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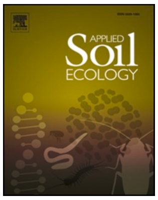

High sensitivity to dietary imidacloprid exposure in early life stages of *Folsomia quadrioculata* (Collembola) populations from contrasting climates

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ABSTRACT

Survival of early life stages is critical for population growth. Understanding the vulnerability of these sensitive stages can therefore improve ecological risk predictions. The globally-used neonicotinoid insecticide imidacloprid does not degrade easily in soil and occurs at concentrations potentially toxic to non-target soil organisms, such as Collembola. Here, we studied the effects of dietary exposure to imidacloprid on the survival of juveniles in an arctic and a temperate population of *Folsomia quadrioculata* (Collembola), a commonly-occurring litter-dwelling species in the Northern Hemisphere. The exposure lasted from <24 h to 30 days since hatching at 15 °C. We assessed whether (1) the arctic population of the test species with faster development rates could be more vulnerable than the temperate population, (2) cumulative mortality could increase substantially with exposure time, and (3) the mortality risk experienced during the juvenile stage could be greater than that experienced by the adults. We found a strong concentration-dependent decline in survival in both populations, with small but significantly different LC50s, despite large differences in multiple life-history traits. Applying a General Unified Threshold model for Survival (GUTS) showed a low threshold for effects, slow damage dynamics, a 6–11-fold decrease in LC50 from 14 to 30 days of exposure, and saturation of damage at the three highest concentrations. In addition, exposure starting soon after hatching caused much more mortality than that starting in the adult stages. Our findings suggest that toxicity tests not emphasizing the neonate stages or not lasting long enough can underestimate the implications for populations of naturally abundant non-target organisms.

1. Introduction

Survival of the vulnerable early life stages is pre-requisite for the persistence and growth of natural populations. In most organisms, these stages are sensitive to stress, and exposure of juveniles to anthropogenic chemicals can drastically reduce their ability to survive (Anderson and Harmon-Threatt, 2019; Tran et al., 2020). Intuitively, survival of the early life stages should represent an important aspect of the risk assessment of chemicals such as insecticides (Fountain and Hopkin, 2001). Standard toxicity tests for soil ecosystems generally recruit juveniles close to maturation (OECD, 2009) but do not specifically measure the performance of the presumably more vulnerable life stages, such as the newly-hatched individuals of terrestrial and soil arthropods.

Neonicotinoids, such as imidacloprid, are popular insecticides used globally as sprays and seed dressings (Douglas and Tooker, 2015; Main et al., 2018; Singh and Leppanen, 2020). Most of the applied volume of these insecticides reach soil, resulting in adverse effects on non-target organisms (e.g., Anderson and Harmon-Threatt, 2019). Neonicotinoids are neurotoxicants that target the nicotinic acetylcholine receptors of insects. However, degradation rates of these photosensitive insecticides are much slower in soil than in the aquatic phase, which has resulted in increasing levels of neonicotinoid residues in agricultural soils over time (Silva et al., 2019). The neonicotinoid imidacloprid is widely used globally (Overton et al., 2021), although its outdoor use is regulated in Europe (Wood and Goulson, 2017); nation- and season-specific exceptions are made, and it is also used in other sectors such as indoors, for pet de-licing, aquaculture, and greenhouses. The environmental footprint of indoor use of imidacloprid, however, needs further study (but see

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Herbertsson et al., 2021). Imidacloprid exposure of important non-target soil organisms therefore attracts wide attention (see Wood and Goulson, 2017).

Collembolans (springtails) play an important role in litter decomposition and nutrient recycling in soil (Lavelle and Spain, 2001; Potapov et al., 2020; Rusek, 1998). Exposure of collembolans to imidacloprid in soil can occur by the uptake of soil pore water through the ventral tube (Ogungbemi and van Gestel, 2018) as well as through the ingestion contaminated organic matter (Potapov et al., 2016, 2019; Seastedt, 1984). Effects of the latter have remained poorly studied in collembolans. Moreover, the collembolan model used in standard toxicity tests — *Folsomia candida* Willem, 1902 (e.g., guideline 232, OECD, 2009) — has been widely studied only in soil-exposure systems, focusing on adult specimens (de Lima e Silva et al., 2021; Idinger, 2002) or 10–12-day old juveniles, which can be much more robust than hatchlings (Alves et al., 2014; Bandeira et al., 2019; de Lima e Silva et al., 2020; Ogungbemi and van Gestel, 2018; van Gestel et al., 2017). Information on the susceptibility of newly hatched collembolans is lacking and that on variation in sensitivity among the life stages is limited (but see Guimarães et al., 2019), largely because soil exposure methods do not allow continuous observation of life events in soil-dwelling collembolans.

