PoshBee

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

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STAKEHOLDER SUMMARIES

This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 773921.

Contents

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Pollen nutrition fosters honeybee tolerance to pesticides

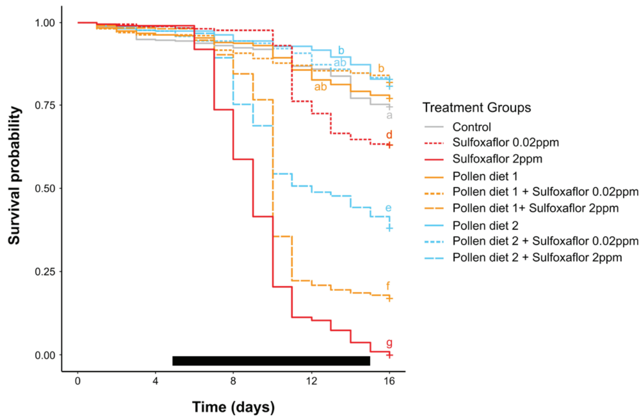
Background

Pollen is a major source of nutrients for honeybees. However, due to differences in their nutrient content, pollens from different floral species are not of the same quality for honeybees. Furthermore, a reduction in the availability of flowers is generally observed in agricultural landscapes, along with widespread exposure to pesticides.

Question

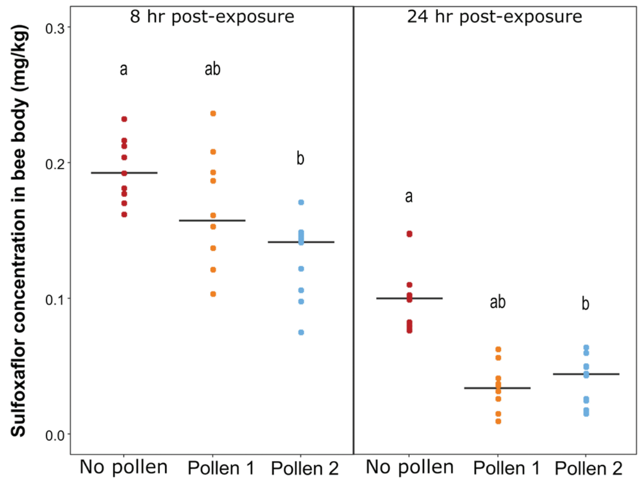
Can variation in pollen diet quality affect the sensitivity of honeybees to pesticides?

Effects of pollen feeding and sulfoxaflor (insecticide) on bee survival



Sulfoxaflor reduced **honeybee survival**, but this reduction was **less** when bees were able to **consume pollen**. In addition, some **pollens** were **better than others** in reducing the sensitivity of honeybees to the insecticide sulfoxaflor.

Effects of pollen feeding on sulfoxaflor detoxification



Different letters indicate significant differences between groups, while the black line denotes the median.

The **consumption of pollen** helped honeybees to **eliminate sulfoxaflor** from their body, and this pesticide elimination was **faster** with **some pollens** than with others.

Take-home message

The toxicity of pesticides might not be correctly estimated if the **quality of pollen** diets is not considered in **pesticide risk assessment tests**.

Promoting **wildflower species** in agricultural landscapes may contribute to **increase the access to pollen diets of different quality** and therefore **reduce their sensitivity** to pesticides.

Source

Barascou L., Sené D., Barraud A., Michez D., Lefevre V., Medrzycki P., Di Prisco G., Strobl V., Yanez O., Neumann P., Le Conte Y., Alaux C., 2021. Pollen nutrition fosters honeybee tolerance to pesticides. *R. Soc. Open Sci*. 8: 210818. **https://doi.org/10.1098/rsos.210818**

Link to French translation: here.

A single dose, but long-term effect: the case of the neurotoxic sulfoxaflor in honeybees

Background

Pesticide risk-assessment guidelines for honeybees require determining the toxicity of a single dose of pesticide over 48h.

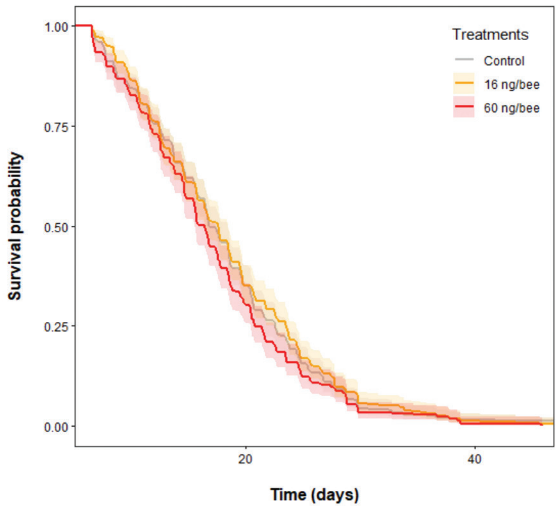
Question

Can a single dose of pesticide cause long-lasting or delayed effects?

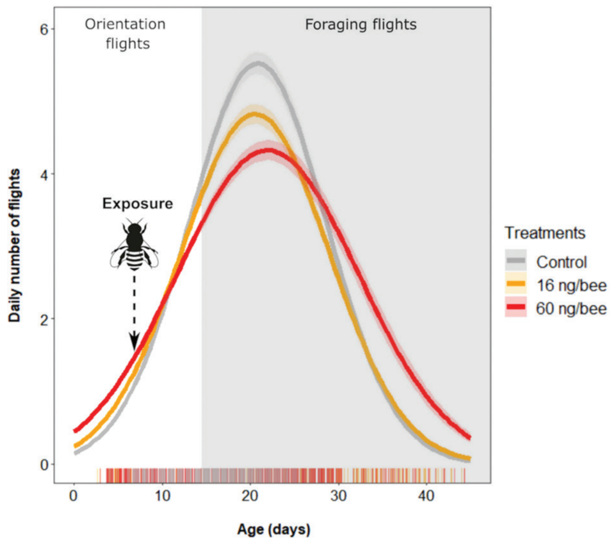
Exposure to **different doses** of **sulfoxaflor**

Automated monitoring of flight activity and lifespan

No effect of sulfoxaflor doses on bee mortality.



However, **a delayed effect** on foraging activity has been observed, i.e. a significant **decrease** in the **foraging activity** appeared, not immediately or within 48h, but one week after the exposure to sulfoxaflor.



Take-home message

Acute exposure in honeybees does not necessarily cause short-term effects but can generate **delayed effects.**

Pesticide risk assessment guidelines for honeybees should require more **long-term tests** (> 48h) for determining the toxicity of a **single dose** of pesticide.

Link to French translation: **here.**

Source

Barascou L., Requier F., Sené 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. **https://doi.org/10.1016/j.scitotenv.2021.150351**

An integrated system for field studies on honey bees

Research on honey bees may be carried out in a laboratory where the bees are constrained, or outdoors, where the bees can fly from and back to their hives. Outdoor studies are classified either as 'semi-field', where bee flight is restricted in a cage or tunnel, or 'field', where bees have unrestricted flight. A key challenge in a semi-field study is ensuring that the food (nectar and pollen) available within the cage is sufficient to sustain the colony, whereas in a field study the challenge is to understand and evaluate the forage available to the colony. In both field and semi-field studies, improved and simplified techniques of observation and recording of impacts are desirable. The use of small colonies in miniature hives is commonplace. However, there is a lack of specialised equipment; bee hives designed for commercial beekeepers may not be effective for research. For example, commercial pollen traps designed to collect kilograms of pollen are disruptive and cumbersome, when only a few grams of pollen are required. We propose an integrated system of research equipment which enables the creation and management of small viable honeybee colonies (say 3,000 adults). Each part of the system is designed and built in a coordinated fashion to improve speed, ease, reliability and accuracy.

