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Analysis of the immune transcriptome of the invasive pest spotted wing drosophila infected by *Steinernema carpocapsae*

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Research Paper

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Abstract

Drosophila suzukii is a pest of global concern due to its great impact on several crops. The entomopathogenic nematode *Steinernema carpocapsae* was highly virulent to the larvae of the fly although some immune mechanisms were triggered along the infection course. Thus, to understand the gene activation profile we performed a comparative transcriptome of *D. suzukii* larvae infected with *S. carpocapsae* and *Xenorhabdus nematophila* to map the differentially expressed genes involved in the defence response. The analysis exposed the induction of genes involved in the humoral response such as the antimicrobial peptides and pattern-recognition receptors while there was a suppression of the cellular defence. Besides, genes involved in melanisation, and clot formation were downregulated hindering the encapsulation response and wound healing. After the infection, larvae were in a stress condition with an enrichment of metabolic and transport functionalities. Concerning the stress response, we observed variations of the heat-shock proteins, detoxification, and peroxidase enzymes. These findings set a genetical comprehensive knowledge of the host-pathogen relation of *D. suzukii* challenged with *S. carpocapsae* which could support further comparative studies with entomopathogenic nematodes.

Introduction

Drosophila suzukii (Diptera: Drosophilidae), widely known as Spotted Wing Drosophila, is a globally invasive pest that significantly impacts soft-skinned and stone fruits such as berries, cherries, and strawberries (Lee *et al.*, 2011; Walsh *et al.*, 2011). Initially introduced in America, it rapidly spread across Europe, causing great economic losses due to its polyphagous nature (Asplen *et al.*, 2015). Furthermore, effective control measures have been challenging due to the quick generation span of the fly which requires frequent interventions. Gress and Zalom (2019) reported resistance of *D. suzukii* to the chemical pesticide Spinosad, indicative of a potent detoxification response mechanism. Therefore, the prospection of biological control agents progressed by pointing to parasitoids and entomopathogenic nematodes (EPNs) as promising candidates to control the pest (Wang *et al.*, 2021). The results with parasitoids varied, with pupal parasitoids being successful, while *D. suzukii* larvae mounted a strong immune response to encapsulate parasitoid eggs (Kacsoh and Schlenke, 2012). In contrast, the EPN *Steinernema carpocapsae* (Steinernema: Panagrolaimida) was highly effective against both larvae and adults of *D. suzukii* under laboratory conditions (Garriga *et al.*, 2018, 2020*b*). Given these differences in larval responses to parasitoid eggs and EPNs, our goal was to prospect the immune response elicited by the nematode-bacterial complex in *D. suzukii*.

EPNs belonging to the families Steinernematidae and Heterorhabditidae are obligate parasites of a wide range of insects used for the biological control of pests (Woodring and Kaya, 1988). The infection process by EPNs is intricate due to the symbiosis complex of nematode and the bacteria *Xenorhabdus nematophila,* which impacts many pathways from the insect immune response (Peña *et al.*, 2015). The infective process of any pathogen involves a wide range of genes tangled together, from those restricting metabolic pathways to those activating stress and defence mechanisms, to focus resources on overcoming the infection. It is therefore expected to observe an activation of the immune-promotor genes shortly after infection with the triggering of the pattern recognitions proteins that identify the foreign body as non-self (Lemaitre and Hoffmann, 2007). Combating the immune response is crucial for the success of the EPNs which evolved with a dual purpose. Firstly, to evade host recognition through camouflage and evasive strategies (Brivio *et al.*, 2018; Brivio and Mastore, 2020). Secondly, to counter the immune response with the secretions produced by both nematode and bacteria (Toubarro *et al.*, 2013; Eliáš *et al.*, 2020; Jones *et al.*, 2022). In the case of *D. suzukii*, the fly was unable to encapsulate the nematode as seen in previous experiments which evaluated the physiological aspects of this infective process (Garriga *et al.*, 2020*a*). During these experiments,

the cellular response was either ineffective or not triggered by the presence of the nematodes, resulting in a high success rate of nematode infection. Nevertheless, the humoral response was activated by the presence of the symbiont bacteria *X. nematophila* in the haemolymph but was unable to block the progress of the nematode-bacterial infection.

