Synthesis report on agrochemical, pathogen and nutritional effects
Deliverable D10.4

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PoshBee

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

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Summary

Together with deliverables D10.3 and D10.5, deliverable D10.4 attempts to contextualise the outputs of the PoshBee project with the wider scientific field. This systematic quantitative literature review presented in this synthesis report summarizes the research on stressor interactions on bee health across the last five years and compares it to the output of the PoshBee project across the same time frame. The types of interactions, populations, tiers and endpoints studied are summarized across the general literature and the PoshBee project. Differences and similarities in research focus are discussed. The bibliometric analysis shows that the PoshBee project has made a disproportionally larger contribution to research on the novel insecticides sulfoxaflor and flupyradifurone, as well as the fungicide azoxystrobin and the herbicide glyphosate. In contrast, the majority of studies in the general literature consider neonicotinoid insecticides. Across both datasets, *Apis mellifera* is still the most researched model organism, but the proportion of papers considering *Bombus* spp. is increasing. Solitary bees of the genus *Osmia* are featured in a handful of studies, but the overall diversity of bee pollinators is still not adequately represented. There is an equal amount of focus on pesticide-pathogen interactions and pesticide-dietary interaction in the general literature across the last five years, with pesticide-pesticide interactions and pathogen-diet interactions being investigated less frequently. Workers are the most frequently studied caste in honey bees in comparison with sexuals, while the diversity of castes and sexes investigated are greater in bumble bees. Colony endpoints were more commonly assessed in bumble bees than in honey bees, while survival was more commonly assessed in honey bees than in bumble bees. The interaction between insecticides and fungicides was the most commonly investigated pesticide-pesticide interaction across both the general literature and PoshBee. The diversity of pathogens investigated was larger in honey bees compared to all other species, with *Nosema ceranae* being the most frequently investigated. Only one study from the general literature and one document from PoshBee investigated pesticide-pathogen interactions in a solitary bee. Papers on interactions between diet and pesticides included a range of papers on specific phytochemicals and their effects on pesticide resistance. Other papers dealt with the addition of specific pollen sources or modulation of the macronutrient balance. Interactions between pathogens and diet were dominated by the interaction between the trypanosome *Crithidia bombi* and sunflower pollen in bumble bees.

Overall, the PoshBee project made significant contributions to the research field by producing valuable data on stressor interactions involving novel pesticides (sulfoxaflor and flupyradifurone) as well as the fungicide azoxystrobin and the herbicide glyphosate. Notably, stressor interaction investigation in semi-field experiments is still rare, and PoshBee provided the only results on the aforementioned compounds from such a set-up over the last five years.

1. Introduction

The PoshBee project was initiated to assess the exposure to, the effects of and the 'omics of stressors on bees, using the project's three model bee species (*Apis mellifera*, *Bombus terrestris* and *Osmia bicornis*). One important objective of the project was to explore the interactions between stressors, and their potential to exacerbate the negative effects of pesticides, increasing the risk to bee health and in turn putting populations at risk.

The aim of this systematic quantitative literature review was to investigate the focus of the academic literature on stressor interactions in bees published since the inception of the PoshBee project, and to determine what areas of stressor interaction research are perhaps saturated and which require more attention. Due to time restrictions, formal meta-analysis and data extraction were not possible. Instead, we conducted a systematic quantitative review (Pickering and Byrne, 2014), ranking populations, type of stressor interaction, stressors and endpoints that had been studied across the last five years (January 2018 – September 2022). The database created from the general literature was then compared to a database containing the outputs of the PoshBee project up to and including December 2022 (the due month of the report at hand).

2. Literature review: methodology

2.1. Identification of scope, relevant keywords and inclusion criteria

We restricted our search to literature published within the same time frame as the PoshBee project (January 2018 – September 2022) since this pinpointed the research focus when comparing PoshBee to the general literature. The timeframe was restricted to September 2022 in order to be able to complete the review at the given due date. Using the method outlined in Pickering and Byrne (2014), we defined i) the target population, ii) relevant stressors, iii) relevant study types, and iv) relevant outcomes. We chose relevant types of stressor interactions based on the overarching research themes outlined in the PoshBee Grant Agreement (agrochemicals, pathogens and dietary quality), thereby excluding irrelevant stressors such as temperature, heavy metals, microplastics etc. The search string was then designed based on the selected stressor categories (Table 1.). We defined search terms based on previous meta-analyses on stressors on bees (Havard et al., 2020; Bird et al., 2021; Siviter et al., 2021) as well as multiple search iterations. The target population included all bee species, managed or wild. All developmental stages (larvae, pupae, adults), castes and populations (workers, queens, males, females, colonies, microcolonies) were included. Stressor strings were divided up into plant protection products (PPPs) and infectious and parasitic agents (IPAs). A string for nutritional and diet-related keywords and an effects/outcomes string were attached as well as a string excluding irrelevant studies (field surveys, non-experimental studies and studies on non-relevant stressors). See Appendix 1 for the full search string.

2.2. Literature search

We searched in the online databases Web of Science Core Collection (WoS) and Scopus for all peer-reviewed articles published January 2018 – September 2022 (last access: 30 September, 2022), representing the time frame during which the PoshBee project has been underway.

