

Guidelines

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Protocol for assessing the impacts of the insecticide Mospilan SG (acetamiprid) and the fungicide Folicur (tebuconazole) and their combination on the solitary bees *Osmia bicornis* and *O. brevicornis* under semi-field conditions

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1 Context and aim of the study

1.1 Objectives

This semi-field experiment aims to determine the effects of the insecticide Mospilan SG (active ingredient (a.i.): acetamiprid) and the fungicide Folicur (a.i.: tebuconazole) and their combination at realistic exposure levels on two *Osmia* species (*O. bicornis* and *O. brevicornis*).

The protocol is based on the EFSA guidelines for risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees; Annex C – Recommendations for higher tier effect studies) (European Food Safety Authority (EFSA) et al., 2023), the study plan for semi-field work for *Osmia bicornis* of the PoshBee project (Albrecht et al., 2019), the protocols for semi-field and field experiments - Milestone 15 document of the PoshBee project (Allan et al., 2023) and follows recommendations by the ring testing of a semi-field study design to investigate potential impacts of plant protection products on the solitary bees *Osmia bicornis* and *O. cornuta* (Franke et al., 2021).

The experiment is part of the EU project WildPosh, which aims to assess the relationships between pesticides and pollinator health using integrated and controlled laboratory and semi-field experiments (Michez et al., 2025). As part of this initiative, the sensitivity of a range of different pollinator species, including the test species of this experiment, *O. bicornis* and *O. brevicornis*, has already been assessed under laboratory conditions. This semi-field experiment complements the laboratory analyses by testing pesticide effects on the two *Osmia* species under field-realistic conditions.

1.2 Background and general study design

The effects of pesticides on different pollinators can vary between different insect pollinator species, as well as within species (Bass et al., 2024; Dewaele et al., 2024; Jütte et al., 2023; Linguadoca et al., 2022; Nagloo et al., 2024). However, most toxicological studies and risk assessments have focused on the western honeybee *Apis mellifera* (Franklin & Raine, 2019; Nagloo et al., 2024). This overlooks the huge diversity of wild bee species of which many have different physiological and behavioral traits which may influence pesticide susceptibility and exposure routes (Raine & Rundlöf, 2024). While the new EFSA guidelines also include other generalist bee species (*Bombus terrestris* and *O. bicornis*) (Williams et al., 2023), oligolectic species still remain underrepresented (Arena & Sgolastra, 2014; Hellström et al., 2023) even though they experienced greater range declines than generalistic species (Biesmeijer et al., 2006; Rasmussen et al., 2022).

Another critical issue is that most studies and risk assessments assess the toxicity of single compounds, while bees are typically exposed to multiple pesticides simultaneously (Honert et al., 2025; Knapp et al., 2023; Lehmann & Camp, 2021). As interactions between pesticides that amplify toxicity (i.e. synergistic effects) may occur (Siviter et al., 2021) leading to an underestimation of the pesticide risks to pollinators (Robinson et al., 2017).

In this study, we aim to compare the toxicological effects of Mospilan SG and Folicur and their interaction at realistic exposure levels on an oligolectic and a polylectic species. As *O. bicornis* is proposed as a new complementary model species for risk assessment (Williams et al., 2023) the generalist species is to be compared with a closely related oligolectic species from the same genus, *O. brevicornis*, which has proved to be a suitable oligolectic model species under laboratory conditions (Hellström et al., 2023).

To address the research aims adults of the solitary bee species (*O. bicornis* and *O. brevicornis*) will be released in flight cages (each approximately 53m² in ground area) located on an experimental field site in Freiburg, Germany.

We will conduct a full-factorial semi-field experiment with a total of 40 flight cages and 4 different treatments, resulting in 10 flight cages per treatment (Mospilan SG, Folicur, combination of Mospilan SG and Folicur, negative control). The formulated products are sprayed according to label recommendations for application on oilseed rape.