The biological assessment and differential expression of a few genes provided an initial understanding of the infection and defence responses, although many gaps remained uncovered (Garriga *et al*., 2023). Hence, the utilisation of mRNA sequencing (RNA-Seq) technology provides a wider and more comprehensive perspective of the infection process. This technique allows for a deeper understanding of the infection dynamics, unveiling intricate physiological details such as the effects in metabolic processes and stimulus-response pathways of the insects as seen in nematode-bacterial infections in *Drosophila melanogaster* (Diptera: Drosophilidae) larvae (Arefin *et al*., 2014; Castillo *et al*., 2015). The sequencing of transcripts further facilitates the identification of evolutionary distinctions when compared to established species, revealing adaptive strategies that potentially enhance the adaptability and proliferation of the pest (Mérel *et al*., 2021). Due to the advances in transcriptome sequencing and gene annotation, insects such as *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Holotrichia parallela* (Coleoptera: Scarabaeidae) can be analysed with this technique to directly study the infective process of EPNs on the insect pest of interest (Huot *et al*., 2019; Li *et al*., 2021). Previous studies have examined the transcriptome of *D. suzukii,* focusing on traits like olfactory receptors, reproduction, detoxification, and diapause (Shearer *et al*., 2016; Mishra *et al*., 2018; Schwanitz *et al*., 2022; Xing *et al*., 2022). These studies have contributed to understanding the evolutionary dynamics of the fly and assisted in predicting and managing its invasion, although none have focused on the immune response to pathogens.

In this perspective, our objective was to use a comparative transcriptomic analysis to elucidate functional changes and gene regulations in *D. suzukii* larvae infected with the nematode complex *S. carpocapsae* – *X. nematophila*. This data could provide a better understanding of host-pathogen interactions and contribute to the development of targeted future pest management strategies.

Material and methods

Insects and EPNs culture

For the study, we used third-instar larvae of *D. suzukii* produced in a laboratory culture established in 2017 from wild specimens. Insects were reared on a modified drosophila diet (Garriga *et al*., 2018) at 25°C with a 12:12 h photoperiod. The infection experiment was conducted under these climatical conditions.

The assay was performed with *S. carpocapsae* (strain B14) isolated from urban garden soil in Barcelona, Catalonia (NE Spain). The native bacteria were replaced by the *X. nematophila* Green Fluorescent Protein (GFP-labelled strain F1D3) provided by the laboratory of Prof. Givaudan (University of Montpellier, France) (Garriga *et al*., 2020*a*). Nematodes were reared in the late instar *G. mellonella* larva at 25°C (Woodring and Kaya, 1988). Once the IJs emerged from the insect, IJs were stored in sterile tap water (STW) at 9°C for a maximum of two weeks. Before use, the nematodes were acclimatised at room temperature for 3 h to ensure maximum activation during the assay.

Infection assay

The infection assay was performed following the methodology described by Garriga *et al*. (2023). Larvae were exposed to 300 IJs in 96-well plates filled with sterile filter paper for 2 h. After the exposition, we rinsed larvae thoroughly with STW and transferred to a new Petri dish with filter paper and a cube of drosophila diet to allow the infection to develop for 2 more hours. After this incubation period, larvae were subjected to a final rinse with STW before being transferred individually to a 1.5 ml tube containing 20 µl of RNAlater and immediately frozen at −80°C. The same procedure of manipulation and rinsing steps were conducted with control larvae which only received STW as exposure treatment. *Drosophila suzukii* larvae were dissected individually in RNAlater to verify the nematode entry by observation of IJs (mean entry of 6.68 ± 4.45 IJs per larvae). Only larvae with confirmed nematode presence were used for RNA extraction. These larvae were pooled into groups of five per treatment for the RNA extraction, and three biological repetitions were performed for both control and infected larvae.

mRNA collection and sequencing

RNA isolation was performed with TRIzol Reagent combined with Invitrogen PureLink RNA Mini Kit (ThermoFisher), following the manufacturer's instructions. Purified RNA was kept at −80°C until the sequencing step. The quality and quantity of RNA were assessed with Nanodrop and the RNA integrity confirmed with Bioanalyzer Samples were sequenced with the Illumina NovaSeq platform using 150 bp paired-end sequence reads with the library for stranded mRNA preparation kit. The reads were trimmed to remove low coverage and adaptors. The quality of the reads was assessed with Fast-QC before continuing with the bioinformatic process (Andrews, 2010).

