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Route-specific ecotoxicogenomic responses of the honey bee *Apis mellifera* to imidacloprid revealed by co-expression analysis

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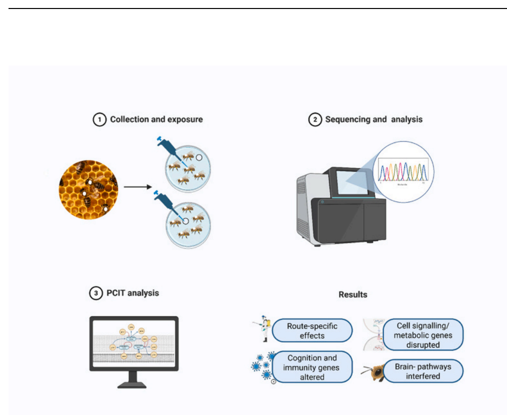
HIGHLIGHTS

- Ecotoxicogenomics reveals molecular effects of imidacloprid in honey bees.
- Co-expression networks expose critical molecular targets of pesticide stress.
- Distinct hub genes identified in ingestion and contact exposure trials
- Imidacloprid alters key pathways linked to cognition, immunity, and metabolism.
- Results support regulatory efforts to reduce pollinator pesticide exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

This study applies an ecotoxicogenomic approach to investigate the molecular impacts of imidacloprid, a systemic insecticide, on *Apis mellifera*, using RNA-sequencing data to construct co-expression gene networks. We hypothesised that oral and contact exposure routes elicit distinct transcriptomic responses, reflected in the structure and composition of route-specific co-expression networks. Imidacloprid exposure triggers alterations in multiple interconnected pathways, reflecting its widespread impact on essential processes. Two distinct networks were derived from ingestion and contact exposure trials, comprising 263 and 249 genes, respectively. Distinct molecular responses and hub genes were observed between ingestion and contact exposure routes, revealing route-specific mechanisms of imidacloprid toxicity in honey bees. Analysis identified key hub genes, such as *Ac3*, *AChE2*, *A4*, and *ACSF2* in the ingestion network, and *Cryl1*, *Apid1*, *Blop*, and *LOC100577632* in the contact network, implicated in essential processes including cellular signalling, energy metabolism, immune regulation,

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and sensory function. Functional enrichment revealed disruptions in critical biological pathways such as G protein-coupled receptor signalling, oxidative phosphorylation, and lipid biosynthesis. These perturbations suggest that chronic exposure to imidacloprid may compromise foraging behaviour, cognitive function, immunity, and overall colony health. By integrating transcriptomic and network-based analyses, this study offers new insights into the potential sub-lethal molecular effects of neonicotinoids on pollinators, reinforcing the need for sustainable pest management strategies and tighter pesticide regulations. Future research should further elucidate the specific roles of hub genes across different exposure scenarios to better inform conservation strategies and regulatory policies.

1. Introduction

The honey bee, *Apis mellifera*, is globally recognized as an important pollinator of crops as well as non-cultivated plants (Hung et al., 2018). Pollination of crops by honey bees and other insects contributes to food production and has been highlighted as a key contribution of nature to human society. In Brazil, pollinators contribute approximately US$12 billion (Giannini et al., 2015), with the Africanized *A. mellifera* being the most prevalent among them.

Modern agricultural practices such as monocultures and intensified land use, combined with climate change, can harm the availability of pollinators (Chaplin-Kramer et al., 2019; Millard et al., 2023). Furthermore, growing evidence indicates that the use of pesticides in crop protection has played a significant role in the rising rates of colony loss (Sánchez-Bayo et al., 2016; Woodcock et al., 2017; Gao et al., 2020; Fent et al., 2020a; Astolfi et al., 2025; de Souza et al., 2025).

Neonicotinoid insecticides, such as imidacloprid, are widely used in agriculture and have become a primary focus of environmental research due to their potential to cause not only increased mortality but also sublethal effects on bees (Simon-Delso et al., 2015; Shi et al., 2017). These neurotoxic insecticides selectively target insect nicotinic acetylcholine receptors, leading to overstimulation of the central nervous system, paralysis, and eventual death (Matsuda et al., 2001).

Neonicotinoids are frequently detected in soil, freshwater, and even air, highlighting their environmental persistence and capacity to contaminate multiple ecological compartments (Mamy et al., 2023). Imidacloprid contains a nitro group, which increases its toxicity, with lethal doses in the ng/bee range (Pisa et al., 2015). The persistent presence of these pesticides in the environment, accumulating in bees, pollen, nectar, and honey, leads to significant contamination (Blacquiere et al., 2012; Simon-Delso et al., 2015). Environmental contamination has a chronic impact on plants, animals and humans.

Imidacloprid is a systemic insecticide capable of translocating through plant vascular tissues, reaching nectar, pollen, and other floral resources that pollinators collect. It can be applied through various methods, including foliar sprays, seed treatments, and soil applications. Approximately 60 % of all neonicotinoid applications globally are estimated to occur as seed or soil treatments (Jeschke et al., 2011). In agricultural environments, environmental contamination occurs via multiple pathways, including drift, dust from treated seeds, runoff, and uptake by non-target plants (Bonmatin et al., 2015). Consequently, bees can be exposed by ingesting contaminated resources (e.g., nectar, pollen, water) and contact with residues on vegetation or within the hive environment. This highlights the importance of conducting both oral and contact exposure tests (Schmuck and Lewis, 2016; Paloschi et al., 2023).

Pollinators, like *A. mellifera*, can be exposed to both lethal and sublethal concentrations of pesticides. While previous studies have reported a wide range of sublethal effects caused by imidacloprid (Henry et al., 2012; Elston et al., 2013; Palmer et al., 2013; Williamson et al., 2013; Derecka et al., 2013; Williamson and Wright, 2013; Williams et al., 2015; Christen et al., 2016; De Smet et al., 2017; Christen and Fent, 2017; Wu et al., 2017; Bovi et al., 2018; Fent et al., 2020a), the majority of these studies have not investigated whether, and to what extent at the molecular level, the effects differ depending on whether exposure occurs via oral or contact routes.

Once inside the hive, pesticides may be redistributed through trophallaxis, food sharing, grooming, and wax contact, exposing multiple castes to different exposure routes and doses (Benuszak et al., 2017; Tremolada and Vighi, 2014). Such complex transmission dynamics make it challenging to determine the origin and pathway of pesticide exposure, especially when both ingestion and contact co-occur (Sponsler and Johnson, 2017). Despite detecting neonicotinoids in pollen, comb, and dead foragers, the exposure routes remain largely undefined in many studies (Krupke et al., 2012). Understanding the physiological and molecular effects specific to each exposure route may help clarify these scenarios.

Our previous studies have shown that both lethal and sublethal doses of imidacloprid, administered through different exposure routes, significantly alter gene expression patterns in *A. mellifera*, revealing sets of differentially expressed genes and gene ontology enrichments (de Castro Lippi et al., 2024a, 2024b, 2025). In this context, identifying route-specific hub genes and gene network architecture could be a valuable tool to differentiate how bees were exposed in field situations. This approach may aid in unravelling in-hive contamination patterns and improve diagnostic accuracy in ecotoxicological assessments. Ecotoxicogenomics integrates molecular biology and ecotoxicology to assess the effects of environmental contaminants on gene expression, particularly in ecologically relevant organisms.

In this study, we used RNA-sequencing data to predict co-expression gene networks, using the PCIT algorithm (Reverter and Chan, 2008). We hypothesise that oral and contact exposure to imidacloprid elicit distinct transcriptomic responses in *A. mellifera*, reflected by the formation of unique co-expression networks and route-specific hub genes. By comparing these responses, we aim to elucidate how different exposure pathways affect physiological systems and to identify molecular markers indicative of each route. Our analysis supports this hypothesis, revealing apparent differences in gene network structures between exposure types. These findings highlight the need to consider exposure routes when assessing pesticide effects and support the development of molecular tools for ecotoxicological studies.

2. Materials and methods

2.1. Animal use and experimental design

The use of bees in this research was approved by the Ethics Committee on the Use of Animals, from the Faculty of Veterinary Medicine and Zootechny of the University of the State of São Paulo (CEUA/FMVZ, UNESP), registered under protocol number 0093/2020.

The experiment was conducted using ten colonies of *A. mellifera* housed in a 5-frame Langstroth nucleus hive. The beehives were standardised to include three brood frames and two frames of food (bee pollen and nectar), all of which contained naturally mated queens. Two frames containing a sealed brood were removed from each experimental colony. Then these frames were carefully wrapped in perforated tissue and then returned to their respective hives until the emergence of the new bees (Camilli et al., 2022). Approximately one thousand newly emerged *A. mellifera* were individually marked on the thorax using a Uni posca pen (Mitsubishi Pencil, Tokyo, Japan). Following the marking

process, the bees were reintroduced into their original colonies (de Barros et al., 2021; Astolfi et al., 2022).

Twenty-one days after reintroduction, 360 marked *A. mellifera* (foraging phase) were carefully gathered using tweezers. Bees exhibiting abnormal behaviours, such as sluggishness, deformities, or physical injuries resulting from the handling, were substituted with other marked bees that were similarly marked and of the same age. The bees were then anaesthetised at low temperatures and placed in plastic Petri dishes (90x20mm) perforated to ensure ventilation. Six Petri dishes per treatment were used, each containing five bees, resulting in 30 bees per treatment, kept inside an incubator (33 ± 1,0 °C and 75 % humidity) (Table 1).

The bees underwent a 3-h fasting period to empty their crop for the ingestion test. The food was provided in plastic troughs, with 250 μl added per trough onto each plastic Petri dish containing five bees for 50 μl per bee, corresponding to the average volume of the crop vesicles (Crane, 1990). Subsequently, the bees were provided with syrup (honey and water, 1:1) containing either a lethal dose (IG50) of 0.081 μg/bee (Bovi et al., 2018) or a sublethal dose (IGSUB) of 0.00081 μg/bee of imidacloprid, reflecting 1/100 of the LD50 (Gonzalez et al., 2022) (Imidacloprid from SIGMA® 37894). A control group of bees was maintained and collected similarly but fed uncontaminated honey syrup.

