PoshBee

PAN-EUROPEAN ASSESSMENT, MONITORING, AND MITIGATION OF STRESSORS ON THE HEALTH OF BEES

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PoshBee Project

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CONTENT

PROJECT COORDINATOR

Professor Mark Brown, Royal Holloway University of London
mark.brown@rhul.ac.uk

DURATION

June 2018 – May 2023

PROJECT OUTCOMES

- PoshBee publications
- Stakeholder summaries
- Practice abstracts
- PoshBee Project Outcomes Collection in the Research Ideas and Outcomes (RIO) Journal

PARTNERS

Biobest: Biobest Group
UMONS: Université de Mons

Pensoft: Pensoft Publishers

AU: Aarhus Universitet

EMÜ: Eesti Maaülikool
EPBKA: Eesti Kutseliste Mesinike Ühing
EPKK: Eesti Põllumajandus-Kaubanduskoda

ANSES: Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
BIOP: Association Plateforme BioPark d'Archamps
CNRS: Centre National de la Recherche Scientifique
INRA: Institut national de la recherche agronomique
UBx: Université de Bordeaux

ALU-FR: Albert-Ludwigs-Universität Freiburg
BV ST: Bauernverband Sachsen-Anhalt e.V.
ISA: Imkerverband Sachsen-Anhalt e.V.
MLU: Martin-Luther-Universität Halle-Wittenberg
UFZ: Helmholtz-Zentrum für Umweltforschung GmbH - UFZ

Hungarian NFCSO: Nemzeti Élelmiszerlánc-biztonsági Hivatal

FIBKA: Federation of Beekeepers of Ireland Associations
TCD: Trinity College Dublin
Teagasc: Teagasc - Agriculture & Food Development Authority

Coldiretti: Confederazione Nazionale Coldiretti
CREA: Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

UNAAPI: Unione Nazionale Associazioni Apicoltori Italiani
UNIUD: Università degli Studi di Udine

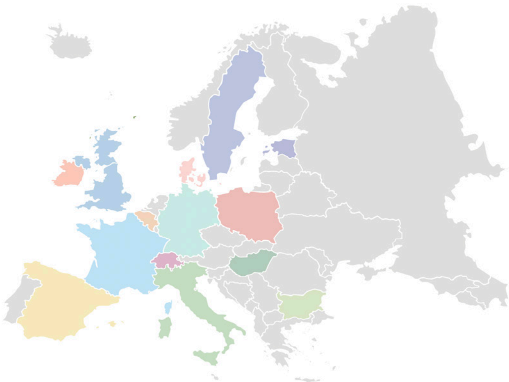
PIWET: Państwowy Instytut Weterynaryjny – Państwowy Instytut Badawczy

ADEA ASAJA: Asociación Regional de Empresas Agrícolas y Ganaderas de la Comunidad Autónoma de Murcia
UM: Universidad de Murcia

BF: Biodlingsföretagarna
LRF: Lantbrukarnas Riksförbund
SLU: Sveriges Lantbruksuniversitet

APIS: apisuisse
BERN: Universität Bern
USP: Union Suisse des Paysans
WBF-Agroscope: Agroscope, Eidgenössisches Departement für Wirtschaft, Bildung und Forschung
WILD: Wildbiene + Partner AG

ATPOLL: Atlantic Pollination Ltd.
BBKA: British Beekeepers Association
NFU: National Farmers Union of England and Wales
RBH: The Red Beehive Company Ltd.
RHUL: Royal Holloway University of London
UREAD: The University of Reading
VITA: Vita (Europe) Ltd.



BACKGROUND

PoshBee is a Horizon 2020 research project composed of academics, governmental organisations, industry, and NGOs. It addressed the impacts of agrochemicals to ensure the sustainable health of bees and their pollination services in Europe. Integrating the knowledge and experience of local beekeeping and farming organisations and academic researchers, the project:

- provided the first pan-European quantification of the exposure hazard of chemicals to managed and wild bees;
- determined how chemicals alone, in mixtures, and in combination with pathogens and nutrition, affect bee health, and;
- supported the need for monitoring tools, novel screening protocols, and practice- and policy-relevant research outputs to local, national, European, and global stakeholders

WP1

A site network for assessing exposure of bees to chemical, nutritional, and pathogen stressors

