

## Beyond generalists: The Brassicaceae pollen specialist *Osmia brevicornis* as a prospective model organism when exploring pesticide risk to bees

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### ABSTRACT

Bees are under threat from agricultural intensification, and species which are pollen specialists (oligolectic) are thought to have declined disproportionately compared to pollen generalists (polylectic). When assessing the risks of dietary pesticide (plant protection products) exposure to non-target beneficial insects such as wild bees, effects on pollen specialist species have seldom been considered. Research and risk assessment on pesticide risk to bees mainly use a small selection of model species, only representing pollen generalist species. Moreover, the foraging preferences of the existing model species are not always adequately matched to the crops investigated, which may lead to incorrect conclusions regarding the risks posed by pesticides in pollen and nectar. Here, we propose *Osmia brevicornis*, an oligolectic European wild bee species specialized on Brassicaceae pollen, as a new model organism suitable for assessment of how pesticides can impact specialist pollinators, especially in oilseed rape, a mass flowering Brassicaceae crop. We demonstrate that *O. brevicornis* can be successfully reared in the field next to oilseed rape and that its nesting success and offspring numbers can be increased by setting out a starting population. In our field assay, nesting tube diameter affected occupation rate and the sex ratio of *O. brevicornis* offspring. We describe a method for housing and controlled oral administration of sucrose solution in the laboratory, facilitating future studies on pesticide exposure. We conclude that *O. brevicornis* is a feasible model for assessing the risk of pesticides in the laboratory and in the field, especially for those compounds used in oilseed rape cultivation, as well as for investigating the general ecology of pollen specialists. By suggesting *O. brevicornis* as a potential model species, we aim to encourage diversification of the species used in agricultural ecology, especially to consider pollen specialists, and encourage attention to the foraging preferences and dietary needs of selected model species when considering pesticide exposure risk and effects.

### 1. Introduction

Insects, including bees, are declining in range, biomass, abundance, and species richness (Seibold et al., 2019; Outhwaite et al., 2020; Zattara and Aizen, 2021), thereby threatening the ecosystem service of crop and wild plant pollination (Potts et al., 2016). Wild bees have been shown to be in decline, although trends vary across species (Cameron et al., 2011; Powney et al., 2019; Wood et al., 2020), while the number of managed honey bee (*Apis mellifera* L.) colonies has increased globally (Osterman

et al., 2021b), likely due to expansion of agricultural crops dependent on pollinators (Aizen and Harder, 2009). In particular, specialist (oligolectic) bees, species that collect pollen from a single family or genus of flowering plants, have shown greater range declines compared to generalist (polylectic) species, collecting pollen from a wide range of plant families (Biesmeijer et al., 2006; Rasmussen and Madsen, 2022). In agricultural landscapes, species that are declining have been shown to collect pollen from fewer plant taxa and do not opportunistically switch host plants as the flora changes due to agricultural intensification (Kleijn

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and Raemakers, 2008). Therefore, breadth and flexibility of diet appear to contribute to the success or failure of bee species under anthropogenic stress. While this trend could be partially explained by the loss of specific host plant taxa (Scheper et al., 2014), it is not yet understood how oligolectic bees are affected by exposure to plant protection products (PPP) such as pesticides, one key threat to both managed honey bees and wild bees (Potts et al., 2016). Oligolectic bees cannot, by definition, utilize diverse food resources, a factor which has been shown to mitigate the detrimental effects of PPP exposure in pollen generalist bees (Klaus et al., 2021; Wintermantel et al., 2022). Oligolectic bee species could also potentially be exposed to higher levels of PPPs when they are specialized on a crop plant family (Willis Chan et al., 2019; Rondeau and Raine, 2022). Thus, empirical research on oligolectic and/or threatened species would contribute to a better understanding of why bee taxa vary in their response to agricultural intensification and subsequent PPP exposure risk.

Academic research as well as environmental risk assessments (ERAs) have almost exclusively used the managed western honey bee *A. mellifera* as a model organism when testing the hazards of pesticides to bees (Franklin and Raine, 2019), using a tiered approach in which laboratory, semi-field and field studies may be employed (Siviter et al., 2023). It has been suggested that the acute hazard of pesticides to wild bee species (1st tier) may be extrapolated from endpoints generated on honey bees when a large enough safety margin is used (Arena and Sgolastra, 2014). However, the diversity of life-history traits among bees, such as phenology, level of sociality, foraging behaviour and habitat preferences, lead to many exposure routes and risk scenarios that cannot be extrapolated from or tested in *A. mellifera* (Sgolastra et al., 2019). Consequently, using *A. mellifera* as the primary model organism for testing risks of pesticides to wild bees has been repeatedly questioned (EFSA, 2013; Franklin and Raine, 2019; Sgolastra et al., 2019; Sponsler et al., 2019; Dietzsch and Jütte, 2020; Rondeau and Raine, 2022; Rondeau et al., 2022). Indeed, when exposed to neonicotinoid insecticide in a field experiment, negative effects were observed in bumble bees and solitary bees, while honey bee colonies showed no decline in fitness, highlighting the greater resilience of managed *A. mellifera* colonies to pesticide exposure (Rundlöf et al., 2015). For this reason, other bee species such as *Bombus terrestris* L. have been suggested for inclusion in environmental risk assessment (EFSA, 2013). Several solitary wild bee species have also been used in ecotoxicological assays; however, only a few taxa are frequently used in semi-field and field studies, e.g. *Osmia bicornis* L. and *Osmia cornuta* L. (Dietzsch and Jütte, 2020). However, these suggested model species are polylectic (Westrich, 2019), and have had stable or increasing populations during the agricultural expansion in Europe over the past 50 years, while other closely related taxa have decreased within the same time period (Woodcock et al., 2016). Research performed solely on robust species may miss effects relevant to other, less common or threatened species, which are important targets of mitigation measures.