In addition to the life stage exposed, adaptation to environmental conditions, such as climate variability, affects responses to anthropogenic stressors like insecticides. Accordingly, conspecific arthropod populations that develop and grow faster may be more susceptible to toxicants, because more metabolic resources are channelized toward maintaining the rapid growth and development, thus limiting the resources available for activating or speeding up detoxifying mechanisms (Debecker and Stoks, 2019; Dinh et al., 2014). However, the relationships of adaptive variation, e.g., in growth and development, among conspecific populations with toxicant exposure effects have remained a poorly-addressed area of ecological risk assessments in general, and soil organisms in particular (Awuah et al., 2020; Bach et al., 2020).

Folsomia quadrioculata Tullberg, 1871 is a Collembola species occurring abundantly across a wide geographical range spanning across the Northern Hemisphere. This species is easy to handle in the laboratory, and in contrast to *F. candida*, reproduces sexually. Previous studies on life-history strategies and thermal adaptation across several arctic and temperate populations have shown that the juvenile life-history traits are strongly affected by temperature, with large variation among populations both within and across climate regions. Thus, a high arctic (Little Slate Island) and a temperate population (Ås) from Norway showed large differences under common garden conditions — the arctic population had a shorter egg development time, attained reproductive maturity earlier, and showed higher fecundity than the temperate population (Sengupta et al., 2016, 2017; differences summarized in Section 2.1.1). The faster development rates in the arctic population could be an adaptation for coping with time constraints imposed by the short and cool growing seasons characterized by stochastic spells of sunny weather (and warmer soil temperatures) at the high-arctic site (Sengupta et al., 2016, 2017), which is a life-history strategy often observed in arctic arthropods (Danks, 1999, 2004; Escribano-Álvarez et al., 2021). Furthermore, a short-term 14-day dietary exposure of adult *F. quadrioculata* to a range of imidacloprid concentrations showed strong sublethal effects on egg production and a potential for recovery in this trait during the depuration phase following the exposure (Sengupta et al., 2021). These findings emphasized that, for improving the realism of population-level predictions, studies on the effects of initiating the exposure during the neonate (hatchling) stages and the consequences of longer exposure periods are needed.

We studied the juvenile life stages of an arctic and a temperate population of *F. quadrioculata* for the effects of a dietary imidacloprid concentration range, which was previously found to be sublethal for the adults (Sengupta et al., 2021). We compared the two populations from contrasting climates (arctic and temperate) and expected that the faster-developing arctic population from the presumably pristine site would be more susceptible to imidacloprid exposure. In addition, we expected that cumulative mortality would increase with exposure duration, leading to several-fold decrease in median lethal concentrations (LC50), with a consistent magnitude of difference between the populations over time. Moreover, we expected that for both the populations, the juveniles exposed since hatching would show greater mortality than that previously reported for the adults. We discuss the importance of the potential effects of dietary exposure on population growth.

2. Material and methods

2.1. Study organisms and cultures

2.1.1. Sampling sites and life-history traits

Folsomia quadrioculata was extracted from randomly-sampled soil cores collected from an arctic and a temperate site. Taxonomic keys of Fjellberg (1994) were used for species identification. The arctic site is a presumably highly pristine small-sized island in northern Svalbard, Little Slate Island (80.321°N, 20.360°E; sampled in 2007). The climate in this barren, wind-exposed island is characterized by harsh cold winters and summer conditions with great variation among years in terms of precipitation and duration of sunshine. This results in highly stochastic conditions between years during the short growing season. Under field conditions, this arctic population takes two summers to complete one generation (Birkemoe and Sømme, 1998).

The temperate population (Ås) was sampled from an agricultural field situated 35 km from Oslo, Norway (59.659°N; 10.754°E; sampled in 2010). Cultivation and pesticide application had been discontinued at this site several years before sampling, and it is unknown whether neonicotinoids had been applied at this site earlier. This site experiences a cold temperate climate, where the ground remains snow-covered and frozen for several months and summers are fairly warm, with mean temperatures from June–August of 15.3 °C, compared to 2.2 °C at the arctic site (Sengupta et al., 2016). The climate conditions are more stable at the temperate than at the arctic site. The field is covered by grass vegetation, and during sunny spells, the ground temperature shows greater fluctuation than air temperatures. This population has a one-year life cycle (Leinaas, 1978).