1 Pan-European site network reveals impacts of surrounding landscape and taxon-specific responses to stressors

A pan-European field site network comprising a total of 128 sites (8 countries, 2 crops per country, 8 sites per crop, representing and gradient of land use intensity) was established, sentinel colonies of honey, bumble and red mason bees were deployed, and common methods were used to make surveys during the spring flowering of the crops in 2019. We found that the surrounding landscape (up to 1km) of the two focal crops (oilseed rape and apple) was quite different, with oilseed rape tending to occur in less diverse landscapes. In addition, at the local scale, half of the boundaries and adjacent fields were characterised by improved grasslands, hedgerows, and other semi-natural habitats that potentially provide additional resources to pollinators. Surveys of wild flower-visiting insects showed that different taxa responded in different ways to this landscape context. For example, more honey bees and solitary bees were associated with oilseed rape fields, and hoverflies with apple orchards. However, less intensively managed habitats positively influenced all insect groups. In addition, in Ireland, the composition of the insect assemblages shifted after the crop flowering period, when floral resources in margins were important food sources. Bees from sentinel honey and bumble bee colonies across Europe showed rapid phenotypic shifts in wing size, shape and asymmetry after being introduced to the sites and experiencing new environmental conditions, but this was not related to pesticide management or landscape composition. Examination of wild solitary bees in Irish sites showed differences among species in terms of emergence date and the diameter of their nest tubes. Preliminary analysis of red mason bees across continental European sites showed that their fitness was connected to both landscape composition and the proportion of non-crop pollen in larval diets. Overall, our results support the importance of preserving non-crop habitats and floral resources in agricultural landscapes, particularly in those containing mass-flowering crops.

Sources

Hodge, S., Schweiger, O., Klein, A.-M., Potts, S.G., Costa, C., Albrecht, M., de Miranda, J.R., Mand, M., De la Rúa, P., Rundlöf, M., et al. (2022). Design and Planning of a Transdisciplinary Investigation into Farmland Pollinators: Rationale, Co-Design, and Lessons Learned. Sustainability 14, 10549. **https://doi.org/10.3390/su141710549**

Bottero, I., Hodge, S., & Stout, J. (2021). Taxon-specific temporal shifts in pollinating insects in mass-flowering crops and field margins in Ireland. *Journal of Pollination Ecology* 28, 90–107. **https://doi.org/10.26786/1920-7603(2021)628**

Hodge, S., Bottero, I., Dean, R., Maher, S., & Stout, J. (2022). Stem-nesting Hymenoptera in Irish farmland: empirical evaluation of artificial trap nests as tools for fundamental research and pollinator conservation. *Journal of Pollination Ecology* 32, 110–123. **https://doi.org/10.26786/1920-7603(2022)697**

Gérard, M., Baird, E., Breeze, T., Dominik, C., & Michez, D. (2022). Impact of crop exposure and agricultural intensification on the phenotypic variation of bees. *Agriculture, Ecosystems & Environment* 338, 108107. **https://doi.org/10.1016/j.agee.2022.108107**

WP2

Measuring chemical exposure, pathogens, and poor nutrition in honey bees, bumble bees and solitary bees

1 Measuring pesticide exposure of honeybees inside the colonies is possible with APISH, a new easy to use tool

Poshbee developed cheap and easy-to-use APISH sensors (Atmospheric Passive Integrated Sampler in Hive) to robustly detect low levels of several bee-related pesticides. Using APISH, Poshbee has shown that bees were exposed to various pesticides inside honey bee colonies according to a citizen science experiment implemented at European level.

2 Pesticides and parasitic and infectious agents strongly affected bee health

Using exposure data collected in the pan-European network, Poshbee demonstrated accumulating effects of multiple pressures on the performance of managed bees (*Apis mellifera*), eusocial wild bees (*Bombus terrestris*), and solitary wild bees (*Osmia bicornis*). The results also demonstrated strong effects of pesticides on colony growth and to a lesser extent on reproduction. Parasitic and infectious agents had consistent and strong negative effects on eusocial bees (*A. mellifera* and *B. terrestris*).