Oilseed rape (*Brassica napus* L., OSR) is a mass-flowering, economically important crop in which PPPs are commonly used for pest management (Lundin, 2021). The use of PPPs in OSR, especially systemic insecticide treatments, is considered a risk for flower-visiting insects, mainly through exposure to contaminated pollen and nectar (EFSA, 2018). In field and semi-field experiments testing the effects of PPP exposure on non-target organisms, it is important to use organisms expected to forage on the target crop. *Osmia bicornis* has repeatedly been used in semi-field and field-based assays (e.g., Schwarz et al., 2022), in particular on OSR (Rundlöf et al., 2016; Ruddle et al., 2018; Bednarska et al., 2021; Klaus et al., 2021). However, when floral resources are not manipulated, *O. bicornis* collects pollen from a wide range of plant taxa, especially from *Quercus*, *Ranunculus*, *Salicaceae*, and *Rosaceae*, and only rarely from Brassicaceae such as OSR (Budde and Lunau, 2007; Haider et al., 2014; Persson et al., 2018). Indeed, *O. bicornis* will seek out pollen from certain trees (particularly *Quercus* spp.) even when mass-flowering OSR is readily available at close range (Peters et al.,

2016; Ruddle et al., 2018; Bednarska et al., 2021; Yourstone et al., 2021). When foraging possibilities are limited to OSR, the production of brood cells by *O. bicornis* females is considerably reduced (Holzschuh et al., 2013; Ruddle et al., 2018; Klaus et al., 2021; Bednarska et al., 2021), suggesting that OSR pollen alone is a poor floral resource for them, although the presence of OSR in the landscape may still benefit them as a nectar resource (Yourstone et al., 2021). Thus, *O. bicornis* is a poor model species for studying the off-target effects of pesticide in OSR systems in the field (Franke et al., 2021). When foraging is limited, any effects seen from pesticide treatment on e.g. brood cell output may be confounded by the fitness disadvantage *O. bicornis* suffers from rearing brood on OSR (Klaus et al., 2021). Such mismatches between the model non-target organism and the studied crop system may produce results that do not reflect a worst-case scenario to the wider community of pollen-collecting insects. Therefore, there is a need for establishing a range of novel model non-target species, including pollen specialists, for assessing the risk of PPPs (Franklin and Raine, 2019). In North America, the pollen specialist *Eucera pruinosa* has been suggested as a model species to study the effects of pesticides in cucurbit crops (Willis-Chan et al., 2019; Rondeau et al., 2022), yet species have not been proposed either in Europe or for OSR crop systems.

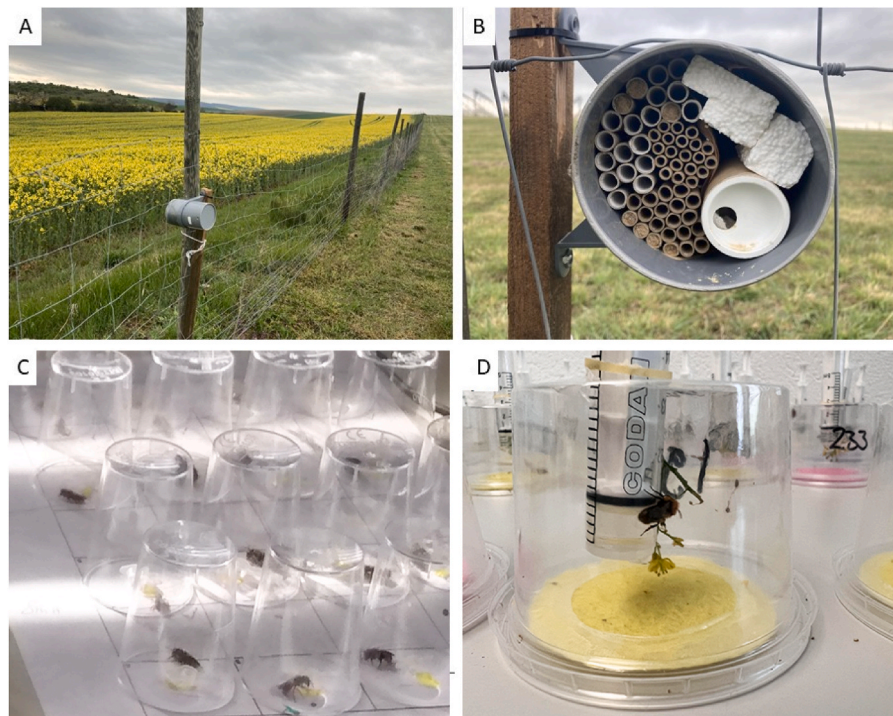
As a response to these issues, we suggest the wallflower mason bee, *Osmia brevicornis* F., as a prospective model species for future research on pollen specialists, especially in the context of PPP exposure through OSR and other Brassicaceae crops. *Osmia brevicornis* is a solitary bee with a range throughout Central and Southern Europe, which is specialized on plant species of the Brassicaceae family for pollen collection (Westrich et al., 2011). It is a cavity-nesting bee that places its eggs in a single, uniform pollen provision, rather than in subdivided brood cells, as practiced by *O. bicornis* and *O. cornuta* (Westrich, 2019). The species has been found in trap nests (Tscharniske et al., 1998; Pereira-Peixoto et al., 2014; Dainese et al., 2018) and has been recorded foraging on OSR (Saure et al., 2003; Westrich, 2019). Schenk et al. (2018) successfully used adult *O. brevicornis* in a semi-field experiment with OSR after trap-nesting cocoons from the field in central Germany. However, the basic husbandry of this species is not yet explored. This study aimed to establish appropriate methods for rearing and handling *O. brevicornis*. We examined whether its population size could be increased by supplementing cocoons to a trap nests, what diameter of cardboard tubes was preferred by *O. brevicornis* females for brood rearing and how the diameter affected body size and sex ratio of offspring. We furthermore assessed food uptake and survival in two laboratory trials. Finally, we compared the proportion of Brassicaceae pollen in provisions gathered by *O. brevicornis* to that of spontaneously nesting *O. bicornis* from our experimental field sites. Based on our findings, we discuss the potential of *O. brevicornis* as a model species for future toxicological laboratory, semi-field and field experiments.