In previous common garden studies, these two focal populations showed large differences in life-history traits. The arctic population had a shorter egg development time (by approx. 2 days [9 %]), attained reproductive maturity approx. 16 days (30 %) earlier, and showed higher fecundity (by approx. 11 eggs per individual [90 %] at the age of 84 days) than the temperate population from Ås (Sengupta et al., 2016, 2017). Taken together, these differences suggest a faster life cycle of the arctic population than the temperate population under common garden conditions.

2.1.2. Stock cultures

Stock cultures of the collembolans were established using 100–200 animals from 5 to 10 soil cores. For minimizing the possibility of genetic drift, large stocks comprising 500–1000 animals were maintained in 5–10 cultures with intermixing 2–3 times per year. Excess animals were removed into fresh culture boxes once every 2–3 months (Sengupta et al., 2016, 2017). Before starting the experiments, we checked for effects of long-term culturing on the two populations and found no effects (see Section A.1 and Table A.1 in the Supplementary Information).

2.1.3. Culturing conditions

The animals (both stock cultures and experimental replicates) were cultured in plastic boxes (diameter 3.4 cm, height 3 cm) with a base made of plaster of Paris and charcoal, at 15 °C in climate cabinets (Sanyo MIR 553, Osaka, Japan), which is the optimal temperature for rearing this species (Sengupta et al., 2016, 2017). Temperature conditions were monitored constantly using HOBO data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA). The plaster was kept moist by adding droplets of distilled water every week to maintain high relative humidity in the culture boxes (close to saturation). To mimic the light regime of the growing season in the high arctic site, we used a 24:0 photoperiod. Food (small pieces of bark covered with crusts of cyanobacteria) was provided ad libitum to the stock cultures, whereas the feeding regime of the experimental replicates are described in Section 2.2.

2.2. Spiking of food

Branches of trees with dark crusts of cyanobacteria collected from the campus of the University of Oslo were defaunated overnight at −70 °C. After attainment of room temperature and air drying, the crusts covering the bark were scraped off, and the fine powdery material obtained was sieved through a 250-μm stainless steel mesh and soaked in imidacloprid solutions (CAS Number 138261-41-3, Sigma-Aldrich, St. Louis, USA; 0, 42.9, 128.7, 429, 1287, and 4290 μg L^{-1}; 3.5 g food soaked in 100 mL spiking solution) overnight, in the dark at 4 °C. Subsequently, the food was filtered out onto polypropylene membranes, dried in the dark for 3 days, and stored in the dark before being provided to the collembolans (Sengupta et al., 2021). Dietary exposure effects of imidacloprid to the hatchlings of *F. quadrioculata* have not been studied earlier, but this spiking method was used for exposing the adults of the collembolan *Hypogastrura viatica* Tullberg, 1872 (Kristiansen et al., 2021) and *F. quadrioculata* (Sengupta et al., 2021). The concentration range, based on Sengupta et al. (2021), was sublethal to adult *F. quadrioculata*. A part of the food was preserved under dark conditions at −20 °C until chemical analysis. Imidacloprid concentration in the food was measured by high-performance liquid chromatography-mass spectroscopy (details in Section A.2 in Supplementary information; Sengupta et al., 2021).

2.3. Exposure and data acquisition

Hatchlings of the focal populations, collected within 24 h of hatching, were exposed to imidacloprid via the diet at 15 °C, which is the optimal temperature for this species. The exposure lasted for 30 days, which covers most of the juvenile life stage at 15 °C for both populations. Twenty randomly-selected hatchlings of *F. quadrioculata* were added to each culture box (henceforth, these culture boxes, which serve as experimental units, are referred to as “replicates”). Experimental replicates were fed with spiked or control food (see Section 2.2 for the method of spiking). Four milligrams of food were provided to each replicate twice a week and distilled water drops were added to keep the plaster substratum moist. The hatchlings were exposed to five imidacloprid concentrations and control for 30 days with four replicates per treatment. Every 3–4 days, dead animals were counted and removed from the replicates while renewing the food. The total number of collembolans alive until the end of the experiment was counted.