Sources

Serra, G., Costa, C., Cardaio I., & Colombo R. (2021). *Report on exposure of bees to agrochemicals*. Deliverable D2.2 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

Babin, A., Schurr, F., Delannoy, S., Fach, P., Chauzat, M.-P., Riviere, M.-P., Dubois, E. (2021). *Report on exposure of bees to pathogens*. Deliverable D2.3 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

Michez, D., Barraud, A., & Lefebvre, V. (2021). *Report on the nutritional quality of pollen*. Deliverable D2.4 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

Schweiger, O. & Dominik, C. (2023). *Factors and processes leading to contamination, and the effects of multiple stressors on bee health*. Deliverable D2.7 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

Kiljanek, T., Niewiadowska, A., Małysiak, M., & Posyniak, A. (2021) Miniaturized multiresidue method for determination of 267 pesticides, their metabolites and polychlorinated biphenyls in low mass beebread samples by liquid and gas chromatography coupled with tandem mass spectrometry. *Talanta*, 235, 122721. **https://doi.org/10.1016/j.talanta.2021.122721**.

WP3

Toxicokinetics, toxicodynamics and interactions among agrochemicals

1 The toxicity of a pesticide is dependent on the exact dosage, but also the exposure route and time, as well as the speed of detoxification and clearance from a body. Our results highlight large variations in degradation rates and sensitivity across bumble bees, solitary bees, and honey bees, and across different castes and sexes. We point out that the generalisation at least over so distinct groups like honey bees, bumble bees and *Osmia* sp. may result in misleading conclusions in ecotoxicological assessments.

Sources

Linguadoca, A., Jurison, M, Hellstroem, S., Straw, E.A., Sima, P., Karise, R., Costa, C., Serra, G., Colombo, R., Paxton, R.J., Mand, M. & Brown, M.J. F. (2022). Intra-specific variation in sensitivity of *Bombus terrestris* and *Osmia bicornis* to three pesticides. *Scientific Reports* 12, ARTN 17311. **https://www.nature.com/articles/s41598-022-22239-4**

2 Chronic effects of pesticides might not be in a linear relationship with doses. We saw that the toxicity of sulfoxaflor was only significant at the lowest and highest concentrations. Our results suggest that regulatory tests should address testing of a large range of concentrations, especially low ones, to fully inform pesticide risk assessment.

Sources

Barascou, L., Requier, F., Sene, D., Crauser, D., Le Conte, Y. & Alaux, C. (2022). Delayed effects of a single dose of a neurotoxic pesticide (sulfoxaflor) on honeybee foraging activity. *Science of the Total Environment* 805, 150351. **https://doi.org/10.1016/j.scitotenv.2021.150351**

Barascou, L., Sene, D., Le Conte, Y. et al. (2022). Pesticide risk assessment: honeybee workers are not all equal regarding the risk posed by exposure to pesticides. *Environ Sci Pollut Res* 29, 90328–90337. **https://doi.org/10.1007/s11356-022-21969-2**

WP4

Development of novel wild bee species for risk assessment

1 Evaluating effects of soil contaminants to ground-nesting solitary bees

We have, for the first time, performed a controlled experiment on a ground-nesting, European bee species (*Anthophora plumipes*) testing agrochemical exposure through soil. We found no significant effects on brood survival or nesting frequency when females and brood were exposed to high concentrations of soil-bound Imidacloprid. Using this newly developed method, the effects of soil exposure can potentially be assessed in larger field and semi-field studies.

Source

Hellström, S., Seidelmann, K., Serra, G., Lora, G., Gaboardi, G., Paxton, R.J. The hairy-footed flower bee (*Anthophora plumipes*) as a novel model species for assessing effects of agricultural soil contaminants on ground-nesting wild bees. In preparation.

2 An exposure protocol adapted for wild bees reveals species-specific impacts of sulfoxaflor

The sensitivity of wild bee species varies considerably from that of honey bees or *Bombus terrestris*; some are more sensitive, others less sensitive, making generalisation difficult.

Source

Barraud, A., Dewaele, J., Hellström, S., Paxton, R.J. & Michez, D. (2022). *Manuscript on the response of novel wild bees to diverse agrochemicals*. Deliverable D4.1 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

3 A novel pollen specialist species as a model for studying diet-pesticide interactions

When experimentally testing pesticide risk to bees, only pollen generalists are used. What about pollen specialist (oligolectic) species? We introduce the wallflower mason bee (*Osmia brevicornis*) as a potential model species to study the effects of pesticides on a bee specialized on the cabbage family, including the common European field crop oilseed rape.

We show that the wallflower mason bee might be a better candidate than the commonly used red mason bee or the orchard mason bee when studying the effects of pesticide use in oilseed rape. While other mason bee species generally do not prefer to gather pollen from oilseed rape (and suffer reduced fitness when they are restricted to it) the wallflower mason bee will.