The system is built around the Study Frame, shown below, which fits the German Mini Plus hive (a small well-insulated hive primarily used for queen rearing). The Study Frame has many features, the key ones being queen excluder covers (to cage the queen on the frame) and the use of plastic foundation (to prevent queens escaping through holes in the comb). By noting the date and time of caging and releasing a queen, the age of her brood can be accurately determined. The system also uses the Converter Hive which accommodates Study Frames as well as large commercial frames in populous colonies. This facilitates the creation of small, equalised colonies by permitting the wide selection of comb, stores, brood and adults. Other features of the system include a mobile photographic apparatus for studying impacts on brood; and a combined floor, stand, varroa tray, dead bee trap and miniature pollen trap. The pollen trap is particularly useful in collecting small amounts of pollen for analysis of agrochemical residues, and measuring the pollen collection rate of colonies, as an indicator of colony activity.

PoshBee Study Frame

Source

Allan M.J. & Dean R.R., 2022. An integrated system for field studies on honey bees, *Journal of Apicultural Research* **https://doi.org/10.1080/00218839.2021.2018107**

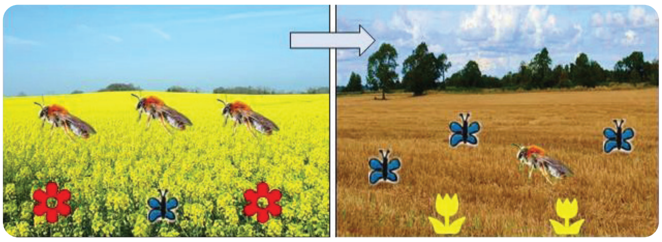
Flowering hedges and edges support pollinating insects throughout the season

Pollinators are animals that feed from flowers, and in doing so, transfer pollen, helping plants to produce fruits and seeds. Flower-visiting insects such as bees, butterflies, and hoverflies, are important pollinators, but some species are declining. One of the causes of these declines is the destruction of natural habitats, which decreases the amount and diversity of flowers that pollinators use as a food source. In agricultural landscapes, natural habitats remain in the form of flowering hedgerows or floral edges along the borders of grass pastures or cultivated crop fields. Mass-flowering crops (cultivated crops that have abundant, but short-lived, flowers) can also act as a food source, but flowers in hedges and edges could be important outside the flowering period of the crop. We studied the relationship between the number and different types of flowers in the hedges and edges of two mass-flowering crops (apples and oilseed rape), on the number of bees, butterflies and hoverflies, and investigated how this changed throughout the season. We selected 11 mass-flowering crop sites (six oilseed rape crop fields and five apple orchards), where we monitored the flowers, and five groups of insects (honeybees, bumblebees, solitary bees, hoverflies and butterflies). To check how the communities changed through time, we did this in three different periods, during and after the flowering periods of the crops (April – August 2019). We sampled insects both in the centre of the crop fields and along their margins. We found seasonal shifts in the number and types of insects and flowers in the hedges and edges of the crops, but there were not predictable relationships between insects and flowers. One exception was that the number of bumble bees increased when there were more different types of flowers in bloom. We discovered that each group of insects changed through the season in different ways – solitary bees declined in abundance through the year, while butterflies were more common at the end of the summer. We also found differences in the abundance of insects in the centre of crops and on the margins. Specifically, butterflies and hoverflies were more common at the edges than in the middle of fields when crops were in flower. This was also true after the crops had ceased to flower, in apple orchards. Our results confirm the importance of natural habitats at the edges of crops for these insects, both as alternative sources of food during the blossoming of the crops, and to fill potential nutritional gaps at the end of the flowering periods of the crops. Overall, our study supports policies that preserve and improve natural habitats (such as flowering hedges and field margins) in agricultural landscapes, to protect pollinating insects and promote sustainable crop production.

Do flowering hedges and edges of crops support pollinating insects in apple and oilseed rape crops?

"Yes, flowers in hedges and edges supported bees, hoveflies and butterflies. In fact, hoverflies and butterflies were more abundant here than in the crop fields themselves."

Does the number and type of insects and flowers vary though the season?



"Yes, insect and plant diversity changed through the season, but not in predictable ways."

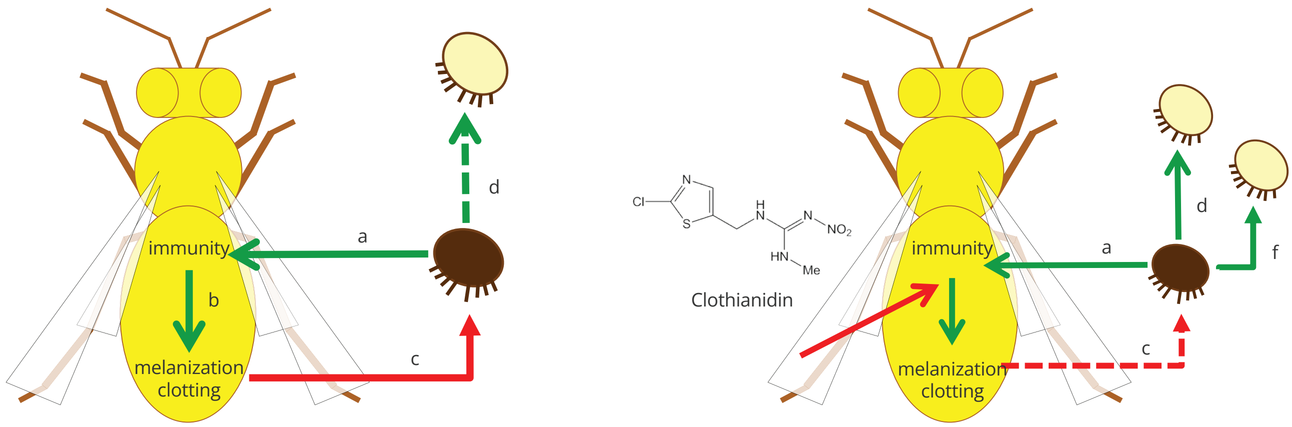
Exception: there were more bumblebees recorded when there were more different types of flowers.

Source

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**

Link to Italian translation: here.

The insecticide Clothianidin increases *Varroa destructor* fertility, with negative implications for honey bee health



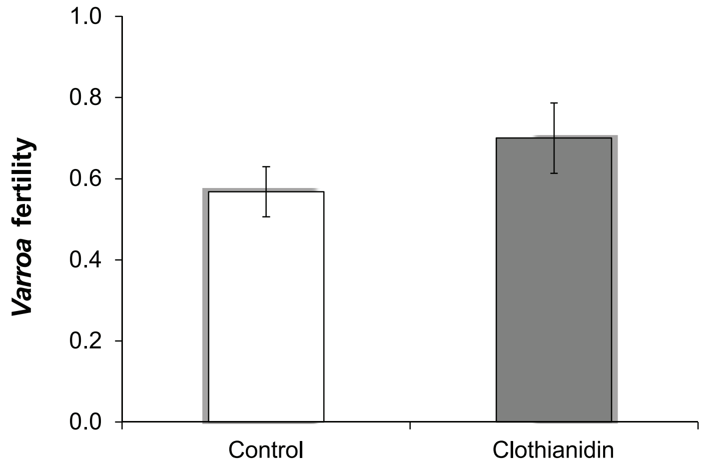
a) *Varroa destructor* feeds on the bee's haemolymph "blood" through a feeding hole.

b) The honeybee immune system reacts by activating two processes: melanization (to encapsulate pathogens, preventing possible infections) and clot formation.

c) This immune response promotes wound healing, limiting mite feeding and reproduction (d).

e) The neonicotinoid Clothianidin impairs immunity and the response to *Varroa* feeding (c).

f) Increased feeding results in enhanced mite reproduction.



Varroa fertility is higher on Clothianidin treated bee pupae. The bars show the average mite fertility in presence of Clothianidin or not, while the error bars illustrate the variability of data.

The difference observed between the two groups is statistically significant.

