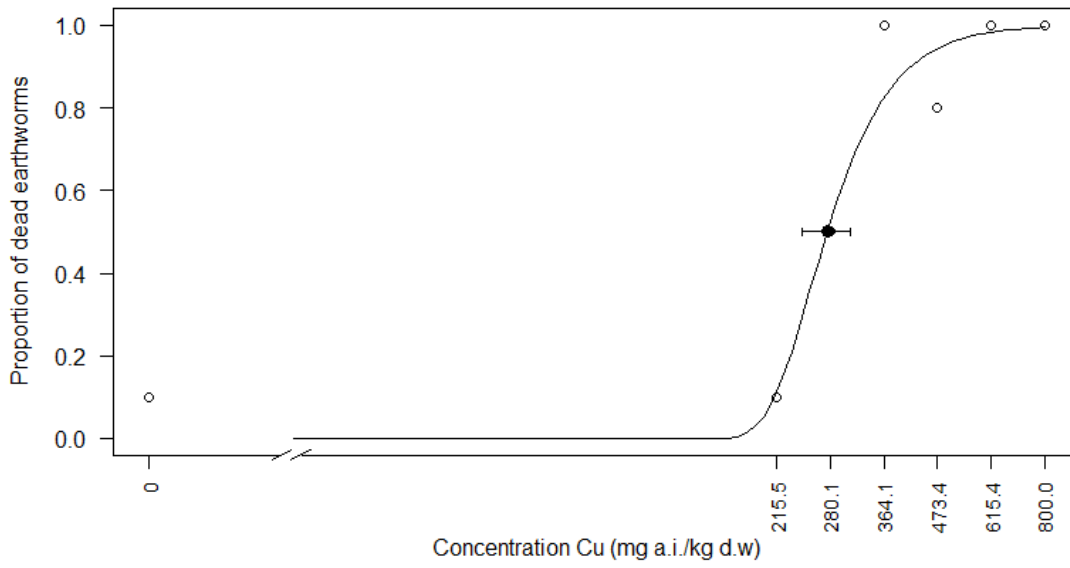
**Figure S17** Dose-response curve from *A. caliginosa* (ID 3; Table S3) 14-day toxicity test with Copper.

### 4. *Allolobophora chlorotica*



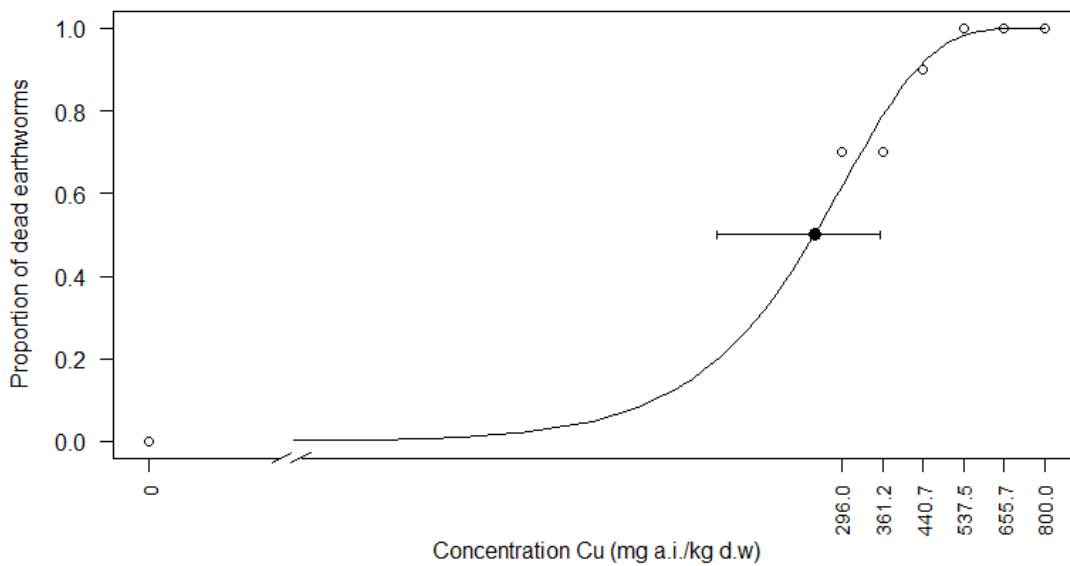
**Figure S18** Dose-response curve from *A. chlorotica* (ID 4; Table S3) 14-day toxicity test with Copper.

5. *Aporrectodea rosea*



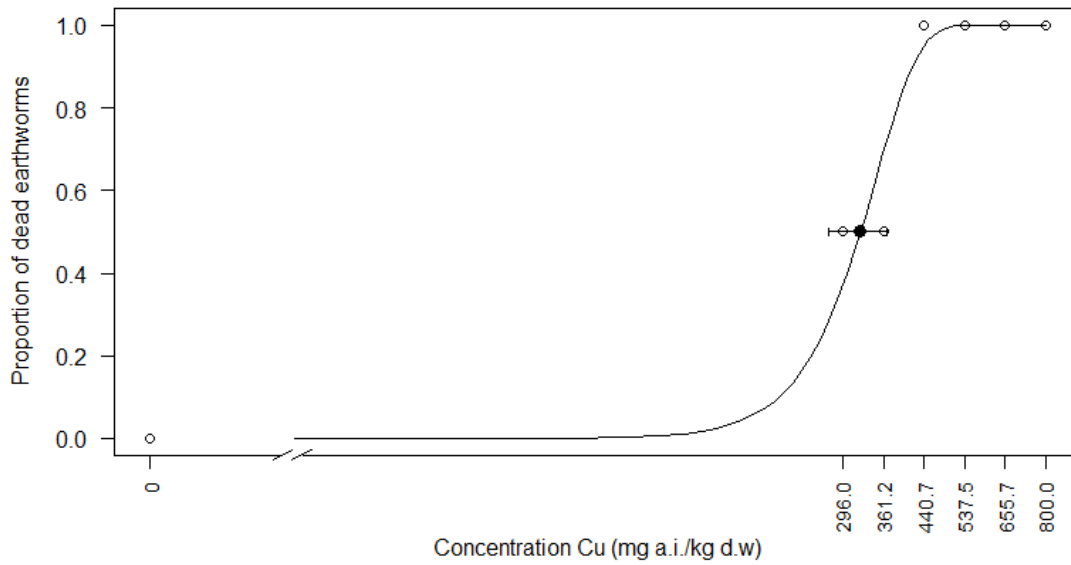
**Figure S19** Dose-response curve from *A. rosea* (ID 5; Table S3) 14-day toxicity test with Copper.

6. *Aporrectodea caliginosa*



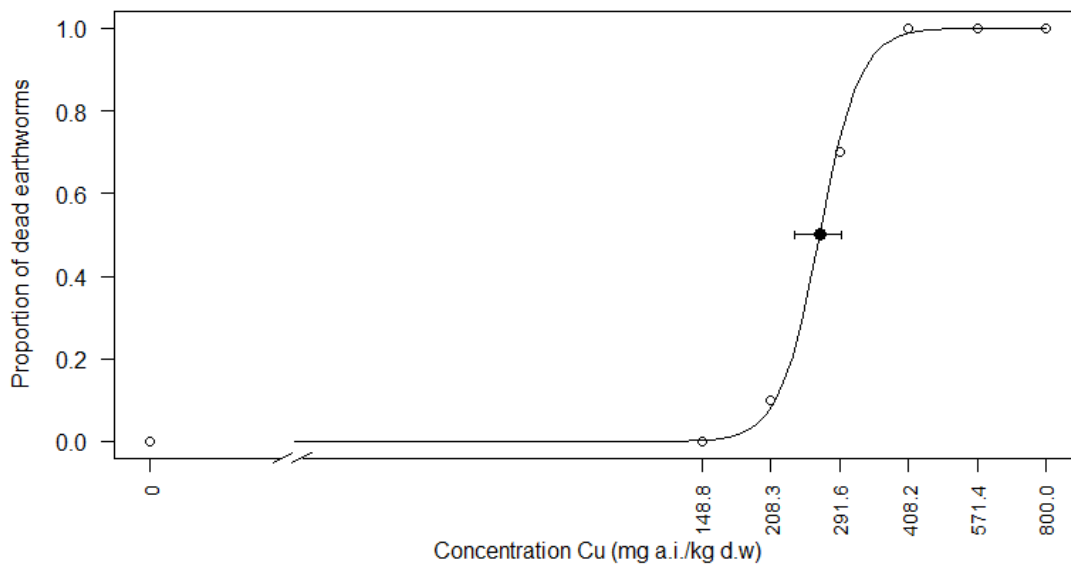
**Figure S20** Dose-response curve from *A. caliginosa* (ID 6; Table S3) 14-day toxicity test with Copper.

7. *Aporrectodea caliginosa*



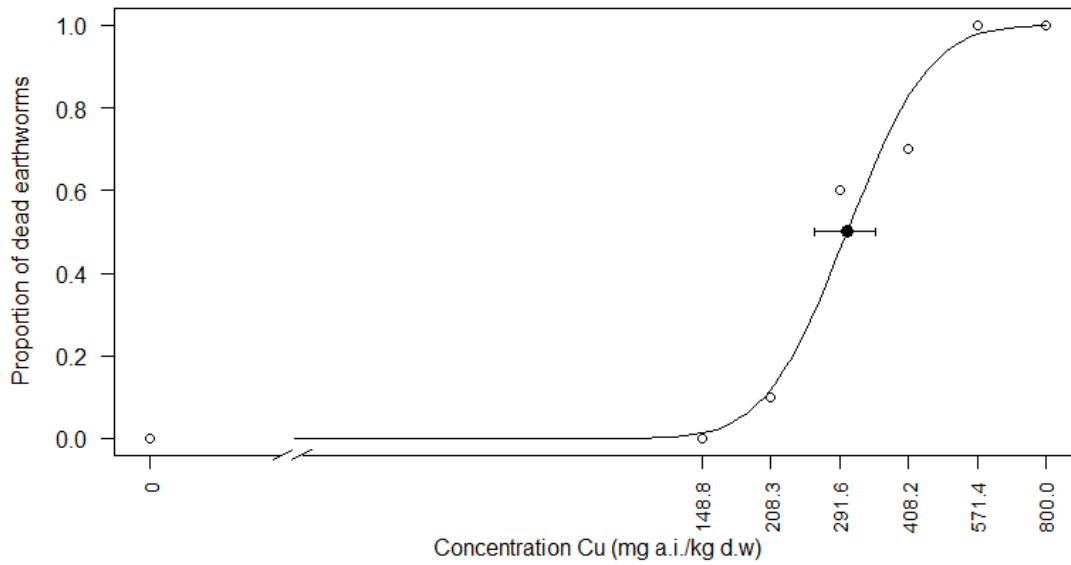
**Figure S21** Dose-response curve from *A. caliginosa* (ID 7; Table S3) 14-day toxicity test with Copper.

8. *Aporrectodea caliginosa*



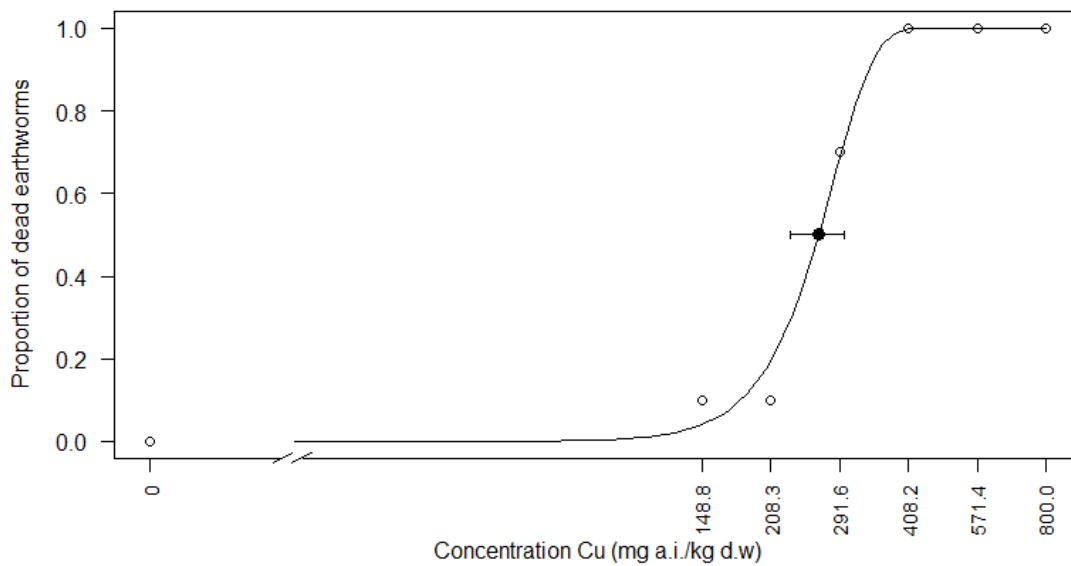
**Figure S22** Dose-response curve from *A. caliginosa* (ID 8; Table S3) 14-day toxicity test with Copper.

9. *Aporrectodea caliginosa*



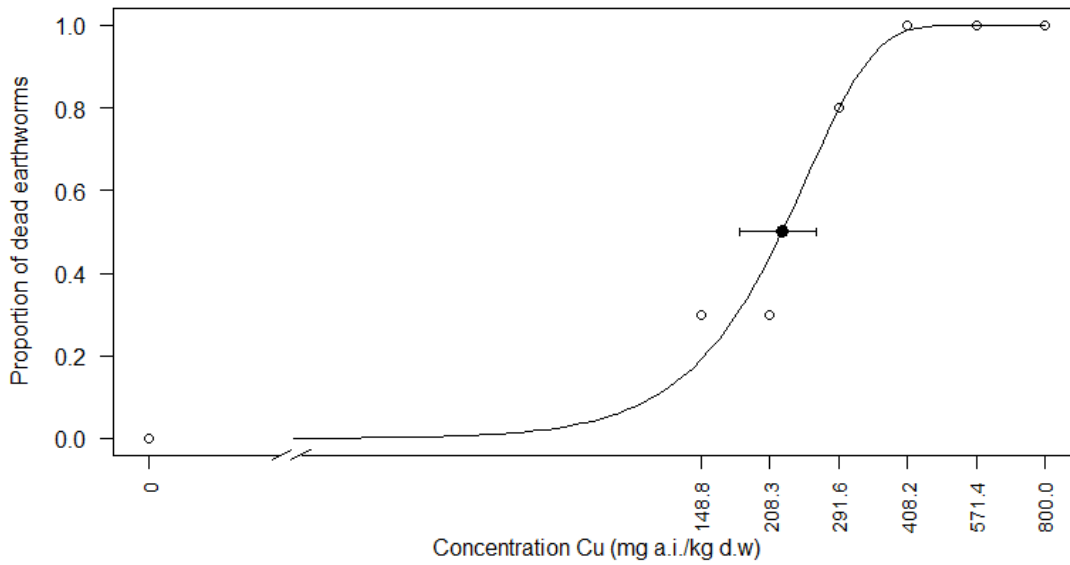
**Figure S23** Dose-response curve from *A. caliginosa* (ID 9; Table S3) 14-day toxicity test with Copper.

10. *Aporrectodea longa*



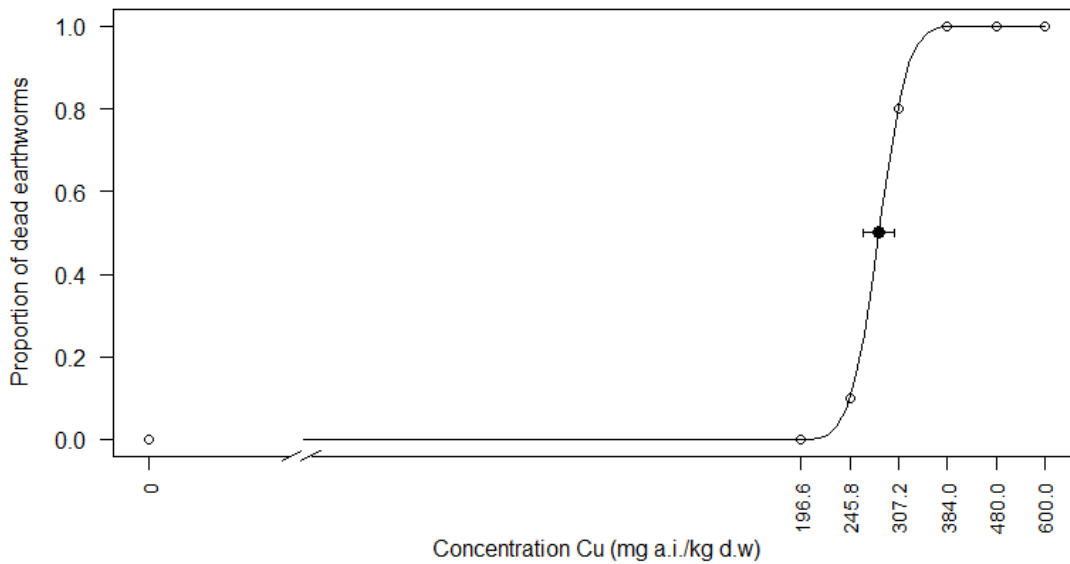
**Figure S24** Dose-response curve from *A. longa* (ID 10; Table S3) 14-day toxicity test with Copper.

11. *Aporrectodea longa*



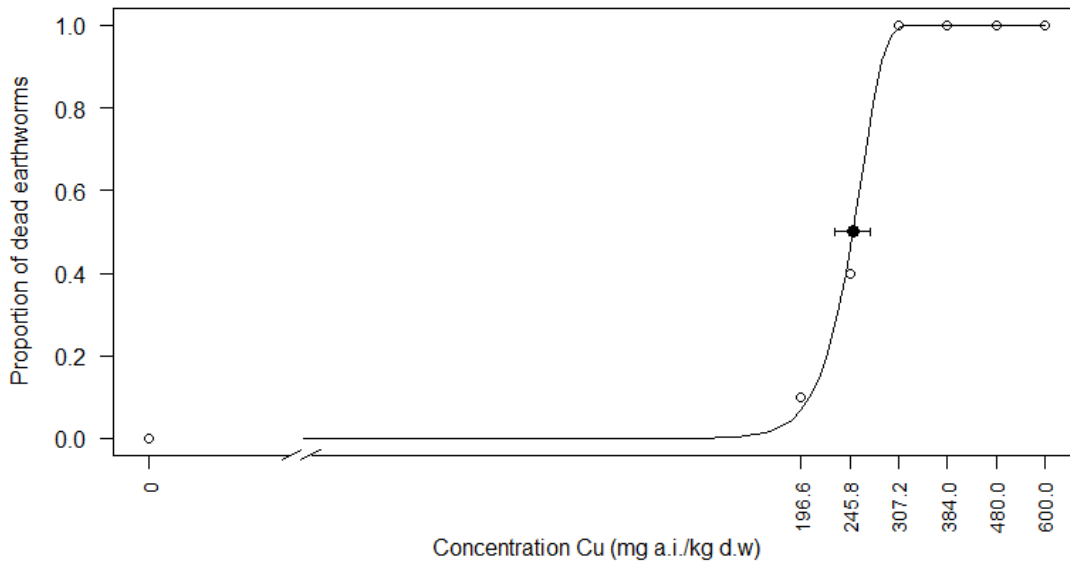
**Figure S25** Dose-response curve from *A. longa* (ID 11; Table S3) 14-day toxicity test with Copper.

12. *Aporrectodea rosea*



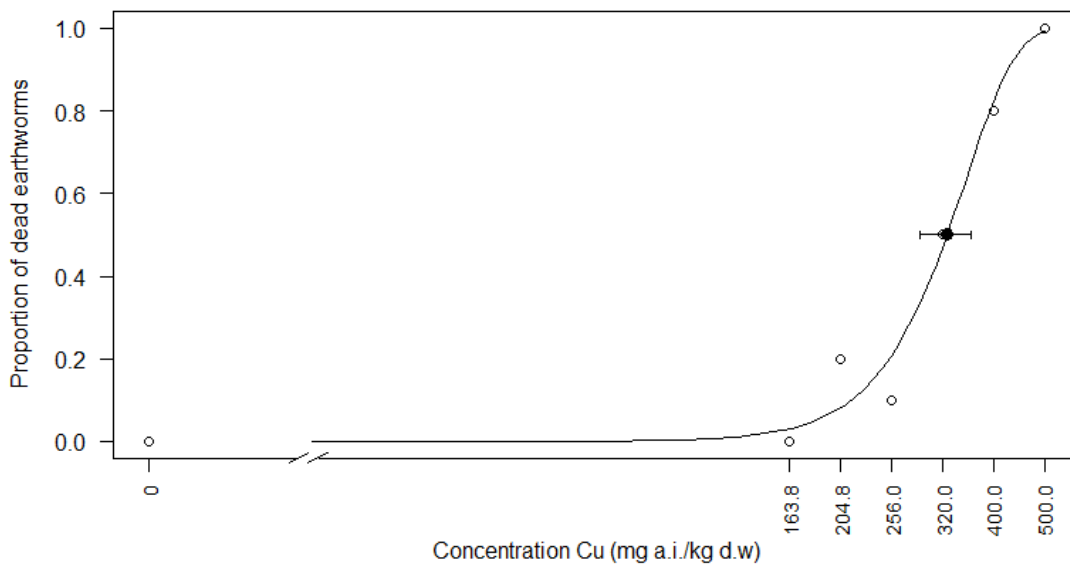
**Figure S26** Dose-response curve from *A. rosea* (ID 12; Table S3) 14-day toxicity test with Copper.

13. *Lumbricus rubellus*



**Figure S27** Dose-response curve from *L. rubellus* (ID 13; Table S3) 14-day toxicity test with Copper.

14. *Eisenia fetida*



**Figure S28** Dose-response curve from *E. fetida* (ID 14; Table S3) 14-day toxicity test with Copper.

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# Chapter 3

Sensitivity of spiders from different ecosystems to lambda-cyhalothrin: effects of phylogeny and climate

*Tomás Duque, Sumaiya Chowdhury, Marco Isaia, Stano Pekár, Kai Riess, Gregor Scherf, Ralf B. Schäfer and Martin H. Entling*



# Sensitivity of spiders from different ecosystems to lambda-cyhalothrin: effects of phylogeny and climate

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## Abstract

**BACKGROUND:** In spite of their importance as arthropod predators, spiders have received little attention in the risk assessment of pesticides. In addition, research has mainly focused on a few species commonly found in agricultural habitats. Spiders living in more natural ecosystems may also be exposed to and affected by pesticides, including insecticides. However, their sensitivity and factors driving possible variations in sensitivity between spider taxa are largely unknown. To fill this gap, we quantified the sensitivity of 28 spider species from a wide range of European ecosystems to lambda-cyhalothrin in an acute exposure scenario.

**RESULTS:** Sensitivity varied among the tested populations by a factor of 30. Strong differences in sensitivity were observed between families, but also between genera within the Lycosidae. Apart from the variation explained by the phylogeny, spiders from boreal and polar climates were more sensitive than spiders from warmer areas. Overall, the median lethal concentration (LC<sub>50</sub>) of 85% of species was below the recommended application rate of lambda-cyhalothrin (75 ng a.i. cm<sup>-2</sup>).

**CONCLUSION:** Our study underlines the high sensitivity of spiders to lambda-cyhalothrin, which can lead to unintended negative effects on pest suppression in areas treated with this insecticide. The strong differences observed between families and genera indicate that the functional composition of spider communities would change in affected areas. Overall, the variation in spider sensitivity suggests that multispecies investigations should be more widely considered in pesticide risk assessment. © 2023 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Supporting information may be found in the online version of this article.

**Keywords:** spiders; acute toxicity; species sensitivity distributions; lambda-cyhalothrin

## 1 INTRODUCTION

Spiders are abundant natural enemies in many terrestrial ecosystems.<sup>1</sup> Most spiders are polyphagous predators and consume a variety of arthropods, including pest species.<sup>2</sup> Therefore, they play a role in pest regulation, which is especially relevant for agro-ecosystems.<sup>3,4</sup> Nevertheless, their beneficial function may be disrupted because of negative effects from the use of agrochemicals, such as pesticides.<sup>5</sup> Among pesticides, synthetic insecticides and acaricides have been shown to be the most toxic compounds for spiders.<sup>6</sup> Since most neonicotinoid insecticides were banned in the European Union (EU) in 2018, other substances have regained importance.<sup>7</sup> Among them are the pyrethroid insecticides, which may affect spider survival,<sup>8,9</sup> feeding behavior<sup>9,10</sup> and locomotor activity,<sup>11</sup> even at concentrations below the recommended field dose. One widespread pyrethroid insecticide is lambda-cyhalothrin, which is known to reduce the abundance and diversity of natural enemies, including spiders, in field scenarios<sup>12,13</sup> and to cause spider mortality under laboratory conditions.<sup>8</sup> Moreover, lambda-cyhalothrin is cataloged as a bioaccumulative and toxic substance for non-target organisms,

and is currently classified as a candidate for substitution in the EU.<sup>14</sup>

Despite their ecological importance, spiders are rarely addressed in pesticide risk assessment.<sup>6,15</sup> This may be because it is challenging to establish efficient rearing techniques<sup>16,17</sup> and bioassay designs.<sup>18,19</sup> Although protocols for testing pesticides on spiders from the genus *Pardosa*<sup>20</sup> and the family Linyphiidae<sup>19</sup> have been developed, no standardized protocol applicable to all spider species has been accepted for risk assessment, because

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spider species belong to diverse hunting guilds.<sup>21</sup> Consequently, ecotoxicological information for spiders has been derived from many different laboratory test designs, with most evaluating only direct mortality<sup>6</sup> and typically testing field doses<sup>22</sup> or pesticide residues.<sup>23</sup> Only about 23% of these studies (United States Environmental Protection Agency; <https://cfpub.epa.gov/ecotox/>) have derived median lethal concentrations (LC<sub>50</sub>) or median lethal doses, which are important endpoints used in risk assessment.

Pesticide sensitivity varies between spider species.<sup>24,25</sup> This variation has mainly been attributed to behavioral and physiological factors. For example, a species' foraging mode plays an important role in the uptake of pesticides, with main differences occurring between free-hunting and web-building species.<sup>26</sup> Free-hunters are expected to have more direct contact with pesticides because of their walking activity, whereas webs can protect spiders from direct contact.<sup>25</sup> This could translate into higher sensitivity of free-hunters compared with web-builders.<sup>25</sup> Other factors that are expected to influence chemical sensitivity include body weight, because smaller species have a higher surface-to-body mass ratio. This could lead to higher pesticide concentration in the body tissue of smaller species, resulting in a higher sensitivity.<sup>27</sup>

Furthermore, spider species vary in their preferred habitat type and climate, with different species occurring in open land, wetland and forest of different climate zones.<sup>28,29</sup> Different habitat and climate with contrasting temperature and humidity may affect the chemical sensitivity of spiders because they influence the cuticular composition of arthropods. For example, spiders from dry or warm habitats are expected to have a stronger cuticle to resist desiccation,<sup>30</sup> which could translate into reduced uptake of pesticides and a higher chemical tolerance. Other morphological traits may also be linked to spider chemical sensitivity. Phylogenetic analyses are needed to account for the statistical non-independence of multiple taxa within clades. In addition, they are useful for identifying sensitive and resistant clades.<sup>31,32</sup>

The aim of this study was to investigate potential relationships between the chemical sensitivity of 28 spider species, phylogenetic signal, traits (foraging mode and weight) and habitat preferences. We sampled spiders from different climate zones and ecosystem types across Europe. Spiders were reared in the laboratory and their spiderlings were subsequently used for acute ecotoxicological testing with the pyrethroid insecticide lambda-cyhalothrin. We conducted 24-h, single-species tests based on previous acute ecotoxicological protocols,<sup>19,33</sup> with some adaptations to allow for LC<sub>50</sub> calculations. In addition, we derived species sensitivity distributions (SSDs) to identify sensitive species, and calculated the hazardous concentrations affecting 5% (HC<sub>5</sub>) of the tested spider species according to their climate zone of origin. We tested the following hypotheses: (i) spider sensitivity in terms of LC<sub>50</sub> varies among the tested species; (ii) variation in pesticide sensitivity can be explained by spider traits, such as foraging mode and body mass, where free-hunters<sup>25</sup> and smaller spiders<sup>27</sup> are expected to be more sensitive; (iii) spider sensitivity is related to habitat characteristics, where spiders collected in dry and warm habitats are less sensitive to lambda-cyhalothrin, because of a stronger cuticle<sup>30</sup> which may reduce pesticide uptake.