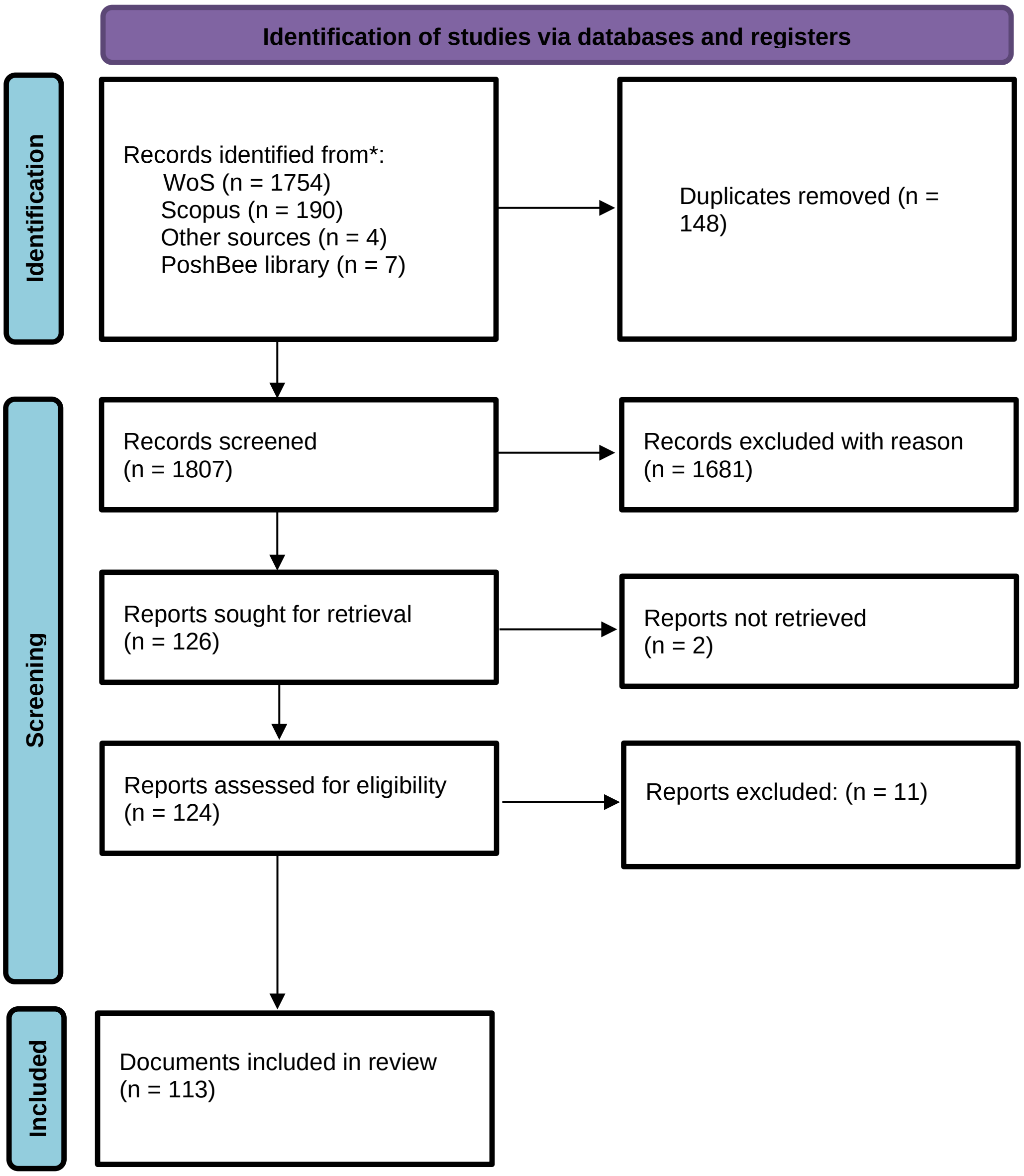

Figure 1. PRISMA flow diagram describing the gathering of the literature.

This yielded 1754 and 190 hits respectively. An additional four relevant papers were included manually and 7 documents were downloaded from the Poshbee repository (www. PoshBee.eu/library). After duplicates were removed, 1807 documents were assessed based on title and/or abstract. Including PoshBee reports, 124 documents were assessed in full for eligibility and 113 documents were included in the final database (Figure 1).

2.3. Review of PoshBee outputs

All relevant reports, milestones and deliverables produced in the PoshBee project up to and including December 2022 were downloaded from the PoshBee repository. Additionally, peer-reviewed articles resulting from PoshBee-funded research identified in the above literature review were included, and thus excluded from the review of non-PoshBee outputs.

Table 1. List of Deliverables from PoshBee included in the database for bibliometric analysis

Year	Report number	Title
2021	D3.3	Manuscript - acute & chronic effects of chemicals
2022	D5.2	Manuscript - nutritional mitigation of chemical effects
2022	D5.3	Manuscript - chemicals effect on nutritional intake
2021	D6.1	Manuscript - pathogen/chemical effect on individual *Apis*
2021	D6.2	Manuscript - pathogen/chemical effect on individual *Bombus*
2022	D6.3	Manuscript - pathogen/chemical effect on Bombus colonies
2022	D6.4	Manuscript - pathogen/chemical effect on individual *Osmia*

2.4. Data extraction

Articles were excluded upon abstract screening if the study was correlational in nature (e.g. if the study addressed the effects of one stressor on the natural occurrence of another stressor) and only included if it presented an experimental setup with controlled stressor application and a control treatment. Studies did not have to be fully factorial (i.e. control, stressor1, stressor2, stressor 1+2) in order to be included. Veterinary intervention studies were not considered relevant, with the exception of nutritional supplementation. Publications resulting from PoshBee-funded research were transferred to a separate database (see above). Stressor combinations were sorted into a set of categories (Table 2). The studies selected for full-text screening were ordered into a database where each row represented one stressor pair and effect endpoint measured. This meant that one paper could result in several rows in the database. General information (authors, year published, DOI, journal) was extracted from each title. Information on the study organism (species, caste/sex, developmental stage), the type of interaction and details on the stressors investigated were extracted based on pre-defined categories (Table 2). The tier at which the study was conducted (laboratory, semi-field or field) was noted. Information on doses used in experiments was not extracted, as it was not relevant for the purposes of this study.

Table 2. Description of organismal categories, stressor classes, interaction types and endpoint classes as assigned in the databases.

Organism categories	**Description**
Worker	*Worker caste of eusocial species*
Queen	*Queen/gyne caste of eusocial species*
Female	*Female adults of non-eusocial species*
Male	*Males, including both non-eusocial and social species (i.e., drones)*
Larvae	*Immatures in all larval stages*
Colony	*Colony parameters measured in eusocial species*
Microcolony	*Reproductive unit of 3-7 workers common in studies of Bombus spp.*

Stressor classes	**Description**
Plant Protection Product (PPPs)	*Any form of herbicides, fungicides, insecticides in pure or formulated form*
Infectious of parasitic agents (IPAs)	*Any form of potential disease-causing agent or parasitoids (e.g. microbe, virus, bacteria, fungus)*
Diet	*Pollen treatments, pollen macronutrient composition, sucrose concentration modulation, starvation or specific nectar secondary compounds or other naturally occurring phytochemicals*

