

# Synthesis report on Omics Deliverable D10.5

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PoshBee

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



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#### Summary

Deliverable D10.5, arising from Task 10.3, reports from the current bibliography on the use of omics (genomics, transcriptomics, proteomics, metabolomics, and their association/combination referred to as multiomics) to monitor the impact of stressors (abiotic and biotic) on the three bee species investigated within PoshBee (Apis mellifera, Bombus terrestris and Osmia bicornis). The bibliometric analysis was centred on the Web of Science and Scopus databases. Fifty-four keywords were used to run the first search and then to refine the study by using filters. Different omics disciplines were investigated in our literature search. Genomics was found to be the most used approach to study effects of stressors on bees followed by metabolomics and transcriptomics. The proteomics studies on the bee species evaluated within PoshBee are only recently emerging in this world of omics. Here, PoshBee presents its innovative input in proteomics to study the effects of (i) the pesticides sulfoxaflor, azoxystrobin, flupyradifurone and glyphosate, (ii) nutrition and (iii) pathogens. The general omics screening showed that Apis is the most studied species, followed by Bombus and Osmia. The larvae and adult workers are the most studied stages/castes. Regarding pesticides, we found that in omics, the insecticides are most studied, followed by the fungicides and the herbicides. Among the pesticides, our screening showed that imidacloprid is the most studied, followed by glyphosate, flupyradifurone, sulfoxaflor and azoxystrobin. The analysis was advanced to a deeper level by focusing on the omics papers (i) reporting the impact of the main pesticides used in PoshBee (sulfoxaflor, azoxystrobin, glyphosate, flupyradifurone and imidacloprid), (ii) the main bee species studied within PoshBee (Apis, Bombus and Osmia) and (iii) the proteins identified to play crucial roles in detoxification, immunity and other responses to stress. Based on our bibliometric analysis, the protein markers that have been most studied belong to the Cytochrome P450 family. The peptides/proteins Vitellogenin, Defensin, Hymenoptaecin, Abaecin, and Apidaecin were mostly reported in Apis with a very few studies on Bombus. Within PoshBee, these markers were investigated in Apis, Bombus and Osmia in order to deepen knowledge about these markers in these three major species. The results from our analysis of the literature outside PoshBee demonstrate that PoshBee objectives and outcomes were complementary to this literature. To our knowledge such omics studies on three major species of pollinators (Apis, Bombus, Osmia) to elucidate and to deepen knowledge on the molecular impact of stressors alone or in synergy in field, semi-field and laboratory conditions has never been achieved in other projects than PoshBee.

## 1. Introduction

Omics refers to high-throughput analyses, for example of metabolites (metabolomics), proteins (proteomics), or genes (genomics/transcriptomics) in a biological system, representing comprehensive studies of the roles, relationships, and actions of various types of molecules in an organism. These system-based approaches can unravel stressor-related processes and are important for biomarker discovery in different contexts (i.e., disease, environmental exposure, reproduction, infection, behaviour) and interestingly in our project in bee health monitoring. This issue is preliminary to any development of tools for health monitoring and evaluation of treatments. The number of omics studies performed with the overall aim of better understanding the effects of pesticides and/or pathogens and/or climate change on bees has increased substantially over the past decade. We review this literature through a formal literature search performed exclusively on scientific manuscripts published between 2018 and 2022, the period covered by the PoshBee project. This deliverable is the results of that formal literature search.

## 2. Material and Methods

#### 2.1. Identification of the scope and relevant keywords

For the omics search, we ran a literature search using the following keywords found to be relevant for studying the impact of stressors in bees at the molecular level:

#### #1 Omics

("biological pathway" OR "Bottom-Up proteomics" OR "differential analysis" OR electrophoresis OR genome OR genomics OR glycomics OR haemolymph OR hemolymph OR "Imaging mass spectrometry" OR "innate immunity" OR "LC-ESI-MS/MS" OR LFQ OR lipidomics OR MALDI\* OR "MALDI BeeTyping" OR "MALDI biotyping" OR "MALDI imaging" OR "marker discovery" OR "marker identification" OR "mass fingerprint" OR "mass spectrometry" OR metabolomics OR detoxification OR "molecular crosstalk" OR "molecular histology" OR "molecular impact" OR "molecular pattern" OR multiomics OR proteomics OR sequencing OR "Top-down proteomics" OR transcriptomics)

## #2 Population

AND ("honey bee\*" OR honeybee\* OR solitary bee\* OR pollinator\* OR Apis OR Bombus OR bumblebee\* OR "bumble bee\*")