The flight cages will be sown with the flowering plants *Sinapis alba* and *Phacelia tanacetifolia* to meet both species nutritional demands. Since *O. brevicornis* is specialized in collecting pollen of Brassicaceae, *Sinapis alba* is used as a main resource for the experiment. In contrast, Klaus et al. (2021) found, that oilseed rape monocultures, a Brassicaceae, negatively affected the amount of brood and their development in *O. bicornis* compared to cages containing oilseed rape and a floral mix. Providing the two bees species with its optimal diets is important to avoid effects of the pesticides being induced by suboptimal diet. Therefore, *Phacelia tanacetifolia* will be used as complementary flowering resource for *O. bicornis* in particular. The plant species is commonly used for pesticide testing on bees (European Food Safety Authority (EFSA) et al., 2023) and has proved to be a suitable model crop in higher tier trials with *O. bicornis* (Franke et al., 2021; Lückmann et al., 2018; Schwarz et al., 2022).

1.3 Hypotheses

The following hypotheses will be tested:

- **H1:** Mospilan SG (a.i. acetamiprid) negatively affects survival and foraging behavior of both bee species and reduces pollination services.
- **H2:** Folicur (a.i. tebuconazole) does not affect survival, foraging behavior and pollination services when applied alone.
- **H3:** Folicur increases the toxicity of Mospilan SG (synergistic interaction).
- **H4:** Compared to *O. bicornis*, *O. brevicornis* is more strongly affected by Mospilan SG or the combination of Mospilan SG and Folicur, showing more pronounced treatment effects on survival and foraging behavior.

2 Study set-up

- Experimental timeline

The study will consist of a pre-exposure, exposure and post-exposure period (detailed timeline see Figure 1).

- Pre-exposure = before pesticide application
 - Exposure period = starting with pesticide application and lasts as long as the adult bees fly inside the flight cages. Duration: a minimum 7 days, better 10 days
 - Post-exposure = starts after the exposure period until the hatching and assessment of F1-generation
- The day of pesticide application will determine day 0 of the experiment.

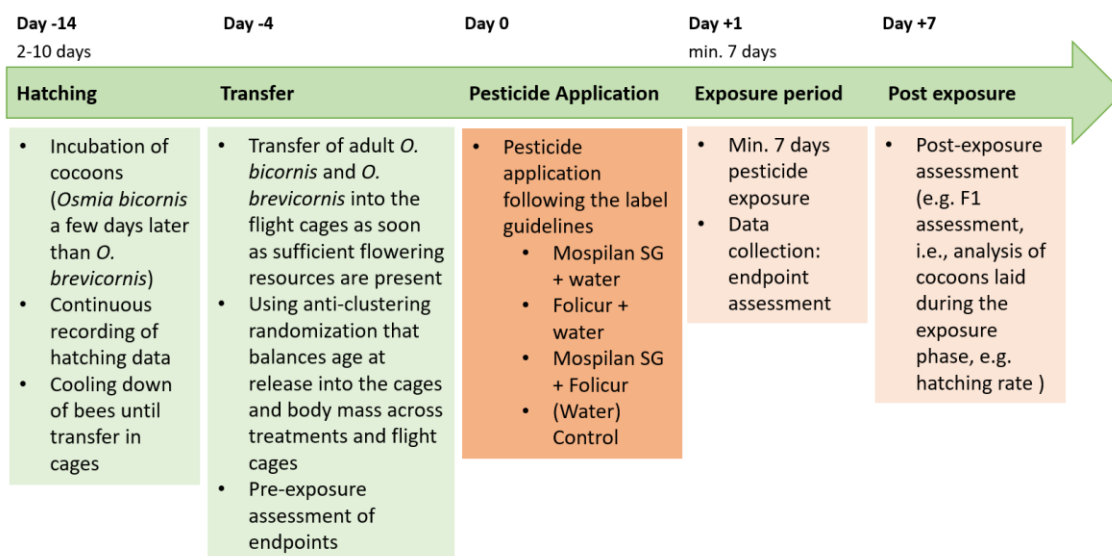


Figure 1. Overview experimental timeline.