## 2 MATERIALS AND METHODS

### 2.1 Source of spiders

We collected adult female spiders with a cocoon or that were visually pregnant in the field. For linyphiids, we collected males and females and allowed them to mate in the laboratory to increase

the chance of reproduction. Spiders were collected by hand, using empty pitfall traps or an inverted leaf-blower (Stihl SH 85; Andreas Stihl, Dieburg, Germany), between spring 2020 and autumn 2021. Sampling sites were selected to cover the four main western European Holdridge life zones (HLZ): polar, boreal, cool temperate and warm temperate.<sup>34</sup> Because spider communities are differentiated mainly by moisture and shading,<sup>28</sup> three main habitat types were selected in each HLZ: open space, wetland and forest. Sampling was done in Denmark, France, Germany, Italy and Switzerland, in natural areas and agroecosystems (Supporting Information, Table S1). None of the collected species (Table 1) are threatened or under special protection in their respective countries.<sup>35</sup> Once collected, spiders were placed individually in a glass jar (35 mL, 44 mm diameter × 42.5 mm height) with a layer (~1 cm) of moistened plaster and transported to the laboratory (iES Landau, Germany).

### 2.2 Rearing of spiderlings under laboratory conditions

In the laboratory, female spiders were individually transferred to larger containers with a layer (1 cm) of moistened plaster. Free-hunters (Table 1) were kept in polypropylene boxes (1 L, 18 cm length × 13.2 cm width × 6.8 cm height), and web-builders (Table 1) were kept in plastic cups (770 mL, 11.8 cm diameter × 11.3 cm height) with four plastic sticks fixed in the plaster to allow for web construction. Spiders were fed *ad libitum* biweekly with a mixture of fruit flies: *Drosophila hydei* (Sturtevant) *Drosophila melanogaster* (Meigen) and springtails (*Coecobrya tenebricosa*, Folsom; see Supporting Information, DNA Barcoding of the tropical springtail population of *Coecobrya tenebricosa*) to maintain a polytypic diet.<sup>17</sup> Because most linyphiids depend on high moisture,<sup>36</sup> they were kept in a climate chamber at 20 ± 1 °C, 100% relative humidity and 16/8 h light/dark cycle, and because of their small size, were only offered springtails as prey. If available, a male was added with each linyphiid female for two nights to facilitate reproduction and later cocoon production. Non-linyphiids were kept at room temperature (~20–25 °C), avoiding direct sunlight. Once spiderlings hatched, they were kept with their mother for approximately 1 week and the supply of springtails was increased. The 1-week-old spiderlings were individually transferred to glass jars with moistened plaster and kept at room temperature, except for linyphiids, which were kept in the climate chamber as described above. Juveniles were fed *ad libitum* with springtails or fruit flies twice per week, depending on the spiderling size. Once spiderlings reached 1 month of age, they were used for acute exposure testing.

### 2.3 Identification of spiders to species level

After juvenile hatching, the mother spider was removed, preserved in ethanol (70%) and identified to species following the identification keys of Roberts<sup>37</sup> and Nentwig *et al.*<sup>38</sup> The cryptic species *Pardosa proxima* (Koch), *Pardosa tenuipes* (Koch) and *Trochosa hispanica* (Simon) were identified using DNA barcoding of the cytochrome c oxidase subunit 1 (COI) gene (see Supporting Information, Phylogenetic tree). It should be noted that *P. tenuipes* sequences are not available in the National Center for Biotechnology Information database and this species is usually identified as *P. proxima*.<sup>39</sup> Thus, following Isaia *et al.*,<sup>39</sup> males from *P. proxima* and *P. tenuipes* were identified morphologically and sequenced as described above. Female sequences were then matched with the male sequences, and females were assigned to species according to the known identity of the males. In addition, males of the cryptic species *Pardosa saltans* (Töpfer-Hofmann) were

**Table 1.** Chemical sensitivity ( $LC_{50} \pm 95\%$  confidence intervals) of collected spider species with their respective habitat and trait characterization

Family	Species	Climate (HLZ)	Habitat	Foraging mode	Fresh weight (mg)		Body length (mm) <sup>a</sup>	$LC_{50}$ (ng a.i. $cm^{-2}$ )
					Mean $\pm$ SD	<i>n</i>		
Clubionidae	<i>Clubiona alpicola</i>	Boreal + polar	Open space	Free	1.54 $\pm$ 0.30	70	6.00	9.00 $\pm$ 3.24
Gnaphosidae	<i>Drassodex heeri</i>	Boreal + polar	Open space	Free	2.09 $\pm$ 0.47	70	12.95	7.60 $\pm$ 4.35
	<i>Haplodrassus signifer</i>	Boreal + polar	Open space	Free	0.64 $\pm$ 0.31	70	8.45	10.75 $\pm$ 5.52
	<i>Zelotes apricorum</i>	Boreal + polar	Open space	Free	0.88 $\pm$ 0.28	24	7.60	16.37 $\pm$ 10.99
Linyphiidae	<i>Diplocephalus graecus</i>	Warm temperate	Open space	Web	0.68 $\pm$ 0.39	65	2.00	29.56 $\pm$ 8.51
	<i>Erigone atra</i>	Cool temperate	Open space	Web	1.01 $\pm$ 0.45	70	2.30	35.93 $\pm$ 10.49
	<i>Erigone dentipalpis</i>	Cool temperate	Open space	Web	1.21 $\pm$ 0.42	35	2.30	22.24 $\pm$ 12.93
	<i>Gnathonarium dentatum</i>	Warm temperate	Wetland	Web	1.79 $\pm$ 0.63	63	2.60	91.44 $\pm$ 28.74
	<i>Mermessus trilobatus</i>	Cool temperate	Open space	Web	1.18 $\pm$ 0.32	70	1.85	75.99 $\pm$ 27.39
	<i>Oedothorax fuscus</i>	Cool temperate	Open space	Free	0.89 $\pm$ 0.33	18	2.55	30.07 $\pm$ 19.22
	<i>Tenuiphantes tenuis</i>	Cool temperate	Open space	Web	1.23 $\pm$ 0.59	56	3.15	48.19 $\pm$ 14.23
Lycosidae	<i>Pardosa agrestis</i>	Cool temperate	Open space	Free	1.25 $\pm$ 0.40	70	5.00	14.75 $\pm$ 5.10
	<i>Pardosa amentata</i>	Boreal + polar	Wetland	Free	1.10 $\pm$ 0.30	140	6.50	6.68 $\pm$ 2.00
	<i>Pardosa amentata</i>	Cool temperate	Wetland	Free	1.25 $\pm$ 0.43	48	6.50	12.81 $\pm$ 7.98
	<i>Pardosa hortensis</i>	Cool temperate	Open space	Free	0.83 $\pm$ 0.35	70	5.50	14.80 $\pm$ 7.48
	<i>Pardosa hortensis</i>	Warm temperate	Forest	Free	2.45 $\pm$ 0.76	20	5.50	12.43 $\pm$ 30.22
	<i>Pardosa morosa</i>	Warm temperate	Wetland	Free	2.27 $\pm$ 0.69	15	8.00	16.79 $\pm$ 14.80
	<i>Pardosa oreophila</i>	Boreal + polar	Open space	Free	0.73 $\pm$ 0.25	30	6.00	9.55 $\pm$ 5.61
	<i>Pardosa proxima</i>	Warm temperate	Open space	Free	0.91 $\pm$ 0.23	36	7.50	16.79 $\pm$ 7.80
	<i>Pardosa riparia</i>	Boreal + polar	Forest	Free	0.91 $\pm$ 0.34	70	5.50	11.99 $\pm$ 3.96
	<i>Pardosa saltans</i>	Cool temperate	Forest	Free	1.84 $\pm$ 0.60	122	6.25	18.13 $\pm$ 11.49
	<i>Pardosa tenuipes</i>	Warm temperate	Open space	Free	1.14 $\pm$ 0.21	24	5.60	13.26 $\pm$ 8.74
	<i>Pardosa wagleri</i>	Warm temperate	Wetland	Free	1.77 $\pm$ 0.48	25	7.10	35.97 $\pm$ 13.03
	<i>Piratula hygrophila</i>	Cool temperate	Forest	Free	2.22 $\pm$ 0.61	140	5.35	117.13 $\pm$ 39.93
	<i>Piratula latitans</i>	Cool temperate	Wetland	Free	2.15 $\pm$ 0.80	70	4.50	123.23 $\pm$ 75.94
	<i>Trochosa hispanica</i>	Warm temperate	Wetland	Free	3.29 $\pm$ 0.92	42	12.20	71.10 $\pm$ 29.11
Pisauridae	<i>Pisaura mirabilis</i>	Cool temperate	Open space	Free	0.95 $\pm$ 0.25	63	13.50	191.22 $\pm$ 51.76
Theridiidae	<i>Enoplognatha ovata</i>	Cool temperate	Forest	Web	0.27 $\pm$ 0.16	23	6.60	67.64 $\pm$ 35.32
Thomisidae	<i>Xysticus desidiosus</i>	Boreal + polar	Open space	Free	0.68 $\pm$ 0.18	42	6.20	21.42 $\pm$ 9.50
Zoropsidae	<i>Zoropsis spinimana</i>	Warm temperate	Open space	Free	4.46 $\pm$ 0.98	70	14.50	14.64 $\pm$ 4.06

Abbreviation: HLZ, Holdridge life zones;  $LC_{50}$ , median lethal concentration; a.i., active ingredient.

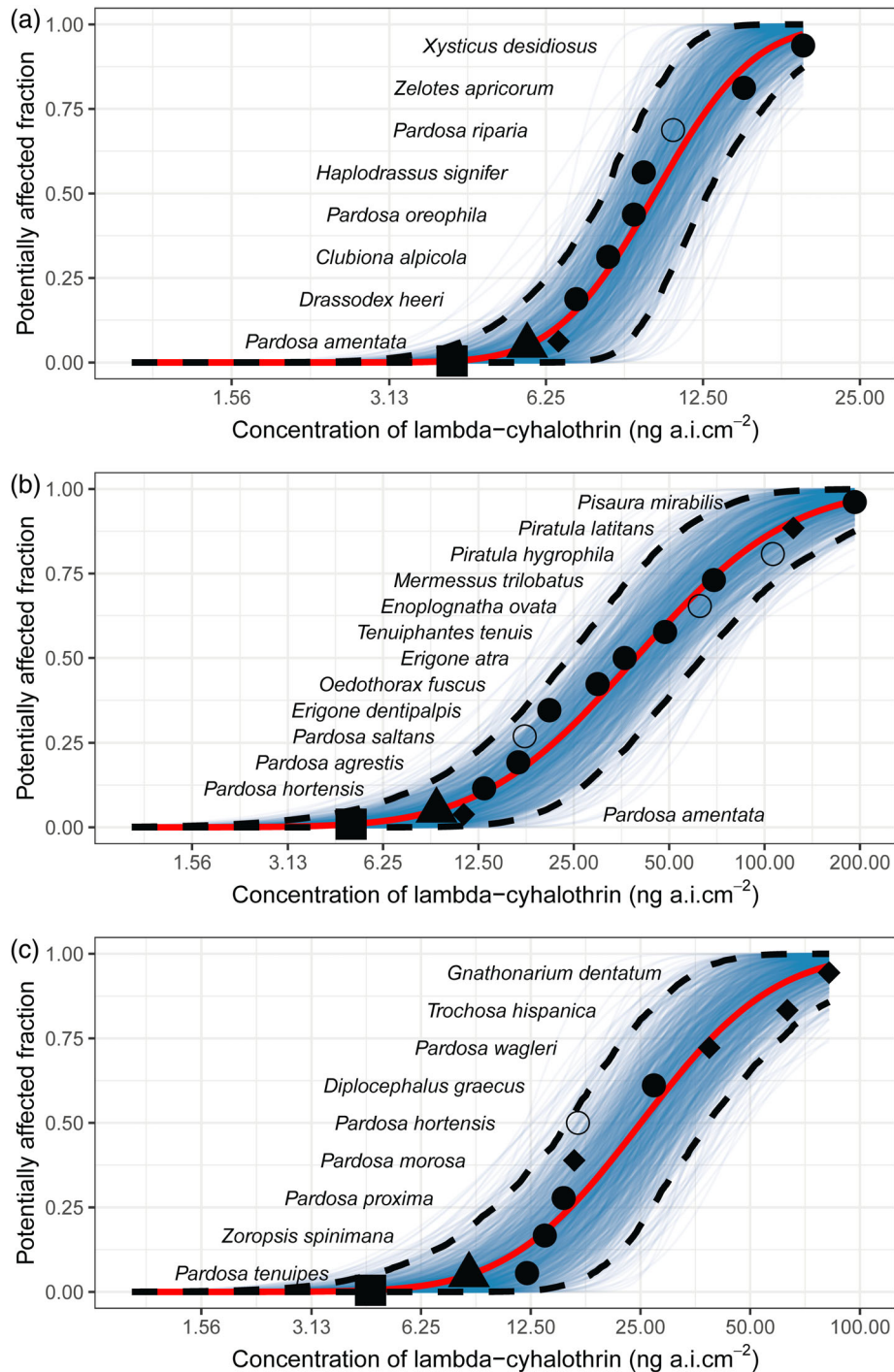
<sup>a</sup> Calculated with the average female body length (max + min/2) from the identification keys of Nentwig *et al.*<sup>36</sup> and Roberts.<sup>35</sup>

identified morphologically<sup>37,38</sup> and females from the same sampling sites were assumed to be the same species.

## 2.4 Ecotoxicological assessment

The spider acute exposure test was designed based on the pesticide exposure assay described by Aukema *et al.*<sup>19</sup> and Tahir *et al.*<sup>33</sup> with modifications to allow for dose–response calculations. Two days before the start of the test, spiderlings of uniform age were individually transferred to glass jars (35 mL, 44 mm diameter  $\times$  42.5 mm height) with moistened plaster, and no food was provided.<sup>25</sup> Juveniles were stored in a climate chamber at  $20 \pm 1$  °C, 100% relative humidity, and 16/8 h light/dark cycle. The insecticide lambda-cyhalothrin [5% active ingredient (a.i.); Hunter® EG, Certis Europe, Hamburg, Germany] was used for acute exposure testing. The insecticide was weighed to the nearest 0.01 mg (AT261 DeltaRange® 205 g/0.01 mg, Metler Toledo, Columbus, Ohio, USA), diluted in ultrapure water, and stock solutions were created using serial dilutions. Range-finding tests for

some species were performed around the recommended application rate of lambda-cyhalothrin (75 ng a.i.  $cm^{-2}$ ; Certis Europe, <https://www.certiseurope.de/produkte/>), which has been reported to decrease spider abundance on fields.<sup>12</sup> Generally, 70 spiderlings were used for one test; however, for some species, juvenile hatching and survival rates were lower and tests had to be done with fewer individuals (Supporting Information, Table S2). In most cases, we used seven concentrations of lambda-cyhalothrin (between 0 and 503 ng a.i.  $cm^{-2}$ ; Supporting Information, Table S2) in a geometric series (Supporting Information, Table S2), including one control, for each species and ecosystem of origin; i.e., where the mother spider was collected (Supporting Information, Table S1). There were ten replicates per concentration. Each replicate consisted of one juvenile, previously weighed (Table 1) to the nearest 0.1 mg (PA214® 210 g/0.0001 g; Ohaus, Parsippany, NJ, USA), placed on a filter paper (MN 615, 90 mm diameter; Macherey-Nagel, Düren, Germany) immediately after insecticide application. Filter papers



**Figure 1.** Species sensitivity distributions for boreal + polar (a), cool temperate (b) and warm temperate climates (c) calculated from multiple spider species sensitivity (red line). The 24-h median lethal test concentration ( $LC_{50}$ ) values of spider species are represented by habitats: open points) forest (open circles) and wetland (black diamonds). Species names are aligned by sensitivity in ascending order from bottom to top on y-axes. The x-axes are on a log scale. Dashed lines enclose parametric bootstrap (95% confidence intervals; 1000 iterations). Blue transparent lines display all parametric bootstrap samples. The black triangle marks the hazardous concentrations affecting 5% of the tested spider species ( $HC_5$  value) and the black square its lower limit. a.i., active ingredient.

were previously treated with an aliquot (1 mL, applied with an Eppendorf pipette) of the desired test concentration in a glass Petri dish (90 mm diameter; Steriplan®, DWK Life Sciences, Wertheim, Germany). Ultrapure water was used as a control. Petri dishes with the spiderlings and moist filter papers were closed and placed back in the climate chamber, and survival was visually assessed and confirmed by testing the spider's reaction to a

gentle mechanical stimulus 24 h after the application. Spiderlings were classified as alive, dead or paralyzed.<sup>11</sup>

## 2.5 Data analysis

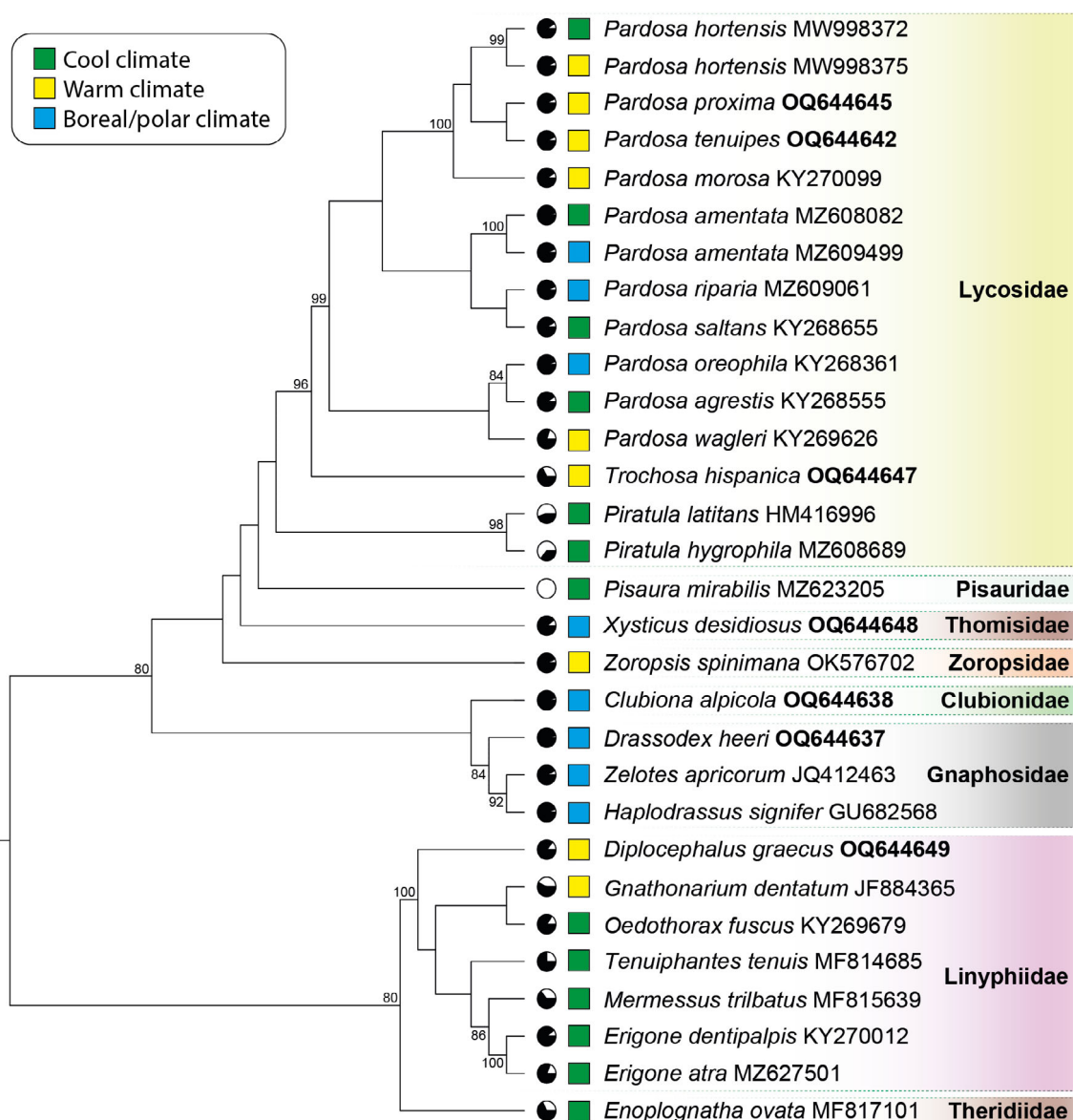
For dose–response calculations, paralyzed individuals were considered as ‘alive’. Because fewer than 12% of all individuals were paralyzed, this had only a minor influence on the  $LC_{50}$  values



calculated (Supporting Information; Table S3). For all tested species, the two-parameter log-logistic model (Supporting Information, The two-parameter log-logistic model for binomial dose-response) was fitted, and  $LC_{50}$  values after 24 h of exposure were calculated following Ritz *et al.*<sup>40</sup> for binomial dose-response data (Supporting Information, Figs S1–S34). Intra-specific variations in sensitivity between spiders from the same species collected in the same climate (Supporting Information, Table S4) were assessed via pairwise comparisons of multiple binomial dose-response curves.<sup>40</sup> Populations of the same species from different climate zones were included separately for the SSDs and the generalized least squares (GLS) analysis (see below). Separate SSDs<sup>41</sup> were fitted for warm temperate and cool temperate climates (Table 1). A joint SSD was fitted for polar and boreal climate because fewer species were available from these zones, and locations were only marginally below or above the

biotemperature limit between the two zones. If multiple  $LC_{50}$  values from the same species collected in the same climate zone were available (Supporting Information, Table S2), the geometric mean  $LC_{50}$  was computed (Table 1). The  $HC_5$  values were derived from the SSDs, and parametric bootstrap 95% confidence intervals (95%CI; 1000 iterations) were calculated to obtain the lower limits of  $HC_5$ .

In addition, the effects of habitat (open space, wetland and forest), climate (boreal + polar, cool and warm temperate), foraging mode and fresh body weight (Table 1) on the sensitivity of spiders ( $LC_{50}$ ) were tested using GLS<sup>42</sup> with phylogenetic covariance structure. Briefly, we estimated a phylogenetic tree based on COI sequences for all study species (657 bp) using the maximum likelihood (ML) approach with combined rapid bootstrapping under the GTRCAT model with 1000 runs in RAxML version 8.2.10.<sup>43</sup> The phylogenetic information using only the COI gene



**Figure 2.** Tree topology based on the partial COI gene (657 bp) using a maximum likelihood (ML) approach from 28 spider species. Branch lengths were estimated using Grafen's method<sup>44</sup> adopting the topology from the spider tree of life.<sup>45</sup> Numbers on branches are bootstrap values obtained from 1000 replicates (only values  $\geq 70$  are shown). Color boxes indicate climate zones. Pie charts represent the chemical sensitivity (black = high sensitivity). GenBank accession number from new sequences generated in this study are given in bold.

was incomplete; thus, branch lengths were estimated using Grafen's method,<sup>44</sup> adopting the topology of the eight families tested from the spider tree of life.<sup>45</sup> Technical details are given in the Supporting Information, Phylogenetic Tree, Phylogenetic correlation analysis. The response variable (LC<sub>50</sub> values) was logarithmically transformed. Pagel's<sup>46</sup>  $\lambda$  phylogenetic structure was used to test the effect of phylogeny, where a value of  $\lambda$  close to 1 indicates a strong effect of phylogeny, and a value close to 0 indicates a weak effect of phylogeny. All statistical analyses were conducted with R version 4.2.2 for Windows<sup>47</sup> together with the add-on packages "drc",<sup>48</sup> "multcomp",<sup>49</sup> "plotrix"<sup>50</sup> for the dose-response modeling, "fitdistrplus",<sup>51</sup> "reshape2",<sup>52</sup> "ggplot2",<sup>53</sup> "ggpubr"<sup>54</sup> for the SSD, "ape"<sup>55</sup> and "nlme"<sup>56</sup> for GLS.

### 3 RESULTS

#### 3.1 Species sensitivity distributions

In total, 34 toxicity tests were performed and included 28 spider species from the families Clubionidae, Gnaphosidae, Linyphiidae, Lycosidae, Pisauridae, Theridiidae, Thomisidae and Zoropsidae (Table 1). Spider LC<sub>50</sub> values ranged from 6.6 to 19.5 ng a.i. cm<sup>-2</sup> for boreal + polar climate species, from 11.2 to 192.6 ng a.i. cm<sup>-2</sup> for cool temperate species, and from 12.2 to 82.3 ng a.i. cm<sup>-2</sup> for warm temperate species (Fig. 1; Supporting Information, Table S5). Spider HC<sub>5</sub> values (95% CI) derived from the SSDs for boreal + polar, cool and warm temperate climates were 5.8 (4.4–8.6), 9.2 (4.7–21.0) and 8.5 (4.8–17.8) ng a.i. cm<sup>-2</sup>, respectively. *Pardosa amentata* (Clerck) was the most sensitive species in boreal + polar and in cool temperate climates, whereas *P. tenuipes* was the most sensitive species in the warm temperate climate.

#### 3.2 Spider sensitivity, phylogenetic correlation and habitat characteristics

The value of  $\lambda$  was 1.08, showing that chemical sensitivity was strongly affected by the phylogeny (Fig. 2). Gnaphosidae were three and four times more sensitive than Lycosidae and Linyphiidae, respectively (Table 1). Among Lycosidae species, the genus *Pardosa* was seven and four times more sensitive than *Pirattula* and *Trochosa*, respectively. Within Linyphiidae, the differences between species were less than a factor of three, whereas Gnaphosidae did not differ by more than a factor of two. In addition to the phylogenetic signal, spider sensitivity was significantly higher for species from boreal + polar climates than for cool and warm temperate climates (Table 2). Boreal + polar spiders were

five and three times more sensitive to lambda-cyhalothrin tested than cool and warm temperate species, respectively (Fig. 3; Table 1). After accounting for phylogeny, the effects of habitat, foraging mode and fresh weight on chemical sensitivity were not significant (Table 2).

## 4 DISCUSSION

#### 4.1 Spider chemical sensitivity and phylogenetic signal

Spiders from the family Linyphiidae were less sensitive than spiders from the genus *Pardosa* and the family Gnaphosidae (Fig. 2). This corresponds to the expectation that web-builders (Linyphiidae) are less sensitive than free-hunters (*Pardosa* and Gnaphosidae). Free-hunters presumably had more contact with lambda-cyhalothrin in the applied area because of their higher mobility in comparison with web-builders, which may result in a higher insecticide uptake. However, differences in sensitivity were also observed between species with similar traits, for example the ground-hunting *Pardosa* spp. were seven times more sensitive than the equally ground-hunting *Pirattula* spp. (Table 1; Fig. 1). Overall, pesticide sensitivity was strongly determined by phylogeny, most likely because evolutionary-stable morphological and anatomical characteristics affect the toxicokinetics and toxicodynamics of pesticides.