#### <u>#3 stressors</u>

AND (pathogen\* OR parasit\* OR disease\* OR virus\* OR pest\* OR fungi OR bacteria\* OR "bee disease" OR varroa\* OR varroa OR "varroa mite" OR "biological stressor\*" OR probiotic\* OR microbiom\* OR nutrition OR protein\* OR pesticide\* OR fungicide\* OR herbicide\* OR insecticide\* OR neonic\* OR "heavy metals" OR metals OR metabolite OR chemical OR agrochemical OR "plant protection product" OR acaricide\* OR glyphosate OR boscalid OR thiacloprid OR azoxystrobin OR imidacloprid OR thiamethoxam OR fipronil OR clothianidin OR deltamethrin OR tebuconazole OR thymol OR amitraz OR acetamiprid OR coumaphos OR cyantraniliprole OR Dimethoate OR tau-fluvalinate OR cypermethrin OR thiacloprid)

#### #4 period

## 2018 to 2022.

This search string was uploaded to two complementary bibliography databases: <u>Web of Science</u> and <u>Scopus</u>, in order to extract scientific papers associated with this topic.

The information extracted from this survey is described below (Table 1):

Publication information	Source, Authors, Article title, Publication, Year, DOI Abstract					
Specific Omics	Detoxification, Lipidomics, Transcriptomics, Omics, Genomics, Glycomics, Metabolomics, Multiomics, Proteomics, Immune system, GC-MS, LC-MS, MALDI, Gene, Mass spectrometry, DNA, RNA,					
Stressor type	Flupyradifurone, Sulfoxaflor, Glyphosate, Azoxystrobin, Imidacloprid, Herbicide, Insecticide, Miticide, Fungicide, Pollen, Varroa, Nosema, Crithidia					
Caste/ colony/ population	Male, Queen, Workers, Larv., Colony,					
Type of experiment	Lab, Field, Semi-field					
Exposure	Acute, Chronic, Field-realistic					
Species	Apis, Bombus, Osmia					

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## **2.2.** Review of PoshBee outputs

The deliverables and milestones produced within PoshBee associated with omics [in Work Package (WP) 9] were included in this bibliographic study in addition to the literature database generated from Web of Science and Scopus. The list of these deliverables and milestones is presented in Table 2:

Year	Report n°	Description			
2018	D9.1	Haemolymph field collection kit and traceability tool			
2010	D9.2	Validated SOPs for MALDI mass fingerprints I			
2019	D9.5	Validated models for bees exposed to stressors I			
2020	D9.3	Validated SOPs for MALDI mass fingerprints II			
2020	D9.6	Validated models for bees exposed to stressors II			
	D9.4	Validated SOPs for MALDI mass fingerprints III			
2021	D9.8	Consolidated peptide/protein databases including markers for application I			
2021	D9.11	Manuscripts on Omics research for model bees I			
	D9.7	Validated models for bees exposed to stressors III			
	D9.9	Consolidated peptide/protein databases including markers for application II			
2022	D9.12	Manuscripts on Omics research for model bees II			
	D9.14	Method for honey bee MALDI imaging			
	MS20	Validation of biomarkers for bee health			
2022	MS21	Validation of MALDI imaging			
	MS22	MALDI BeeTyping <sup>®</sup> prototype			

# 3. Results

Using this procedure, we recovered 1999 peer-reviewed articles (entries) with Web of Science and 3999 with Scopus. Then we merged both datasets and the duplicates were removed based on title and DOI. Using this refinement 4797 unique entries were conserved. The flow diagram of the process and the obtained number of papers is presented in Figure 1.





## 3.1. General Bibliometric screening

Based on the general screening (n=2171), the number of publications per year showing scientific production in the field of pollinator omics was explored and is reported in Figure 2. It shows a number of publications more or less constant over the five years.



Figure 2: Number of published papers related to omics and stressors in bees per year during the five past years (2018-2022)

The screening search allowed us to classify the publications according to the publication year and the keywords we selected and used. In omics, the highest number of published papers was in genomics (n=557), then metabolomics (n=389), transcriptomics (n=176) and proteomics (116). Only one paper was found to report lipidomics data and no papers mentioned glycomics (Figure 3).



Figure 3: Number of published papers in omics per omics category/discipline

In these omics papers, we investigated the techniques used, such as mass spectrometry-based (proteomics and metabolomics) and those dealing with RNA, DNA or genes (genomics and transcriptomics). The details are presented in Figure 4.