For the contact exposure, the fasting period was not applied. Bees were topically exposed to imidacloprid (SIGMA® 37894) on the pronotum using 2 μl of distilled water containing either a lethal dose (CONT50) of 0.063 μg/bee, a sublethal dose (CONTSUB) of 0.00063 μg/bee, or distilled water alone for the control group.

After 1 and 4 h of exposure for both ingestion and contact, two live bees were collected randomly from the Petri dishes, resulting in 12 bees per treatment, for transcriptome analyses. These bees were immediately frozen at −80 °C in an ultra-freezer until processing for RNA extraction (Astolfi et al., 2022).

2.2. RNA extraction, library preparation, and sequencing

Each *A. mellifera* head was dissected using stereoscopic microscopes, tweezers, and a sterilised scalpel to remove the compound eyes and antennae (Astolfi et al., 2022; da Luz Scheffer et al., 2024). For the transcriptome analysis, a pool was created using the heads of the bees, with three heads per pool for each treatment, totalling four samples per treatment. The total RNA was extracted from the bee brain using TRIzol reagent (Invitrogen, USA) (Chomczynski and Sacchi, 1987). RNA quality and quantity were evaluated using a Qubit fluorometer (Invitrogen, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., USA) (Schroeder et al., 2006). For constructing cDNA libraries, 200 ng of total RNA was utilised, following the manufacturer's instructions in the SureSelect Strand Specific RNA Library Preparation Kit (Agilent Technologies, Santa Clara, USA). Subsequently, the library products were subjected to sequencing on an Illumina Nextseq platform (Illumina, San Diego, USA) in a single-end run with read lengths of 150 bp (Wang et al., 2009).

Table 1
Treatments used in the experiment. Each Petri dish contained five bees, totalling 6 Petri dishes per treatment (n = 30).

Treatment no.	Mode of exposure	Duration	Dose type	Number of bees
1	Ingestion	1 h	Lethal	30
2	Ingestion	1 h	Sublethal	30
3	Ingestion	1 h	Control	30
4	Ingestion	4 h	Lethal	30
5	Ingestion	4 h	Sublethal	30
6	Ingestion	4 h	Control	30
7	Contact	1 h	Lethal	30
8	Contact	1 h	Sublethal	30
9	Contact	1 h	Control	30
10	Contact	4 h	Lethal	30
11	Contact	4 h	Sublethal	30
12	Contact	4 h	Control	30

2.3. Sequencing data processing and differentially expressed genes

The sequencing data is available at NCBI (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1017469, BioProject code PRJNA1017469). The FastQC program (Andrews et al., 2010) was employed to evaluate the sequencing quality of the raw reads. Quality control for sequencing data was conducted using the Trimmomatic software (Bolger et al., 2014). The following filters were applied: elimination of identified adapters, exclusion of bases with a quality score below 20 (Phred Score), discarding reads shorter than 40 base pairs, and trimming the initial 10 base calls.

The sequencing data was aligned using the Burrows-Wheeler Aligner (BWA) software (Li and Durbin, 2009), with the reference sequence Amel_HAv3.1 (NCBI accession number GCF_003254395). Using the GTF annotation from Amel_HAv3.1, the feature count matrix was generated using the HTSeq program (Anders et al., 2015). The tidyverse ecosystem R package was utilised for data processing, analysis, and plotting (Wickham et al., 2019). The differential expression analysis was performed using the edgeR v3.38.4 package, employing a binomial generalised log-linear model. Genes with a count per million lower than one in at least two samples were excluded. The counts were normalised using the TMM normalisation. Multiple tests were corrected using the Benjamini-Hochberg false discovery rate (FDR) procedure. Differentially expressed genes (DEGs) were deemed significant when the FDR-adjusted P-value was less than 0.05, and the absolute value of Log_2 fold change was equal to or greater than 1. The DEGs were identified based on differential expression relative to the control group. All treatments were contrasted against the control, and genes were considered upregulated or downregulated when their expression patterns significantly differed from those of the control condition (Supplementary materials, Table 5).

2.4. Gene co-expression comparison between contact and ingestion group samples

For the co-expression analysis, we used two sample groups: one with 24 samples in the ingestion group and one with 22 samples in the contact group, independent of the doses or the time of the exposure to Imidacloprid. This sample size was achieved by combining the six ingestion treatments and six contact treatments, each consisting of four samples. However, two samples from the contact treatment did not meet the quality control criteria for sequencing, resulting in two fewer samples in this group.

This analysis aimed to identify genes previously reported as differentially expressed (de Castro Lippi et al., 2024a, 2025) that also exhibit differential co-expression between the ingestion and contact exposure groups. For each group's expression data, separately, we filtered out the genes not expressed, normalised the data with the TMM method, using the EdgeR R package and corrected the data for time of exposure (one or four hours) with the Limma R package (Leek et al., 2006; Ritchie et al., 2015). The PCIT algorithm (Reverter and Chan, 2008) was used to identify significant correlations among the expressed genes for each group. The contact group analysis yielded 243 significant correlations, while the ingestion group yielded 261 correlations. To investigate whether the differentially expressed genes (DEGs) display distinct co-expression patterns compared to the rest of the transcriptome, we compared the network structures formed by DEGs to those formed by non-DEGs. This approach evaluated whether DEGs are embedded within more tightly co-regulated or functionally specialised subnetworks. The correlation matrices were filtered to retain only strong correlations: higher than 0.85 or lower than −0.85 involving DEGs. These filtered correlations were then used to construct co-expression networks in Cytoscape v.3.10.1 (Shannon et al., 2003). The Network Analyzer tool in Cytoscape was employed to determine the connectivity degree of each

gene within the networks. This connectivity degree was used to identify hub genes, defined as those whose connectivity degree exceeds the network's average connectivity degree by twice the standard deviation. In simple terms, the network's genes with the highest number of neighbours were seen as hub genes.

2.4.1. Gene annotation and functional enrichment analysis

Functional enrichment analysis was performed using the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database, version 12.0 (https://string-db.org) (Szklarczyk et al., 2023). Two approaches were employed to identify the primary biological functions associated with the proteins of interest:

1) Single protein enrichment: Functional enrichment was conducted for the hub gene by entering its identity into the STRING platform and analysing the available enrichment categories, including Gene Ontology (GO) biological processes, molecular functions, cellular components, and KEGG pathways and Reactome Pathways.
2) Multiple proteins enrichment: To assess the functional associations of the hub gene cluster, multiple protein enrichment analysis was performed by inputting all proteins within the cluster into STRING. The analysis considered the same enrichment categories as described above.

The interaction network was generated using the minimum required interaction score of 0.400 (medium confidence), and functional enrichment was evaluated with a False Discovery Rate (FDR) correction set to medium stringency (5 %) to minimise false positives (Sun et al., 2025). Known interactions from experimental and predictive databases were considered for the analysis.

3. Results

Two co-expression networks were created, one based on the ingestion data (Fig. 1) and another using data from the contact treatment (Fig. 2). Results are presented for each network separately.

3.1. Selected DEGs

A set of the significant DEGs used as input for network construction is summarised in Supplementary Table 5. These genes were identified based on differential expression relative to the control group, with all treatments contrasted against the control. Genes were considered up- or downregulated when their expression patterns significantly differed from control treatments.

Fig. 1. Co-expression gene network constructed from the complete set of significant correlations with differentially expressed genes (DEGs). Edges in the network represent significant correlations between differentially expressed genes, represented as circles, labelled with gene symbols. The hub genes are marked in green, and the genes that connect with two hubs are marked in blue.

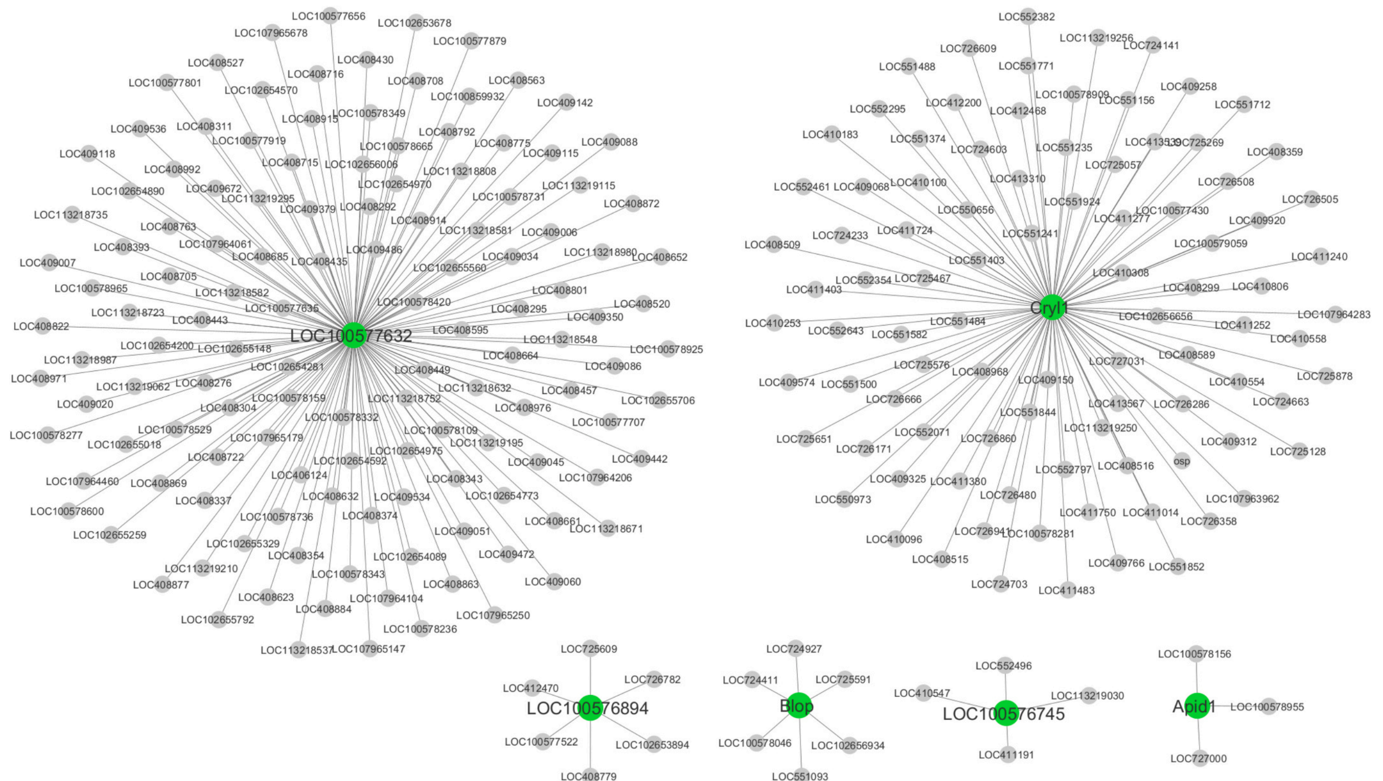

Fig. 2. Co-expression gene network constructed from the complete set of significant correlations with differentially expressed genes (DEGs). Edges in the network are significant correlations between differentially expressed genes, represented as circles, labelled with gene symbols. The hub genes are marked in green.

