Environ Monit Assess (2025) 197:1260
https://doi.org/10.1007/s10661-025-14741-9

RESEARCH

Are cover crops a potential threat for pollinators due to clothianidin residues in floral resources?

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Received: 9 August 2025 / Accepted: 20 October 2025

Abstract Cover crops are now mandatory in areas at risk of nitrogen leaching into groundwater. Many late-flowering entomophilous species used in these cover crops provide critical floral resources (pollen and nectar) for pollinators in early autumn. However, pesticide residues such as neonicotinoids can transfer to cover plants and their floral resources, posing a potential threat to pollinators. We investigated the transfer of clothianidin, a neonicotinoid insecticide, from soil to plants and floral resources in three common cover species (phacelia, white mustard, and faba bean). The study was conducted both in fields conditions (three years after clothianidin treatment on sugar beet) and under controlled growth chamber conditions (74 days post-treatment). We analysed clothianidin concentrations in soils, vegetative parts (stems and leaves), flowers, pollen, and fruits. Our results demonstrated that clothianidin persisted across the soil–plant–floral resource continuum in both field (soil concentration of 2.79 ng g^{-1}) and growth chamber (soil concentrations of 34.4–106 ng g^{-1}) experiments. Clothianidin residues accumulated in floral resources (ranging from 0.55 to 66.0 ng g^{-1} in pollen) posed a potential risk to pollinators through pollen consumption exposure (hazard quotient close to 1), and an even greater risk through soil contact exposure (hazard quotient > 1, reaching up to several hundred). These findings highlight that neonicotinoid residues in soil, and to a lesser extent in cover crop pollen, may threaten pollinator health.

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Published online: 27 October 2025

Keywords Pollinator exposure · Neonicotinoids · Pesticide residues · Ecotoxicological risk · Bee health

Introduction

The European Union (EU) is the world's leading producer of beet sugar, accounting for approximately 50% of the global production, including 4.55 megatonnes annually in Belgium (FAO, 2022; Statista, 2022). Like other intensively cultivated crops, sugar beet is vulnerable to pests and pathogens. Among the most damaging diseases, the beet yellows virus, transmitted by aphids, has particularly severe effects on yield (Dewar & Qi, 2021; Francis et al., 2022; Hossain et al., 2021). Prophylactic control typically involves insecticidal seed treatments, particularly neonicotinoids (Hauer et al., 2017; Jeschke et al., 2011). Clothianidin, introduced in 2001 and one of the breakdown products of thiamethoxam, has been widely used due to its efficacy against a broad spectrum of insect pests (Jiang et al., 2018).

However, neonicotinoid residues have been detected in 7% of European agricultural soils at concentrations ranging from < 1 to 60 ng g^{-1} (Silva et al., 2019). Their presence depends on their soil half-lives spanning from 191 days for imidacloprid to 545 days for clothianidin (Alsafran et al., 2022; Lewis et al., 2016). The high water solubility of neonicotinoids facilitates root uptake and systemic distribution throughout plant tissues (Bredeson & Lundgren, 2018; Zhang et al., 2023). Consequently, these compounds have been detected in various plant species and their floral resources (nectar and pollen), both in treated crops and wild plants near treated fields, indicating potential transfer to untreated plants (Botías et al., 2016; Krupke et al., 2012; Viric Gasparic et al., 2020; Zhang et al., 2023; Zioga et al., 2020). Such contamination exposes beneficial non-target organisms, with documented direct impacts of neonicotinoids on both invertebrates and vertebrates in terrestrial and aquatic ecosystems (Klatt et al., 2023; Willis Chan & Raine, 2021; Willis Chan et al., 2019), threatening both biodiversity and associated ecosystem services (Humann-Guilleminot et al., 2019). In response, the EU restricted the use of three neonicotinoids—clothianidin, imidacloprid, and thiamethoxam—as seed coatings starting December 1, 2013, with further restrictions in 2018 (CE 1107/2009/EU 2018/784). However, derogations have permitted limited use for sugar beet seed coatings in Belgium (2019–2022) and France (2020–2023), accompanied by a five-year restriction on cultivating pollinator-attracting species after sugar beet production (European Commission, 2021, 2018).

Nevertheless, pollinator-attracting species are still promoted as cover crops—plants sown on arable land to manage erosion, soil fertility, weeds, pests, etc.—between harvest and sowing periods. In Belgium, for example, the typical crop succession after sugar beet cultivation is winter wheat, generally followed by a cover crop. These cover crops often include highly attractive entomophilous species, such as white mustard (*Sinapis alba*), phacelia (*Phacelia tanacetifolia*), faba bean (*Vicia faba*), radish (*Raphanus sativus*), and sunflower (*Helianthus annuus*; Mallinger et al., 2019; Ouvrard et al., 2018). While these mass-flowering cover crops provide valuable food resources for pollinators, they may also act as ecological traps if foraging insects are exposed to harmful pesticides like neonicotinoids. Detrimental effects of neonicotinoid seed treatments on pollinators have been well-documented (e.g., Boyle et al., 2019; Pisa et al., 2021; Willis Chan & Raine, 2021; Zioga et al., 2020).