- Spray treatments

In total 4 spray treatments will be applied to assess pesticide toxicity to *Osmia* under semi-field conditions:

1. Negative control group (two water applications)
2. Mospilan SG (a.i. acetamiprid) and water
3. Folicur (a.i. tebuconazole) and water
4. Combined treatment: Mospilan SG + Folicur (a.i. acetamiprid + a.i. tebuconazole)

As no positive control is included, residues will be taken for validation of treatments (see below 11.).

- Replicates

In total 40 flight cages and 4 treatments will be conducted, which will lead to 10 replicates per treatment.

- Test organisms

Osmia bicornis and *O. brevicornis* females and males will be used as test organisms simultaneously in the flight cages (depending on their natural occurrence and the flowering of floral resources the testing period for both species will be in May/June). Cocoons were purchased from Pollinature (Wildbiene + Partner AG; *O. bicornis*) and from Imkereij Bienenwiese Priester (*O. brevicornis*). For both species, pre-exposure from pesticides during larvae stage cannot be excluded, but exposure possibilities should not vary within species. Adult mortality and other endpoints (see below 9.) will be assessed during exposure phase.

All life stages (F1-generation) will be assessed post exposure and with photo evaluation (see 9.3.).

- Flight cages

The flight cages are from Howitec (9 m × 5.9 m). All cages will be covered with nylon nets (mesh size = 0.95 × 1.35 mm), which will be dug into the ground in order to prevent escaping of bees.

- Nesting units

In each flight cage, two nesting units (one per species and with different cavity diameters) will be installed at approximately 1.5m height. A housing structure that protects each nesting block from rain will be installed. To minimize nesting competition 4 cavities per released female will be offered (minimum of 1.1 to 3.6 cavities per released female; Franke et al., 2020). As soon as the assessment of nesting behavior (see 9.1) shows that the cavities are becoming too full of nests, more nesting layers are added in the flight cage.

Co-nesting of both species can occur but is expected to be reduced by providing two different holes sizes based on their respective nesting preferences (Hellström et al., 2023). For *O. brevicornis* cavities with an inner diameter of 5 mm are used, which lies at the lower end of the suitable range for *O. bicornis* (Hellström et al., 2023). For *O. bicornis* cavities of 8 mm are used as their optimal range are 8 - 10 mm (Hellström et al., 2023; Seidelmann et al., 2016) which should keep the co-nesting rate low. However, cases of co-nesting will be recorded (see below 9.1).

The nesting blocks for *O. bicornis* will contain eight layers (1.6cm x 16cm x 16cm MDF layer) each with ten 8 mm cavities, resulting in a total of 80 cavities per nesting block (20 females per flight cage expected). Nesting blocks for *O. brevicornis* will contain four layers (1.9 cm x 15 cm x 15 cm MDF layer) with ten 5 mm cavities each, resulting in 40 cavities in total (10 females per flight cage expected). Each nesting layer is open on top and covered with a transparent (acetate) sheet (labeled with a unique ID incl. cage ID, species and layer ID) to observe nesting progress and sleeping individuals (see below 9.1).

The labeling scheme will be:

- Cage IDs: C10 – C49 depending on the position of the cage
 - Row 1: C10-19
 - Row 2: C20-29
 - Row 3: C30-39
 - Row 4: C40-49
- Species:
 - *O. bicornis* = OBI
 - *O. brevicornis* = OBR
- Layer IDs:
 - *Osmia brevicornis* layer: OBR_B1, OBR_B2, OBR_B3, OBR_B4
 - *Osmia bicornis* layer: OBI_B1, OBI_B2, OBI_B3, OBI_B4, OBI_B5, OBI_B6, OBI_B7, OBI_B8
- As an example: **C10_OBR_B1** (Cage No. 10, *O. brevicornis* layer (5mm cavities diameter), board No. 1 out of 4)