## 2. Methods

### 2.1. Field trial

#### 2.1.1. Field sites and trap nest properties

The field study was conducted around the city of Halle (Saale) in the federal state Saxony-Anhalt in Central Germany in spring and summer 2021. The state is dominated by agricultural land (60%) and OSR is a commonly grown crop, occupying approximately 10% of the arable land in the state (Statistisches Landesamt Sachsen-Anhalt, 2022). We selected five independent OSR fields at which we placed two trap nests each (i.e., five pairs), one containing only empty cardboard tubes as nesting material (control) and one supplemented with a starting population of *O. brevicornis* cocoons (treatment). Trap nests were mounted at 1.5 m height <2 m from OSR fields, facing southwards (Fig. 1A). The paired trap nests were separated by at least 500 m, as this is the common foraging distance for solitary bees (Gathmann and Tscharniske, 2002) and installed in mid-April 2021, before OSR bloom (Fig. S1).



**Fig. 1.** (A) Trap nest mounted on a pole next to an oilseed rape field and (B) a trap nest consisting of 60 cardboard tubes of varying inner diameter. (C) *Osmia brevicornis* in an individual oral exposure assay and (D) during individual housing in the laboratory.

The inner diameter of nesting tubes preferred by *O. brevicornis* has been proposed to be 5 mm, which is at the lower range of the 5–9 mm diameters preferred by *O. bicornis* (Westrich, 2019). To quantify the effect of inner tube diameter on nest initiation, brood number, sex ratio and body size of offspring, we stocked trap nests with 20 commercially available cardboard tubes each (length 14 cm) of 4, 6, and 8 mm inner diameter ( $N = 60$  tubes per trap nest; LBV Naturshop, Hilpoltstein, Germany; Fig. 1B). Trap nests in the cocoon supplementation treatment were equipped with a container housing 51 *O. brevicornis* cocoons (Fig. 1B). These cocoons were collected from a trap nest adjacent to an OSR field in the previous year in the federal state of Thuringia, Germany, where *O. brevicornis* had nested spontaneously. As sex dimorphism is common in *Osmia* species, whereby small cocoons generally contain males and large cocoons contain females (Seidelmann et al., 2010), smaller and larger cocoons were evenly distributed between the starting populations.

### 2.1.2. Population dynamics of *O. brevicornis*

At the end of September 2021, all trap nests were collected from the field (Fig. S1). The trap nests from two sites (i.e., four trap nests) were damaged and therefore excluded from further analysis. Cardboard tubes from the remaining three pairs (i.e., six trap nests) were cut open and their contents examined. Nest occupation rate (i.e., number of tubes with *O. brevicornis* brood) and the number of cocoons per tube were recorded. The occupation rate by species other than *O. brevicornis* was also noted. We also counted the number of empty cocoons (i.e., successfully emerged adults) from the starting populations set out together with the trap nests. Collected *O. brevicornis* cocoons were then stored in paper bags and hibernated under a rain-cover outside at ambient temperature until February and thereafter brought into a 4 °C cooling chamber (relative humidity 30–40%) in order to control the timing of adult emergence (Fig. S1).

### 2.1.3. Proportion of Brassicaceae in pollen provisions

The proportion of Brassicaceae pollen collected by *O. bicornis* and *O. brevicornis* nesting at the same sites were assessed as a measure of

differences in their foraging preferences. To our knowledge, OSR pollen cannot easily be morphologically distinguished from other Brassicaceae (personal communication, Sawyer, 1981), and thus pollen grains were only determined to family level. Three pollen samples per species and trap nest ( $N = 9$  per species) were examined. Provision residues or frass were mixed on a microscopic slide in a water droplet and examined in a polarized light microscope at  $\times 400$  magnification. The proportion of Brassicaceae-like pollen was determined based on 200 pollen grains per sample, counted from left to right at a randomly selected part of the microscopic slide.

## 2.2. Laboratory trials

### 2.2.1. Feeder design and survival under group housing in the laboratory

In order to establish best practices for first tier (laboratory-based) studies, three separate laboratory trials were performed, one feeder design assay, one testing survival during group housing and one testing survival under individual housing. The first two assays were performed in May 2022. A batch of 197 *O. brevicornis* cocoons was taken from the 4 °C cooling chamber, separated per nest tube into Petri dishes and incubated at 21 °C in darkness. Emergence was monitored daily, and freshly emerged individuals were kept dormant at 4 °C for a maximum of four days until enough bees had emerged to populate the feeder design assay.

Establishing an appropriate feeder design is essential for future oral exposure to known quantities of compounds (e.g. pesticides) in the laboratory, since enticing solitary bees to feed in the laboratory is often difficult (e.g. Tadei et al., 2022). Using a modified version of the “flower method” introduced by Ladurner et al. (2005), we examined whether a colour cue or a cue in the form of a petal would enhance sucrose consumption success compared to a feeder without visual cues. In the feeder cue assay, bees were taken out from 4 °C and immediately presented with a 10  $\mu$ L droplet of 50% [w/v] sucrose solution in a small plastic ampoule with either a petal from a flowering weed (*Diploaxis tenuifolia*, family Brassicaceae), a yellow piece of tape or no visual cue (Fig. 1C). Bees were sorted into treatments based on time since emergence, and it



was the first opportunity to ingest sucrose solution for all individuals. The trial took place under natural light conditions at ambient temperature (24 °C). Bees which had consumed the solution within 2 h were counted as feeders and the others as non-feeders. The trial was performed separately for males (N = 36) and females (N = 47).