3.2. *Co-expression network analyses: ingestion treatment*

The ingestion co-expression network filtered for DEGs is formed by 263 genes, divided into two sub-networks (Fig. 1). Each sub-network had two main clusters and one gene that connected both clusters. The hub genes in the ingestion network were: *Ac3*, *AChE2*, *A4*, and *ACSF2*.

3.3. *Co-expression network analyses: contact treatment*

The contact co-expression network filtered for DEGs had 249 genes, divided into six sub-networks (Fig. 2). Each sub-network had one clear hub gene. The hub genes in the contact network were: *LOC100577632*, *Cryl1*, *LOC100576894*, *Blop*, *LOC100576745*, and *Apid1*.

3.4. *Gene annotation and functional enrichment analysis*

Functional annotation and enrichment results for hub genes and their clusters in both oral and contact exposure groups are summarised in Table 2. These findings demonstrate that imidacloprid modulates distinct molecular pathways depending on the exposure route. Genes not included in the table did not present relevant annotations or significant enrichment. Full details of all annotations and enrichment analyses are available in Supplementary Tables 1, 2, 3 and 4.

These results highlight the functional significance of hub genes and clusters in metabolism, immune response, neural function, and sensory perception pathways, emphasising the broad molecular impact of imidacloprid exposure in *A. mellifera*.

4. Discussion

Although differential expression analysis helps assess pesticide effects, it overlooks the complexity of gene–gene interactions. Network-based approaches, such as Partial Correlation Information Theory (PCIT), provide a more integrated view of molecular responses (de la Fuente, 2010). To our knowledge, this is the first study to apply PCIT to investigate pathways and gene networks associated with pesticide exposure in honey bees, making it a pioneering contribution to ecotoxicology.

Hub genes, which have significantly more connections in a network, play a central role in biological processes. They impact various pathways governing essential cellular functions (Zhang et al., 2023) and act as pivotal regulatory nodes, controlling information flow between genes and their networks (Lau et al., 2020). Studying hub genes provides insights into core mechanisms driving complex biological functions, especially under environmental stressors like pesticide exposure (Sohrabi et al., 2020). This discussion focuses on hub genes identified in ingestion and contact co-expression networks from RNA-seq data of *A. mellifera*.

Notably, networks might help to discover gene roles and functions for genes that are not yet characterized. Using "guilt by association" logic, when uncharacterized genes are part of enriched functional clusters, this evidence suggests their potential biological roles (Tian et al., 2008), generating a hypothesis that can be tested during subsequent functional studies that directly test gene interactions and uncover their contributions within the predicted networks.

4.1. *Ingestion co-expression network hubs and their functional roles*

4.1.1. *Hub gene: adenylyl cyclase 3 - Ac3 (408363)*

The Ac3 gene encodes an enzyme involved in cyclic adenosine monophosphate (cAMP) production. In *A. mellifera*, cAMP signalling pathways are involved in gustatory response, long-term memory, and habituation. From a physiological standpoint, all transmembrane adenylate cyclases play an important role as targets in G protein-coupled

Table 2
Summary of functional annotation and enrichment results for hub genes and their respective gene clusters in *Apis mellifera* following oral and contact exposure to imidacloprid. Annotations were obtained from Gene Ontology (GO), KEGG, and Reactome databases. Pathways are grouped by treatment type and analytical level (hub gene or gene cluster). Differentially expressed genes (DEGs) were deemed significant when the p-value was less than 0.05. Clusters are a group of genes; each has its own p-value and regulation. Genes not included did not show significant enrichment or annotation. Full enrichment outputs are available in Supplementary Tables 1–4, and complete DEGs information are available in Supplementary Table 5.

Treatment	Level	Hub gene/ cluster	Database	Enriched pathways/terms	DEGs p-value	Regulation
Ingestion	Hub gene	*Ac3*	Reactome	G protein-coupled receptor signalling (GPCRs)	$p < 0.001$	Downregulated
Ingestion	Cluster	*Ac3*	KEGG	Oxidative phosphorylation, ribosome, metabolic pathways	–	–
Ingestion	Hub gene	*AChE2*	Reactome	Glycerophospholipid biosynthesis, phospholipid metabolism	$p < 0.001$	Downregulated
Ingestion	Cluster	*AChE2*	GO	Regulation, signalling, cellular communication	–	–
Ingestion	Cluster	*A4*	KEGG	Ribosome, drug metabolism (other enzymes), oxidative phosphorylation, metabolic pathways	–	–
Ingestion	Hub gene	*Acsf2*	Reactome	Mitochondrial function	0.002	Upregulated
Ingestion	Cluster	*Acsf2*	Reactome	Mitochondrial fatty acid β-oxidation, fatty acid metabolism	–	–
Contact	Cluster	*LOC100577632*	GO, Reactome	Regulation of transcription (DNA-templated), RNA polymerase II regulation, RNA binding, mRNA splicing	–	–
Contact	Hub gene	*Cryl1*	GO, KEGG	Fatty acid beta-oxidation, fatty acid metabolism and degradation, lipid metabolic processes, valine, leucine, and isoleucine degradation	$p < 0.001$	Upregulated
Contact	Cluster	*Cryl1*	GO	Translation, peptide metabolism, macromolecule biosynthesis and processes	–	–
Contact	Hub gene	*Blop*	GO	Phototransduction, sensory perception, GPCR signalling, regulation of rhodopsin-mediated signalling, and photoreceptor cell maintenance	$p < 0.001$	Downregulated
Contact	Hub gene	*Apid1*	GO	Innate immune response, bacterial defence, response to external stimuli	$p < 0.001$	Upregulated

receptor (GPCR)-mediated signalling (Fuss et al., 2010).

Among the key GPCR-mediated signalling pathways enriched for Ac3 are opioid, gamma-aminobutyric acid (GABA), phototransduction, and Hedgehog signalling. In insects, GPCRs are activated by various ligands, such as biogenic amines, neuropeptides, glutamate, and GABA, as well as light stimuli in rhodopsins (Calkins et al., 2019). These pathways regulate a wide range of physiological processes, including neuronal signalling, hormone release, feeding behaviour, pheromone synthesis, reproduction, learning, and memory (Fuss et al., 2010; Hauser et al., 2006; Calkins et al., 2019). During stress, such as acute pesticide exposure, GPCRs modulate cAMP or calcium signalling to drive lipid mobilisation and support metabolic adaptation (Arrese and Soulages, 2010).

The enrichment analysis of the *Ac3* gene cluster identified pathways such as oxidative phosphorylation, ribosome, and metabolic pathways, which are directly linked to fundamental cellular processes essential for organisms' survival and proper functioning (Liu et al., 2022). As observed in multiple hubs, including Ac3 and A4, oxidative phosphorylation has been consistently linked to foraging efficiency and neural function in bees (Bezabih et al., 2017; Christen et al., 2018). Disruption of this pathway has been linked to impaired foraging and reduced lifespan in *A. mellifera* and has been shown to affect mitochondrial function and structure, emphasising oxidative phosphorylation (Christen, 2023; Christen et al., 2018).

Similarly, pesticides can impair protein synthesis by downregulating ribosomal genes, as observed with flumethrin exposure (Liu et al., 2022). Thiamethoxam has also been shown to reduce expression of genes involved in oxidative phosphorylation and ribosomal function (Shi et al., 2017).

Beyond mitochondrial damage, insecticides such as fipronil and neonicotinoids alter key metabolic pathways, compromising energy balance, brain function, and colony fitness (Belzunces et al., 2012; Lima et al., 2024; de Castro Lippi et al., 2024a). Dysregulation of lipid metabolism and fatty acid biosynthesis may impair energy storage and longevity, contributing to caste-related and behavioural shifts (Hansen et al., 2013; Kunieda et al., 2006). Additionally, pesticide exposure can affect detoxification processes, including xenobiotic metabolism and monooxygenase activity (Ranganathan et al., 2022). These results suggest that *Ac3* dysregulation by imidacloprid may impair cAMP signalling, affecting memory, feeding behaviour, and flight capacity, ultimately compromising foraging performance and colony productivity.

4.1.2. Hub gene: acetylcholinesterase 2 - AChE2 (406104)

Under normal conditions, acetylcholine (ACh) binds to its receptor (AChR), activates ion channels, and is rapidly hydrolysed by acetylcholinesterase (AChE). Neonicotinoids mimic acetylcholine and bind irreversibly to its receptors, causing continuous neuronal stimulation. While they do not directly inhibit AChE, this overstimulation can indirectly disrupt AChE function and may impair its regulatory role in synaptic signalling. Imidacloprid exposure may change AChE gene expression, suggesting persistent stimulation of nicotinic receptors and disrupting neural signalling. This continuous excitation impairs nervous system function, leading to behavioural changes, muscular dysfunction, and compromised locomotion (Azevedo-Pereira et al., 2011), likely affecting foraging and overall energy balance.

The hub gene AChE2 was annotated in the glycerophospholipid biosynthesis and phospholipid metabolism pathway. Neural membranes consist of phospholipids, sphingolipids, cholesterol, and proteins. Glycerophospholipids and sphingolipids act as precursors of lipid mediators (Farooqui et al., 2007). They are associated with maintaining the cellular permeability barrier, regulating the activities of membrane-associated proteins, and controlling intracellular signalling by acting as precursors for signalling molecules (Liu et al., 2023).