3 Pesticide test items

- **MOSPILAN SG** (formulated product)

Active ingredient: **Acetamiprid** (insecticide)

registration number: 005655-00

Content of a.i. nominal: 200 g/kg acetamiprid (20.0 % by weight)

Intended usage: Insecticide

Manufacturer: FMC Cheminova

Risk symbols: H302, H361d, H400, H410 (Mospilan SG)

Storage conditions: Keep the container tightly closed in a dry, well-ventilated place. Carefully close opened containers and store upright to prevent any leakage. Electrical installations/equipment must comply with the state of the art in safety technology. Storage instructions: Store separately from foodstuffs and animal feed. Do not store together with strong oxidizing agents. Further information on storage conditions: Protect from heat and direct sunlight. Keep out of reach of children.

- **FOLICUR** (formulated product)

Active ingredient: **Tebuconazole** (fungicide)

registration number: 034028-00

Molecular weight: 307.82 g·mol⁻¹

Intended usage: Fungicide

Manufacturer: Bayer CropScience

Risk symbols: H302, H332, H318, H335, H361d, H400, H410 (Folicur)

Storage conditions: Store in the original container. Keep container tightly closed in a dry, cool and well-ventilated place. Store in a place accessible only to authorized persons. Protect from direct sunlight. Protect from frost. Keep away from food, drink and animal fodder. Keep away from sources of heat and ignition. Storage period: Folicur has a shelf life of at least two years, see imprint on the packaging.

4 Study design

- The study site will be in Freiburg, Germany (48°01'08.5"N 7°49'31.2"E) on a university-owned experimental field. The total area of the study land will be approximately 0.7 ha. There was no pesticide application over the last four years on the study site.
- A total of 40 flight cages (each approximately 53 m²) will be set up. All flight cages entrances and entrances of the nesting units will be facing in the same direction.
- Every flight cage will be provided with the same flower resources: The flowering resource will be *Phacelia tanacetifolia* (Balo) and *Sinapis alba* (Attack) to provide a sufficient food resource for both species. Since *O. brevicornis* is specialized on Brassicaceae and *O. bicornis* is a generalist, proportionally more of *S. alba* compared to *P. tanacetifolia* is sown. The seeds were purchased from ZG Raiffeisen Agrar (Endingen, Germany) and sown together on 7th April 2025 as a mixture: 10kg/ha *S. alba* and 3 kg/ha *P. tanacetifolia*. Eleven days after the first sowing using a tractor, the same seed mixture of the two plant species will be sown again by hand (0,6g/qm) to extend the flowering period, synchronize the flowering of crops and ensure consistent flower density. The flight cages will be set up 4 weeks after sowing.
- The spray treatments will be allocated to the flight cages using an anti-clustering randomization. The individuals within the *Osmia* species will be allocated to the flight cages using anti-clustering randomization that balances age at release into the cages and body mass after hatching across treatments and flight cages.
- In each flight cage, there will be a hole dug in the soil in proximity to the nesting units which will be filled with water to provide mud for the females for nest construction (see Knauer et al., 2022).

5 Pre-exposure phase: Hatching of *Osmia*

Cocoons will be placed in an incubator to trigger hatching of the bees. Incubation of *O. bicornis* cocoons will start around six days before the release into the cages (incubation can take place separated for females/males due to differences in cocoon size for *O. bicornis*). Incubation of *O. brevicornis* cocoons will start around 8 to 9 days before release into the cages.