Dietary exposure through contaminated pollen and nectar is considered a major exposure route, posing significant risks to pollinators (Bireley et al., 2019; Zioga et al., 2020). In temperate regions, bees—including honeybees and wild bees—are the primary pollinators of both crops and wild species (Ollerton et al., 2011). Neonicotinoids negatively impact bee health by altering both individuals and colonies, leading to reduced lifespans or indirect mortality depending on the exposure duration and consumption mode (Di Prisco et al., 2013; Douglas et al., 2015; Simon-Delso et al., 2015; Singla et al., 2021; Wintermantel et al., 2018). Ground-nesting solitary bees face an additional exposure route when excavating soil to build their nests (Willis Chan et al., 2019). The acute toxicity of neonicotinoids on pollinators is measured using the lethal dose for 50% of the population (LD_{50}), while chronic toxicity over 10 days is assessed via the median lethal dietary dose (LDD_{50}; EFSA, 2018; OECD, 2017; Phytoweb, 2021).

In this study, we quantified clothianidin concentrations in soils, vegetative parts (stems and leaves), flowers, pollen floral resources, and fruits of three key entomophilous cover crops (phacelia, white mustard,

and faba bean) under the field and growth chamber conditions. Our research addressed two questions: (i) Do clothianidin residues persist in soils and accumulate along the soil – plant – pollen continuum in these cover crop species? (ii) Do clothianidin concentrations found in pollen pose a potential toxic risk to honeybees and solitary bees compared to exposure through soil contact?

Materials and methods

Plant material

Three species were considered in this study according to their frequent use as cover crops and high attractiveness and visitation rates to bee pollinators (e.g., *Apis mellifera*, *Bombus* spp., species from Andrenidae, Anthophoridae, and Halictidae families): phacelia, white mustard, and faba bean (Marzinzig et al., 2018; Nuessly et al., 2004; Ouvrard et al., 2018; Petanidou, 2003). Phacelia (*Phacelia tanacetifolia*, Boraginaceae) produces up to 5000 flower per plant, offering abundant nectar and pollen resources: each flower provides an average of 0.56 mg of pollen (Kumova & Korkmaz, 2013). White mustard (*Sinapis alba*, Brassicaceae) presents 300–600 flowers per plant, with pollen production ranging from 0.12 to 1.2 mg per flower, depending on cultivar, season, and environmental conditions (Masierowska & Piętka, 2014; Ouvrard et al., 2018). Faba bean (*Vicia faba*, Fabaceae) produces 50–80 flowers per plant, yielding 0.1 to 1.0 mg of pollen per flower, varying by cultivar and environmental conditions (Bailes et al., 2018).

Field experiment

The field experiment was conducted at the UCLouvain experimental farm (FERM platform, Corroy-le-Grand, Belgium; 50°40′05″ N 4°38′20″ E). Soils are silty loam with a soil pH_{KCl} of 6.6 and an organic carbon content of 1.1%. The field was initially sown with clothianidin-coated sugar beet seeds (Lisanna KWS® treated with Poncho Beta®, field application rate of 60 g ha^{-1} of clothianidin) in Avril 2018. In October 2019, we established cover crops in single-species strips of *P. tanacetifolia* (Balo®), *S. alba* (Severka®), and *V. faba* (Julia®). Following a potato crop in July 2020, the same cover crop species were replanted in April 2021. No further clothianidin was applied after the initial sugar beet seeding in 2018.

Soil sampling was performed in August 2021. A composite sample was performed by collecting 3 subsamples across the field using an auger (top 20 cm depth) for a total weight of about 200 g. Soil samples were oven-dried (Binder ED, Tuttlingen, Germany) at 40 °C for 76 h before storage at room temperature.

Vegetative parts (stems and leaves), flowers, and fruits of the three cover species were randomly collected in July 2021. For pollen collection, flowers were shaken over containers; only pollen from *S. alba* was obtained in sufficient quantities for chemical analysis. Only one replicate was considered for plant matrices, prioritizing the diversity of species and matrices analysed. All plant samples were oven-dried (Binder ED, Tuttlingen, Germany) at 35 °C for 24 h. Pollen samples were sieved through a 50-µm mesh (full height, brass/stainless, USA) to separate pollen from stamen tissues. Missing data for specific matrices resulted from insufficient collected biomass (pollen) or a sample handling loss (vegetative parts and fruits).

Growth chamber experiment

An experiment was carried out in 2021 in the UCLouvain cultivation platform (SEFY platform, Louvain-la-Neuve, Belgium; 50°39′58″ N 4° 37′9″ E).

Sugar beet pre-treatments

Sugar beet seeds (Lisanna KWS®) coated with clothianidin (Poncho Beta®) were sown in 10 L pots filled with a 1:1 (v/v) mix of sand (0/5 size, M PRO, Amsterdam, The Netherlands) and universal peat compost (DCM, Amsterdam, The Netherlands). The sugar beet seedlings were cut at the 3-leaf stage, i.e., 6 days after sowing, which is when we transplanted the cover crops. Note that the seed remained in the pot until the end of the experiment. The experimental design included four treatments for each species, representing different clothianidin exposure levels: (1) untreated control (i.e., 0 sugar beet seeds per pot); (2) dose 1 (1 sugar beet seed per pot, equivalent to field application rate of 60 g ha^{-1} of clothianidin); (3) dose 2 (2 sugar beet seeds per pot); and (4) dose 4 (4 sugar beet seeds per pot).