#### 4.2 Spider chemical sensitivity, traits and habitat characteristics

Contrary to our expectations, we did not find any relationship between chemical sensitivity and spider traits, such as foraging mode and body mass (Table 2). However, this may be partly because the foraging mode varies mostly between families,<sup>21</sup> and differences in chemical sensitivity between families was already taken into account by the phylogenetic analysis. When phylogeny was not considered, web-builder spiders were significantly less sensitive than free-hunters (data not shown), in accordance with a previous meta-analysis across pesticides.<sup>6</sup> Because of the link between traits and phylogeny, our results should not be taken as evidence against the effects of traits on chemical sensitivity. Instead, our results show that for a proper test of traits, more pairs of closely related species with contrasting traits need to be selected. With regard to web building, *Aulonia* and *Pardosa* (both Lycosidae), *Metellina* and *Pachygnatha* (both Tetragnathidae) and *Mermessus* and *Oedothorax* (both Linyphiidae) exemplify such species pairs, where the respective second-mentioned genus has abandoned web building. Among the Linyphiidae that we tested in cool temperate climate (Fig. 1(b)), the two strictly web-building species *Mermessus* and *Tenuiphantes* are indeed less sensitive than *Oedothorax* and *Erigone*, which at least partly hunt outside webs.<sup>57</sup>

Unexpectedly, typically agrobiont species (Supporting Information, Table S1), such as *Diplocephalus graecus* (Pickard-Cambridge), *Erigone dentipalpis* (Wider) and *Pardosa agrestis* (Westring), were generally more sensitive than related species sampled in non-agricultural ecosystems. Thus, we found no indication for a possible development of pesticide tolerance in spiders from agricultural ecosystems. The high sensitivity of agrobiont species indicates that pesticide exposure can negatively affect biological control, an important ecological function of spiders.<sup>4</sup>

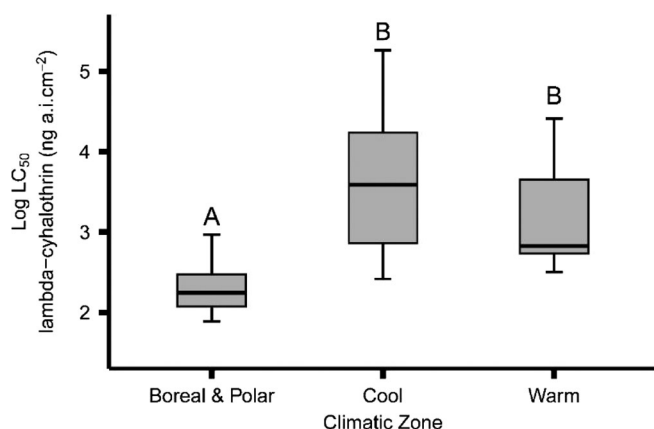
As hypothesized, our results showed that spider sensitivity varied with the climate from which spiders originated (Fig. 3; Table 2). Although 62% of the collected species are widely

**Table 2.** Summary of type III sums of squares from the generalized least squares model between spider traits, habitat characteristics, and chemical sensitivity

Coefficients	log (LC <sub>50</sub> lambda-cyhalothrin)		
	df	X <sup>2</sup>	P-value
Habitat	2	4.27	0.12
Climate	2	24.71	<b>&lt;0.001</b>
Foraging mode	1	0.87	0.35
Fresh weight	1	2.69	0.10

Abbreviation: LC<sub>50</sub>, median lethal concentration; df, degrees of freedom.

Note: Statistically significant differences ( $P < 0.05$ ) are shown in bold.



**Figure 3.** Boxplots showing the comparison between spider chemical sensitivity (on a logarithmic scale), and climate. Different letters show significant differences ( $P < 0.05$ ).  $LC_{50}$ , median lethal concentration; a.i., active ingredient.

distributed across Europe (Supporting Information, Table S1), our results suggest that characteristics of their habitat of origin influenced their response to pesticides. For example, *P. amentata* collected in a boreal + polar climate was approximately twice as sensitive as *P. amentata* collected in a cool temperate climate, similar to the intra-specific pattern that we found (Fig. 2; Table 1). These differences may be related to biological traits not analyzed in this study, such as the structure or chemical composition of the cuticle. Arthropods adapted to warm and dry conditions are expected to have a greater amount of cuticular hydrocarbons that help them prevent desiccation.<sup>30</sup> This adaptation may also reduce pesticide effects, because water depletion is an important cause of mortality in spiders exposed to pyrethroids.<sup>58</sup> Moreover, a stronger cuticle can also reduce the uptake of the pesticide, if direct contact is the main exposure route. Correspondingly, the least sensitive spider in our study, *Pisaura mirabilis* (Clerck), carries mostly hydrocarbons on its cuticle,<sup>59</sup> whereas less-hydrophobic substances dominate in many other spiders.<sup>60</sup>

### 4.3 Implications for risk assessment

#### 4.3.1 Current non-target arthropod risk assessment

Pesticide risk assessment for non-target arthropods is regulated in the EU with Commission Regulations 283/2013 and 284/2013. The potential risk of a substance on non-target arthropods is determined using a hazard quotient (HQ). The HQ is the ratio between an exposure concentration and an ecotoxicological endpoint; e.g., the recommended application rate/ $LC_{50}$ . Adverse effects of pesticides on non-target arthropods are expected if HQ values are  $>1$ . Moreover, risk assessment follows a tiered approach, starting from laboratory tests (Tier 1) and if a  $HQ > 1$  is obtained, a refinement to (semi-) field (Tier 2) studies are performed for a more realistic calculation of the risk. Following the EU risk assessment methodology, we evaluated the potential risk of the recommended application rate of lambda-cyhalothrin ( $75 \text{ ng a.i. cm}^{-2}$ ; Certis Europe) on spiders, as a Tier 1 approach. The HQ values of 85% of the species tested ( $LC_{50}$ ) and the HQ values derived with the  $HC_5$  values for all climate zones (Fig. 1) were  $>1$  (between 1.1 and 11.3). Thus, our results suggest a high mortality risk to spiders when exposed to the recommended field dose, which can partly explain the effects of lambda-cyhalothrin observed in field

studies<sup>12,61</sup> where the abundance and diversity of spiders were reduced after application. EU Regulation 1107/2009 newly includes environmental and climatic conditions in the risk assessment framework. The EU is divided into three zones: north, central and south, each with specific criteria for the approval and authorization of pesticides. The differences in chemical sensitivity between climate zones observed in our study partly support such regional approaches to pesticide risk assessment, although the differences that we observed between climate zones are covered by current safety factors.

#### 4.3.2 Future perspectives: ecosystem services and risk assessment

The European Food Safety Authority is aiming to shift the focus of the current risk assessment framework by incorporating ecosystem services, multiple stressors and environmental compartments.<sup>62,63</sup> Therefore, ecotoxicological data will be used to identify and protect sensitive communities and their ecosystem services.<sup>64,65</sup> An important ecosystem service provided by spiders and numerous other natural enemies is biological control.<sup>4,66</sup> However, non-target arthropod risk assessment is mainly focused on the acute and chronic responses of only two species of natural enemies [the wasp *Aphidius rhopalosiphi* (De Stefani-Perez) and the mite *Typhlodromus pyri* (Scheuten)]. In this framework, the results of the current study can offer valuable insights, suggesting the inclusion of additional taxa in risk assessment to safeguard a wider range of natural enemies and their beneficial functions in ecosystems.

Moreover, sublethal effects of pesticides on non-target arthropods also need to be considered, as they occur at lower concentrations than mortality effects. For example, prey consumption of the spider *Pardosa birmanica* (Simon) was significantly reduced after an exposure to lambda-cyhalothrin at one-twentieth of the recommended field dose ( $3.75 \text{ ng a.i. cm}^{-2}$ ).<sup>10</sup> This concentration is half of the  $LC_{50}$  of *P. amentata* from a boreal climate, the most sensitive species tested in this study (Table 1). As a consequence, sublethal effects on natural enemies may have similar negative consequences than mortality.<sup>6</sup> For example, if sublethal effects affect spider predatory performance<sup>10</sup> or lead to emigration, they may be of similar relevance as lethal effects. Spider migration translates to a reduction in spider diversity and abundance,<sup>12,61</sup> and consequently to a reduction in the ecosystem service of biological control. Another factor to be considered in future risk assessment is the effect of pesticide additives, such as surfactants, on non-target organisms. Previous research has shown that pesticide additives can induce mortality<sup>67</sup> and affect the predatory performance<sup>68</sup> of spiders, which may pose a risk for the ecosystem services provided from these organisms.

## 5 CONCLUSION

Spiders showed high sensitivity to lambda-cyhalothrin under laboratory conditions, which may reduce the abundance of most species under field scenarios.<sup>12,61</sup> This could change the spider communities in affected areas, and alter ecosystem functions such as biological control. Furthermore, our results demonstrated that spider chemical sensitivity varies depending on phylogenetic relationship and climate. The variation in spider chemical sensitivity suggests that multispecies studies should be more widely considered in risk assessment framework.



## AUTHOR CONTRIBUTIONS

TD, SP, RBS and MHE conceptualized the study and developed the methods. RBS and MHE supervised the study, provided study resources and acquired funding. TD, SC, GS, KR, MI, SP and MHE. Undertook the investigation. TD performed the formal analysis and wrote the original draft. KR, SP, MI, RBS, MHE provided editorial advice. All authors contributed to manuscript revisions and agreed on the version submitted for publication.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

R codes for toxicity data calculations are available from Figshare: <https://doi.org/10.6084/m9.figshare.23094023>. Data, associated metadata, and calculation tools are available from the corresponding author ([duque@uni-landau.de](mailto:duque@uni-landau.de)).

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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## Supporting Information

**Table S1** Additional information on the habitat characterization of collected spider species.

ID	Species	Country	Coordinates (WGS 84)	Distribution <sup>†</sup>	Habitat preferences <sup>†</sup>	Temperature (°C) <sup>‡</sup>	Elevation (m.a.s.l.)
1	<i>Pardosa saltans</i>	Germany	49.2813, 8.2819	E	Forests	10.2	116
2	<i>Pardosa amentata</i>	Germany	49.2535, 7.9620	E	Damp areas	9.1	233
3	<i>Piratula latitans</i>	Germany	49.2535, 7.9620	E	Open and damp areas	9.1	233
4	<i>Piratula hygrophila</i>	Germany	49.0401, 8.2551	E	Bog forest - Riparian zone	10.3	121
5	<i>Piratula hygrophila</i>	Germany	49.2813, 8.2819	E	Bog forest - Riparian zone	10.2	116
6	<i>Enoplognatha ovata</i>	Germany	49.3537, 8.1983	E	Bushes – Forests (Heiko & Wilker, 2016)	10.1	131
7	<i>Pardosa agrestis</i>	Germany	49.2048, 8.0927	E	Open areas	10.0	149
8	<i>Diplocephalus graecus</i>	Italy	38.0762, 12.7304	SE	Open areas	16.3	291
9	<i>Pardosa proxima</i>	Italy	45.1502, 7.6076	SE, EE	Open and damp areas	12.0	283
10	<i>Pardosa tenuipes</i>	Italy	45.1502, 7.6076	SE, CE	Open and damp areas (Isaia et al., 2018)	12.0	283
11	<i>Pardosa wagleri</i>	Italy	45.1548, 7.5563	SE, CE, EE	Gravel banks (Mountains)	11.9	287
12	<i>Pisaura mirabilis</i>	Germany	49.2014, 8.1381	E	Open and damp areas (bushes)	10.2	139
13	<i>Gnathonarium dentatum</i>	Switzerland	45.9939, 8.9252	E	Open and damp areas	11.5	338
14	<i>Pardosa hortensis</i>	Germany	49.1900, 8.0997	E	Open and damp areas	10.1	161
15	<i>Trochosa hispanica</i>	Italy	45.1490, 7.5947	SE, EE	Damp areas (Mediterranean)	12.0	296
16	<i>Pardosa hortensis</i>	France	42.4943, 3.0144	SE, CE, EE	Open and damp areas	12.9	339
17	<i>Pardosa morosa</i>	France	42.4707, 3.1187	SE, CE, EE	Damp areas	15.1	22
18	<i>Pardosa saltans</i>	Germany	49.0389, 8.2165	E	Forests	10.2	128
19	<i>Pardosa saltans</i>	Germany	49.2339, 8.3253	E	Forests	10.3	122
20	<i>Pardosa amentata</i>	Switzerland	46.5406, 8.7132	E	Damp areas	3.1	1990
21	<i>Oedothorax fuscus</i>	Denmark	57.2659, 10.3063	E	Open and damp areas	7.7	12
22	<i>Erigone dentipalpis</i>	Denmark	57.2659, 10.3063	E	Open areas	7.7	12
23	<i>Pardosa amentata</i>	Switzerland	46.5737, 8.5584	E	Damp areas	3.1	1959
24	<i>Pardosa oreophila</i>	Switzerland	46.5594, 8.5585	SE, CE	Open and damp areas (mountain)	2.8	2100
25	<i>Clubiona alpicola</i>	Switzerland	46.5580, 8.5699	SE, CE, EE	Open areas (mountain block fields)	2.6	2122
26	<i>Tenuiphantes tenuis</i>	Denmark	57.4782, 10.4071	E	Eurytopic	7.8	10

**Table S1 continued.**

ID	Species	Country	Coordinates (WGS 84)	Distribution <sup>†</sup>	Habitat preferences <sup>†</sup>	Temperature (°C) <sup>‡</sup>	Elevation (m.a.s.l.)
27	<i>Erigone atra</i>	Denmark	57.2659, 10.3063	E	Eurytopic	7.7	12
28	<i>Pardosa riparia</i>	Switzerland	46.5408, 8.7109	E	Damp areas and forests	3.1	1951
29	<i>Xysticus desidiosus</i>	Switzerland	46.5491, 8.7381	SE, CE, EE	Open areas (mountain block fields)	2.2	2290
30	<i>Drassodex heeri</i>	Switzerland	46.5491, 8.7381	SE, CE	High to nival zone (Alps)	2.2	2290
31	<i>Haplodrassus signifer</i>	Switzerland	46.5491, 8.7381	E	Eurytopic	2.2	2290
32	<i>Zelotes apricorum</i>	Switzerland	46.5580, 8.5699	E	Open areas and forests	2.6	2122
33	<i>Mermessus trilobatus</i>	Denmark	57.4782, 10.4071	CE, EE	Open areas	7.7	12
34	<i>Zoropsis spinimana</i>	Germany	49.1933, 8.1164	SE, CE	Open forests (under stones), synanthropic	-	-

ID = identifier for ecotoxicological assessment, see figures S1-S34.

<sup>†</sup> Spider distribution and habitat preferences according to the World Spider Catalog (World Spider Catalog, 2023). Spider distribution is only considered for Europe (E) and its regions: South (SE), Central (CE) and East (EE).

<sup>‡</sup> Temperature was retrieved using the mean monthly values for Europe from the WorldClim dataset (Fick & Hijmans, 2017)

## **DNA Barcoding of the tropical springtail population of *Coecobrya tenebricosa***

Springtails were purchased from the company MyAnts.de (Weiden, Germany). Since it was unknown which species of Collembola was provided to the spiders, we identified the population in a DNA barcoding approach. Total DNA was isolated using the innuPREP DNA/RNA Mini Kit (IST Innuscreen, Berlin) following the manufacturer's instructions. The cytochrome c oxidase subunit 1 (COI) gene was amplified using the primers UEA3 and UEA8 (Zhang & Hewlitt, 1997) with FIREPol® DNA Polymerase (Solis BioDyne, Tartu, Estland) and an annealing temperature of 48°C. The sample was sequenced externally by GENEWIZ (Leipzig, Germany) using the PCR primers. The sequence obtained (924 bp) was compared in the BLAST database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) with only 82% percent identity for the best result of *Entomobrya unifasciata*. Therefore, two datasets were assembled: (i) A dataset including sequences from all members of the family Entomobryidae available in GenBank (924 bp). (ii) A dataset including all sequences of the genus *Coecobrya* (393 bp) which was the best match (i). Both datasets were aligned using MAFFT 7.450 under the E-INS-i algorithm (Kato et al., 2005). Amino acid codons were identified manually using the annotated sequence KM610126 of *Entomobryoides dissimilis* in CLC Main Workbench 23.0.2 (QUIAGEN, Aarhus, Denmark). Phylogenetic trees using maximum likelihood (ML) with combined rapid bootstrapping under the GTRCAT model were computed from 1000 runs with RAxML 8.2.10 (Stamatakis, 2006). Trees were midpoint rooted and visualized using FigTree 1.3.1 (Rambaut A: FigTree Drawing Tool, <http://tree.bio.ed.ac.uk/software/figtree>). Finally, the springtail culture analyzed belongs to the species *Coecobrya tenebricosa* with maximum bootstrap support (data not shown). The sequence obtained was uploaded to GenBank under accession number OQ733367.

## The two-parameter log-logistic model for binomial dose-response (Ritz et al., 2019)

Log-logistic models for modelling dose-responses are constructed via a cumulative distribution function:

$$f(x) = c + (d - c)F(x, b, c, d, e, \dots)$$

Where the parameter  $b$  reflects the rate of change of the dose-response curve between the lower ( $c$ ) and upper ( $d$ ) limits, and  $e$  acts as a scaling factor of the magnitude of the doses. For binomial dose-response data; e.g., binary response: 0/1 or alive/dead; the parameters  $c$  and  $d$  are fixed at 0 and 1, respectively, and the two-parameter log-logistic model is obtained:

$$f(x, (b, e)) = \frac{1}{1 + \exp(b(\log(x) - \log(e)))}$$

Where the parameter  $e$  corresponds to the dose producing a response half-way between the upper (1) and lower limit (0), in our case  $e$  is the  $LC_{50}$ . The parameter  $b$  is the steepness of the dose-response curve at the  $LC_{50}$ .

**Table S2** Additional information on spider ecotoxicological assessments. LC<sub>50</sub> values with 95% CI

ID	Species	Test date	Concentrations tested (ng a.i. cm <sup>-2</sup> )	n <sup>†</sup>	LC <sub>50</sub> (ng a.i. cm <sup>-2</sup> )
1	<i>Pardosa saltans</i>	5 Aug 2020	0, 4.53, 21.35, 100.33, 471.55	10	15.83 ± 10.33
2	<i>Pardosa amentata</i>	5 Aug 2020	0, 0.97, 4.53, 21.35, 100.33, 471.55	10	12.81 ± 7.98
3	<i>Piratula latitans</i>	19 Aug 2020	0, 15.72, 31.44, 62.87, 125.75, 251.49, 502.99	10	123.23 ± 75.94
4	<i>Piratula hygrophila</i>	19 Aug 2020	0, 15.72, 31.44, 62.87, 125.75, 251.49, 502.99	10	108.78 ± 44.63
5	<i>Piratula hygrophila</i>	26 Aug 2020	0, 15.72, 31.44, 62.87, 125.75, 251.49, 502.99	10	126.13 ± 35.72
6	<i>Enoplognatha ovata</i>	26 Aug 2020	0, 31.44, 62.87, 125.75, 251.49, 502.99	10	67.64 ± 35.32
7	<i>Pardosa agrestis</i>	14 Oct 2020	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	14.75 ± 5.10
8	<i>Diplocephalus graecus</i>	13 Jan 2021	0, 12.77, 25.54, 51.09, 102.17, 204.34, 408.68	10	29.56 ± 8.51
9	<i>Pardosa proxima</i>	9 Jun 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75	6	16.79 ± 7.80
10	<i>Pardosa tenuipes</i>	9 Jun 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75	4	13.26 ± 8.74
11	<i>Pardosa wagleri</i>	30 Jun 2021	0, 15.75, 31.44, 62.87, 125.75	5	35.97 ± 13.03
12	<i>Pisaura mirabilis</i>	7 Jul 2021	0, 15.72, 31.44, 62.87, 125.75, 251.49, 502.99	9	191.22 ± 51.76
13	<i>Gnathonarium dentatum</i>	7 Jul 2021	0, 15.72, 31.44, 62.87, 125.75, 251.49, 502.99	9	91.44 ± 28.74
14	<i>Pardosa hortensis</i>	14 Jul 2021	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	14.80 ± 7.48
15	<i>Trochosa hispanica</i>	14 Jul 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75, 251.49	6	71.10 ± 29.11
16	<i>Pardosa hortensis</i>	14 Jul 2021	0, 15.75, 31.44, 62.87, 125.75	4	12.43 ± 30.22
17	<i>Pardosa morosa</i>	14 Jul 2021	0, 15.75, 31.44, 62.87, 125.75	3	16.79 ± 14.80
18	<i>Pardosa saltans</i>	28 Jul 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75	3	22.22 ± 10.99
19	<i>Pardosa saltans</i>	28 Jul 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75	8	16.93 ± 13.37
20	<i>Pardosa amentata</i>	15 Sep 2021	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	6.65 ± 2.09
21	<i>Oedothorax fuscus</i>	15 Sep 2021	0, 31.44, 62.87, 125.75, 251.49, 502.99	3	30.07 ± 19.22
22	<i>Erigone dentipalpis</i>	15 Sep 2021	0, 15.72, 31.44, 62.87, 125.75, 251.49, 502.99	5	22.24 ± 12.93
23	<i>Pardosa amentata</i>	15 Sep 2021	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	6.72 ± 1.92
24	<i>Pardosa oreophila</i>	15 Sep 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75	5	9.55 ± 5.61
25	<i>Clubiona alpicola</i>	15 Sep 2021	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	9.00 ± 3.24
26	<i>Tenuiphantes tenuis</i>	22 Sep 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75, 251.49	8	48.19 ± 14.23
27	<i>Erigone atra</i>	22 Sep 2021	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	35.93 ± 10.49
28	<i>Pardosa riparia</i>	22 Sep 2021	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	11.99 ± 3.96
29	<i>Xysticus desidiosus</i>	22 Sep 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75, 251.49	6	21.42 ± 9.50
30	<i>Drassodex heeri</i>	22 Sep 2021	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	7.60 ± 4.35
31	<i>Haplodrassus signifer</i>	6 Oct 2021	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	10.75 ± 5.52
32	<i>Zelotes apricorum</i>	6 Oct 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75	4	16.37 ± 10.99
33	<i>Mermessus trilobatus</i>	18 Nov 2021	0, 7.86, 15.75, 31.44, 62.87, 125.75, 251.49	10	75.99 ± 27.39
34	<i>Zoropsis spinimana</i>	2 Mar 2022	0, 3.93, 7.86, 15.75, 31.44, 62.87, 125.75	10	14.64 ± 4.06

† Number of replicates per concentration.

## Influence of paralyzed spiders in dose-response calculations

A multinomial dose-response approach following Ritz et al., (2019) was used to analyze the influence of paralyzed spiderlings in LC<sub>50</sub> calculations. LC<sub>50</sub> values derived from counting “paralyzed” spiderlings as “alive” were compared statistically to LC<sub>50</sub> values derived from merging “paralyzed” and “dead” spiderlings into one category. Since no significant differences were found (Table S3), paralyzed spiderlings were counted as alive for LC<sub>50</sub> calculations.

**Table S3** Influence of paralyzed spiderlings in dose-response calculations

ID	Species	Alive	Dead	Paralyzed	% Paralyzed	t-value	p-value
1	<i>P. saltans</i>	33	27	0	0.00	-	-
2	<i>P. amentata</i>	20	29	1	2.00	0.33	0.74
3	<i>P. latitans</i>	43	26	1	1.43	0.04	0.97
4	<i>P. hygrophila</i>	38	29	3	4.29	0.66	0.51
5	<i>P. hygrophila</i>	40	26	4	5.71	1.03	0.30
6	<i>E. ovata</i>	26	34	0	0.00	-	-
7	<i>P. agrestis</i>	31	34	5	7.14	1.12	0.26
8	<i>D. graecus</i>	24	44	2	2.86	0.65	0.51
9	<i>P. proxima</i>	15	21	0	0.00	-	-
10	<i>P. tenuipes</i>	9	15	0	0.00	-	-
11	<i>P. wagleri</i>	12	11	2	8.00	0.72	0.47
12	<i>P. mirabilis</i>	45	17	1	1.59	0.33	0.74
13	<i>G. dentatum</i>	30	28	5	7.94	1.28	0.20
14	<i>P. hortensis</i>	33	37	0	0.00	-	-
15	<i>T. hispanica</i>	26	15	1	2.38	0.45	0.65
16	<i>P. hortensis</i>	5	15	0	0.00	-	-
17	<i>P. morosa</i>	4	10	1	6.67	0.16	0.87
18	<i>P. saltans</i>	8	9	1	5.56	0.65	0.52
19	<i>P. saltans</i>	19	26	3	6.25	0.37	0.71
20	<i>P. amentata</i>	21	46	3	4.29	0.73	0.47
21	<i>O. fuscus</i>	4	14	0	0.00	-	-
22	<i>E. dentipalpis</i>	9	25	1	2.86	0.33	0.74
23	<i>P. amentata</i>	21	48	1	1.43	0.39	0.69
24	<i>P. oreophila</i>	6	21	3	10.00	0.34	0.73
25	<i>C. alpicola</i>	24	44	2	2.86	0.56	0.57
26	<i>T. tenuis</i>	32	23	1	1.79	0.29	0.77
27	<i>E. atra</i>	47	23	0	0.00	-	-
28	<i>P. riparia</i>	30	40	0	0.00	-	-
29	<i>X. desidiosus</i>	12	25	5	11.90	1.18	0.24
30	<i>D. heeri</i>	21	45	4	5.71	0.84	0.40
31	<i>H. signifer</i>	29	41	0	0.00	-	-
32	<i>Z. apricorum</i>	9	14	1	4.17	0.41	0.69
33	<i>M. trilobatus</i>	39	24	7	10.00	1.74	0.08
34	<i>Z. spinimana</i>	33	37	0	0.00	-	-

**Table S4** Intra-specific pairwise comparisons of LC<sub>50</sub>s values via analysis of multiple binomial dose-response curves.

HLZ	Species	Location 1	Location 2	LC <sub>50</sub> difference	t-value	p-value
Boreal + Polar	<i>P. amentata</i>	46.5406, 8.7132	46.5737, 8.5584	0.59	0.34	0.73
Cool temperate	<i>P. saltans</i>	49.2813, 8.2819	49.0389, 8.2165	-6.56	-0.97	0.33
		49.2813, 8.2819	49.2339, 8.3253	0.18	0.02	0.98
		49.0389, 8.2165	49.2339, 8.3253	6.74	0.67	0.51
	<i>P. hygrophila</i>	49.0401, 8.2551	49.2813, 8.2819	-21.68	-0.82	0.41



## Phylogenetic tree

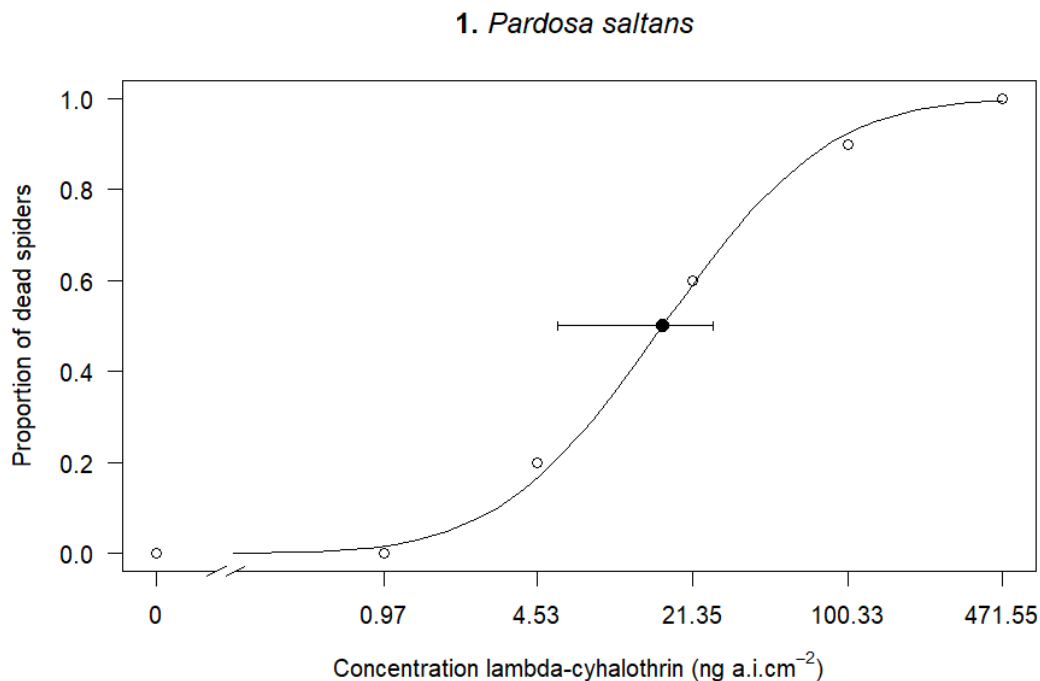
To test for a possible phylogenetic signal in pesticide sensitivity, a sequence dataset including one or two representatives per species was compiled. Since data was not available for all species in NCBI GenBank, we generated sequences for the missing spider species *Clubiona alpicola*, *Diplocephalus graecus*, *Drassodex heeri*, *Pardosa proxima*, *Pardosa tenuipes*, *Trochosa hispanica*, and *Xysticus desidiosus* as follows: DNA was extracted using a 'high-salt' extraction method (Aljanabi & Martinez, 1997) or the innuPREP DNA/RNA Mini Kit (IST Innuscreen, Berlin) following the manufacturer's instructions. The cytochrome c oxidase subunit 1 (COI) gene was amplified using the primer combinations LCO1490/HCO2198 (Folmer et al., 1994) or LepF1/LeR1 (Hebert et al., 2004) with FIREPol® DNA Polymerase (Solis BioDyne, Tartu, Estonia) and annealing temperatures of 50°C or 48°C, respectively. Samples were sequenced in both directions by GENEWIZ (Leipzig, Germany) using the PCR primers. The dataset including all species tested in this study was aligned using MAFFT 7.450 under the E-INS-i algorithm (Kato et al., 2005). Amino acid codons were identified manually using the annotated sequence GU682568 of *Haplodrassus signifer* in CLC Main Workbench 23.0.2 (QUIAGEN, Aarhus, Denmark). The phylogenetic tree was constructed using maximum likelihood (ML) with combined rapid bootstrapping under the GTRCAT model were computed from 1000 runs with RAxML 8.2.10 (Stamatakis, 2006). The final tree was midpoint rooted and visualized using FigTree 1.3.1 (Rambaut A: FigTree Drawing Tool, <http://tree.bio.ed.ac.uk/software/figtree>), see Fig. 2. The sequences obtained were uploaded to NCBI GenBank under accession numbers OQ644637-OQ644638 and OQ644640-OQ644649.