Source

Hellström, S., Strobl, V., Straub, L., Osterman, W. H. A., Paxton, R. J., Osterman, J. (2023). Beyond generalists: The Brassicaceae pollen specialist *Osmia brevicornis* as a prospective model organism when exploring pesticide risk to bees. *Environmental and Sustainability Indicators* 18, 100239. **doi:10.1016/j.indic.2023.100239**.

WP5

Effects of agrochemical-nutrition interactions on bee health in the laboratory

Overall, our results highlight the importance of diverse floral resources for bee development as well as low pesticide exposure to keep a low level of stress and an optimal food intake for bee species. Moreover, we show the need for targeted studies of pesticide exposure alone, and in combination with variable nutrition qualities, on all life history stages of bees.

1. Our study confirmed that the nutritional requirements across bee species are different, and that a low-quality diet for one species is not necessarily low-quality for another one. While honey bees are negatively impacted by diets with a high protein content (~40%), bumble bees and mason bees develop normally on these diets but struggle on diets with a low total amino acid and sterol content.
2. Our study revealed that pollen quality can influence the ability of honey bees to metabolise pesticides and withstand their detrimental effects at high doses, providing another strong argument for the restoration of suitable foraging habitats.
3. We found a drastic reduction in the volume of ingested sugar solution by solitary mason bees after exposure to the insecticide sulfoxaflor. Interestingly, we also found an antagonistic interaction between this insecticide and the fungicide azoxystrobin, showing that the reduction in nectar intake was only pronounced in the absence, but not in the presence of the fungicide azoxystrobin. This finding suggests that the two substances might interact with each other directly at the molecular level, or that bee physiological mechanisms could contribute to this antagonistic interaction.

Sources

Barraud, A., Barascou, L., Lefebvre, V., Sene, D., Le Conte, Y., Alaux, C., Grillenzoni, F.-V., Corvucci, F., Serra, G., Costa, C., Vanderplanck, M. & Michez D. (2022). Variations in Nutritional Requirements Across Bee Species. *Frontiers in Sustainable Food Systems* 6, 824750. **https://doi.org/10.3389/fsufs.2022.824750**

Barascou, L., Sene, D., Barraud, A., Michez, D., Lefebvre, V., Medrzycki, P., Di Prisco, G., Strobl, V., Yañez, O., Neumann, P., Le Conte, Y. & Alaux, C. (2021). Pollen nutrition fosters honeybee resistance to pesticides. *Royal Society Open Science* 8, 210818. **https://doi.org/10.1098/rsos.210818**

WP6

Effects of agrochemical-pathogen interactions on bee health in the laboratory

The data show that the type of interaction between agrochemicals and bee pathogens is individual (negative, positive; additive or synergistic) and specific to the chemical (class), pathogen (species) and bee (species). The following specific discoveries were made:

1. Acute exposure of honey bee larvae to field-realistic concentrations of the insecticide sulfoxaflor can enhance the reproduction of the ectoparasite *Varroa destructor*.

Sources

University of Udine & University of Bern. (2022). *Sulfoxaflor can benefit Varroa destructor and might interact with a commonly used acaricide*. Published on the Horizon Results Platform. <**https://ec.europa.eu/info/funding-tenders/opportunities/portal/screen/opportunities/horizon-results-platform/45829;keyword=Varroa%20destructor%20**>. Accessed on 06/02/2023.

Strobl, V., Yañez, O., Neumann, P., Urueña, A., Blasco-Lavilla, N., De la Rúa, P., Le Conte, Y., Alaux, C., Frizzera, D., Seffin, E., Zanni, V., Annoscia, D., Nazzi, F., Onorati, P., Forsgren, E., & De Miranda, J. (2021). *Agrochemical and pathogen effects on individual honey bee health*. Deliverable D6.1 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

2. The fungicide azoxystrobin has a synergistic negative interaction with the bacterium *Paenibacillus larvae* (etiologic factor of the American Foulbrood disease), enhancing the mortality of *P. larvae* to honey bee larvae. The effects of sulfoxaflor (insecticide) and glyphosate (herbicide) on *P. larvae*-induced mortality are additive, i.e. the sum of the separate mortalities caused by *P. larvae* and the chemical agent. Sulfoxaflor had a strong negative effect on larval survival while glyphosate had no effect on larval survival.