Cover crop cultivation

The seeds of *P. tanacetifolia* (Balo®), *S. alba* (Severka®), and *V. faba* (Julia®) were placed in germination plates containing universal peat compost (DCM, Amsterdam, The Netherlands). The plates were maintained in a germination chamber (Economic Delux model ECD01E; Snijders Scientific, Tilburg, The Netherlands) at a constant temperature of 20 °C with a 12 h light/12 h dark photoperiod. Once the seedlings reached the 3-leaf stage, they were transplanted into 10 L pots containing pre-treated beet soil and placed in a growth chamber maintained at 20 °C during the day and 16 °C at night, with a light intensity of 120 µmol m^{-2} s^{-1} and 60% relative humidity. Plants were watered with rainwater every two days, ensuring occasional drainage from the bottom of the pots. This controlled watering regime aimed to simulate a key leaching process found in field conditions, while acknowledging that the simplified pot system does not fully capture the spatial and temporal heterogeneity of a natural agricultural environment. To control aphid infestations on the untreated *S. alba* control plants, an additional treatment with Provado® (Bayer) was applied. This product contains imidacloprid as its active substance at a concentration of 25.8 g kg^{-1}.

Plant and soil sampling

After a maximum of 74 days of cultivation (from May 16th to July 29th 2021), pollen was collected from flowering plant of all three cover crop species using forceps. Following pollen collection, we sampled the entire flowers along with stems, and leaves from each plant. For each treatment, we pooled samples (pollen, flowers, and vegetative parts) from each pot and stored them at − 20 °C until chemical analysis. Data for plant matrices represented single samples, as the sampling strategy prioritized a broad screening of species and matrices over replication. After completing the plant sample collection, three samples of homogenized soil (for each dose treatment and each species) were sampled. These soil samples were dried at 40 °C for 76 h in an oven (Binder ED, Tuttlingen, Germany) before storage at room temperature in preparation for chemical analyses.

Chemical analysis

All collected samples (10 g of soil samples and 2 g of plant samples, except for pollens where the sample size varied from 3 to 446 mg depending on the collected samples) were analysed by the GIRPA contaminant analysis laboratory (Beaucouzé, France). The extraction was performed by a 20-min mechanical agitation using 10 mL of water and 10 mL of acetonitrile, except for pollen (25 min using 10 mL of water and 20 mL of acetonitrile). A mixture of $MgSO_4$/NaCl/buffer salts were added for all matrices. The solution was mixed for 5 min by mechanical agitation before centrifugation for 5 min (or 10 min for pollen) at 4000 rpm. A purification step using a dispersive solid phase extraction (dSPE; 150 mg of PSA and 900 mg of $MgSO_4$) with mechanical agitation for 10 min was used for all samples except pollen, followed by centrifugation for 5 min at 4000 rpm. For pollen, the dSPE purification was performed (1200 mg of $MgSO_4$, 400 mg of PSA, 400 mg of C18, and 50 mg of GCB) with mechanical agitation for 5 min followed by centrifugation for 5 min at 4000 rpm before flushing the supernatant with a 50% dilution in ultrapure water. For pollen samples, the concentration step for pollen samples was performed on a Büchi Syncore evaporator (water bath at 55 °C and pressure at 145 mbar). The recovery was made in acetonitrile (including 5% of formic acid)/ultrapure water in a volumetric ratio of 50:50, followed by centrifugation for 10 min at 10,000 rpm before injection.

Clothianidin concentrations were measured for all samples by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS; Sciex 7500). The limits of quantification (LOQ) were 0.01 ng g^{-1} for soils and flowers, 0.05 ng g^{-1} for leaves, stems, and fruits, and between 0.045 and 6.7 ng g^{-1} for pollens. The mobile phase consisted of 100% ultrapure water, 0.1% acetic acid, 5 mM ammonium acetate and 100% methanol, 0.1% acetic acid, and 5 mM ammonium acetate. The column used was the C18 Synergi Hydro-RP 100 A (100 mm; 3 mm ID; 2.5 µm) with a flow rate of 700 µL min^{-1}. The column oven was at 60 °C and the injection volume was 5 µL. The analysis transitions were as follows (parent/ion product): 247.9/57.9—247.9/46.0—250.0/58.1.