Bioinformatic and statistical procedure

The reads were assembled with the *D. suzukii* genome of reference GCF_013340165.1 from the NCBI database. The assembly was done with the software Bowtie2 altogether with the software RSEM to make the counts of the transcripts (Li and Dewey, 2011). Differential analysis was conducted using the *DESeq2* package to obtain the significant differentially expressed genes (DEG) with a cut-off of False Discovery Rate (FDR) less than 0.1 and an absolute Log2 Fold-Change of 0.5 (Love *et al*., 2014). The functional annotation was done using the Sma3s software with the Uniref90 database for the assignment of the Gene Ontology (GO) Terms (Casimiro-Soriguer *et al*., 2017). The pathway information was obtained from the Kyoto Encyclopaedia of Genes and Genomes (KEGG) through the EggNOG platform as well as the KASS platform (Moriya *et al*., 2007; Cantalapiedra *et al*., 2021). We discriminated the enriched terms from the DEG compared to the complete set of mRNA using the *ClusterProfiler* and *TopGO* packages (Wu *et al*., 2021; Alexa and Rahnenfuhrer, 2023). A manual curation helped by the GO terms was used to filter the genes of immune defence and stimulus-response for a closer assessment of these pathways. The visualisation of results was done with the *ggplot2* package. All statistical analysis packages were conducted with RStudio (R Core Team, 2017).

Results

Sequencing results and differential analysis

Infection with EPN was observed in 75% of the larvae dissected in the present test. We sequenced the complete transcriptome of

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Table 1. Summary statistics of transcriptome sequencing

Summary	
Mean number of reads	71,408,529 reads
Average length of raw reads	150 bp
Genes identified	15,567 sequences
Blast gene identification	13,861 sequences
Annotated GO terms (Sma3)	12,806 sequences
Annotated GO terms (Eggnog)	10,610 sequences
Annotated KEGG terms (Eggnog)	8149 sequences
Annotated KEGG terms (KAAS)	7533 sequences

infected *D. suzukii* larvae with *S. carpocapsae* B14 and the symbiont bacteria *X. nematophila* F1D3. From the sequencing process, a mean number of 71 million reads was obtained per sample once the adaptors and low-quality were trimmed (table 1). The sequenced reads can be found in the NCBI SRA database with the code PRJNA910932. The mapped reads reached a percentage of 65 to 74% across samples with a total of 15,567 identified genes through the assembly with the *D. suzukii* reference genome. The transcriptome of *D. suzukii* was functionally annotated for GO terms reaching an identification of 12,806 transcripts, while 8149 transcripts were identified using the KEGG database.

In the principal component analysis (PCA), the PC2 explains 31% of the variance which include transcripts that separate the samples of nematode-infected larvae from the control ones (fig. S1). From the identified sequences, 242 transcripts were differentially expressed genes (DEG) comparing control and nematode-infected larvae. From these genes, 104 were upregulated and 138 were downregulated (fig. 1, table S1). Among the DEG, our analysis identified 40 genes annotated in the defence response category while 50 genes belong to the stress response system. The metabolism of larvae presented many alterations (93 DEG) mainly related to secondary metabolism as well as significant variations of the transmembrane transportation (21 DEG). The genes of immune defence and stress altered after the EPNs infection will be discussed in detail further below.

Functional analysis

The functional analysis displayed enrichment of the GO terms corresponding to the categories Biological Process (BP), Molecular Function (MF), and Cell Component (CC) (fig. 2). The most enriched functionalities from the BP were related to the defence response and immune reaction. Among those, we highlight the enrichment of the antibacterial response, humoral immune mechanisms, and inflammatory response in line with an infection course. A general stress activation was also observed by the enrichment of the processes related to responses to other organisms, cold, fungi, and biotic stimuli. Moreover, we observed the involvement of metabolic functions such as the diol process, the carboxylic acid, and the organic acid biosynthetic. Regarding the MF, the more enriched terms were the structural constituent of cuticle and the iron ion binding, which are categories characteristically tight to pathogen infection. We also detected an enrichment of the activity aldo-keto reductase (NAPD), oxidoreductase, monooxygenase, and catalytic categories. The alteration of these enzymatic processes is linked to the metabolic swift due to the stress condition of larvae. In the cell component category (CC), the extracellular space was enriched indicating the increase of the transport components across the cellular space and haemolymph can be related to humoral and detoxification processes.

Regarding the pathway KEGG categories (level 2) that were altered after the infection of nematodes were related to metabolism and ABC transporters, with the enrichment of the biosynthesis of secondary metabolites, and the metabolism of ether lipid, sphingolipid, ascorbate and aldarate, porphyrin, steroid biosynthesis, and retinol (fig. 3A). The KEGG analysis at the gene level (level 3) further revealed that the genes Cecropin and Diptericin were strongly enriched together with the immune-related enzyme Aminopeptidase N (fig. 3B). Genes coding for metabolic enzymes such as ceramide galactosyltransferase, glucuronosyltransferase, and choline dehydrogenase were also enriched. The enrichment of the gene DnaJ homologue evidences the implication of translation processes and heat shock proteins.

