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Effects of agricultural landscape structure, insecticide residues, and pollen diversity on the life-history traits of the red mason bee *Osmia bicornis*

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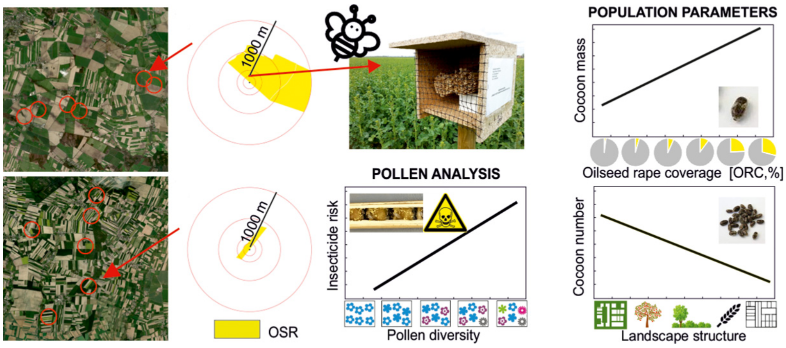
HIGHLIGHTS

- Landscape structure around bee nests affects diet diversity and insecticide risk.
- More diverse diets have higher insecticide risk levels in bee-collected pollen.
- The more natural features in the landscape, the more cocoons are produced.
- Cocoon mass positively depends on oilseed rape coverage around the nests.
- Sensitivity of bees to Dursban 480 EC not related to environmental variables.

GRAPHICAL ABSTRACT



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ABSTRACT

Agricultural landscapes have changed substantially in recent decades, shifting from the dominance of small fields (S) with diverse cropping systems toward large-scale monoculture (L), where landscape heterogeneity disappears. In this study, artificial nests of the red mason bee, *Osmia bicornis*, were placed in S and L landscape types on the perimeter of oilseed rape fields representing different oilseed rape coverages (ORC, % land cover). The local landscape structure around each nest was characterised within a 100, 200, 500, and 1000 m radius using ORC and 14 landscape characteristics, which were then reduced by non-metric multidimensional scaling (nMDS) to two axes: nMDS1 characterised the dataset primarily according to land fragmentation and the main crop, whereas nMDS2 captured the prevalence of more *natural* areas in the landscape. Pollen diversity and insecticide risk levels in the pollen provisions collected by the bees were analysed, and their dependence on the landscape structure was tested. Thereafter, the effects of pollen diversity, insecticide risk, and landscape structure on the life-history traits of bees and their sensitivity to topically applied Dursban 480 EC were determined. Pollen taxa richness in a single nest ranged from 3 to 12, and 34 pesticides were detected in the pollen at concentrations of up to 320 ng/g for desmedipham. The *O. bicornis* foraging range was relatively large, indicating that the landscape structure within a radius of ~1000 m around the nest is important for this species. Pollen diversity in the studied areas was of minor importance for bee performance, but the ORC or landscape structure significantly

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affected the life-history traits of the bees. Contamination of pollen with insecticides affected the bees by decreasing the mass of newly emerged adults but their sensitivity to Dursban 480 EC was not related to environmental variables.

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1. Introduction

Widespread arthropod decline has occurred in recent years, and agricultural intensification at the landscape level is suggested to be the major driver of the decrease in biomass, abundance, and the number of arthropod species (Seibold et al., 2019). The ongoing loss of semi-natural habitats, land-use change (Hallmann et al., 2017), and farming practices such as insecticide application (Geiger et al., 2010) are blamed for the decline in flying insect biomass (Hallmann et al., 2017) and the diversity of insect pollinators, including bees (Biesmeijer et al., 2006; Potts et al., 2010). Bees provide an important ecological and agricultural service by pollinating wild plants (Ollerton et al., 2011) and crops (Klein et al., 2007). There are approximately 20,000 known bee species, among which the honeybee, *Apis mellifera*, is most commonly and widely used for crop pollination. Nevertheless, wild bees, including the red mason bee, *Osmia bicornis* (=*O. rufa*), also represent a considerable economic value by pollinating various crops. In some cases, wild bee species exceed the crop pollination contribution of managed honey bees (Winfree et al., 2008). Solitary bees are important pollinators of many crops globally; in Europe, they play a key role as pollinators in oilseed rape (*Brassica napus*) cultivation (Holzschuh et al., 2013).

Landscape heterogeneity in agroecosystems is deemed important for biodiversity conservation in general (Tscharntke et al., 2005) and for wild bee conservation in particular (Kremen et al., 2007). In agricultural landscapes, all types of non-cultivated habitats, including field margins, hedgerows, meadows, and wetlands, play important roles as reservoirs for many plant species, as they provide a natural food source for pollinators (Long and Krupke, 2016). Therefore, maintaining pollinator abundance and diversity in agricultural landscapes largely depends on the abundance and quality of such non-cultivated areas (Stopes et al., 1995; Tscharntke et al., 2002). However, due to agricultural intensification, land use has changed substantially in the last few decades, shifting from small-scale family-run farms with diverse cropping systems toward large-scale monoculture farming where landscape heterogeneity disappears. Such large-scale crop monoculture is not attractive to pollinators because it represents a lack of floral resources, which can increase female foraging trip time (Westphal et al., 2006), thus increasing the time the nest is open, making it more vulnerable to predation and parasitism (Goodell, 2003). The availability of diverse floral resources is considered the major driving force that directly regulates the abundance and diversity of wild bee communities (Potts et al., 2003; Roulston and Goodell, 2011), which are likely to decline in response to insufficient nutrition from less diverse diets (Donkersley et al., 2017; Roulston and Cane, 1999).

Mass-flowering crops provide reliable, although short-lived, nectar and pollen resources for wild pollinators and may be particularly important for population growth in early season solitary bees that are able to produce sexuals during the mass-flowering period (Jauker et al., 2012; Labruyere et al., 2016). Oilseed rape (*B. napus*) is a prominent example of such a resource, as it is the most important oil plant pollinated by bees in Poland and many other European countries. Indeed, some studies have found a positive effect of the proximity of oilseed rape cultivation on the number of nesting *O. bicornis* (Holzschuh et al., 2013; Jauker et al., 2012). However, mass-flowering crops are usually intensively treated with pesticides, including neonicotinoids, which appear to play a significant role in the decline of bees (Goulson et al., 2015) as bees are attracted to the blooming crops and use the contaminated nectar and pollen as food for themselves and their larvae. Residues of different pesticides have been found not only in the pollen and nectar of flowering crops (Dively and Kamel, 2012) but also in wildflowers growing in agricultural field margins (Botías et al., 2015; David et al., 2016).

The overall aim of our study was to evaluate the effect of agricultural landscape structure on pesticide residues in pollen and on the diversity of pollen collected by the solitary red mason bee, *O. bicornis*. We also investigated how landscape structure, pesticide residues in pollen, and pollen diversity affect the life-history traits of *O. bicornis*, and the sensitivity of this species to an insecticide. We hypothesised that the surrounding agricultural landscape and the percentage of oilseed rape cover in the vicinity of the nests indirectly influence the bees by (i) reducing pollen diversity or (ii) increasing insecticide risk (determined from levels of insecticide residues in bee-collected pollen). We expected (iii) reduced pollen diversity to increase insecticide risk levels in bee-collected pollen, as diets with a higher proportion of oilseed rape pollen are more likely to be contaminated with pesticides. Finally, we tested (iv) the effect of the surrounding landscape, pollen diversity, and insecticide risk on the life-history traits of bees, such as the number of cocoons produced, mean cocoon mass, the emergence of adults, sex ratio, body mass, and sensitivity to Dursban 480 EC, an insecticide containing the organophosphate chlorpyrifos. We chose *O. bicornis* for our study because it is widely distributed across Europe and is commonly used for commercial agriculture (especially in orchards) as well as wild plant pollination. *Osmia bicornis* has been also proposed as an additional species for pesticide risk assessment in Europe (EFSA, 2013) as a good surrogate for most solitary bees (Schmolke et al., 2021; Sgolastra et al., 2019).

2. Methods

2.1. Selection and characteristics of studied landscapes and sites

The study was conducted in two 10 × 10 km agricultural landscapes in central Poland (Wielkopolska Province), spaced approximately 60 km apart to guarantee similar climatic and edaphic conditions. The two landscapes were similar in terms of arable land cover (~80%) and other landscape features such as forests, meadows, and built-up areas (Table S1) but differed in terms of agricultural management systems. One landscape was dominated by large-scale (L) agriculture, with 69% of the arable land covered by agricultural parcels greater than 10 ha, whereas the second landscape was dominated by small-scale (S) agriculture, with 70% of the agricultural parcels smaller than 10 ha (Table S1, Fig. 1). In each landscape, six winter-sown oilseed rape fields of different sizes were selected, and one *O. bicornis* nest was placed on the margin of each field. The nest locations (sites) were selected to represent different oilseed rape coverage (ORC, % land cover) within a circular area with a 100, 200, 500, and 1000 m radius (further referred to as *buffers*) around the nests (Fig. 1). The ORC ranged from 17.2% to 75.8% for the 100 m buffer and from 2.0% to 51.4% for the 1000 m buffer (Table 1). To limit the possibility of migration of bees among the nests, these were placed at least 200 m apart from one another, as it is commonly accepted that *O. bicornis* does not fly far to forage if sufficient resources are available in the nest vicinity (Hofmann et al., 2020).