## Phylogenetic correlation analysis

Following Pekár & Brabec (Pekár & Brabec, 2016), we tested the null hypothesis that chemical sensitivity in terms of  $LC_{50}$  is independent among spider species using the Pagel's correlation structure derived from the Brownian motion model (Pagel, 1999) as follows:

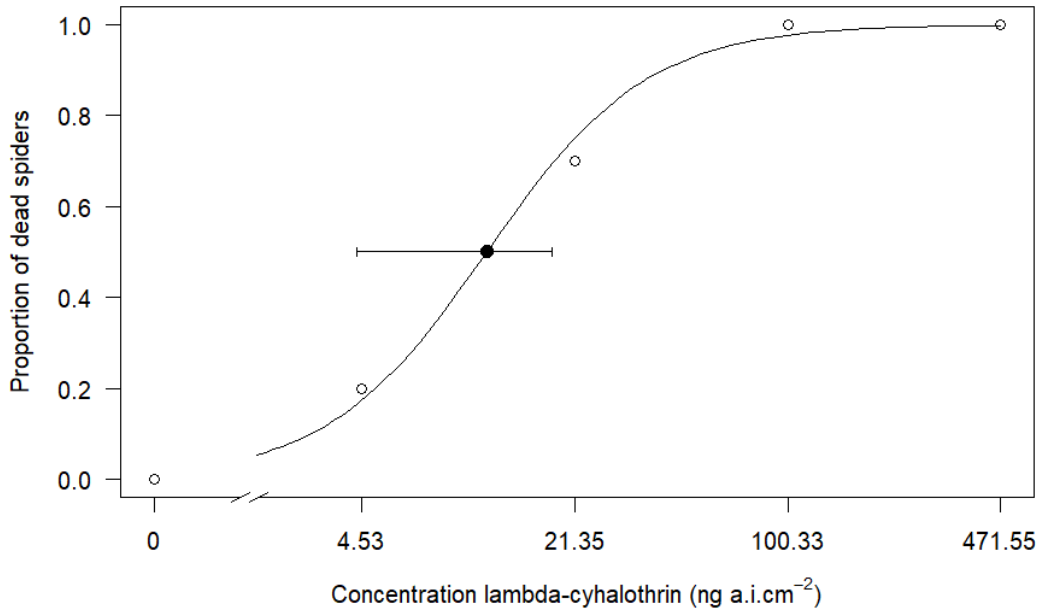
$$\sigma_{kk'} = \sigma_{\gamma}^2 \mathbf{D}_{kk'} \lambda$$

Where  $\sigma_{kk'}$  is the covariance among the species  $k$  and  $k'$ ,  $\sigma_{\gamma}^2$  is the rate of evolutionary divergence,  $\mathbf{D}_{kk'}$  the phylogenetic distances between the species  $k$  and  $k'$ ,  $k \neq k'$ , and  $\lambda$  the strength of the phylogenetic signal. Values of  $\lambda \geq 1$  indicate a strong effect of phylogeny. At  $\lambda = 1$ , the Brownian motion model is suggested. If  $0 < \lambda < 1$ , there is a weak effect of phylogeny; and  $\lambda = 0$ , suggests an independent evolution of a trait.



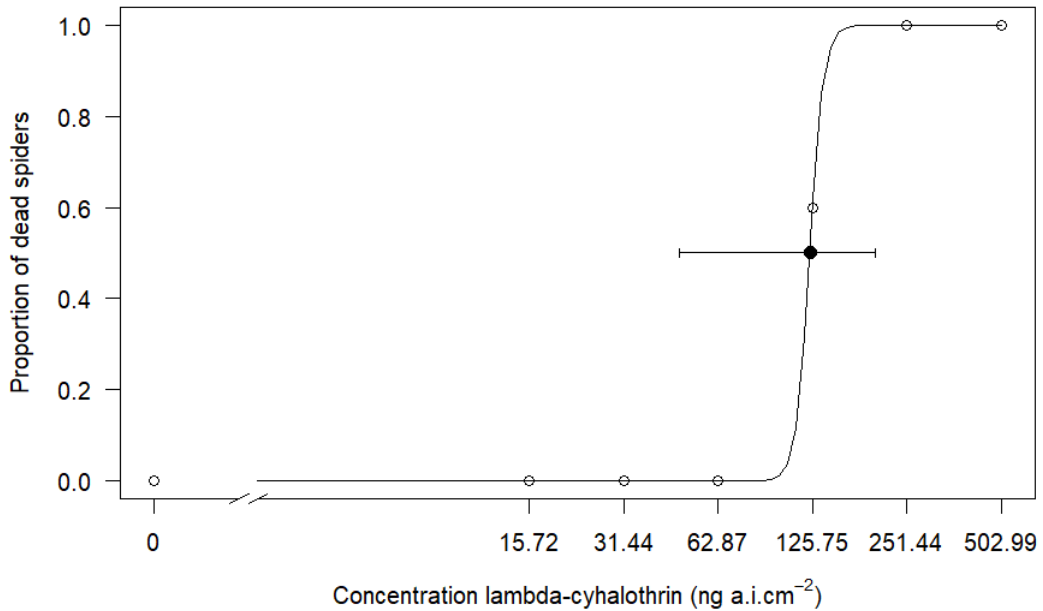
**Figure S1** Dose-response curve for *P. saltans* (ID 1, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

### 2. *Pardosa amentata*



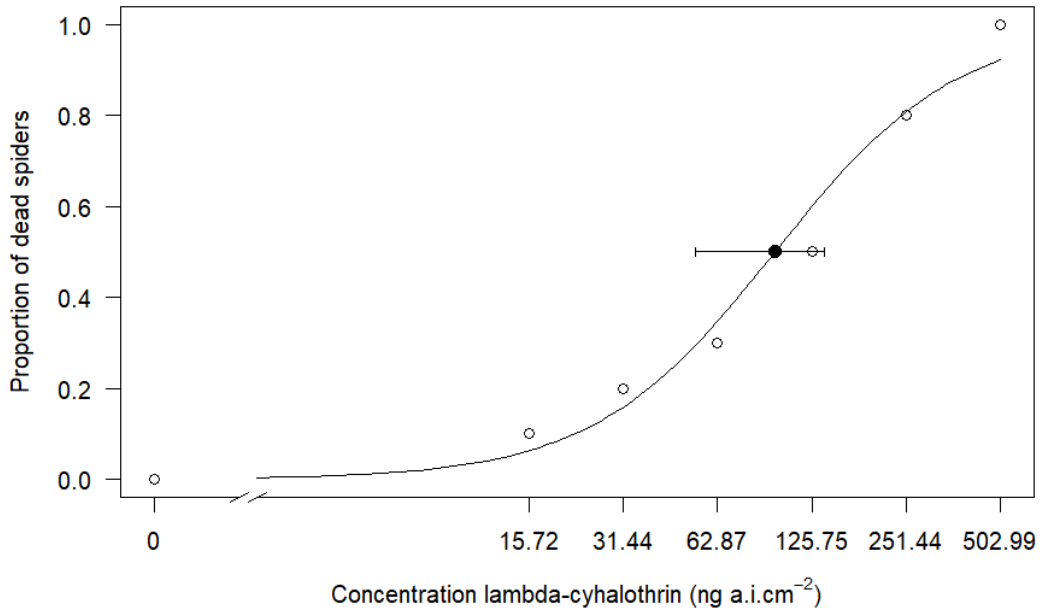
**Figure S2** Dose-response curve for *P. amentata* (ID 2, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

### 3. *Piratula latitans*



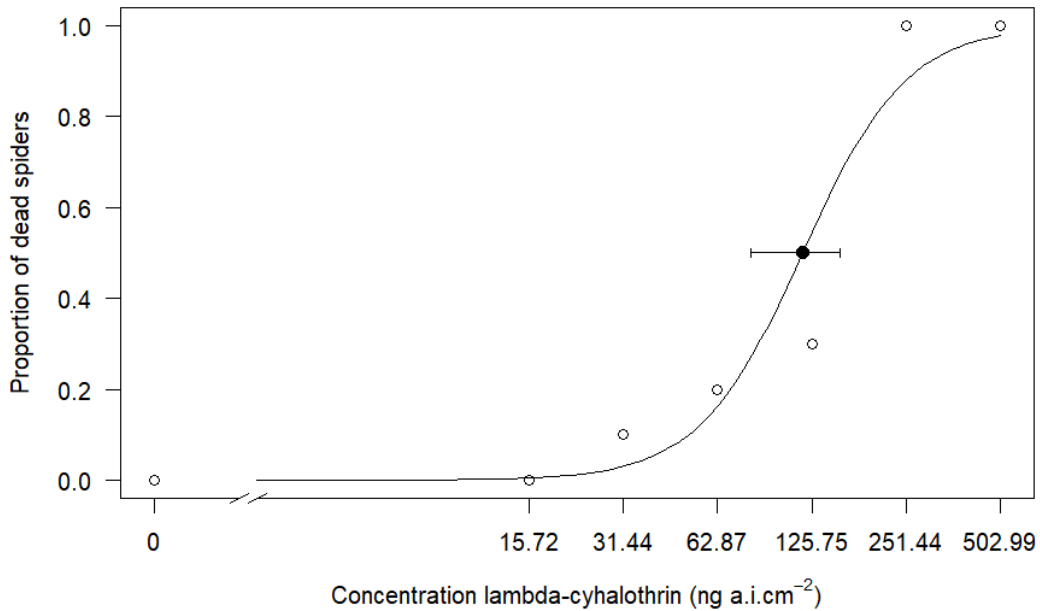
**Figure S3** Dose-response curve for *P. latitans* (ID 3, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

4. *Piratula hygrophila*



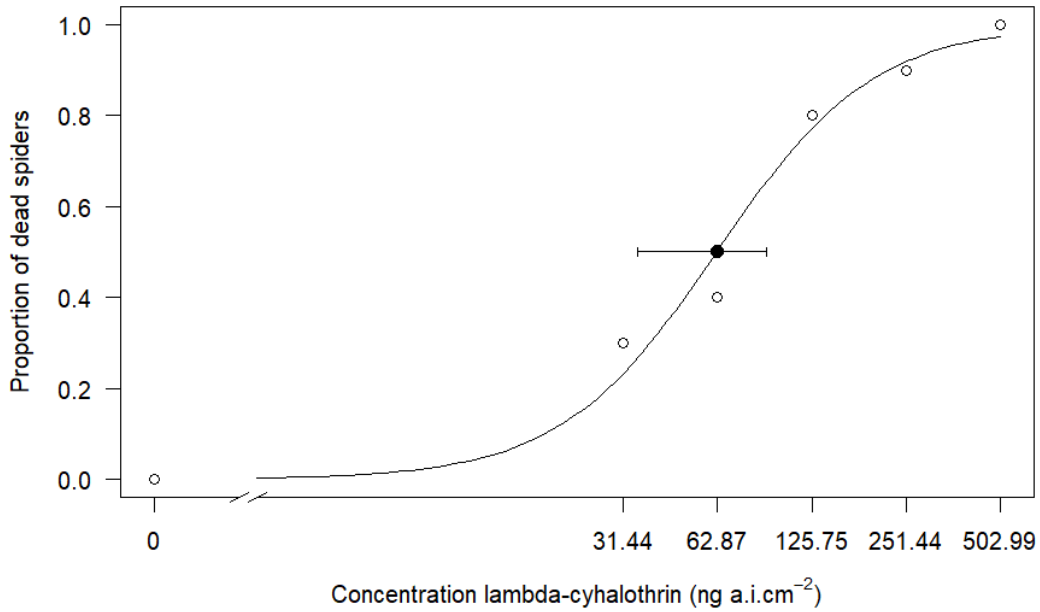
**Figure S4** Dose-response curve for *P. hygrophila* (ID 4, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

5. *Piratula hygrophila*



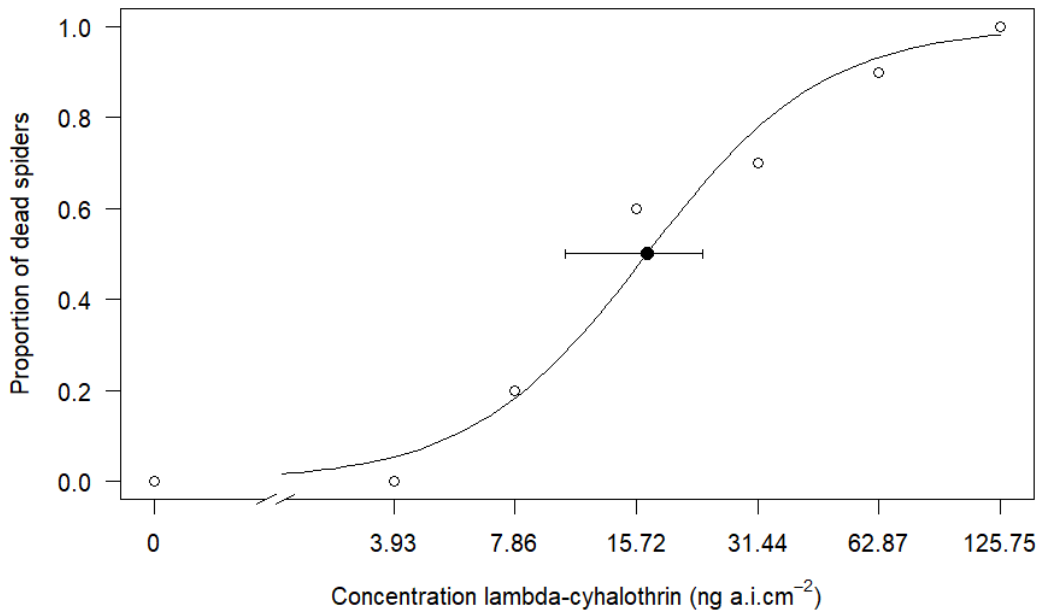
**Figure S5** Dose-response curve for *P. hygrophila* (ID 5, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

6. *Enoplognatha ovata*



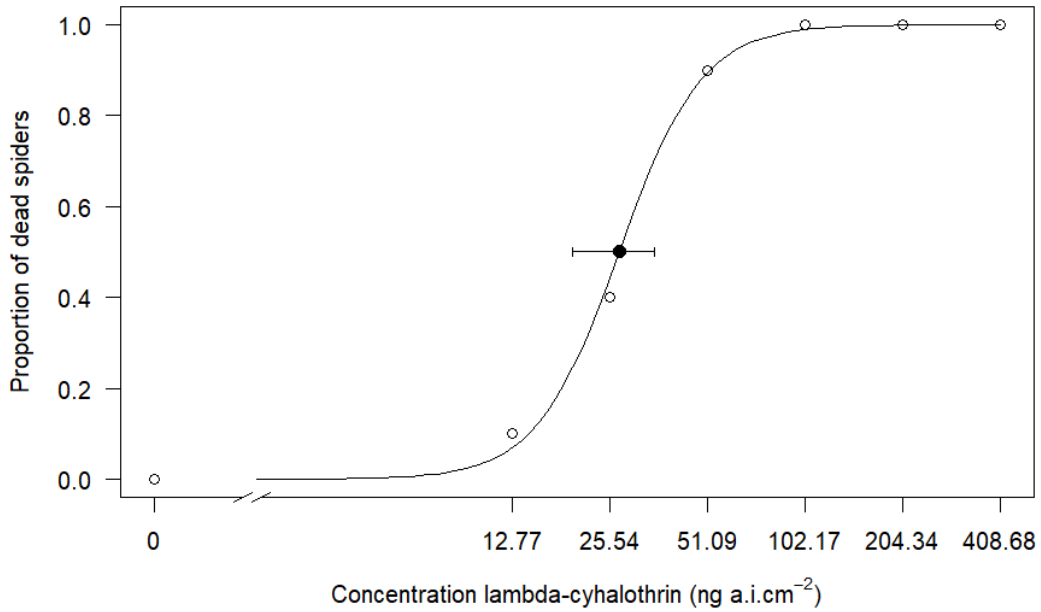
**Figure S6** Dose-response curve for *E. ovata* (ID 6, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

7. *Pardosa agrestis*



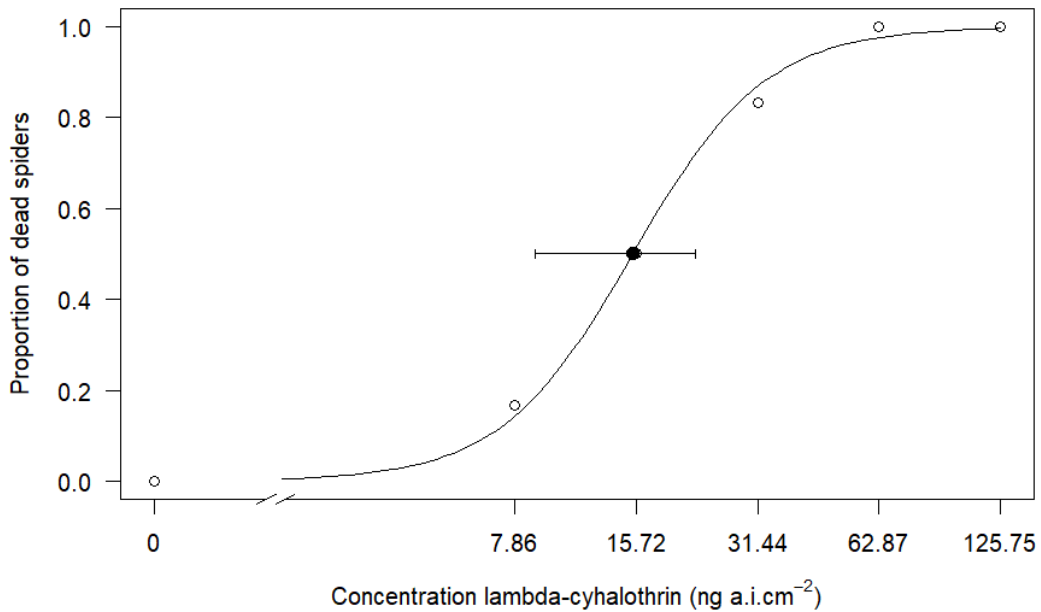
**Figure S7** Dose-response curve for *P. agrestis* (ID 7, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

8. *Diplocephalus graecus*



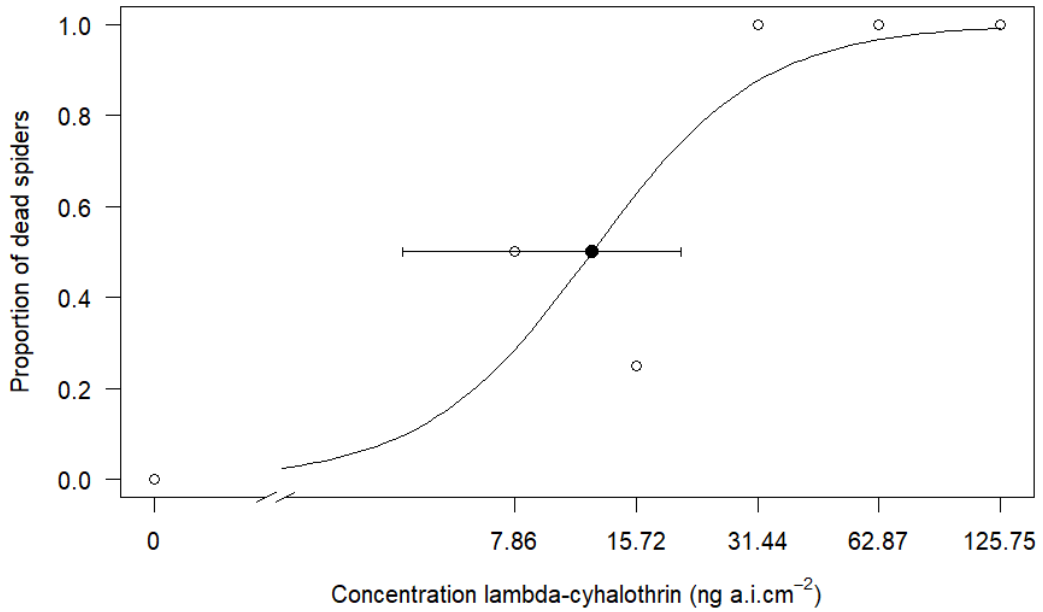
**Figure S8** Dose-response curve for *D. graecus* (ID 8, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

9. *Pardosa proxima*



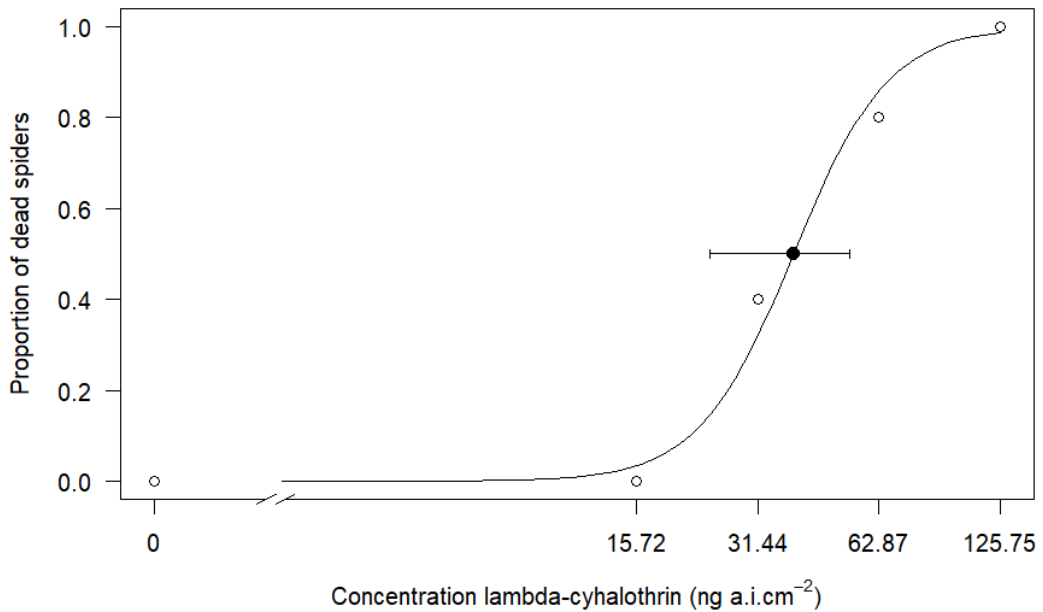
**Figure S9** Dose-response curve for *P. proxima* (ID 9, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

10. *Pardosa tenuipes*



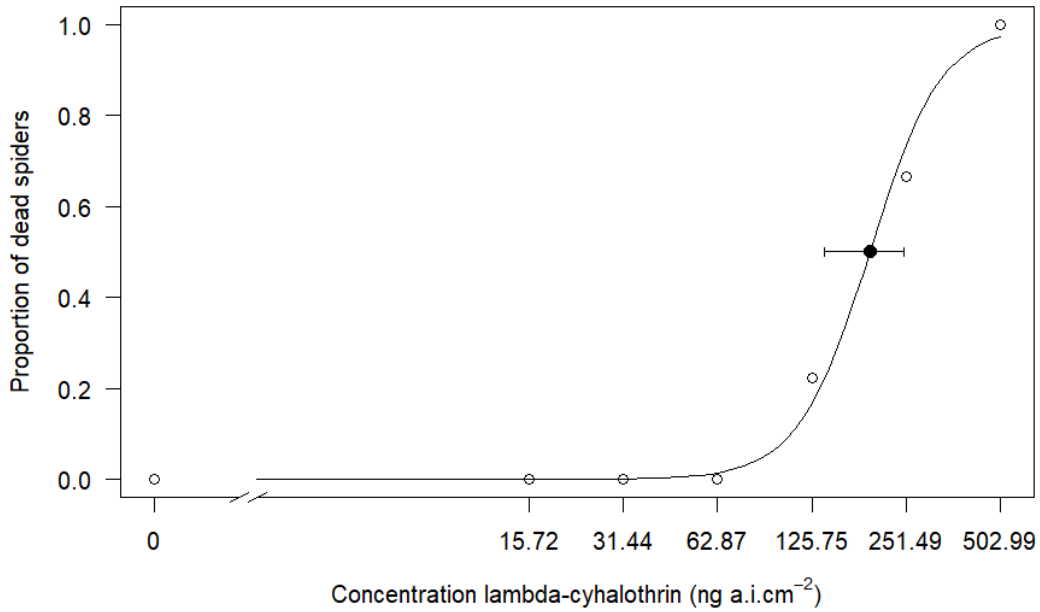
**Figure S10** Dose-response curve for *P. tenuipes* (ID 10, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

11. *Pardosa wagleri*



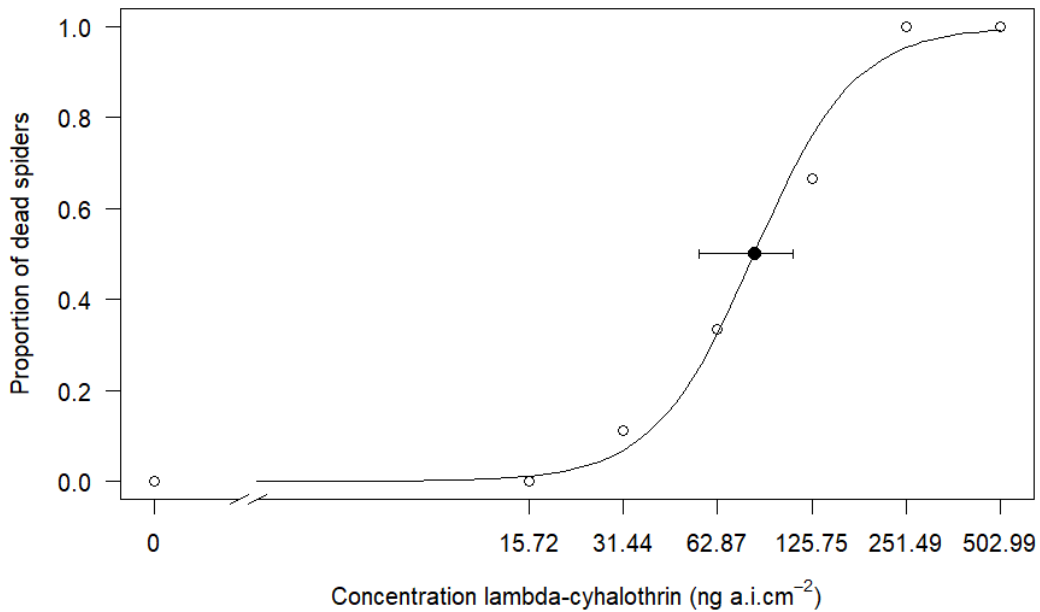
**Figure S11** Dose-response curve for *P. wagleri* (ID 11, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