Type of interaction	**Description**
PPP - PPP	*Interaction between PPP classes only (i.e. insecticides, fungicides, herbicides)*
PPP - IPA	*Interaction between any PPP class and any infective unit (IPAs)*
PPP - Diet	*Includes pollen treatments and phytochemicals*
IPA - Diet	*Interaction between any infectious treatment and pathogens/parasites*

Endpoint class	**Description**
Survival	*dose-response, ld50, lc50, survival during any interval*
Behaviour	*PER, food intake, foraging trips, field observations, locomotion*
Physiology	*Body size or mass measures, organ/tissue size*
Immunity	*Hemocyte count, melanization etc.*
Gene expression	*Relative gene expression*
Pathogen	*Prevalence/replication/intensity of pathogens in vivo*
Reproduction	*No. offspring/gynes or sperm and oocyte assessment*
Colony	*Colony parameters, e.g., weight gain*
Molecular	*Protein titres*
Detoxification	*The physiological degradation of active ingredients in bee bodies or tissues*

2.5. Analysis

Since an investigation of the original data and evaluation of the statistical methods presented in the articles was not performed, we did not assess the effects of specific stressor combinations. The systematic, quantitative review consisted of comparisons of ratios of papers and endpoints between datasets, bee genera and interaction types. Ratios between two groups were compared using a 2-sample test for equality of proportions (chi^2) with continuity correction, or a Fisher's Exact Test.

3. Results: Comparison between general literature and PoshBee studies

3.1. Dataset parameters

In total 113 documents (106 peer-reviewed articles and seven reports) were included in the datasets (Figure 2). The general literature dataset contained 92 peer-reviewed articles from which 415 unique endpoint/stressor combinations were extracted. The PoshBee dataset contained 14 peer reviewed articles and seven publicly available Deliverable reports. A total of 145 endpoint/stressor combinations were extracted from the PoshBee articles and reports. The number of studies published per year has remained relatively stable across the last five years (Figure 2).

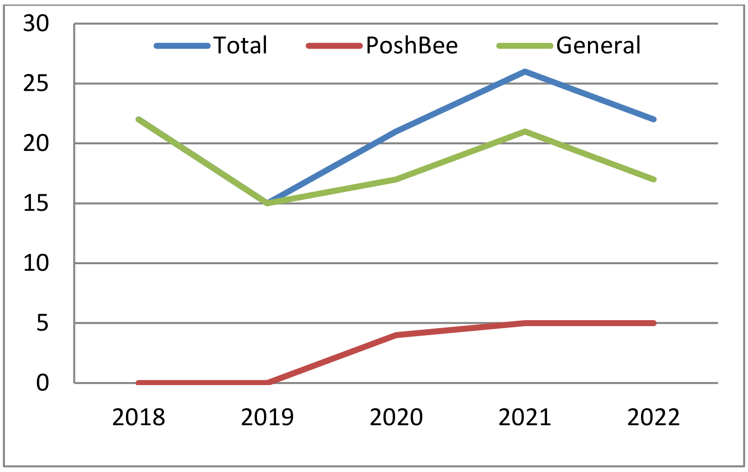

Figure 2. Number of studies in the general literature which were PoshBee-funded or not per year.

3.2. Type of interactions investigated

In the general literature, a similar percentage of studies investigated PPP-IPA interactions (34%) and PPP-diet interactions (30%). PPP-PPP interactions were investigated in 21% of studies and the remainder concerned IPA-diet interactions. In the PoshBee dataset, the majority of documents investigated PPP-IPA interactions (52%), followed by PPP-diet interactions (29%) and PPP-PPP interactions (14%); (Figure 3).

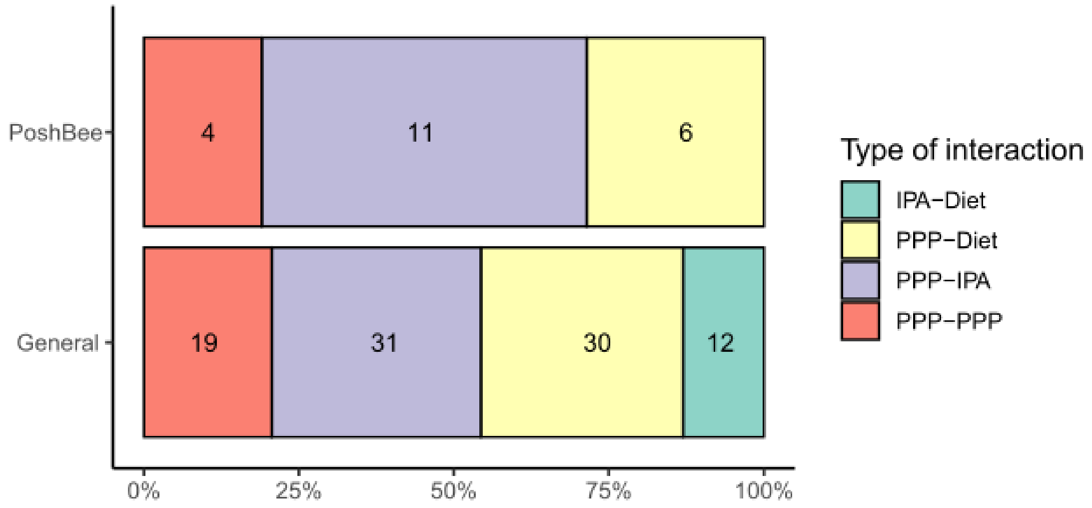

Figure 3. Barplot showing the proportion of papers across the four interaction types in the PoshBee dataset and the general literature.

3.3. Species and castes/sexes investigated

In the general literature, 63% of papers used *A. mellifera* (n = 58) as a study species, five of which used Africanized *A. mellifera.* Two studies used the eastern honey bee *Apis cerana*. 23% of the studies used *Bombus* spp.*,* with 12 papers using the North American species *Bombus impatiens* and nine papers using the European *Bombus terrestris.* The third most common species group was the solitary bee genus *Osmia,* which was utilized in nine studies, with *Osmia bicornis* (n = 5 studies), *Osmia lignaria* (n = 3) and *Osmia cornifrons* (n = 1) represented. Three studies utilized stingless bees (*Melipona quadrifasciata* n = 2; *Melipona colimana* n = 1). One study used the solitary bee *Megachila rotundata*, and one study the solitary *Tetrapedia diversipes*.