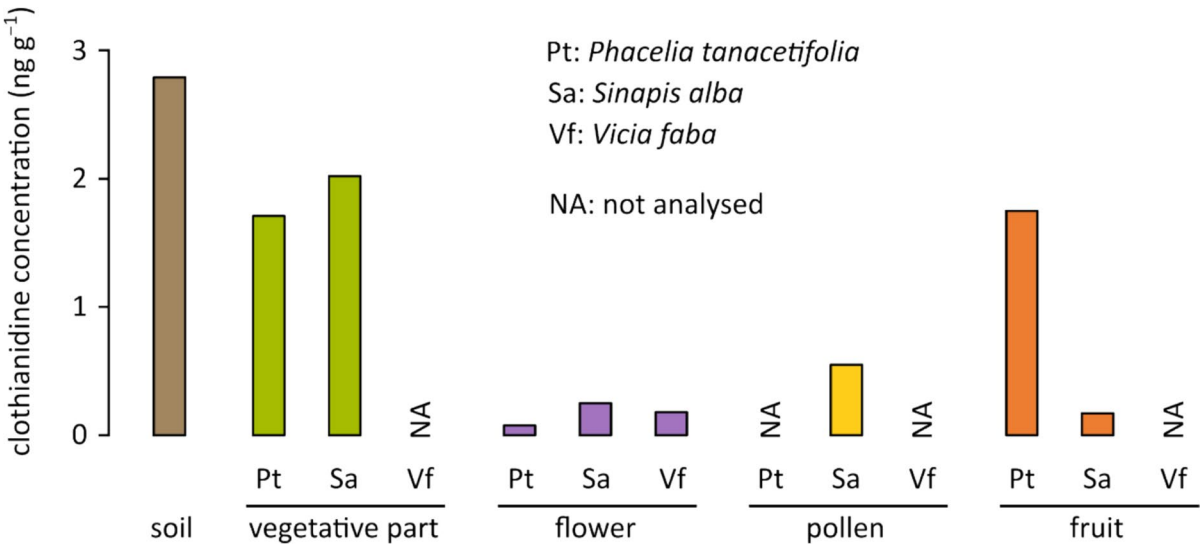

Fig. 1 Clothianidin concentrations measured in soil and plant materials of *P. tanacetifolia*, *S. alba*, and *V. faba* in the field experiment three years (2021) after the clothianidin treatment. Missing data for specific matrices resulted from insufficient collected biomass (pollen) or a sample handling loss (vegetative parts and fruits)

Risk assessment

To assess the risk for bee pollinators (honeybees and solitary bees), we evaluated both soil contact and oral pollen exposure using data from field and growth chamber experiments, complemented by a review of published literature (EFSA et al., 2023); EFSA, 2018; Willis Chan et al., 2019). For chronic exposure through soil contact, we used the measured clothianidin concentration in soil and an estimated soil contact/consumption of 33.5 g of soil per bee over 30 days; Willis Chan et al., 2019). The hazard quotient (HQ) was then calculated by comparing this exposure to the LD_{50} values for honeybees and solitary bees (35.88 and 3.588 ng clothianidin per bee, respectively). For chronic exposure through oral pollen consumption, we used the measured clothianidin concentrations in pollen and daily pollen consumption rate of 11.6 mg per honeybee and 6.0 mg per solitary bee. The HQ was then calculated by comparing these exposures to the LDD_{50} (0.95 and 0.395 ng clothianidin per bee for honeybee and solitary bee, respectively; EFSA et al., 2023); EFSA, 2018, 2013).

Given limited data on pollen mixture composition in bee diets, we assumed that bees exclusively foraged on a single contaminated cover crop pollen source for 10 consecutive days. This scenario is ecologically relevant, particularly for honeybee workers, during the late season when cover crops provide abundant floral resources compared to other common entomophilous species. Moreover, honeybees are known to exhibit strong floral fidelity (Ramalho et al., 1994; Sanderson & Wells, 2005).

Results

Field experiment

Three years after clothianidin treatment, clothianidin residues were detected in all samples, from soil to plants, including both vegetative and reproductive parts (Fig. 1 and Table 1). Soil contained the highest clothianidin concentration at 2.79 ng g^{-1}, slightly exceeding levels in vegetative parts (i.e., stems and leaves), which measured 1.71 ng g^{-1} for *P. tanacetifolia* and 2.02 ng g^{-1} for *S. alba*. Flower samples showed lower clothianidin residues concentrations: 0.08 ng g^{-1} for *P. tanacetifolia*, 0.25 ng g^{-1} for *S. alba*, and 0.18 ng g^{-1} for *V. faba*. Pollen concentrations were higher, showing values of 0.55 ng g^{-1} for *S. alba*, the only species for which we had pollen data due to the limited amount of pollen available. Finally, clothianidin was also detected in fruits, reaching

Table 1 Clothianidin concentrations measured in soil and plant materials of *P. tanacetifolia*, *S. alba*, and *V. faba* in the field experiment three years (2021) after the clothianidin treatment and in the growth chamber experiment after different clothianidin treatments (control untreated, dose 1 equivalent to 30 µL m^{-2}, dose 2 equivalent to 60 µL m^{-2}, and dose 4 equivalent to 120 µL m^{-2}). NA: not analysed; < LOQ: below limit of quantification (0.01 ng g^{-1})

	n	Clothianidin concentration (ng g^{-1})				
		Field exp	Chamber exp			
			Untreated	Dose 1	Dose 2	Dose 4
Phacelia tanacetifolia						
Soil	3	2.79	0.20	106	60.8	200
Vegetative part	1	1.71	3.84	71.5	76.9	138
Flower	1	0.08	38.8	56.2	140	210
Pollen	1	NA	2.23	9.91	15.5	29.6
Fruit	1	1.75				
Sinapis alba						
Soil	3	2.79	0.75	34.4	125	167
Vegetative part	1	2.02	1.39	30.7	83.1	89.8
Flower	1	0.25	< LOQ	294	210	490
Pollen	1	0.55	8.57	66.0	93.3	112
Fruit	1	0.17				
Vicia faba						
Soil	3	2.79	0.13	59.8	94.0	158
Vegetative part	1	NA	2.03	160	159	310
Flower	1	0.18	0.33	46.0	51.1	122
Pollen	1	NA	8.93	41.4	93.2	195
Fruit	1	NA				

1.8 ng g^{-1} for *P. tanacetifolia* and 2.02 ng g^{-1} for *S. alba*.