As a complementary analysis, we were interested in the representation of bee species within our dataset; we found that *Apis* was the most investigated species with more than 1000 publications (n=1144), then *Bombus* with 128 publications and finally *Osmia* with 17 publications (Figure 5).



Figure 5: Number of publications on the three main bee species Apis, Bombus and Osmia

At this stage, to complete the study of the species, we focused on the castes/life stages of honey bees and found that the highest number of studies was conducted on larvae (n=304), workers (n=302) and then at the colony level (n=301); 184 studies were performed on queens and 171 on males (Figure 6).



Figure 6: Number of publications on the different castes/life stages of bees

Regarding the type of stressors, the first topic of the analysis was related to pesticides, namely their target (classification) and name. The classifications were insecticide (n=188 papers), fungicide (n=41) and herbicide (n=25). Only two papers were on miticides (n=2) (Figure 7).



Figure 7: Number of publications per class of pesticide

Imidacloprid was the most studied pesticide, with 84 papers, followed by Glyphosate (n=19), Flupyradifurone (n=10), Sulfoxaflor (n=9), and Azoxystrobin (n=4) (Figure 8).





The second topic of investigation of the stressors was about viruses (generic name) and parasites (*Crithidia, Nosema* and *Varroa*). The highest number of scientific contributions was found on viruses (n=247), followed by *Varroa* (n=168), *Nosema* (n=111) and finally *Crithidia* (n=24) (Figure 9).



Figure 9: Number of publications per pathogen and parasites

The studies reported in our analysis relied on two different criteria (1) the doses used and (2) the duration of exposure to stressors. The literature mainly addressed acute (n=94), chronic (n=84) and field-realistic (n=17) doses (Figure 10).



Figure 10: Number of publications according to the type of bee exposure to stressors Field-realistic means that studies were performed using concentrations of chemicals generally used in the field.

In relation to the combination of factors (combination between abiotic and biotic factors), most of the studies focused only on one factor (n=578), less on two (n=135) and even fewer on a combination of three factors (n=69) (Figure 11).





## **3.2.** Stressors in omics

To complete this bibliometric study, we analysed the distribution of papers in omics according to the stressor(s) investigated within PoshBee (sulfoxaflor, azoxystrobin, glyphosate, *Nosema*, *Crithidia*,

*Varroa* and viruses), complemented with Imidacloprid since it is a widely investigated insecticide and was occasionally employed in PoshBee studies. A total of 47 papers were investigated to extract information on the bee model, the type of experiment conducted, the results obtained and the conclusions.

#### **3.2.1.** Abiotic stressors:

Azoxystrobin was investigated within PoshBee. According to the literature, no study was found to investigate azoxystrobin in omics. Within PoshBee, metabolomics was used to chase Cytochrome P450 and vitellogenin expression on *A. mellifera* following exposure to azoxystrobin (Barascou et al., 2021). Additional value was added as the omics analyses (Molecular mass fingerprints (MFP) and proteomics) on bee haemolymph were performed to study the effects of this pesticide not only on *Apis* but also on *Bombus* and *Osmia* in semi-field and laboratory experiments. The results obtained by PoshBee are reported for the first time in the international literature.

Sulfoxaflor is known as one of the latest systemic insecticides developed to circumvent the toxicity of previous formulations using neonicotinoids to pollinators. According to the literature only Zhou et al. in 2022 reported a metabolomics study on the molecular impact of this pesticide (Zhou et al., 2022). Within PoshBee, LC-MS/MS was used to investigate the effects of sulfoxaflor on honey bees (Barascou et al., 2022). Furthermore, multiple experiments were conducted within PoshBee at three levels (field, semi-field and laboratory) on the three main species (*Apis, Bombus* and *Osmia*). Moreover, the effects on the novel wild species investigated were also of interest. Our omics analyses were based on innovative tools, namely the MALDI MFP (MALDI BeeTyping), bottom-up proteomics and the IMS. The results reported by PoshBee have not been reported before.

Regarding the butanolide insecticide flupyradifurone, comparative studies of its neurotoxicity in *Apis* species have been undertaken. qPCR and UPLC-MS/MS are the common approaches reported to study the effects of this insecticide (Haas et al., 2022, 2021). In PoshBee, the investigation was performed on *Osmia* species through laboratory and semi-field experiments. Here again, the MFP and the proteomics were conducted exclusively within PoshBee. Interestingly, the effects of flupyradifurone were studied in combination with other factors such as the parasite *Crithidia* and the nutrition (buckwheat and phacelia).