2.4. Data treatment

We analyzed the patterns of survival over time and estimated median lethal concentrations (LC50s) at 10, 14, 20, and 30 days by applying the General Unified Threshold model for Survival (GUTS; Jager et al., 2011). The GUTS model explains survival as a function of concentration and time. Therefore, after calibrating the model to a data set, it can extrapolate to other (untested) concentrations and time points (Jager and Ashauer, 2018). These responses were quantified as effects of exposure to imidacloprid concentrations measured in the food. We extended the GUTS model with a saturation module (Bechmann et al., 2019; detailed descriptions of the model in Sections A.3 and A.4 in Supplementary information) to account for a specific observed pattern in the data: a lower-than-expected increase in effect with increasing imidacloprid exposure concentration. This extension introduces one additional parameter to the simple (reduced) GUTS model: a half-saturation concentration (C_K). GUTS is usually applied to data from acute effects studies, in which the animals are not fed, and hence do not grow. Here, the animals grew, which has the potential to affect toxicokinetics, and possible damage dynamics as well. It is possible to extend the GUTS model with feedback mechanisms, as also proposed for toxicokinetic-toxicodynamic analysis of sub-lethal effects (Jager, 2020), such as dilution of the internal concentration by growth. However, in the present study, we did not have observations on body size over time. Furthermore, preliminary testing with various feedback options, using growth curves from previous experiments, did not lead to improved fits. Therefore, we assumed that growth did not substantially affect damage dynamics of imidacloprid in the populations studied.

In principle, population-specific data sets can be fitted separately, with their own sets of parameters. However, since we had five free model parameters, and a data set with limited information content, there is a strong potential to obtain a good fit for the wrong reasons. Even though the effect patterns were quite similar for both populations, fitting them separately led to very different sets of parameter estimates, which seems unrealistic. Preliminary model exploration showed that a very good fit could be obtained by fitting the two populations together, allowing only two parameters to differ: the dominant rate constant (k_d) and the half-saturation concentration (C_K). Likelihood-ratio testing ($\alpha = 0.05$) showed that the resulting fit with seven parameters was not significantly worse than the fit on the two populations separately (involving 10 free parameters), but much better than the fit with all parameters common (involving 5 free parameters). This selection is plausible, as it assumes that both populations have the same intrinsic sensitivity, but differ slightly in their toxicokinetics and/or their damage dynamics. The alternative case (no difference in toxicokinetics and damage dynamics, but a difference in sensitivity) led to a worse fit. Further studies would be needed for elucidating the nature of the between-population differences, but these assumptions are sufficient for capturing the survival patterns over time, and produce reliable estimates for LC50.

The model was implemented in the BYOM framework under Matlab (http://debtox.info/byom.html), in a likelihood framework. The appropriate likelihood function for survival data over time derives from the multinomial distribution (see Jager et al., 2011; Jager, 2021). All statistical inference was based on likelihood-ratio testing, thus comparing two nested models. This comparison uses Wilk's theorem: −2 times the ratio of two likelihood values is assumed to follow a chi-square distribution, with as degrees of freedom the number of free parameters in which both models differ. This approach was used to generate confidence intervals on model parameters and model predictions using likelihood profiling (Kreutz et al., 2013), as well as to compare different model simplifications (see above). In BYOM, we fitted the model using the parameter-space algorithm (Jager, 2021). This algorithm maps parameter space yielding a robust global optimum as well as a sample to be used for the construction of likelihood-based confidence intervals on model parameters and model predictions; note that the LC50 as function of exposure time is a model prediction. Apart from confidence intervals on the LC50s, also prediction profile likelihoods were generated for the difference in LC50 between both populations, at each time point (explained in detail in Section A.3 in Supplementary information). This way, we have a formal likelihood-ratio test for significance of the differences between the populations.

3. Results

3.1. Imidacloprid content of food

The mean imidacloprid concentrations quantified were 0, 1.35, 3.70, 11.8, 40.3, and 121.7 mg kg^{-1} dry food after the food for the collembolans had been spiked with 0, 42.9, 128.7, 429, 1287, and 4290 μg L^{-1} imidacloprid solutions, respectively (Table A.2), and were thus within our targeted range of dietary imidacloprid concentrations. The lowest imidacloprid treatment level was well above the limit of quantification (0.1 ng g^{-1} imidacloprid in dry food). All effects reported in the present study are based on the imidacloprid concentrations measured in the food.