The brain lipid composition of *A. mellifera* predominantly comprises glycerophospholipids (88.89 %), sphingolipids (9.23 %), and glycerolipids (1.88 %) (Morfin et al., 2022). Dysfunction in glycerophospholipid metabolism is closely associated with structural and functional damage to cell membranes, potentially impairing transmembrane transport during energy production and the removal of toxic molecules (Farooqui et al., 2000; Ventura et al., 2022). This phenomenon could intensify the effects of neonicotinoid insecticides. Moreover, a relationship between changes in brain phospholipids and the intensity of grooming behaviour after sublethal doses of clothianidin exposure has been observed (Morfin et al., 2022).

The GO analysis of the AChE2 hub gene cluster revealed enrichment in regulation, signalling, and cellular communication pathways. In insects, acetylcholinesterase is associated with promoting apoptosis in neurons, a regulated process that results in the death and elimination of individual cells without causing significant damage to surrounding

tissue. This mechanism contributes to tissue renewal and removing defective or dysfunctional cells (Knorr et al., 2020). In insects of the order Hymenoptera, such as bees, AChE2 is predominantly expressed as the primary catalytic enzyme. It plays a crucial role in hydrolysing the neurotransmitter acetylcholine at cholinergic synapses and neuromuscular junctions, thus regulating communication between nerve cells (Kim and Lee, 2013). This suggests that neonicotinoid exposure disrupts cholinergic signalling, potentially impairing neural communication and contributing to affected bees' cognitive and behavioural deficits.

4.1.3. Hub gene: A4 - apolipophorin-III-like protein (552391)

The *A4* gene is involved in lipid transport and plays an important role in innate immunity. In *Apis cerana*, the Apolipophorin-III protein demonstrated antibacterial activity against Gram-negative and Gram-positive bacteria, suggesting its role in the innate immune response following bacterial infection (Kim and Jin, 2015).

The KEGG pathway related to ribosomes was enriched for this hub gene cluster. Ribosomes are molecular machines synthesising proteins in the cell, composed of amino acid chains linked by peptide bonds (Rodnina et al., 2007). Homeostatic imbalance and immune system stimulation are closely tied to ribosomal function (Harapas et al., 2022), and its activity is critical for normal cell proliferation while being highly responsive to metabolic, physiological, and environmental challenges (Bianco and Mohr, 2019).

Other KEGG pathways enriched for the *A4* hub gene cluster included drug metabolism-other enzymes, oxidative phosphorylation, and metabolic pathways. Exposure to sublethal and lethal doses of imidacloprid altered the expression of genes related to metabolism and the antioxidant system (De Smet et al., 2017; de Castro Lippi et al., 2024a; Moreira et al., 2025). Antioxidant reactions involve enzymes such as cytochrome P450 monooxygenases, catalases, glutathione-S-transferases, and superoxide dismutase, which are crucial for detoxifying reactive oxygen species and metabolising xenobiotics and pesticides (Gong and Diao, 2017; Shi et al., 2017).

4.1.4. Hub gene: Acsf2 - Acyl-CoA synthetase family member 2 (552475)

The *Acyl-CoA synthetase family member 2* gene is involved in the fatty acid (FA) metabolic process. FAs are fundamental for metabolism and the maintenance of *A. mellifera* colonies. These lipids are primarily obtained from pollen, which determines the body FA composition in bees (Wright et al., 2018). Pollen consumption and lipid increases are associated with improved bee health, promoting greater survival (Annoscia et al., 2017) and reducing insecticide susceptibility (Crone and Grozinger, 2021).

In addition to serving as chemical precursors for pheromones and secondary metabolites, FAs are essential for energy demands. They are also mobilized into the hemolymph during immune challenges, playing a role in the synthesis and activity of lipid transport proteins (Zdybicka-Barabas and Cytryńska, 2013; Domínguez et al., 2024).

The Reactome pathways annotated for this gene are related to mitochondria. In *Bombus terrestris*, genes involved in mitochondrial function were differentially expressed in response to neonicotinoid exposure (Colgan et al., 2019). These findings highlight the potential effects of neonicotinoids on the bees' energy metabolism, which could impair their foraging ability and compromise colony survival.

The Reactome pathways of Mitochondrial Fatty Acid Beta-Oxidation and Fatty Acid Metabolism were enriched for this hub gene cluster. This process involves several enzymatic steps resulting in the production of acetyl-CoA, NADH, and FADH2, which are utilised in the electron transport chain to generate ATP (Talley and Mohiuddin, 2020). Exposure of *A. mellifera* to the pesticide carbendazim altered the metabolomic pathways of fatty acid oxidation (Chen et al., 2021). Fatty acid oxidation in mitochondria plays a vital role in energy metabolism, and genetic disturbances in this pathway can lead to metabolic diseases (Modre-Osprian et al., 2009).

4.2. Contact co-expression network hubs and their functional roles

4.2.1. Hub gene: phosphopantothenoylcysteine decarboxylase subunit VHS3 (LOC100577632)

No annotation was found for the gene *100577632*, which encodes phosphopantothenoylcysteine decarboxylase, an enzyme involved in coenzyme A (CoA) biosynthesis (Wei et al., 2022). CoA is essential for cellular metabolism, with around 4 % of enzymes depending on CoA, its thioesters, or 4′-phosphopantetheine. As a key cofactor, 4′-phosphopantetheine acts as an acyl group carrier in fatty acid, polyketide, and nonribosomal peptide synthesis, enabling Claisen reactions and the production of peptides and esters. Acetyl-CoA, a central molecule in energy metabolism, fuels the tricarboxylic acid cycle (Krebs cycle) (Strauss et al., 2001).

CoA participates in over 100 catabolic and anabolic reactions, including those involved in the metabolism of lipids, carbohydrates, proteins, ethanol, bile acids, and xenobiotics, such as pesticides and other environmental stressors (Czumaj et al., 2020). This suggests that CoA and its associated enzymes may play a role in mitigating oxidative stress and cellular damage caused by environmental challenges. In the context of *A. mellifera*, exposure to imidacloprid could similarly trigger stress responses that involve CoA-related pathways. For instance, the differential expression of genes linked to CoA biosynthesis, such as *phosphopantothenoylcysteine decarboxylase*, observed in bees exposed to sublethal doses of clothianidin (Ramirez, 2018), may indicate an adaptive mechanism to counteract pesticide-induced oxidative stress. This process could involve enhanced CoA production to support cellular detoxification and energy metabolism, helping bees survive pesticide exposure.

The cluster associated with *LOC100577632* was enriched for transcription-related processes, including DNA-templated transcription, RNA polymerase II activity, and mRNA splicing. These functions suggest that imidacloprid may interfere with core regulatory mechanisms of gene expression. This observation is consistent with previous studies reporting that pesticide exposure can alter transcriptional regulation in *A. mellifera* (Christen et al., 2017; Fent et al., 2020b; Astolfi et al., 2022).

Transcriptional pausing of RNA polymerase II has been proposed as an innate immune response in *Drosophila melanogaster*, priming the host genome by increasing the accessibility of promoter regions associated with virally induced genes, enabling a faster response (Xu et al., 2012). The innate immune system rapidly activates gene expression programs to combat pathogens. A key example is the LPS-induced macrophage response, which occurs in two phases: immediate-early gene induction (primary response) and subsequent protein synthesis-dependent gene expression (secondary response). Transcription initiation regulates these phases, as pathogen recognition activates transcription factors that recruit RNA polymerase II and general transcription factors to promoters, driving gene expression (Medzhitov and Horng, 2009; Smale, 2010). Disruption of these transcriptional processes in pesticide-exposed bees may compromise their immune responsiveness, making them more vulnerable to infections and reducing colony resilience.

4.2.2. Hub gene: Cryl1 - beta hydroxy acid dehydrogenase 1 (406119)

The gene 406119 was frequently enriched for fatty acid-related processes, including biological process terms for GO, such as fatty acid beta-oxidation, fatty acid metabolic process, and lipid metabolic process. KEGG pathways included fatty acid degradation, fatty acid metabolism, and valine, leucine, and isoleucine degradation. The Cryl1 gene plays a crucial role in fatty acid (FA) beta-oxidation and metabolic processes, similar to the Acsf2 gene. Dysregulation of fatty acid balance in worker *A. mellifera* impairs their ability to recognize odours, affecting flower selection and pollination efficiency (Bennett et al., 2022). Transcriptomic analyses revealed that the Cryl1 gene is upregulated, potentially increasing fatty acid degradation rates and leading to deficiencies in bees. Stressed bees may exhibit reduced energetic capacity as energy reserves are mobilized to fuel stress responses (Arrese and

Soulages, 2010).

Fatty acid deficiencies have been linked to reduced bacterial growth inhibition (Maya-Aguirre et al., 2024). Additionally, oxidative stress negatively impacts fatty acid metabolism in the brain, contributing to neurodegenerative disorders (Montesinos et al., 2020). A study on *A. mellifera* exposed to the pesticide tebuconazole, an azole-type fungicide, revealed significant alterations in brain fatty acid metabolism, supporting the hypothesis that pesticides disrupt fatty acid metabolism and harm bee health (Mackei et al., 2023).

For the gene cluster, GO enrichment included translation, peptide metabolism, and macromolecule biosynthesis. A study on the proteome of *A. mellifera* exposed to environmentally relevant doses of pesticides found downregulation of proteins involved in RNA translation and/or transport processes, suggesting a reduction in protein synthesis in exposed bees (Zaluski et al., 2020). This study, through protein expression profiling, could confirm that the observed transcriptomic changes were indeed reflected at the protein level.

These findings demonstrate that vital proteins and metabolic processes are impaired in *A. mellifera*. Cryl1 upregulation indicates altered fatty acid metabolism, potentially reducing bees' energy reserves and impairing olfactory-driven behaviours crucial for efficient pollination.