Only two studies investigated more than one species, with Azpiazu et al. (2021) using the three model species *A. mellifera, B. terrestris* and *O. bicornis* also represented in PoshBee. Fowler et al. (2022) investigated three additional wild bumble bee species together with the model *B. impatiens.*

For the three commonly investigated PoshBee model species (*A. mellifera, B. terrestris* and *O. bicornis*), the most common tier at which to study interactions was the laboratory. For bees of the genus *Osmia*, a larger proportion of endpoints were derrived from semi-field experiments when compared to bumble bees and honey bees (Fisher's Exact Test; p-value = 0.001). No field-level studies have been performed on solitary bees, while two field-level studies were performed on bumble bees and five on honey bees (Figure 4).

In order to better portray the content of the unpublished reports in the PoshBee dataset, often containing multiple experiments from multiple institutions, in the following paragraphs we present the number of endpoints recorded in the dataset rather than papers. When considering *Apis*, *Bombus* and *Osmia* spp., there were differences in the castes, sexes and developmental stages at which the endpoints were measured. In both the general literature and in PoshBee, the largest variety of castes and sexes was seen in bumble bees, where 47% and 43% of endpoints were derrived from workers, while 12% and 17%, respectively were derrived from sexuals (queens or males). In contrast, in honey bees the majority of experimental endpoints were derrived from workers (82% in the literature and 76% in PoshBee) and only small fractions (2 and 4%) were devoted to sexuals (Figure 5). In the general literature, 29% of bumble bee endpoints were concerned with the colony level whereas 19% of the PoshBee endpoints did the same. For honey bees only 4% of endpoints concerned the colony level, published in six papers. No effects of combined stressors at colony level were recorded in PoshBee. Effects on brood/larvae were most commonly assessed in honey bees (X^2 = 11.068, df = 1, p-value = 0.0004).

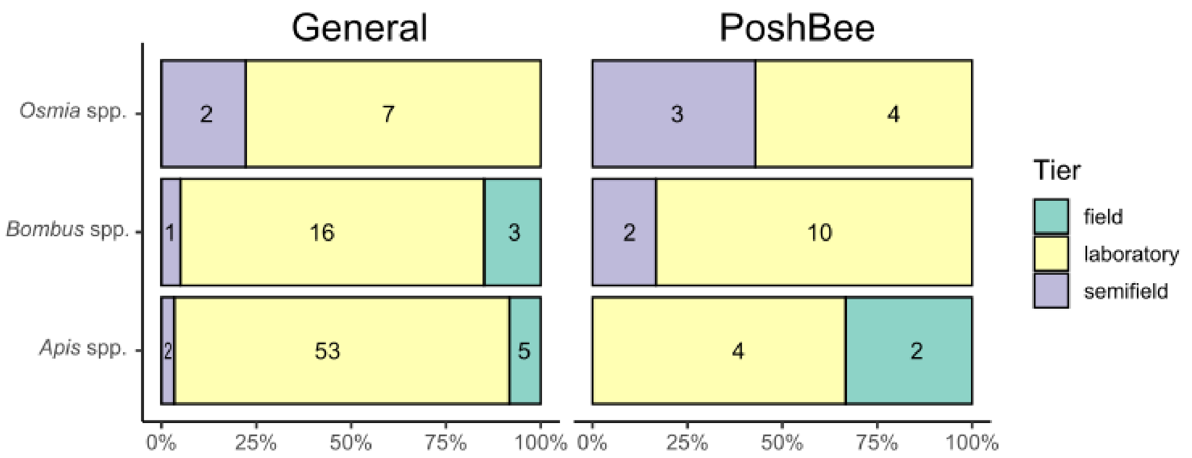

Figure 4. Barplot showing the proportions of papers investigating laboratory, semi-field and field scale impacts across the three model genera in the general literature (left) and the PoshBee dataset (right).

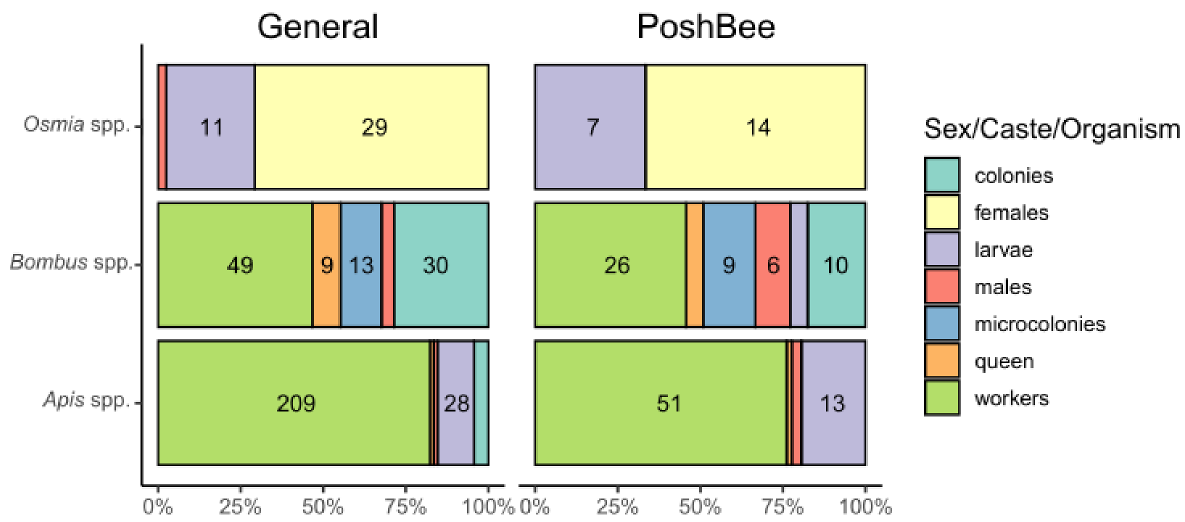

Figure 5. Barplot showing the proportions of endpoints investigating different sexes, castes and developmental stages across the three model genera in the general literature (left) and the PoshBee dataset (right).

3.4. Interaction types per species

For *A. mellifera*, the PPP-IPA interactions were the most common interaction category in both the PoshBee dataset and the general literature (Figure 6). These were followed by PPP-diet and PPP-PPP interactions in the general literature, with a smaller focus on IPA-diet interactions. IPA-diet was the most common interaction type for *Bombus* spp. in the general literature followed by the PPP-diet interaction. In contrast, the PoshBee dataset contained more PPP-IPA endpoints for *Bombus* than the general literature. For solitary bees of the genus *Osmia,* there was equal emphasis on PPP-diet and PPP-PPP interaction endpoints in the general dataset, but only one instance of a PPP-IPA interaction, while the PoshBee dataset featured five PPP-IPA interactions. IPA-diet interaction in *Osmia* spp. or other species was not investigated in either database.