Source

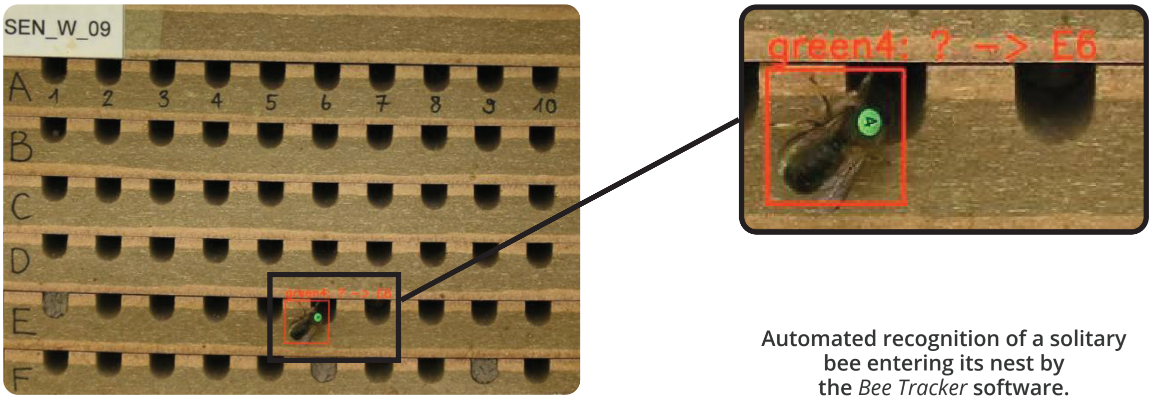
Annoscia D., Di Prisco G., Becchimanzi A. et al., 2020. Neonicotinoid Clothianidin reduces honey bee immune response and contributes to *Varroa* mite proliferation. *Nat Commun*, 11: 5887. **https://doi.org/10.1038/s41467-020-19715-8**

Link to Italian translation: here.

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

Background

How successful bees are at foraging and nesting can provide important information on bee health and is of interest for risk and impact assessment of environmental stressors. While radio-frequency identification (RFID) technology is an efficient tool increasingly used for the collection of behavioural data in social bee species such as honey bees, behavioural studies on solitary bees still largely depend on direct observations, which is very time-consuming.



Automated recognition of a solitary bee entering its nest by the *Bee Tracker* software.

Bee Tracker software

Bee Tracker is a novel software package for the automated analysis of foraging and nesting behaviour of numerous cavity-nesting solitary bees based on video recordings of nesting units. The software can detect bees that enter or leave their nest and recognize individual IDs on the bees' thorax as well as the IDs of their nests according to their positions in the nesting unit.

The software is able to identify each nest of each individual nesting bee, which enables measurement of individual-based measures of reproductive success. Moreover,the software can quantify nest recognition and flight duration. The success rate in measuring these parameters was 96% in the analysed videos.

Source

Knauer A., 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:e8575. **https://doi.org/10.1002/ece3.8575**

Link to Germany translation: here.

Take-home message

The machine learning based software could be adapted to various experimental setups by training it to a representative set of videos. The software is provided free and open-source including the underlying Python code along with a user manual, which makes the software accessible to users who have no programming background.

The method presented enables the efficient collection of large amounts of data on cavity-nesting solitary bee species and represents a promising new tool for the monitoring and assessment of behaviour and reproductive success under laboratory, semi-field and field conditions.

A new frontier for visualising the impact of stressors in honey bees: proteins in pictures

A cutting-edge technique of scanning opens perspectives for pathology research and bee health monitoring

In health care (animal and human), imaging techniques such as radiology, echography and scanning by magnetic resonance imaging (MRI) have contributed hugely to improving the prognosis and diagnosis of diseases by veterinarians and doctors.

Imaging mass spectrometry (IMS) provides unique opportunities for analysing tissues, organs, and even whole organisms at an unprecedented level of detail. We have adapted IMS for use in honey bees, enabling us to produce images of drugs/chemicals, metabolites, sugars, lipids (fats) and protein distributions across organs. This is illustrated below in scans of protein distribution in the body of honey bees that are infected, or not, by a fungal parasite, the causative agent of nosemosis.

How to get imaged organs with protein distributions: just scan slices of a bee

The diagram shows the work flow for producing IMS images, which bridge the gap between visual examination and targeted molecular analyses.

Source

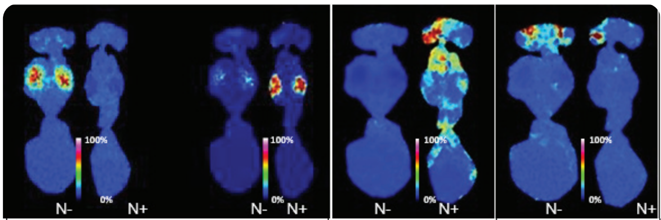
Houdelet C., Arafah K., Bocquet M. & Bulet P., 2022. Molecular histoproteomy by MALDI mass spectrometry imaging to uncover markers of the impact of Nosema on Apis mellifera. *Proteomics*. 22: 2100224. **https://doi.org/10.1002/pmic.202100224**

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

Link to French translation: here.

A cutting-edge technique demonstrated on bees inoculated with spores of the fungal parasite *Nosema*

IMS analysis of a non-infected bees (N-) and of a bee inoculated with *Nosema* spores (N+): examples of protein images; two that are differentially expressed in the flight muscles (left panel) and two others (right panel) in the bee blood (Apidaecin a marker of an activated immune response) and in the head (Royalisin, less present in the head of bees developing nosemosis), respectively. The absence or presence of a biomolecule is representative of a modification of the health status of the bee, confirming the impact of nosemosis on bee health.



Two proteins are imaged in the flight muscles, 1 in the N- bee and a different one in the N+ bee	Apidaecin imaged in the blood of the N+ bee	Royalisin imaged in the head of the N- bee

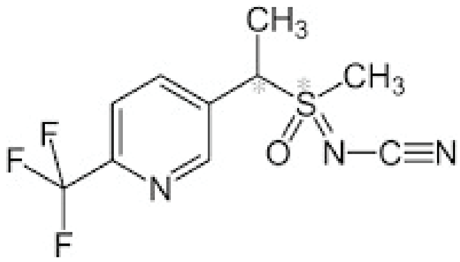
Lessons to learn from pictures

- As Confucius, the Chinese Philosopher, said "a picture is worth a thousand words". Just imagine a general practitioner facing a broken leg without the image provided by a radiogram.
- The unique technique of IMS bridges the gap between visual examination and targeted molecular analyses.
- A new frontier to discover protein signatures of an organ and a body in response to stressors.
- New generation of mass spectrometers are compatible for high-throughput screening of the spatial distribution of proteins, lipids, metabolites and drugs in any type of tissue or the entire body of a honey bee.
- It is well known that nosemosis impacts gut morphology and physiology. When applied to a honey bee facing nosemosis, IMS also highlighted the nosemosis' impact on the flight muscles located in the thorax, on the gland secreting the Royalisin protein, and on the immune response triggered by Nosema (presence of the Apidaecin protein) in bee blood.
- A versatile technique applicable to other pollinators.

Novel insecticide reduces egg-laying and reproductive success in bumble bees

Sulfoxaflor, a novel insecticide, was introduced to replace neonicotinoid pesticides in agricultural systems.