4.2.3. Hub gene: keratin, type II cytoskeletal 74 (LOC100576894)

No significant enrichment was detected for this hub gene or its cluster. However, keratins are known to be the primary intermediate filament proteins in epithelial cells, playing a crucial role in maintaining cellular structure and integrity (Ehrlich et al., 2019). For terrestrial animals, including *A. mellifera*, the presence of rigid structural proteins like keratin or sclerotin is essential to protect against desiccation and mechanical stress, as their rigidity relies on numerous cross-linkages (Wigglesworth, 1948).

Exposure to contact with imidacloprid may have altered the expression of this structural related gene in *A. mellifera*. This disruption might affect the bee's ability to maintain its physical barrier against environmental stressors, potentially leading to increased vulnerability to dehydration, pathogens, or other external threats (Micas et al., 2016). While no direct enrichment was observed, the potential alteration of this gene highlights the broader impact of pesticide exposure on cellular structures and protective mechanisms in *A. mellifera*, which could contribute to their overall decline in health and resilience. Studies, such as those by Nozal et al. (2003), have shown that xenobiotics like oxalic acid can penetrate bee keratin.

4.2.4. Hub gene: Blop blue-sensitive opsin (406128)

The functions of the Blop blue-sensitive opsin gene are like the above-mentioned gene: like those of Ac3. The enrichment of this hub gene was associated with GO terms related to *A. mellifera* vision, including biological process: phototransduction, sensory perception, G protein-coupled receptor signalling pathway, regulation of rhodopsin-mediated signalling pathway, and photoreceptor cell maintenance.

A. mellifera possess compound eyes composed of approximately 5500 ommatidia, each containing a lens, a crystalline cone, and nine photoreceptor cells (Streinzer et al., 2013). These cells detect light and convert it into electrical signals essential for vision. A key structure within each ommatidium, the rhabdom, houses opsins—light-sensitive proteins that undergo conformational changes in response to light (Chapman, 1998; Wakakuwa et al., 2005). This process, known as phototransduction, enables bees to perceive their surroundings and navigate efficiently (Martin et al., 2021; Geng, 2022). It has been observed that phototactic behaviour correlates with gustatory responsiveness (Erber et al., 2006). Reduced phototactic behaviour in *A. mellifera* due to pesticide exposure may impair their ability to navigate, locate food sources, and respond to environmental cues, ultimately affecting pollination, colony efficiency and survival. For this hub gene, no enrichment was detected for the cluster.

4.2.5. Hub gene: leucine-rich repeat-containing protein 26 (100576745)

No significant enrichment detected for the hub gene nor for the cluster.

Leucine-rich repeats (LRRs) are 20–29-residue sequence motifs present in several proteins with diverse functions. Their primary role seems to be providing a flexible structural framework that facilitates protein–protein interactions (Kobe and Kajava, 2001). Leucine-rich repeat motif-containing proteins were described as an essential component of insect immunity (Albert et al., 2011).

4.2.6. Hub gene: Apid1-apidaecin 1 (406140)

The GO analysis of this gene revealed enrichment in biological processes related to innate immune response, defence response to bacteria, and response to external stimuli. Similar to antimicrobial peptides (AMPs), apidermins (APDs)—recognized as structural cuticular proteins in insects—exhibit antimicrobial properties and share several similarities with AMPs (Danihlík et al., 2015; Kim et al., 2022). In another study conducted by our group (de Castro Lippi et al., 2025), exposure to lethal and sublethal doses for 4 h led to the upregulation of the *apidaecin* (*Apid1*) transcript. Disruptions in genes related to immune function may lead to consequences comparable to those previously mentioned, including reduced immune responsiveness and increased vulnerability to pathogens. For this hub gene, no enrichment was detected for the cluster.

5. Conclusion

Different exposure routes to imidacloprid triggered distinct gene co-expression networks, each characterized by unique hub genes. These findings highlight the route-specific molecular mechanisms of toxicity, emphasising the importance of considering exposure pathways in ecotoxicological risk assessments. This disruption suggests that exposure to neonicotinoids could lead to energetic imbalances, metabolism alteration, deficient immune response, and physiological stress, increasing the vulnerability of *A. mellifera* populations to environmental challenges.

These results emphasize the urgent need for stricter pesticide regulations and sustainable agricultural practices to mitigate the detrimental effects of neonicotinoids on pollinators. By integrating transcriptomic data with network-based approaches, this study contributes to the field of ecotoxicogenomics, offering a systems-level understanding of pesticide-induced stress in a key pollinator species. Given the ecological and economic importance of *A. mellifera* in pollination services, these molecular-level findings may have broader implications for ecosystem functioning. The decline of pollinators can lead to ecological imbalances, including reduced plant diversity, which in turn threatens species that depend on diverse vegetation (Nath et al., 2023). It potentially also compromises agricultural productivity through decreased pollination, ultimately endangering global food security.

Future research should focus not only on the long-term effects of pesticide exposure but also on characterizing unannotated or poorly understood genes within the co-expression networks. This will enable a more comprehensive understanding of how gene networks are interconnected and how they respond to pesticide intoxication, ultimately improving our ability to develop targeted conservation strategies for *A. mellifera* populations.

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CRediT authorship contribution statement

Isabella Cristina de Castro Lippi: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Juliana Afonso:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Jaine da Luz**

Scheffer: Resources, Methodology, Investigation. **Yan Souza Lima:** Resources, Methodology, Investigation. **Marcus Vinícius Niz Alvarez:** Methodology, Investigation, Formal analysis. **Marina Rufino Salinas Fortes:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Ricardo de Oliveira Orsi:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Ethics statement

The use of bees in this research was approved by the Ethics Committee on the Use of Animals (CEUA/FMVZ), registered under protocol number 0093/2020.

Author's statement

We declare that the manuscript has not been previously submitted to another journal.

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Data availability

Data will be made available on request.

References

Albert, Š., Gätschenberger, H., Azzami, K., Gimple, O., Grimmer, G., Sumner, S., Mueller, M.J., 2011. Evidence of a novel immune responsive protein in the Hymenoptera. Insect Biochem. Mol. Biol. 41, 968–981. https://doi.org/10.1016/j.ibmb.2011.09.006.

Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinform 31 (2), 166–169. https://doi.org/10.1093/bioinformatics/btu638.

Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C., Wingett, S., 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data. Babraham Bioinform.

Annoscia, D., Zanni, V., Galbraith, D., Quirici, A., Grozinger, C., Bortolomeazzi, R., Nazzi, F., 2017. Elucidating the mechanisms underlying the beneficial health effects of dietary pollen on honey bees (Apis mellifera) infested by Varroa mite ectoparasites. Sci. Rep. 7, 6258. https://doi.org/10.1038/s41598-017-06488-2.

Arrese, E.L., Soulages, J.L., 2010. Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomol. 55, 207–225. https://doi.org/10.1146/annurev-ento-112408-085356.

Astolfi, A., Kadri, S.M., de Castro Lippi, I.C., Mendes, D.D., Alonso, D.P., Ribolla, P.E.M., de Oliveira Orsi, R., 2022. Field relevant doses of the fipronil affects gene expression in honey bees Apis mellifera. Apidologie 53, 69. https://doi.org/10.1007/s13592-022-00978-7.

Astolfi, A., de Souza, G.D.F., Moreira, I.D.R.C., de Castro Lippi, I.C., da Luz Scheffer, J., Arruda, R.A., de Oliveira Orsi, R., 2025. A systematic review on the effects of the insecticide Thiamethoxam and the fungicide Difenoconazole on the health of bees Apis mellifera L. Observ. Econ. Lat. Am. 23, e8631. https://doi.org/10.55905/oelv23n1-096.

Azevedo-Pereira, H.M.V.S., Lemos, M.F.L., Soares, A.M., 2011. Effects of imidacloprid exposure on *Chironomus riparius Meigen* larvae: linking acetylcholinesterase activity to behaviour. Ecotoxicol. Environ. Saf. 74 (5), 1210–1215. https://doi.org/10.1016/j.ecoenv.2011.03.018.

Belzunces, L.P., Tchamitchian, S., Brunet, J.L., 2012. Neural effects of insecticides in the honey bee. Apidologie 43, 348–370. https://doi.org/10.1007/s13592-012-0134-0.

Bennett, M.M., Welchert, A.C., Carroll, M., Shafir, S., Smith, B.H., Corby-Harris, V., 2022. Unbalanced fatty acid diets impair discrimination ability of honey bee workers to damaged and healthy brood odors. J. Exp. Biol. 225, jeb244103. https://doi.org/10.1242/jeb.244103.

Benuszak, J., Laurent, M., Chauzat, M.P., 2017. The exposure of honey bees (*Apis mellifera;* hymenoptera: apidae) to pesticides: room for improvement in research. Sci. Total Environ. 587, 423–438. https://doi.org/10.1016/j.scitotenv.2017.02.062.

Bezabih, G., Cheng, H., Han, B., Feng, M., Xue, Y., Hu, H., Li, J., 2017. Phosphoproteome analysis reveals phosphorylation underpinnings in the brains of nurse and forager honeybees (*Apis mellifera*). Sci. Rep. 7 (1), 1973. https://doi.org/10.1038/s41598-017-02192-3.

Bianco, C., Mohr, I., 2019. Ribosome biogenesis restricts innate immune responses to virus infection and DNA. Elife 8, e49551. https://doi.org/10.7554/eLife.49551.

Blacquiere, T., Smagghe, G., Van Gestel, C.A., Mommaerts, V., 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotoxicology 21, 973–992. https://doi.org/10.1007/s10646-012-0863-x.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environ. Sci. Pollut. Res. 22, 35–67. https://doi.org/10.1007/s11356-014-3332-7.

Bovi, T.S., Zaluski, R., Orsi, R.O., 2018. Toxicity and motor changes in Africanized honey bees (Apis mellifera L.) exposed to fipronil and imidacloprid. An. Acad. Bras. Ciênc. 90, 239–245. https://doi.org/10.1590/0001-3765201820150191.

Calkins, T.L., Tamborindeguy, C., Pietrantonio, P.V., 2019. GPCR annotation, G proteins, and transcriptomics of fire ant (Solenopsis invicta) queen and worker brain: an improved view of signalling in an invasive superorganism. Gen. Comp. Endocrinol. 278, 89–103. https://doi.org/10.1016/j.ygcen.2018.12.008.