To quantify the widely used herbicide glyphosate and its main metabolite AMPA in honey bee, LC-MS/MS has been successfully reported as a straightforward method (Kasiotis et al., 2018). Most of the literature studies (n=9), based on omics, report glyphosate impacts on honey bees (Zhao et al., 2020 and Faita et al., 2022). In Poshbee, the glyphosate effects were investigated on the three model bee species and at three levels. We analysed the bee haemolymph of bees exposed to glyphosate in field, semi-field and laboratory experiments in association with nutritional or chemical stress.

Imidacloprid, a neonicotinoid class of pesticides, was a widely applied insecticide in the control of pests in agricultural fields, and studies investigating its impact on bees (i.e., honey bees, bumble bees) are quite numerous. Using transcriptomics, several studies (n=9) investigated the effects of imidacloprid on honey bees (Almasri et al., 2020, 2022; Chen et al., 2021a, 2021b; Christen et al., 2018; Gao et al., 2020; Kim et al., 2022; Ma et al., 2018; Murawska et al., 2021). They focused on the impacts on several enzymes involved in toxin metabolism, detoxification, stress and immunity. In addition, proteomics was used to follow the degradation and toxicity of pesticides and the distribution by MALDI mass spectrometry imaging of proteins in the brain of *A. mellifera* (Catae et al., 2018). They also showed that imidacloprid also caused damage in the midgut (Catae et al., 2018). In 2022, a proteomics study was conducted on haemolymph of *A. mellifera* (workers versus drones) (McAfee et al., 2022). Several papers were published referring to metabolomics studies for monitoring pesticide residues and metabolites in *A. mellifera* (Ardalani et al., 2021a, 2021b; Haas and Nauen, 2021; Kasiotis et al., 2021; Shuai et al., 2022; Zaworra et al., 2019). Only a few studies examined the effect of imidacloprid on bumble bees using metabolomics (Manjon et al., 2018), transcriptomics (Erban et al., 2019b), and proteomics (Rothman et al., 2020). As shown here, the work on this pesticide is already well advanced and therefore was not introduced by PoshBee. Interestingly, using omics tools, we will contribute to deepening the knowledge of this pesticide through investigations within PoshBee using our original tools and analytical strategy.

#### 3.2.2. Biotic stressors

According to our literature search, the impacts of the parasite *Crithidia* (*C. bombi* and *C. expoeki*) on bees have been studied from the perspectives of genomics (n=6), transcriptomics (n=1), and metabolomics (n=1). No papers in proteomics were found during the past five years. Omics were used on bumble bees (de Sousa Pereira et al., 2019; Gerasimov et al., 2019; Schmid-Hempel et al., 2018). Furthermore, a metatranscriptomic study of eight social and solitary wild bee species was performed, aiming to discover novel viruses and parasites (Schoonvaere et al., 2018). In addition, the effect of two trypanosomatid species, *Lotmaria passim* and *C. mellificae* on honey bees was investigated (Liu et al., 2019). More recently, one study examined the effects of *Crithidia* on *A. mellifera* and *A. ceranae* (Gancarz et al., 2021). Within PoshBee, using omics tools, the effects of *Crithidia* on *Bombus* and *Osmia* and also the interaction with the pesticides were analysed. This is an endpoint that has not been analysed before PoshBee.

Based on the literature, Nosema was investigated in genomics (n=21), metabolomics (n=19), transcriptomics (n=4) and proteomics (n=4). Here we focus only on proteomics. The main proteomic studies conducted on the impact of Nosema on bees are from our team at BIOPARK (Houdelet et al., 2022, 2021a, 2021b) and were in part performed within PoshBee (Houdelet et al., 2022). In these studies, we first demonstrated that MALDI BeeTyping is an effective approach to decipher and assess the identity of the honey bee pathogen N. ceranae (Houdelet et al., 2021a), complementary to PCR analysis. Then, we used an approach of quantification termed label-free quantification (LFQ) to investigate the effect of this parasite on anatomical sections of the gut of Nosema-infected A. mellifera (Houdelet et al., 2021b). A third study to decipher the impact of Nosema was carried out using MALDI IMS (Houdelet et al., 2022). In this paper, MALDI IMS was introduced as an effective approach to monitor the impact of N. ceranae infection on honey bees (Houdelet et al., 2022). This innovative approach of imaging was never used before our project to follow the impact of stressors on whole sections of honey bees. MALDI imaging was also used within PoshBee in collaboration with the INRA team. The experiment exposed honey bees to sulfoxaflor and viruses to follow their response using pictures. Using MALDI-TOF MS, Los et al. (2020) investigated the manifestation of bacterial and fungal microorganisms in pollen load on O. bicornis. This technique is similar to the MALDI BeeTyping we initiated on A. mellifera (Arafah et al., 2019) and applied to Apis, Bombus, Osmia and other novel bee models within PoshBee.