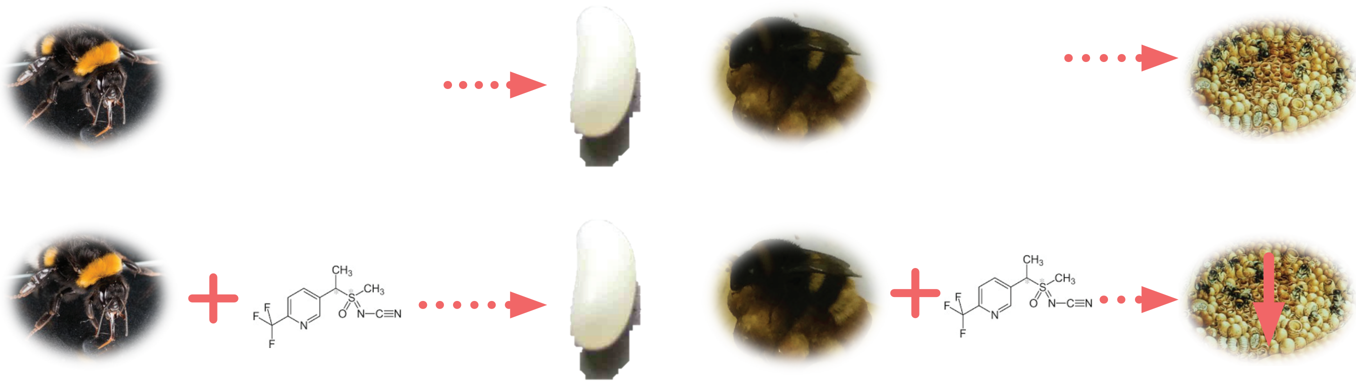
Sulfoxaflor chemical formula



To test the potential impacts of sulfoxaflor on bumble bee health, we conducted two experiments: 1) small groups of bumblebees were exposed to varying levels of sulfoxaflor and reproduction was measured; 2) newly-founded colonies were exposed for two weeks to low levels of sulfoxaflor and then placed in the field, where we measured colony growth and reproduction.

1) Does exposure impact egg-laying?

2) Does exposure impact colony health?



Results

Our results show a risk to bumble bee health if they are exposed to low doses of sulfoxaflor, with egg-laying and colony reproduction both being reduced. Since these experiments, the EU has banned use of sulfoxaflor outside of greenhouses.

Source

Siviter H., Horner J., Brown M.J.F. & Leadbeater E., 2019. Sulfoxaflor exposure reduces egg laying in bumblebees *Bombus terrestris*. *Journal of Applied Ecology.* 57: 160-169. **https://doi.org/10.1111/1365-2664.13519**

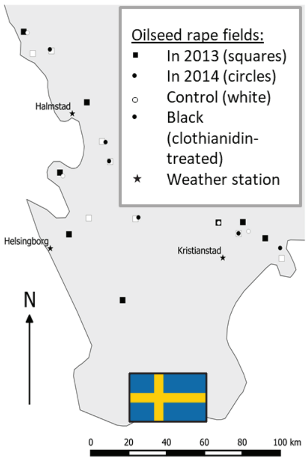
Siviter H., Brown M.J.F. & Leadbeater E., 2018. Sulfoxaflor exposure reduces bumblebee reproductive success. *Nature*. 561: 109–112. **https://doi.org/10.1038/s41586-018-0430-6**

How does an insecticide seed-treatment affect honeybee and bumblebee colonies and their pathogens, parasites and beneficial bacteria?

Background

Neonicotinoids are systemic pesticides when applied as a seed-treatment, meaning their active compounds can be found in all plant parts including nectar and pollen, potentially threatening bees. In addition to direct effects on bees, exposure to this type of insecticide might promote **pathogens and parasites of bees** as neonicotinoids have been shown to reduce immune gene expression, antimicrobial activity and hygienic behaviour. Here we investigate this question using the neonicotinoid clothianidin.

Experimental setup



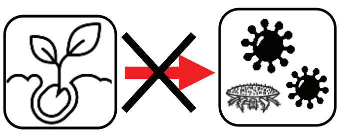
Honeybees
(*A. mellifera*)
2013 & 2014

Bumblebees
(*B. terrestris*)
2013

Results

Clothianidin exposure did not increase disease levels

We found **no negative effects** of clothianidin on honeybee or bumblebee health with respect to the prevalence and abundance of **RNA viruses**, ***Nosema spp, Crithidia bombi*** (only tested in bumblebees), ***Varroa destructor*** (only tested in honeybees), and **beneficial bacteria**, or **immune gene expression** (only tested in honeybees).

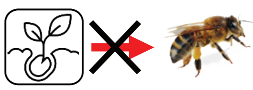


No negative effects

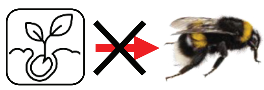
Clothianidin negatively impacted bumblebee reproduction and bodysize, but no adverse effects in honeybees

Honeybees
No impacts on swarming, overwinter mortality, honey production. No impacts on number of adult bees and brood in the first year but more bees and more brood in the second year when exposed to clothianidin.

Bumblebees
Negative impacts of clothianidin on bumblebee reproduction: Reduced worker size (-5%), reduced male pupal body mass (-23%), fewer males (-66%), fewer queens (-74%) but no impact on the number of adult bees.



No negative effects



Severe negative effects

Further support for testing the effect of pesticides on more species than just the honeybee!

Honeybee colonies consisting of several thousands of individual bees might be **more resilient** to stressors in the environment **than the smaller bumblebee colonies**. This research indicates that **pollinator groups can react differently** to pesticide exposure, and this should be considered in future research.

Link to German translation: here.

Source

Rundlöf M., Andersson G., Bommarco R. et al., 2015. Seed coating with a neonicotinoid insecticide negatively affects wildbees. *Nature* 521: 77-80. **https://doi.org/10.1038/nature14420**

Wintermantel D., Locke B., Andersson G.K.S. et al., 2018. Field-level clothianidin exposure affects bumblebees but generally not their pathogens. *Nat Commun.* 9: 5446. **https://doi.org/10.1038/s41467-018-07914-3**

Osterman J., Wintermantel D., Locke B. et al., 2019. Clothianidin seed-treatment has no detectable negative impact on honeybee colonies and their pathogens. *Nat Commun,* 10: 692. **https://doi.org/10.1038/s41467-019-08523-4**

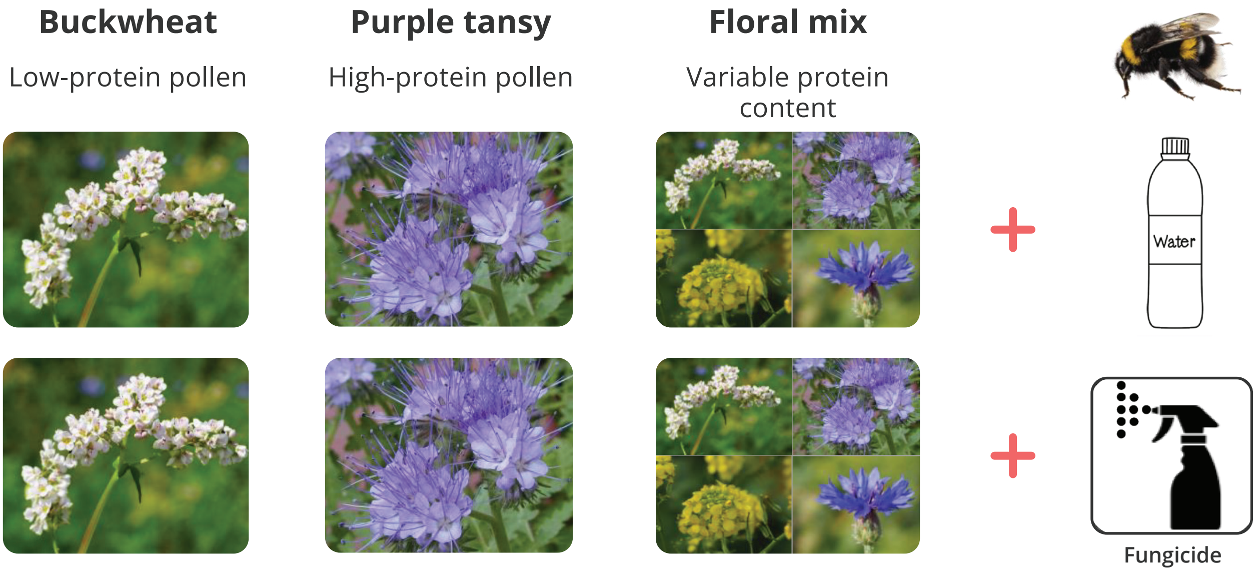
Diet matters: The effect of a common fungicide on bumblebees depends on floral resources

Agricultural practices and bee health

Fungicides are commonly applied to crops in bloom, therefore their effects on bees should be investigated. Another stressor for bees is the reduced floral abundance or a lack of floral diversity that can be seen in agricultural landscapes. Flowering plants differ in the nutrients they provide. **Monocultures** consisting of only one crop plant might therefore be another stressor for bee health. How multiple stressors affect bees in **combination** is rarely studied.