Camilli, M.P., Kadri, S.M., Alvarez, M.V.N., Ribolla, P.E.M., Orsi, R.O., 2022. Zinc supplementation modifies brain tissue transcriptome of Apis mellifera honeybees. BMC Genomics 23 (1), 282. https://doi.org/10.1186/s12864-022-08464-1.

Chaplin-Kramer, R., Sharp, R.P., Weil, C., Bennett, E.M., Pascual, U., Arkema, K.K., Daily, G.C., 2019. Global modeling of nature's contributions to people. Science 366, 255–258. https://doi.org/10.1126/science.aaw3372.

Chapman, R.F., 1998. The Insects: Structure and Function. Cambridge University Press.

Chen, H., Wang, K., Ji, W., Xu, H., Liu, Y., Wang, S., Ji, T., 2021. Metabolomic analysis of honey bees (Apis mellifera) response to carbendazim based on UPLC-MS. Pestic. Biochem. Physiol. 179, 104975. https://doi.org/10.1016/j.pestbp.2021.104975.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162 (1), 156–159.

Christen, V., 2023. Different effects of pesticides on transcripts of the endocrine regulation and energy metabolism in honey bee foragers from different colonies. Sci. Rep. 13, 1985. https://doi.org/10.1038/s41598-023-29257-w.

Christen, V., Fent, K., 2017. Exposure of honey bees (Apis mellifera) to different classes of insecticides exhibits distinct molecular effect patterns at concentrations that mimic environmental contamination. Environ. Pollut. 226, 48–59. https://doi.org/10.1016/j.envpol.2017.04.003.

Christen, V., Mittner, F., Fent, K., 2016. Molecular effects of neonicotinoids in honey bees (Apis mellifera). Environ. Sci. Technol. 50, 4071–4081. https://doi.org/10.1021/acs.est.6b00678.

Christen, V., Bachofer, S., Fent, K., 2017. Binary mixtures of neonicotinoids show different transcriptional changes than single neonicotinoids in honey bees (*Apis mellifera*). Environ. Pollut. 220, 1264–1270. https://doi.org/10.1016/j.envpol.2016.10.105.

Christen, V., Kunz, P.Y., Fent, K., 2018. Endocrine disruption and chronic effects of plant protection products in bees: can we better protect our pollinators? Environ. Pollut. 243, 1588–1601. https://doi.org/10.1016/j.envpol.2018.09.117.

Colgan, T.J., Fletcher, I.K., Arce, A.N., Gill, R.J., Ramos Rodrigues, A., Stolle, E., Wurm, Y., 2019. Caste- and pesticide-specific effects of neonicotinoid pesticide exposure on gene expression in bumblebees. Mol. Ecol. 28, 1964–1974. https://doi.org/10.1111/mec.15047.

Crane, E., 1990. Bees and Beekeeping: Science, Practice and World Resources. Heinemann Newnes.

Crone, M.K., Grozinger, C.M., 2021. Pollen protein and lipid content influence resilience to insecticides in honey bees (*Apis mellifera*). J. Exp. Biol. 224, jeb242040. https://doi.org/10.1242/jeb.242040.

Czumaj, A., Szrok-Jurga, S., Hebanowska, A., Turyn, J., Swierczynski, J., Sledzinski, T., Stelmanska, E., 2020. The pathophysiological role of CoA. Int. J. Mol. Sci. 21, 9057. https://doi.org/10.3390/ijms21239057.

da Luz Scheffer, J., Lima, Y.S., de Castro Lippi, I.C., Lunardi, J.S., Kadri, S.M., Alvarez, M.V.N., de Oliveira Orsi, R., 2024. Glyphosate contact alters the expression of genes in the head of Africanized *Apis mellifera* bees. Observ. Econ. Lat. 22 (11), e7697. https://doi.org/10.55905/oelv22n11-083.

Danihlík, J., Aronstein, K., Petřivalský, M., 2015. Antimicrobial peptides: A key component of honey bee innate immunity: physiology, biochemistry, and chemical ecology. J. Apic. Res. 54 (2), 123–136. https://doi.org/10.1080/00218839.2015.1109919.

De Barros, D.C., Camilli, M.P., Martineli, G.M., Justulin, L.A., Orsi, R.O., 2021. Effect of organic and inorganic zinc supplementation on the development of mandibular glands in *Apis mellifera*. Bull. Insectol. 74 (2), 209–212.

de Castro Lippi, I.C., da Luz Scheffer, J., de Lima, Y.S., Lunardi, J.S., Astolfi, A., Kadri, S. M., de Oliveira Orsi, R., 2024a. Intake of imidacloprid in lethal and sublethal doses alters gene expression in *Apis mellifera* bees. Sci. Total Environ., 173393 https://doi.org/10.1016/j.scitotenv.2024.173393.

de Castro Lippi, I.C., Lima, Y.S., da Luz Scheffer, J., Do Rosário, I., Moreira, C., Kadri, S. M., de Oliveira Orsi, R., 2024b. Imidacloprid exposure disrupts gene expression in *Apis mellifera*, potentially threatening pollination efficiency. Bull. Insectol. 77 (2), 245–252.

de Castro Lippi, I.C., Lima, Y.S., da Luz Scheffer, J., Lunardi, J.S., Kadri, S.M., Alvarez, M. V.N., de Oliveira Orsi, R., 2025. Transcriptomic analysis of the head reveals molecular mechanisms underlying topical imidacloprid effects on *A. mellifera* forager bees. Apidologie 56 (2), 1–17. https://doi.org/10.1007/s13592-025-01170-3.

de la Fuente, A., 2010. From 'differential expression'to 'differential networking'–identification of dysfunctional regulatory networks in diseases. Trends Genet. 26 (7), 326–333. https://doi.org/10.1016/j.tig.2010.05.001.

De Smet, L., Hatjina, F., Ioannidis, P., Hamamtzoglou, A., Schoonvaere, K., Francis, F., de Graaf, D.C., 2017. Stress indicator gene expression profiles, colony dynamics and tissue development of honey bees exposed to sub-lethal doses of imidacloprid in laboratory and field experiments. PLoS One 12 (2), e0171529. https://doi.org/10.1371/journal.pone.0171529.

de Souza, G.D.F., de Castro Lippi, I.C., do Rosário Corrêa Moreira, I., da Silva, S.M., da Luz Scheffer, J., Astolfi, A., de Oliveira Orsi, R., 2025. Is sulfoxaflor a safe alternative to neonicotinoids for *Apis mellifera*? A systematic review. Apidologie 56 (2), 1–17. https://doi.org/10.1007/s13592-025-01175-y.

Derecka, K., Blythe, M.J., Malla, S., Genereux, D.P., Guffanti, A., Pavan, P., Stöger, R., 2013. Transient exposure to low levels of insecticide affects metabolic networks of honey bee larvae. PLoS One 8 (7), e68191. https://doi.org/10.1371/journal.pone.0068191.

Domínguez, E., Giardini, P.M., Quintana, S., de la Paz Moliné, M., Chierichetti, M., Medici, S.K., Damiani, N., 2024. Fatty acid profile of *Apis mellifera* workers in the face of an immune challenge. Apidologie 55, 15. https://doi.org/10.1007/s13592-024-01059-7.

Ehrlich, F., Fischer, H., Langbein, L., Praetzel-Wunder, S., Ebner, B., Figlak, K., Eckhart, L., 2019. Differential evolution of the epidermal keratin cytoskeleton in terrestrial and aquatic mammals. Mol. Biol. Evol. 36 (2), 328–340. https://doi.org/10.1093/molbev/msy214.

Elston, C., Thompson, H.M., Walters, K.F., 2013. Sub-lethal effects of thiamethoxam, a neonicotinoid pesticide, and propiconazole, a DMI fungicide, on colony initiation in bumblebee (*Bombus terrestris*) micro-colonies. Apidologie 44, 563–574. https://doi.org/10.1007/s13592-013-0206-9.

Erber, J., Hoormann, J., Scheiner, R., 2006. Phototactic behaviour correlates with gustatory responsiveness in honey bees (*Apis mellifera* L.). Behav. Brain Res. 174 (1), 174–180. https://doi.org/10.1016/j.bbr.2006.07.023. Get rights and content.

Farooqui, A.A., Horrocks, L.A., Farooqui, T., 2000. Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. Chem. Phys. Lipids 106 (1), 1–29. https://doi.org/10.1016/S0009-3084(00)00128-6.

Farooqui, A.A., Horrocks, L.A., Farooqui, T., 2007. Interactions between neural membrane glycerophospholipid and sphingolipid mediators: a recipe for neural cell survival or suicide. J. Neurosci. Res. 85 (9), 1834–1850. https://doi.org/10.1002/jnr.21268.

Fent, K., Schmid, M., Christen, V., 2020a. Global transcriptome analysis reveals relevant effects at environmental concentrations of cypermethrin in honey bees (*Apis mellifera*). Environ. Pollut. 259, 113715. https://doi.org/10.1016/j.envpol.2019.113715.

Fent, K., Schmid, M., Hettich, T., Schmid, S., 2020b. The neonicotinoid thiacloprid causes transcriptional alteration of genes associated with mitochondria at environmental concentrations in honey bees. Environ. Pollut. 266, 115297. https://doi.org/10.1016/j.envpol.2020.115297.

Fuss, N., Mujagic, S., Erber, J., Wachten, S., Baumann, A., 2010. Biochemical properties of heterologously expressed and native adenylyl cyclases from the honey bee brain (*Apis mellifera* L.). Insect Biochem. Mol. Biol. 40 (8), 573–580. https://doi.org/10.1016/j.ibmb.2010.05.004.

Gao, J., Jin, S.S., He, Y., Luo, J.H., Xu, C.Q., Wu, Y.Y., Diao, Q.Y., 2020. Physiological analysis and transcriptome analysis of Asian honey bee (*Apis cerana cerana*) in response to sublethal neonicotinoid imidacloprid. Insects 11 (11), 753. https://doi.org/10.3390/insects11110753.