*Varroa destructor* is one of the most prevalent parasites in honey bee hives. It has also been studied by many researchers in omics (n=65). This parasite was mainly investigated in genomics (n=44), then metabolomics (n=16), transcriptomics (n=12) and proteomics (n=4). In this section, we focus only on

proteomics. In proteomics, 4 papers were identified in our search (Erban et al., 2019a; Genath et al., 2021; Słowińska et al., 2019; Ward et al., 2022). LFQ was used in two studies (Erban et al., 2019a; Ward et al., 2022) to investigate the effects on *A. mellifera*. Using another kind of approach, 2D-DIGE proteomics, Słowińska et al. revealed that varroa infestation affected many biological pathways in *Apis* (Słowińska et al., 2019). The study of Genath et al. (2021) focused on the *Varroa* proteome when infesting the honey bees and reported dysregulation of the proteostasis, oxidative stress, detoxification proteins and cellular defence mechanisms (Genath et al., 2021). In PoshBee, we used LFQ to study the effects of all stressors on *Apis, Bombus* and *Osmia. Varroa* was recorded in *Apis* hives from the field work and correlations between *Varroa* presence and impact on MFPs were performed.

Another important input is that within PoshBee, we performed experiments on bacterial challenge to study the immune responses of *Apis*, *Bombus* and *Osmia* using proteomics as tools. According to the literature, this kind of research was only previously reported for *Apis* and *Osmia* using bacterial infection models different from those used within PoshBee. This demonstrates an additional endpoint of PoshBee in the understanding of bee health in response to biotic stressors. Importantly, the unique work on *Apis* using MALDI BeeTyping to study the effects of bacteria was published by our team in 2019 (Arafah et al., 2019).

Moreover, regarding nutrition, the literature search identified many omics papers (n=64) reporting effects of pollen. These studies are mainly on *Apis* and *Osmia* aiming to test the quality of the pollen and to study its metabolites (Li et al., 2018; Lu et al., 2022). Interestingly, one study investigated the impact of different pollen types on honey bee immunity under laboratory conditions (Danihlík et al., 2018). However, overall, the objectives of the published studies are different from those undertaken within PoshBee, where we analysed the interaction between pollen and pesticides in different contexts, namely laboratory and semi-field.

#### 3.3. Protein markers

Here, in our bibliographic survey, we looked for the identification of protein markers involved in pesticide/Reactive Oxygen Species (ROS) detoxification and those contributing to the immune response of the host to stressors. The information recovered (Table 3) demonstrated that the most studied markers are the Cytochrome P450 family (n=65), followed by Vitellogenin (n=58), and the immunopeptides Defensin (n=33), Hymenoptaecin (n=23), Abaecin (n=21) and Apidaecin (n=14). Interestingly, within PoshBee, we demonstrated that in addition to these markers, the Chymotrypsin inhibitor (AMCI, (Houdelet et al., 2022)) represents a promising marker. Only three papers mentioned this enzyme (Felicioli et al., 2018; Jasso-Martínez et al., 2021; Kim et al., 2021).

Cytochrome P450	65
Vitellogenin	58
Defensin	33
Hymenoptaecin	23
Abaecin	21
Apidaecin	14
Chymotrypsin	3

#### Table 3: List of the markers identified in the literature search

These detoxification and immune response markers were studied mainly in *Apis*, as shown in the Venn diagrams (see below **Figure 12**).



Figure 12: Protein markers investigated according to the three main bee species *Apis, Bombus* and *Osmia* 

## 4. Discussion

Within PoshBee, using omics tools (MFP, proteomics and IMS), we studied the impacts of the different stressors (pesticides, nutrition, pathogens) and focused on the three main species (*Apis, Bombus* and *Osmia*). Based on MFP, we followed the proteins involved in the immune response (Hymenoptaecin, Apidaecin, Abaecin, Defensin and Chymotrypsin inhibitor). Results were reported for *Apis* and *Bombus*. Additional work is in progress for validation of the homologs in *Osmia*. Variation of some of these proteins was also demonstrated with MALDI IMS in bee tissues in the context of experimental infections with spores of different isolates of *Nosema spp*. (Houdelet et al., 2022). Interestingly, the proteomics-based LFQ approach allowed us to identify the proteins cited above in our databases and to follow the variation of the two proteins Vitellogenin and Cytochrome P450 when detected in the haemolymph samples. This contributed to reinforce our knowledge regarding the role of these proteins in bee health.