In order to investigate if *O. brevicornis* could be housed in the laboratory for acute ( $\leq 96$  h; e.g. OECD, 1998) and chronic PPP exposure assays (10 days; e.g., OECD, 2017), we examined their survival during group housing using metal cages with a removable sliding door of clear plastic designed for maintaining honey bees in the laboratory (5.5 × 10 × 10 cm). Bees were housed in groups of 5–8 individuals, separated by sex. Each cage was equipped with *ad libitum* 50% [w/v] sucrose solution in a 2 mL Eppendorf tube with a 2 mm hole drilled in the tube's bottom, with a petal (see above) attached as a cue and a piece of tissue paper for protection (Strobl et al., 2019). Cages were kept at 21 °C under 16:8 light:dark cycle. Survival was assessed at 96 h after start of housing and then weekly for 21 days.

### 2.2.2. Feeding rate and survival during individual housing

A third laboratory trial was performed to determine survival rates of *O. brevicornis* during individual housing and to monitor daily sucrose consumption. In June 2022, a second batch of cocoons (N = 245) was incubated at ambient temperature and emergence was monitored daily. Bees were housed individually (Fig. 1D) following Strobl et al. (2021). In brief, transparent cages (80 cm<sup>3</sup>) were equipped with a syringe (5 ml CODAN) containing 50% [w/v] sucrose solution and maintained at room temperature (24 °C) with indirect natural light. To promote feeding success, a flower petal of *Sinapis arvensis* (Brassicaceae) was attached to the tip of the 5 ml syringe. Sucrose consumption and adult mortality were recorded on a daily basis. An additional starvation trial was performed on females in which survival without food was monitored until all individuals were dead.

A random subset of bees from both batches was weighed after emergence to the nearest mg (males, n = 226; females, n = 175), and these data were used to examine the effects of sex and tube size diameter on body mass. In both batches, cocoons that had not emerged after 20 days were opened manually to determine their survival status, sex and eventual presence of parasites.

### 2.3. Statistical analysis

All analyses were performed with R version 4.1.2 (R Core Team, 2021). To test whether *O. brevicornis* cocoon supplementation or nesting tube diameter affected a trap nest's occupation rate, we compared the proportion of tubes occupied by *O. brevicornis* with or without supplemented cocoons with respect to tube diameter using a generalized linear mixed-effects model (GLMM) with binomial error structure using the package *lme4* (Bates et al., 2015). Trap nest occupation by other species was included as an explanatory variable as they could have influenced the nesting behavior of *O. brevicornis*. Pair identity (of trap nests at the same site) was included as a random factor. A Tukey post-hoc comparison was used to test for differences in nest occupancy between diameters using the R package *multcomp* (Hothorn et al., 2008). Using the same approach, we analyzed differences in the occupancy by other species, with occupation of *O. brevicornis* this time as an explanatory variable. We compared the numbers of *O. brevicornis* cocoons per occupied nest tube between treatments using a GLMM with negative binomial error structure and pair identity as a random factor. A Tukey post-hoc comparison was used to test for differences between diameters. Differences in the proportion of Brassicaceae pollen between *O. brevicornis* and *O. bicornis* were examined using a Kruskal-Wallis test to account for non-normality of residuals.

Differences in the sex ratio between nest tube diameters were tested using a linear mixed-effects model (LMM, package *lme4*) with trap nest as a random factor. To study the effect of nest tube diameter on body size between males and females we again used an LMM with the interaction

of sex and diameter as fixed factors and trap nest identity as a random factor. We performed pairwise comparisons on all sex (female/male) and diameters (4/6/8 mm) combinations (Tukey post-hoc test).

To assess the effect of feeding treatment (i.e., none, tape, petal) and sex of the individual on the feeding probability, we used a generalized linear model (GLM, package *lme4*) with a binomial error distribution. Survival between the group and individual housing trials were compared at 96 h for both sexes with a Fisher's exact test. In the individual housing trial, survival probability between sexes and feeding treatments for females (sucrose/starvation) was analyzed with a Cox proportional hazards model using the package *survival* (Therneau, 2022) and Kaplan-Meier survival plots were fitted and illustrated using *survminer* (Kassambara et al., 2021). Pairwise comparisons were performed using a Tukey post-hoc test. We tested model assumptions for all linear models and obtained residual diagnostic plots using the test 'Dispersion' and the 'simulateResiduals' functions with the package *DHARMa* (Hartig, 2022); assumptions were upheld in all models.

## 3. Results

### 3.1. Field trial

#### 3.1.1. Population establishment in the field

From the supplemented start populations in spring 2021, 45% of cocoons emerged (Table 1). In summer 2021, 627 cocoons of *Osmia brevicornis* were collected from 96 nesting tubes of a total of 358 nesting tubes originally placed out in the three pairs of trap nests (i.e., six trap nests from three sites). Trap nests supplemented with cocoons in April had a higher percentage of tubes occupied by *O. brevicornis* in summer 2021 (32%; N = 57/178) compared to trap nests that were not supplemented (22%; N = 39/180; GLMM, Z = 2.545, p = 0.011; Fig. 2A). There were on average 55% more cocoons in the cocoon-supplemented trap nests than in the control trap nests (Table 1) because of the greater occupancy of nesting tubes in trap nests supplemented with cocoons in early spring. The number of cocoons produced per occupied tube did not differ between treatments (Supplemented:  $\bar{x}$  = 6.7, SD = 4.4; Control:  $\bar{x}$  = 6.4, SD = 5.2; GLMM, Z = 0.518, p = 0.604).

Additionally, 342 cocoons of other species, including *O. bicornis*, *O. cornuta* and *Megachile* spp., were found in the trap nests. In total, 96 tubes were occupied by *O. brevicornis* and 88 by other species (Table 1). The percentage of tubes occupied by other species was significantly higher in the supplemented nests (42%, N = 75/178) compared to control nests (7%, N = 13/180; GLMM, Z = 7.365, p < 0.001). Though both *O. brevicornis* and other solitary bee species were often found nesting in the same trap nest, the nesting rate (i.e., percentage of tubes occupied) of other solitary bee species was not statistically related to the nesting rate of *O. brevicornis* (GLMM, Z = -1.653, p = 0.098). Co-nesting of *O. brevicornis* and other species occurred in 10% of the total number of occupied tubes (N = 19/184).