Geng, H., 2022. Molecular Analyses of Visual Processing and Learning in Honey Bees. Université Paul Sabatier - Toulouse III. https://theses.hal.science/tel-03889969 (PhD Thesis).

Giannini, T.C., Cordeiro, G.D., Freitas, B.M., Saraiva, A.M., Imperatriz-Fonseca, V.L., 2015. The dependence of crops for pollinators and the economic value of pollination in Brazil. J. Econ. Entomol. 108 (3), 849–857. https://doi.org/10.1093/jee/tov093.

Gong, Y., Diao, Q., 2017. Current knowledge of detoxification mechanisms of xenobiotic in honey bees. Ecotoxicology 26, 1–12. https://doi.org/10.1007/s10646-016-1742-7.

Gonzalez, V.H., Hranitz, J.M., McGonigle, M.B., Manweiler, R.E., Smith, D.R., Barthell, J. F., 2022. Acute exposure to sublethal doses of neonicotinoid insecticides increases heat tolerance in honey bees. PLoS One 17 (2), e0240950. https://doi.org/10.1371/journal.pone.0240950.

Hansen, M., Flatt, T., Aguilaniu, H., 2013. Reproduction, fat metabolism, and life span: what is the connection? Cell Metab. 17 (1), 10–19. https://doi.org/10.1016/j.cmet.2012.12.003.

Harapas, C.R., Idiiatullina, E., Al-Azab, M., Hrovat-Schaale, K., Reygaerts, T., Steiner, A., Masters, S.L., 2022. Organellar homeostasis and innate immune sensing. Nat. Rev. Immunol. 22 (9), 535–549. https://doi.org/10.1038/s41577-022-00682-8.

Hauser, F., Cazzamali, G., Williamson, M., Blenau, W., Grimmelikhuijzen, C.J.P., 2006. A review of neurohormone GPCRs present in the fruitfly *Drosophila melanogaster* and the honey bee *Apis mellifera*. Prog. Neurobiol. 80 (1), 1–19. https://doi.org/10.1016/j.pneurobio.2006.07.005.

Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J.F., Aupinel, P., Decourtye, A., 2012. A common pesticide decreases foraging success and survival in honey bees. Science 336 (6079), 348–350. https://doi.org/10.1126/science.1215039.

Hung, K.L.J., Kingston, J.M., Albrecht, M., Holway, D.A., Kohn, J.R., 2018. The worldwide importance of honey bees as pollinators in natural habitats. Proc. R. Soc. B 285 (1870), 20172140. https://doi.org/10.1098/rspb.2017.2140.

Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global strategy for neonicotinoids. J. Agric. Food Chem. 59 (7), 2897–2908. https://doi.org/10.1021/jf101303g.

Kim, B.Y., Jin, B.R., 2015. Apolipophorin III from honey bees (*Apis cerana*) exhibits antibacterial activity. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 182, 6–13. https://doi.org/10.1016/j.cbpb.2014.11.010.

Kim, Y.H., Lee, S.H., 2013. Which acetylcholinesterase functions as the main catalytic enzyme in the Class Insecta? Insect Biochem. Mol. Biol. 43 (1), 47–53. https://doi.org/10.1016/j.ibmb.2012.11.004.

Kim, B.Y., Kim, Y.H., Choi, Y.S., Lee, M.Y., Lee, K.S., Jin, B.R., 2022. Antimicrobial activity of apidermin 2 from the honey bee *Apis mellifera*. Insects 13 (10), 958. https://doi.org/10.3390/insects13100958.

Knorr, D.Y., Georges, N.S., Pauls, S., et al., 2020. Acetylcholinesterase promotes apoptosis in insect neurons. Apoptosis 25, 730–746. https://doi.org/10.1007/s10495-020-01630-4.

Kobe, B., Kajava, A.V., 2001. The leucine-rich repeat as a protein recognition motif. Curr. Opin. Struct. Biol. 11 (6), 725–732. https://doi.org/10.1016/S0959-440X(01)00266-4.

Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G., Given, K., 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7 (1), e29268. https://doi.org/10.1371/journal.pone.0029268.

Kunieda, T., Fujiyuki, T., Kucharski, R., Foret, S., Ament, S.A., Toth, A.L., Maleszka, R., 2006. Carbohydrate metabolism genes and pathways in insects: insights from the honey bee genome. Insect Mol. Biol. 15 (5), 563–576. https://doi.org/10.1111/j.1365-2583.2006.00677.x.

Lau, L.Y., Nguyen, L.T., Reverter, A., Moore, S.S., Lynn, A., McBride-Kelly, L., Fortes, M. R., 2020. Gene regulation could be attributed to TCF3 and other key transcription factors in the muscle of pubertal heifers. Vet. Med. Sci. 6, 695–710. https://doi.org/10.1002/vms3.278.

Leek, J.T., Monsen, E., Dabney, A.R., Storey, J.D., 2006. EDGE: extraction and analysis of differential gene expression. Bioinformatics 22, 507–508. https://doi.org/10.1093/bioinformatics/btk005. L.

Li, H., Durbin, R., 2009. Fast and accurate short read alignment with burrows-wheeler transform. Bioinformatics 25 (14), 1754–1760.

Lima, Y.S., de Castro Lippi, I.C., da Luz Scheffer, J., Lunardi, J.S., Alvarez, M.V.N., Kadri, S.M., de Oliveira Orsi, R., 2024. Food contamination with fipronil alters gene expression associated with foraging in Africanized honey bees. Environ. Sci. Pollut. Res. 1. https://doi.org/10.1007/s11356-024-34695-8.

Liu, C., Wu, X., Yang, H., Yu, L., Zhang, Y., 2022. Effects of larval exposure to the insecticide flumethrin on the development of honey bee (*Apis mellifera*) workers. Front. Physiol. 13, 1054769. https://doi.org/10.3389/fphys.2022.1054769.

Liu, X., Li, C., Chen, Y., Xue, Z., Miao, J., Liu, X., 2023. Untargeted lipidomics reveals lipid metabolism disorders induced by oxathiapiprolin in Phytophthora sojae. Pest Manag. Sci. 79, 1593–1603. https://doi.org/10.1002/ps.7334.

Mackei, M., Sebők, C., Vöröházi, J., Tráj, P., Mackei, F., Oláh, B., Mátis, G., 2023. Detrimental consequences of tebuconazole on redox homeostasis and fatty acid profile of honey bee brain. Insect Biochem. Mol. Biol. 159, 103990. https://doi.org/10.1016/j.ibmb.2023.103990.

Mamy, L., Pesce, S., Sanchez, W., Aviron, S., Bedos, C., Berny, P., Leenhardt, S., 2023. Impacts of neonicotinoids on biodiversity: a critical review. Environ. Sci. Pollut. Res. (6), 1. https://doi.org/10.1007/s11356-023-31032-3.

Martin, G.J., Lower, S.E., Suvorov, A., Bybee, S.M., 2021. Molecular evolution of phototransduction pathway genes in nocturnal and diurnal fireflies (Coleoptera: Lampyridae). Insects 12 (6), 561. https://doi.org/10.3390/insects12060561.

Matsuda, K., Buckingham, S.D., Kleier, D., Rauh, J.J., Grauso, M., Sattelle, D.B., 2001. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends Pharmacol. Sci. 22 (11), 573–580. https://doi.org/10.1016/s0165-6147(00)01820-4.

Maya-Aguirre, C.A., Torres, A., Gutiérrez-Castañeda, L.D., Salazar, L.M., Abreu-Villaça, Y., Manhães, A.C., Arenas, N.E., 2024. Changes in the proteome of *Apis mellifera* acutely exposed to sublethal dosage of glyphosate and imidacloprid. Environ. Sci. Pollut. Res. 31 (33), 45954–45969. https://doi.org/10.1007/s11356-024-34185-x.

Medzhitov, R., Horng, T., 2009. Transcriptional control of the inflammatory response. Nat. Rev. Immunol. 9 (10), 692–703.

Micas, A.F.D., Ferreira, G.A., Laure, H.J., Rosa, J.C., Bitondi, M.M.G., 2016. Proteins of the integumentary system of the honey bee, *Apis mellifera*. Arch. Insect Biochem. Physiol. 93 (1), 3–24. https://doi.org/10.1002/arch.21336.

Millard, J., Outhwaite, C.L., Ceaușu, S., Carvalheiro, L.G., da Silva E Silva, F.D., Dicks, L. V., Ollerton, J., Newbold, T., 2023. Key tropical crops at risk from pollinator loss due to climate change and land use. Sci. Adv. 9 (41). https://doi.org/10.1126/sciadv.adh0756.

Modre-Osprian, R., Osprian, I., Tilg, B., Schreier, G., Weinberger, K.M., Graber, A., 2009. Dynamic simulations on the mitochondrial fatty acid beta-oxidation network. BMC Syst. Biol. 3, 2. https://doi.org/10.1186/1752-0509-3-2.

Montesinos, J., Guardia-Laguarta, C., Area-Gomez, E., 2020. The fat brain. Curr. Opin. Clin. Nutr. Metab. Care 23 (2), 68–75. https://doi.org/10.1097/MCO.0000000000000634.

Moreira, I.D.R.C., de Castro Lippi, I.C., Lima, Y.S., da Luz Scheffer, J., Kadri, S.M., Alvarez, M.V.N., de Oliveira Orsi, R., 2025. Pesticides modulate the expression of genes in the antioxidant system of Africanized *Apis mellifera* bees in the forager phase. Observatório de la economía latinoamericana 23 (5), e9949. https://doi.org/10.55905/oelv23n5-089.

Morfin, N., Fillier, T.A., Pham, T.H., Goodwin, P.H., Thomas, R.H., Guzman-Novoa, E., 2022. First insights into the honey bee (Apis mellifera) brain lipidome and its neonicotinoid-induced alterations associated with reduced self-grooming behaviour. J. Adv. Res. 37, 75–89. https://doi.org/10.1016/j.jare.2021.08.007.

Nath, R., Singh, H., Mukherjee, S., 2023. Insect pollinators decline: an emerging concern of Anthropocene epoch. J. Apic. Res. 62 (1), 23–38. https://doi.org/10.1080/00218839.2022.2088931.

