

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

Tomás Duque,<sup>a\*</sup> Sumaiya Chowdhury,<sup>a</sup> Marco Isaia,<sup>b</sup> Stano Pekár,<sup>c</sup> Kai Riess,<sup>a</sup> Gregor Scherf,<sup>a</sup> Ralf B Schäfer<sup>a</sup> and Martin H Entling<sup>a</sup>



## 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

\* Correspondence to: T Duque, iES Landau, Institute for Environmental Sciences, RPTU University of Kaiserslautern-Landau, Fortstraße 7, 76829, Landau, Germany. E-mail: [duque@uni-landau.de](mailto:duque@uni-landau.de)

a iES Landau, Institute for Environmental Sciences, RPTU University of Kaiserslautern-Landau, Landau, Germany

b Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy

c Department of Botany and Zoology, Faculty of Sciences, Masaryk University, Brno, Czech Republic

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 <sup>-2</sup> ) |
|-------------|------------------------------|----------------|------------|---------------|-------------------|----------|-------------------------------|---------------------------------------|
|             |                              |                |            |               | 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.

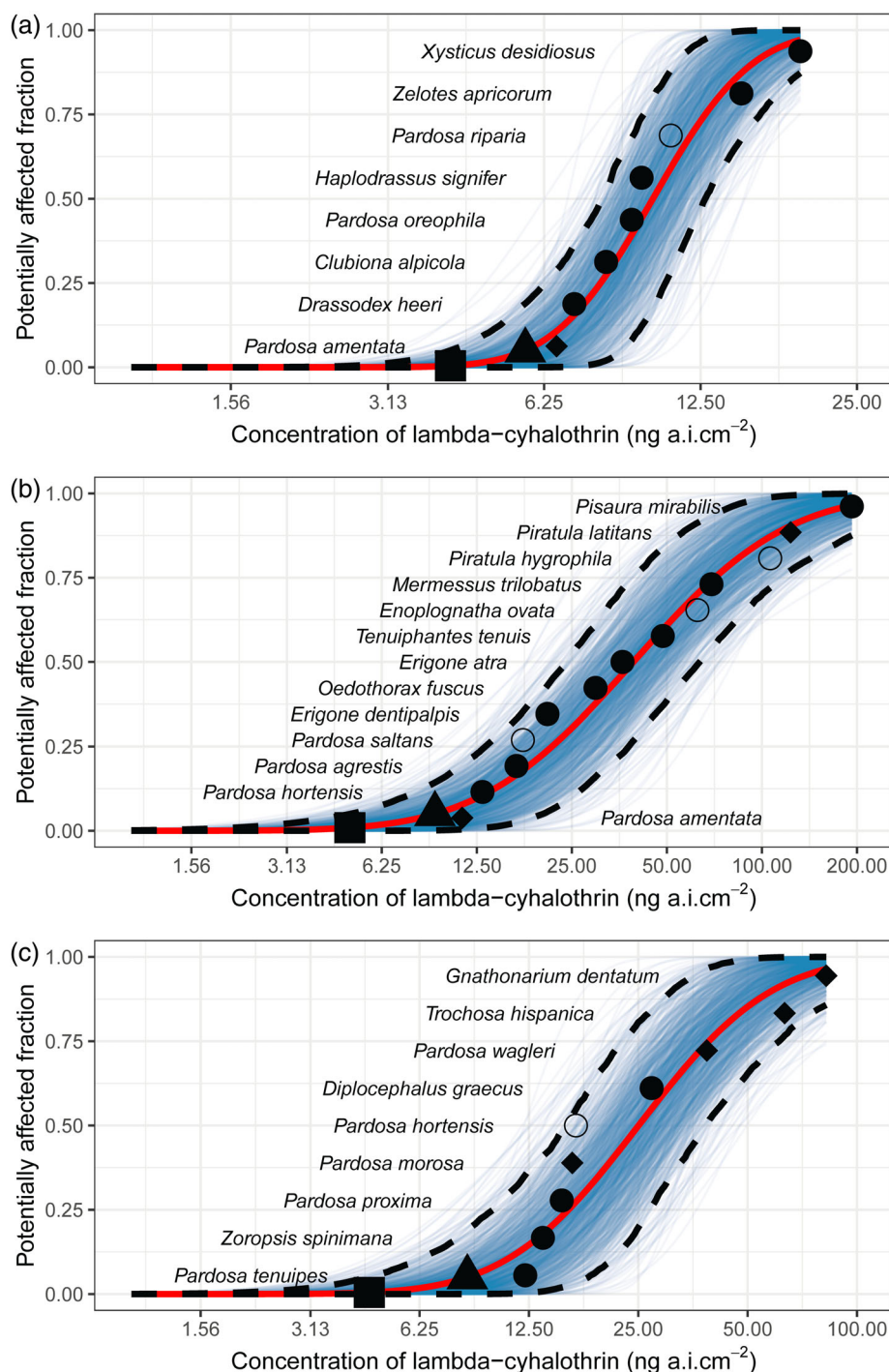
<sup>a</sup> Calculated with the average female body length (max + min/2) from the identification keys of Nentwig *et al.*<sup>36</sup> and Roberts.<sup>35</sup>

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<sup>-2</sup>; 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<sup>-2</sup>; 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 concentration ( $LC_{50}$ ) values of spider species are represented by habitats: open space (black 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>