#### 3.1.2. Tube diameter effects on occupancy rate, sex ratio and body size of offspring

Cardboard tubes with inner diameters of 4 and 6 mm were more often occupied by *O. brevicornis* compared to 8 mm tubes (Tukey post-hoc, 4–8 mm, Z = -2.343, p = 0.049; 6–8 mm, Z = -3.677, p < 0.001; Fig. 2B), while we found no significant difference between 4 and 6 mm tubes (Tukey post-hoc, Z = 0.343, p = 0.343; Fig. 2B). No significant difference was found in the number of cocoons in occupied tubes between diameters (4 mm:  $\bar{x}$  = 6.2, SD = 3.5; 6 mm:  $\bar{x}$  = 5.9, SD = 2.7; 8 mm:  $\bar{x}$  = 9, SD = 4.6; Tukey post-hoc, 4–6 mm, Z = -0.279, p = 0.958; 4–8 mm, Z = 1.674, p = 0.212; 6–8 mm, Z = 1.986, p = 0.114). For other solitary bee species, the occupancy rate of nesting tubes was lower in tubes with 4 mm diameter compared to 6 or 8 mm (Tukey post-hoc, 4–6 mm, Z = 4.235, p < 0.001; 4–8 mm, Z = 5.150, p < 0.001), while no difference was found between 6 and 8 mm tubes (Tukey post-hoc, Z = 1.270, p = 0.410).

**Table 1**

Description of the treatments and contents of the six trap nests investigated in the field trial.

Pair	Treatment	Coordinates	<i>Osmia brevicornis</i>		Other species		
			Number of cocoons supplemented/ emerged	Tubes occupied	Number of cocoons	Tubes occupied	Number of cocoons
Aseleben	Control	51°29'00.7"N 11°39'29.6"E	0	29% (17/58)	78	10% (6/58)	32
Aseleben	Supplement	51°28'53.1"N 11°39'52.7"E	51/22 (43%)	32% (19/60)	104	42% (25/60)	127
Fienstedt	Control	51°34'00.2"N 11°47'52.1"E	0	18% (11/60)	113	12% (7/60)	32
Fienstedt	Supplement	51°33'54.3"N 11°47'14.8"E	51/23 (45%)	27% (16/60)	100	68% (41/60)	123
Süsser See	Control	51°31'25.3"N 11°39'47.0"E	0	18% (11/60)	55	0% (0/60)	0
Süsser See	Supplement	51°31'01.1"N 11°39'39.2"E	51/24 (47%)	37% (22/60)	177	15% (9/60)	28
<b>Total</b>	<b>Control</b>		<b>0</b>	<b>22% (39/ 178)</b>	<b>246</b>	<b>7% (13/178)</b>	<b>64</b>
	<b>Supplemented</b>		<b>153/69 (45%)</b>	<b>32% (57/ 180)</b>	<b>381</b>	<b>42% (75/180)</b>	<b>278</b>
	<b>Overall</b>		<b>153/69 (45%)</b>	<b>27% (96/ 358)</b>	<b>627</b>	<b>25% (88/358)</b>	<b>342</b>

The average sex ratio of *O. brevicornis* per nest (females/total) was skewed towards males at 4 mm diameter (99.9% males; 0.1% females; Fig. 2C), while 6 and 8 mm diameter tubes resulted in similar average sex ratios of 57% and 52% female offspring per nest, respectively (Tukey post-hoc, 4–6 mm,  $Z = 8.210$ ,  $p < 0.001$ ; 4–8 mm,  $Z = 4.589$ ,  $p < 0.001$ ; 6–8 mm,  $Z = -1.131$ ,  $p = 0.488$ ; Fig. 2C). Body mass among successfully emerged individuals varied significantly across tube diameters. Males developing in 4 mm tubes were significantly lighter ( $\bar{x} = 20.3$ ,  $SD = 5.2$  mg) than males from 6 mm ( $\bar{x} = 29.7$ ,  $SD = 8.8$  mg) and 8 mm ( $\bar{x} = 32.5$ ,  $SD = 7.5$  mg) tubes (Tukey post-hoc, 4–6 mm,  $F = 5.688$ ,  $p < 0.001$ ; 4–8 mm,  $F = 5.691$ ,  $p < 0.001$ ; Fig. 2D), while the weight of males from 6 mm tubes did not differ from those developing in 8 mm tubes (Tukey post-hoc,  $F = 1.278$ ,  $p = 0.698$ ; Fig. 2D). Females developing in 8 mm tubes ( $\bar{x} = 56.1$ ,  $SD = 9.8$  mg) were significantly heavier than females developing in 6 mm tubes ( $\bar{x} = 41.8$ ,  $SD = 11.6$  mg; Tukey post-hoc,  $F = 8.338$ ,  $p < 0.001$ ; Fig. 2D). Sex dimorphism was evident at both 6 and 8 mm diameters, with females being significantly heavier than males (Tukey post-hoc, 6 mm:  $m - f$ ,  $Z = -7.544$ , 8 mm:  $m - f$ ,  $Z = -10.091$ ,  $p < 0.001$ ; Fig. 2D).

### 3.1.3. Proportion of Brassicaceae pollen between species

The proportion of Brassicaceae pollen in provisions differed significantly between species (Kruskal-Wallis:  $\chi^2 = 13.574$ ,  $df = 1$ ,  $p < 0.001$ ), averaging 99.5% ( $SE = 0.2\%$ ) for *O. brevicornis* and 34% ( $SE = 9.2\%$ ) for *O. bicornis* (Fig. S2) when nesting at the same sites.