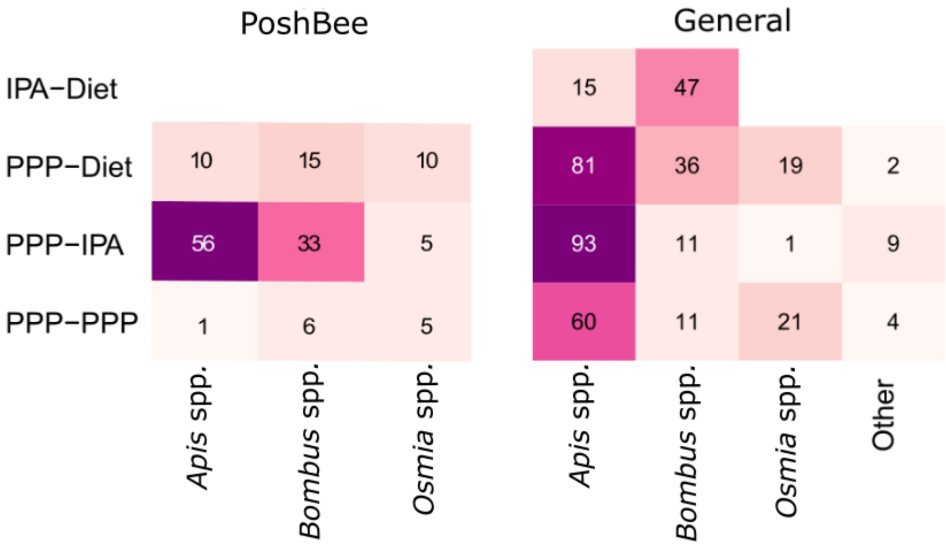

Figure 6. Heatmap showing the proportions of papers investigating different sexes, castes and developmental stages across the three model genera in the general literature (right) and the PoshBee dataset (left).

3.5. PPP classes investigated

In total, 52 compounds of 26 chemical classes were studied within the literature. The most frequently investigated class was neonicotinoids. In the general literature, 51% of the endpoints investigated were on a stressor combination including neonicotinoids. In the PoshBee-funded dataset, only 7% of endpoints tested the effects of a neonicotinoid. The most frequently investigated insecticides were imidacloprid followed by thiamethoxam, clothianidin (all neonicotinoids) and acephate (an organophosphate).

Within the PoshBee dataset, nine compounds belonging to seven chemical classes were experimentally investigated. The bulk (74%) of the 145 endpoints presented within PoshBee were conducted on the two novel insecticides sulfoxaflor (sulfoximines) and flupyradifurone (butelonide) and the fungicide azoxystrobin (strobilurine) (Figure 7). Overall, PoshBee-funded research contributed to 90% of the endpoints in the total (combined) literature using sulfoxaflor and 63% of the endpoints using flupyradifurone in combination with other stressors. PoshBee thus contributed disproportionally more to expanding the knowledge base on novel classes of pesticides in combination with other stressors during the considered timespan (Figure 7).

In the general literature, fungicides were investigated in 37% of studies (n = 35). The most investigated fungicidal compounds were pyraclostrobin, tebuconazole and boscalid. The PoshBee dataset contained 100% of the research conducted on azoxystrobin in combination with other stressors.

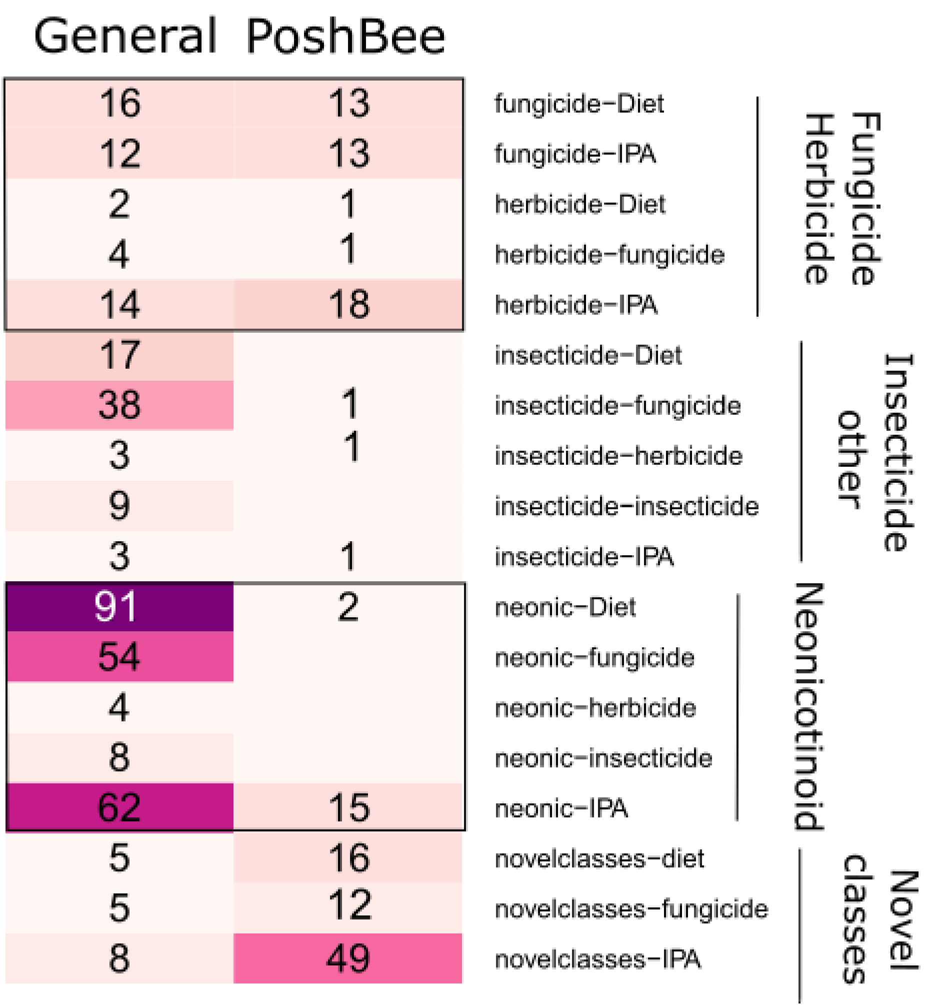

Figure 7. Heatmap showing the proportions of endpoints investigating different chemical classes in the general literature (left) and the PoshBee dataset (right). Novel classes include sulfoximines and butenolides.