To characterise the local habitat structure around the bee nests (sites), we used bee-specific landscape characterisation maps, with a spatial resolution of 1 m, constructed using two main sources: (1) the Polish National Database of Topographic Objects BDOT (1:10,000) and

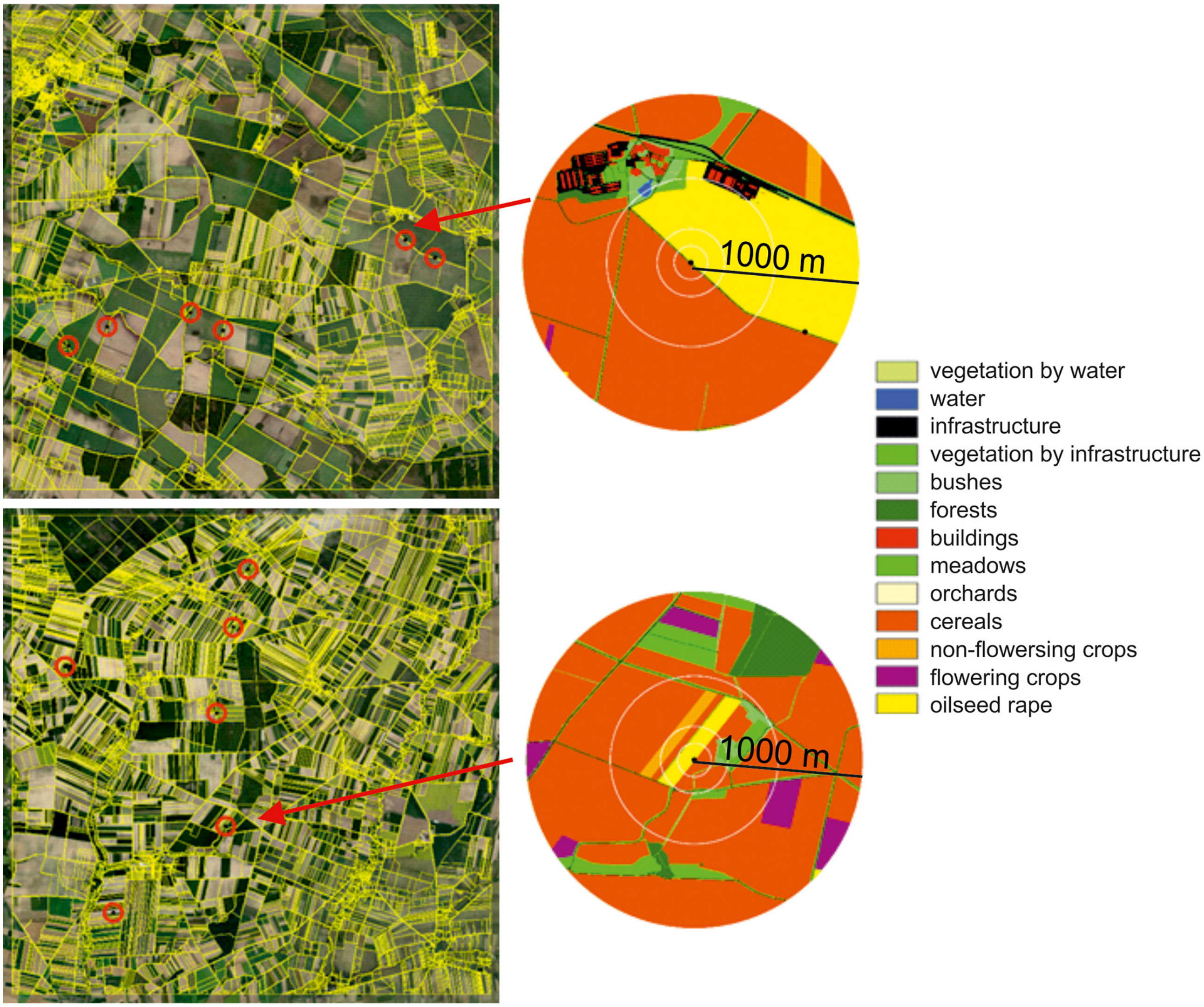

Fig. 1. Location of study sites within 100 m buffers depicted by red circles in two landscapes, one dominated by large fields (upper landscape) and the other by small fields (lower landscape), and examples of the 1000 m buffer with the smaller buffers that were considered in the study depicted by white, concentric circles.

(2) cadastral data combined with the agricultural register for 2017 obtained from the Polish Agency for Restructuring and Modernisation of Agriculture, as described by Mikołajczyk et al. (2021). Thirteen non-overlapping classes of landscape elements were used for local landscape characterisation of each buffer: vegetation close to water bodies and courses; water bodies and courses; concrete and infrastructure; vegetation close to infrastructure; bushes; forests; buildings; meadows; orchards; and four agricultural field subdivisions based on crop types: cereals (cultivated, anemophilous grasses); non-flowering crops (cultivated plants harvested before blooming or not blooming at all); flowering crops (cultivated plants harvested after blooming and visited by pollinators, such as maise, soybean, or berry plantations); and oilseed rape (selected separately because of its importance for pollinators) (Table S2). These landscape element classes were chosen to reflect the landscape as perceived by the red mason bee in relation to its biological and ecological needs (Mikołajczyk et al., 2021). Additionally, two linear features were calculated: (i) the length of field-to-field borders as a proxy for average plot size and land fragmentation and (ii) the length of borders between fields and natural habitats, such as forests and bushes (field-to-natural borders) (Table S2). These metrics (not including the area of oilseed rape that was treated as a separate variable) were further subjected to non-metric multidimensional scaling (nMDS) using the metaMDS function from the Vegan package (Oksanen et al., 2019) to characterise the local habitat within each of the analysed buffers around the *O. bicornis* nests. All metrics were standardised and shifted to display positive values only, and the number of nMDS axes chosen for further analysis was based on two criteria with the aim of: (i) minimising the stress value (thus ensuring adequate representation of the dataset in a low-dimensional space), and (ii) achieving a landscape-related, meaningful interpretation of each axis (Mikołajczyk et al., 2021). The axes were rotated according to the planes of greatest variance. Finally, two axes (nMDS1 and nMDS2) were chosen for all four buffers, yielding stress values ≤ 0.11. Within the 200 m buffer, there were no *flowering crops* registered (except oilseed rape), and within the 100 m buffer *buildings*, *orchards*, *flowering crops*, and *non-flowering crops* were missing; consequently, these categories were removed from the analysis of these two buffers. The axis loadings of the observations were used as variables to describe the buffers around the nests. Spatial data were manipulated using ArcMap 10 (ESRI, 2020), and the non-metric ordination method was applied in the R environment (R Core Team, 2017).

2.2. Study species and experimental design

Osmia bicornis is a solitary polylectic (pollen generalist) bee species, with a univoltine life cycle and main flight period from mid-April until the end of June in Central Europe (Steffan-Dewenter and Schiele,

Table 1

Population parameters of *Osmia bicornis* determined from sites located in landscapes dominated either by large (L) or small fields (S), with different oilseed rape coverage (ORC, % land cover) within a 1000 m radius around the bee nests. Each nest is additionally characterised by pollen diversity expressed as pollen effective number of species (PENS) and insecticide risk expressed as toxic units (TUs) (the PENS and TU calculations are described in Sections 2.3 and 2.6, respectively).

Nest ID	ORC [%]	PENS	TU (×0.001)[a]	No. reed tubes[b]	No. cocoons	No. cocoons per reed tube	Mean (±SD) cocoon mass[c] [mg]	Adult emergence rate[d] [%]	Emerged females [%]	Emerged males [%]	Sex ratio (f/m)	Mean (±SD) mass of emerged adults[e] [mg]	Mean (±SD) mass of emerged females	Mean (±SD) mass of emerged of males
L1	29.1	2.31	1.36	95	628	6.61	94.24 ± 30.842	0.94	0.49	0.51	0.95	64.62 ± 20.974	83.49 ± 19.923	52.11 ± 8.743
L2	24.2	2.34	1.19	100	833	8.33	102.73 ± 34.711	0.94	0.65	0.35	1.86	82.04 ± 26.724	96.75 ± 19.634	55.31 ± 14.208
L3	27.2	2.15	0.64	99	713	7.20	105.66 ± 29.690	0.92	0.55	0.45	1.23	81.55 ± 23.552	96.48 ± 15.771	58.92 ± 13.001
L4	51.4	2.59	5.1	93	610	6.56								
L5	44.9	3.85	0.69											
L6	24.5	3.99	6.51											
S1	6.6	5.75	9.97	97	520	5.36	106.03 ± 31.128	0.93	0.54	0.46	1.18	71.55 ± 23.030	90.81 ± 15.923	55.11 ± 13.361
S2	7.4	2.06	4.26											
S3	2.0	1.79	0.004	100	825	8.25	93.99 ± 29.301	0.94	0.46	0.54	0.84	62.09 ± 21.583	85.59 ± 16.795	50.09 ± 11.556
S4	10.3	2.20	0.12	99	679	6.86	97.29 ± 30.565	0.96	0.42	0.58	0.72	70.09 ± 25.548	98.57 ± 15.847	55.01 ± 14.369
S5	2.1	4.63	0.43	115	650	5.65	92.08 ± 30.327	0.94	0.41	0.59	0.70	75.53 ± 25.004	95.8 ± 114.241	53.28 ± 11.753
S6	4.4	2.41	0.57	94	499	5.31	90.63 ± 28.358	0.93	0.40	0.60	0.68	66.89 ± 22.880	91.60 ± 13.162	53.42 ± 14.102

[a] Values for TU should be multiplied by 0.001.

[b] Number of reed tubes suitable for colonization.

[c] Mean cocoon mass based on all cocoons extracted from reed tubes in the nest (truncated data).

[d] Emergence rate estimated based on 486 cocoons per nests.

[e] Mean mass adults (males and females) that emerged from 162 cocoons.

2008). Under natural conditions, females nest above the ground inside pre-existing cavities, such as hollow plant stems or beetle borings in deadwood. Nests of *O. bicornis* consist of a series of sequentially arranged brood cells that are separated by mud partitions. Before egg laying, each cell is provisioned with a mixture of pollen and nectar by a female bee. The larva that hatches in each cell consumes the provisions before spinning a cocoon and pupating. Offspring overwinter as adults and emerge the following spring. Females usually prefer to return to and build cells in their natal nests rather than establishing nests in equivalent new nest cavities (Bosch and Kemp, 2002).