## 3.2. Laboratory trials

### 3.2.1. Feeding experiment

Attaching a petal to the feeder increased the ratio of individuals feeding ( $N = 20/29$  feeding) compared to tape ( $N = 6/27$  feeding; Tukey post-hoc,  $Z = 3.349$ ,  $p = 0.002$ ; Fig. 3) or when only the feeder was presented without a visual cue ( $N = 3/27$  feeding; Tukey post-hoc,  $Z = 3.985$ ,  $p < 0.001$ ; Fig. 3). The ratio of individuals feeding did not differ between tape and no visual cue (Tukey post-hoc,  $Z = 1.173$ ,  $p = 0.467$ ; Fig. 3). Males were more successful at feeding than females ( $N = 17/36$  males vs.  $N = 12/47$  females; GLM,  $Z = 2.252$ ,  $p = 0.024$ ).

### 3.2.2. Emergence and survival during group and individual housing

In the first incubation period from 3 to 17 May, the emergence rate was 49.2%, with the majority of non-emerged cocoons ( $N = 77/192$ ) containing dead adults, except for three cases of insect parasitism (by *Monodontomerus* sp., a parasitic wasp). Males emerged on days 1–5 ( $Mdn = 3$ ) after the start of incubation and females on days 5–12 ( $Mdn =$

8).

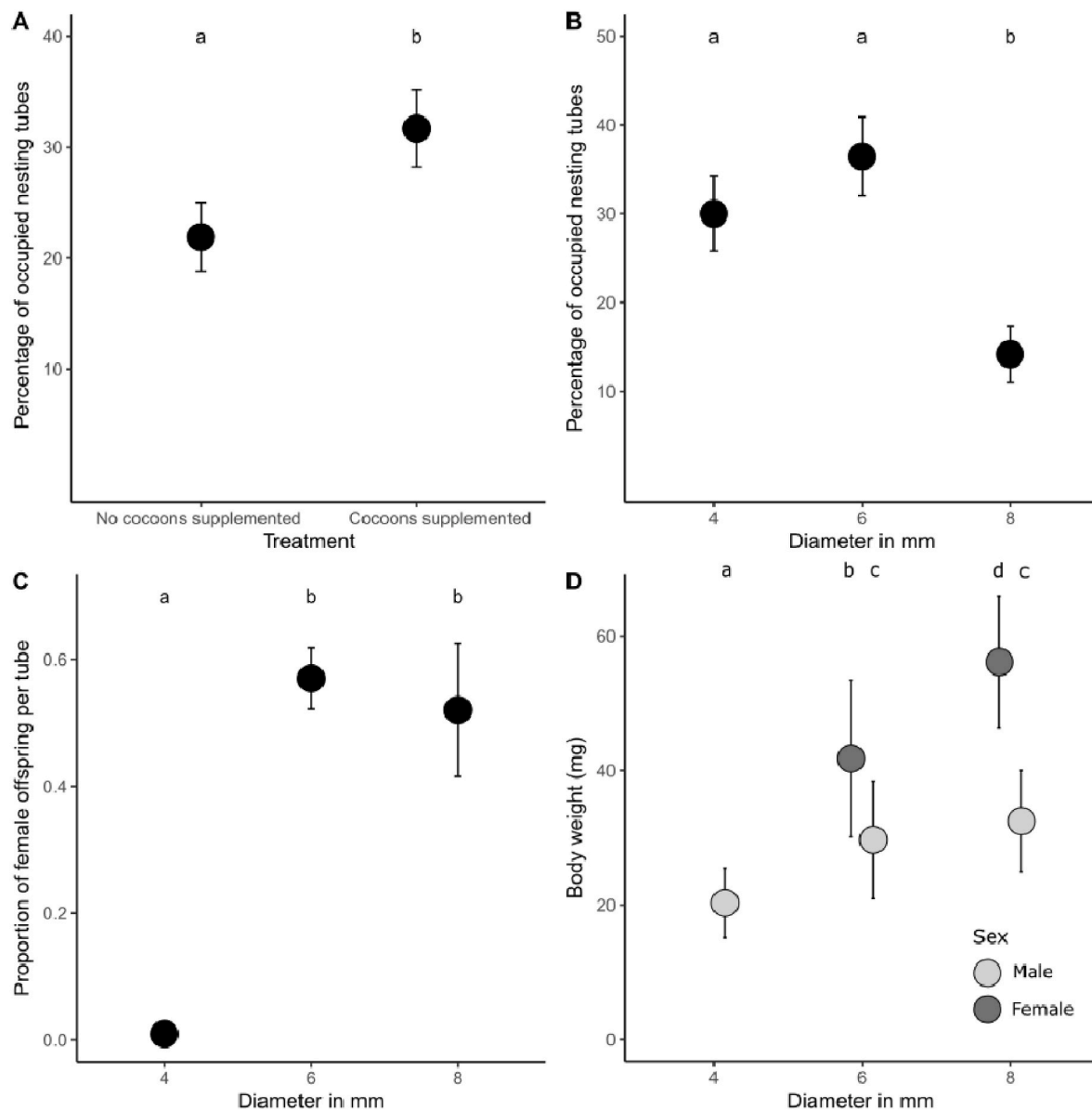
The emergence rate was higher later in spring, with 85.4% emergence from 11 to 17 June. Of the non-emerged cocoons ( $N = 36/245$ ), 13 cocoons contained dead larvae and the remaining contained dead adults. Here, males emerged 1–4 ( $Mdn = 4$ ) days after the start of incubation and females on days 4–7 ( $Mdn = 6$ ).