3.2. *Survival*

Survival was high in the controls for both populations (> 95 %), but decreased sharply with an increasing imidacloprid exposure concentration (Fig. 1). Survival at the age of 0–7 d was >95 % in all the treatments for both populations. In the 3.7 mg kg^{-1} treatment, survival started to decrease at the age of 25 d but remained higher than 80 % in both populations until the end of the experiment. However, at concentrations >3.7 mg kg^{-1}, a sharp decline in survival with age was evident in the two populations studied, with <50 % survival at the age of 20 d.

The GUTS model provided a good explanation of the survival data over time ($r^2 > 0.98$; fits are shown in Fig. 1). The fitted parameters for the reduced GUTS model for stochastic death are shown in Table 1. Very similar fits and LC50s were obtained with the alternative individual tolerance model (Fig. A.2; Tables 2, A.3, A.4). The no-effect threshold was estimated at 1.44 mg kg^{-1}. However, due to the saturation applied in the model, this parameter value should not be interpreted as a no-effect level for the concentration in food (discussed in Section 4.3). The low dominant rate constant (k_d) values for both populations (Table 1) reflects rather slow damage dynamics of imidacloprid, which results in the long exposure time until a steady state of damage is reached. From the k_d estimates, we can calculate that it takes 23 days to reach 95 % of steady state for the temperate population, and 50 days for the arctic. These models predicted a 6–11-fold reduction in LC50 values over time between 14 and 30 days of exposure (LC50 values in Table 2). In contrast to our expectations, LC50s were lower in the temperate than in the arctic population during the early stages, but this order was reversed in the latter part of the test (Table 2). The confidence intervals for the LC50s of the two population overlap at each time point. Nevertheless, no significant differences were observed for LC50s at 14 and 20 days, and for the incipient LC50s (i.e., after infinite exposure duration, see Table 2). This discrepancy relates to correlations between the parameters.

Table 1
Model parameters with 95 % likelihood-based confidence intervals of the General Unified Threshold model for Survival (GUTS) for stochastic death used for analyzing the effects of imidacloprid exposure through food on the survival of *Folsomia quadrioculata* juveniles from an arctic and a temperate population. k_d: dominant rate constant, m_w: threshold for effects on mortality, h_b: background hazard rate, b_w: effect strength (killing rate), and C_K: half-saturation constant.

Parameter	Temperate	Arctic	Units
k_d	0.128 (0.103, 0.155)	0.0591 (0.0363, 0.114)	d^{-1}
m_w[a]	1.44 (1.26, 1.63)		mg kg^{-1}
h_b[a]	0.00123 (0.000754, 0.00190)		d^{-1}
b_w[a]	0.275 (0.213, 0.455)		kg mg^{-1} d^{-1}
C_K	2.71 (2.03, 3.49)	4.26 (2.23, 6.70)	mg kg^{-1}

[a] Common parameters for the two populations.

Table 2
Median lethal concentrations (LC50) in mg kg^{-1} dry food with 95 % likelihood-based confidence intervals estimated using a GUTS model for analyzing the imidacloprid effects on juvenile *Folsomia quadrioculata*. The corresponding survival patterns are shown in Fig. 1. All values are based on imidacloprid content measured in the food. Asterisks mark the time points at which the LC50 for the arctic population differs significantly from the temperate population (α = 0.05).

Time (d)	Temperate population	Arctic population
14	27.5 (21.4, 38.5)	49.5 (35.7, 76.7)*
20	7.77 (7.26, 8.43)	9.02 (8.01, 10.6)*
30	4.72 (4.42, 5.06)	4.54 (4.33, 5.15)
Infinite	3.08 (2.75, 3.36)	2.18 (1.79, 2.97)*