Sources

Strobl, V., Yañez, O., Neumann, P., Urueña, A., Blasco-Lavilla, N., De la Rúa, P., Le Conte, Y., Alaux, C., Frizzera, D., Seffin, E., Zanni, V., Annoscia, D., Nazzi, F., Onorati, P., Forsgren, E., & De Miranda, J. (2021). *Agrochemical and pathogen effects on individual honey bee health*. Deliverable D6.1 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

3

Of the three pesticides (azoxystrobin, sulfoxaflor and glyphosate) tested (alone or in combination) only sulfoxaflor increased mortality in honey bees. None of the pesticides affected the production of *Nosema ceranae* spores in the gut of the workers. In honey bees exposed to sulfoxaflor and infected with *N. ceranae*, increased sugar water intake and altered immune and detoxification gene expression were observed.

Sources

Strobl, V., Yañez, O., Neumann, P., Urueña, A., Blasco-Lavilla, N., De la Rúa, P., Le Conte, Y., Alaux, C., Frizzera, D., Seffin, E., Zanni, V., Annoscia, D., Nazzi, F., Onorati, P., Forsgren, E., & De Miranda, J. (2021). *Agrochemical and pathogen effects on individual honey bee health*. Deliverable D6.1 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

4

Exposure of bumble bees to glyphosate and infections with the gut parasite, *Crithidia bombi*, had no significant impact alone or in combination. Similarly, there were no significant effects of the parasite (*Crithidia mellificae*) and insecticide flupyradifurone alone or in combination on the solitary bee *Osmia bicornis*.

Sources

Straw, E.A., & Brown, M.J.F. (2021). No evidence of effects or interaction between the widely used herbicide, glyphosate, and a common parasite in bumble bees. *PeerJ* 9, e12486. **https://doi.org/10.7717/peerj.12486**

Hellström, S., Albrecht, M., Paxton, R. J. (2022). *Manuscript on agrochemical and pathogen effects on health in the solitary bee Osmia bicornis*. Deliverable D6.4 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

Hellström, S., Wintermantel, D., Paxton, R. J., Albrecht, M. No evidence of detrimental interaction between a trypanosome parasite and a butenolide insecticide in a solitary bee. In preparation.

WP7

Effects of chemicals and their interactions with other stressors on bees tested in semi-field and field experiments

1 Pesticide effects on bees are resource dependent

In two separate semi-field experiments (see Figure 1 and description below), pesticide effects on bees were dependent on the flowering resources provided, possibly because flowering resources differ in nutritional properties that influence pesticide detoxification. Also, resource type itself was shown to play a major role in offspring production in *Osmia* as well as in the development of bumble bees.

Figure 1: Semi-field experiments consist of large flight cages that enclose bees with flowering resources that are either pesticide-treated or not. This allows for assessing (interactive) effects of flowering resources and pesticides applied according to label guidelines.

In the first experiment, the solitary bees ***Osmia bicornis*** exposed to Sivanto (active ingredient: flupyradiforone; FPF) and nutritional stress had reduced offspring production, flight activity, flight duration and flower visitation frequency but only when Sivanto was applied in buckwheat (Knauer ***et al***., 2022). In the second experiment, bumblebees exposed to the fungicide Amistar (active ingredient: azoxystrobin) in ***Phacelia*** had reduced colony growth and fewer males produced. In buckwheat and floral mix cages, no effect of the fungicide was observed compared to the control (Wintermantel ***et al***., 2022).

These results highlight that interactions between nutrition and pesticides can occur. This interaction might be dependent on the bee species and its life-history traits and/or nutritional niche. By providing diverse and abundant floral resources, negative effects of pesticides might be buffered.

Sources

Knauer, A., *et al*. (2022). Nutritional stress exacerbates impact of a novel insecticide on solitary bees` behaviour, reproduction and survival. *Proceedings of the Royal Society B: Biological Sciences* 289, 20221013. **https://doi.org/10.1098/rspb.2022.1013**

Wintermantel, D., *et al*. (2022): Flowering resources modulate the sensitivity of bumblebees to a common fungicide. *Science of the Total Environment* 829, 154450. **https://doi.org/10.1016/j.scitotenv.2022.154450**

Output: Based on the publication of Wintermantel *et al.* (2022) a stakeholder summary has been produced: Diet matters: The effect of a common fungicide on bumblebees depends on floral resources. **https://poshbee.eu/documents/7/**

2 Negative detectable effect of two pesticides found on bumblebees but not on honeybees or *Osmia*

Under semi-field conditions, the fungicide Amistar (active ingredient: azoxystrobin) as well as the insecticide Closer (active ingredient: sulfoxaflor) reduced flower visitation rate by bumblebees (Tamburini ***et al***., 2021) and bumblebee colony growth (Tamburini ***et al***., 2021; Wintermantel ***et al***., 2022), while no detectable negative effects of either pesticide was found on ***Osmia*** or honeybees (Tamburini, Wintermantel ***et al***. 2021; Schwarz, ***et al***. 2022).