At the end of March 2017, artificial nests were set up on the margins of the oilseed rape fields in both study landscapes. Each blockboard nest (~30 × 30 × 30 cm) had a plastic grid on one side (grid squares: 1 × 1 cm), and was attached to a wooden pole at a height of ~1.5 m above the ground, with the front (grid-side) facing southeast (Fig. S1). Each nest box was provided with two bundles of reed tubes (~20 cm long, with nodes at one end) made from stems of the common reed *Phragmites australis* (~100 tubes per bundle), and a carton containing 400 *O. bicornis* cocoons (purchased from Biodar, Poznan, Poland, and kept at 4 °C until placed in the field). At the end of May 2017, after completion of oilseed rape blooming and bee nesting, one bundle of reed tubes from each of the 12 nests, was removed for analysis of pesticide residues and palynological studies of the bee-collected pollen. These reed bundles were transported to a laboratory and stored at −20 °C to preserve pesticide residues and kill bee eggs or larvae (if any) before they could consume the pollen. The remaining bundles of reed tubes were collected in October 2017 and used to assess population parameters (see Table S3 for reed tube numbers collected for each purpose). Not all reed tubes were used in the analyses as two nests from landscape L (nests L5 and L6) and one from landscape S (S2) were lost in the field due to unknown random events. In addition, the L4 nest was found overturned on the ground, and since falling to the ground may have affected the survival of pupae or cocooned adults, it was also excluded from the analysis of population parameters.

2.3. Chemical and palynological analysis of pollen provisions

During winter, the pollen provisions were extracted from each reed tube. Each provision was placed into a separate Eppendorf tube, weighed, and stored at −20 °C until further analysis. Because of the large amount of pollen needed to screen for pesticide residues, the pollen extracted from cells in selected reed tubes was pooled for each nest. The maximum number of pollen provisions per reed tube was 14; however, 66% to 89% of the reed tubes contained from 4 to 10 cells, and these reed tubes were only used to pool pollen provisions for further analysis. Thus, 55 to 62 reed tubes were selected from each nest to obtain a similar number of pollen provisions (412–433 cells) per nest. The selected provisions per nest were then thoroughly mixed to create a combined representative sample for the entire nest. Each combined sample was divided into three subsamples: two of ~35 g and one of ~10 g.

The two 35 g subsamples from each nest were screened for residues of 510 different molecules using LC-MS/MS and GC–MS/MS techniques (see Supplementary materials for a description of the methods and Tables S4–S7 for results of the screened pesticides and their limits of quantification and recovery). All detected pesticides in the pollen were categorised as fungicides, herbicides, or insecticides. The results were reported as the mean value of two parallel determinations for each nest (Table S8) and used to calculate the insecticide risk levels in bee-collected pollen (see Section 2.6).

The 10 g subsample from each nest was used for palynological analysis following the method described in the Supplementary materials. Oilseed rape (*B. napus*) pollen was identified at the species level and other taxa at the genus or family level. All detected pollen types for the two parallel determinations for each nest site are presented in Table S9. Pollen diversity was expressed as the pollen effective number of species (PENS), calculated as exp. (H′), where H′ is the Shannon-Wiener diversity index (Jost, 2006, 2007).

After taxonomic identification, the distance from the nest to the nearest individual plant identified in the pollen samples was measured in the field using a GPS tracker (Garmin Ltd., Schaffhausen, Switzerland) and expressed in metres (m) (Table S10) to reflect the minimum linear foraging distances of the bees in the studied landscapes.

2.4. Assessment of population parameters

The reed tubes collected at the end of October 2017 were stored in a climatic chamber for 4 weeks at 15 °C, then for 4 weeks at 10 °C, followed by overwintering at 4 °C until emergence of adult bees in April 2018. During December and January, cocoons were extracted from the reed tubes. The diameter of the reed tubes at both ends was measured using callipers, then the tubes were carefully cut open, and the number of cells containing cocoons as well as pollen provisions or parasites was recorded. Each cocoon was weighed and placed in a labelled Eppendorf tube with a hole in the lid for ventilation and stored at 4 °C for further overwintering. In April 2018, all Eppendorf tubes with cocoons were placed under the following conditions: 20 °C, 60% ± 5% relative humidity (RH), and 16:8 h light:dark (L:D) to support the emergence of adult bees, and this was done in two series: first 162 cocoons (9 April), and a few weeks later (4 May), another 324 cocoons per nest. The tubes were controlled daily for two weeks, and the number of emerged adults and their sex was recorded. Upon emergence, bees in the first series that were younger than 24 h were weighed and released. The bees that emerged in the second series were transferred to plexiglass boxes (46 × 30 × 17 cm) with air flow provided from the top. Males and females from each nest were kept separately. The males from all nests started to emerge on the second day, and females on the third or fourth day, after being transferred to 20 °C. Over 95% females from each nest emerged within 3–4 days and were kept until testing for sensitivity to Dursban 480 EC (Dow AgroSciences, Indianapolis, Indiana, USA) (see Section 2.5), whereas males were released. The bees were fed ad libitum with 33% (*w*/w) sucrose solution placed in 2 mL Eppendorf tubes without lids but with cotton wool inside to prevent the bees from entering the tubes. A small square piece of yellow sponge-cloth was wrapped around each tube to attract the bees to the food source (Fig. S2A and B). The bees in the group housing were also provided with small cardboard grilles as hides (Fig. S2B).

2.5. Sensitivity of bees to Dursban 480 EC

Unmated female bees at the age of at least 4 days were used to avoid a cohort effect on the duration of bee survival in the experiment. The cohort effect results in a loss of portion of the population early during husbandry, while the remaining bees could be kept alive for 10 days and beyond (Robinson et al., 2017; personal observation). Hence, after an excess of females emerged in each nest, they were kept unexposed for at least 4 days and bees still alive after this time were then subsequently used in the experiments. Because between 95.0 and 99.4% females emerged within 3–4 days, they were mostly at the age of 4 to 8 days when experiment started. We used the insecticide Dursban 480 EC, which contains chlorpyrifos as an active ingredient (480 g/L), and which was commonly used by farmers in Poland in 2017. Nevertheless, for testing the sensitivity of newly emerged bees to insecticides, other formulation might as well have been used, especially that we were not interested in the effect of a particular formulation on bees but rather in testing whether the newly emerged bees that developed on less diverse and more contaminated pollen are handicapped in terms of their resistance to additional stressor (here an insecticide). Based on the recommended field application rate for oilseed rape and recommended dilution (0.6 L diluted in 300 L per hectare), the recommended application concentration (RAC) of Dursban 480 EC was prepared in 0.01% Triton X-100 (Sigma-Aldrich, Poznań, Poland), which facilitates adhesion of the applied solution. Bees at the age of at least four days were exposed topically to 1 μL of either 0.01% Triton X-100 solution as the control, or the insecticide solution at 0.36 RAC, which is approximately equivalent to the 48 h LD_{50} estimated by Mokkapati et al. (2021a) at 0.34 μg/bee (95% CI 0.28–0.47), both applied to the dorsal thorax of the bees using a Hamilton microsyringe with repeater (Hamilton Company, Bonaduz, Switzerland) (Fig. S2C and D). Approximately 1 h before treatment, 60 bees (30 for the insecticide and 30 for the control treatments) were taken from the cages, weighed individually, placed in glass Petri dishes, and anaesthetised at 4 °C for ~20 min to ensure accurate insecticide application and to avoid the spread of the solution to the neck or wing hinges. The bees were left in the Petri dishes for ~20 min to ensure infiltration of the treatment droplet and then transferred to individual treatment boxes (disposable plastic boxes (500 mL) with flat lids with holes for air supply and Eppendorf tubes attached to the bottom of the boxes for food supply; Fig. S2E) and kept in a climatic chamber (20 ± 2 °C, 60% ± 5% RH, 16:8 L:D). The bees were fed ad libitum with 33% (*w*/w) sucrose solution. The survival of the bees was checked daily for 39 days until the death of the last bee. The sensitivity of bees toward Dursban 450 EC was expressed for each nest as the median lethal time (LT_{50}).

2.6. Insecticide risk levels in bee-collected pollen

The insecticide risk to the bees was expressed in toxic units (TUs) estimated for each nest. Because neither larval nor adult LD_{50} values specific to *Osmia* sp. were available, the oral acute 48 h LD_{50} values for adult honey bees were used to calculate TUs. The TUs estimated in this way could, thus, be used only to compare risk to the bees across sites, but did not predict the exact *O. bicornis* larval mortality. Because LD_{50} values for honey bees were not available for most fungicides and herbicides detected in the pollen samples (values for many of the less toxic ones were reported in the database only as greater than some threshold value), the TUs were calculated either for insecticides only (using the exact LD_{50} values) or for all detected pesticides (using conservative approach), with the highest tested doses taken as LD_{50} for those fungicides and herbicides for which only "greater than" values were reported (Table S8). Further statistical analyses were, however, based on TUs estimated for insecticides only, as the values obtained from both approaches were highly correlated ($r = 0.99$, $p \le 0.0001$). Moreover, a previous study on honey bees showed that although fungicides accounted for 94% of the total residues in beebread, insecticides represented the majority of pesticide risk to bees, accounting for ~98 of the pollen hazard quotients (McArt et al., 2017). The oral acute 48 h LD_{50} values for adult honey bees (Table S8) were available in the Pesticide Properties Database (https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/321.htm) for all detected insecticides except flonicamid, for which the data were available in Minnesota Department of Agriculture (https://www.mda.state.mn.us/protecting/bmps/pollinators/beetoxicity).

The TU for each nest was calculated as the sum of the products of the concentration of each active ingredient and the mean pollen amount consumed per larvae divided by the LD_{50} of that particular pesticide for adult honey bees, using a modified equation from Centrella et al. (2020):

$$TU = \sum \frac{Acive\ ingredient\ \left[\frac{ng}{g}\right] \times \frac{pollen\ [g]}{larvae}}{LD_{50}\,\frac{ng}{bee}}$$

The mean weight of pollen still available for larvae in each nest was calculated from the provision weights collected for pollen analysis (Section 2.3) after truncating the data by removing two minimum and two maximum provision weights from each nest (Table S3). The truncation eliminated provisions <30 mg (presumably unfinished provisioning or pollen largely eaten by the larvae) and >535 mg (possibly two provisions not separated from each other).

