Report on exposure of bees to pathogens

Deliverable D2.3

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PoshBee

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

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Summary

High-throughput real-time quantitative PCR methods have been developed and validated for the detection and quantification of 11 pathogens (2 bacteria, 3 microsporidia and 6 viruses) in honey bees, bumble bees and solitary bees exposed to pesticides in field-realistic conditions. The 582 bee samples collected in 2019 by the PoshBee's Work Package 1 teams were analysed, and produced 6,402 results describing the pathogen profiles of managed and wild bees. The data are available in PoshBee's database (PoshBee deliverable D2.1) and will be used to assess the risk for bee health of co-exposure to pathogens and pesticide.

1. Field samples

Bee samples were collected in the field by the teams of Work Package 1 to measure the exposure to pathogens under field-realistic conditions.

1.1. Field sites selection

Eight European countries were involved in the sampling of honey bees (*Apis mellifera*) and wild bees (bumble bees - *Bombus terrestris* and solitary bees - *Osmia bicornis*): the United Kingdom, Ireland, Germany, Switzerland, Spain, Italy, Estonia, and Sweden.

In each of these eight countries, 16 sampling sites were selected on two crops (oil seed rape fields (OSR) and apple orchards (APP)) based on the land-use intensity gradient in a 3-km radius (PoshBee deliverable D1.2 Report on landscape context of field sites). Among the selected sites, eight were OSR fields (labelled 01 to 08) and eight were APP orchards (labelled 09 to 16). The OSR sites showed a gradient of pesticide use. The APP sites randomly belonged to various crop management systems, from conventional to environment-friendly and organic.

1.2. Bee installation

Prior to the plant flowering period, three hives of honey bees, three colonies of commercially-purchased bumble bees, and three nests of commercially-purchased solitary bees were installed on each site on the appropriate side of the crop field/orchard. Solitary bee nests were installed on the sites of six countries, but not in Ireland or in the United-Kingdom.

1.3. Bee sampling

On each site, bees of the three species (*A. mellifera*, *B. terrestris* and *O. bicornis*) were sampled in the hive/colony/nest installations, as presented in Figure 1 below. For each bee species, one bee sample corresponded to a pool of bees collected in the three hives, colonies or nests.

Honey bees and bumble bees were sampled at two time points of the flowering period: "before" their installation on the sites (for pathogen loads, quantification before exposure to field conditions), and during or after the flowering period (for pathogen loads, quantification after exposure). In order to reduce the impact of the sampling on bee emergence and to maximise the number of adults emerging on the sites, only one initial sample of 10 solitary bee females was collected for each of the six countries before exposure.

This sampling process produced a total of 631 expected samples, including 256 samples of honey bees, 256 samples of bumble bees and 119 samples of solitary bees.