Glyphosate was the only researched herbicide in combination with other stressors across the last five years, except for one study using paraquat. In the general literature, three studies investigated glyphosate in *A. mellifera* in combination with IPAs (*N. ceranae*) or with pollen treatments. In the PoshBee dataset, glyphosate-stressor interactions were tested in five documents, generating 19 endpoints. The PoshBee dataset contained the only research on stressors in combination with glyphosate, or any herbicide, on *B. terrestris* and *O. bicornis* present in the total (combined) literature.

3.6. Endpoints studied

Survival endpoints were those most commonly assessed in honey bees across all interaction types, with 79% of papers (n = 42) assessing survival, compared to 45% (n = 9) of papers on bumble bees (X^2 = 5.2672, df = 1, p-value = 0.01; Figure 8). In 70% of cases where survival was assessed in honey bees, long-term survival was assessed (i.e., longer than standard acute assessments of 96 hours). In 15% of cases, acute (<96h) and pre-eclosion mortality was assessed. The most commonly assessed behavioural endpoint across all genera was food consumption (i.e., the amount of sucrose or pollen consumed within a pre-determined time frame; Figure 8). Other behavioural endpoints included foraging, learning (e.g., proboscis extension reflex (PER)) and locomotion. Colony endpoints were more commonly assessed in bumble bees than in honey bees, with only two studies investigating colony endpoints in honey bees compared to six studies in bumble bees. Since sexuals were rarely assessed in honey bees, reproduction endpoints (e.g., number of offspring produced, ovary or sperm assessment) were not assessed in honey bees in the general dataset or in the PoshBee data. On the other hand, reproduction endpoints in bumble bees were assessed 20 times in five studies in the general literature and nine times in three articles and two reports within PoshBee.

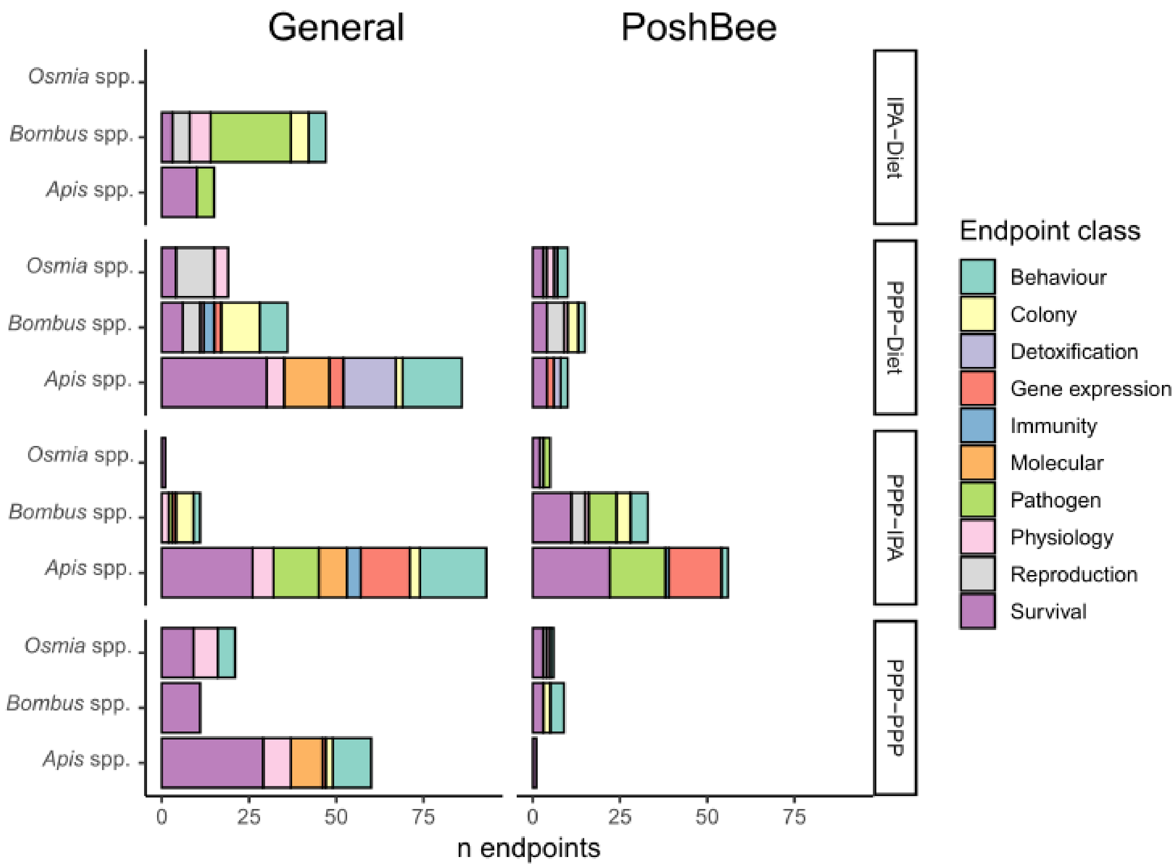

Figure 8. Barplot showing the number of different endpoint classes across the three model genera in the general literature (left) and the PoshBee dataset (right). The four interaction types are displayed.

Gene expression was assessed in 30% (n = 16) of studies with the honey bee as a study species, while only two studies (20%) assessed gene expression in the bumble bee (Fig. 8). Colony endpoints were more often assessed in bumble bees than in honey bees (X^2 = 6.1145, df = 1, p-value = 0.007; Fig. 8).

3.7. PPP-PPP interaction

In the general literature, 74 % (n = 72) of interaction endpoints investigated insecticide interactions with fungicides, with 42% (n = 40) investigating neonicotinoid interactions with fungicides. Four studies investigated the interaction between PPPs on *Osmia* sp. and one investigated neonicotinoid-fungicide interactions in the solitary bee *Tetrapedia diversipes.* It should be noted that the PPP-PPP interaction on *A. mellifera* within PoshBee either was not successfully conducted (Tamburini et al., 2021b) or data have not yet been made public.