2.7. Data analysis

To determine how the pollen diversity (expressed as PENS) and the insecticide risk (expressed as TUs for the insecticides found in the pollen) depended on local landscape characteristics, we performed, separately for each buffer, multiple regression analysis with all landscape variables (i.e., nMDS1, nMDS2, and ORC). The landscape variables were standardised, and the backwards-selection process was performed to consecutively remove non-significant variables from the model so that only variables significant at $p \leq 0.05$ remained. The normal distribution of residuals was formally tested for each model using the Shapiro-Wilk test.

Simple regression and a comparison with alternative models were performed to analyse the relationship between the proportion of pollen source dominated in nests and the distance between the nests and the nearest source of that pollen. Moreover, the relationship between pollen diversity and insecticide risk was analysed using reduced major axis (RMA) regression to test whether reduced pollen diversity increases insecticide risk levels in bee-collected pollen. The reduced major axis was used instead of standard least-squares regression to handle errors in both the x and y variables.

The landscape variables (nMDS1, nMDS2, and ORC) for the 1000 m buffer, pollen diversity index (PENS), and insecticide risk (TUs) were used as explanatory variables for all measures of *O. bicornis* life-history parameters (i.e., mean cocoon number, mean cocoon mass, emergence of adults, adult mass, and sex ratio) and bee sensitivity to Dursban 480 EC expressed as LT_{50}. Multiple regression analyses were performed on both non-standardised and standardised explanatory variables. After running the initial model, the non-significant terms were consecutively removed from the model (backward stepwise selection), starting with those with the highest F value until only variables significant at $p \leq 0.05$ remained.

Survival curves of females treated with Dursban 480 EC versus those in the control group were compared within each nest (pair-wise comparisons, $p \leq 0.05$) using the log-rank test and the body mass of females used in the test was compared using a *t*-test. The Bonferroni correction for multiple comparisons was applied.

All analyses apart from RMA regression were performed using Statgraphics Centurion XVIII (StatPoint, Herndon, VA, USA; http://www.statgraphics.com); the RMA regression was performed using PAST 3 software for Windows (https://softfamous.com/past/).

3. Results

3.1. Landscape analysis

The multidimensional scaling analysis yielded adequate levels of data representation for all the buffers, and the stress value for each buffer was as follows: 1000 m: 0.11, 500 m: 0.10, 200 m: 0.07, and 100 m: 0.09. Here, a more detailed description of the 1000 m buffer is provided because it was for this buffer, only that significant relationships were found for some of the studied variables (see Section 3.4); the characteristics of nMDS axes for all buffers are presented in Fig. S3. In the 1000 m buffer (Fig. 2), nMDS1 characterised the dataset according to the field-to-field border length (a proxy for agricultural land fragmentation) and main crop (cereals) as confronted with landscape naturalness (i.e., length of borders between fields and natural habitats, and share of orchards and bushes), whereas nMDS2 captured the prevalence of *semi-natural* landscape features such as vegetation close to water, but also the length of borders between fields and natural habitats, as confronted with features related to built-up areas (e.g., buildings, concrete, and vegetation close to infrastructure) (Fig. 2). Taken together, both axes clearly distinguished between the L and S landscape types.

3.2. Pollen provisions and pollen diversity versus local landscape characteristics

The number of reed tubes in the bundles used for pollen analysis at the nesting sites ranged from 96 to 100. At least one pollen provision

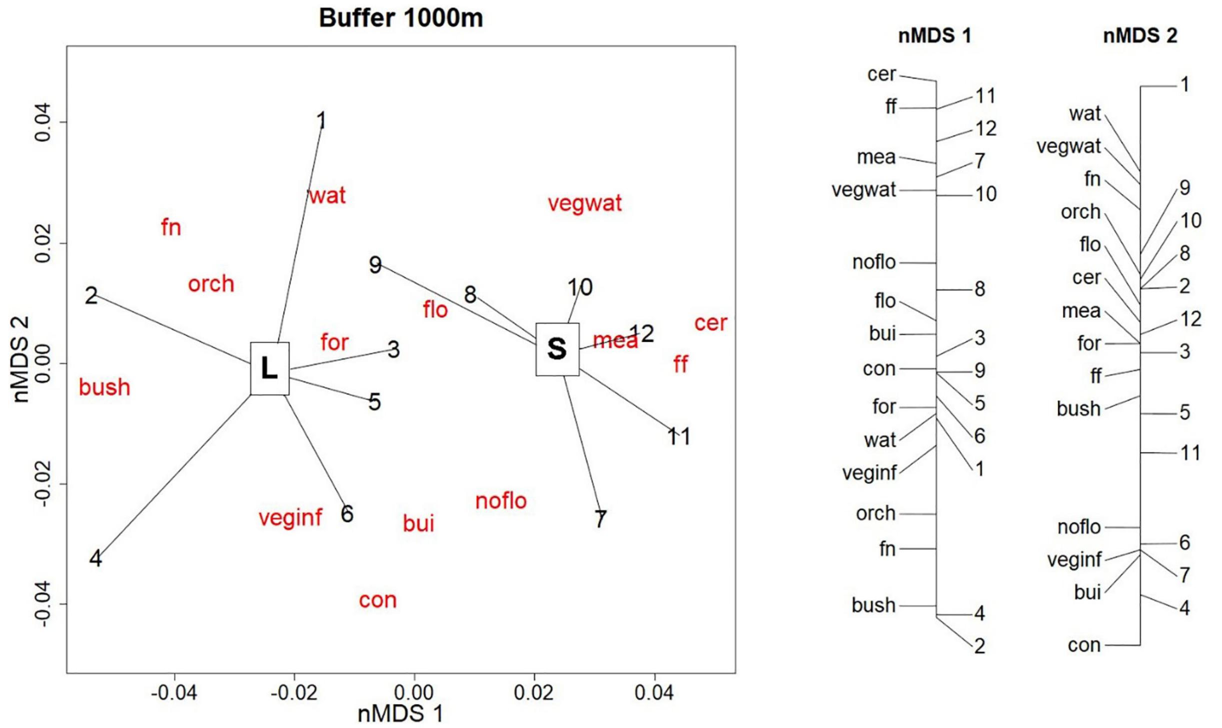

Fig. 2. Left: ordination plots depicting results of non-metric multidimensional scaling (nMDS) analysis for the 1000 m buffer with the site (black numbers) and variable (red lettering) scores plotted over an ordination plane with axes representing the first two nMDS factors. Sites are grouped according to their agricultural landscapes: dominated by large fields (L) and small fields (S). Right: Site and variable scores spread on the respective axes.

was found in 83% to 100% of the tubes in the L landscape and 81% to 99% in the S landscape (Table S3). The number of pollen provisions per nest ranged from 594 to 894 in the L landscape and from 529 to 855 in the S landscape (Table S3). The average weight of the provisions in a nest ranged from 189.5 ± 68.74 mg (mean ± SD) to 242.5 ± 86.14 mg, with no significant differences between the landscapes (mean values for the L and S landscapes were 203.1 ± 8.87 mg and 214.7 ± 17.38 mg, respectively, $p = 0.17$, *t*-test). Table S3 shows the site-specific details of the number of occupied and empty nest reeds, pollen provisions, and mean pollen weights.

In provisioning their nests, the bees collected pollen from 17 floral taxa. The provisions were dominated by *Quercus* sp. and *B. napus* which constituted 11% to 85% and 9% to 73%, respectively. Pollen identified as *other* comprised less than 2% (1.44% to 1.98%). Floral taxon richness in a single nest ranged from 3 to 12 (Table S9). Because *Quercus* sp. was the most dominant species in the pollen, the relationship between the percentage of *Quercus* sp. in the pollen and the distance to the nearest oak tree was tested and, of all the models fitted, the logarithmic-Y squared-X model yielded the highest R^2 value of 56.0%, $p = 0.005$ and $r = -0.75$, Fig. S4.

Pollen diversity was negatively related to nMDS2 ($p = 0.02$, $R^2 = 42.7\%$; Fig. 3A), but only for the 1000 m radius area, indicating that pollen diversity decreased with the share of vegetation close to water bodies and borders between fields and natural habitats, and increased with the share of built-up areas (i.e., the share of concrete and buildings, and in-between vegetation) around the nest. No relationship between the percentage of oilseed rape in the pollen and any of the explanatory variables was found.