Nozal, M.J., Bernal, J.L., Gómez, L.A., Higes, M., Meana, A., 2003. Determination of oxalic acid and other organic acids in honey and in some anatomic structures of bees. Apidologie 34 (2), 181–188. https://doi.org/10.1051/apido:2003001.

Palmer, M.J., Moffat, C., Saranzewa, N., Harvey, J., Wright, G.A., Connolly, C.N., 2013. Cholinergic pesticides cause mushroom body neuronal inactivation in honey bees. Nat. Commun. 4, 1634. https://doi.org/10.1038/ncomms2648.

Paloschi, C.L., Tavares, M.H.F., Berte, E.A., Model, K., Rosa, K.M., da Conceição, F.G., Potrich, M., 2023. Imidacloprid: impact on Africanized *Apis mellifera* L. (Hymenoptera: Apidae) workers and honey contamination. Chemosphere 338, 139591. https://doi.org/10.1016/j.chemosphere.2023.139591.

Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Downs, C.A., Goulson, D., Wiemers, M., 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ. Sci. Pollut. Res. 22, 68–102. https://doi.org/10.1007/s11356-014-3471-x.

Ramirez, Morfin, 2018. Effect of Sublethal Doses of Clothianidin and/or *V. destructor* on Honey Bee (*Apis mellifera* L.) Health, Behavior and Associated Gene Expression. University of Guelph (PhD thesis).

Ranganathan, M., Narayanan, M., Kumarasamy, S., 2022. Importance of metabolic enzymes and their role in insecticide resistance. In: Mandal, S.D., Ramkumar, G., Karthi, S., Jin, F. (Eds.), New and Future Development in Biopesticide Research: Biotechnological Exploration. Springer, Singapore. https://doi.org/10.1007/978-981-16-3989-0_10.

Reverter, A., Chan, E.K., 2008. Combining partial correlation and an information theory approach to the reversed engineering of gene co-expression networks. Bioinformatics 24 (21), 2491–2497. https://doi.org/10.1093/bioinformatics/btn482.

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., Smyth, G.K., 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43 (7), e47. https://doi.org/10.1093/nar/gkv007.

Rodnina, M.V., Beringer, M., Wintermeyer, W., 2007. How ribosomes make peptide bonds. Trends Biochem. Sci. 32 (1), 20–26. https://doi.org/10.1016/j.tibs.2006.11.007.

Sánchez-Bayo, F., Goulson, D., Pennacchio, F., Nazzi, F., Goka, K., Desneux, N., 2016. Are bee diseases linked to pesticides?—a brief review. Environ. Int. 89, 7–11. https://doi.org/10.1016/j.envint.2016.01.009.

Schmuck, R., Lewis, G., 2016. Review of field and monitoring studies investigating the role of nitro-substituted neonicotinoid insecticides in the reported losses of honey bee colonies (Apis mellifera). Ecotoxicology 25 (9), 1617–1629. https://doi.org/10.1007/s10646-016-1734-7.

Schroeder, A., Mueller, O., Stocker, S., Salowsky, R., Leiber, M., Gassmann, M., Ragg, T., 2006. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol. Biol. 7, 1–14. https://doi.org/10.1186/1471-2199-7-3.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504. https://doi.org/10.1101/gr.1239303.

Shi, T.F., Wang, Y.F., Liu, F., Qi, L., Yu, L.S., 2017. Sublethal effects of the neonicotinoid insecticide thiamethoxam on the transcriptome of the honey bees (Hymenoptera: Apidae). J. Econ. Entomol. 110 (6), 2283–2289. https://doi.org/10.1093/jee/tox262.

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Chagnon, M., Downs, C., Wiemers, M., 2015. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ. Sci. Pollut. Res. 22, 5–34. https://doi.org/10.1007/s11356-014-3470-y.

Smale, S.T., 2010. Selective transcription in response to an inflammatory stimulus. Cell 140 (6), 833–844. https://doi.org/10.1016/j.cell.2010.01.037.

Sohrabi, S.S., Sohrabi, S.M., Rashidipour, M., Mohammadi, M., Fard, J.K., Najafgholi, H. M., 2020. Identification of common key regulators in rat hepatocyte cell lines under exposure of different pesticides. Gene 739, 144508. https://doi.org/10.1016/j.gene.2020.144508.

Sponsler, D.B., Johnson, R.M., 2017. Mechanistic modeling of pesticide exposure: the missing keystone of honey bee toxicology. Environ. Toxicol. Chem. 36 (4), 871–881. https://doi.org/10.1002/etc.3661.

Strauss, E., Kinsland, C., Ge, Y., McLafferty, F.W., Begley, T.P., 2001. Phosphopantothenoylcysteine synthetase from Escherichia coli: identification and characterization of the last unidentified coenzyme A biosynthetic enzyme in bacteria. J. Biol. Chem. 276 (17), 13513–13516. https://doi.org/10.1074/jbc.C100033200.

Streinzer, M., Brockmann, A., Nagaraja, N., Spaethe, J., 2013. Sex and caste-specific variation in compound eye morphology of five honeybee species. PLoS One 8 (2), e57702. https://doi.org/10.1371/journal.pone.0057702.

Sun, S., Ding, Y., Yang, D., Shen, J., Zhang, T., Song, G., Chen, R., 2025. Identification of potential hub genes and drugs in septic kidney injury: a bioinformatic analysis with preliminary experimental validation. Front. Med. 12, 1502189. https://doi.org/10.3389/fmed.2025.1502189.

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Von Mering, C., 2023. The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 51 (D1), D638–D646. https://doi.org/10.1093/nar/gkac1000.

Talley, J.T., Mohiuddin, S.S., 2020. Biochemistry, fatty acid oxidation. In: StatPearls. StatPearls Publishing, Treasure Island (FL) (Internet).

Tian, W., Zhang, L.V., Taşan, M., Gibbons, F.D., King, O.D., Park, J., Roth, F.P., 2008. Combining guilt-by-association and guilt-by-profiling to predict Saccharomyces cerevisiae gene function. Genome Biol. 9, 1–21. https://doi.org/10.1186/gb-2008-9-s1-s7.

Tremolada, P., Vighi, M., 2014. Mathematical models for the comprehension of chemical contamination into the hive. In: Silico Bees, pp. 153–178.

Ventura, R., Martínez-Ruiz, I., Hernández-Alvarez, M.I., 2022. Phospholipid membrane transport and associated diseases. Biomedicines 10 (5), 1201. https://doi.org/10.3390/biomedicines10051201.

Wakakuwa, M., Kurasawa, M., Giurfa, M., Arikawa, K., 2005. Spectral heterogeneity of honeybee ommatidia. Naturwissenschaften 92, 464–467. https://doi.org/10.1007/s00114-005-0018-5.

Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. 10 (1), 57–63. https://doi.org/10.1038/nrg2484.

Wei, Y., Liu, S., Xiong, D., Xiong, Z., Zhang, Z., Wang, F., Huang, J., 2022. Genome-wide association study for non-photochemical quenching traits in Oryza sativa L. Agronomy 12 (12), 3216. https://doi.org/10.3390/agronomy12123216.

Wickham, H., Averick, M., Bryan, J., Chang, W., Mcgowan, L.D.A., François, R., Yutani, H., 2019. Welcome to the Tidyverse. J. Open Source Softw. 4 (43), 1686. https://doi.org/10.21105/joss.01686.

Wigglesworth, V.B., 1948. The insect cuticle. Biol. Rev. 23 (4), 408–451. https://doi.org/10.1111/j.1469-185X.1948.tb00566.x.

Williams, G.R., Troxler, A., Retschnig, G., Roth, K., Yañez, O., Shutler, D., Gauthier, L., 2015. Neonicotinoid pesticides severely affect honey bee queens. Sci. Rep. 5, 14621. https://doi.org/10.1038/srep14621.

Williamson, S.M., Wright, G.A., 2013. Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honey bees. J. Exp. Biol. 216 (10), 1799–1807. https://doi.org/10.1242/jeb.083931.

Williamson, S.M., Moffat, C., Gomersall, M.A., Saranzewa, N., Connolly, C.N., Wright, G. A., 2013. Exposure to acetylcholinesterase inhibitors alters the physiology and motor function of honey bees. Front. Physiol. 4, 13. https://doi.org/10.3389/fphys.2013.00013.

Woodcock, B.A., Bullock, J.M., Shore, R.F., Heard, M.S., Pereira, M.G., Redhead, J., Pywell, R.F., 2017. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science 356 (6345), 1393–1395. https://doi.org/10.1126/science.aaa1190.

Wright, G.A., Nicolson, S.W., Shafir, S., 2018. Nutritional physiology and ecology of honey bees. Annu. Rev. Entomol. 63 (1), 327–344. https://doi.org/10.1146/annurev-ento-020117-043423.

Wu, M.C., Chang, Y.W., Lu, K.H., Yang, E.C., 2017. Gene expression changes in honey bees induced by sublethal imidacloprid exposure during the larval stage. Insect Biochem. Mol. Biol. 88, 12–20. https://doi.org/10.1016/j.ibmb.2017.06.016.

Xu, J., Grant, G., Sabin, L.R., Gordesky-Gold, B., Yasunaga, A., Tudor, M., Cherry, S., 2012. Transcriptional pausing controls a rapid antiviral innate immune response in Drosophila. Cell Host Microbe 12 (4), 531–543. https://doi.org/10.1016/j.chom.2012.08.011.

Zaluski, R., Bittarello, A.C., Vieira, J.C.S., Braga, C.P., Padilha, P.D.M., Fernandes, M.D. S., Orsi, R.D.O., 2020. Modification of the head proteome of nurse honey bees (Apis mellifera) exposed to field-relevant doses of pesticides. Sci. Rep. 10 (1), 2190. https://doi.org/10.1038/s41598-020-59070-8.

Zdybicka-Barabas, A., Cytryńska, M., 2013. Apolipophorins and insects immune response. Invertebr. Surviv. J. 10 (1), 58–68.

Zhang, X., Zhang, B., Zhang, Y., Zhang, F., 2023. Association analysis of hepatocellular carcinoma-related hub proteins and hub genes. Proteomics Clin. Appl. 17 (5), 2200090. https://doi.org/10.1002/prca.202200090.