Sources: same as number 1 and 3

3 No detectable interaction between the insecticide Closer and the fungicide Amistar

Interactive effects between insecticide and fungicide were not detected in any of the semi-field studies including honeybees (Tamburini, Wintermantel ***et al***. 2021), bumblebees (Tamburini ***et al***., 2021) and ***Osmia*** (Schwarz et al., 2022); this means there was no indication that the fungicide increased the toxicity of the insecticide or vice versa.

Honeybees

Tamburini*, G., Wintermantel*, D. *et al*. (2021). Sulfoxaflor insecticide and azoxystrobin fungicide have no major impact on honeybees in a realistic-exposure semi-field experiment. *Science of the Total Environment* 778: 146084. **https://doi.org/10.1016/j.scitotenv.2021.146084** *These authors contributed equally.

Output: Stakeholder summary: Sulfoxaflor insecticide and azoxystrobin fungicide have no major impact on honeybees. **https://poshbee.eu/documents/7**

Bumble bees

Tamburini, G., *et al*. (2021). Fungicide and insecticide exposure adversely impacts bumblebees and pollination services under semi-field conditions. *Environment International 157*: 106813. **https://doi.org/10.1016/j.envint.2021.106813**

Output: Stakeholder summary: Fungicide and insecticide exposure adversely impact bumblebee health and behaviour. **https://poshbee.eu/documents/7**

Osmia

Schwarz, J.M., *et al.* (2022). No evidence for impaired solitary bee fitness following pre-flowering sulfoxaflor application alone or in combination with a common fungicide in a semi-field experiment. *Environment International* 164: 107252. **https://doi.org/10.1016/j.envint.2022.107252**

WP8

Systems and agent-based modelling approaches to assess the synergistic effects of multiple stressors on bee health

1 We elaborated a new working definition of 'bee health'

We present a practical, working definition of 'health' from the bee's own perspective, and identify the parameters important for their well-being and survival. We have conceptualised bee health as a hierarchical set of interdependent homeostatic layers, or systems, that individually and together protect the bee (i.e., provide resilience) against short-, medium- and long-term fluctuations in its environment. These homeostatic systems are dynamic, flexible resources, to be used and replenished in the service of the life of the bee.

We subsequently identified several major dimensions (molecular, microbial, parasitic, nutritional, social-behavioural, environmental and population-genetic) that are key to managing bee life and health at different scales within these systems. For each of these dimensions we then identified a number of key indicators for measuring the status of the health dimension at any one time.

The conceptual models are similar to those developed for health management in other organisms and biological systems, and are compatible with the WHO OneHealth concept, where the health of any one organism is contingent on the health of the environment it depends on for survival.

Sources

de Miranda, J.R., Rundlöf, M. & Nazzi, F.(2022). *Definition of bee health and set of key health indicators for each of the three model bee species*. Deliverable D8.1 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

2 We created a conceptual model of honey bee health that enabled us to interpret contrasting field results on pesticide impact on honey bees

While there is widespread concern regarding the impact of pesticides on honey bees, well-replicated field experiments, to date, have failed to provide clear insights on pesticide effects. We adopted a systems biology approach to gain insights into the web of interactions among the factors influencing honey bee health. We demonstrated that the immune-suppressive capacity of a widespread pathogen of bees can cause bistability, meaning that honey bees under similar initial conditions can experience different consequences when exposed to the same stressor, including prolonged survival or premature death. Our conclusions reconcile contrasting field-testing outcomes and have important implications for the application of field studies to complex systems.