12. *Pisaura mirabilis*



**Figure S12** Dose-response curve for *P. mirabilis* (ID 12, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

13. *Gnathonarium dentatum*



**Figure S13** Dose-response curve for *G. dentatum* (ID 13, Table S1) 24-hour toxicity test with lambda-cyhalothrin.



14. *Pardosa hortensis*

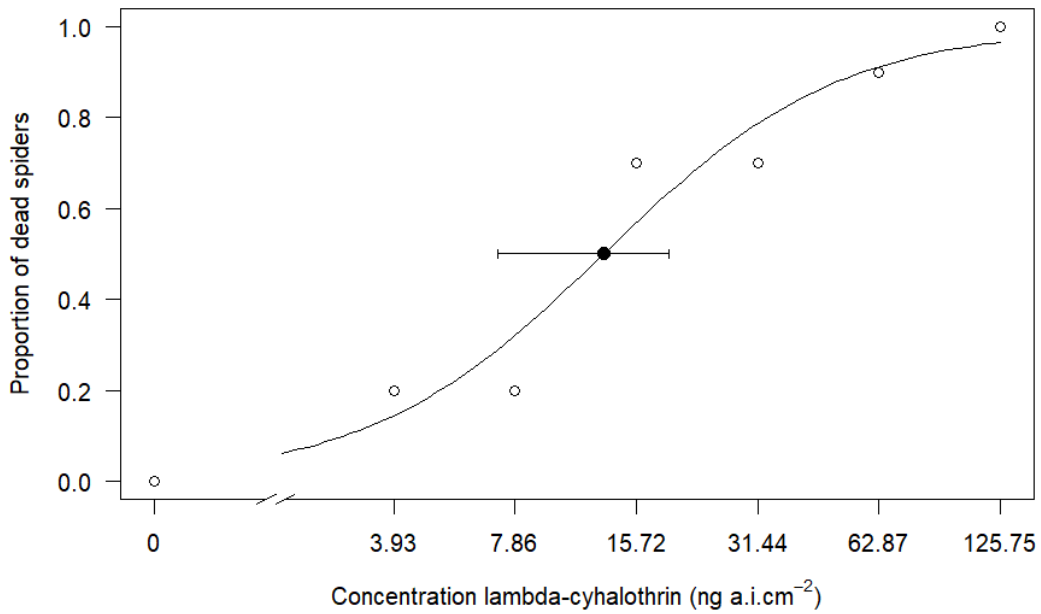


Figure S14 Dose-response curve for *P. hortensis* (ID 14, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

15. *Trochosa hispanica*

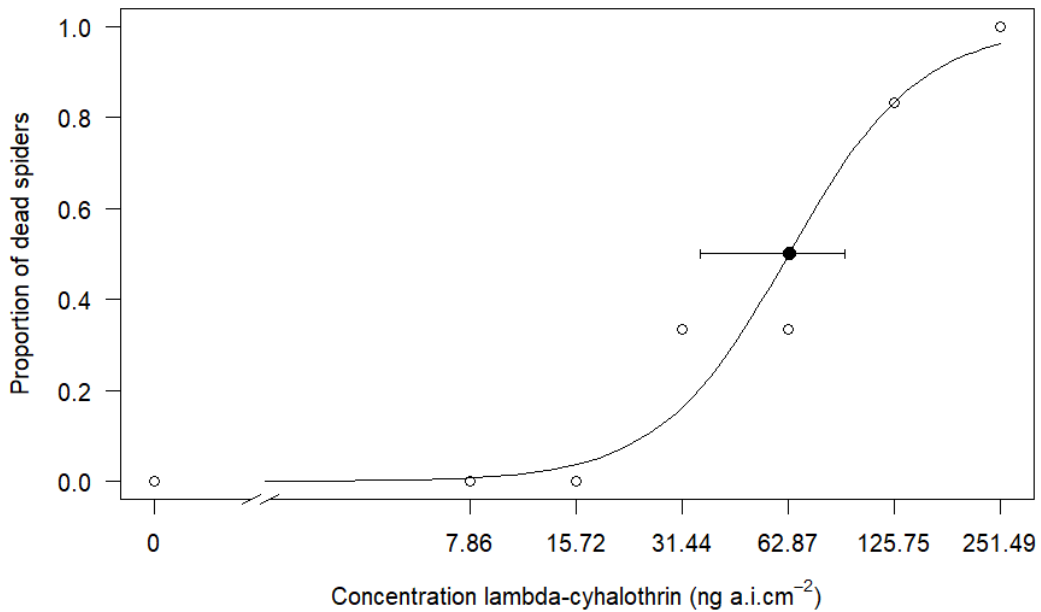
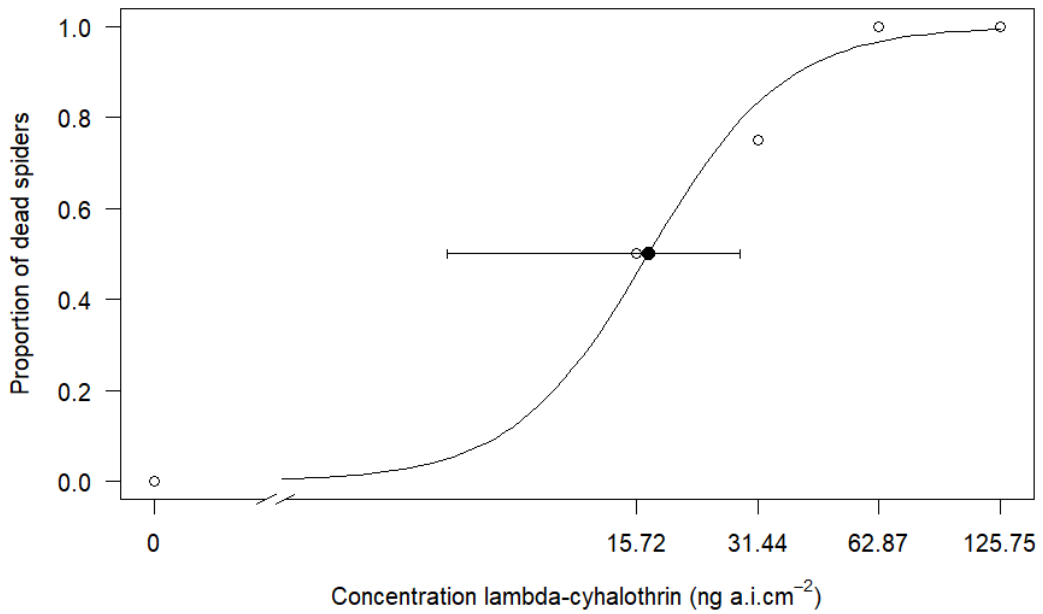


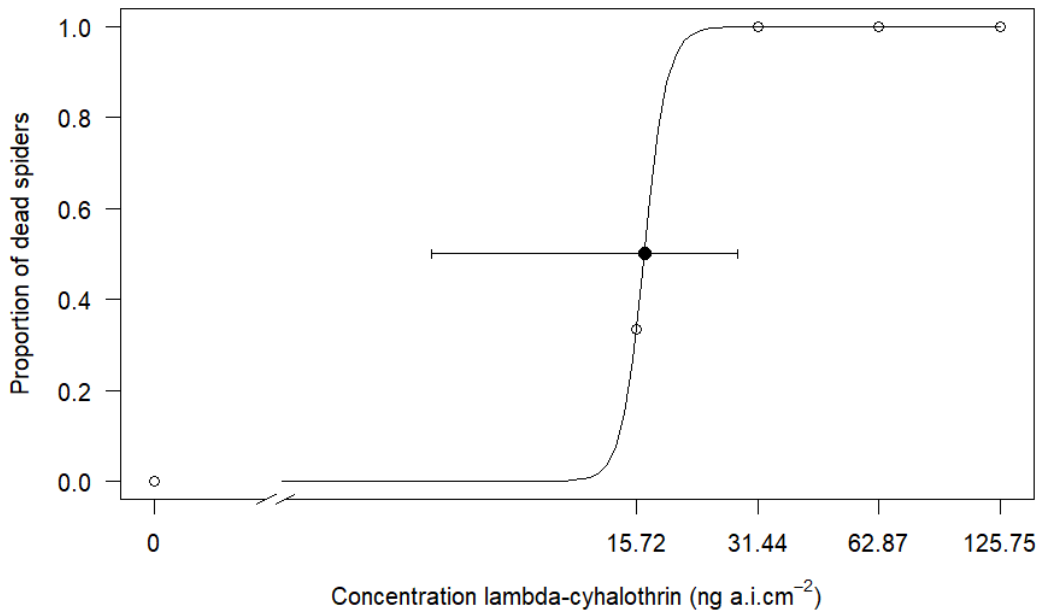
Figure S15 Dose-response curve for *T. hispanica* (ID 15, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

16. *Pardosa hortensis*



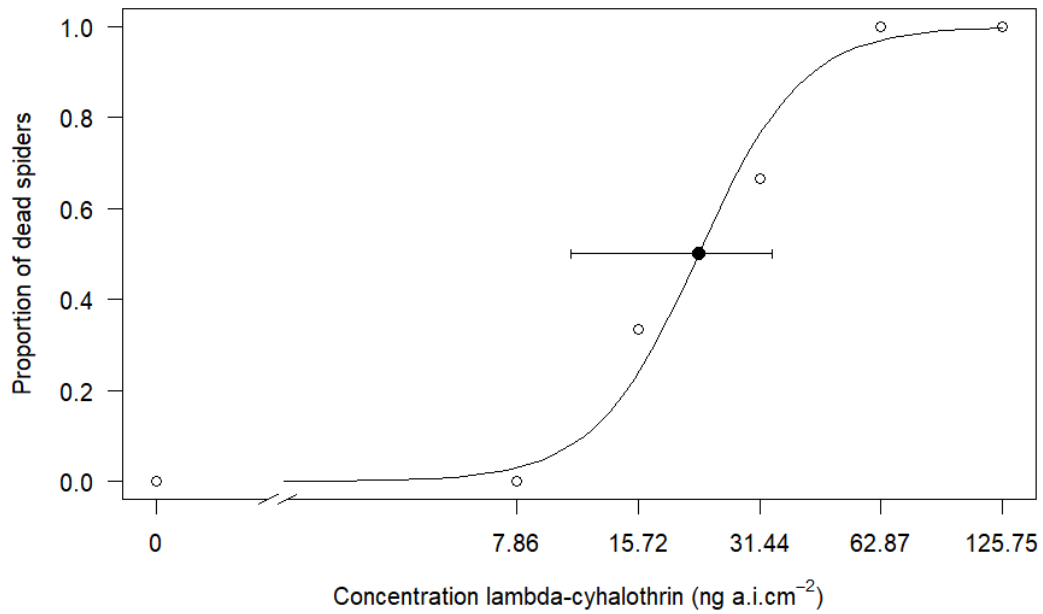
**Figure S16** Dose-response curve for *P. hortensis* (ID 16, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

17. *Pardosa morosa*



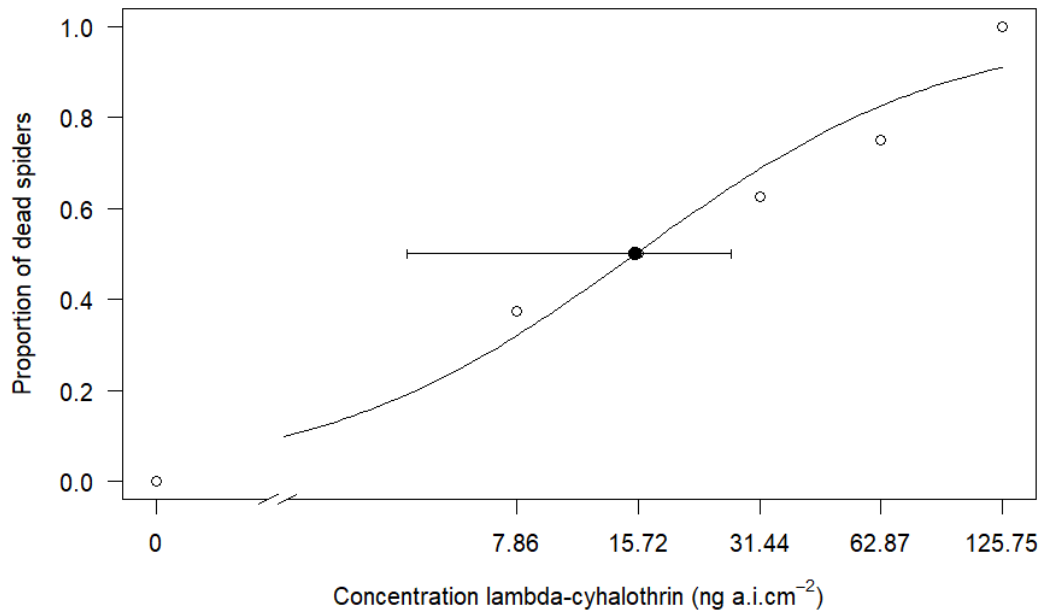
**Figure S17** Dose-response curve for *P. morosa* (ID 17, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

18. *Pardosa saltans*



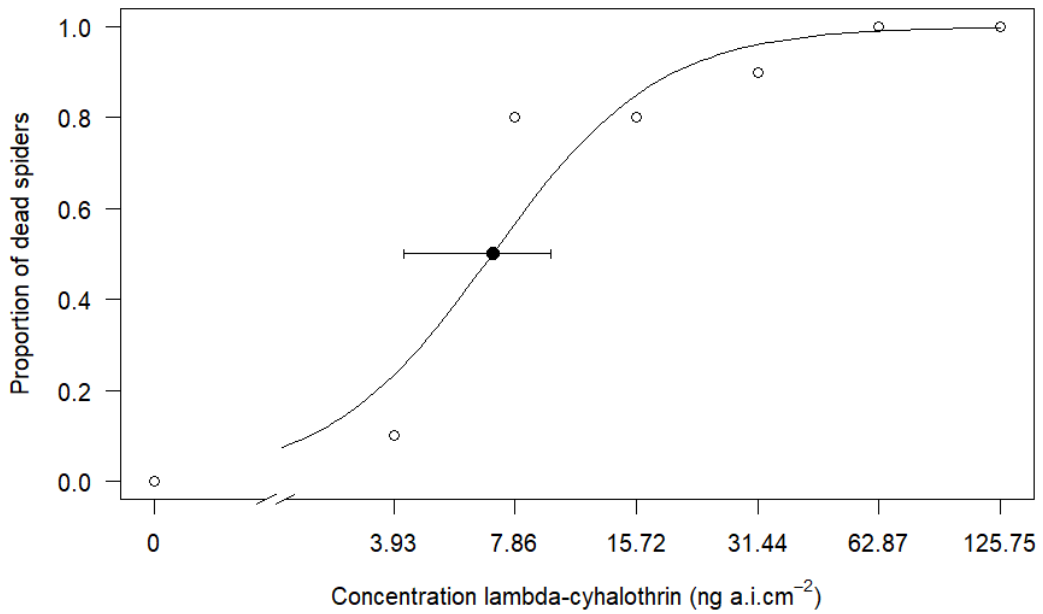
**Figure S18** Dose-response curve for *P. saltans* (ID 18, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

19. *Pardosa saltans*



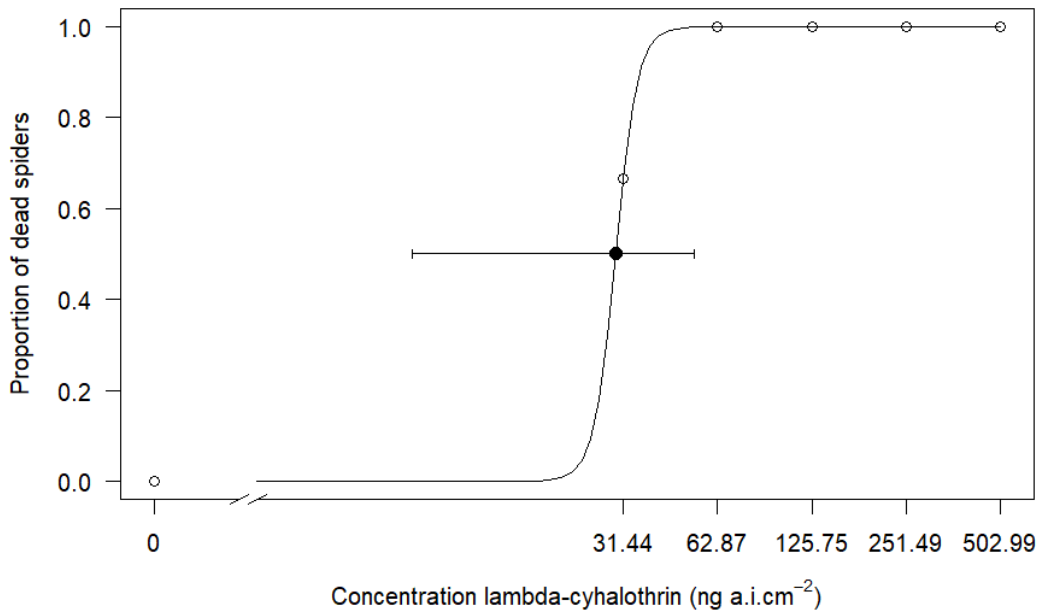
**Figure S19** Dose-response curve for *P. saltans* (ID 19, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

20. *Pardosa amentata*



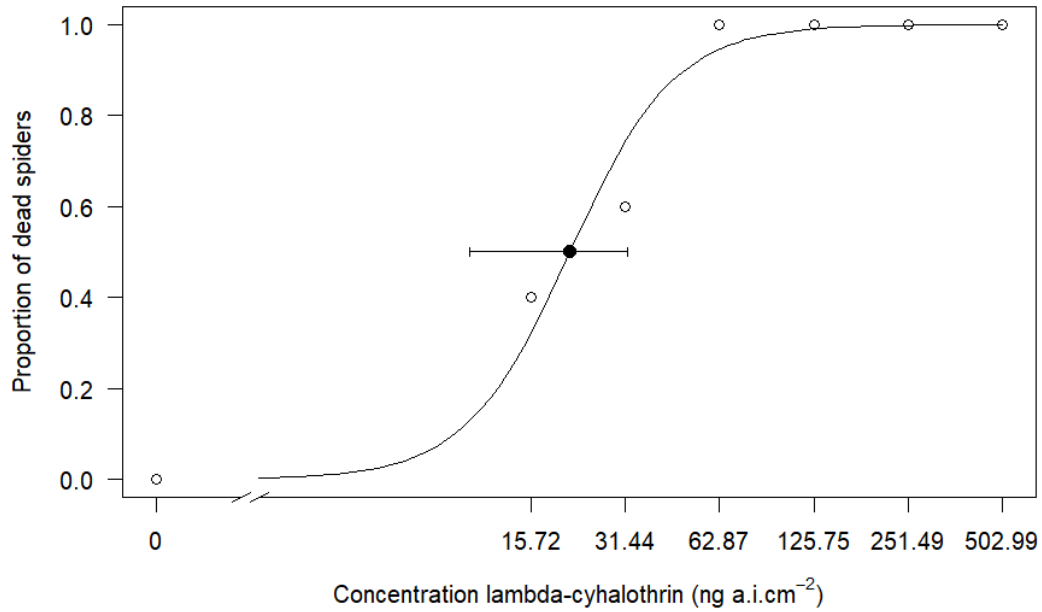
**Figure S20** Dose-response curve for *P. amentata* (ID 20, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

21. *Oedothorax fuscus*



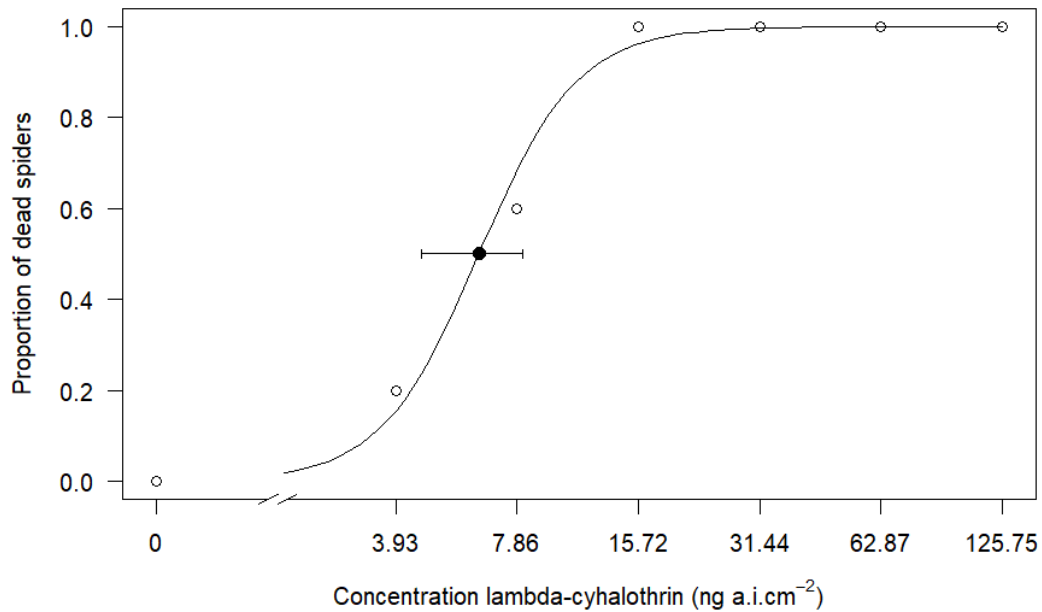
**Figure S21** Dose-response curve for *O. fuscus* (ID 21, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

**22. *Erigone dentipalpis***



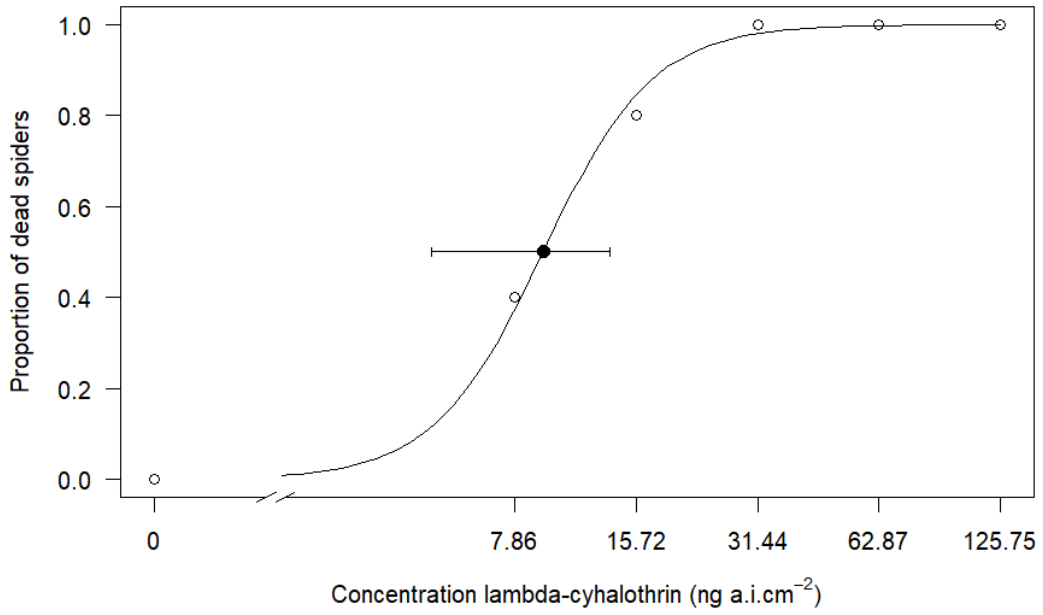
**Figure S22** Dose-response curve for *E. dentipalpis* (ID 22, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

**23. *Pardosa amentata***



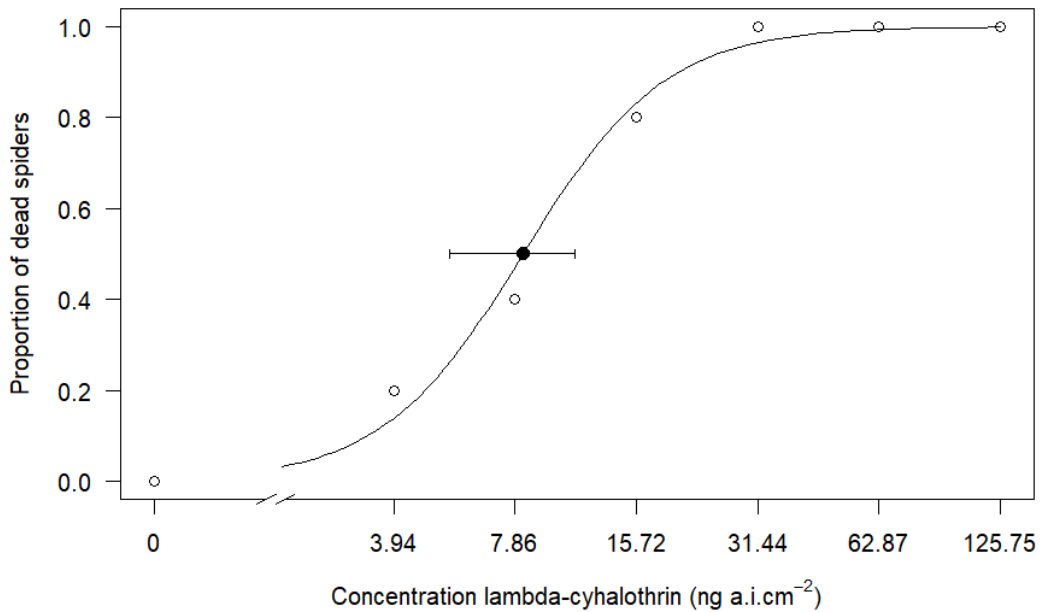
**Figure S23** Dose-response curve for *P. amentata* (ID 23, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

24. *Pardosa oreophila*



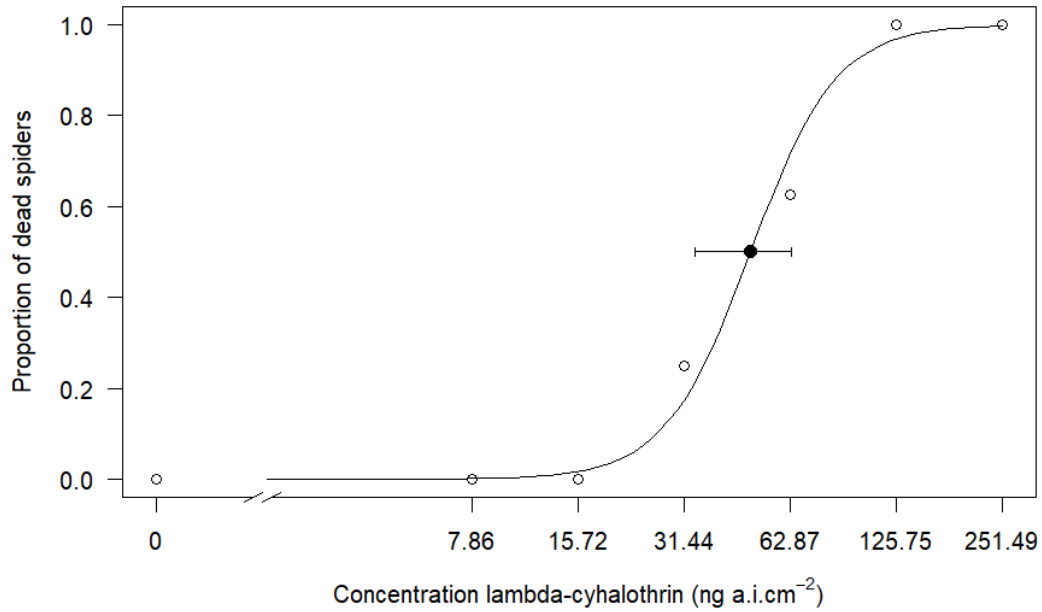
**Figure S24** Dose-response curve for *P. oreophila* (ID 24, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

25. *Clubiona alpicola*



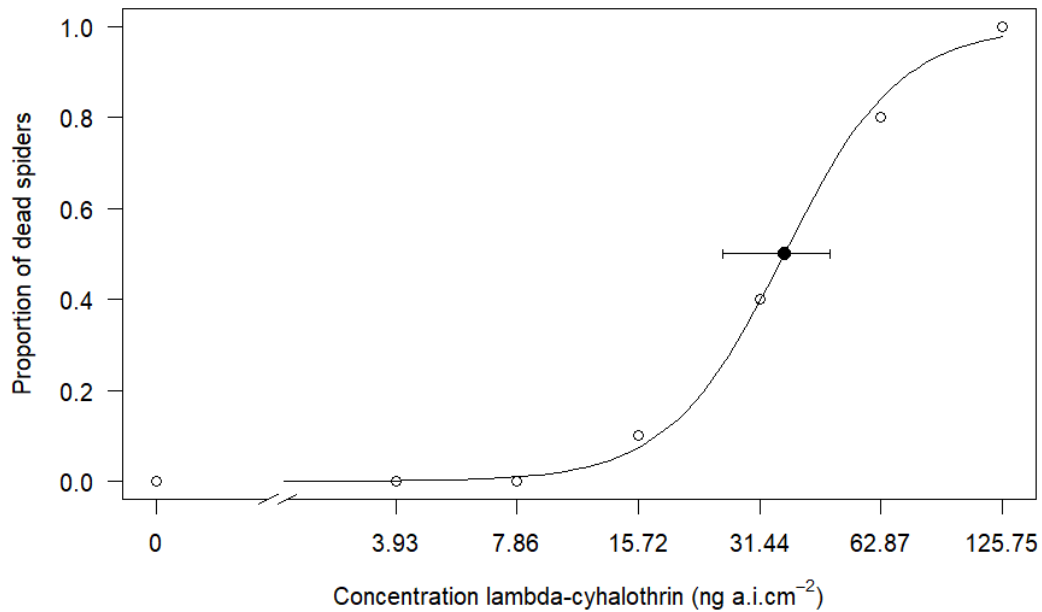
**Figure S25** Dose-response curve for *C. alpicola* (ID 25, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