DEG of immune defence

The immune defence is a complex system that involves many branched processes, so the basal GO term immune system process

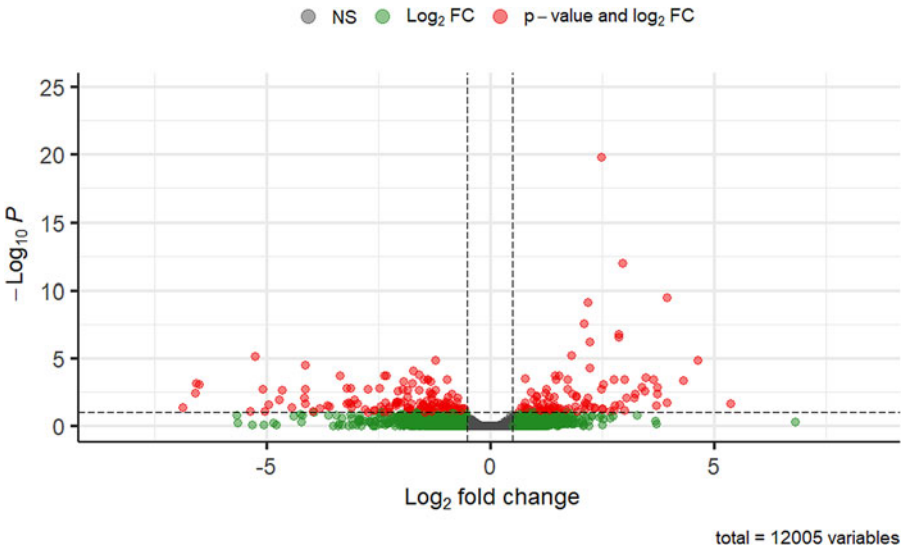

Figure 1. Volcano plot representation of the differentially expressed genes comparing the transcriptome of infected and control larvae.

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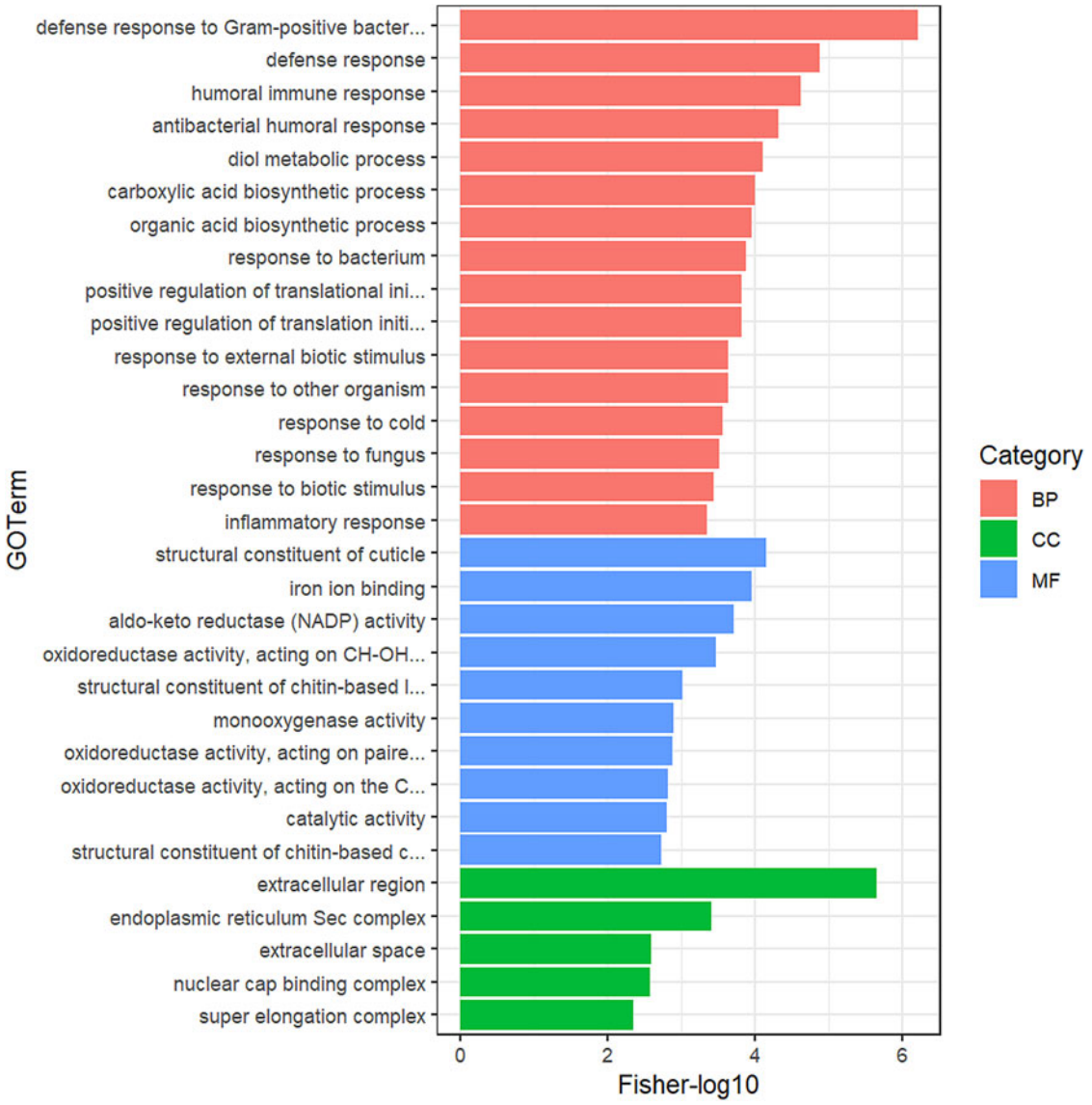