3.8. PPP-IPA interaction

The interaction between PPP and IPAs was explored in 31 documents in the general literature and 11 documents in the PoshBee dataset (Figure 3). In both datasets, the diversity of IPAs researched was larger in honey bees than in other species. The most commonly assessed IPA in honey bees was the microsporidian *Nosema ceranae* followed by *Varroa destructor* (Figure 9). In bumble bees, one study assessed *Nosema bombi* exposure in a field setup (Botías et al., 2021) whereas most endpoints employing *B. terrestris* in the PoshBee dataset concerned the trypanosome *Crithidia bombi.* In solitary bees, only one study assessed the interaction between a fungal pathogen and fungicide (Krichilsky et al., 2021). One document investigated the effects of the trypanosome *Crithidia mellificae* on *O. bicornis* in the PoshBee project (Deliverable 6.4).

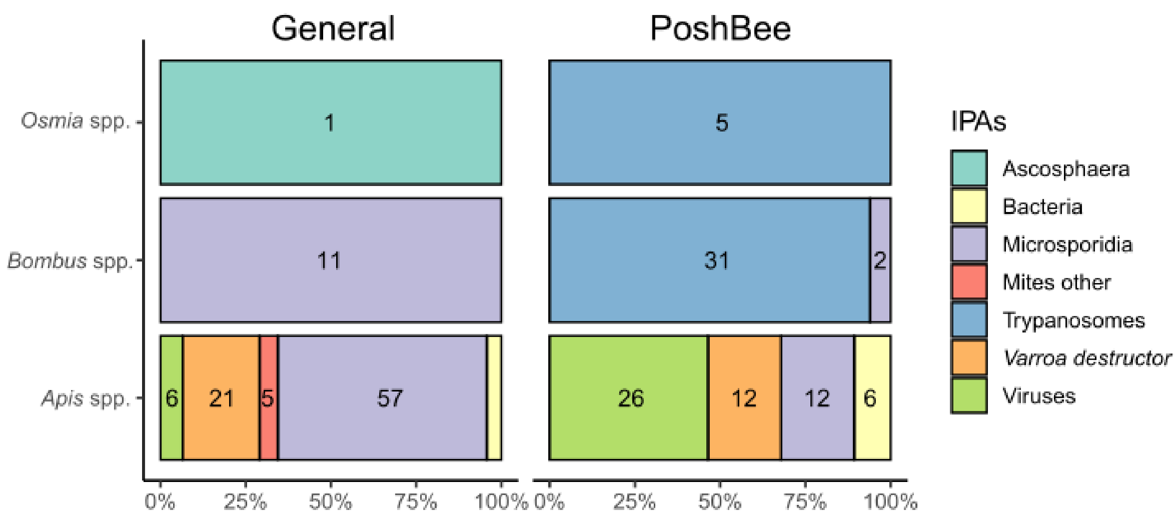

Figure 9. Barplot showing the proportion of endpoints investigating different IPAs across the three model genera in the general literature (left) and the PoshBee dataset (right).

3.9. PPP-Diet interaction

In honey bees, the effects of dietary addition of various nectar metabolites on PPP resistance were explored in ten studies, generating 39 endpoints (Figure 10). Quercetin, caffeine, thymol and b-coumaric acid were the most researched metabolites. Within PoshBee, specific nectar metabolites or phytochemicals were not investigated. One study investigated the addition of floral strips on honey bee health in a semi-field setup (Castle et al., 2022). In *Bombus* spp.*,* there were two notable studies exploring the presence/absence of specific crops in a field setting, finding that flowering crops may offset the effects of PPPs (Rundlöf and Lundin, 2019; Knapp et al., 2022). The fitness effects of floral diversity on *Osmia sp.* with and without PPP exposure were investigated in two semi-field studies (Stuligross and Williams, 2020; Klaus et al., 2021), echoing the investigation on nutrition and flupyradifurone exposure on *O. bicornis* in a semi-field setting within the PoshBee project (Knauer et al., 2022).

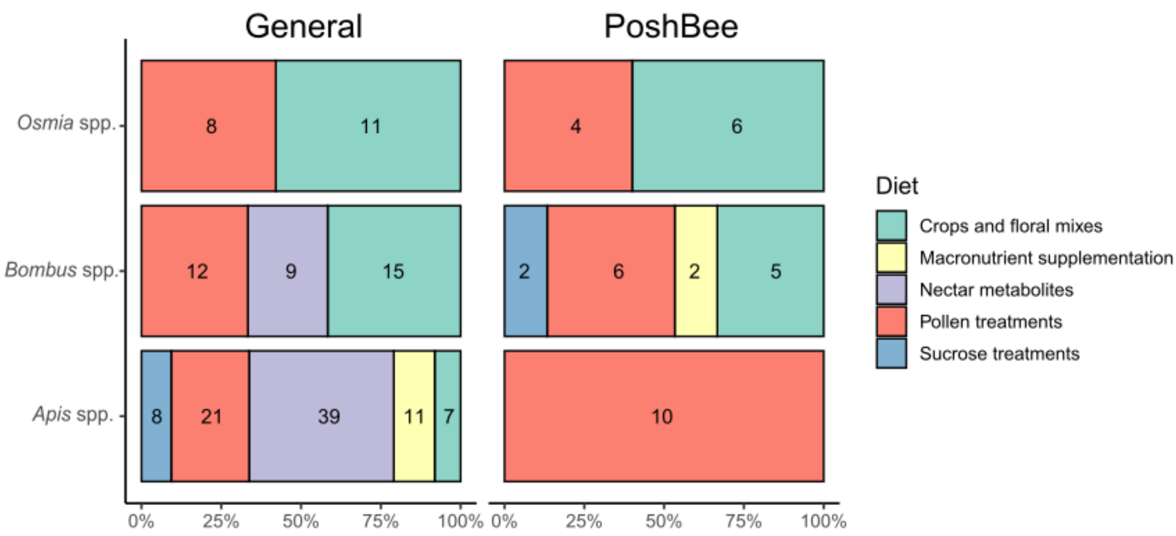

Figure 10. Barplot showing the proportions of endpoints investigating different diet treatments across the three model genera in the general literature (left) and the PoshBee dataset (right).