Testing the effect of nutrition and a fungicide on bumblebees

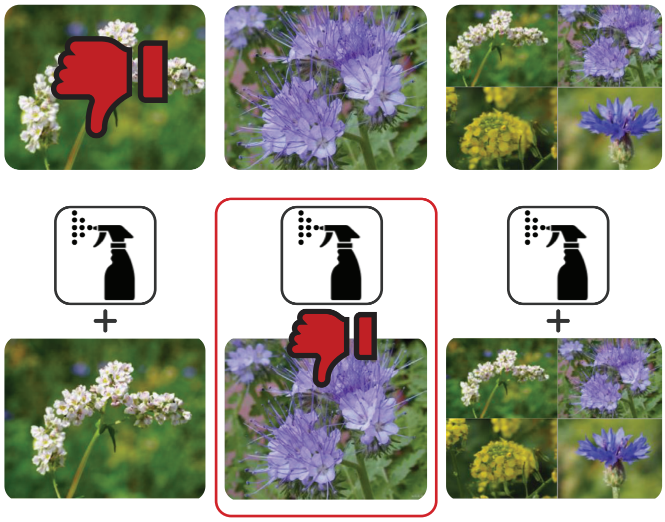
In 39 large flight cages (a semi-field experiment) either buckwheat, purple tansy or a floral mixture was grown. In each cage one colony of the buff-tailed bumblebee was placed. About half of the cages were treated with a common fungicide (**amistar**, active ingredient: **azoxystrobin**) and the others were sprayed with water only.



Results

Nutrition: bumblebee colonies in buckwheat monocultures generally developed poorly compared to colonies foraging on purple tansy or a floral mixture.

Effect of fungicide exposure: The fungicide applied in purple tansy reduced colony growth and the number of males produced. Also the body mass of workers was lower compared to colonies in purple tansy cages sprayed with only water. In buckwheat and floral mix cages, no effect of the fungicide Purple tansy was observed.



Diverse habitats are urgently needed to buffer negative effects of pesticides!

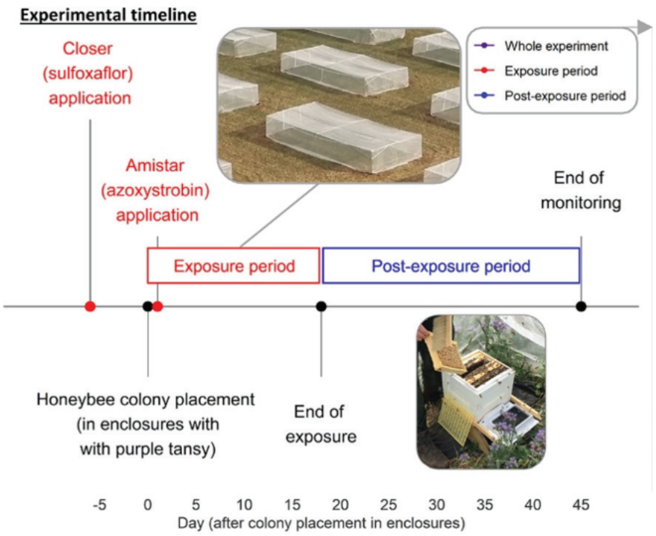
Bumblebee colonies only performed consistently well in the flowering mix. In contrast, monocultures either reduced bumblebee health directly or failed to buffer the negative effects of a fungicide. If we bring diverse flowering habitats back into agricultural landscapes, we can help bumblebees and other bees by making them more resistant to pesticides.

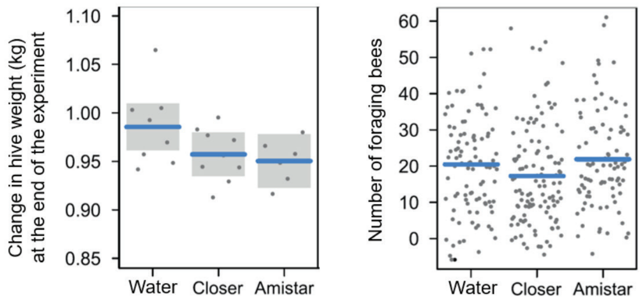
Source

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

Link to German translation: here.

Sulfoxaflor insecticide and azoxystrobin fungicide have no major impact on honeybees





Effects of spray application of the product Closer (sulfoxaflor) and Amistar (azoxystrobin) compared to the control treatment (water) on honeybees. Plots display model prediction (horizontal lines), and data variability (dots and bands). There are no significant differences between Closer, Amistar and control (water) treatments.

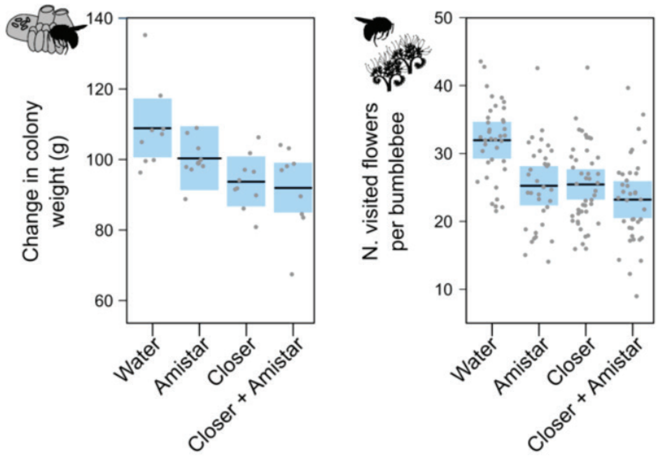
Exposure to pesticides is considered a major threat to bees and several neonicotinoid insecticides were recently banned in cropland within the European Union because of their potential negative effects. However, bees remain exposed to many pesticides whose effects are poorly understood. Recent evidence suggests that one of the most prominent replacements of the banned neonicotinoids – the insecticide sulfoxaflor - harms bees. Similarly to neonicotinoids, sulfoxaflor is an insecticide that is first absorbed by treated crops and then it spreads throughout plant tissues, and can contaminate their pollen and nectar. However, experiments testing bee response to spray application of sulfoxaflor under real-world conditions are scarce. Moreover, fungicides have received less attention than insecticides, as they are often viewed as relatively non-toxic to bees. Some experiments have however shown that fungicides alone or mixed with other pesticides can have negative effects on bees. Here, we assess the impact of the product Closer, containing the insecticide sulfoxaflor, and the widely used Amistar, containing the fungicide azoxystrobin, on honeybees. We set up 30 large cages (12 m × 5.9 m, height:2 m, covered by nets), each one containing purple tansy (a plant often used in pollinator studies) and a small honeybee colony (approximately 3000 adult bees). The products were applied according to the then current regulations: Closer before and Amistar during the bloom of purple tansy. In this study, Closer was applied six days before bloom. The health and growth of treated colonies were compared to those of colonies placed in cages where only water was applied. We found no significant effects of Closer or Amistar on the development of honeybee colonies (e.g., growth in colony weight, adult bee mortality, change in number of adults and brood cells, brood failure) or foraging activity (number of bees entering the hive and visiting flowers, daily pollen collection). Our study suggests that these pesticides pose no notable risk to honeybees when applied in isolation and following stringent label instructions. The findings on Closer indicate that a safety period of 5–6 days between application and bloom, which is only prescribed in a few EU member states, may prevent its impacts on honeybees. However, to conclude whether Closer and Amistar can safely be applied, further realistic-exposure studies should examine their effects in combination with other chemical or biological stressors on various pollinator species.