Growth chamber experiment

Clothianidin concentrations were measured in soil and plant material samples from the growth chamber experiment 74 days after clothianidin treatment (Fig. 2 and Table 1). Soil samples showed increasing clothianidin concentrations with higher application doses, ranging from an average of 66.8 ng g^{-1} at dose 1 (equivalent to the field rate) to 175 ng g^{-1} at dose 4 (i.e., 4-times the field rate). The clothianidin concentration at dose 4 was 2.6-times higher than those at dose 1. Trace amount of clothianidin ($\leq$ 0.75 ng g^{-1}) were detected in the untreated control, representing concentrations 3–20-times lower than those measured in the field soil (Table 1).

In vegetative parts, clothianidin concentrations were generally lower than in the soil for *P. tanacetifolia* (from 71.5 ng g^{-1} at dose 1 to 138 ng g^{-1} at dose 4) and *S. alba* (from 30.7 ng g^{-1} at dose 1 to 89.8 ng g^{-1} at dose 4). In contrast, *V. faba* showed higher concentrations in vegetative parts than in the soil (from 160 ng g^{-1} at dose 1 to 310 ng g^{-1} at dose 4). Specifically, *V. faba* accumulated 2- and 5-times more clothianidin than *P. tanacetifolia* and *S. alba*, depending on the dose. Additionally, concentrations at dose 4 were 2- to 3-times higher than at dose 1.

Flower samples contained clothianidin concentrations ranging from 132 ng g^{-1} (dose 1) to 274 ng g^{-1} (dose 4), exceeding soil concentrations. Differences between species were notable, with *S. alba* flowers presenting 4- to 6-times higher concentrations than *V. faba*, depending on the dose. As observed in soil and vegetative part samples, clothianidin concentrations increased with dose (2- to 4-times higher at dose 4 than dose 1).

Pollen samples presented clothianidin concentrations from 39.1 ng g^{-1} at dose 1 to 112 ng g^{-1} at dose 4, which were lower than flowers, except for *V. faba*. For a given dose, *P. tanacetifolia* pollen contained 5- to 6-times less clothianidin than *V. faba* and *S. alba*. Finally, our growth chamber results demonstrated that pollen retained clothianidin at concentrations > 10 ng g^{-1} at dose 1 (equivalent to field-treated sugar beet dose) and up to 66 ng g^{-1} (in *S. alba*).