Figure 2. Significantly enriched terms of Gene Ontology (GO) after the analysis of the DEG using TopGO, considering the categories: biological process (BP), Molecular Function (MF), and Cell Component (CC).

(GO 0002376) was our base point to analyse the annotated transcripts as such. After the filtering of low counts, 1024 transcripts were included in this GO term with 40 significant DEG (table 2). As pointed out by the enrichment of the defence process, the most upregulated genes after the nematode infection belong to the Imd pathway. Among them, there were two PGRP receptors homologues to PGRP-SB and -SD of *D. melanogaster* which were significantly upregulated. Furthermore, the activation reached the AMP effector genes in at least seven different genes of the Imd pathway (AttB, CecB, CecC, Cec1, AttB, DptB, Dpt) with a fold-change greater than 2. In the Toll pathway, we identified the upregulation of the two AMP effector genes Drosocin and Metchnikowin after the nematode infection. Moreover, there were upregulated genes with signalling roles or humoral compounds from these pathways' cascades such as edin (XM_017067854), slif (XM_017071440), IDGF (XM_017082031), sno (XM_017089152), and ficolin-1 (XM_017072621).

While the antimicrobial pathways were activated up to the effector genes, other immune responses more specialised in cellular defence were suppressed. This is the case of the effector gene TurandotB (XM_017086060) from Jak-STAT strongly downregulated and the negative feedback *socs4* (suppressor cytokine XM_017090197) which was upregulated. Genes belonging to the PPO pathway did not present a significant variation and the serine protease Sp7 (XM_017073757) that activates the melanisation response was downregulated. Additionally, the transglutaminase gene (XM_017087002) which plays an essential role as a clotting factor was also downregulated. Besides, the downregulation of two chitinase genes (XM_036818866 and XM_036817002) associated with wound healing could indicate a compromised capacity for healing tissues after nematode infection.

DEG of the stress response

The transcripts related to stress and stimulus-response represented a large portion of the 242 DEG indicating the stress condition of the larvae after the infection (table S2). Among these, we highlight the significant upregulation of the heat-shock genes

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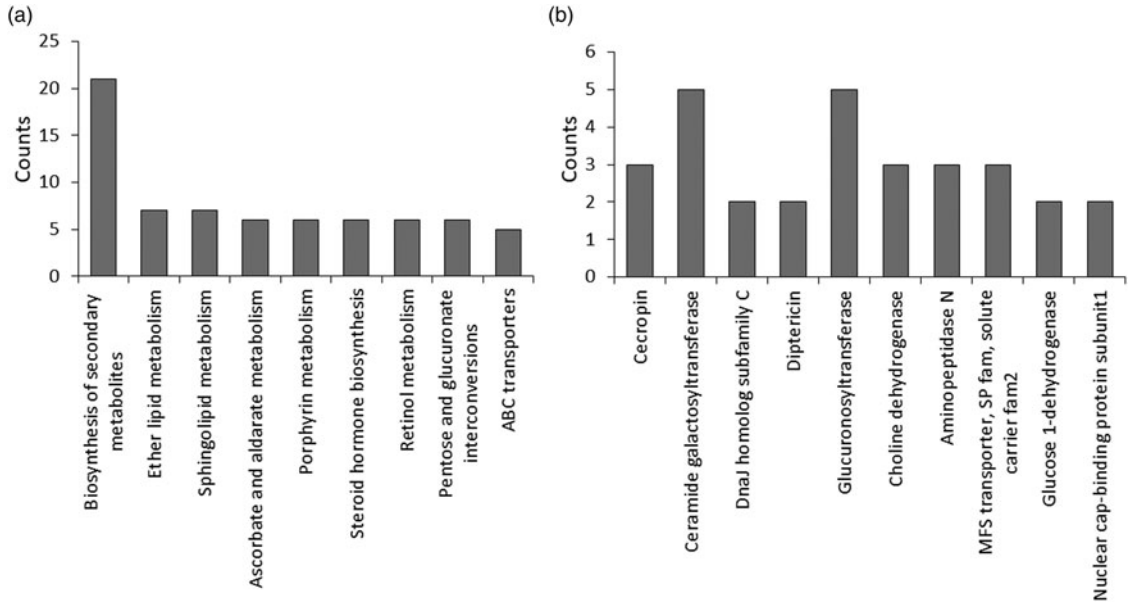