3.3. Pesticide residues in pollen and insecticide risk versus local landscape characteristics

Altogether 34 pesticides (out of 510 tested agrochemicals), including active ingredients and major metabolites, were detected in bee-collected pollen at concentrations ranging from 0.015 ng/g for trifluralin to 320 ng/g for desmedipham. The full list of pesticides, their uses, and levels found in the studied nests are presented in Table S8. Ten

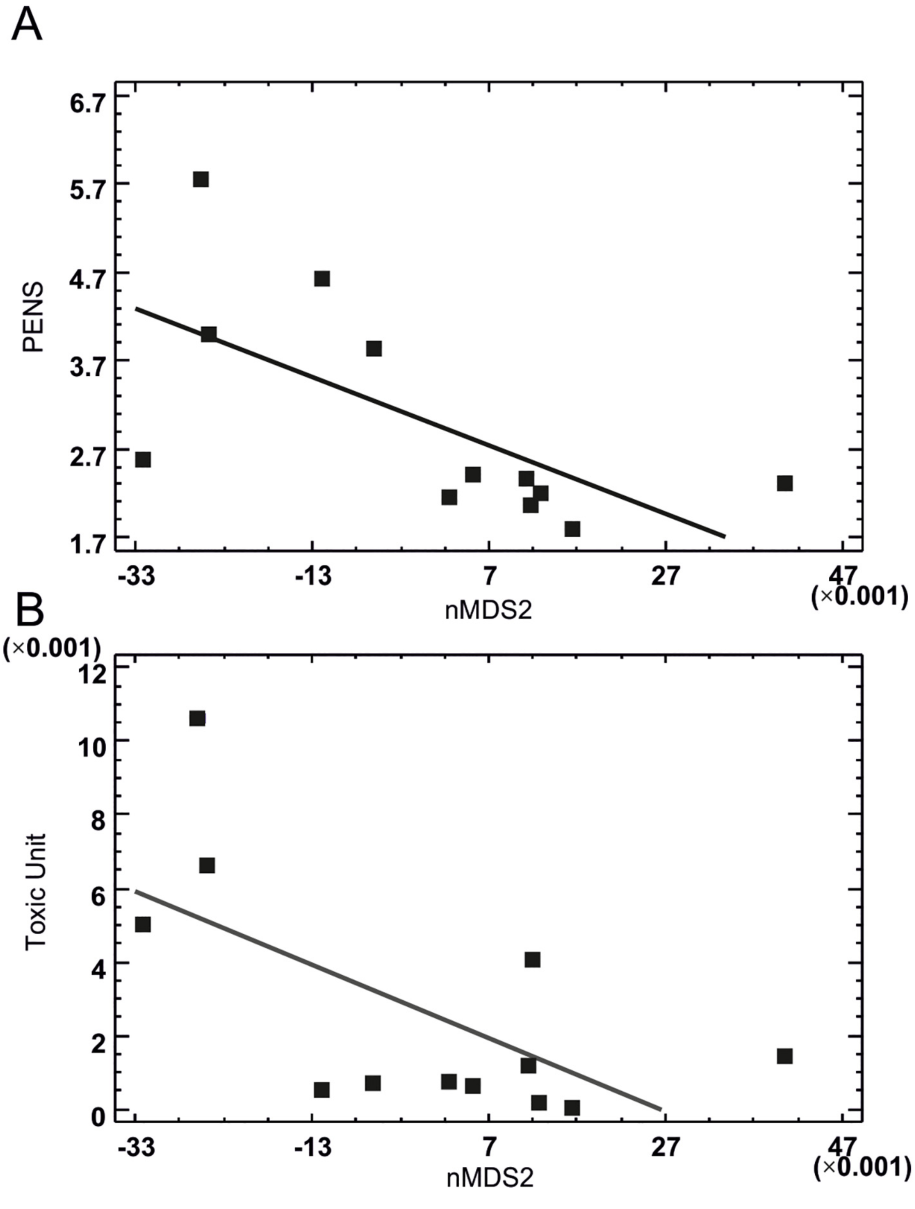

Fig. 3. Results of the multiple regression analysis for the 1000 m buffer: effect of nMDS2 on (A) diversity of pollen expressed as pollen effective number of species (PENS) ($p = 0.02$, $R^2 = 42.7\%$) and (B) insecticide risk expressed as toxic units (TUs) ($p = 0.027$, $R^2 = 40\%$) as analysed in pollen provisions collected by *Osmia bicornis* for their larvae in 12 nests located in landscapes dominated by large fields or small fields.

pesticides were detected in all studied nests (four fungicides, five herbicides, and one insecticide). Among the insecticides, flonicamid was detected in the pollen from all 12 nests. Acetamiprid (10 nests) and chlorpyrifos-ethyl (8 nests) were the most frequently detected insecticides.

As for pollen diversity, the multiple regression analysis indicated that insecticide risk, expressed in TUs for insecticides found in the pollen, was also negatively related to nMDS2 ($p = 0.027$, $R^2 = 40\%$; Fig. 3B), for the 1000 m radius only. A significant positive relationship ($r = 0.63$, $p = 0.029$; Fig. 4) between pollen diversity and insecticide risk was found, showing that the more diverse the food sources, the higher the insecticide risk level, although it must be noted that the significance of this relationship was driven by the single nest (S1) with the highest toxic unit value of ~0.01.

3.4. Population parameters versus local landscape characteristics

The initial number of reed tubes in the bundle used for the analysis of population parameters at nest sites ranged from 94 to 117. Between 91% and 99% and 81% and 98% were colonised in the L and S landscapes, respectively (Table 1). The mean inner diameter of the occupied reed nest tubes ranged from 5.25 cm (nest S3) to 5.71 cm (nest S1). No more than two reed tubes per nest were excluded as uninhabitable (too small in diameter to be colonised by *O. bicornis*). The lowest number of cocoons was found in nest S6 (499 cocoons at an average of 5.3 cocoons per tube suitable for colonization), and the highest was in nest L2 (833 cocoons at an average of 8.3 cocoons per reed tube) (Table 1). The nest S6 cocoons also had the lowest mean mass (90.6 ± 28.36 mg, mean ± SD), whereas the highest mean mass was found for cocoons from nest S1 (106.3 ± 31.13 mg). The emergence rate was high in all nests (92%–96%), and between 40% and 65% of emerged adults were females, with a mean body mass ranging from 85.6 to 98.6 mg (Table 1).

Multiple regression analysis for the mean number of cocoons per reed tube showed a negative relationship with nMDS1 ($p = 0.034$, $R^2 = 55.5\%$, Fig. 5) for the 1000 m buffer. This indicates that the number of cocoons produced per reed tube decreased with agricultural land fragmentation and cereal crop domination and increased with the length of borders between fields and natural habitats, with a high share of orchards and bushes around the nest.

The mean cocoon mass was positively related to ORC ($p = 0.03$) and negatively related to nMDS2 ($p = 0.03$); the model was significant at $p = 0.045$ and explained 71% of the variability (Table S11, Fig. 6). Thus, although on average more cocoons were produced when the share of field-to-natural borders was high around the nest, they were smaller than those produced when the share of built-up areas (i.e., share of concrete and buildings and in-between vegetation) was high around the nest.

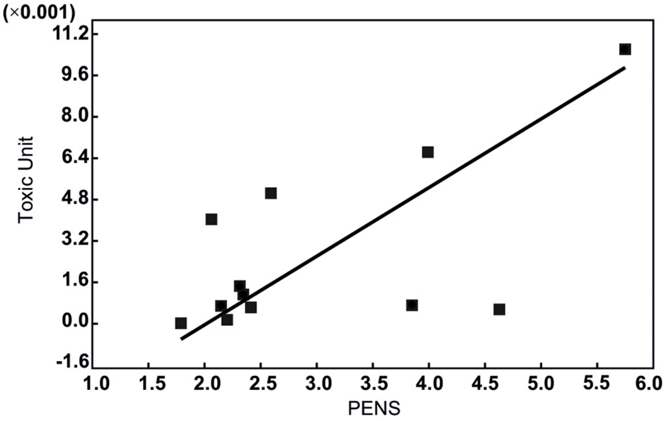

Fig. 4. The significant positive relationship ($r = 0.63$, $p = 0.029$) between the diversity of pollen expressed as pollen effective number of species (PENS) and insecticide risk expressed as toxic units (TUs) as analysed in pollen provisions collected by *Osmia bicornis* for their larvae in 12 nests located in landscapes dominated by large fields or small fields.

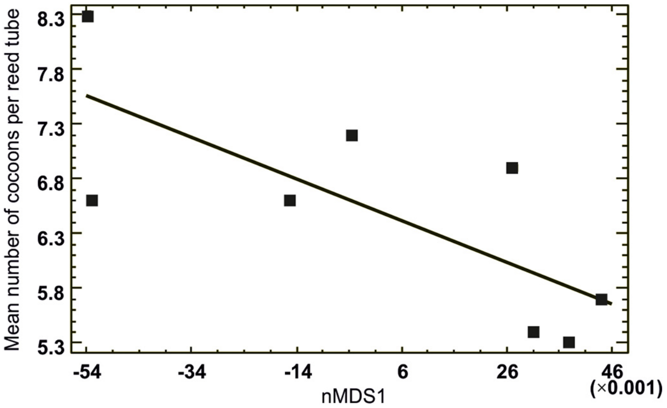

Fig. 5. Results of multiple regression analysis for the 1000 m buffer: effect of nMDS1 on the mean number of cocoons per reed tube ($p = 0.034$, $R^2 = 55.5\%$) in *Osmia bicornis* nests in eight nests located in landscapes dominated by large fields or small fields.

No significant relationship between the percentage of emerged adults and any of the explanatory variables was found. However, the mean mass of newly emerged adults, similar to the mean cocoon mass, was negatively related to nMDS2 ($p = 0.0045$) and positively related to ORC ($p = 0.005$). Additionally, a negative relationship was found between adult mass and insecticide risk levels ($p = 0.03$), and the model including all three significant variables was significant at $p = 0.014$, explaining 91.2% of the variability in adult mass (Table S11, Fig. 7). However, as shown in Fig. 7, the significance of the relationship between the mean mass of newly emerged adults and insecticide risk was mostly driven by the single nest (S1) with the highest TU of ~0.01. After excluding nest S1 from the multiple regression analysis, the insecticide risk was no longer significant, and the model including the negative relationship of the mean mass of newly emerged adults on nMDS2 ($p = 0.004$) and the positive relationship on ORC ($p = 0.006$) became significant at $p = 0.008$; $R^2 = 91.3\%$.

The female to male sex ratio was negatively dependent on both nMDS1 ($p = 0.0005$) and nMDS2 ($p = 0.006$) where $p = 0.001$ for the model, $R^2 = 92.9\%$ (Fig. 8). Thus, whereas more females than males were produced in nests at sites with a high share of fields to natural borders, orchards, and bushes than in those with a high share of field-to-field borders, built-up areas dominated by infrastructure and related vegetation also shifted the sex ratio toward females.

The parameters of the multiple regression models for the mean number of cocoons per reed tube, the mean cocoon mass, the emergence rate, the mean mass of newly emerged adults, and their sex ratio on both standardised variables (parameter β allowing for comparisons model estimates) and non-standardised (parameter b) variables are presented for the 1000 m buffer in Table S11.