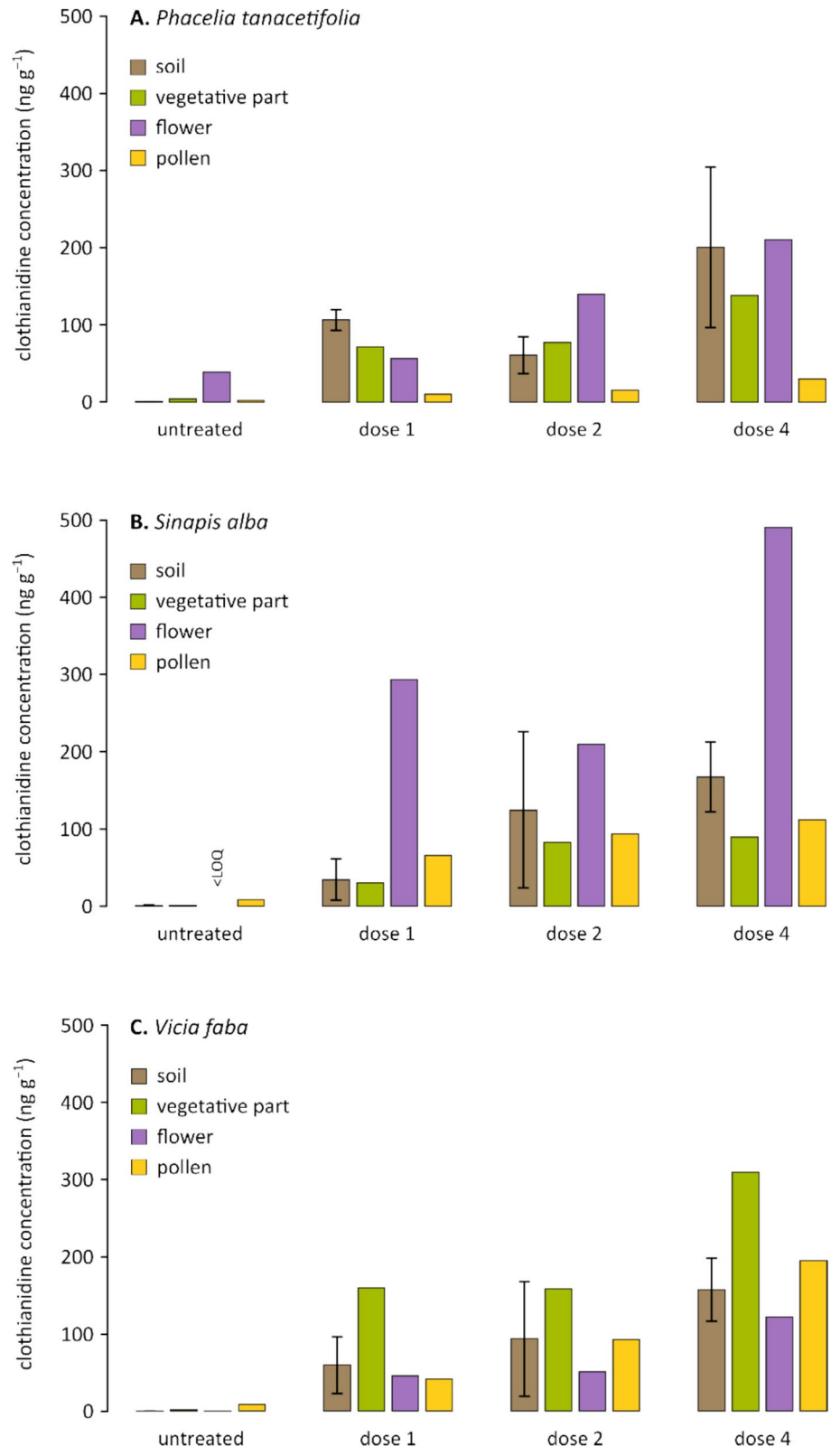

Fig. 2 Clothianidin concentrations measured in soils and plant vegetative parts, flowers, and pollen of *P. tanacetifolia* (**A**), *S. alba* (**B**), and *V. faba* (**C**) in the growth chamber experiment after different clothianidin treatments: control untreated, dose 1 (field dose, i.e., 30 µL m^{-2}), dose 2 (60 µL m^{-2}), and dose 4 (120 µL m^{-2}). < LOQ: below limit of quantification 0.01 ng g^{-1}

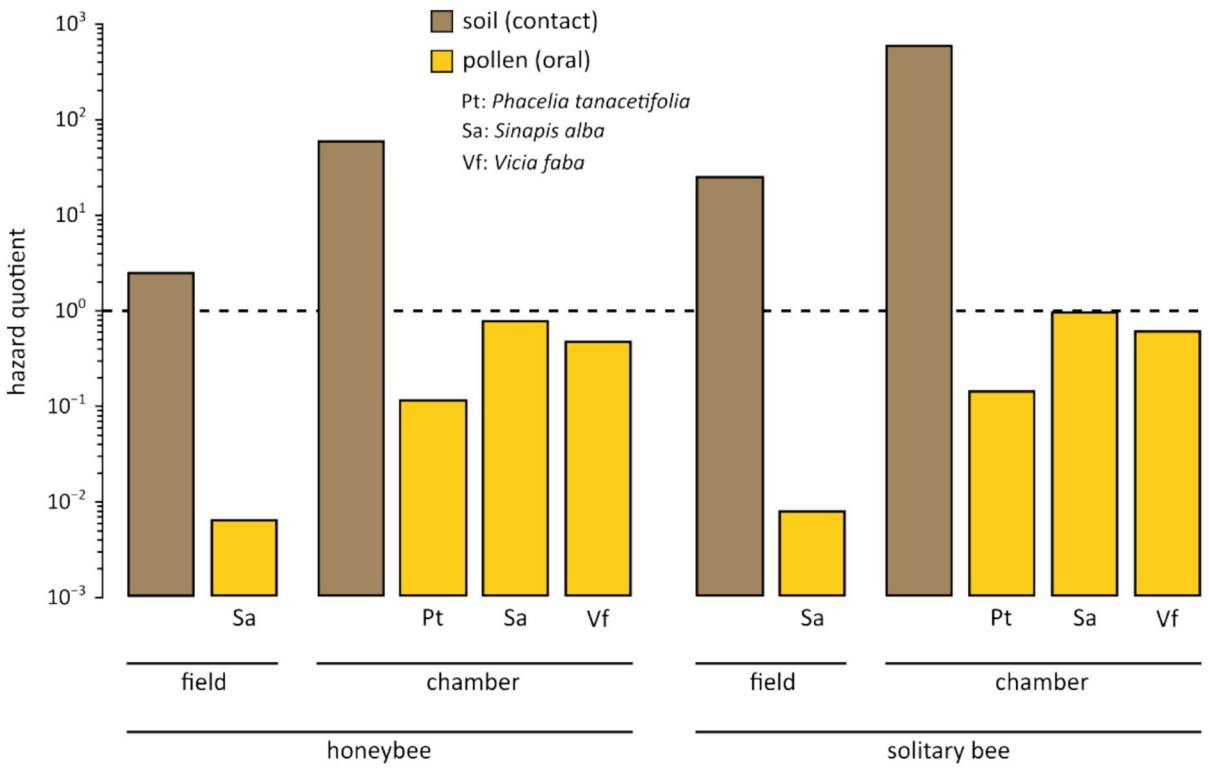

Fig. 3 Hazard quotients (HQ) for honeybee and solitary bee individuals under contact (soil) and oral (pollen) exposure, based on field and growth chamber experiment data. Values of HQ < 1 indicate no risk, whereas HQ > 1 indicate potential risk