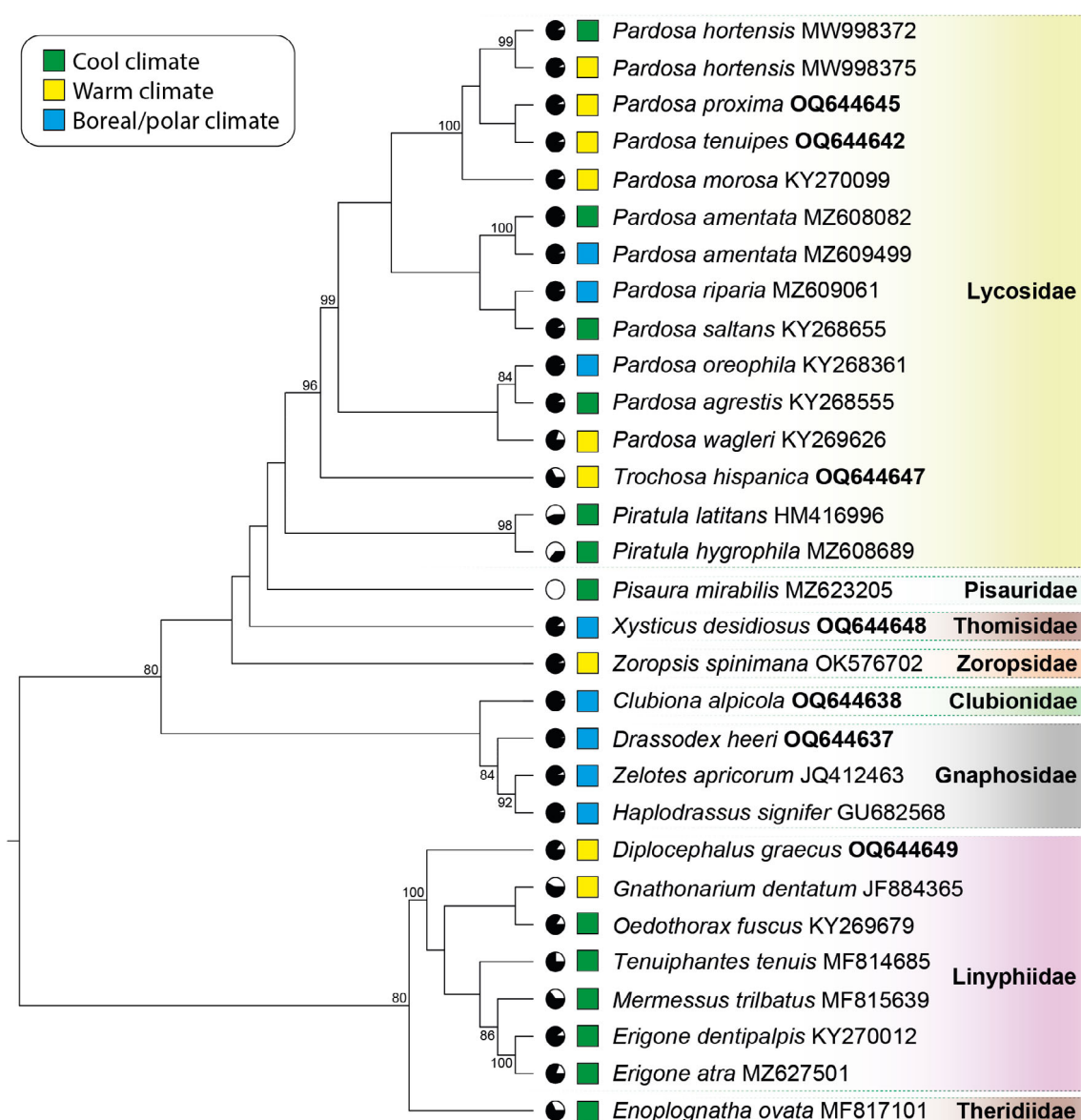
## 2.5 Data analysis

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 ( $LC_{50}$  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  $LC_{50}$  values ranged from 6.6 to 19.5 ng a.i.  $cm^{-2}$  for boreal + polar climate species, from 11.2 to 192.6 ng a.i.  $cm^{-2}$  for cool temperate species, and from 12.2 to 82.3 ng a.i.  $cm^{-2}$  for warm temperate species (Fig. 1; Supporting Information, Table S5). Spider  $HC_5$  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.  $cm^{-2}$ , 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 *Piratula* 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 *Piratula* 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>

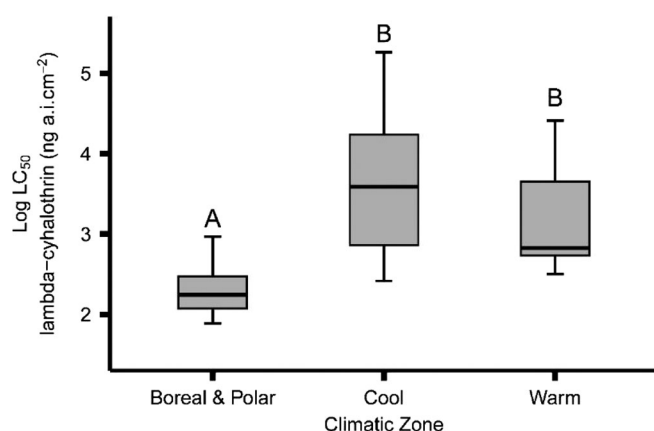
As hypothesized, our results showed that spider sensitivity varied with the climate from which spiders originated (Fig. 3; Table 2). Although 62% of the collected species are widely

**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 ( $LC_{50}$ lambda-cyhalothrin) |          |                  |
|---------------|-------------------------------------|----------|------------------|
|               | df                                  | $\chi^2$ | 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:  $LC_{50}$ , 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 rhopalosiphii* (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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