Source

Tamburini G., Wintermantel D., Allan M.J., Dean R.R., Knauer A., Albrecht M., Klein A-M., 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**

Link to Italian translation: here.

Fungicide and insecticide exposure adversely impact bumblebee health and behaviour



Effects of spray application of the product Closer (sulfoxaflor) and Amistar (azoxystrobin) compared to the control treatment (water) on bumblebees. Closer had a negative effect on colony weight and both products decreased the number of flowers visited by bumblebees. Plots display model prediction (horizontal lines), and data variability (dots and bands).

Exposure to pesticides is considered a major threat to bees and several neonicotinoid insecticides were recently banned in cropland within the European Union because of their potential negative effects. However, bees remain exposed to many pesticides whose effects are poorly understood. Recent evidence suggests that one of the most prominent replacements of the banned neonicotinoids – the insecticide sulfoxaflor - harms bees. Similarly to neonicotinoids, sulfoxaflor is an insecticide that is first absorbed by treated crops and then it spreads throughout plant tissues, and can contaminate their pollen and nectar. However, experiments testing bee response to spray application of sulfoxaflor under real-world conditions are scarce. Moreover, fungicides have received less attention than insecticides, as they are often viewed as relatively non-toxic to bees. Some experiments have however shown that fungicides alone or mixed with other pesticides can have negative effects on bees. Here, we assess the impact of the product Closer, containing the insecticide sulfoxaflor, and the widely used Amistar, containing the fungicide azoxystrobin, and both pesticides on bumblebees (*Bombus terrestris*). We set up 40 large cages (12 m × 5.9 m, height: 2 m, covered by nets), each one containing purple tansy (a plant often used in pollinator studies) and a bumblebee colony. The products were applied according to the then current regulations: Closer before and Amistar during the bloom of purple tansy. In this study, Closer was applied two days before bloom. The health and growth of treated colonies were compared to those of colonies placed in cages where only water was applied. We found that both the product Closer and the product Amistar negatively affected the individual foraging performance of bumblebees (measured as the number of flowers visited by bumblebees). The insecticide also reduced colony growth (measured as the weight of the colony) whereas the fungicide decreased the ability of bumblebees to transport pollen. The limited amount of flower resources in the cages during the experiment might have exacerbated pesticide effects on bumblebee colonies. Our work demonstrates that field-realistic applications of the product Closer (active ingredient: sulfoxaflor) can adversely impact bumblebees. Applying this insecticide only shortly before crop flowering (two days) may be insufficient to prevent its negative impacts on pollinators. Moreover, fungicide use during bloom could reduce bumblebee foraging performance and pollination services.

Source

Tamburini G., Pereira-Peixoto M-H., Borth J., Lotz S., Wintermantel D., Allan M.J., Dean R., Schwarz J.M., Knauer A., Albrecht M., Klein A-M., 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**

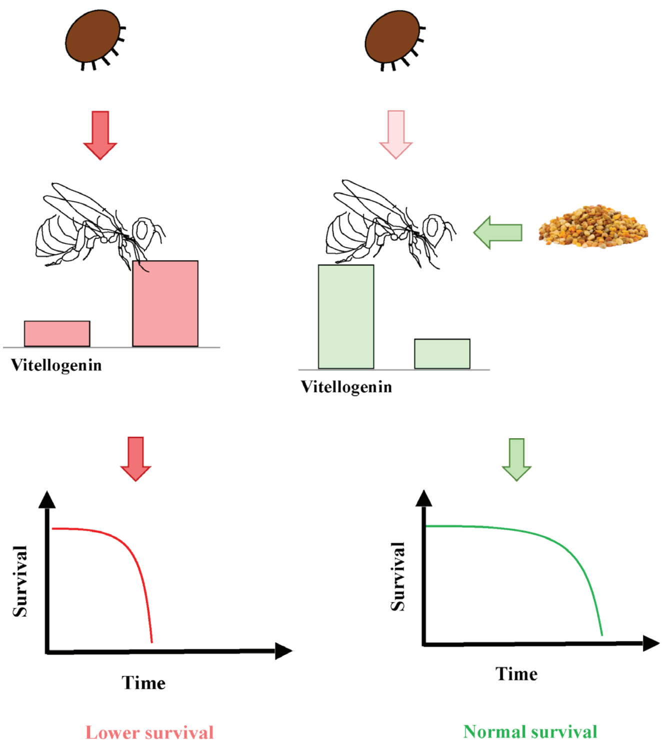
Link to Italian translation: here.

Pollen slows down the aging induced by Varroa mites

Pollen is the only source of proteins, fats, amino acids and vitamins for honey bees and is essential for colony survival. Recently, it has been demonstrated that pollen can also mitigate the deleterious effect of *Varroa destructor*: the most important parasite of the honeybee.

Varroa accelerates the aging of honey bees influencing proteins and hormones. In particular, the mite inhibits the production of a specific protein (i.e. Vitellogenin), typical of younger bees and stimulates the synthesis of a hormone typical of the older ones (i.e. Juvenile hormone). This impairment reduces the lifespan of the honey bees.

We show that pollen increases the lifespan of mite-infested bees restoring the natural balance of Vitellogenin and Juvenile hormone. This counteracts the faster aging induced by the parasite. Together with previous studies, these data highlight the importance of pollen feeding for honey bees.



Source

Tamburini G., Pereira-Peixoto M-H., Borth J., Lotz S., Wintermantel D., Allan M.J., Dean R., Schwarz J.M., Knauer A., Albrecht M. & Klein A-M., 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

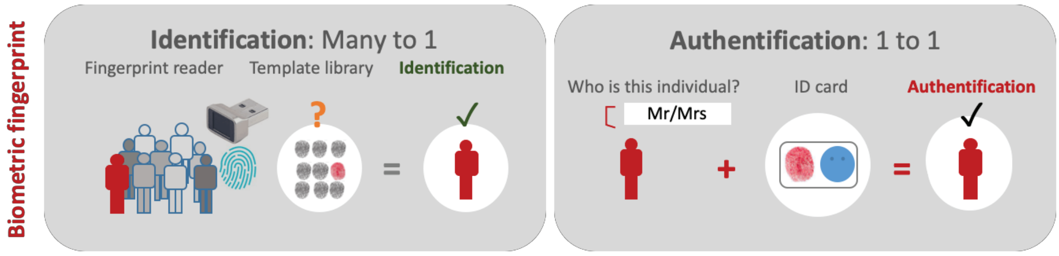
Link to Italian translation: here.

An individual "blood test" to monitor the impact of stressors on bee health

An individual blood test to track the health status of bees

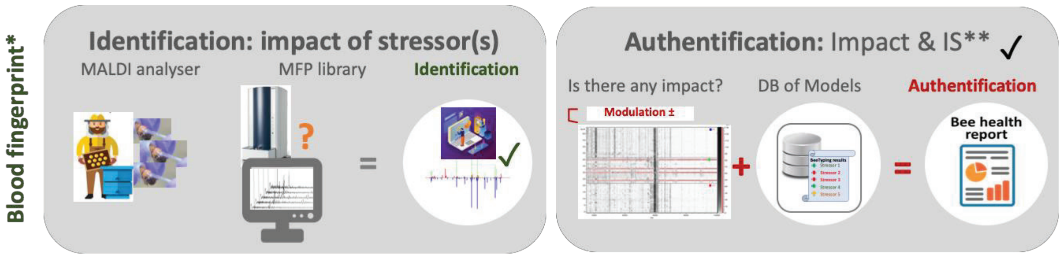
In health care, a blood test is done to check how organisms (animal and human) cope, for example, with infection, medication or pathology. If the blood test results are abnormal, it gives indications of how to treat or prevent future problems.