Risk assessment for pollinators

Considering the field experiment results (i.e., three years after clothianidin treatment), soil contact exposure represented 93.5 ng of clothianidin per bee, corresponding to a HQ for chronic toxicity of 2.6 for honeybees and 26 for solitary bees (Fig. 3). Results from pollen consumption indicated a daily exposure of 0.0064 and 0.0033 ng of clothianidin for honeybees and solitary bees, respectively, corresponding to a HQ for chronic toxicity of 0.01 for both species.

In contrast, the growth chamber experiment (i.e., 74 days after clothianidin treatment, dose 1) revealed higher clothianidin concentrations in soil and pollen, leading to greater soil contact exposure (HQ = 62.3 for honeybees and HQ = 623 for solitary bees) and increased pollen consumption exposure (HQ = 0.12–0.81 for honeybees and HQ = 0.15–1.00 for solitary bees, depending on the plant species: *P. tanacetifolia* < *V. faba* < *S. alba*).

Discussion

Pesticide residues in the soil–plant–floral resource continuum

Results from both field (Fig. 1) and growth chamber (Fig. 2) experiments confirmed the presence of clothianidin (> 0.07 ng g^{-1}) across the entire soil–plant–floral resource continuum (despite pollen samples from some species were not analysed due to insufficient collected biomass). In the field experiment, detectable soil concentrations persisted three years after treated sugar beet cultivation (2.7 ng g^{-1}), consistent with literature values (~6 ng g^{-1}) following annual clothianidin seed treatments (Schaafsma et al., 2016). This persistence aligns with clothianidin's reported half-life of 545 days (Lewis et al., 2016), though degradation rates vary widely from a few months to several years depending on soil properties, such as organic carbon content, pH, temperature, and moisture (Bonmatin et al., 2015; Li et al., 2018; Schaafsma et al., 2016; Wu et al., 2020; Zhou et al., 2021). In contrast,

growth chamber soils after 74 days of cultivation exhibited significantly higher clothianidin concentration (34.4 – 106.2 ng g^{-1} at dose 1) than field soils. This discrepancy reflects either higher soil clothianidin sorption in the growth chamber experiment, driven by higher soil organic carbon content (ca. 13–17% resulting from the universal peat compost), or reduced degradation under controlled conditions (Zhou et al., 2021), potentially due to shorter experiment duration and differences in microbial activity (Xu et al., 2016).

Notable differences emerged between field and growth chamber conditions in clothianidin distribution across plant species. Soils serve as a primary pesticide reservoir, facilitating transfer to subsequent plants and floral resources (Viric Gasparic et al., 2020; Zheng et al., 2020; Zhou et al., 2021). However, plant species influenced both accumulation patterns (e.g., higher floral accumulation in *S. alba* vs. vegetative accumulation in *V. faba*) and compartment-specific concentrations (e.g., pollen levels in *S. alba* and *V. faba* exceeded those in *P. tanacetifolia*). Previous studies reported similar clothianidin residues in *P. tanacetifolia* foliage near treated fields (up to 55 ng g^{-1}; Mogren & Lundgren, 2016).

In the field experiment, *S. alba* pollen contained 0.55 ng g^{-1} clothianidin, comparable to literature values for maize (*Zea mays*; 1.8 ng g^{-1}) and rapeseed (*Brassica napus*; 0.6 ng g^{-1}) pollen after clothianidin application as sugar beet coated seeds (2 – 11 and 2 – 4 years, respectively; Xu et al., 2016). However, direct pesticide quantification in pollen (on flowers or on bees) remains methodologically challenging due to the large sample requirements (> 1 g) for chemical analysis (Zioga et al., 2020), limiting broader assessments of pollinator exposure.

Risk assessment for pollinators

Our field data indicated that clothianidin exposure three years post-treatment represented an unacceptable chronic risk for both honeybees and solitary bees, but only through soil contact (HQ of 2.6 and 26, respectively; Fig. 3). Exposure through pollen consumption exposure (based on *S. alba* pollen data) was 2 to 3 orders of magnitude lower than soil contact exposure, indicating a low risk. However, growth chamber data (i.e., 74 days post-treatment) revealed higher chronic toxicity. On the one hand, soil contact exposure showed markedly higher HQ values (62.3 for honeybees and 623 for solitary bees), indicating an extremely high risk linked to elevated soil concentrations, likely representative of early post-seeding periods in sugar beet cultivation. The chronic risk through pollen consumption also increased compared to the field data, reaching HQ values between 0.12 and 1.00, depending on the plant species and bee species, and approaching or reaching values close to 1 (e.g., for solitary bees feeding on *S. alba* pollen; Fig. 3). Despite potential overestimation due to the short interval between sugar beet harvest and cover crop seeding, these results underscore a clear potential threat to pollinators from neonicotinoid residues in cover crop floral resources. Conversely, this risk may be underestimated, as data on contact exposure through pollen and vegetative parts were not considered (Willis Chan et al., 2019). It is noteworthy that these data were obtained from the growth chamber experiment with limited replication, which showed higher concentrations than field experiments, likely reflecting conditions limiting pesticide degradation.