26. *Tenuiphantes tenuis*



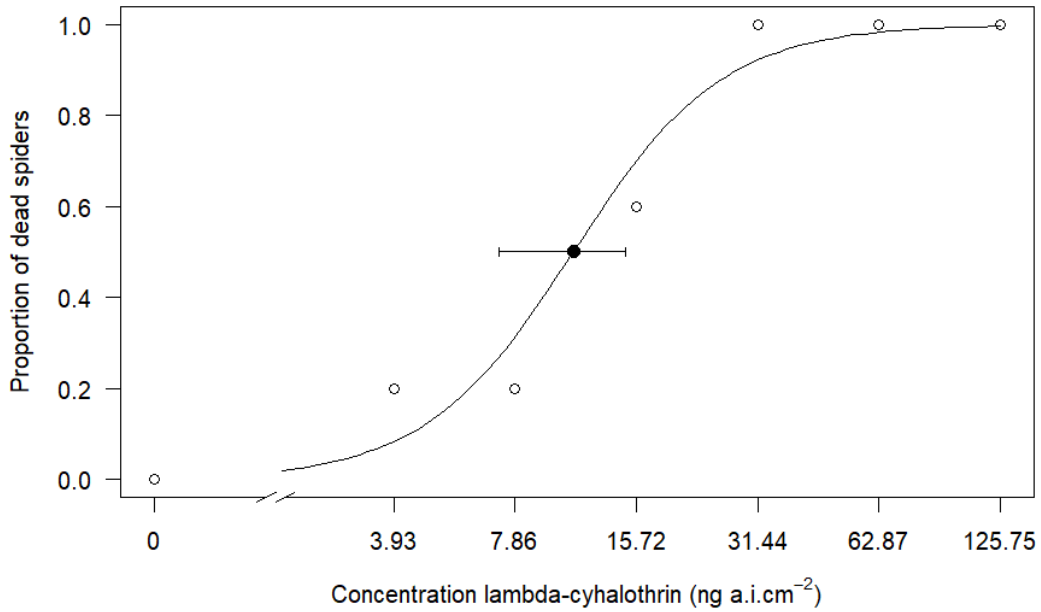
**Figure S26** Dose-response curve for *T. tenuis* (ID 26, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

27. *Erigone atra*



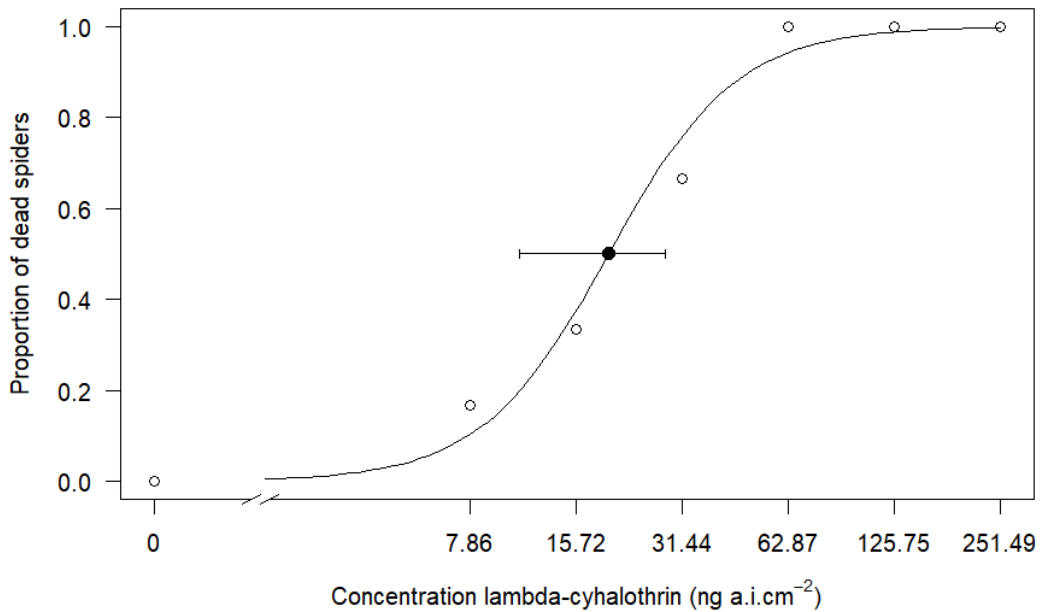
**Figure S27** Dose-response curve for *E. atra* (ID 27, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

28. *Pardosa riparia*



**Figure S28** Dose-response curve for *P. riparia* (ID 28, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

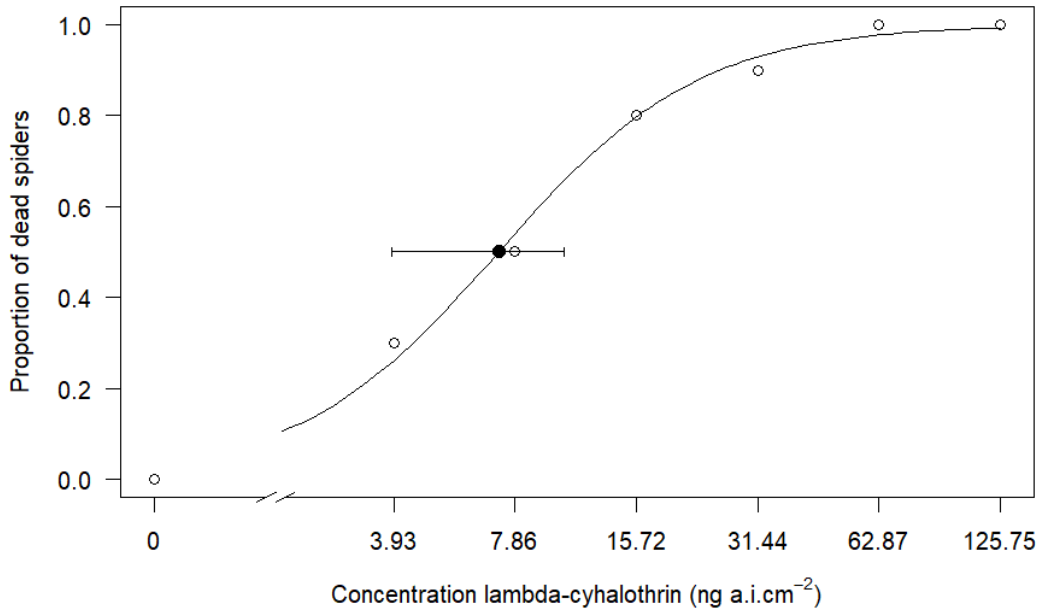
29. *Xysticus desidiosus*



**Figure S29** Dose-response curve for *X. desidiosus* (ID 29, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

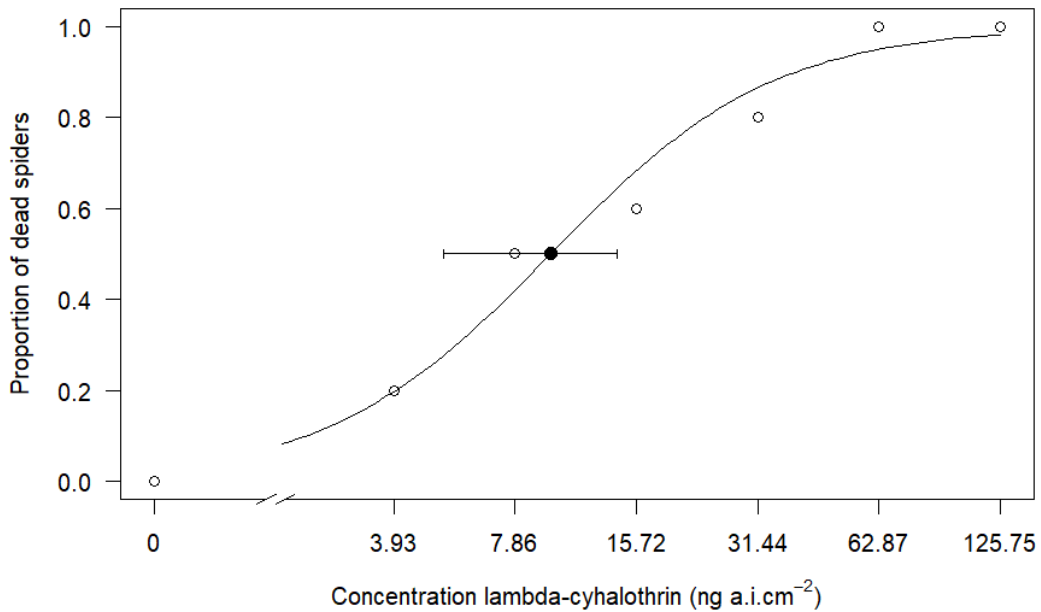


30. *Drassodex heeri*



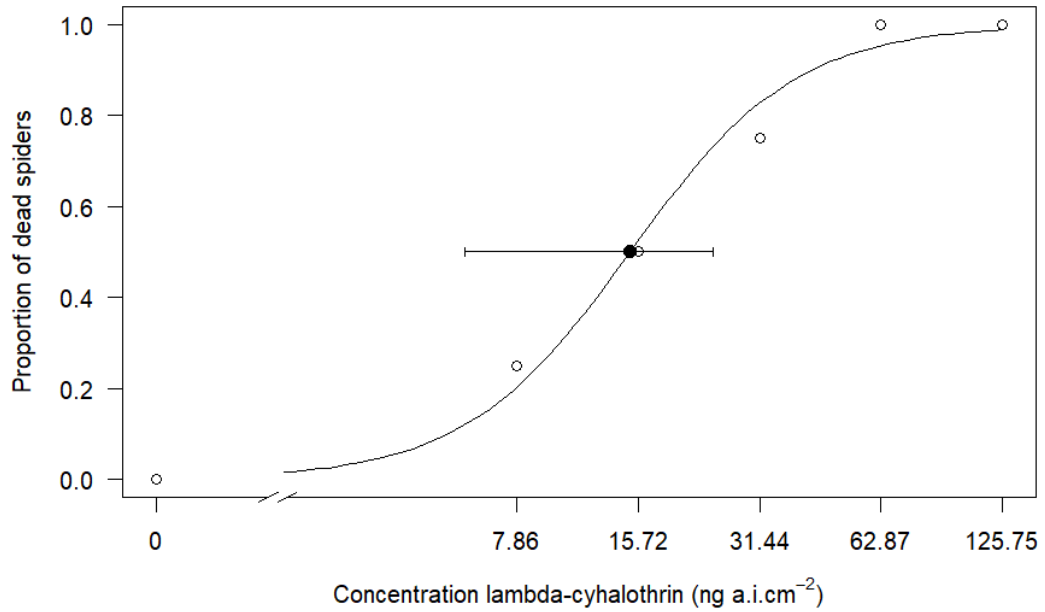
**Figure S30** Dose-response curve for *D. heeri* (ID 30, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

31. *Haplodrassus signifer*



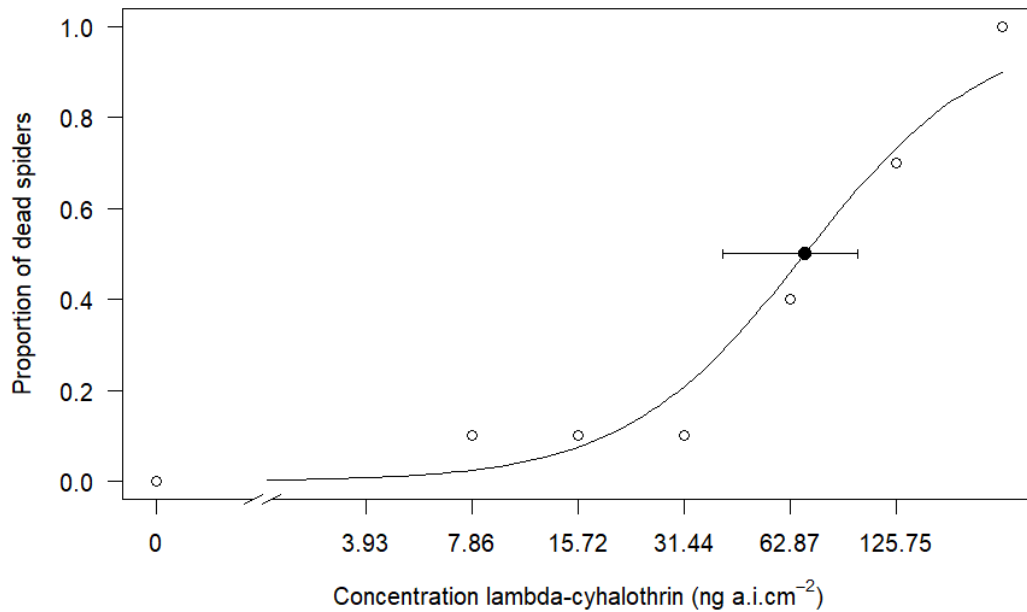
**Figure S31** Dose-response curve for *H. signifer* (ID 31, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

32. *Zelotes apricorum*



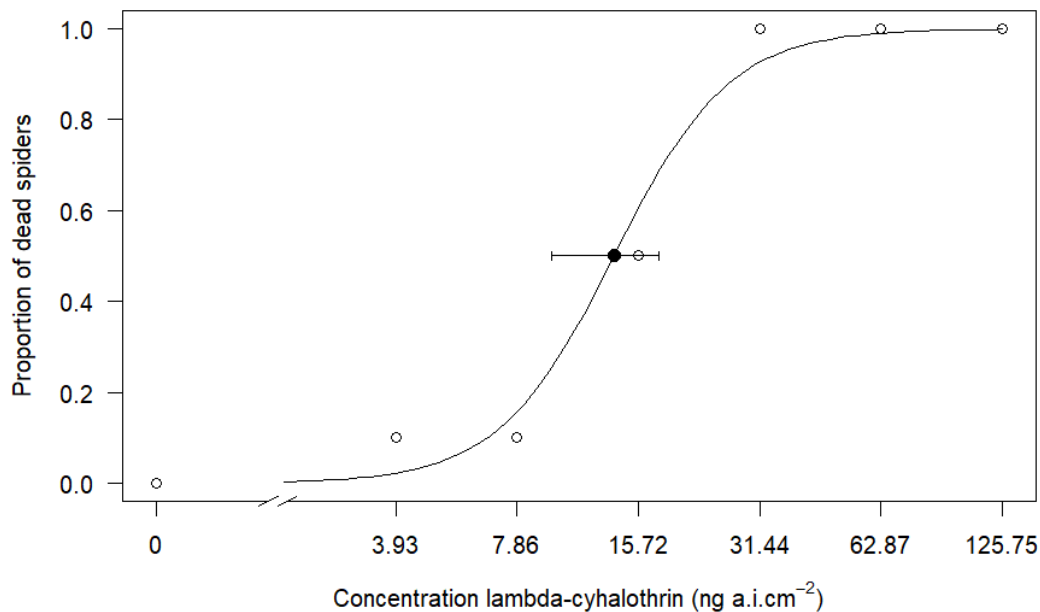
**Figure S32** Dose-response curve for *Z. apricorum* (ID 32, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

33. *Mermessus trilobatus*



**Figure S33** Dose-response curve for *M. trilobatus* (ID 33, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

### 34. *Zoropsis spinimana*



**Figure S34** Dose-response curve for *Z. spinimana* (ID 34, Table S1) 24-hour toxicity test with lambda-cyhalothrin.

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# Chapter 4

Which Temperature Matters? Effects of Origin, Rearing and Test Conditions on the  
Chemical Sensitivity of *Pardosa amentata*

*Tomás Duque, Ralf B. Schäfer and Martin H. Entling*

# Which temperature matters? Effects of origin, rearing and test conditions on the chemical sensitivity of *Pardosa amentata*

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## Abstract

Spiders may be adversely affected by pesticides, yet they are not included in regulatory risk assessment and a related standard guideline to test their sensitivity to chemicals is lacking. Different laboratory setups, including test temperature and relative humidity, have been shown to influence the sensitivity of spiders. The climate from which spiders originate and the rearing conditions in the laboratory prior to ecotoxicological testing may also alter their sensitivity to chemicals, potentially in interaction with test conditions. We investigated the influence of population origin, rearing and test temperature on the chemical sensitivity of the spider *Pardosa amentata* towards lambda-cyhalothrin. We collected female *P. amentata* carrying egg sacs from two climates, i.e., boreal and cool temperate. Spiders were kept in the laboratory and their offspring were reared and tested at 15, 20 and 25°C. Hatching of egg sacs largely failed at 15°C, while a moderate spiderling mortality (40%) was recorded at 20°C. At 25°C, mortality increased (63%) and a faster developmental rate was observed. Rearing and test temperature had no significant effects on spider chemical sensitivity. However, spider chemical sensitivity differed between populations, with spiders from boreal climate being 38% more sensitive than spiders from cool temperate climate. A higher sensitivity towards lambda-cyhalothrin increases the risk of population reduction in treated areas, with potential alterations of ecosystem functions such as biological control. Our results suggest that the climatic origin of test organisms deserves stronger attention in ecotoxicological research.

## KEYWORDS

acute toxicity, lambda-cyhalothrin, Lycosidae, temperature

## 1 | INTRODUCTION

Spiders are important predators in many terrestrial ecosystems (Wise, 1993). They also provide a beneficial function as they consume a wide range of insects, including pest species (Nyffeler, 1999). This is especially relevant for agroecosystems (Michalko et al., 2019;

Riechert, 1999). However, pesticide applications may affect this key role (Reiff et al., 2023; Theiling & Croft, 1988). Moreover, pesticides can unintentionally reach areas outside agroecosystems (Kaur & Garg, 2014) and may also affect other spider species along with the agrobiont spiders. Among pesticides, acaricides and synthetic pyrethroids are the most toxic compounds for spiders (Pekár, 2012).

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Most importantly, pyrethroids can reduce spider survival (Navarro-Silva et al., 2010; Shaw et al., 2006), prey capture (Shaw et al., 2006; Tahir et al., 2015), and locomotor activity (Baatrup & Bayley, 1993) even at concentrations below the recommended application dose. As a consequence, areas treated with pyrethroids exhibited reduced abundance and diversity of spiders and other natural enemies (Fritz et al., 2013; Rodrigues et al., 2013). This may translate into a reduction of their capacity for biological control (e.g., Hanna & Hanna, 2013; Tahir et al., 2019), an important ecological function provided by spiders (Michalko et al., 2019).

Despite the known adverse effects of pesticides, spiders have received little attention in ecotoxicology compared to other non-target arthropods (EFSA Panel on Plant Protection Products and their Residues, 2015; Pekár, 2012). Moreover, spiders are not routinely included as test organisms for the risk assessment of pesticides in Europe (European Commission, 2013a, 2013b). Although methods for testing pesticides on spiders from the family Linyphiidae (Aukema et al., 1990) and the genus *Pardosa* (Wehling et al., 1998) have been proposed, so far, no standard protocol applicable to all spider taxa has been accepted for regulatory risk assessment. Hence, the available spider ecotoxicological data have been derived from many different test designs (Pekár, 2012), mainly focusing on the lethal and sublethal effects of field doses or pesticide residues. Spider pesticide sensitivity varies between different test designs, and this variation has been mainly attributed to the abiotic parameters from the test setup (Jagers Op Akkerhuis et al., 1997). In particular, higher spider mortality is expected at low test temperatures and low relative humidity (Everts et al., 1991). We hope that the current research will provide valuable information for the potential development of a standardised protocol to test the chemical sensitivity of spiders, which could improve the reproducibility and comparability of ecotoxicological studies.

Apart from test temperature, the climatic origin and rearing conditions may also influence the pesticide sensitivity of spiders. For example, climatic factors influence the cuticular structure and composition of arthropods. Spiders from warm and dry ecosystems are expected to have a stronger cuticle to resist desiccation (Sprenger et al., 2018), which could translate to a reduced uptake and higher pesticide tolerance. In a multi-species comparison, spiders from boreal climates were indeed more sensitive to lambda-cyhalothrin than spiders from warmer climates (Duque et al., 2023). Moreover, when comparing the chemical sensitivity between spiders of

different origins, differences may arise because standard test or rearing temperatures are differently amenable for different species. For example, a test or rearing temperature of 25°C could represent heat stress for a spider adapted to cold environments, while it may represent the optimum temperature for spiders from a warm temperate climate. Although the relationship between spider pesticide sensitivity and the test temperature has been previously evaluated (Everts et al., 1991; Michalko & Košulič, 2016), information about potential interactions of test temperature, rearing temperature and climatic background on spider sensitivity is lacking.

The aim of the present study was to investigate the influence of climatic origin, rearing and test temperature on the chemical sensitivity of a spider species. We used the wolf spider *P.amentata* (Clerck, 1757) as a test organism, which is sensitive to pyrethroids (Baatrup & Bayley, 1993; Shaw et al., 2006). *P.amentata* is common and widely distributed in Europe, preferring damp habitats in open areas (Nentwig et al., 2023). It is common in agricultural field margins where pesticide application may directly affect it. We collected 81 females of *P.amentata* carrying egg sacs in two European climates: 36 in boreal and 45 in cool temperate, respectively. Spiders were reared in the laboratory at three different temperatures (rearing; 15, 20 and 25°C) and their spiderlings were used for ecotoxicological assessment using the pyrethroid insecticide lambda-cyhalothrin. To derive median lethal concentrations (LC<sub>50</sub>), we conducted 24-h acute tests in a crossed-treatment design with test temperatures of 15, 20 and 25°C. We addressed the following research questions: (1) Are spiders originating from boreal climate more sensitive to lambda-cyhalothrin than spiders from cool temperate climate? (2) Is spider sensitivity negatively related to rearing temperature? (3) Is spider sensitivity negatively related to test temperature, particularly for individuals originating from boreal climate?

## 2 | MATERIALS AND METHODS

### 2.1 | Source of test organisms

In total, 85 adult female spiders carrying egg sacs were collected from wetlands away from agricultural areas in both climates: a subalpine bog with a boreal climate and an open riparian wetland with cool temperate climate, according to the western European

TABLE 1 Development of *Pardosa amentata* under laboratory conditions (mean values with  $\pm$  standard deviation).

Females captured	Climate of origin	Rearing temperature (°C)	Female mortality (%)	Egg sacs hatched (%)	Spiderlings hatched <sup>a</sup>	Spiderlings mortality (%)	Time to reach 2nd instar (days)
12	Cool temperate	15	8	0	-	-	-
12		20	0	58	48 $\pm$ 14	44	25 $\pm$ 6
12		25	8	100	50 $\pm$ 16	68	21 $\pm$ 6
15	Boreal	15	0	13	47 $\pm$ 1	17	-
15		20	0	73	35 $\pm$ 17	36	26 $\pm$ 7
15		25	0	100	37 $\pm$ 10	57	22 $\pm$ 6

<sup>a</sup>Average spiderlings hatched per egg sac.

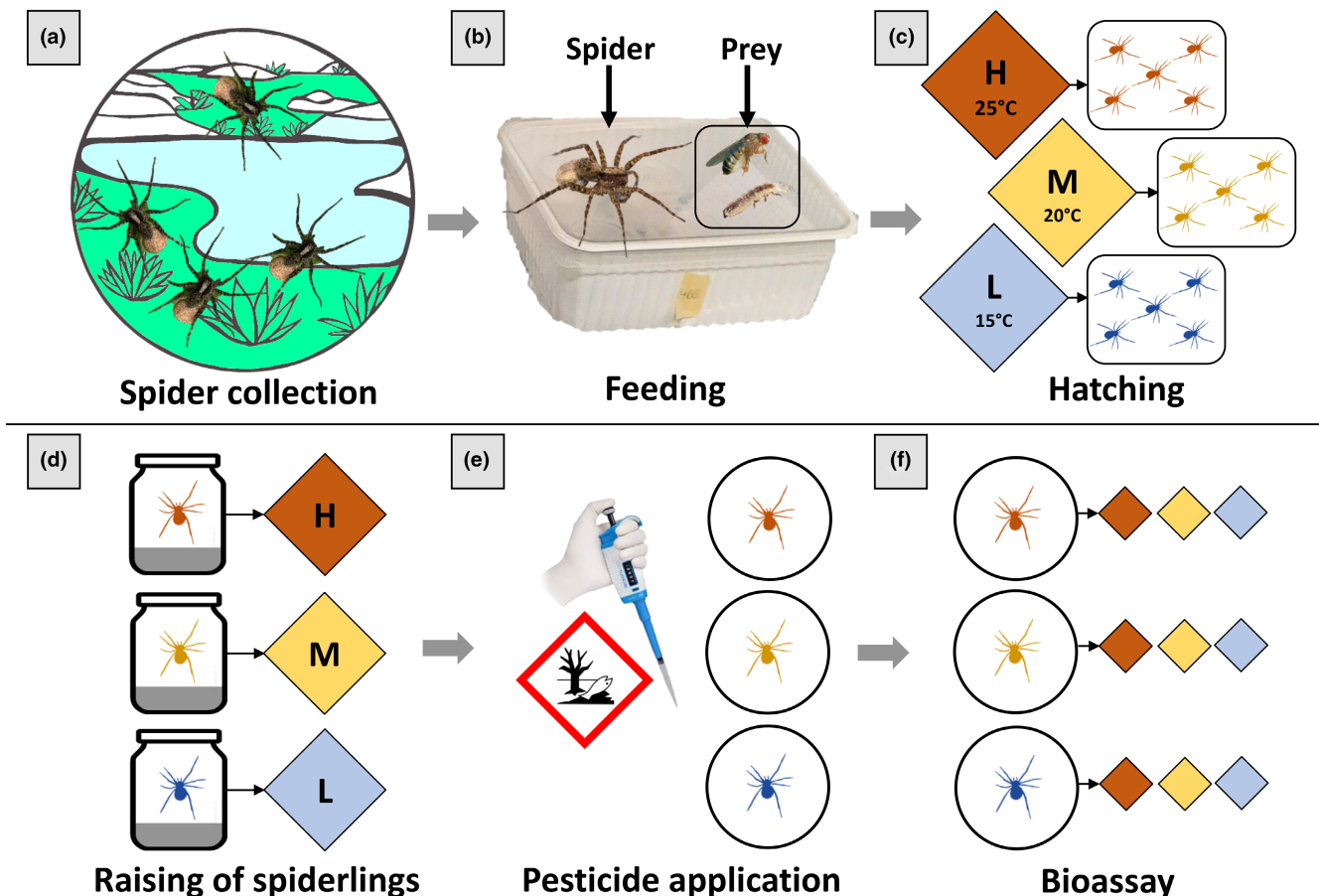
Holdridge life Zones (HLZ; Holdridge, 1967). At least 36 females were collected from each climatic zone (Table 1). The collection at boreal climate was done in July 2022 at the Gotthard Pass in the Swiss Alps (46°34'25.6" N, 8°33'30.7" E). Due to earlier phenology, collection at cool temperate climate was done in late May 2022 at the Eußerthal Ecosystem Research Station in the Palatinate Forest in Germany (EERES; 49°15'16.4" N, 7°57'42.4" E). *P.amentata* is not a threatened species or under special protection in Germany or Switzerland (Milano et al., 2021). Collected spiders were pre-identified visually in the field (Roberts, 1995) and placed individually into a glass jar (35 mL, 44 mm Ø×42.5 mm height) with a layer of moistened plaster for transport to the laboratory (iES Landau, Germany).

## 2.2 | Rearing *Pardosa amentata* under laboratory conditions

In the laboratory, *P.amentata* females were transferred individually into polypropylene boxes (1 L, 18 cm length×13.2 cm width×6.8 cm height) with a layer (~1 cm) of moistened plaster. To maintain a

polytypic diet (Uetz et al., 1992), spiders were fed ad libitum twice per week (Figure 1b) with a mixture of fruit flies (*Drosophila hydei* and *D.melanogaster*; b.t.b.e. Insektenzucht, Bad Wörishofen, Germany) and springtails (*Coecobrya tenebricosa*, MyAnts.de, Weiden, Germany). Spiders were kept avoiding direct light exposure in three climate chambers at constant relative humidity (100%), light/dark cycle (16/8 h), and illuminance (300 lux), but with different temperatures. One-third of the spiders (~12; Table 1) collected from each location were bred at 15°C (Treatment L; Figure 1c), the second third at 20°C (Treatment M; Figure 1c) and the remaining females at 25°C (Treatment H; Figure 1c) until the juveniles hatched. During the rearing period, we recorded female mortality, the number of egg sacs hatched, the number of hatched spiderlings and the survival of spiderlings (Table 1).