For bees, a "blood/haemolymph test" performed by mass spectrometry (MALDI BeeTyping®) produces a record of molecular fingerprints (MFP) representative of a bee's physiology, in the same way that a biometric fingerprint is exclusive to one human (and can be used both for identification and authentication, see diagram on the right).



How to track the impact of a stressor on bee health?

A molecular fingerprint is generated and compared to a library of reference MFPs obtained under different stress conditions (e.g., bacteria, parasites, bad nutrition, pesticides). MALDI BeeTyping® enables classification of bees according to their responses to stressors and gives the immune status of the bee.



* A molecular fingerprint (MFP)

**IS: immune status; ± : grey line intensity is the level of modulation

From a "haemolymph test" to a Health card

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. This MALDI BeeTyping® approach is designed as a user-friendly read-out of bee health status.

The lab report can be interpreted by the beekeeper or bee veterinary services, who can integrate it with complementary analytical measurements (detection of viruses, residues of chemicals, etc.) and field observations of bee hive health.

Compared to other molecular approaches that look at gene expression in bees, MALDI BeeTyping® is fast (< 5 min), reliable and cost-effective, and thus could provide a valuable tool for bee health.

Source

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**

Link to French translation: **here.**

Novel insecticides and viral pathogens act independently on worker honey bees in the laboratory

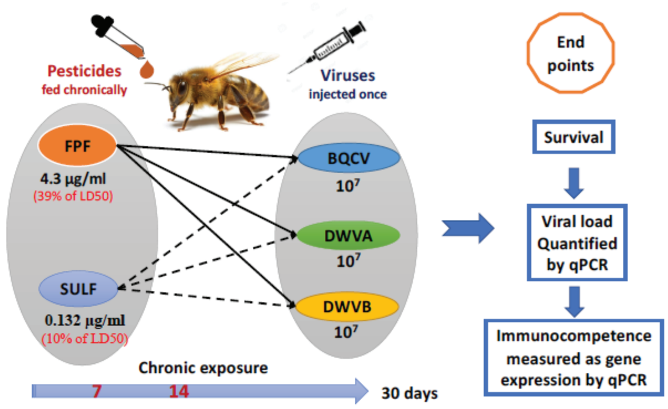
Background

The decline of insect pollinators threatens global food security. A major potential cause of decline is considered to be the interaction between environmental stressors, particularly pesticides and pathogens, that may amplify the impact of each.

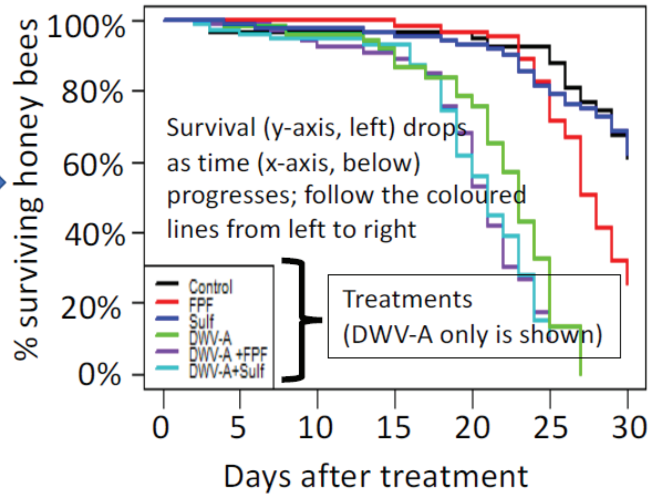
Question

Do novel insecticides flupyradifurone (FPF) and sulfoxaflor (SULF) interact with honey bee viral pathogens: deformed wing virus variant A (DWV-A), variant B (DWV-B) and black queen cell virus (BQCV), to amplify harm to bees?

Experimental set-up



Effect on Survival - same pattern for all 3 viruses



Major result: Pesticide + pathogen: do not interact to amplify bee mortality; viral loads remain unchanged, but bee immunocompetence is modulated (data not shown).

Take-home messages

- Sublethal doses of FPF fed for 30 days impact honey bee survival
- Common viral pathogens are a major threat to honey bees
- Co-exposure to novel insecticides does not significantly amplify viral impacts

Source

Al Naggar Y. & Paxton R. J., 2021. The novel insecticides flupyradifurone and sulfoxaflor do not act synergistically with viral pathogens in reducing honey bee (*Apis mellifera*) survival but sulfoxaflor modulates host immunocompetence. *Microbial biotechnology*. 14(1): 227-240. **https://doi.org/10.1111/1751-7915.13673**

New molecular method to detect and quantify three microsporidia infecting bees, *Vairimorpha* (*Nosema*) *apis**, *Vairimorpha* (*Nosema*) *ceranae** and *Vairimorpha* (*Nosema*) *bombi**

**Nosema apis*, *N. ceranae* and *N. bombi* have been reassigned in 2020 from the genus *Nosema* to the genus *Vairimorpha*

Background

- These three microsporidia are **intracellular parasites of bees**: *V. apis* and *V. ceranae* are honey bee parasites, *V. bombi* is a bumble bee parasite.
- Current molecular methods do not allow the **quantification of the three *Vairimorpha* species.**

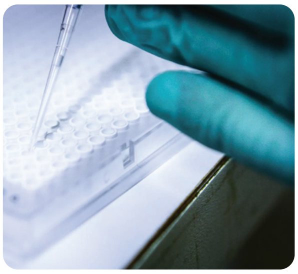
Objective

To develop and validate a **harmonised molecular method** (quantitative PCR) enabling the specific detection and quantification of these three parasites in honey bees, bumble bees and mason bees.

Development

Based on the single-copy gene RPB1

- *In silico* development (bioinformatics)
- Assay *in vitro*



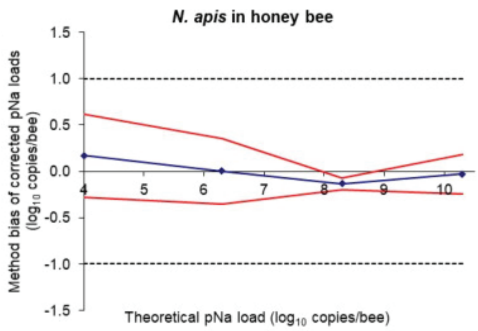
Validation

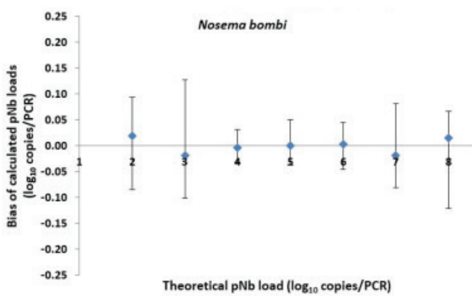
- Performances and specificities of the quantitative PCR
- Method performances:

in *Apis mellifera*,

in *Bombus terrestris*,

in *Osmia bicornis*





Perspectives

- Study accurately *Vairimorpha* (co)infections, especially those devoid of clear clinical signs
- Study the microsporidia spillover between bee species
- Study the dynamics of coinfection/synergism with other pathogens and parasites
- Transfer to high-throughput methods for the analysis of big sample sets

Source

Babin A., Schurr F., Rivière M-P., Chauzat M-P. & Dubois E., 2022. Specific detection and quantification of three microsporidia infecting bees, Nosema apis, Nosema ceranae, and Nosema bombi, using probe-based real-time PCR. *European Journal of Protistology*. 86: 125935. **https://doi.org/10.1016/j.ejop.2022.125935**

Link to French translation: **here.**

Haemolymph Proteomics: A New Approach to Monitoring Bee Health

A technology used in molecular medicine for protein biomarker identification: perspectives for pathology research and health monitoring in bee models

In health care (animal and human), modern approaches in the discovery of protein biomarkers have hugely contributed to improve the prognosis and diagnosis of diseases by veterinarians and doctors. The discovery of blood protein biomarkers through proteomics is one of the existing tools.