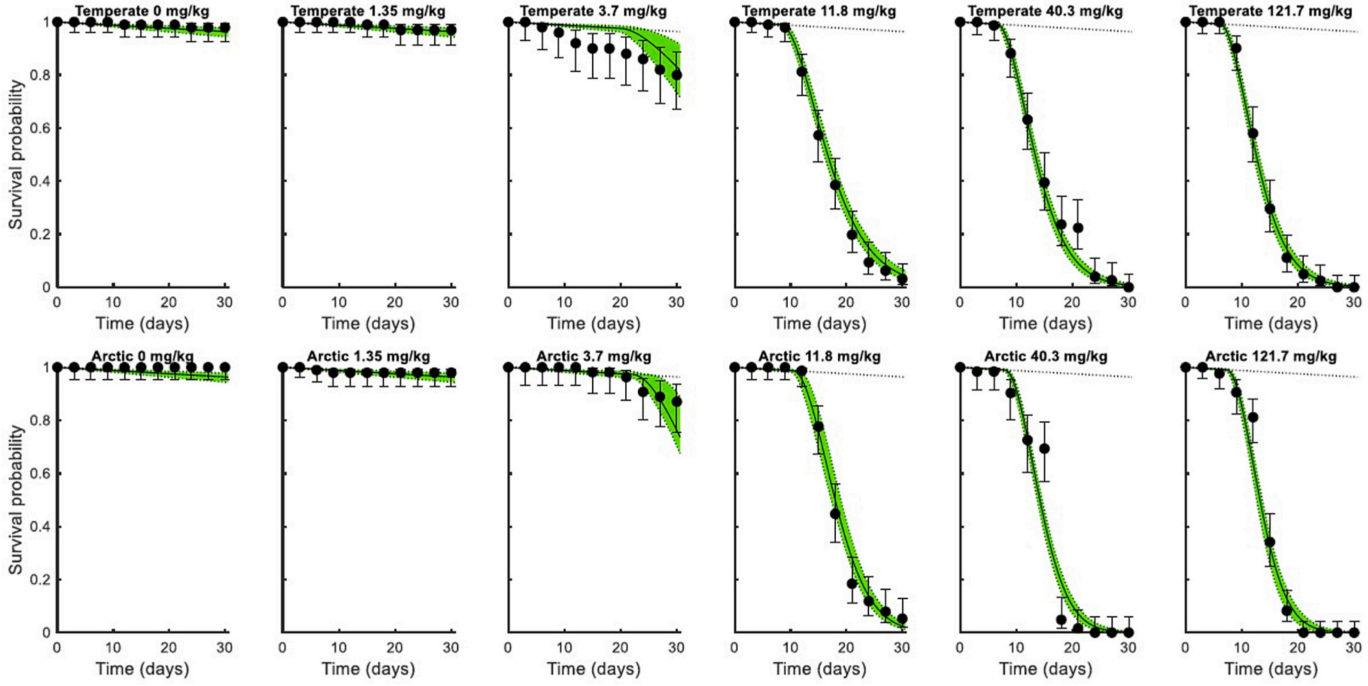

Fig. 1. Survival probability over time of juvenile *Folsomia quadrioculata* from two populations as a result of continuous exposure to imidacloprid through the diet. The points are the observed survival probability and error bars represent Wilson score intervals. The black solid line and green-shaded area represent the model prediction and likelihood-based confidence intervals, respectively, from a GUTS model for stochastic death. The black dotted lines represent survival probability in the control predicted by the model.

4. Discussion

Within-species variation in the response of soil organisms to toxicant challenges, and how these responses are affected by differential adaptation to the ambient environment, needs to be better understood (Bach et al., 2020). Contrary to our expectations, the present study on dietary imidacloprid exposure in arctic and temperate Collembola populations resulted in small but significant differences in LC50 values between the two focal populations. Moreover, there was a tendency for the slower-developing temperate population to be more susceptible than the arctic population, at least for the first 20 days of exposure. Our hypothesis assuming that the faster-developing arctic population would be more susceptible than the temperate was thus not supported by the present comparison, particularly by the similar 30-day LC50 estimates of the populations. The generally small (but significant) differences in LC50s of the two populations were unexpected, as it indicates no clear links of evolutionary and physiological constraints arising from their large differences in thermal adaptation (specified in Section 2.1) on tolerance to a stressor (the neonicotinoid imidacloprid).