Sources

Breda, D., Frizzera, D., Giordano, G., Seffin, E., Zanni, V., Annoscia, D., Topping, C.J., Blanchini, F. & Nazzi, F. (2022). A deeper understanding of system interactions can explain contradictory field results on pesticide impact on honey bees. *Nat Commun*. 29: 5720. **https://doi.org/10.1038/s41467-022-33405-7**

3 We built a descriptive model of the bumble bee colony which will be integrated with other models for honey bees and solitary bees to develop a multi-species Environmental Risk Assessment tool for evaluating the potential effects of agrochemicals or farming practices on bees

The new Bombus model is grounded in the biology of bumble bees. Created as both a laboratory and full lifecycle landscape version, the model allows for a deeper understanding of eusocial bumble bees generally as well as landscape-scale risk assessment of bee health. Parameterised for *Bombus terrestris*, the model is used, with the honey bee and solitary bee models, to assess the effects of parasites and diseases, in combination with landscape management and pesticide application. All three models are highly detailed agent-based models operating in highly realistic landscape simulations created in collaboration with three Horizon 2020 projects.

Sources

Chetcuti, J., Stout, J., Brown, M. & Topping, C.J. Formal Model for a complex agent-based simulation of laboratory bumble bees. *Submitted to Food and Ecological Systems Modelling Journal*.

WP9

OMICS of agrochemical responses in bees

1 **A blood test** to follow the impact of stressors through molecular mass fingerprints: **MALDI BeeTyping®** and associated databases (molecular fingerprints models)

We have generated a **database of reference spectra (Molecular mass Fingerprints, MFPs)** from laboratory and semi-field experiments: Outcomes: MALDI BeeTyping is fast (less than 5 min), reliable (at least 3 MFPs per sample) and cost-effective analytical tool compared to PCR for example. We shall achieve TRL6 level by the end of PoshBee and we are looking for a demonstration in an operational level (level 7) and achieve a marketable service in 2025. In summary, based on this approach **>300 samples/day/technician** can be analysed and **5-15 bees/hive** are sufficient. The results interpretation can be done on the base of a laboratory report either by the beekeeper or the sanitary services, who will aggregate the BeeTyping results with field observations on the apiary and other potential analyses done on honeybees.

With this technique:

- we have been able inside the PoshBee project to make distinction between control conditions and mono or pluri-stressed bees (nutrition, pathogens, pesticides), even when survival has not been affected, showing the accuracy of the method, for lab and semi field experiments;
- we provide a view on the immune response of the different bee species, and specifically the peptide production (not only gene expression delivered by PCR);
- we demonstrate in field experiments, a large variability of hemolymph profiles among the different sites, opening the possibility to use specific markers at the landscape scale for diagnostic purpose;
- we show that the hemolymph of all the pollinators studied have a species specific profile.

This is routine in medicine and clinical microbiology

Sources

Arafah, K., Voisin, S.N., Masson, V., Alaux, C., Le Conte, Y., Bocquet, M. & Bulet, P. (2019). MALDI–MS Profiling to Address Honey Bee Health Status under Bacterial Challenge through Computational Modeling. *Proteomics*, 19: 1900268. **https://doi.org/10.1002/pmic.201900268**

Houdelet, C. (2020). Analyse de l'immunoprotéome de l'abeille en réponse à différents stress environnementaux. **https://www.theses.fr/2020GRALV009**

Houdelet, C., Bocquet, M. & Bulet, P. (2020). Matrix-assisted laser desorption/ionization mass spectrometry biotyping, an approach for deciphering and assessing the identity of the honeybee pathogen *Nosema*. *Rapid Communications in Mass Spectrometry*, 35: e8980. **https://doi.org/10.1002/rcm.8980**

2 **A bottom-up proteomics workflow (blood/organs)** to decipher physiological pathways: **LC-ESI-MS/MS** to identify and characterise molecular markers to develop/propose ELISA, PCR, colorimetric tests.

Three protein databases were provided on the three major pollinators: APIDBase (v1_1934 accession numbers, 1581 distinct proteins of *Apis mellifera*), BOMDBase (v1_1353 accession numbers, corresponding to 1042 distinct proteins of *Bombus* species (646 from *Bombus terrestris*)) and OSMDBase (dedicated to *Osmia bicornis*). Bottom-up proteomics by LC-ESI-MS/MS allows to decipher pathways that are dysregulated in response to stressors. It is a widely considered approach to identify and characterise biomarkers from complex biological matrices (robust and sensitive). LC-ESI-MS/MS is a fast, quantitative and reliable tool for monitoring variations of identified markers, it is compatible with high-throughput analyses in dedicated laboratories and label free quantification is also routine (differential analysis). This is also a gold standard approach for pharmacokinetic-pharmacodynamic (PKPD) studies. In relation to PoshBee, this approach is routine and applicable to different pollinators, with a perspective of the characterisation of biomarkers as a first step in the proposal of diagnostic/prognosis tools/kits for assessing the health status of bees in their environment.