- Hatching in the incubator: *O. bicornis* at 22/23°C; *O. brevicornis* at > 25°C
- Daily checks for hatched individuals
- Hatching data is recorded continuously
- After hatching, bees will be separated by sex and immediately transferred to transparent boxes, supplied with syrup solution and placed in a cold chamber at 4 °C to transfer all adults at the same time to the flight cages and to minimize variability between the individuals
- Before assigning (using anti-clustering randomization) the bees to the flight cages based on the age and body mass (females), the body mass is determined by weighing all females individually and then dividing them into four weight classes based on previous data collected in 2024
- Number of individuals per spray treatment (might slightly change depending on the hatching rate of the species, which is expected to be > 70% for *O. bicornis* and around 50% for *O. brevicornis* based on hatching data of a laboratory experiment conducted in Freiburg in 2024):
 - *Osmia bicornis*: Per cage appr. 20 females and 20 - 30 males will be released.
 - *Osmia brevicornis*: Per cage appr. 10 females and 10 males will be released (with a total of 10 flight cages per treatment).

The female:male ratio will be between 1:1 and 1:1.5 depending on hatching rate and sex ratio of hatched individual.

- Sufficient floral food resources (i.e., open flowers; at least 25% of *S. alba* have reached BBCH 61) have to be present in the flight cages when the bees will be released.
- Optional: Depending on the development and behavior of the species, as many males as possible can be removed from the flight cage after they mated to eliminate them as a disturbing factor for behavioral parameter assessment as endpoints are not assessed for males.

6 Spray application

- The application of spray treatments will take place after females start to nest and determine Day 0 of the experiment (Application Day), which will be approx. 3-4 days after releasing the bees into the flight cages.
- First, Folicur will be applied at daytime (following the label guidelines) in the cages assigned to the treatments of Folicur and the combined treatment (20 cages, 10 single treatment Folicur, 10 combination treatment). Simultaneously the other cages (10 cages water control and 10 cages Mospilan SG) are sprayed with the water control with the same amount of L/ha. Mospilan SG will be applied in the evening of the same day (following the german label guidelines) in the cages assigned to the treatments of Mospilan and the combined treatment (10 cages single treatment, 10 cages combined treatment). Simultaneously the other cages (10 cages of water control and 10 cages of Folicur) are sprayed with the water control with the same amount of L/ha.
- The application rates follow the recommendations for oilseed rape/*Sinapis* (Table 1). According to OECD No. 75, treatments will be applied at the highest recommended field rate (g/ha) in order to produce a worst-case exposure scenario.

Table 1. Application rates for the treatments.

Treatment	Application rate
Acetamiprid (product Mospilan SG)	200g/ha in 400L/ha water
Tebuconazole (product Folicur)	1.5L/ha in 300L/ha water
Combined treatment (Mospilan SG and Folicur)	1.5L/ha Folicur in 300L/ha water (daytime) and 200g/ha Mospilan SG in 400L/ha water (evening)
Water (negative control)	300 L/ha (during Folicur application), 400L/ha (during Mospilan SG application)

- Products will be applied in the flight cages with hand-held spray equipment, when there is no wind (< 2m/s) outside the flight cage (OECD, 2014). The sprayer will be filled with the exact amount of spray liquid (Table 1) which is needed for the area, so that the exact amount of treatment will be applied.
- Nesting units and water reservoirs will be covered with plastic foil during spraying to prevent spray drift into nests. Flight cages will be covered with plastic sheets during the application to avoid spray drift. Spraying of the covering gauze is avoided (OECD, 2014). To avoid cross-contamination the spray tanks and spray equipment will be cleaned after each treatment application.
- To confirm pesticide exposure, residue analyses of pollen, nectar and bees will be conducted on samples taken on day +1 of the pesticide application(s) (see below 11.).

7 Meteorological data

During the whole testing period daily recording of data (OECD, 2014):

- Temperature (min, max, mean)
- Rel. humidity (min, max, mean)
- Rainfall (total daily)
- Wind speed during product application (In and outside of the flight cages)
- Cloudiness (during assessment of flying bees)

8 Assessment of experimental conditions

- **Flower abundance:** number of open flowers (for both species) within three 1 x 1m quadrats will be counted (can be estimated based on the number of inflorescence and the average number of open flowers per inflorescence estimated on five inflorescences). For the position of the quadrats, the ground area of the flight cage is divided into 6 plots and the quadrats are placed in each assessment in 3 plots that are randomly selected without replacement. After two assessments, all plots will have been assessed and random selection without replacement is repeated. The assessment will be done before exposure and after exposure approximately every three days, in order to ensure sufficient pollen/nectar sources.

