Report on PoshBee external protocols, tools, best practice guides

Deliverable D10.7

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PoshBee

Pan-european assessment, monitoring, and mitigation of stressors on the health of bees

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Preface

PoshBee's aim is to support sustainable pollinators and sustainable pollination services in European agri-ecosystems. One way it will achieve this is through the production of an array of public- and stakeholder-oriented protocols, tools, and best practice guides for practitioners. This deliverable provides a list of these key outputs, with a guide to their availability and content.

Summary

PoshBee, as a multi-actor project, aims to support managed and wild pollinator populations and sustainable pollination services in agricultural ecosystems across the European Union and associated countries. A key goal in PoshBee is to produce an array of valuable protocols, tools, and best practice guides to support stakeholders and practitioners in their pollinator-focused work. To make these easily available, we reviewed the production of such protocols, tools, and practice guides across PoshBee, and summarise their status, availability and aims herein.

1. EIP-Agri Practice Abstracts

PoshBee has produced over 15 Practice Abstracts, covering a broad range of topics, which are accessible at https://ec.europa.eu/eip/agriculture/en/find-connect/projects/pan-european-assessment-monitoring-and-mitigation

2. Experimental methodology protocols

2.1. Protocol for managing a ground-nesting bee (*Anthophora plumipes*) for field and laboratory experiments

The resulting manuscript (expected to be submitted in spring 2023) describes the use of the protocol for testing the impact of neonicotinoid soil-exposure for this ground-nesting bee.

The protocol can be used for 'trap nesting' a widespread ground-nesting bee (*Anthophora plumipes*) in the field and subsequently to move its brood cells into the laboratory. This allows the fitness (growth, survival) of the offspring to be measured as end-points in pesticide risk assessment applied in the field e.g. to the soil nesting medium.

2.2. Protocol to measure pesticide effects on *Bombus terrestris* colonies in dose-response in-hive feeding with declining pesticide concentrations

Available at: https://www.nature.uni-freiburg.de/ressourcen/publikationen-pdfs/protocol-to-measure-pesticide-effects-on-bombus-terrestris-colonies-in-a-dose-response-in-hive-feeding-experiment-with-declining-concentrations.pdf

This protocol details how to conduct an experiment on the impacts of a pesticide on bumble bee colonies, whereby the colonies are allowed to forage freely but have access to a syrup container inside their hive that contains differing concentrations of the pesticide. One set of colonies receives a field-realistic sequence of pesticide concentrations (i.e., over time lower concentrations are administered to mimic degradation) and additional sets of colonies receive either a multiple or a fraction of these concentrations. Syrup consumption and colony development is monitored regularly, and up to daily.

2.3. Protocol for field assessments of *Bombus terrestris* colonies

Available at: https://www.nature.uni-freiburg.de/ressourcen/publikationen-pdfs/protocol-for-field-assessments-of-bombus-terrestris-colonies.pdf

This protocol describes how to conduct field assessments to study effects of pesticides and other stressors on bumblebee colonies. Assessed are several colony development parameters such as colony weight or number of dead/alive bees and wax cover building (weight and area).

2.4. Novel protocols for ecotoxicology of bumble bees and solitary bees in the laboratory

This is available as Deliverable 3.2 on the PoshBee website.

Precise protocols designed to test the toxicokinetics and toxicodynamics of the agrochemicals sulfoxaflor, azoxystrobin and glyphosate in bumble bees and solitary bees, and across sexes and castes.

2.5. Protocols for assessing impacts of multiple stressors on honey bees, bumble bees and solitary bees in semi-field and field studies

This will become available in 2023 through ARPHA preprints, with a link to the PoshBee Open Science collection.

These protocols provide detailed and practical guidance on the design of studies looking at impacts of stressors on honey bees, bumble bees and solitary bees. The bees may be in the open (field studies) or in large cages (semi-field studies). The stresses may include exposure to chemicals (primarily plant protection products); inadequate diet; exposure to pathogens. The impacts are measured as reduced or altered activity, and increased mortality. The document includes details of novel techniques and equipment generated for PoshBee.

2.6. An archive of reference photos of honey bee brood and adults on Mini Plus Beuten Frames to assist in assessment in studying the population of a colony

This will become available through the PoshBee website (www.poshbee.eu) in 2023.

Measuring the populations of honey bee colonies is challenging. Frequently, field technicians are trained by estimating numbers of adults, young brood and old brood using photos, checking answers and repeating the process (this is known as the ColEval method). The biases of each technician are calculated and compensated for. This photo archive (which is not available elsewhere) shows bees on small (Mini Plus Beuten) frames in small colonies, which are more appropriate for use in ecotoxicological studies.

3. Monitoring tool protocols

3.1. APISH (Atmospheric Passive Integrated Sampler in Hive)

The tool is not currently available for people to use as the results have not been published yet. However, we aim at submitting to a journal the results of citizen science use of this tool in the first half of 2023.

APISH (Atmospheric Passive Integrated Sampler in Hive) is a cheap and easy-to-use tool to robustly detect low levels of bee-related pesticides in honey bee and bumble bee colonies and in mason bee nests. Exposure is assessed by simply placing APISH inside colonies or nests for one week, and then

sending it back to the laboratory where the samples are analysed. The design and workflow for this will be publicly available, enabling others with access to chemical analytical labs to use it.