Figure 3. Significantly enriched terms (*x*-axis) using the KEGG database and the transcript count (*y*-axis) after the analysis of the DEG, considering (A) the level 2 pathways and (B) the level 3 genes.

hsp22 and mcm5-hsp70 (XM_017077979 and XM_017071045). Furthermore, several genes of multidrug resistance proteins (XM_017070631 and XM_036818694) and the drug transmembrane transport Mdr49 (XM_017085672) were upregulated, which also explains the functional enrichment of the ABC transporters. However, the detoxification genes related to UDP-glycosyltransferase activity (XM_017075039, XM_036819290, and XM_036816950) were downregulated. Besides, the downregulation of two peroxidase genes and oxidoreductase limited the operation of the ROS mechanism. Concerning cellular regulation, the genes NimCs and the gene encoding for the protein Croquemort were downregulated after the nematode infection. These genes are implicated in the process of cell death, phagocytosis, and haemocyte regulation.

Discussion

EPNs undergo a dynamic relationship with insects, leading to a constant coevolution of defensive mechanisms to combat infection. The *S. carpocapsae* infection induced a distinct activation of the immune mechanisms in *D. suzukii* larvae, based on our previous physiological assessment (Garriga *et al.*, 2020*a*). Thus, the results of the current work with transcriptomic sequencing provide a wider view and help to uncover the gaps concerning the genes regulating these physiological mechanisms.

Through the analysis of the comparative transcriptome, we identified a total of 242 genes induced after infection. Our results suggest that infected larvae reduce metabolic processes to focus resources on confronting the infection. Nevertheless, only a portion of the immune responses was triggered, primarily activating antimicrobial defence relying on humoral components, while concurrently restraining other mechanisms like cellular defence. These defence actions of *D. suzukii,* pointed out by the RNA-Seq, are in concordance with the results from the q-PCR technique, which serve as validation of the current work (Garriga *et al.*, 2023). There was recognition of the pathogen by the Imd PGRP receptors-SB, -SD, and -LC while PGRP-LF was not regulated in either q-PCR or RNASeq results. In accordance with our findings, *D. melanogaster* infected with *S. carpocapsae* showed an upregulation of only some PGRP genes (Yadav *et al.*, 2017). The upregulation of pattern recognition genes conveyed the enrichment of the extracellular matrix region, also reported in infected *D. melanogaster* (Arefin *et al.*, 2014). The AMPs Cecropin, Dipericin, and Attacin were upregulated, which indicates the detection of nematodes or at least their symbiotic bacteria, in agreement with q-PCR results within 4 h after infection (Garriga *et al.*, 2023). The Imd pathway upregulation is typically associated with nematode-bacterial complexes, as when only axenic nematodes infect a fly, a lower activation of the AMP gene was achieved (Peña *et al.*, 2015; Yadav *et al.*, 2017). Concerning the Toll pathway, Defensin was not significantly altered at 4 h post-infection in both analyses, although q-PCR results at 14 h showed a huge increase, suggesting later gene activation (Garriga *et al.*, 2023). Transcriptome data revealed the upregulation of the Metchnikowin and Drosocin genes, which belong to the Toll pathway. Previous works reported a large upregulation of AMPs in this pathway during EPNs infection course (Castillo *et al.*, 2015; Huot *et al.*, 2019). Indeed, the Toll pathway is associated not only with infection of Gram-positive bacteria but also with metazoan pathogens such as nematodes (Shan *et al.*, 2023). These results may also hint the presence of additional bacteria in the insect haemolymph besides *X. nematophila*, which could originate from the microbiome of the nematode called pathobiome (Ogier *et al.*, 2020).