Survival after 96 h was higher during group housing than individual housing, both for males (group housing: 97.6%,  $N = 41/42$  vs. individual housing: 46.2%,  $N = 12/26$ ; Fisher's exact test,  $p < 0.001$ ) and females (group housing: 100%,  $N = 48/48$  vs. individual housing: 69.2%,  $N = 18/26$ ; Fisher's exact test,  $p < 0.001$ ). Survival during group housing was high across 21 days, with 61.3% ( $N = 19/31$ ) of males and 72.9% ( $N = 35/48$ ) of females surviving across the assessment period. During individual housing, survival between males and females with access to sucrose solution did not differ (Tukey post-hoc,  $Z = 1.381$ ,  $p = 0.350$ ; Fig. S3), with a mean lifespan of 5.2 ( $SD = 2.2$ ;  $N = 26$ ) days for males and 4.4 ( $SD = 1$ ;  $N = 26$ ) for females. Starved females had a significantly shorter lifespan ( $\bar{x} = 3.6$ ;  $SD = 1$ ;  $N = 25$ ) than females with access to sucrose (Tukey post-hoc,  $Z = 2.606$ ,  $p = 0.025$ ; Fig. S3). On average, females consumed 16.6 ( $SE = 1.7$ ) mg sucrose solution per day, while males consumed 14.1 ( $SE = 1.2$ ) mg.

## 4. Discussion

We here demonstrate that *O. brevicornis* can be successfully reared in trap nests, and that 1st tier PPP laboratory studies as well as 2nd and 3rd tier field studies may be possible to perform on this non-target insect species in its native range. Rearing this species brings an opportunity to study the effects of pesticide exposure on an oligolectic pollinator in Europe. In particular, *O. brevicornis* can be used for studies on pesticide use in OSR, which is one of the most intensively farmed crops in Europe (Lundin, 2021). Both sexes can survive in the laboratory and can be induced to consume sucrose solution. A flower petal used as a nectar guide increased feeding success and group housing appears to be more suitable than individual housing. This may enable their use in the assessment of acute and chronic oral toxicity to compounds (e.g., pesticides). Yet, additional research is needed to find optimal conditions for maintenance of the species in the laboratory.

*Osmia brevicornis*'s propensity to use trap nests enables the study of fitness parameters normally recorded in other *Osmia* species, such as cocoon number and size, occupation rate, emergence rate, and sex ratio of offspring. We also show that *O. brevicornis* collects mainly Brassicaceae pollen when placed next to OSR fields, in stark contrast to *O. bicornis* nesting at the same site. We therefore suggest it is suitable to



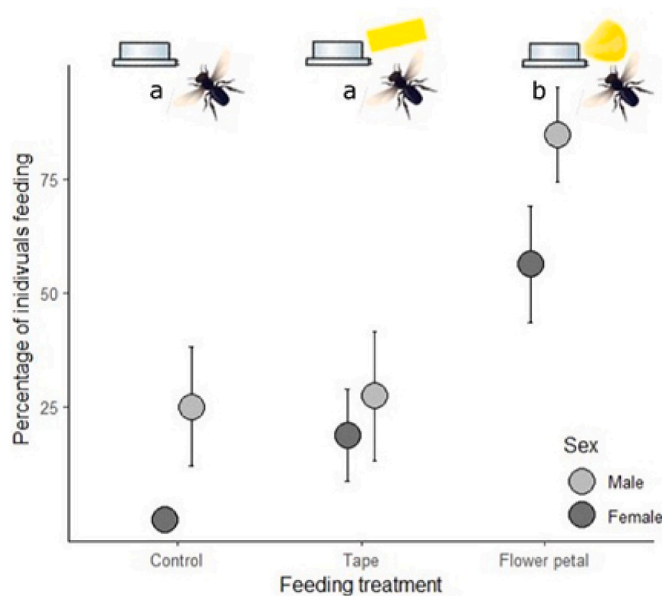
**Fig. 2.** (A) Percentage of cardboard tubes occupied by *Osmia brevicornis* without (No cocoons supplemented) or supplemented with 51 cocoons (Cocoons supplemented) of *O. brevicornis* before the start of the flight period; (B) percentage of occupied cardboard tubes per diameter; (C) Proportion of female offspring per tube for the different diameters; Mean (dot) and standard error (whiskers) are displayed (A–C); (D) body weight in mg of males (light grey) and females (dark grey) from nest tubes of three diameters. The single female found at 4 mm diameter has not been weighed. Mean (dot) and standard deviation (whiskers) are displayed. Different letters indicate significant differences ( $p < 0.05$ ).

be used in field studies investigating the impact of PPPs on non-target insects visiting Brassicaceae crops such as OSR, where *O. bicornis* would be less appropriate (Franke et al., 2021).

The nesting tube inner diameter affected occupation rate, sex ratio and offspring body size of *O. brevicornis*. Inner diameters of 4 and 6 mm were more often occupied by *O. brevicornis* than tubes of 8 mm diameter, which supports Westrich et al. (2011) observations that *O. brevicornis* prefer a diameter of 5 mm. Cocoons found in tubes with a diameter of 4 mm contained almost exclusively males, while the sex ratio in other tubes were almost equal (Fig. 2C). This indicates that nesting females adjust the offspring sex ratio in accordance with nesting tube diameter, as known in other Megachilidae (Stephen and Osgood, 1965; Tepedino and Torchio, 1989; Seidelmann et al., 2016). Similar to *Osmia lignaria* (Tepedino and Torchio, 1989), *O. brevicornis* female offspring possess a greater potential to increase in body size with tube diameter compared to males, as female, but not male, offspring size increased between 6 mm

and 8 mm diameters (Fig. 2D). It is interesting to note that sex dimorphism persists in *O. brevicornis*, despite this species not using physically separated brood provisions of varying sizes for males or females, in contrast to other species of the genus *Osmia* in which brood provisions of individual offspring are separated from each other within the same nest (Westrich, 2019). Because the size of male bees, unlike females, is kept constant regardless of tube diameter, it suggests that sex dimorphism in *O. brevicornis* may be driven by sex-specific genetic factors rather than food or space availability. Based on these findings, we recommend using nesting material with an inner diameter of 6 mm, as nesting tubes of this diameter had a higher occupancy rate than 8 mm tubes and a more balanced sex ratio compared to tubes of 4 mm diameter.