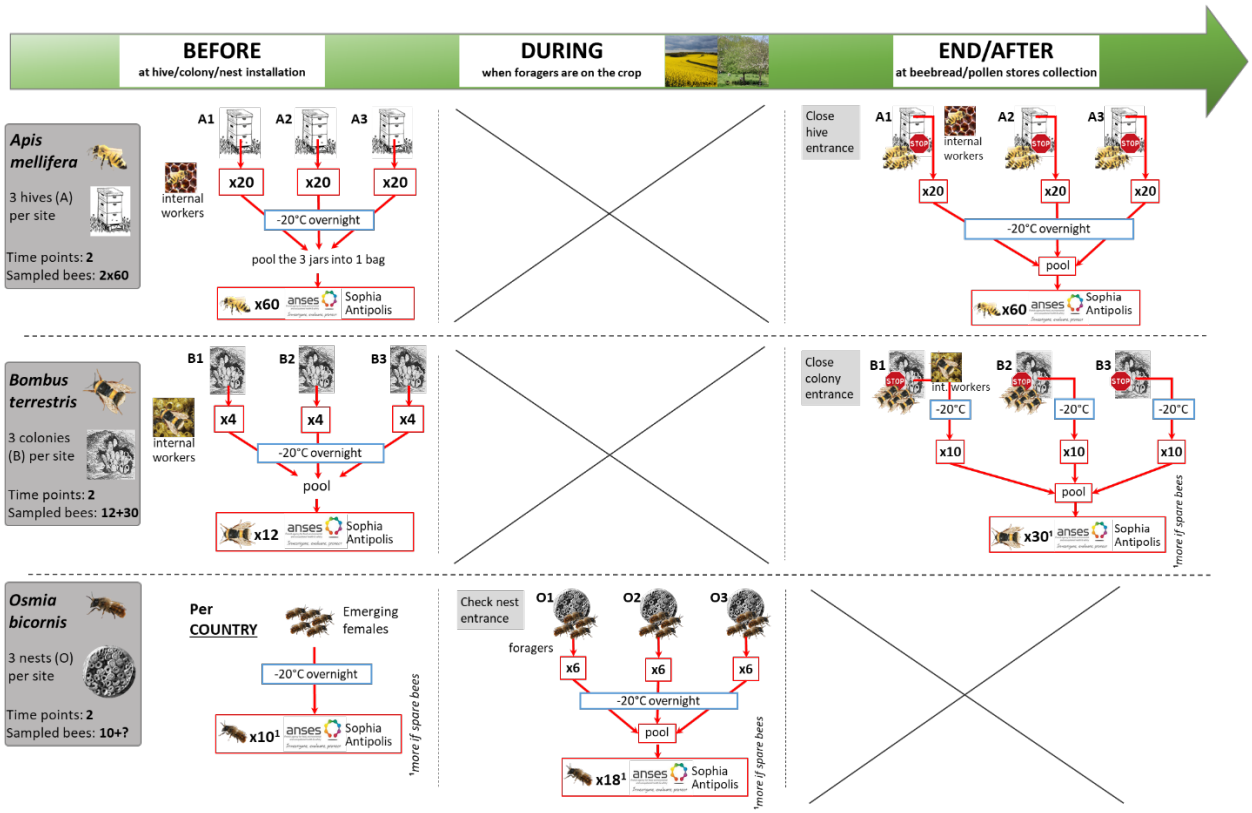

Figure 1: Outline of the bee sampling for pathogens analysis at each site and at the three time points of crop flowering period

2. Development and validation of the molecular quantification method

2.1. Targeted pathogens

The molecular screening focused on 11 honey bee pathogens: 6 viruses (Acute bee paralysis virus - ABPV, Black queen cell virus - BQCV, Chronic bee paralysis virus - CBPV, Deformed wing virus types A and B - DWV-A and –B, and Sacbrood virus - SBV), the 2 bacterial agents of foulbrood (*Paenibacillus larvae (Pl)* and *Melissococcus plutonius(Mp)*), and 3 *Nosema* microsporidia (*Nosema apis (Na), Nosema ceranae (Nc),* and *Nosema bombi (Nb)*).

2.2. Molecular quantification method

The 11 pathogens were quantified by a harmonised high-throughput real-time quantitative PCR (qPCR). Before being used on the field bee samples, the performance of the methods (limit of detection, limits of quantification, specificity, trueness, and precision) were validated according to the standard NF-U47-600, part 2 (Afnor, 2015).

2.2.1. Sample preparation

Ahead of the real-time qPCR analysis, samples were ground for nucleic acid extraction (see Figure 2). Due to the wide diversity of samples, the grinding method was adjusted to the sample bee species and volume (*e.g.* Ultra-turrax grinding for the largest bee samples or Tenbroeck device for single bee samples). Nucleic acids (RNA and DNA) were purified on spin columns (Machery-Nagel) with the high-throughput automated extraction of nucleic acids (TECAN pipetting robot).