9 Assessment of endpoints

Assessment will be done separately for both bee species. Where possible, an assessment will be carried out prior to pesticide application (pre-exposure phase when the bees are already in the flight cages). All endpoints will be assessed at least two times during exposure period if weather conditions allow assessment.

9.1 Assessments on *Osmia* fitness i.e. survival and reproduction

- **Establishment of females/count of nesting females:** the number of nesting females will be counted at night after 9 pm, starting at least at the day before pesticide application (day -1) until the exposure period ends (this can be done after the exposure phase with photo evaluation). If possible, this assessment is made at least every second night.
- **Adult mortality:** mortality of adult females (additionally adult males in the first assessment) will be calculated: number of females roosting in the layers during the night (see establishment of females/count of nesting females) minus the number of females roosting at the following assessment.
- **Nesting behavior and egg/larval/pupal assessment:** will be assessed by photographing of nest layers (open on top and covered with a transparent sheet (labeled with a unique ID incl. cage ID, species and layer ID) to observe nesting progress). Shortly before exposure the extent of nest construction (brood cells for *O. bicornis*, pollen provision for *O. brevicornis*) up to that point will be marked with a permanent marker. It is then assumed that the nests provided prior to that point will not be exposed to the applied product. At each assessment after day 0 the extent of nest/cell production is marked on the acetate cover of each layer with a waterproof marker and photographed thereafter. To make the photo evaluation easier, different colors are used depending on the day of assessment. If possible, this assessment is made at least every second night. Labels on nest layers need to be visible in each picture. With the photographs for each flight cage, nesting unit ID and layer ID the following parameters are measured:
 - Number of (new) brood cells (*O. bicornis*) produced since the last check; number of nests/eggs produced since the last check (*O. brevicornis*)
 - Additionally, co-nesting (i.e. number of brood cells (*O. bicornis*) or number of nests/eggs (*O. brevicornis*) of the species nesting in the layer which was provided for the other species) will be recorded.
 - *Osmia bicornis*: Status of each brood cell: each brood cell is given a unique ID (layerID + cavities ID + position): record for each brood cell, whether it (a) contains no pollen, it contains (b) only pollen (no egg visible); (c) an intact egg, (d) a dead egg, (e) a living larvae, (f) a dead larva; (g) a living pupa, (h) a dead pupa, (i) a cocoon; also record disease signs: record if brood cell contains: (n) a natural enemy, (s) signs of natural enemy attack visible, (m) or if mold is present.
 - *Osmia brevicornis*: status of each provided nest: (a) number of intact eggs, (b) number of dead eggs, (c) number of living larvae, (d) number of dead larvae, (e) number of living pupae, (f) number of dead pupae, (g) number of cocoons; (n) a natural enemy, (s) signs of natural enemy attack visible, (m) or if mold is present.
 - The following parameters are determined with this assessment:
 - failure to lay egg (pollen provision but egg is lacking)
 - egg mortality
 - larval mortality
 - pupal mortality
 - offspring production per female
 - parasitism rate
- **Offspring assessment:** F1 generation will be assessed post exposure (when F1 generation will hatch triggered by incubating the cocoons, probably April/May 2026). Following parameters will be assessed (see below 9.3): **Hatching rate, Mass of offspring, Sex ratio of offspring.**

9.2 Assessment on *Osmia* foraging behavior and pollination services

All assessments regarding pollination services will be conducted during daytime (10 am – 4 pm when bees are flying; on the same day for all treatments).