Once the spiderlings hatched, they were kept with their mother in the container and more springtails were offered as food. Approximately 1 week after, when spiderlings became independent, i.e., left their mother's back, they were transferred for individual rearing into 35 mL glass jars (44 mm Ø×42.5 mm height) with moistened plaster and fed ad libitum with springtails twice per week. The temperature treatment of the mothers was maintained for rearing



**FIGURE 1** Scheme of experimental procedure for one climate (cool temperate or boreal). (a) Sampling of *Pardosa amentata* females with egg sac. (b) Feeding of spiders in the laboratory and (c) temperature treatment in the climate chambers at 25°C (H), 20°C (M) or 15°C (L) until hatching of the spiderlings. (d) Separation of spiderlings and rearing at the same temperatures. (e) Pesticide application and (f) ecotoxicological assessment.



the spiderlings (Treatment L, M or H; Figure 1d) until they reached their second instar after being independent (Table 1). The rearing at 15°C (Treatment L, Figure 1c) resulted in low hatching success, for boreal and cool temperate origin, and was omitted from further analysis. Each mother spider was preserved in 70% ethanol and identified to species level to confirm the field identification using Nentwig et al., 2023.

## 2.3 | Ecotoxicological assessment

To assess the influence of climatic origin, rearing and test temperatures on the sensitivity of *P. amentata*, we performed a full-factorial experiment. For spiders from both climatic origins, boreal and cool, spiderlings reared at 20 and 25°C were tested at 15, 20 and 25°C (Table 2). This resulted in a full 2×2×3 factorial design with 12 temperature combinations. For each temperature combination, we performed a 24-h acute exposure toxicity test for spiders as in Aukema et al. (1990), with modifications to allow for dose–response calculations. Moreover, every test was performed with six different concentrations of lambda-cyhalothrin plus a blank control, each one with five replicates, i.e., five spiderlings. Two days before the ecotoxicological assessment, at least 105 spiderlings reared at the same temperature and originating from the same climate, were transferred into glass jars with moistened plaster, but without food (Pekár, 1999). During this time, spiders were stored at the same temperature treatments in which they had developed.

The insecticide lambda-cyhalothrin (Hunter® EG, CERTIS Europe, Hamburg, Germany, 5% active ingredient [a.i.]) was used for acute exposure testing. The product was weighed to the nearest 0.01 mg (AT261 DeltaRange® 205 g/0.01 mg, Metler Toledo, Columbus, Ohio, USA), diluted in ultra-pure water, and stock solutions were created using serial dilutions. Stock solutions were homogenized with magnetic stirrers for 10 min at room temperature.

Range-finding tests had previously been performed around the concentration of 75 ng a.i./cm<sup>2</sup> (Duque et al., 2023), which has been reported to decrease spider abundance in fields (Rodrigues et al., 2013). On the test day, spiderlings were weighed to the nearest 0.1 mg (PA214® 210 g/0.0001 g, OHAUS, New Jersey, USA) and only spiders with a similar weight (1±0.3 mg; Table 2) were used in the test. Thirty-five spiderlings were used for each test and were exposed to seven concentrations of lambda-cyhalothrin in a geometric series between 0 (control) and 125.75 ng a.i./cm<sup>2</sup> (with a separation factor of 2), with 5 replicates per concentration. One replicate consisted of a filter paper (MN 615, 90 mm Ø, Macherey-Nagel, Düren, Germany) previously treated with an aliquot (1 mL, directly applied with an Eppendorf pipette) of the desired test concentration inside a glass petri dish (90 mm Ø, Steriplan®, DWK Life Sciences, Wertheim, Germany). Ultra-pure water was used for the control. Immediately after the lambda-cyhalothrin or control application, one spiderling was placed on each filter paper (Figure 1e), and petri dishes were closed and placed in the respective climate chamber to have a crossed treatment (Figure 1f). *P. amentata* survival was visually assessed after 24 h of the application. Spiderlings were classified as alive, dead or paralysed (Baatrup & Bayley, 1993).

## 2.4 | Data analysis

For dose–response calculations, paralysed individuals were considered as “alive”. Less than 9% of all spiderlings were paralysed, having only a minor influence on the LC<sub>50</sub>s calculated (Table S1). As per the analysis of binomial dose–response data (Ritz et al., 2019) the two-parameter log-logistic model was fitted for all tests, and LC<sub>50</sub>s after 24 h of exposure were calculated (Figures S1–S12). The distribution of the resulting LC<sub>50</sub> values was checked via quantile–quantile plots as well as Shapiro–Wilk tests and variance homogeneity by using residual plots and Levene’s test. Then, the distribution of

Climate of origin	Rearing temperature (°C)	Test temperature (°C)	Fresh weight (mg) mean ± SD	LC <sub>50</sub> (ng a.i./cm <sup>2</sup> )
Cool temperate	25	25	1.1±0.2	11.11±3.90
		20	1.1±0.2	10.52±6.46
		15	1.0±0.2	16.81±7.6
	20	25	0.9±0.2	7.06±3.58
		20	0.9±0.1	9.64±3.66
		15	0.9±0.1	9.38±4.94
Boreal	25	25	0.9±0.2	8.23±3.98
		20	1.0±0.2	7.06±3.58
		15	1.0±0.2	5.64±3.80
	20	25	0.9±0.2	4.61±2.16
		20	0.9±0.2	8.77±5.99
		15	0.8±0.1	5.51±2.02

TABLE 2 Chemical sensitivity (LC<sub>50</sub>±95% CI) and fresh weight of spiderlings used for the ecotoxicological assessment.

LC<sub>50</sub> values was normalized by applying the natural-log transformation. The interactive effect of climate (boreal or cool temperate), rearing temperature (20 or 25°C) and test temperature (15, 20 or 25°C) was analysed via linear modelling. All statistical analyses and figures were performed with R Version 4.3.0 for Windows (R Core Team, 2023), together with the add-on packages “drc” (Ritz et al., 2015), “plotrix” (Lemon, 2006) for dose-response modelling, “car” (Fox & Weisberg, 2019), “MuMIn” (Bartoń, 2022) and “ggplot2” (Wickham, 2016) for the linear modelling.

### 3 | RESULTS

#### 3.1 | Rearing success

In total, 81 spiders with egg sacs were collected, 36 in cool temperate and 45 in boreal climate. At the rearing temperature of 15°C, no hatching was observed for spiders collected in cool temperate climate, whereas two out of fifteen egg sacs hatched for the boreal spiders (Table 1). All egg sacs hatched at 25°C, while 58 and 73% of egg sacs from spiders collected in cool temperate and boreal climate, respectively, hatched at 20°C. A moderate spiderling mortality (~40%) was recorded at 20°C, while mortality increased to ~63% at 25°C. Spiderlings reached their 2nd instar 26 days after hatching when reared at 20°C, and 22 days after hatching when reared at 25°C (Table 1).

#### 3.2 | Chemical sensitivity

Since rearing at 15°C largely failed (Table 1), only the tests for the rearing treatments at 20 and 25°C were performed (Table 2). Spider LC<sub>50</sub>s ranged from 7.06 to 16.81 ng a.i./cm<sup>2</sup> for spiders collected in the cool temperate climate (Table 2; Figures S1–S6), and from 4.61 to 8.77 ng a.i./cm<sup>2</sup> for the boreal climate (Table 2; Figures S7–S12). For both climatic zones, the treatment of spiderlings reared at 20°C with a test temperature of 25°C resulted in the most sensitive endpoints (Table 2). However, neither rearing temperatures nor test temperatures or any of their interactions had a significant effect on spider

TABLE 3 Effects of climatic origin, rearing and test temperature on spider chemical sensitivity.

Factor <sup>a</sup>	df	F	p
Climate of origin	1	9.96	<b>0.03</b>
Rearing temperature	1	2.81	0.17
Test temperature	1	0.46	0.53
Climate:Rearing	1	0.67	0.46
Climate:Test	1	1.49	0.29
Rearing:Test	1	0.34	0.59
Climate:Rearing:Test	1	0.87	0.40

Note: Statistically significant effects ( $p < 0.05$ ) are printed in bold.

<sup>a</sup>Interaction term for factors are represented by “:”.

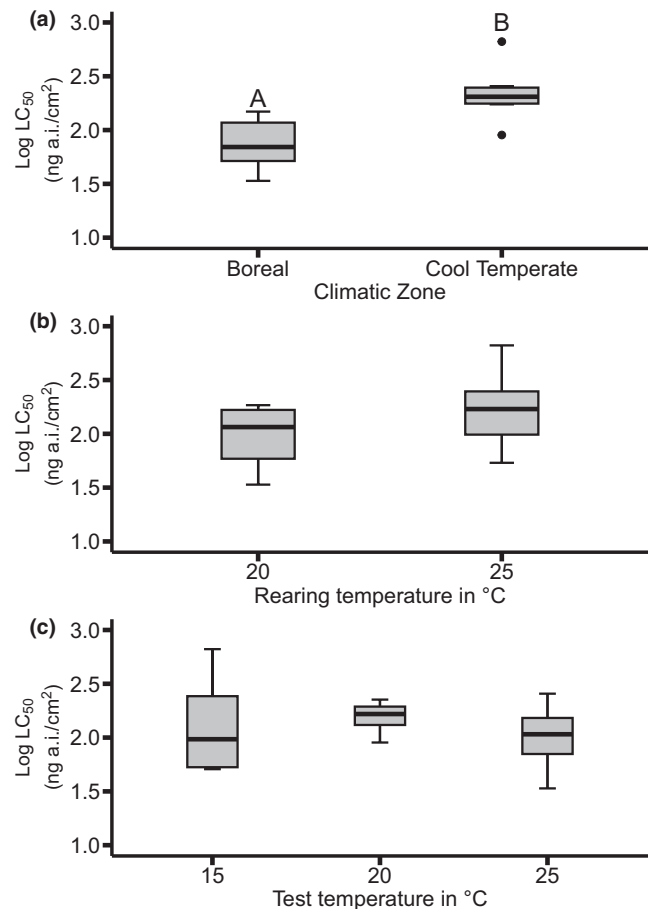


FIGURE 2 Chemical sensitivity of spiders towards lambda-cyhalothrin expressed as median lethal concentration: Effects of (a) climate of origin, (b) rearing temperature and (c) test temperature. Different letters show significant differences ( $p < 0.05$ ). LC<sub>50</sub> = median lethal concentration; a.i. = active ingredient.

chemical sensitivity (Table 3, Figure 2b,c). *P. amentata* chemical sensitivity differed exclusively between the climatic zones ( $F_{(1,4)} = 9.96$ ,  $p = 0.03$ ; Table 3). The LC<sub>50</sub>s of spiderlings from the boreal climate were on average 38% lower than those originating from the cool temperate climate (Figure 2a).

## 4 | DISCUSSION

### 4.1 | Chemical sensitivity

Our results suggest that the climatic origin of *P. amentata* influenced their sensitivity to lambda-cyhalothrin, independent of rearing or test temperatures. The higher sensitivity of the population from the boreal climate is in accordance with a generally higher sensitivity of multiple spider species from boreal and polar climates when compared to spiders from a temperate climate after exposure to the same insecticide (Duque et al., 2023). Nevertheless, the high sensitivity of both populations of *P. amentata* to lambda-cyhalothrin which is reflected in LC<sub>50</sub> values far

below the recommended application dose of lambda-cyhalothrin (i.e., 75 ng a.i./cm<sup>2</sup>), indicates a high potential risk of mortality. Indeed, a reduction of spider abundance in fields treated with this insecticide has been reported by Rodrigues et al. (2013). As a consequence, ecosystem functions such as biocontrol may be reduced (Hanna & Hanna, 2013; Tahir et al., 2019), an important function provided by spiders, especially free hunters, such as *P.amentata* (Michalko et al., 2019).

The differences in chemical sensitivity between spiders from different climates may be explained by differences in biological traits related to climate adaptation, such as metabolism or desiccation resistance. To prevent desiccation, arthropods living in dry or warm habitats can adapt the chemical composition of their cuticular hydrocarbons (Sprenger et al., 2018). This adaptation may also reduce pesticide uptake when direct contact is the main exposure route. Moreover, a waterproofing cuticle may reduce pesticide effects, as water depletion appears to contribute to the mortality of spiders exposed to pyrethroids (Jagers Op Akkerhuis et al., 1997). This mechanism could explain sensitivity differences in the direction observed in the current study. In addition, reduced chemical sensitivity in warmer climates may reflect higher tolerance to natural plant secondary metabolites that are ingested e.g., with herbivore prey. While relationships of metabolite concentrations with temperature are variable, higher concentrations at warmer temperatures seem to prevail (Pant et al., 2021; Yang et al., 2018). This could lead to a higher natural exposure of spiders in warmer climates to plant secondary compounds.

Lastly, the adaptation of spiders to lower environmental temperatures in a boreal climate may influence their internal response to lambda-cyhalothrin. Such adaptation can be related to a higher nerve sensitivity and changes in the receptor binding, as has been documented in other arthropods (Ahn et al., 1987). Information on the internal effect of pesticides on populations from different habitats may help to understand the climate effect observed in our study. This would require toxicogenomic (Hamadeh et al., 2002) studies analysing other ecotoxicological endpoints, such as the activity of detoxification enzymes (Zhou et al., 2019), the binding of active ingredients to target and non-target receptors (Narahashi et al., 2007), and gene expression (Giambò et al., 2021).

As each of the two climatic origins was only represented by one location, we cannot exclude that other factors than climate contributed to the observed differences. For example, it could be expected that pesticides were more common in the temperate climate, since no crops are grown at the altitude where the boreal population was sampled (Ding et al., 2023). We tried to avoid confounding effects of background pollution and possible adaptation by sampling the temperate climate population in a location with no agriculture within at least a 3 km radius. Furthermore, a study comparing the chemical sensitivities of 28 spider species found no indication that species from agricultural areas (e.g., *Pardosa agrestis*) were more sensitive than related species from more natural areas (Duque et al., 2023). Also, in this multi-species comparison that comprised 25 sampling locations, spiders from boreal to arctic climates were

more sensitive to lambda-cyhalothrin than spiders from warmer climates. Thus, we assume that climate is the most likely explanation for the higher sensitivity of spiders from colder climate also in the current experiment.

Contrary to our expectations, spider sensitivity showed no significant response to rearing or test temperature. No interactive effects of climate origin and rearing or test temperature were detected. It could be expected that high test temperatures are stressful for spiders adapted to a boreal climate. However, the high reproduction success and developmental rate of spiders from the Alps at 25°C does not indicate any temperature stress. In contrast to our results, linyphiid spider mortality from pyrethroids increased from 30°C towards 10°C test temperature at high air humidity (Everts et al., 1991). In addition, the mortality of *Philodromus* when exposed to lambda-cyhalothrin was higher at 31°C in comparison with lower test temperatures (Michalko & Košulič, 2016). Previous research suggests that the range of temperatures that we tested (15–25°C) may have been too small to detect test temperature effects on spider sensitivity. The elevated spider mortality at high temperatures, i.e., >30°C, observed by Everts et al. (1991) and Michalko and Košulič (2016) was beyond the conditions tested in our study. Nevertheless, our results indicate that rearing and test conditions have no major effect on chemical sensitivity, as long as the conditions used are favourable for spider reproduction.

## 4.2 | Rearing success

Surprisingly, the rearing temperature of 15°C was too low for hatching, even for spiders from boreal climate, which are expected to be better adapted to lower temperatures. At 15°C, the majority of the spiders lost their egg sacs and spiderlings did not hatch (Table 1). In the field, *P.amentata* are known to reproduce at mean air temperatures during the day between 17 and 23°C (Nyffeler, 2000). However, we observed the highest hatching and developmental success at 25°C, indicating a shift of optimal rearing conditions to higher temperatures compared to those observed in the field. Given their diurnal activity and occurrence in open habitats, it is likely that *P.amentata* females can heat their bodies and egg sacs above air temperatures in their natural environment using microhabitat selection, e.g., through sun basking or by staying on warmed-up surfaces. Thus, the poor reproduction at 15°C in the lab may be due to low light intensities and a lack of microclimatic heterogeneity available to the spiders in the climate chambers. Body temperature has a strong influence on arthropod metabolism and development (Mirhosseini et al., 2017). Specifically for spiders, a fast development at higher temperatures is an indicator of warm season adaptation (Li & Jackson, 1996), wherein *P.amentata* reproduction takes place (Vlijm et al., 1963).

The spiderling mortality observed during the rearing period (Table 1) can be partially explained by the monotypic diet of springtails offered during the rearing. *P.amentata* is a generalist predator,

which may feed on a variety of arthropods including other spiders. A polytypic diet could have enhanced the survival of our spiderlings (Uetz et al., 1992), but was not provided here as early instar *P. amentata* were not able to prey on *Drosophila*.

## 5 | CONCLUSION

We provided a detailed method on how to rear *P. amentata* juveniles under laboratory conditions, and subsequently use them for ecotoxicological testing. Our results suggested an optimal temperature of egg hatching of 25°C, while juvenile survival was highest at 20°C. *P. amentata* spiders from a boreal population were more sensitive to lambda-cyhalothrin than from a population in a cool temperate climate independent of rearing and test conditions. The high sensitivity of *P. amentata* is in accordance with the generally high sensitivity of spiders to this insecticide (Duque et al., 2023), which may negatively affect their capacity for biocontrol. Hence, we suggest that the climatic background of organisms should be more widely considered in ecotoxicological and ecological research.

### AUTHOR CONTRIBUTIONS

**Tomás Duque:** Conceptualization; methodology; investigation; writing – original draft; writing – review and editing; formal analysis. **Ralf B. Schäfer:** Conceptualization; methodology; supervision; resources; funding acquisition; writing – review and editing. **Martin H. Entling:** Conceptualization; methodology; resources; supervision; funding acquisition; investigation; writing – review and editing.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

### DATA AVAILABILITY STATEMENT

R codes for toxicity data calculations are available from Figshare: <https://doi.org/10.6084/m9.figshare.23096423>. Data, associated metadata and calculation tools are available from the corresponding author ([martin.entling@rptu.de](mailto:martin.entling@rptu.de)).

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#### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Duque, T., Schäfer, R. B., & Entling, M. H. (2023). Which temperature matters? Effects of origin, rearing and test conditions on the chemical sensitivity of *Pardosa amentata*. *Journal of Applied Entomology*, 00, 1–9. <https://doi.org/10.1111/jen.13219>



## Supporting Information

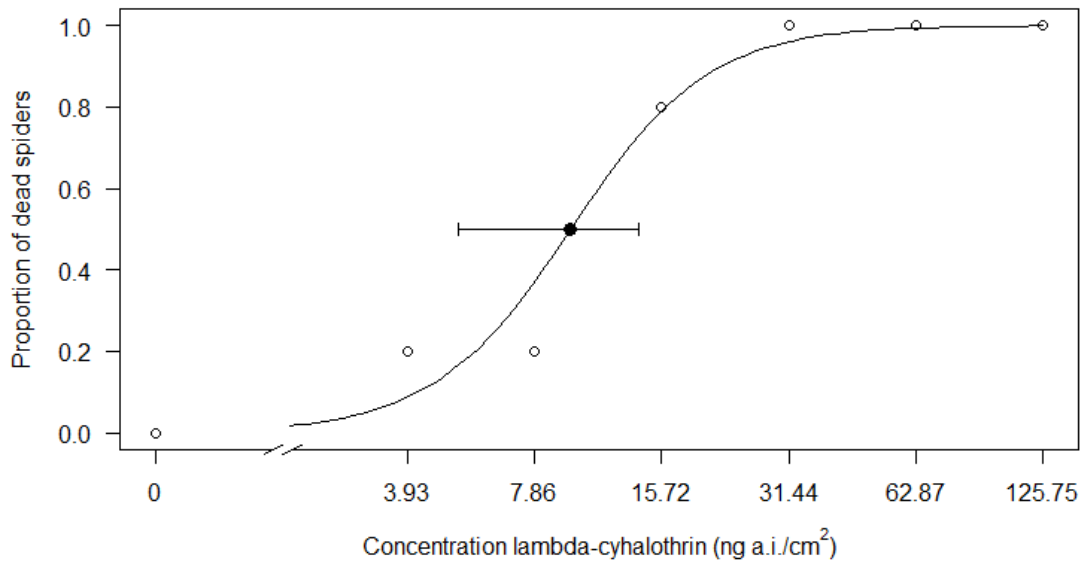
### Influence of paralyzed spiders in dose-response calculations

A multinomial dose-response approach following Ritz et al., (2019) was used to analyze the influence of paralyzed spiderlings in LC<sub>50</sub> calculations. LC<sub>50</sub> values derived from counting “paralyzed” spiderlings as “alive” were compared statistically to LC<sub>50</sub> values derived from merging “paralyzed” and “dead” spiderlings into one category. Since no significant differences were found (Table S1), paralyzed spiderlings were counted as alive for LC<sub>50</sub> calculations. Temp. = Temperature.

**Table S1.** Influence of paralyzed spiderlings in dose-response calculations

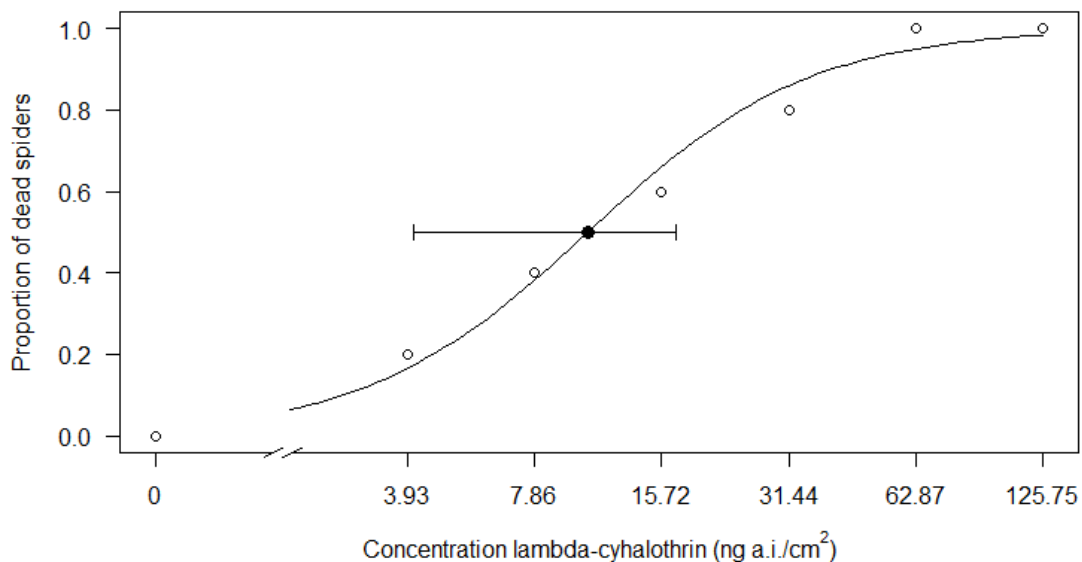
Climate of origin	Rearing temp.	Test temp.	Alive	Dead	Paralyzed	% Paralyzed	t-value	p-value
Cool temperate	25°C	25°C	14	20	1	2.86	0.48	0.63
		20°C	15	20	0	0.00	-	-
		15°C	18	17	0	0.00	-	-
	20°C	25°C	12	23	0	0.00	-	-
		20°C	14	21	0	0.00	-	-
		15°C	14	21	0	0.00	-	-
Boreal	25°C	25°C	12	22	1	2.86	0.46	0.65
		20°C	9	23	3	8.57	0.98	0.32
		15°C	10	24	1	2.86	0.32	0.75
	20°C	25°C	8	26	1	2.86	0.50	0.62
		20°C	11	21	3	8.57	0.70	0.49
		15°C	8	25	2	5.71	1.12	0.26

### 1. Cool temperate, Rearing 25°C, Test 25°C



**Figure S1** Dose-response curve for *P. amentata* (Climate: Cool temperate, Rearing temperature: 25°C, Test temperature: 25°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

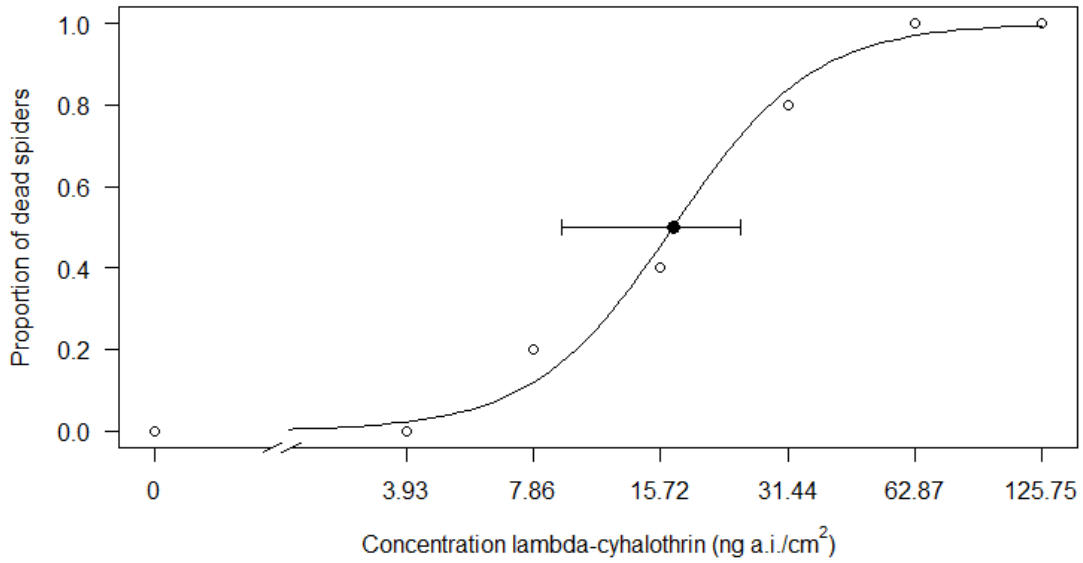
### 2. Cool temperate, Rearing 25°C, Test 20°C



**Figure S2** Dose-response curve for *P. amentata* (Climate: Cool temperate, Rearing temperature: 25°C, Test temperature: 20°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