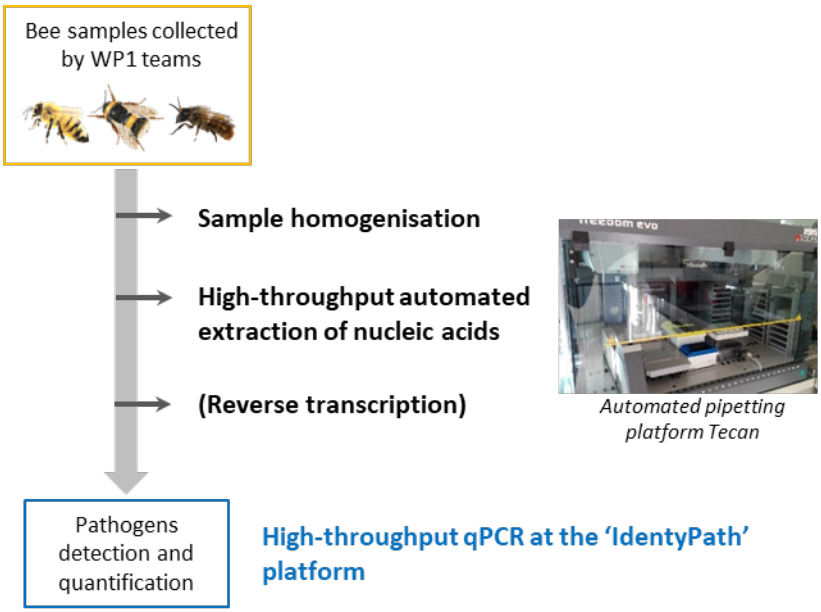

Figure 2: Outline of the molecular quantification method for the analysis of bee pathogens

The sample preparation and nucleic acid purification (and reverse transcription of viral RNA) were performed at Anses Sophia Antipolis before being shipped to the Anses analytical platform "IdentyPath" (Maisons-Alfort, France) for pathogen quantification by a LightCycler® 1536 thermocycler (Roche), except for ABPV and DWV-B viruses. These last viruses were quantified with a Quant Studio 5 thermocycler (Applied Biosystems) at Anses Sophia Antipolis.

2.2.2. Quantification of viruses and bacteria

For both viruses and bacteria, standard qPCR protocols validated by the European reference laboratory for diagnosis (www.eurl-bee.anses.fr) were optimised with the reagents and the parameters for high-throughput qPCR (e.g., for the American foulbrood quantification, primers were modified to perform the qPCR with the same temperature parameters as those used for the other pathogens).

2.2.3. Quantification of *Nosema* microsporidia

For the three microsporidia species, relevant molecular targets for an accurate quantification by real-time qPCR were selected from the literature. This step was followed by an *in silico* (bioinformatics) analysis to design the molecular primers and probes to run the qPCR experiments. Recombinant plasmids including the targeted sequences were also produced to serve as material for standard curves of qPCRs. *In vitro* assays of candidate primers and probes allowed the selection of efficient candidates for the method characterisation and validation.

3. Results summary

3.1. Analysed samples

A total of 582 bee samples were delivered in autumn 2019 to the Anses – Laboratory of Sophia Antipolis for the analysis of their pathogens. This represents a satisfactory 90% of the expected samples. This amounted to 255 samples of honey bees (128 before field exposure and 127 after), 250 samples of bumble bees (128 before field exposure and 122 after), and 77 samples of solitary

bees (8 before field exposure and 69 after; corresponding to 65% of expected samples because of poor nest performances on the field).

3.2. Data description

Prior to the exposure to field conditions, 10 out of the 11 screened pathogens were detected in honey bee samples with varying prevalence, the most prevalent being the viruses. The exposure to field conditions increased the prevalence with varying amplitudes depending on the pathogen (Table 1). For bumble bee samples, only viruses and one microsporidia were initially detected. After exposure to field conditions, a similar panel of pathogens was detected but with higher prevalence (Table 2). None of the pathogens were initially detected in solitary bee samples. However, a few of them, mainly viruses, were detected after the solitary bees had been exposed to field conditions (Table 3).

Table 1: Prevalence (in %) of each pathogen in *Apis mellifera* bees before and after exposure to field conditions.