This bibliometric study based on omics and more precisely on proteomics in bees was performed with the aim of having a better understanding of current literature and the input of PoshBee in terms of innovation and deeper knowledge on stressor impacts on bee health. In omics, we used a series of keywords to compile a complete list of what has been published on these topics over the last 5 years (the same timeframe as PoshBee).

Based on the results of our literature search, we observed that most omics investigations were conducted largely to study the impact of stressors on honey bees (Apis). This is not surprising since A. mellifera is the bee species most frequently subject to human management due to its economic value worldwide (Osterman et al., 2021). The second most studied species was bumble bees (Bombus), which is also linked to their importance in terms of pollination and economical value (Osterman et al., 2021). The third ranked species, which are the most recent species of interest for pollination, are solitary bees in the genus Osmia. Regarding the stressors tested on bees, we identified the insecticide imidacloprid as the most studied pesticide. This is due to the fact that this insecticide is used worldwide in agriculture due to its selective toxicity for insects. Imidacloprid was generally not employed in the PoshBee project as a pesticide since it is well documented in the literature and was banned from use in the EU soon after the initiation of PoshBee. The main pesticides investigated by PoshBee are glyphosate (herbicide), the two insecticides flupyradifurone and sulfoxaflor, and the fungicide azoxystrobin. A literature survey reporting on these pesticides is limited, showing that PoshBee contributed substantially to improve our knowledge on the use of omics approaches to monitoring the impacts of pesticides on bee health. In terms of route, acute and chronic exposure were the most documented. 'Field-realistic' was given more emphasis in PoshBee to study the toxicity of pesticides on the three main species Apis, Bombus and Osmia. Regarding pathogens, as expected the Varroa mite (Arachnida), the world's most devastating pest of honey bees due to their potential to transmit viruses such as DWV (Deformed Wing Virus), was the most investigated parasite followed by the flagellate Crithidia (Kinetoplastea) and the microsporidia Nosema. Within PoshBee, more interest was given to Crithidia and Nosema because they target the gut tissue and may be in interaction with pesticides. Moreover, no semi-field or laboratory experiments from PoshBee studied the effects of Varroa, Thus, omics studies are needed to be carried out in the future to better evaluate the effects of Varroa on bee health. Protein markers linked to stressor exposure have also been investigated in this bibliometric search. The Cytochrome P450 family was the most documented, then Vitellogenin and the immunopeptide Defensin. These molecules are widely shown to be involved in bee response to chemicals or pathogens, followed by the immunopeptides Abaecin and Apidaecin. Chymotrypsin and its inhibitor (AMCI) also play roles in host defence. Through the omics approaches we developed and applied to *Apis, Bombus* and *Osmia*, the AMCI appeared as a potential marker to follow the bee health. All protein markers were mostly investigated in *Apis*. Within PoshBee, WP9 "Omics of agrochemical responses in bees" analysed haemolymph from pollinators to study the molecular response of bees to stressors (protein markers, deregulated pathways and modulated molecular mechanisms). Using proteomics, these proteins (AMPs and AMCI) were identified, quantified and found to be impacted following different types of exposures across the three bee species, which will help to elucidate the common and specific characteristics of the response of a bee species to them. Overall, PoshBee has made important and significant contributions to the omics of stressors in pollinators that are largely complementary to and novel when compared with the published literature in this area (see Table 4).

**Table 4:** Comparison between the general literature and PoshBee according to the experiments(laboratory, semi-field and field), the stressors and the species. Green: Done; red: not done.

		Molecular mass f				fingerprints (MALDI MFP)			
		General					Posł	Bee	
	Stressor	Apis	Bombus	Osmia	NWS*	Apis	Bombus	Osmia	NWS
	Nutrition								and the second s
	Crithidia						and the second sec	and the second s	
tic	Nosema	1. The second				1. The			
Bio	Varroa					Ô			
	Bacteria	<b>A</b>				and the second se	<b>A</b>	and the second sec	
	Viruses								
	Sulfoxaflor (insecticide)								LIN
Ę	Flupyradifurone (insecticide)							area a	
bio	Azoxystobin (fungicide)								<b>ST</b>
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