3.5. Sensitivity to Dursban 480 EC

The LT_{50} values of bees exposed to Dursban 480 EC were low (1–2 days) for all nests, in contrast to the broad range of LT_{50} values (4–12 days) found for the control nests (Table S12). Pair-wise comparison between insecticide-treated and control bees indicated significantly higher mortality of the treated bees from all nests ($p \leq 0.00007$), apart from nests S3 ($p = 0.035$, which after applying the Bonferroni correction for multiple comparisons appeared to be non-significant) and S5 ($p = 0.08$) (Table S12). The S3 nest was the only one for which a significant difference in body mass was observed between insecticide-treated (88.5 ± 15.0 mg) and control bees (101.4 ± 15.0 mg) ($p = 0.002$, t-test).

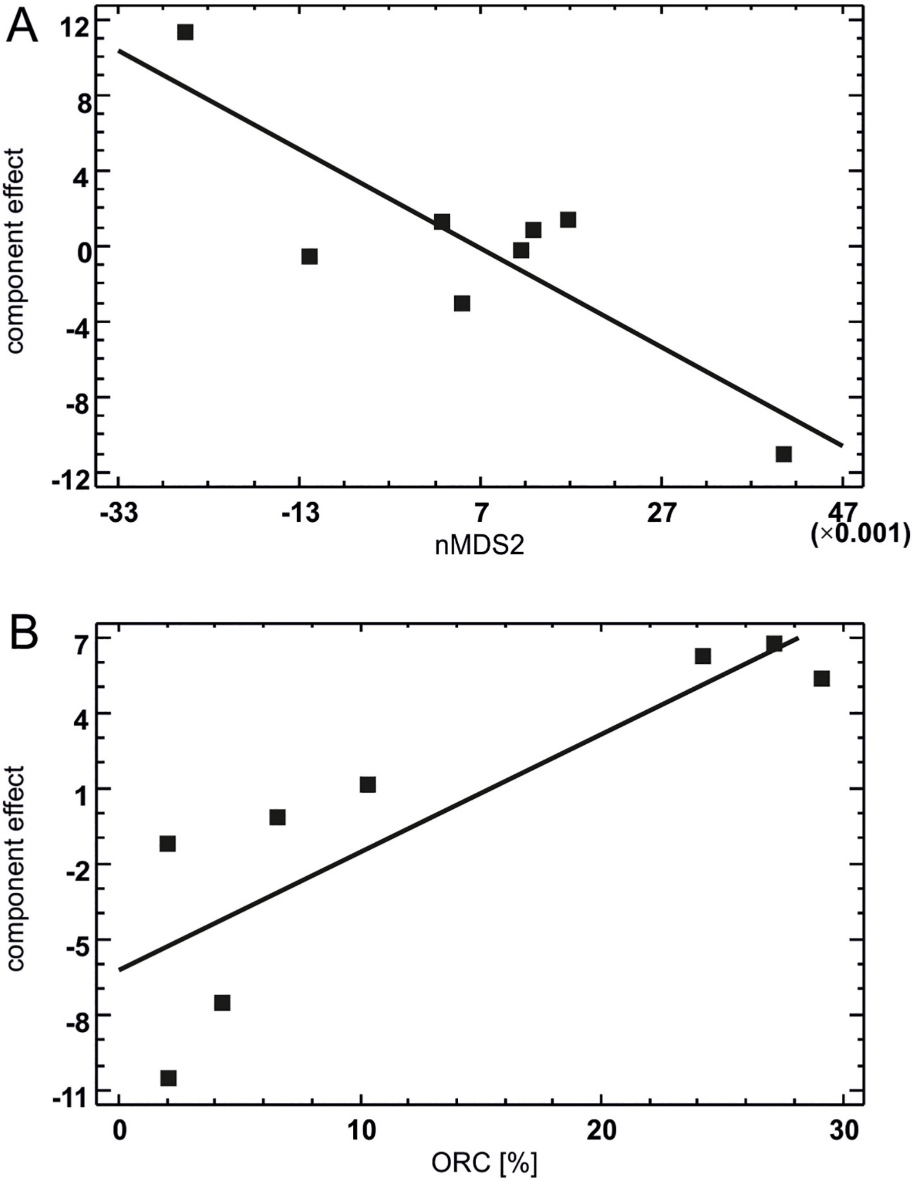

Fig. 6. Results of multiple regression analysis for the 1000 m buffer: effect of (A) nMDS2 ($p = 0.03$) and (B) oilseed rape coverage, ORC ($p = 0.03$) on the mean cocoon mass of *Osmia bicornis* from different sites in landscapes dominated by large fields or small fields. The line shows the relative change in the predicted values of mean cocoon mass that occurs when changing (A) nMDS2 or (B) ORC over their observed ranges. Each point (site) is then plotted by adding its residuals to a line. The overall model including both variables was significant at $p = 0.045$ and explained 71% of the variability.

No significant relationship between the LT_{50} values for Dursban-treated bees and any explanatory variables was found in the multiple regression analysis. Although the survival of control (LT_{50}) females was positively related to pesticide risk ($p = 0.04$, $R^2 = 53.7\%$; Fig. S5), the regression was significant, clearly due to only one nest (S1) with an exceptionally high TU value compared to other nests. After excluding nest S1 from the multiple regression analysis, no relationship between the LT_{50} values for the control bees and any of the explanatory variables was found.

4. Discussion

To the best of our knowledge, this is the first field study in which around-nest landscape characteristics, floral resources, and insecticide risk have been studied for their effects not only on the number of brood cells (Rundlöf et al., 2015; Woodcock et al., 2017) but also on the subsequent life stage of solitary bees. We showed that *O. bicornis* can fly large distances (up to 1000 m from the nest) to reach floral resources and that the structure of the landscape around the nest influences the diversity of the pollen collected and the insecticide risk encountered by bees, which appear to be lower in more *natural* landscapes (i.e., with a high share of field-to-natural borders and bushes). Contrary to our expectations, the more diverse the pollen, the higher the insecticide risk levels in the bee-collected pollen. The influence of local landscape characteristics or ORC on life-history traits depended on the trait. Based on the data for 12 nests from which between 499 and 833 cocoons were obtained, we showed that different life-history traits (number of cocoons, cocoon mass, and sex ratio of emerged adults) might be differently affected by the agricultural landscape characteristics or ORC in the vicinity of *O. bicornis* nests, but not by floral diet diversity. Similar studies on the effects of agricultural landscapes on the life stages of solitary bees are lacking or, as in the case of Klaus et al. (2021), rely on a very small number of individuals.

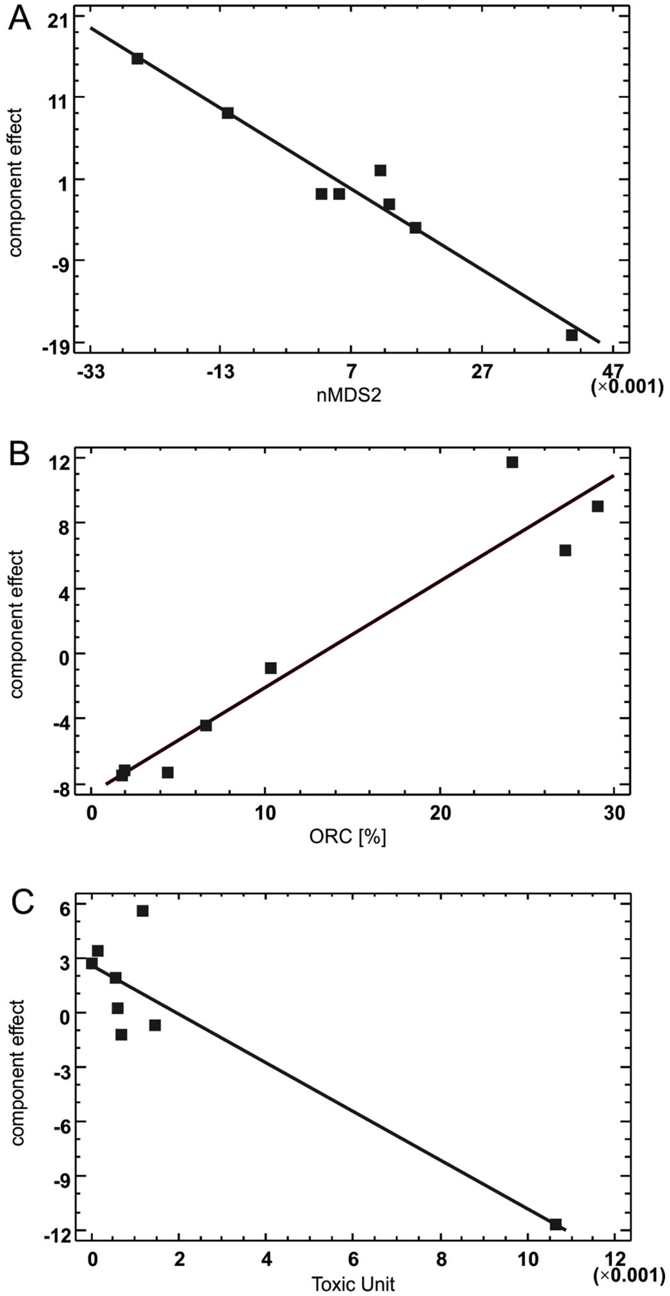

Fig. 7. Results of multiple regression analysis for the 1000 m buffer: effect of (A) nMDS2 ($p = 0.0045$), (B) oilseed rape coverage, ORC ($p = 0.005$), and (C) insecticide risk expressed as toxic units, TUs ($p = 0.03$) on the mean mass of newly emerged adults of *Osmia bicornis* from different sites in landscapes dominated by large fields or small fields. The line shows the relative change in the predicted values of mean cocoon mass that occurs when changing (A) nMDS2, (B) ORC, or (C) TUs over their observed ranges. Each point (site) is then plotted by adding its residuals to a line. The overall model, including both variables was significant at $p = 0.014$ and explained 91.1% of the variability. Please note that the significance of the relationship between the mean mass of newly emerged adults and TUs is driven by the single nest with the highest TU value of ~0.01.