4.1. Difference in susceptibility between populations

Imidacloprid concentration-dependent declines in survival over time were observed in both the populations. The response of the arctic population to low imidacloprid concentrations (LC50 at infinite time in Table 2) is not surprising. This population from a remote arctic island is unlikely to have ever been exposed to the insecticide imidacloprid. The distance from the temperate population and the geographical barriers suggest only the likelihood of reproductive isolation and strong between-population genetic differences in responses to toxicant challenges. However, the higher susceptibility of the temperate population during the first 20 days of exposure contrasted the expectations based on differences between the focal populations for important life-history traits (specified in Section 2.1.1), which was surprising. Nevertheless, the lack of evolution of insecticide tolerance has been reported in populations sampled from agricultural areas, evidenced by the lack of congruence of insecticide sensitivity with gradients of application of neonicotinoids in other arthropod taxa (Rackliffe and Hoverman, 2020), in addition to a collembolan (Arthur et al., 2020). Lower tolerance than expectations based on historical exposure events are possible if, for instance, the site of insecticide application can be recolonized by populations with low insecticide resistance that survive in surrounding uncontaminated microhabitats (Rackliffe and Hoverman, 2020; Shahid et al., 2018). Although the ability of *F. quadrioculata* to colonize habitats (Hågvar, 2010) and to withstand changes in microhabitat conditions (Krab et al., 2010) are known, the role of recolonization and gene flow in shaping the imidacloprid tolerance/susceptibility in the field is unknown. Presently, the limited understanding of dispersal patterns of collembolans and other soil mesofauna (Potapov et al., 2020) constrains further predictions regarding the roles of these processes in shaping insecticide tolerance.

4.2. Contribution of the dietary exposure route to the total toxic effects in natural intact soil

The present dietary imidacloprid exposure starting with hatchlings of *F. quadrioculata* resulted in lesser toxicity (i.e., higher LC50 values) than expectations based on several types of soil exposure methods. *F. quadrioculata* adults exposed to imidacloprid in single-species soil microcosms (intact field-collected soil cores) under the same laboratory conditions as the present study showed 28-day LC50 values of approximately 0.2 mg kg^{-1} dry soil (Sengupta et al., unpublished results), consistent with the findings of a mesocosm-based soil community study under field conditions at the sampling site of our temperate population in Ås, Norway (Konestabo et al., 2022). In line with the present findings, Kristiansen et al. (2021), by simultaneously applying the same dietary method of imidacloprid exposure alongside exposure via a standard test soil in adult *H. viatica*, found the former exposure method less effective. Studies comparing dietary and soil exposure effects of insecticides are scarce; however, some studies focusing on other types of contaminants such as metals also indicate a lower efficacy of dietary exposure (Bruus Pedersen et al., 2000; Fountain and Hopkin, 2001). A plausible cause of incongruence between the effects of dietary and soil exposure may be that the diffusion of imidacloprid-containing pore water through the ventral tube is a major route of exposing collembolans in soil (Ogungbemi and van Gestel, 2018). Furthermore, in line with arguments presented in the context of exposure to metals (e.g., Fountain and Hopkin, 2001), food avoidance can reduce imidacloprid uptake during dietary exposure, whereas in soil exposure systems, exposure through soil pore water can continue even if feeding rates decrease (Kristiansen et al., 2021). Nonetheless, owing to the multiple exposure routes encountered by collembolans in natural intact soil, the relatively low toxicity indicated by dietary toxicity tests, especially those involving highly water-soluble chemicals such as imidacloprid, illustrates the contribution of this exposure route to the total exposure effects in field soil.

4.3. Response to imidacloprid exposure over time

Our hypothesis predicting a concentration-dependent increase in cumulative mortality with exposure duration was supported by a time-dependent decrease in LC50 values. Therefore, LC50s determined at a fixed exposure time are a poor measure of the susceptibility of the organisms, and are difficult to compare between populations, species, and test conditions. A more meaningful measure of susceptibility is the threshold for effects, which can be estimated from the GUTS model. In most cases, the threshold parameter in the model (m_w) is a true no-effect level, and equals the incipient LC50. However, since we extended the model with a saturation module, we needed to consider the half-saturation constant (C_K) as well. We estimated the no-effect thresholds for the temperate and arctic population as 3.1 and 2.2 mg kg^{-1} dry food, respectively (the LC50 estimate for infinite exposure time in Table 2). Thus, after prolonged imidacloprid exposure, the arctic population would be slightly more susceptible than the temperate population. As this GUTS analysis simultaneously uses the information in the entire data set (both populations, all treatments, and all time points), and explains the effect patterns over time with only seven parameters, it is more efficient than repeated fitting of dose-response curves for each time point. Furthermore, GUTS analysis allows for meaningful mechanistic interpretation of the model parameters, whereas concentration-response models can only provide a description. Nevertheless, as already explained in Section 2.4, the data were limited with respect to fitting the GUTS model with the saturation extension. We needed to take several parameters common to both populations to obtain meaningful fits. However, these difficulties did not affect the LC50s (Table 2), but they do limit the ability to extract mechanistic insights about the nature of potential sensitivity differences between the populations studied. The current analysis suggests that the populations differ with respect to their damage dynamics, although more detailed experimental work is needed to thoroughly test this finding.