The transcriptomic data confirmed previous physiological observations that the cellular response is constrained during infection. The cell metabolic enzyme AMP deaminase was downregulated, as well as two defensive genes encoding chitinase enzymes, which are crucial for wound healing during nematode infection (Kucerova *et al.*, 2016). Furthermore, two apoptotic genes were downregulated in response to nematode entry, as previously reported in *S. frugiperda* (Huot *et al.*, 2019). There was a strong downregulation of Turandot B, which could be correlated with the TotC expression observed by q-PCR which suggests a lack

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Table 2. List of significant DEG associated with immune defence responses upon *S. carpocapsae* infection, with the mRNA identification (ID RNA), the protein identification (ID Protein), and the gene name obtained during the annotation step

ID RNA	ID Protein	log2FC	Gene Name	Description
XM_ 017074305.2	XP_016929794.1	5.3749	AttB	Attacin
XM_017083107.2	XP_016938596.1	4.6333	CecB	Cecropin
XM_017070308.2	XP_016925797.2	3.9599	CecC	Cecropin
XM_017083120.2	XP_016938609.1	3.7412	Cec1	Cecropin
XM_017074307.1	XP_016929796.1	3.7215	Dro	Drosocin
XM_017067854.2	XP_016923343.2	3.6424	edin	Elevated during infection
XM_017081368.2	XP_016936857.1	3.4798	FAS	Fatty acid synthase
XM_017073965.2	XP_016929454.1	3.4538	AttB	Attacin
XM_017085064.2	XP_016940553.1	3.3890	DptB	Diptericin
XM_017085179.2	XP_016940668.1	3.2161	Mtk	Metchnikowin
XM_017071440.2	XP_016926929.1	2.6759	slif	Amino acid transporter
XM_017072621.2	XP_016928110.1	2.2159	Dwil\GK22020	Ficolin-1
XM_017085063.2	XP_016940552.1	2.1930	Dpt	Diptericin
XM_036819242.1	XP_036675137.1	2.0997	CG1315	Argininosuccinate synthase
XM_017082031.2	XP_016937520.2	1.9402	IDGF	Imaginal disc growth factor 2 (Idgf2)
XM_017068991.2	XP_016924480.2	1.7445	Dmoj\GI13119	Agronaute 2like
XM_017070436.2	XP_016925925.1	1.4232	PGRP	PGRP receptor
XM_017070081.2	XP_016925570.1	1.4140	ninaD	Neither inactivation nor afterpotential D
XM_017090197.2	XP_016945686.1	1.4129	socs4	Suppressor of cytokine signalling 5
XM_036818497.1	XP_036674392.1	1.3371	P58IPK	LD25575p
XM_017089152.2	XP_016944641.1	1.2743	sno	Strawberry Notch
XM_017070210.2	XP_016925699.2	1.2719	PGRP	PGRP receptor
XM_036818936.1	XP_036674831.1	1.2622	e2f1	Transcription factor E2F2
XM_017084488.2	XP_016939977.2	1.1597	P58IPK	LD25575p
XM_017085591.2	XP_016941080.2	0.7889	scb	Encoder of α-PS3 Integrin
XM_036817332.1	XP_036673227.1	−0.5740	Klp98A	Kinesin-like protein
XM_017073757.2	XP_016929246.1	−0.7874	Swim	RE01730p
XM_036819192.1	XP_036675087.1	−0.8729	Sp7	Serine protease 7, isoform A
XM_017067668.2	XP_016923157.1	−0.9389	sev	Protein sevenless
XM_017070065.2	XP_016925554.2	−0.9473	AMPD2	AMP deaminase
XM_017085675.2	XP_016941164.1	−0.9903	Atg7	Autophagy-related 7, isoform A
XM_017075283.2	XP_016930772.1	−1.0803	GGT1	Gamma-glutamyltranspeptidase 1
XM_017076944.2	XP_016932433.1	−1.3134	Ho	Heme oxygenase
XM_036816433.1	XP_036672328.1	−1.3760	Dsim\GD13037	GD13037
XM_036818866.1	XP_036674761.1	−1.4923	chiB4	Chitinase B4
XM_036817002.1	XP_036672897.1	−1.5253	Cht5	Chitinase 5
XM_017087002.2	XP_016942491.1	−1.5767	Tg	Transgultaminase
XM_017074051.1	XP_016929540.1	−3.6362	CG34215	GL15427
XM_017086060.2	XP_016941549.1	−4.7044	TotB	TurandotB
XM_017082231.1	XP_016937720.1	−5.3587	Dsec\GM24472	Salivary glue protein Sgs-3

of activation of the Jak/STAT pathway (Garriga *et al.*, 2023). This observation aligns with findings in *D. melanogaster* where infection with *S. carpocapsae* also exhibited lower induction of the JNK and Jak/STAT pathways compared to the humoral pathways (Yadav *et al.*, 2017). However, in contrast to *D. suzukii*, the fruit fly exhibited higher activation of the Turandot family (Castillo