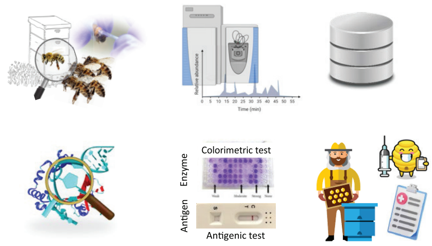
Biomarker discovery research based on proteomics is advanced in different human and animal diseases such as infections, cancer, cardiovascular disorders, while providing opportunities to work with limited to non-invasive methods by the use of blood samples. Blood analysis can indicate if certain elements circulating in the blood stream are, or are not in a normal range, enabling the prediction and detection of pathologies.

How to get a protein marker? Modern proteomics applied to blood samples is a recognised method

The diagram below shows a conventional workflow for protein biomarker discovery, which bridges the gap between visual examination and targeted molecular analyses.

From haemolymph proteomics to bee health monitoring using individual “blood tests”

This six-step workflow can be applied to bee health, taking advantage of the biomarker discovery research principles. Haemolymph is directly collected by the beekeeper and sent off for processing and analysis.



A modern approach of protein biomarker discovery demonstrated on bee haemolymph experimentally infected with microbes

The monitoring report is based on the molecular results observed by color intensity evaluation (color intensity being related to the activity level)/antigenic tests: one band (see “C” figure above) is a negative test, an additional band at T (see fig. above) reflects the presence of the antigen as for a CoVid test. The report can be interpreted by the beekeeper or bee veterinary services, who can integrate it with complementary analytical measurements (detection of viruses, residues of chemicals, etc.) and field observations of bee hive health.

Compared to other molecular approaches looking at gene expression in bees, colorimetric kits and strip-based lateral flow assays (i) have cost-effective advantages, (ii) can be user-friendly and applicable from laboratories to the real-world for prognosis and diagnosis of health problems, (iii) are already available for beekeepers for AFB & EFB monitoring, and (iv) when appropriate, can be developed for transportable devices interfaced with smartphone applications for in field monitoring.

Source

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

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**

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**

Link to French translation: here.

Assessing the effects of agricultural landscape and environment on bee size, shape and asymmetry

Background

The effects of overuse of pesticides or changes in agricultural landscapes on bee morphology, for example bee size and shape, require more investigations. This topic matters because size and shape changes can have negative effects on important aspects of bee lives, such as their flight and ability to visit flowers. We measured three features of the wings that have importance for flight – size, shape, and the asymmetry between the two forewings. These wing features are important because changes in these features can affect the flight which could lead to changes in the ability of bees to pollinate efficiently.

Question

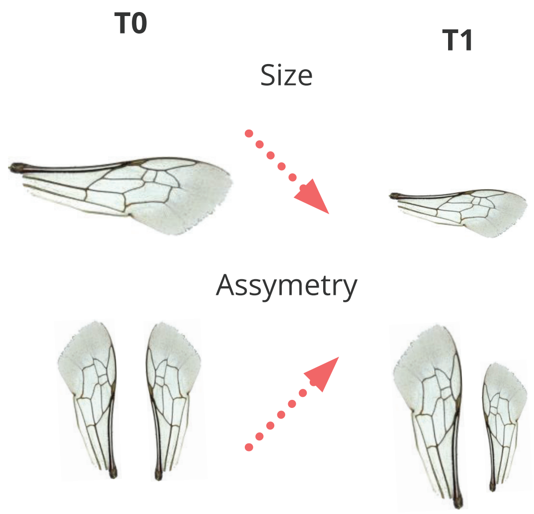
Do environment, pesticides and agricultural landscape affect size, shape, and asymmetry of the wings?

Experimental set-up

Two species: Buff tailed bumblebee (*Bombus terrestris*) and honeybee (*Apis mellifera*)

- 8 countries and 2 type of crops: oilseed rape fields and apple orchards;
- ~ 1000 individuals sampled when the hives/nests were placed in the field by researchers (**T0**);
- ~ 1000 individuals sampled several weeks/months after being exposed to the studied fields (**T1**);
- Parameters evaluated: fragmentation of habitat, the proportion of grassland and urban area, the quantity of pesticides used by hectares, latitude, etc.

Example of changes in bumblebees between T0 – T1



Impact of the parameters evaluated on the wings?

- None of the parameters related to agricultural landscape had any general effect. The quantity of pesticide or the fragmentation of habitat did not affect the wings.

Results and Take-home messages

Changes in size, shape and asymmetry can occur **in a very short period**, after encountering new field conditions, in both species.

However, **no strong effect of the pesticides/habitat fragmentation** on wing size, shape or asymmetry.

Source

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

Link to French translation: **here.**

Deformed wing virus (DWV) makes it hard to predict the impact of stress on honey bees

Background

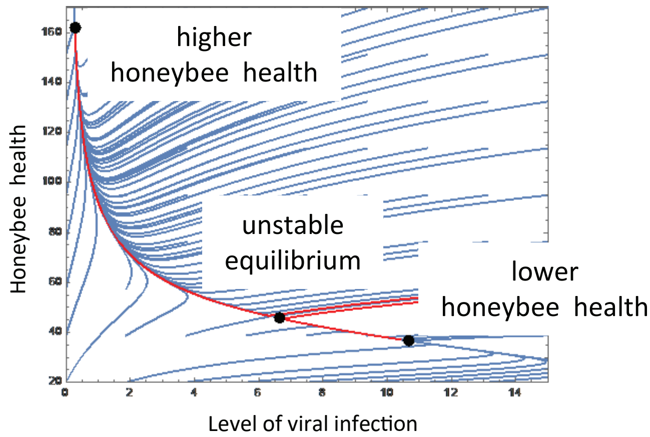
Pesticides, as well as many other stressors, affect honey bee health and hive conditions. However, while the negative impact of some pesticides has been shown convincingly in the laboratory, studies in real-world conditions are contradictory.

Question

What is the cause of these contradictory results? Are harmful pesticides in the lab similarly harmful under realistic field conditions or not?

Methods

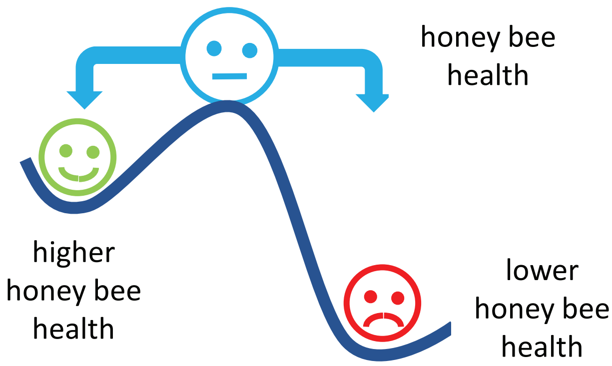
We developed a model of honey bee health as affected by parasites, pathogens, pesticides, suboptimal temperatures and food availability. We analyzed this system to study its possible equilibria (the possible outcome in terms of honey bee health when it does not change any longer).



Results

DWV can suppress honey bee immunity. This generates a situation where the honey bee has two possible stable health points, either low or high.

Thus bee health can be compared to a ball sitting on the top of a hill separating two adjacent valleys. The ball can either fall into one or the other valley, depending on any small initial perturbation.



Implications

If DWV is present, the addition of a second stressor, like a pesticide, can result in either satisfactory or low bee health. This will depend upon tiny differences in the initial state of the honey bee. This explains why real-world studies can produce conflicting results, and makes understanding of how stressors impact real-world honey bee health challenging!

Source

Breda D., Frizzera D., Giordano G. et al., 2022 A deeper understanding of system interactions can explain contradictory field results on pesticide impact on honey bees. *Nat Commun* 13: 5720. **https://doi.org/10.1038/s41467-022-33405-7**