3.10. IPA– Diet interaction

The majority of IPA-diet interaction studies (n=10) investigated the interaction between the trypanosome *C. bombi* and sunflower (*Helianthus annuus*) pollen or its various nectar metabolites in *Bombus* spp. Only two studies explored the interaction between diet (nectar metabolites) and IPAs on *A. mellifera*. The IPA-diet interaction was not investigated in PoshBee.

4. Discussion

PoshBee has contributed significantly to the knowledge base on two novel insecticide classes: sulfoximines and butelonides, producing the majority of the world's scientific research on these compounds in interaction with other stressors on bees across the last five years. The research conducted within PoshBee on the fungicide azoxystrobin is unique, since no other strobilurin fungicide has been investigated in this context in the general literature. Within PoshBee, these compounds were investigated in semi-field conditions in combination with other stressors (the fungicide azoxystrobin and nutritional stress, respectively) for the first time (Tamburini et al., 2021a; Knauer et al., 2022; Schwarz et al., 2022; Wintermantel et al., 2022). PoshBee also contributed disproportionally more to the research on stressor interactions and glyphosate. In the general literature, the focus has remained on neonicotinoid insecticides. The most commonly researched chemical in the general literature, imidacloprid, was banned in the EU before the inception of the PoshBee project, although it is still widely used globally.

The diversity of pathogens investigated within the literature is higher in honey bees and low in other genera. The PPP-IPA interaction in genera other than *Bombus* and *Apis* is still largely unexplored.

Since the pathogens of honey bees are better known than those of other genera, there may still be much groundwork necessary in the form of single-stressor studies before interaction effects can be further explored in these other genera.

While honey bees are by far the most commonly researched genus, there is a marked lack of studies on honey bees at the field level. This may be due to the costliness of maintaining colonies in a sufficiently high number to enable fully factorial studies, as well as the inherent variability among colonies muddying interpretation (Tamburini et al., 2021b). A technical invention, used in the PoshBee project (Tamburini et al., 2021b), the Mini Plus frame system for honey bee colonies comprising 4000 workers, may make well-replicated semi-field studies more affordable. The reliance on laboratory endpoints performed on worker bees may, however, overestimate the harmful effects of stressors, as the buffering effect of the colony is not considered (Havard et al., 2020). There was also a marked lack of studies on sexuals (queens and males) in honey bees, leading to a lack of reproduction endpoints assessed. This was much more readily investigated in bumble bees. One reason may be that the use of microcolonies allows the assessment of reproduction endpoints in bumble bees in a relatively inexpensive manner, evident from the use of such setups in four studies.

In studies assessing dietary factors on stressor resilience, PoshBee research focused on macronutrient ratios (ratios of proteins, lipids and carbohydrates) while not engaging with additions or subtractions of specific metabolites or phytochemicals. In the general literature, the addition of naturally occurring phytochemicals to diets of bees has been readily studied as a treatment to control pathogens as well as enhancing detoxification and PPP exposure resilience. One specific combination that emerges from our literature review is the ameliorating effect of sunflower pollen on trypanosome infection in bumble bees, which has been investigated in 11 studies across the last five years.

The ameliorating effects of diet on pathogen resilience are not explored within PoshBee. Pollen nutritional profile and its effects on PPP resilience is, though, explored in a series of PoshBee experiments on all three model species. In solitary bees, the effects of pollen diet are explored in larvae, and in a semi-field setup its effects are studied in adult females. The results from the semi-field study (Knauer et al. 2022) indicate that poor diet exacerbates the detrimental effects of flupyradifurone spray-over on *O. bicornis*. This is echoed in two semi-field studies from the general literature (Stuligross and Williams, 2020; Klaus et al., 2021), which both find evidence of a detrimental interaction between resource stress and PPP exposure on the reproduction of solitary bees (*O. bicornis* and *Osmia lignaria* respectively). Within Work Package 7 of PoshBee, sulfoxaflor-fungicide interactions were explored in all three model species, although only two experiments successfully studied the interaction between the two pesticide classes (Schwarz et al., 2022; Wintermantel et al., 2022). Such datasets comprising multiple model species are still largely lacking and would be a welcome addition to the literature documenting impacts of PPPs on bees.

5. Conclusion

There is always a trade-off between the lack of realism of cheaper, laboratory-based assays and the costs of large experiments at the semi-field and field scale. The data presented here show the importance of including field and semi-field assessments, and pollinator species other than honey bees or commercially available bumble bees, in order to correctly judge the effects of stressor combinations across the life cycle of individuals, (for social species) colonies, and populations. There is a marked lack of research on PPP-IPAs interaction on species other than honey bees. The knowledge of IPAs in solitary bees is still in its early stages, and we can expect further advances in this field and stressor interactions in general on solitary bees. It is of utmost importance to thoroughly investigate novel and potentially widely used classes of insecticides, and the considerable effort within PoshBee to elucidate the impacts of sulfoxaflor and flupyradifurone on three model species across multiple tiers is so far unique in the scientific literature.

It should be noted that PoshBee's output is not yet complete, and additional material may be made public beyond the time frame discussed in this report.

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7. Appendix 1.

Search string as used in Web of Science. The use of quotation marks is advised when the keyword contains more than one word.

TS=((“honey bee*” OR honeybee* OR bee OR bees OR beehive* OR pollinator* OR Apis OR Bombus OR bumblebee* OR “bumble bee*”) AND (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) 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 lipid* OR sugar* “fat body” OR pollen OR nutrient* OR diet* OR “floral resources” OR nectar OR "bee bread" OR beebread OR beeswax OR sterol* OR amino acid* OR sucrose) AND (survival OR mortality OR fitness OR fecundity OR “reproductive output” OR offspring OR “colony collapse” OR “colony fitness” OR “colony growth” OR “colony health” OR detoxification OR development OR dose-response OR flight OR foraging OR gyne* OR male OR queen OR mushroom bod* OR navigation OR “olfactory learning” OR orientation OR ovary OR “over-winter mortality” OR “overwinter mortality” OR performance OR “proboscis extension reflex” OR learning OR “sex ratio” OR sperm OR sublethal OR sub-lethal OR “sub lethal” OR apoptosis OR hibernation OR reproduction) NOT ("exposure assessment" OR "pesticide residue*" OR spectrometry))