- **Individual foraging performance and flower handling** (i.e., number of flower visits per bee, time per flower visit): number of individual flowers visited by a bee during a 4-minute period will be counted. This will be done for at least three females per flight cage. This will be assessed only under the following conditions: no rain, wind < 2 m/s. Additionally, the time the individual bee spends on a flower (flower handling) and the flower species (*S. alba* or *P. tanacetifolia*) will be noted. If the bee is not found any more after < 2 minutes or does not continue its flight, the recording is discarded, and a new bee is observed. If the bee is not found after > 2 minutes the observation is kept, and the remaining time will be covered with another bee.
- **Flower visitation rate and forager rate** (i.e., number of foraging bees per area and time and number of flower visits per area and time): number of females foraging within a square (2 x 2 m) will be counted for a 4-minute period. Bees that leave the square and re-enter and can be identified as the same bee are not counted twice. This will be done for at least three different selected squares per flight cage. For the position of the quadrats, the ground area of the flight cage is divided into 6 plots and the quadrats are placed in each assessment in 3 plots that are randomly selected without replacement. After two assessments, all plots will have been assessed and random selection without replacement is repeated. The position of the plots correspond with the position of the plots for the assessment of flower abundance (which will be done at the same day if possible). Additionally, flower visits in the quadrats will be counted. These assessments will be done only under the following conditions: no rain, wind < 2 m/s.
- **Fruit set/seed set**: number of fruits per flower of *Sinapis alba* (optionally: additionally for *Phacelia tanacetifolia*) will be assessed. Therefore, in the evening in each flight cage inflorescences will be marked which have about 5 flowers that are about to open. All flowers that are already open at this point will be removed. On the following day, all flowers that are still closed will be removed and the number of freshly opened flowers is noted. After about 2 weeks, when the fruits of the marked inflorescences have ripened, the number of developed fruits (ratio of opened flowers/developed fruit) as well as the number of seeds per fruit will be assessed. This assessment will be done one time pre-exposure when the bees will be inside the flight cages and if the weather allows 2 times after pesticide application.
 - **Seed mass and seed oil content** (optional parameters): 20 seeds per flower will be randomly selected to measure seed dry mass, therefore will be dried and then weighted.

Exposure period will end when there are only few or no nesting females or when floral resources risk to get scarce, after a minimum of 7 days.

9.3 Post-exposure assessment

After exposure period the females of both species will be removed from the nesting units/layers and the nests remain at the test site covered with to prevent parasitism. During winter the cocoons will be stored in a cooling chamber at 4°C. Therefore, all developed cocoons will be removed from the nesting units (using a tweezer or other tools) and separated into boxes/petri dishes per flight cage (per sex for *O. bicornis*) in order to assess offspring separately for each flight cage (labeled with species, flight cage-ID and layer-ID). Before removing the cocoons for each layer, the status of each brood cell (*O. bicornis*) and each nest/egg (*O. brevicornis*) will be assessed again (see above).

After overwintering at 4°C the F1-Generation is assessed:

- **Weighing of cocoons:** Cocoons will be weighed individually.

Cocoons will be incubated after weighing in order to trigger hatching (probably in April/May 2026). The following parameters will be assessed:

- **Hatching rate:** number of hatched offspring/total number of cocoons. Hatching rate will be assessed for each flight cage/treatment separately (cocoons which will not emerge after 14 days will be counted as “not emerged”).
- **Body mass of offspring** (weighing of hatched offspring): A random subset of the hatched offspring will be weighed individually and the average weight of offspring for each treatment will be estimated.
- **Sex ratio of offspring:** Ratio of hatched females:males will be estimated for each treatment.
- **Natural enemies:** If natural enemies will appear they will be allowed to emerge, counted and identified to an appropriate taxonomic level.

10 Evaluation of the test and analysis plan

Evaluation of the results will be done by comparing the results in the pesticide treatments to the water control (OECD, 2014) and by comparing the combined pesticide treatments to both the single pesticide treatments (or their predicted additive effect) and the water control.

If any treatment effects are found, the effects in the combined spray treatment are compared with a predicted additive effect (based on the effects of the single spray treatments) to analyze possible synergistic or antagonistic interactions between the pesticides. Effect levels of the treatments on *O. bicornis* and *O. brevicornis* will be compared.