Apis mellifera	Sampling time	ABPV	BQCV	CBPV	DWV-A	DWV-B	SBV	*Pl*	*Mp*	*Na*	*Nc*	*Nb*
UK	Before	31.3	100	31.3	25.0	100	100	0	43.8	50.0	87.5	0
	After	0	100	37.5	37.5	100	100	0	31.3	37.5	81.3	0
Spain	Before	12.5	100	81.3	100	100	93.8	0	0	25.0	62.5	0
	After	31.3	100	43.8	100	100	43.8	0	0	0	43.8	0
Switzerland	Before	37.5	100	6.3	0	100	100	0	0	0	100	0
	After	31.3	100	50.0	18.8	93.8	100	0	0	0	100	0
Italy	Before	0	100	93.8	6.3	68.8	100	0	0	0	50.0	0
	After	0	100	56.3	6.3	87.5	100	0	0	0	87.5	0
Germany	Before	6.3	100	37.5	0	93.8	62.5	0	0	0	68.8	0
	After	12.5	100	56.3	0	100	93.8	0	0	0	87.5	0
Ireland	Before	0	93.8	0	31.3	100	25.0	6.3	0	18.8	0	0
	After	0	100	0	50.0	100	25.0	6.3	0	12.5	12.5	0
Estonia	Before	31.3	100	31.3	87.5	100	81.3	6.3	0	68.8	81.3	0
	After	6.7	100	33.3	80.0	73.3	80.0	0	0	60.0	80.0	6.7
Sweden	Before	0	100	0	100	100	100	0	0	6.3	12.5	0
	After	12.5	100	0	100	100	100	0	0	6.3	50.0	0

Table 2: Prevalence (in %) of each pathogen in *Bombus terrestris* bees before and after exposure to field conditions.

Bombus terrestris	Sampling time	ABPV	BQCV	CBPV	DWV-A	DWV-B	SBV	*Pl*	*Mp*	*Na*	*Nc*	*Nb*
UK	Before	0	50.0	0	0	0	25.0	0	0	0	0	0
	After	6.3	100	18.8	0	100	100	0	0	0	0	6.3
Spain	Before	25.0	43.8	12.5	6.3	0	37.5	0	0	0	0	0
	After	0	100	0	62.5	87.5	12.5	0	0	0	0	0
Switzerland	Before	18.8	37.5	0	0	12.5	12.5	0	0	0	0	0
	After	0	93.8	0	0	87.5	100	0	0	0	0	0
Italy	Before	62.5	100	87.5	18.8	62.5	100	0	0	0	0	0
	After	6.7	100	26.7	6.7	20.0	100	0	0	0	0	0
Germany	Before	0	12.5	18.8	6.3	0.	0	0	0	0	0	0
	After	0	100	0	0	62.5	100	0	0	0	0	6.3
Ireland	Before	31.3	50.0	0	0	62.5	18.8	0	0	0	0	0
	After	0	68.8	0	0	100	6.3	0	0	0	0	0
Estonia	Before	0	75.0	0	12.5	6.3	50.0	0	0	0	6.3	0
	After	9.1	100	0	18.2	18.2	100	0	0	0	0	0
Sweden	Before	6.3	81.3	0	18.8	37.5	6.3	0	0	0	0	0
	After	6.3	100	0	31.3	93.8	100	0	0	0	0	12.5

Table 3: Prevalence (in %) of each pathogen in *Osmia bicornis* bees after exposure to field conditions (none of the pathogens was detected before exposure).

Osmia bicornis	ABPV	BQCV	CBPV	DWV-A	DWV-B	SBV	*Pl*	*Mp*	*Na*	*Nc*	*Nb*
Spain	0	57.1	0	50.0	64.3	0	0	0	0	0	0
Switzerland	0	50.0	0	0	50.0	83.3	0	0	0	0	0
Germany	0	46.2	0	0	38.5	69.2	0	0	0	0	0
Estonia	0	93.3	0	13.3	13.3	86.7	0	0	0	6.7	0
Sweden	0	80.0	0	33.3	66.7	93.3	0	0	0	0	0

The 6,402 results have been loaded into the PoshBee database to be available to each PoshBee partner in order to investigate the links between pesticide exposure and bee health. At the end of the project, the data will be available for relevant stakeholders to PoshBee.

4. Acknowledgements

We acknowledge all the teams involved in the WP1 field sampling of bees.

5. References

AFNOR, 2015. NF U47-600, Part 2: Animal health analysis methods - PCR - Part2: Requirements and recommendations for the development and the validation of veterinary PCR, French association for standardisation (AFNOR), www.afnor.fr, pp. 1-51.