4.4. Importance of the life stage exposed

The higher susceptibility to imidacloprid of the juveniles, from hatching until 30 days, than of the adults of *F. quadrioculata* using the same dietary exposure method (Sengupta et al., 2021) supports our hypothesis that the juveniles would encounter greater mortality risks than the adults. The adult specimens in Sengupta et al. (2021) had >90 % survival during a 14-d exposure to 290 mg kg^{-1} imidacloprid in dry food. By contrast, the 14-d LC50 for the juvenile animals corresponded to 6–11-fold lower concentrations (Table 2). This finding is consistent with that of a cadmium-exposure study showing that *F. candida* juveniles are more sensitive than adults or eggs (Guimarães et al., 2019). Higher sensitivity of the immature life stages to imidacloprid and other neonicotinoids has been reported in other arthropods, including several-fold lower lethal imidacloprid doses to newly-emerged worker honey bees as compared with mature worker honey bees (Baines et al., 2017). Similarly, greater susceptibility of younger life stages to toxicity challenges has been reported in other classes of toxicants, e.g., chlorpyrifos exposure in *Culex pipiens* (Tran et al., 2020). These results across taxa imply the need of ensuring the inclusion of sensitive life stages in future risk assessments.

4.5. Temporal trends and the role of damage dynamics

The observed time course of imidacloprid-induced mortality suggests that short-term tests for assessing the risks posed by imidacloprid to *F. quadrioculata* populations would underestimate chronic sublethal toxic effects. The decrease in LC50 of *F. quadrioculata* juveniles over time (since hatching) may be partly explained by the strong binding affinity of imidacloprid to nicotinic acetylcholine receptors (Abbink, 1991; Ware and Whitacre, 2004). We found a later-than-expected attainment of a steady state in the extent of damage caused by imidacloprid, which is reflected by the slow damage dynamics (low values for k_d). Previously, slow damage dynamics linked to imidacloprid exposure in *Daphnia magna* identified reduced feeding as a possible underlying cause (Agatz et al., 2013). In collembolans, imidacloprid exposure impairs mobility (Ogungbemi and van Gestel, 2018), which can lead to inefficient food acquisition (Kristiansen et al., 2021). Reduced feeding upon imidacloprid exposure may limit the imidacloprid uptake (Kristiansen et al., 2021), thus explaining the observed saturation of the damage dynamics: the three highest exposure treatments showed only very little increase in effect. However, this saturation would likely not be relevant if the collembolans are exposed through soil pore water, as explained above in Section 4.2. Accordingly, for avoiding the risks of underestimating chronic exposure effects owing to slow damage dynamics and damage saturation, the duration of toxicity tests should be selected carefully.

4.6. Implications for safety factors risk assessments

Previous studies on arthropods with high sensitivity to neonicotinoids report that a 10-fold safety factor around estimated toxicity values could account for population-level differences in sensitivity (see Rackliffe and Hoverman, 2020 and references therein). However, the large difference in survival between the juvenile and adult *F. quadrioculata* (specified in Section 4.4) and the time-dependent decrease in LC50 values (Section 4.3) suggest that the application of safety factors in environmental risk assessments should be done with great caution. Additionally, the present findings highlight the importance of comparing several populations with known life histories for suggesting a suitable buffer around the predictions regarding population growth and persistence across wide geographical regions.

5. Conclusions

The time course of toxicity emphasizes that toxicity tests not lasting sufficiently long can underestimate chronic exposure risks for recruitment, population persistence, and population growth. Overall, the effects of imidacloprid on juvenile life stages over time may explain severe population depletions under realistic exposure scenarios in field conditions. Our findings show that early life stages of both temperate and arctic *F. quadrioculata* populations are highly sensitive to imidacloprid, suggesting ecological consequences at a large geographical scale. Whereas the extrapolation from mechanistic, laboratory-based observations to ecological consequences under field conditions may be complicated by the influence of interacting drivers and stressors, environmental stochasticity and heterogeneity, and biotic factors, our results are essential for demographic modeling and field studies aimed at a holistic assessment of population-level risks.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.104880.

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