We will use (G)LMs or (G)LMMs with cage ID as random factor (where multiple observations are taken per cage and timepoint or to account for overdispersion). These models will contain treatment as fixed factor/predictor along with other co-variables. These may include day since application, time of day (for variables on flight behavior), flower abundance, number of adult females (for offspring) and/or pre-exposure estimates of relevant endpoints. Interactions between treatment and time are included for variables where growth over time is regarded. The choice of independent variables (fixed effects) will depend on their expected impact on the dependent variable (response) and model fits.

Models will be evaluated using the `emmeans` function of the *emmeans* package to obtain estimated marginal means. In addition, uncertainties in differences between the two *Osmia* species and differences between predicted additive effects and observed effects of the combined treatment will be obtained from non-parametric bootstraps.

11 Samples to collect

To confirm pesticides exposure different samples will be collected at day +1 after pesticide application, and pesticide residues will be analyzed (Table 2). All samples will be collected following the protocol of WP1 of the WildPosh project.

Pollen will be collected from the flowers of both plant species using a vacuum device. One sample consists of 50 mg of pollen per plant species from any flowers in the specific cage. Nectar will be collected with micro-capillary tubes and need to happen early in the day under dry conditions. One sample consists of 20 µL nectar per plant species. Samples need

to be placed over ice packs in a cold box after transferred into a centrifuge tube. For the bee samplings, living bees of both species will be caught and stored directly at -20°C in labeled tubes. For the pesticide solutions, samples are filled directly after application and stored in a cool place (4°C).

- Pollen, bees and solutions are to be shipped to Tomasz Kiljanek (Panstwowy Instytut Weterynaryjny - Panstwowy Instytut Badawczy (PIWET)) for residue analysis to confirm pesticide exposure (number and sample size Table 2).
- Nectar is to be shipped together to Marion Laurent (Agence Nationale De La Sécurité Sanitaire De L'alimentation De l'environnement Et Du Travail (ANSES)) for residue analysis to confirm pesticide exposure (number and sample size Table 2).

The samples will be labeled using a scheme including the Working Package, treatment, Species, Cage-ID, sampling type and sampling date to be best possible in accordance with samplings of WP1:

- Sample type:
 - N = Nectar
 - P = Pollen
 - Bees = T (Transect bee)
- As an example: **WPosh2_Mospilan_Phacelia_C10_N01_26-05-2025** (Pollen and Nectar), **WPosh2_Mospilan_OBI_C10_N01_26-05-2025** (Bees)

Table 2. Samples to collect to confirm pesticide exposure.

Treatment	Pollen	Nectar	Bees	Pesticide solutions
Acetamiprid (Mospilan)	3 x 50mg Phacelia 3 x 50mg Sinapis	3 x 20µL Phacelia 3 x 20µL Sinapis	3 x <i>O. bicornis</i> 3 x <i>O. brevicornis</i>	1 x 2mL
Tebuconazole (Folicur)	3 x 50mg Phacelia 3 x 50mg Sinapis	3 x 20µL Phacelia 3 x 20µL Sinapis	3 x <i>O. bicornis</i> 3 x <i>O. brevicornis</i>	1 x 2mL
Combination (Aceta. + Tebu.)	3 x 50mg Phacelia 3 x 50mg Sinapis	3 x 20µL Phacelia 3 x 20µL Sinapis	3 x <i>O. bicornis</i> 3 x <i>O. brevicornis</i>	-
Control (water)	3 x 50mg Phacelia 3 x 50mg Sinapis	3 x 20µL Phacelia 3 x 20µL Sinapis	3 x <i>O. bicornis</i> 3 x <i>O. brevicornis</i>	-
Total	24 (12 x 50mg per plant species)	24 (12 x 20µL per plant species)	12 Individuals per Species	2 x 2mL

12 Materials

[Material list for semi-field study 2025 Link](#)

13 Acknowledgment

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