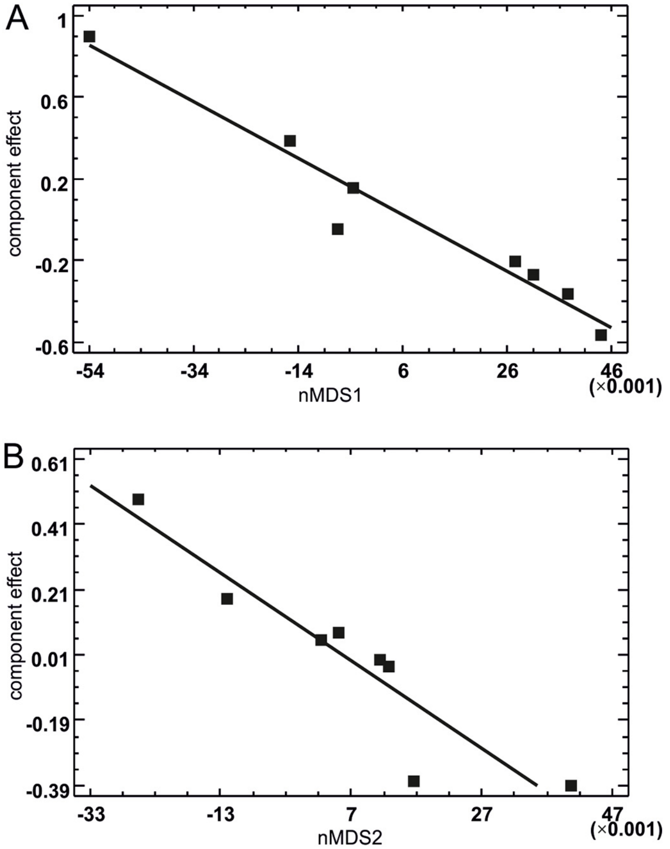

Fig. 8. Results of multiple regression analysis for the 1000 m buffer: effect of (A) nMDS1 ($p = 0.0005$) and (B) nMDS2 ($p = 0.006$) on the female/male sex ratio of *Osmia bicornis* from different sites in landscapes dominated by large fields or small fields. The line shows the relative change in the predicted values of the female/male sex ratio that occurs when changing (A) nMDS1 or (B) nMDS2 over their observed ranges. Each point (site) is then plotted by adding its residuals to a line. The overall model including both variables was significant at $p = 0.001$ and explained 92.9% of the variability.

4.1. Pollen diversity and its dependence on local landscape characteristics

A recent study by Baude et al. (2016) revealed a shortage of nectar and pollen sources in arable land in contrast to semi-natural habitats. In agricultural landscapes, where alternative food resources are limited, mass-flowering crops such as oilseed rape create large spatiotemporal pulses of nectar and pollen for both wild and managed insect pollinators (Stanley and Stout, 2013; Requier et al., 2015), increasing their within-season abundance (Westphal et al., 2003; Williams et al., 2012) and between-year populations (Jauker et al., 2012; Holzschuh et al., 2013; Riedinger et al., 2015). In our study, oilseed rape pollen was found in all bee nests and accounted for 9% to 73% of all the pollen sampled per nest.

To date, only a few studies have examined how *O. bicornis* uses different habitats across agricultural landscapes (e.g., Steffan-Dewenter and Schiele, 2008; Teper and Biliński, 2009; Jauker et al., 2012; Courdian et al., 2016), and their findings are not clear. For example, Courdian et al. (2016) and Jauker et al. (2012) found that mass-flowering oilseed rape were not visited for pollen collection but provided abundant nectar sources for adult *O. bicornis*. This may be related to the poor quality of *B. napus* pollen for larval development, as individuals raised on pure *B. napus* pollen showed behavioural failures (Dobson et al., 2012). However, Courdian et al. (2016) situated *O. bicornis* nests centrally in low-intensity grasslands, and the study sites were selected according to the percentage of woody habitats (3.6%–74.2%), not oilseed rape areas; consequently, the bees had limited opportunity to collect oilseed rape pollen. However, the Brassicaceae has been indicated as a source of *O. bicornis* pollen by Haider et al. (2014). In addition, in a study by Peters et al. (2016), in which pollen collected by *O. bicornis* was sampled from nesting blocks located in a landscape with oilseed rape fields treated with clothianidin seed dressing or untreated, oilseed rape pollen accounted for 10.6% ± 6.8% (mean ± SD) and 21.4% ± 13.2%, respectively, of the pollen, collected by the bees. Teper and Biliński (2009) found an even higher nest cell contents of oilseed rape pollen, up to 46% on average, and up to 100%

in some nest cells. However, only the pollen collected by bees during the oilseed rape full-bloom period was analysed in this study. In contrast to the study by Teper and Biliński (2009), we setup our artificial nests in the field at the end of March, ~4–5 weeks before the oilseed rape blooming time (COBORU, 2017), meaning that the bees in our study could have had access to pollen types other than oilseed rape early after emergence. Nevertheless, the proportion of oilseed rape in the pollen collected by the bees in our study was relatively high in all nests but not related to the ORC, neither in close proximity to the nests (within a 100 m radius) nor at larger scales (within the 200, 500, and 1000 m buffers). The lack of a relationship between the percentage of oilseed rape in provisions and its coverage in our study is not unexpected: all the nests were located on the perimeter of oilseed rape fields, and even a small ORC around the nest was probably enough to provide the bees with pollen for their larvae. The proportion of oilseed rape pollen collected by *O. bicornis* was also not related to landscape characteristics assessed in the different buffers around the nests (i.e., nMDS1 and nMDS2). Similarly, in their study on pollen collected by honeybees from 23 commercial apiary sites located in southeastern Estonia, Raimets et al. (2020) found that the presence of oilseed rape pollen in samples did not correlate with the percentage of any of the land-use types (forest, cultivated land, and grasslands) within a 2 or 4 km radius of the hives.

In addition to *B. napus*, we found that oak (*Quercus* sp.) was a substantial source of pollen collected by the bees, and in seven out of 12 nests, the proportion of *Quercus* sp. pollen exceeded that of *B. napus*. *Quercus* sp. pollen constituted the largest or second-largest amount of pollen in all nests, except for nest S1, where *Salix* sp. pollen was second in abundance (18.7%). We noted that *Salix* was closer to nest S1 (~10 m) than the nearest oak tree (~730 m). Some of the bees flew relatively long distances to collect *Quercus* pollen; those from nest S6, in which *Quercus* accounted for 18.1% of the pollen, had to fly 810 m from this nest to reach the nearest oak tree, a distance close to the maximum foraging distance considered for the red mason bee (Gathmann and Tscharntke, 2002). We found a negative relationship between the percentage of *Quercus* pollen and the distance to the nearest oak tree, and this high contribution of *Quercus* to larval provisions of red mason bees in our study is in accordance with Šlachta et al. (2020), who found that 65%–100% of larval provisions were collected from oaks in both gardens and orchards in Germany and with Ruddle et al. (2018) who found oak contributing up to 86% of the pollen sampled from nests located on winter oilseed rape grown from thiametoxam-treated seed. Radmacher and Strohm (2009) have documented an *O. bicornis* preference for pollen from *Quercus* and *Ranunculus*. Being polylectic, *Osmia* species can forage for pollen produced by a wide taxonomic variety of plants, including fruit trees (*Prunus* sp., and *Malus* sp.) and non-crop plants, such as *Juglans* and *Ranunculus* sp. and, to a lesser extent, *Betula*, *Sorbus*, *Aesculus*, *Pinus*, *Centaurea jacea*, *Crepis*, Asteraceae, and *Trifolium repens* (Šlachta et al., 2020). Most of the above-mentioned taxa were found in the pollen provisions analysed in our study as well. We noted that the bees in our study mixed pollen from more distant species despite substantial added foraging costs, a finding similar to that for the blue orchard bee *Osmia lignaria* (Williams and Tepedino, 2003).

The negative relationship between pollen diversity and nMDS2 found for the 1000 m buffer indicated that the diversity of pollen decreased with longer lengths of the borders between fields and natural habitats (field-to-natural borders) and increased with the share of built-up areas (i.e., buildings, concrete, and vegetation close to infrastructure) around the nest. Although the longer length of borders between fields and natural habitats usually means that more habitats provide a continuous supply of food resources (Oliver et al., 2010), bees may forage across more habitat types and take advantage of spatial and temporal shifts in resource availability in landscapes with a high share of vegetation by infrastructure and throughout the season; for example, woodlands or grasslands that might contain early season floral resources before the flowering season of oilseed rape (Bertrand et al., 2019).