Recent studies demonstrate that wild plants near conventional agricultural fields often experience higher and more prolonged pesticide exposure than crops themselves (Wood et al., 2019; Zioga et al., 2020). These non-crop floral resources, available for longer periods than crop blooms, can accumulate pesticide more extensively. For instance, thiamethoxam (chemically similar to clothianidin) was detected in 100% of pollens samples stored by bumblebees in rural areas, with wildflower pollen containing higher residues (average of 18 ng g^{-1}) than oilseed rape pollen (David et al., 2016). Mass-flowering cover crop species, which bloom in autumn when alternative forage is scarce, may therefore extend pollinator exposure windows (Botías et al., 2016; Ward et al., 2022). While residues also occur in nectar (Barraud et al., 2020), pollen typically accumulates higher concentrations, likely due to its physical adsorption properties (Zioga et al., 2020).

Current neonicotinoid risk assessments mainly focus on individual bees, neglecting colony-level effects in honeybees (up to 50,000 individuals) and bumblebees (50–500 individuals; Thomson, 2004). Nevertheless, population are affected by neonicotinoids, especially ground-nesting solitary bee species (Willis Chan & Raine, 2021). Moreover, bees may experience a cocktail effect when exposed to multiple

pesticide residues (Willis Chan et al., 2019; Zioga et al., 2023). Current LD_{50} values, used to characterize clothianidin toxicity, assess only individual active substances and fail to account for potential interactions with other pesticides, including herbicides, fungicides, and pesticide degradation products. Growing evidence indicates that combined pesticide exposure can produce synergistic effects, where toxicity exceeds the simple additive effects of individual substances, in both honeybees and other bee species (Iverson et al., 2019; Thompson et al., 2014; Ward et al., 2022). Standardized protocols for multi-residue analyses are therefore needed to better assess these cumulative risks (Zioga et al., 2020).

Conclusion

This study investigated clothianidin concentrations in soil and three cover crop species: phacelia (*Phacelia tanacetifolia*), white mustard (*Sinapis alba*), and faba bean (*Vicia faba*). Both field and growth chamber experiments demonstrated that these plant species accumulate clothianidin in soil and plant matrices. In particular, we detected clothianidin residues three years after treatment in the field and 74 days after application under controlled condition. The presence of clothianidin across all compartments exposes both honeybees and solitary bees to potential risks, primarily through soil contact ($HQ > 1$), but also through pollen consumption, particularly for solitary bees, where HQ values approached 1. The uptake of clothianidin by cover crops—especially late-flowering, pollinator-attractive species—contribute to potential risks for pollinators foraging on their floral resources. Nevertheless, soil remains the dominant exposure pathway for clothianidin. These findings suggest that neonicotinoid residues in cover crop floral resources may threaten pollinators, especially when combined with other pesticides and degradation products in the environment. Further measurements are needed to confirm the observed trends. While current pollinator conservation strategies often focus on enhancing floral resources, our results indicate that promoting late-flowering cover crops in areas with recent neonicotinoid applications may be counterproductive. To safeguard pollination services and maintain pollinator biodiversity, reducing pesticide use and accelerating comprehensive risk assessments are critical steps toward achieving conservation goals.

Acknowledgements We thank H. Falys for field access, M. Migon for greenhouse maintenance, C. Boumal, E. Coninck, and O. Imbrecht for their help during field data collection, and C. Buyens for laboratory assistance. We also warmly thank C. Géfard, E. Géfard, P. Géfard, and A. Lecouturier Géfard for transporting all frozen samples from Louvain-la-Neuve to the GIRPA lab. We thank three anonymous reviewers for their valuable comments.

Author contributions Inès Gancedo Tarano : Investigation, Writing - Original Draft. Thomas Boumal : Investigation, Writing - Original Draft. Marc De Toffoli : Conceptualization, Funding acquisition, Methodology, Writing- Reviewing and Editing, Supervision. Maxime Buron : Writing - Review & Editing. Tomasz Kiljanek : Writing - Review & Editing. Anne-Claire Martel : Writing - Review & Editing. Anne-Laure Jacquemart : Conceptualization, Funding acquisition, Methodology, Writing- Reviewing and Editing, Supervision. Yannick Agnan : Conceptualization, Visualization, Writing- Reviewing and Editing.

Funding This research received was funded by Protect'Eau (Walloon Region, Belgium). This work constitutes the Master's thesis of I.GT and T.B.

Data Availability The data supporting the findings of this study are provided within the manuscript and are available from the corresponding author upon request.

Declarations

Ethical approval All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Competing interests The authors declare no competing interests.

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