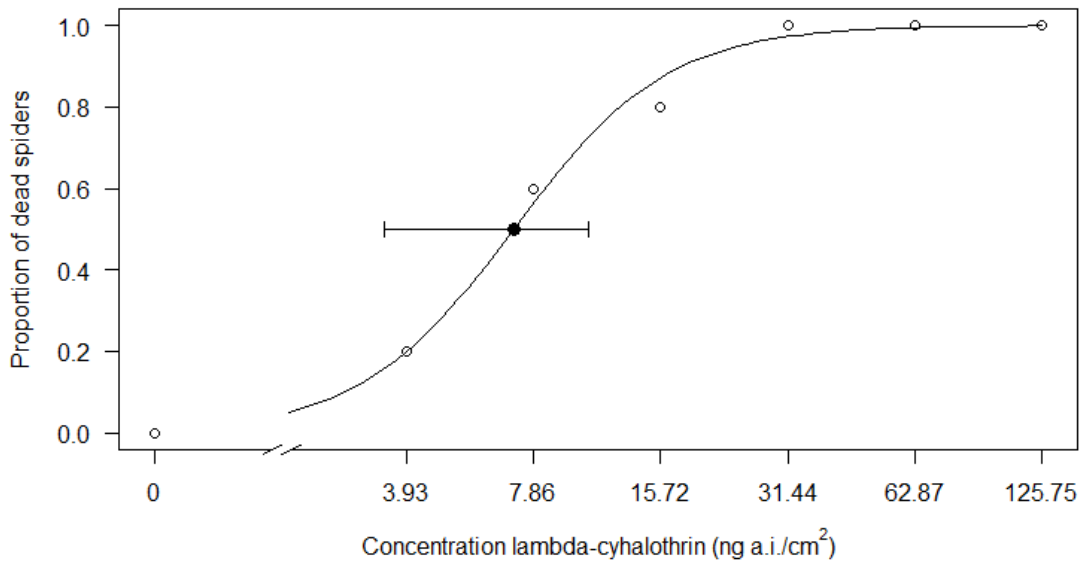


### 3. Cool temperate, Rearing 25°C, Test 15°C



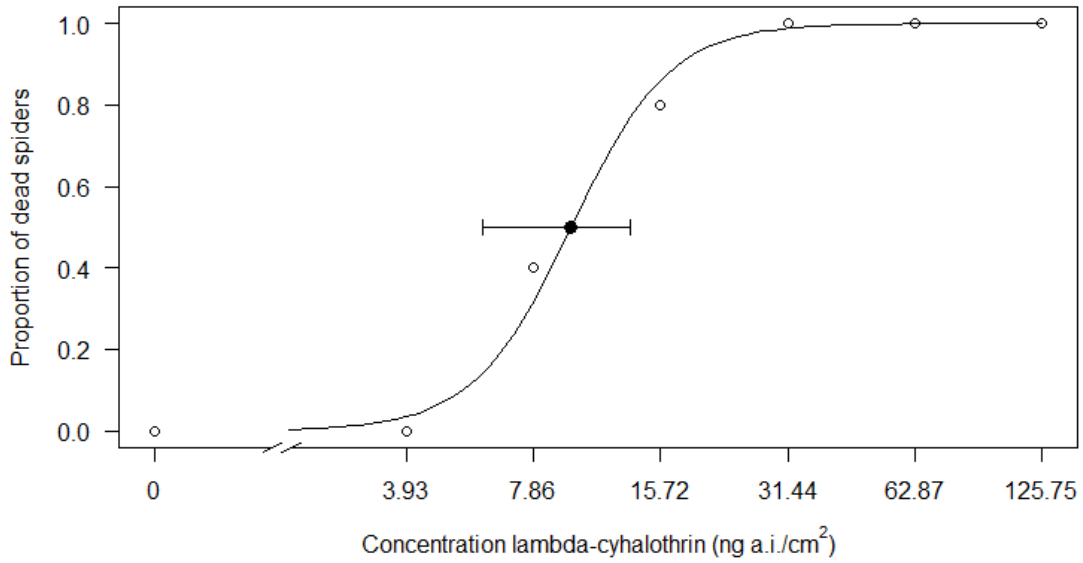
**Figure S3** Dose-response curve for *P. amentata* (Climate: Cool temperate, Rearing temperature: 25°C, Test temperature: 15°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

### 4. Cool temperate, Rearing 20°C, Test 25°C



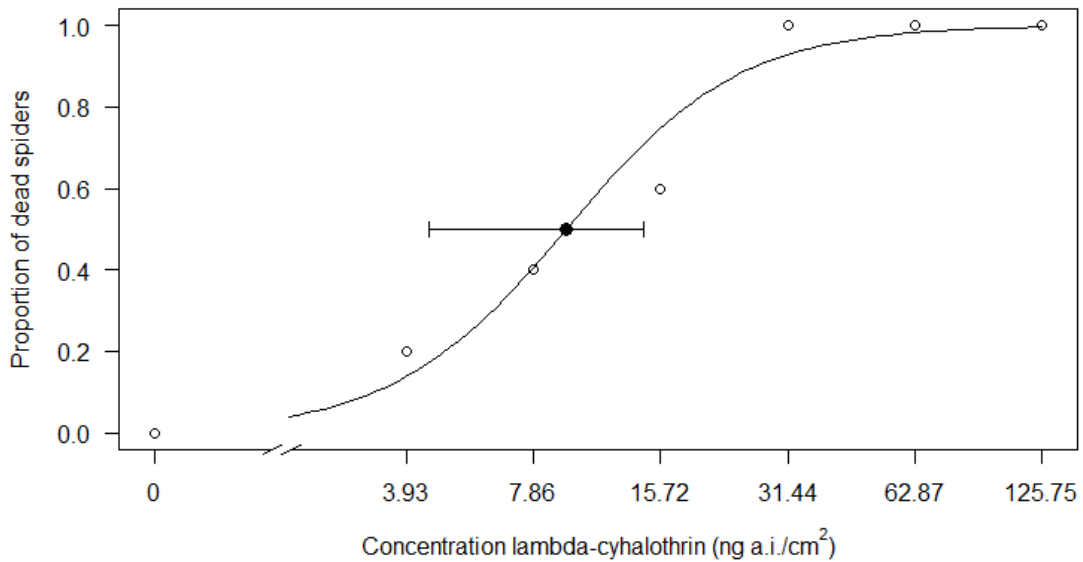
**Figure S4** Dose-response curve for *P. amentata* (Climate: Cool temperate, Rearing temperature: 20°C, Test temperature: 25°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

### 5. Cool temperate, Rearing 20°C, Test 20°C



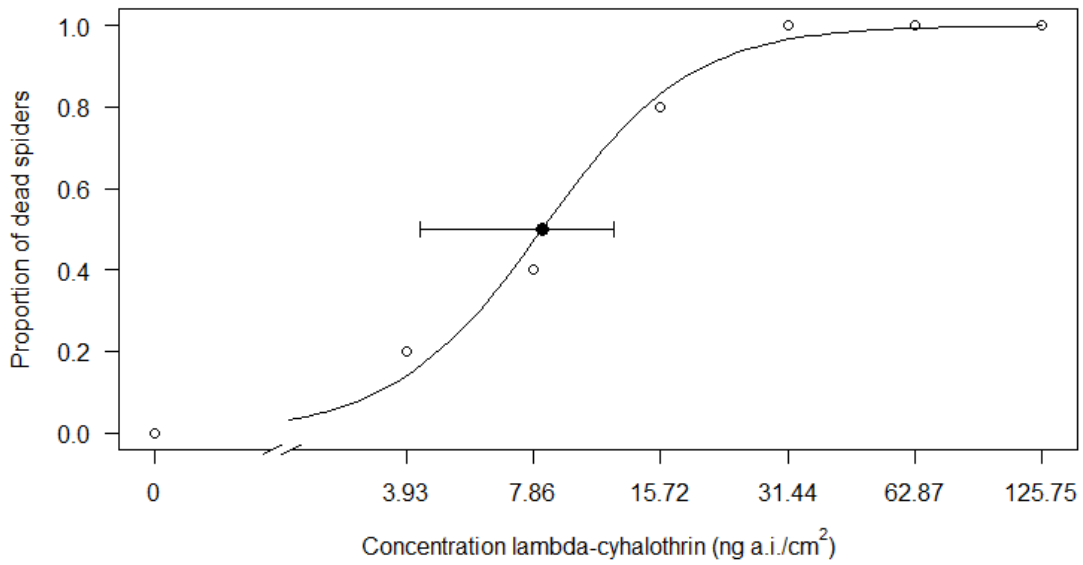
**Figure S5** Dose-response curve for *P. amentata* (Climate: Cool temperate, Rearing temperature: 20°C, Test temperature: 20°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

### 6. Cool temperate, Rearing 20°C, Test 15°C



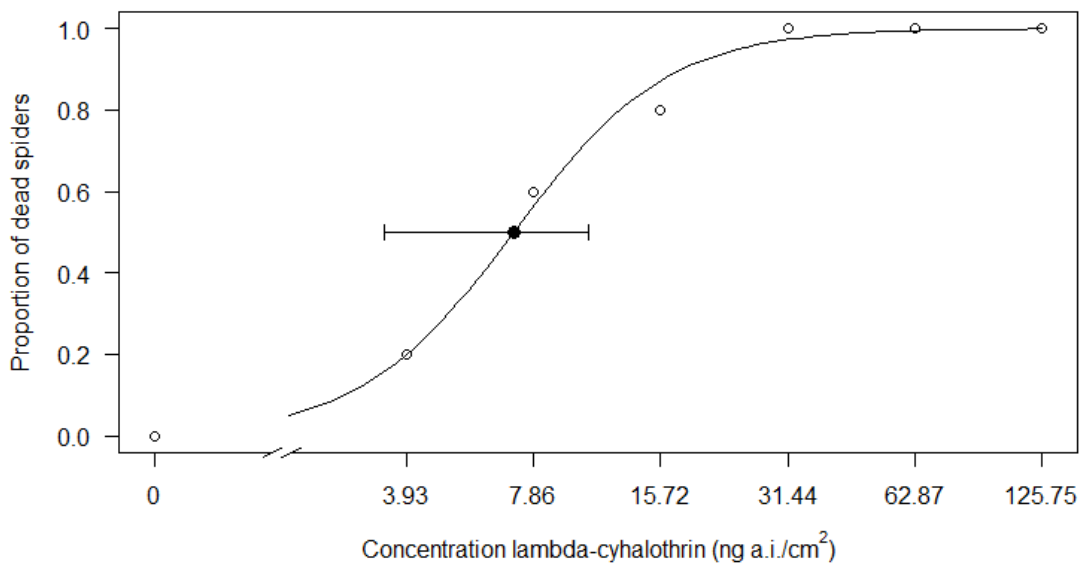
**Figure S6** Dose-response curve for *P. amentata* (Climate: Cool temperate, Rearing temperature: 20°C, Test temperature: 15°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

### 7. Boreal, Rearing 25°C, Test 25°C



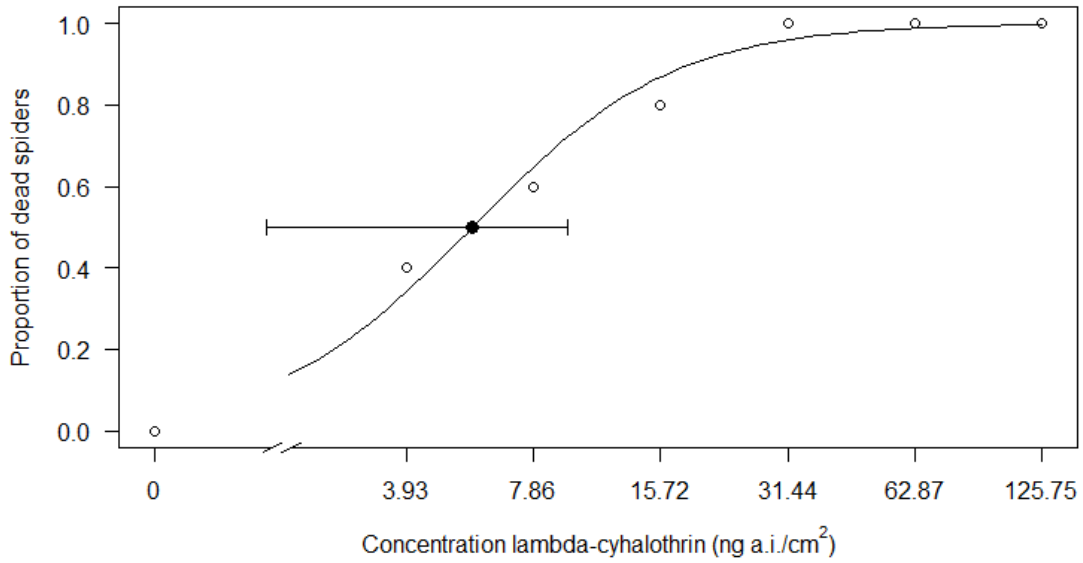
**Figure S7** Dose-response curve for *P. amentata* (Climate: Boreal, Rearing temperature: 25°C, Test temperature: 25°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

### 8. Boreal, Rearing 25°C, Test 20°C



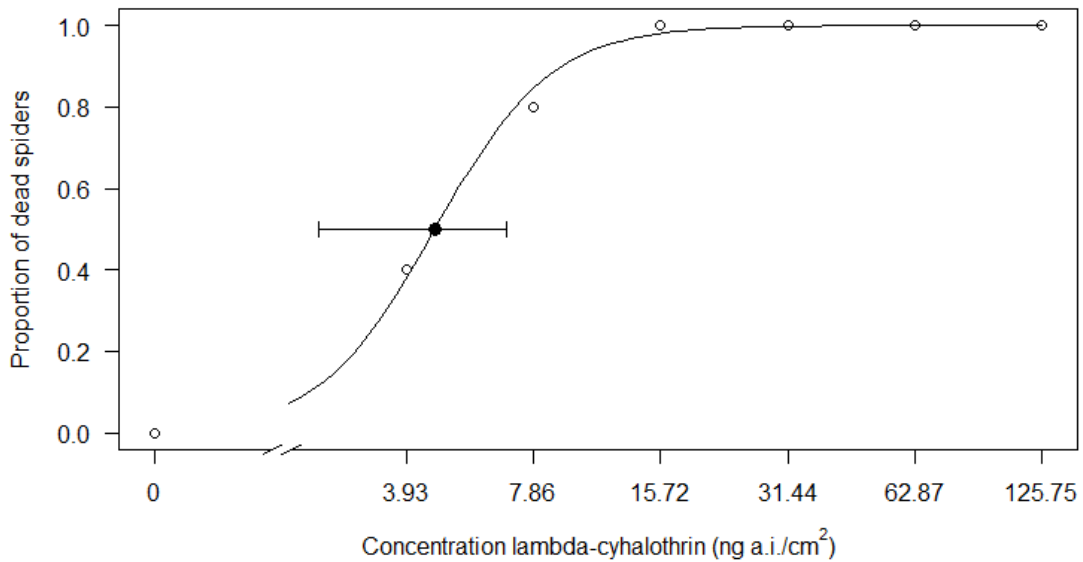
**Figure S8** Dose-response curve for *P. amentata* (Climate: Boreal, Rearing temperature: 25°C, Test temperature: 20°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

### 9. Boreal, Rearing 25°C, Test 15°C



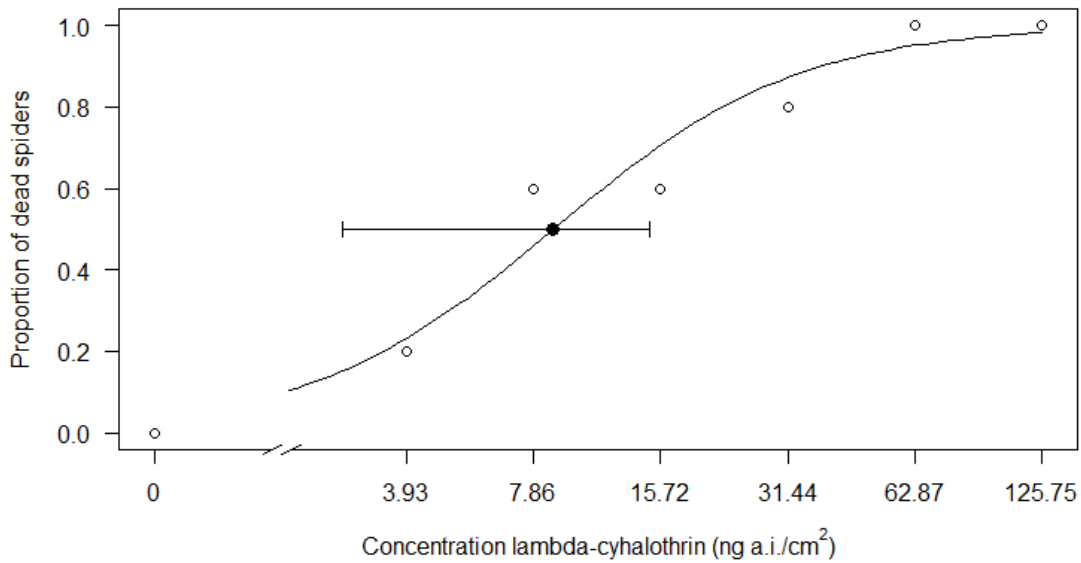
**Figure S9** Dose-response curve for *P. amentata* (Climate: Boreal, Rearing temperature: 25°C, Test temperature: 15°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

### 10. Boreal, Rearing 20°C, Test 25°C



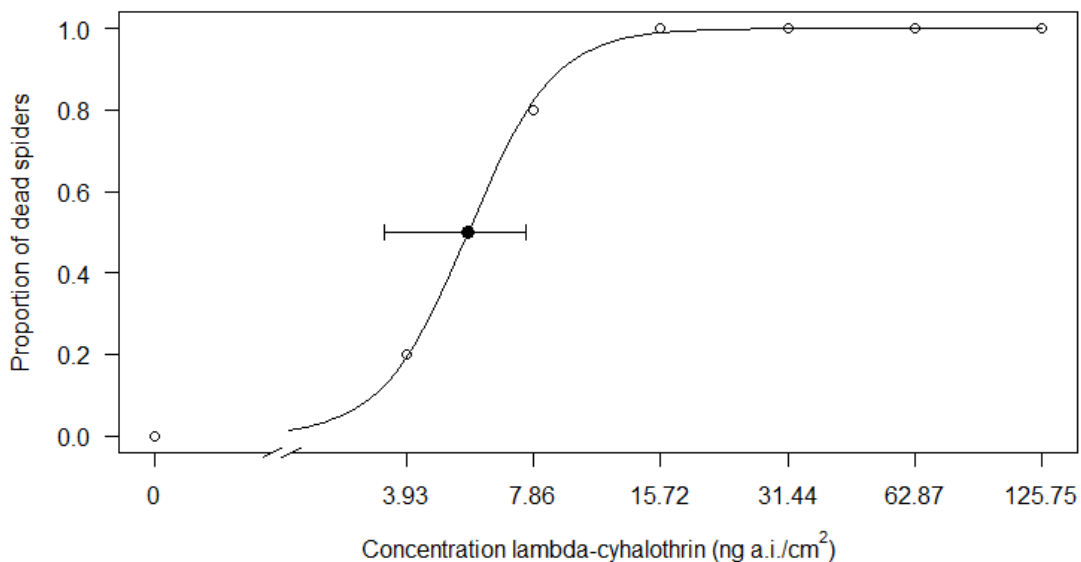
**Figure S10** Dose-response curve for *P. amentata* (Climate: Boreal, Rearing temperature: 20°C, Test temperature: 25°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

### 11. Boreal, Rearing 20°C, Test 20°C



**Figure S11** Dose-response curve for *P. amentata* (Climate: Boreal, Rearing temperature: 20°C, Test temperature: 20°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

### 12. Boreal, Rearing 20°C, Test 15°C



**Figure S12** Dose-response curve for *P. amentata* (Climate: Boreal, Rearing temperature: 20°C, Test temperature: 15°C, Table 2) 24-hour toxicity test with lambda-cyhalothrin.

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# Chapter 5

Synthesis and outlook

*Tomás Duque*

## Earthworms

We found no relationship between earthworm chemical sensitivity, traits (weight) and habitat preferences (forest, grassland or wetland). Our results suggest that earthworm sensitivity varies with soil pH and between ecotypes (Chapter 2). Earthworms adapted to a highly acidic soil (pH <4.3) were less sensitive to copper than earthworms adapted to slightly acidic to neutral soils (pH between 6.2 and 6.8). This variation may be related to other biological traits not analyzed in this work. For example, earthworms living in highly acidic soils may have a higher body wall thickness (Briones & Álvarez-Otero, 2018), which translates to a reduced pesticide sensitivity via dermal contact.

In addition, we found that anecic and endogeic earthworms were more sensitive to imidacloprid than epigeic species. Similarly, a previous meta-analysis between earthworms and pesticides found the same variation, especially with exposure to insecticides (Pelosi et al., 2014). Thus, the higher sensitivity of non-epigeic earthworms may affect ecosystem services, such as bioturbation, i.e., reworking of the soil (Meysman et al., 2006). Anecic and endogeic earthworms contribute meaningfully to this process (Lee & Foster, 1991). As a consequence, pesticide application may lead to a reduction in earthworm populations and affect the ecosystem services provided by these soil organisms.

## Spiders

Our results showed that spider sensitivity was strongly determined by phylogeny and the climate from which species originated (Chapter 3). The phylogenetic analysis showed that spider pesticide sensitivity varies mostly between families. For example, web-building families showed a lower sensitivity than free-hunting families. In addition, differences in sensitivity were also observed between species from the same family, e.g., *Pardosa* spp. was seven times more sensitive than *Piratula* spp. The intraspecific differences between spider species could not be fully explained by the experiments and analyses performed. Thus, the identification of spider receptors associated with sensitivity (Torkkeli et al., 2015) together with toxicogenomic analysis (Hamadeh et al., 2002) would clarify and better explain the intraspecific differences between spider species.



On the other hand, we found that spiders coming from boreal climates were more sensitive than spiders from cool temperate and warm temperate climates (Chapter 3). For example, *Pardosa amentata* collected in a boreal climate was twice as sensitive as *P. amentata* collected in a cool temperate climate (Chapter 3 & 4). In addition, we found no evidence that rearing and test temperatures, between 15 and 25°C, have a major effect on spider sensitivity (Chapter 4). Thus, habitat characteristics appear to influence spider sensitivity to pesticides. This variation may be related with other biological traits not analyzed in the experiments, such as the structure and composition of the cuticle (e.g., Sprenger et al., 2018).

Furthermore, our results suggest a high mortality risk for spiders under field conditions in areas treated with lambda-cyhalothrin. The LC<sub>50</sub>s of 85% of the species tested were below the recommended application rate of lambda-cyhalothrin (Chapter 3). These results can be connected with the reduction of spider abundance observed in field studies after lambda-cyhalothrin application (Niehoff et al., 1994; Rodrigues et al., 2013). Moreover, agrobiont species, such as *Diplocephalus graecus*, *Erigone dentipalpis*, *Pardosa agrestis* and *Pardosa tenuipes*, were generally more sensitive than related species sampled in non-agricultural ecosystems. The high sensitivity of agrobiont species indicates that pesticide exposure can negatively affect the biological control of insect pests, an important ecological function of spiders (Michalko et al., 2019).

## Conclusions

The protection of the ecosystem services and functions provided by earthworms would require the inclusion of more ecologically relevant and sensitive species in risk assessment (Forbes et al., 2021; ISO, 2023). Moreover, earthworm chemical sensitivity varied with soil pH and between ecotypes. Thus, the protection of a region-specific soil community and its ecological roles would require considering the soil characteristics. Additionally, spiders were highly sensitive to lambda-cyhalothrin, where the most sensitive species were found in boreal areas. This may result in a change in spider communities in affected areas, and alter the ecosystem functions of biocontrol. Thus, spiders could be a potential organism group to include in a future risk assessment focused on preserving important ecosystem functions.

The outcomes of this thesis offer valuable insights into the potential effects of pesticides on terrestrial organism groups, and how their differences in sensitivity relate to habitat characteristics, such as soil pH and climate. Variation in chemical sensitivity is currently assumed to be covered by assessment factors when testing effects of pesticides according to standard regulatory framework. However, relying on assessment factors alone could result in under-protection of non-target organisms. These biases can spread even more when moving from single species to the community level, where the local extinction of a key species can lead to dramatic top-down or bottom-up effects on the whole ecological network. In accordance with our study, the local loss of sensitive earthworm or spider species may alter local processes such as bioturbation and soil fertility or pest control. In addition, it should also be considered that multiple chemicals, or more generally, multiple stressors, can co-occur and interact with each other, often with unpredictable effects of these new mixtures on organisms' sensitivity (Schäfer et al., 2023). In this framework, this thesis represents one step forward in improving our understanding of the variability in chemical sensitivity of non-target organisms and its ecosystem-level consequences.

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<https://doi.org/10.1371/journal.pone.0138068>

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# Appendix

**Status and author contributions of publications included in the thesis**

**Author affiliations**

**Curriculum vitae**

**Declaration**

## Status and author contributions of publications included in the thesis

### Chapter 2

Duque, T., Nuriyev, R., Römbke, J., Schäfer, R. B., & Entling, M. H. (2023). Variation in the Chemical Sensitivity of Earthworms from Field Populations to Imidacloprid and Copper. *Environmental Toxicology and Chemistry*. 42(4). 939-947. <https://doi.org/10.1002/etc.5589>

See Chapter 2 for author contribution statements.

### Chapter 3

Duque, T., Chowdhury, S., Isaia, M., Pekár, S., Riess, K., Scherf, G., Schäfer, R. B., & Entling, M. H. (2023). Sensitivity of spiders from different ecosystems to lambda-cyhalothrin: effects of phylogeny and climate. *Pest Management Science*, In press. <https://doi.org/10.1002/ps.7818>

See Chapter 3 for author contribution statements.

### Chapter 4

Duque, T., Schäfer, R. B., & Entling, M. H. (2023). Which Temperature Matters? Effects of Origin, Rearing and Test Conditions on the Chemical Sensitivity of *Pardosa amentata*. *Journal of Applied Entomology*, In press. <https://doi.org/10.1111/jen.13219>

See Chapter 4 for author contribution statements.

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## Curriculum vitae



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## Education

- 2019 – 2023      **Ph.D. in Natural Sciences.**  
*RPTU - University of Kaiserslautern-Landau.*  
Dissertation: “Chemical sensitivity of spiders and earthworms: effects of ecology, phylogeny and habitat”  
Graduation grade: good (1.7) *cum laude*
- 2016 – 2019      **Master of science in Ecotoxicology.**  
*University of Koblenz-Landau.*  
Master Thesis: “Direct & indirect effects of the herbicide diuron on a periphyton-grazer-system”  
Graduation grade: good (1.6)
- 2007 - 2012      **Bachelor degree in Environmental Engineering.**  
*Universidad Santo Tomás de Aquino. Bogotá, Colombia.*  
Bachelor Thesis: “Correlation between automatic and manual particulate matter samplers at the IDEAM air quality station”  
Graduation grade: 4.03 on a 5.0 scale (German equivalent 2.3)

---

## Relevant Work Experience

- September 2023 – Present      **Chemservice GmbH**  
Regulatory affairs advisor for chemicals regulatory compliance. Support on ecotoxicological and environmental assessments
- April 2019 – June 2019      **EUROFINS AGROSCIENCE GmbH**  
Student assistant for earthworm field studies in soil biology / ecotoxicology

April 2018 – May 2018

**INSTITUT FÜR GEWÄSSERSCHUTZ  
MESOCOSMS GmbH**

Internship: Support in aquatic and terrestrial ecotoxicology investigations

August 2015 – December 2015

**WCO S.A.S – Antioquia, Colombia**

Environmental and social management in the construction of “Parque Educativo Los Manglares” in the city Arboletes, Antioquia, Colombia

November 2014 – January 2015

**ANTEA GROUP COLOMBIA S.A.S – Bogotá, Colombia**

Environmental studies: Support in the development and analysis of geographic information systems

October 2013 – October 2014

**MCS CONSULTORÍA S.A.S – Bogotá, Colombia**

Environmental studies: Support and reports of noise and air quality monitoring

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## ***Scientific Publications***

### 2023

**Duque, T., Schäfer, R. B., & Entling, M. H. (2023).** Which temperature matters? Effects of origin, rearing and test conditions on the chemical sensitivity of *Pardosa amentata*. *Journal of Applied Entomology*. In press, <https://doi.org/10.1111/jen.13219>

**Duque, T., Chowdhury, S., Isaia, M., Pekár, S., Riess, K., Scherf, G., Schäfer, R. B., & Entling, M. H. (2023).** Sensitivity of spiders from different ecosystems to lambda-cyhalothrin: effects of phylogeny and climate. *Pest Management Science*. In press. <https://doi.org/10.1002/ps.7818>

**Duque, T., Nuriyev, R., Römbke, J., Schäfer, R. B., & Entling, M. H. (2023).** Variation in the Chemical Sensitivity of Earthworms from Field Populations to Imidacloprid and Copper. *Environmental Toxicology and Chemistry*. 42(4). 939-947. <https://doi.org/10.1002/etc.5589>

### 2021

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### 2022

**Duque, T.**, Chowdhury, S., Scherf, G., Pékar, S., Isaia, M., Schäfer, R. B., & Entling, M. H. (2022). Variation in pesticide sensitivity of twenty-eight European spiders. SFE2, GfÖ & EEF Joint Meeting, International Conference on Ecological Sciences 2022, Metz, France. (Onsite oral presentation)

**Duque, T.**, Nuriyev, R., Römbke, J., Schäfer, R. B., & Entling, M. H. (2022). Chemical sensitivity of earthworms: effects of morphology, phylogeny and habitat. Society of Environmental Toxicology and Chemistry Europe 32nd Annual Meeting 2022, Copenhagen, Denmark. (Onsite oral presentation)

### 2021

**Duque, T.**, Chowdhury, S., Scherf, G., Schäfer, R. B., & Entling, M. H. (2021). Chemical sensitivity of spiders: effects of morphology, phylogeny and habitat. American Arachnological Society 2021. (Online poster presentation)

**Declaration according to §8 of the Promotionsordnung des Fachbereichs 7:  
Natur- und Umweltwissenschaften der Rheinland-Pfälzische Technische  
Universität Kaiserslautern-Landau, Campus Landau vom 14.06.2013 i.d.F. vom  
19.08.2014**

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Landau, 13<sup>th</sup> of July 2023



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Tomás Duque