There was a high rate of nest occupation of *O. bicornis* and *O. cornuta* at some of our sites (Table 1), which could potentially interfere with nesting by *O. brevicornis* in the field. However, we did not detect an effect of the occupation rate by other species on the nest tube occupation



**Fig. 3.** Effect of feeding treatment (feeder design) on percentage of individuals successfully consuming sucrose solution for females (dark grey) and males (light grey). Mean (dot) and standard error (whiskers) are displayed. The letters refer to significant differences between feeding treatments (GLM;  $p < 0.05$ ).

rate of *O. brevicornis*. In fact, it was not uncommon to find co-use of *O. brevicornis* and other *Osmia* species in the same cardboard tube, even when empty tubes were still available.

We found a low rate of emergence of *O. brevicornis* supplemented cocoons in the field when compared to other *Osmia* species. While in this study only 45% of the supplemented cocoons emerged (i.e., were successfully opened by the adult insect within), the emergence rate for *O. cornuta* has been reported to be consistently above 90% (Bosch et al., 2021). In our study, supplemented cocoons were removed from natal nests and sorted into starting populations prior to placement in the field. The cocoons of *O. brevicornis* sit tightly packed together within a nest, in contrast to those of e.g., *O. bicornis* which are divided by mud walls. Thus, the risk of mechanical damage during extraction of *O. brevicornis* cocoons from a nest may be greater than for congeners, and best handling practices should be further developed for *O. brevicornis*. In May 2022, we found low adult emergence rates in the first laboratory trial (49.2%), with a large proportion of dead adults, similar to what we found in the field trial in 2021. Conversely, the emergence rate was high in the trial taking place one month later in June 2022 (85.4%). This suggests that pre-incubation treatment as well as timing and incubation conditions (i.e., temperature, humidity) may increase the rate of emergence, and this should be investigated further. As we show that *O. brevicornis* emergence can be artificially induced at different time points, this could further contribute to the usefulness of the species as a non-target species for testing PPPs, especially for OSR crop systems, as emergence can be artificially timed with OSR bloom.

While we cannot rule out that *O. brevicornis* may have foraged on Brassicaceae plants other than OSR, we demonstrate that *O. bicornis* collected significantly less Brassicaceae pollen compared to *O. brevicornis* when nesting at the same location. We can, however, not rule out that the differences in pollen preference between species was dependent on when the species were active in relation to OSR bloom, as no direct observations were performed during the flowering of OSR.

We could demonstrate here for the first time that *O. brevicornis* can be kept under laboratory conditions and that its survival is higher under group housing compared to individual housing, both at 96 h and longer. The relatively short lifespan of both males and females seen during the individual housing trial may be due to inadequate use of the feeder, as

the starvation trial with females confirms that females with access to food had only a marginally longer lifespan compared to starved females (maximum six days and five days, respectively), and that the average consumption across individuals was  $<20$  mg sucrose solution per day. In contrast, group housing in cages led to high survival rates across three weeks for both sexes, which may be the result of improved feeding, although food consumption was not directly monitored. From our experiments, we cannot determine if the low survival of individually housed bees was due to the differences in feeder design, the presence of multiple individuals in the same cage and potential learning between individuals, or the timing at which the trial took place. We therefore encourage further experiments to fine-tune the design of cages and feeders in order to support optimal survival, as small changes in feeder design or photoperiod may lead to large changes in survival among solitary bees housed in the laboratory (Tadei et al., 2022).

Requirements for a species to be used for pesticide exposure experiments includes easily measurable fitness endpoints and life history traits representative of a larger group of bees and, ideally, commercial availability to enable large enough sample sizes (Sgolastra et al., 2019). To date, *O. brevicornis* is not commercially reared, thus requiring trap-nesting prior to an experiment. This is feasible in its natural range, as both Schenk et al. (2018) and our study demonstrate. Other species that are not commercially available have been used in pesticide effect studies, although their use in ERAs may be limited (Dietzsch and Jütte, 2020; Willis-Chan et al., 2019). Despite its differences in foraging preferences, *O. brevicornis* nevertheless belongs to the same family (Megachilidae) as several other solitary bee species commonly used in pesticide risk assessment. If we are to assess the impact of PPPs on a wider spectrum of flower visitors, bee species from other families should also be considered for use in pesticide testing, including species nesting below ground (Franklin and Raine, 2019).

In this study, we have confirmed the feasibility of using *O. brevicornis* as an experimental model organism that in the future could be used to study the response of a non-target beneficial insect to exposure to pesticides used in OSR, as well as to study pollen specialists' general ecology. The interplay between nutrition and agrochemical hazard is receiving increasing attention (Klaus et al., 2021; Knauer et al., 2022; Wintermantel et al., 2022), and using pollen specialist species further expands this important topic from a conservation perspective (Rasmussen and Madsen, 2022). For such a diverse group of species as the bees, the reliance on a handful of study species for ecotoxicological experiments may limit the scope of what the research community can achieve in terms of general conclusions. We suggest that empirical research into oligolectic species should be expanded, where practicality allows. We also stress the importance of matching one's test organism to the system under study, thus avoiding the drawing of inaccurate conclusions.

#### Author contribution

S.H.: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - Original Draft. W. H. A. O.: Conceptualization, Methodology, Investigation, Writing - Review & Editing. V. S.; L. S.: Methodology, Investigation, Data Curation, Writing - Review & Editing. R. J. P.: Funding Acquisition, Writing - Review & Editing. J. O.: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - Original Draft, Supervision, Project administration.

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## Data accessibility statement

The raw data files supporting the conclusions of this article are available for download at: <https://doi.org/10.5281/zenodo.7625615>.

## Declaration of competing interest

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

## Data availability

The raw data files supporting the conclusions of this article are available for download at: <https://doi.org/10.5281/zenodo.7625615>.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.indic.2023.100239>.

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