3.2. From a haemolymph test to a Bee Health Card

Not currently available for use, but the aim is for it to be available as a 'test' product in the middle of 2023. TRL (Technology Readiness Level) 6 (prototype system verified) will be achieved at the end of Poshbee, as expected, and additional steps will be needed (TRL 7 to 9) to go to the commercial application. For *Apis mellifera*, the publication related to this blood test is already published (Arafah et al., 2019; https://hal.science/hal-02411819/). For *Bombus terrestris*, the publication was submitted on December 2nd, 2022, while for the third pollinator model, *Osmia bicornis*, the manuscript will be submitted during 2023.

This test will ultimately be available at commercial rates to beekeepers and monitoring programmes. The result of this "blood test" will be provided as a series of impact scores that indicate how closely the blood profile matches to a library of stressors. The overall impact level and the immune status of the bee will be represented by an appropriate "traffic light" colour code: green, yellow and red for low, medium and high impact, respectively.

3.3. Risk assessment model for bumble bees

Under development as part of 3.4 below. The bumble bee model is available in ODdox format at https://sess_ac.gitlab.io/a1mass/ALMaSS_all/_bombus_page.html.

The model will form a living model, under continuous improvement as part of the set of bee models provided to EFSA as a basis for future risk assessment. It will allow *Bombus* populations to be modelled in realistic landscapes across Europe, providing the basis to develop both pesticide risk assessment but also to evaluate other managements on *Bombus*.

3.4. Multi-species environmental risk assessment tool

Not yet available. The delivery will be provided to EFSA as a basis for developing their own tool as needed at the end of the project. This will be a package of landscape inputs and scenarios that allow the use of *Bombus*, *Apis* and *Osmia* models in the ALMaSS systems for risk assessment in six European countries. These will be Denmark, Poland, Germany, France, Finland and The Netherlands. For each country, ten landscapes will be supplied together with the necessary pollen and nectar resource models which are needed to drive the three bee models. Each model will include the capability to simulate exposure and effects of pesticide exposure.

This package will provide, for the first time, a set of landscapes for use in modelling risk assessment. This will allow the standardisation of scenarios in these landscapes and thus direct comparisons between pesticides, species and managements will be possible. Combined with the three bee models this will provide the basis for development of risk assessment tools for regulatory use. Further development of this package will continue under the auspices of the EFSA MUST-B working group towards a first regulatory tool for the honey bee in 2024.

4. Technological tools

4.1. Bee Tracker—an open-source machine learning-based video analysis software for the assessment of nesting and foraging performance of cavity-nesting solitary bees

Available along with a user guide: Knauer, A. C., Gallmann, J., & Albrecht, M. (2022). Bee Tracker—an open-source machine learning-based video analysis software for the assessment of nesting and foraging performance of cavity-nesting solitary bees. Ecology and Evolution, 12(3), e8575. https://doi.org/10.1002/ece3.8575. Link to the manual: https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fece3.8575&file=ece38575-sup-0001-Supinfo.pdf

The Bee Tracker software provides a novel automated methodological approach of individually and simultaneously tracking and analysing video recorded foraging and nesting behaviour of numerous cavity-nesting solitary bees. In combination with data on offspring production per nest, this enables the assessment of impacts of drivers studied under (semi-)field conditions on the reproductive success of these bees.

4.2. Integrated system for field studies on honey bees

Available from the Journal of Apicultural Research - open access (https://www.tandfonline.com/doi/full/10.1080/00218839.2021.2018107?tab=permissions&scroll=top)

Data collection from honey bee colonies is often complicated by the logistics of dealing with full-size colonies. This article describes a system of integrated components to enable better data collection from honey bees using smaller colonies, and comprises a full suite of tools built around a honey bee study frame, and includes details of photographic equipment.

4.3. Bumble bee pollen trap

Available from the USDA https://www.ars.usda.gov/pacific-west-area/logan-ut/pollinating-insect-biology-management-systematics-research/docs/pollen-traps/

This is a 3D printed bumble bee pollen trap, which has great potential for bumble bee research. Although this unit is designed for use with North American species, the SVG files could be modified to accommodate European species.

4.4. Laser etching rig for honey bee study frame

Available from RIO Open Access (https://riojournal.com/topical_collection/91/) – accessible from March 2023

Photographs and construction detail for the unit used to laser etch serial numbers on frames. Possible application in other areas of bee research requiring an indelible marking system to enable identification of components in beehives.

4.5. Construction details for photographic rig to record data from honey bee study frame

Available from RIO Open Access (https://riojournal.com/topical_collection/91/) - accessible from March 2023

The photographic rig is used with the honey bee study frame and enables consistent high quality photos to be taken of frames to track brood development throughout the duration of a study.

4.6. Construction drawings for bumble bee handling unit

Available from RIO Open Access (https://riojournal.com/topical_collection/91/) - accessible from March 2023

The bumble bee handling unit fits over a bumble bee colony and enables the operator to open the nest unit in relative comfort.

5. Conclusion

PoshBee will deliver on a range of protocols, tools, and best-practice guides as described above. By making these available to a broad array of stakeholders and end-users, we will deliver impacts that support sustainable pollinator populations and sustainable pollination in agricultural landscapes across the EU.

6. Acknowledgements

Thank you to everyone in PoshBee who enabled the development of these tools, protocols, and best practice guides.