With this technique:

- we decipher the main pathways involved in the response of the hemolymph to the different stressors, alone or in combination, in field, semi-field and laboratory conditions;
- we build three extensive databases of proteins found in hemolymph of each of the three bee species (*Apis mellifera*, *Bombus terrestris*, *Osmia bicornis*).

This is routinely used in medicine for marker discovery

Sources

Houdelet, C., Sinpoo, C., Chantaphanwattana, T., Voisin, S.N., Bocquet, M., Chantawannakul, P., & Bulet P. (2020). Proteomics of Anatomical Sections of the Gut of Nosema-Infected Western Honeybee (*Apis mellifera*) Reveals Different Early Responses to *Nosema* spp. *Isolates. J. Proteome Res*, 20: 804–817. **https://doi.org/10.1021/acs.jproteome.0c00658**

3 A multidimensional drawing of molecular organs: MALDI imaging mass spectrometry (IMS) to scan molecules (metabolites, lipids, peptides/ proteins) within organs.

IMS opens perspectives for bee health monitoring. It is a versatile technique applicable to any pollinator, with as perspective a method for PKPD studies (no need to have labelled molecules, direct visualisation of the active ingredient and its metabolites is possible).

With this technique:

- we demonstrate specific tissues responses to stressors giving new insights in the localisation of the bee response;
- we open the possibility to elaborate a histomolecular atlas of bees..

This is an already considered promising approach to assist clinicians

Sources

Houdelet, C., Arafah, K., Bocquet, M. & Bulet P. (2022). Molecular histoproteomy by MALDI mass spectrometry imaging to uncover markers of the impact of *Nosema* on *Apis mellifera*. *Proteomics*, 22: 2100224. **https://doi.org/10.1002/pmic.202100224**

All these three analytical technologies (BeeTyping®, bottom-up proteomics and MALDI IMS) will serve to implement a Bee Health Card, (1) giving an easy and affordable mean to diagnose the effect of the stressors, (2) to decipher the physiological processes implied in these effects, and (3) to localize this response in the bee body. These three techniques, used in concert, may give new solutions to monitor the impact of the main pollinators stressors.

WP10

Knowledge Exchange and Impact Strategy

1 Response options for multiple stressors on bee health

PoshBee has identified a series of farm-scale response options for mitigating the impact of multiple stressors on bees. This was achieved by combining high-quality scientific project outputs on multiple stressors and expert knowledge to collectively identify the most appropriate mitigations, relevant to both policymakers and practitioners.

Sources

Willcox, B., Senapathi, D., & Potts, S. (2023). *Identification of the most effective policy and practice responses to the multiple stressor effects on bees*. Deliverable D10.6 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921. and other potential analyses done on honeybees.

2 Knowledge synthesis and policy briefings

PoshBee has produced a wide range of high-quality scientific outputs, most of which have strong policy and practice relevance. Three key areas of knowledge where PoshBee has made substantial contributions to the state of the art include: (i) exposure of managed bees to pesticides, pathogens and nutritional stress; (ii) effects pesticides, pathogens and nutritional stress on managed bees; and, (iii) Omics tools to monitor bee health. These topics inform a briefing targeted at EU and national policymakers. The recent scientific literature on stressor exposure, stressor interactions, and 'omics in response to stressors were reviewed as a basis for a peer-reviewed publication.

Sources

Chauzat, M.P., et al. (2023). *Synthesis report on agrochemical, pathogen and nutritional exposure*. Deliverable D10.3 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

Hellström, S., & Paxton, R. (2023). *Synthesis report on agrochemical, pathogen and nutritional effects*. Deliverable D10.4 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921

Askri, D., et al. (2023). Synthesis report on omics. Deliverable D10.5 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.

3 Horizon scan

PoshBee organised a 'horizon scan' workshop to identify upcoming threats and opportunities directly impacting managed bee health within European agricultural systems over the next decade. Using established horizon scan methodologies 21 internationally recognised experts assessed current knowledge and ranked the future potential risks and opportunities for European-managed bee health.

Sources

Willcox, B., Senapathi, D., & Potts, S. (2023). *Horizon scan reports on future risks and opportunities*. Deliverable. D10.9 EU Horizon 2020 PoshBee Project, Grant agreement No. 773921.