4.2. Residues of pesticides in pollen and insecticide risk to bees in differently structured landscapes

We found a positive relationship between pollen diversity and insecticide risk levels in bee-collected pollen. This indicates that insecticide residues in bee-collected pollen can be high despite non-crop sources accounting for most pollen. These results suggest that non-crop sources of pollen may become contaminated with pesticide residues in agricultural landscapes (Botías et al., 2015), as pesticides from both forage and non-forage crops can spread to wild flowers and trees around fields due to pesticide drift during spraying or by leaching into the soil (Chifflet et al., 2011). Another explanation may be that small amounts of focal crop pollen collected by bees could lead to substantial pesticide risk (Long and Krupke, 2016). It has been shown that pollen and nectar from wildflowers at field margins of chemically-treated oilseed rape are important sources of exposure to neonicotinoid insecticides and fungicides for honeybees and bumblebees (Botías et al., 2015; David et al., 2016), as the pollen from these wildflowers contains similar mixtures of pesticides as the pollen from oilseed rape, but generally at lower concentrations. Up to 14 different compounds were detected by Zioga et al. (2020) in winter *B. napus*, and in general, the median concentrations of the compounds found in the cultivated plant families appear to be higher than those in the wild plant families. Whether insecticide (and other pesticides) residues found in our study came from contaminated oilseed rape flowers, wildflowers along field margins, other non-focal crops, or other sources is unknown, as wide pesticide screening could be performed only on mixed pollen from ~400 provisions from each nest. It has to be stressed, however, that the positive relationship between the diversity of pollen and insecticide risk was driven mostly by a single nest (S1) which had the greatest pollen diversity (12 different pollen sources), the highest proportion of *Salix* pollen (18.7%), and the lowest proportion of *Quercus* pollen (11.1%), but also the highest insecticide risk (TU ~ 0.01) among all the studied nests. This high insecticide risk resulted specifically from the highest concentration of chlorpyrifos-ethyl (12.1 ng/g), which was found in pollen from nest S1, this insecticide being highly toxic to both adults (Mokkapati et al., 2021a) and larvae of *O. bicornis* (Mokkapati et al., 2021b), and with an oral acute 48 h LD_{50} value of 0.25 μg/bee for honeybees. Similarly, the concentration of tau-fluvalinate (18.63 ng/g) in pollen from the S1 nest was the highest among all nests. Tau-fluvalinate is, however, less toxic to honeybees (oral acute 48 h LD_{50} value of 12.6 μg/bee); consequently, its contribution to the insecticide risk was not that large.

Our results confirmed the findings of other studies that pollinators are exposed to a wide spectrum of pesticides in agricultural landscapes, as indicated in honeybee case studies (e.g., Mullin et al., 2010; Raimets et al., 2020) and in a solitary bee pollen provisions study (Šlachta et al., 2020). Although there is a lack of studies showing the level of contamination of pollen provisions collected by solitary bees, it may be assumed that many solitary bees forage on plants growing near their nests due to an often small foraging radius (Gathmann and Tscharntke, 2002; Hofmann et al., 2020). This means they may not have a choice between collecting pollen from contaminated or non-contaminated plants. Although most of the insecticides (residues of which were detected in the pollen analysed in our study) may be used in oilseed rape production, they are also permitted for treatment of most other crops. This may be the reason for the lack of relationship between the insecticide levels in the pollen and the percentage of ORC in the foraging territory (within 100–1000 m around the nests) in our study. However, insecticide risk levels were negatively related to nMDS2 for the 1000 m radius buffer, showing that insecticide risk decreased with a high share of borders between fields and natural habitats around the nests.

4.3. Effect of pollen diversity, insecticide risk, and local landscape characteristics on population parameters and sensitivity of bees to Dursban 480 EC

Our study has shown that the foraging range of *O. bicornis* is relatively large; however, although the bees collected pollen of various kinds in the studied areas, pollen diversity was of minor importance for bee performance. None of the studied life-history traits (i.e., number of brood cells, number of cocoons, cocoon mass, emergence rate, the mass of emerged adults, sex ratio, and sensitivity of newly emerged females to Dursban 480 EC) were affected by pollen diversity. There is ongoing research to determine which of diversity, identity, or quantity of floral resources is more important for pollinators (Westphal et al., 2009; Blüthgen and Klein, 2011; Fründ et al., 2013). In a recent semi-field study, Klaus et al. (2021) showed that the production of offspring by *O. bicornis* increased due to plant diversity and plant species identity effects. However, bee development (from the larval to the adult stage) was negatively affected only when oilseed rape was the sole resource available, that is, in resource-poor environments of oilseed rape monocultures (Klaus et al., 2021). For the duration of our field study, the bees had the opportunity to collect pollen from plants other than oilseed rape, which they did: floral taxa richness in a single nest ranged from 3 to 12. Thus, even at sites where oilseed rape dominated around the nest the bees had access to diverse resources or key plant species (i.e., plant species with specific traits such as pollen with a high nutritional value Filipiak, 2019) that promoted bee reproduction and offspring development.

Our findings suggest that landscape characteristics within a 1000 m radius around the nests or ORC are more important than pollen diversity for *O. bicornis* in the landscapes we studied, affecting at least some life-history traits of the bees. For example, the reproduction of bees expressed as the mean cocoon number per reed tube increased in areas with a high share of field-to-natural borders, orchards, and bushes and decreased in areas with a high share of field-to-field borders and cereal crops. However, the positive effect of field-to-natural borders on the number of cocoons did not translate into a positive effect on the mean mass of cocoons. In fact, cocoon (as well as newly emerged adult) mass decreased with a high share of field-to-natural borders and was higher if built-up areas (i.e., areas dominated by concrete, buildings, and non-flowering crops but with a high share of vegetation close to infrastructure) were present around the nests. At the same time, both the mean mass of cocoons and the mean mass of adults increased with increasing ORC, suggesting that *O. bicornis* benefited from the presence of oilseed rape around the nest. Such positive effect of oilseed rape on *O. bicornis* has been reported by Holzschuh et al. (2013): the presence of oilseed rape fields correlated positively with the number of brood cells in adjacent trap nests. In contrast, for the same solitary bee species, Persson et al. (2018) did not find significant effects of the area of oilseed rape within 500 m of nests, or landscape type (conventional, organic farming, or pasture rich), or the length of field borders on the number of brood cells and proportion of female offspring. Although we found that the mean body mass of adults decreased with increasing insecticide risk, these results should be treated with caution as this relationship was predominantly influenced by one nest (S1) with an exceptionally high insecticide risk. No significant effects from exposure to oilseed rape grown from thiamethoxam-treated seed under tunnel or field conditions were found by Ruddle et al. (2018) from nest establishment through cell production to adult *O. bicornis* emergence. In general, the skewing of the sex ratio toward females increased at sites with a high length of field-to-natural borders and the presence of orchards and bushes around the nest (as confirmed by a significant negative relationship between the female/male sex ratio and nMDS1). However, if orchards and bushes were not present around the nest, then the importance of built-up areas with a high share of vegetation close to infrastructure prevailed (as indicated by a significant negative relationship between the female/male sex ratio and nMDS2).

In this study, we also confirmed the sensitivity of bees to Dursban 480 EC. Although chlorpyrifos usage, an active ingredient of Dursban 480 EC, was prohibited in the European Union in 2020, its use was still permitted in Poland during the year of this study (2017), but with recommendation that it should not have been used immediately before flowering and during flowering of winter oilseed rape. Our results are of particular importance outside the EU, where chlorpyrifos-based agrochemicals continue to be heavily used in flowering crops, potentially threatening pollinating insects (Urlacher et al., 2016). Although significant differences in survival of Dursban-treated females were found between nests, no relationship between LT_{50} of Dursban-treated females and any of the explanatory variables was found, mostly because the estimated LT_{50} values were all similar, that is, equal to or lower than 2 days. However, although the survival curves of the control females (Triton-treated) from all nests were similar, the estimated LT_{50} values of the control females were positively related to insecticide risk levels in bee-collected pollen. The better survival of adult females that developed from larvae provisioned with contaminated pollen was, however, driven by the high value of toxic unit found for one nest (S1). Unlike for the insecticide risk calculation, only eight of the 12 nests were available for life-history trait analysis, affecting the strength of the relationship between insecticide risk in bee collected pollen and life-history traits. Thus, for future studies, a larger number of nests/sites is recommended in cases of nest loss due to unpredictable random events. The loss of nests L4, L5, L6, and S2 was especially unfortunate, as three of these nests were characterised by high toxic unit values (0.00404, 0.00504, and 0.00663 for S2, L4, and L6, respectively), substantially higher than the range of values found for the other nests (0.00002–0.00145), except for nest S1 (~0.01).

5. Conclusions

Owing to the ease of obtaining bee cocoons from commercial distributors, the number of studies on solitary bees artificially introduced into agricultural landscapes has increased (e.g., Steffan-Dewenter and Schiele, 2008; Courdian et al., 2016; Woodcock et al., 2017). In relation to these previous studies, our study is unique because, for the first time, the effects of landscape structure, floral resources, and insecticide risk have been determined, not only on the number of brood cells (Rundlöf et al., 2015; Woodcock et al., 2017) but also on the subsequent life stages of solitary bees. Pollen diversity was of minor importance for bee performance, but ORC or landscape structure affected different life-history traits of the bees differently. Contamination of pollen with insecticides also affected the bees by decreasing the mass of newly emerged adults. The sensitivity of newly emerged females to Dursban 480 EC was not related to environmental variables. Regardless of their origin, all the bees were extremely sensitive to Dursban 480 EC, an insecticide containing chlorpyrifos, the use of which has been prohibited in the European Union since 2020, but this insecticide continues to be heavily used in flowering crops in other parts of the world.

Bees located in differently structured landscapes collected pollen from three to 12 floral taxa, including a high share of *Quercus* sp. and *Salix* sp., which highlights the important role of trees in the nutrition of *O. bicornis* larvae (Bertrand et al., 2019). The pollen provisions collected by the bees were contaminated with 16–21 different agrochemicals, and pollen diversity was positively related to insecticide risk, suggesting contamination of plants in non-crop areas. Further research is needed to determine whether the insecticide risk originated from contaminated oilseed rape, wildflowers in field margins, other crops, or other sources, as an understanding the importance of these sources is essential for sound environmental risk assessment (McArt et al., 2017).

CRediT authorship contribution statement

Agnieszka J. Bednarska: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Funding acquisition. **Łukasz Mikołajczyk:** Methodology, Formal analysis, Data curation, Writing – review & editing, Visualization. **Elżbieta Ziółkowska:** Methodology, Formal analysis, Data curation, Writing – review & editing. **Karolina Kocjan:** Investigation. **Agnieszka Wnęk:** Investigation. **Jaya Sravanthi Mokkapati:** Investigation. **Dariusz Teper:** Investigation. **Piotr Kaczyński:** Investigation. **Bożena Łozowicka:** Investigation. **Renata Śliwińska:** Investigation, Project administration. **Ryszard Laskowski:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151142.

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