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et al., 2013; Yadav *et al*., 2018). In the biological tests performed with *D. suzukii* haemocytes, the cells were unable to detect *S. carpocapsae* and adhere to the nematode surface (Garriga *et al*., 2020*a*). This evasive strategy, based on the composition of the nematode's cuticle, was previously described in *G. mellonella* and added to the secretion of proteases from *S. carpocapsae* facilitate the evasion of the cellular encapsulation (Mastore and Brivio, 2008; Toubarro *et al*., 2013). Another fundamental aspect of the cellular response involves the processes of melanisation and haemolymph coagulation, which should be rapidly activated upon infection. However, the genes encoding serine proteases and transglutaminase, which promote melanisation and clot formation (Lindgren *et al*., 2008; Shan *et al*., 2023), were downregulated in our EPN-infected larvae. The absence of PPO genes in the DEG supports the lack of differential regulation observed by q-PCR amplification of the genes PPO1 and PPO2 in *D. suzukii*. The PPO pathway is usually associated with EPN infection due to the formation of melanin clots and contribution to the encapsulation process, although PPO activation was restricted in other nematode–insect infections (Huot *et al*., 2019; Dziedziech *et al*., 2020). In *D. melanogaster*, it was also hypothesised that wound healing involves post-transcriptional products already present in the haemolymph, with no gene expression needed during infection (Arefin *et al*., 2014).

Following *S. carpocapsae* infection, the induction of stress status in *D. suzukii* larvae was apparent, with upregulation of several stress indicators and the functional enrichment of stimulus and stress response categories. This induction was also reported in *D. melanogaster* adults infected with *H. bacteriophora* (Yadav *et al*., 2017). A high upregulation was confirmed for two Hsp genes, crucial for stress response mechanisms (Sorensen *et al*., 2005). These genes indicate cellular stress and modulate the inflammatory process, and were already reported to be upregulated during EPN infection (Yadav *et al*., 2017; Huot *et al*., 2019). The activation of enzymes associated with lipid metabolism could also indicate internal tissue damage caused by nematodes and an intent to repair it (McIntire *et al*., 2009). In contrast, infected *D. suzukii* exhibited downregulation of peroxidase and oxidoreductase genes, which contribute to antimicrobial defence by generating reactive oxygen species (ROS). Concerning detoxification mechanisms, we detected that while three annotated genes as UDP-glycosyltransferase were downregulated, other genes were strongly upregulated such as the multidrug resistance genes from the ABC transporter superfamily which acts as efflux for toxins from cells. In addition, *D. suzukii* presented alterations in iron binding functionalities, an indicator of pathogen presence, as ferric compounds are crucial resource during infections (Akinbosede *et al*., 2022; Liu *et al*., 2022). The upregulation of these genes could result from nematode and bacterial secretions and may be part of the stress response mechanisms linked to specific tissues, as previously reported in *S. frugiperda* (Huot *et al*., 2019). We observed a functional alteration of the DNA translation process in *D. suzukii*, which were also pointed out in *D. melanogaster* infected with the nematode *H. bacteriophora* (Castillo *et al*., 2015). These results may indicate the nematodes' capacity to disrupt gene transcription within the fly, although further experiments should be done to explore this ability.

Transcriptome data represents only a snapshot of the infection course, and a previous or subsequent activation of the cellular response could still occur. For this reason, biological assessments are important to evidence that *D. suzukii* haemocytes are unable to recognise and encapsulate *S. carpocapsae* (Garriga *et al*., 2020*a*). The implications of this type of study extend beyond the description of affected functions and implicated genes. With enhanced knowledge of transcriptomic alternations, we can unravel novel pathways or regulatory networks affected by the nematode-bacterial complex, contributing to our understanding the host-pathogen interactions. Moreover, this genetic knowledge provides hints for designing future biological control tools such as genetic targeting using interference RNA or selecting EPNs to surpass the immune system of the insect.

In conclusion, the transcriptomic analysis aligned with the physiological assessments, as *D. suzukii* infected with *S. carpocapsae* exhibited distinct immune activation, focusing on humoral components, while restraining cellular defence. Stress responses, including upregulation of heat shock protein, and alterations in detoxification and DNA translation processes, indicated broader physiological impacts of the infection. These findings emphasise the importance of understanding host-pathogen interactions, as despite the activation detected in the immune system, EPNs succeeded in the infection. These results contribute to a better understanding of immune mechanisms targeted by EPNs and provide valuable insights for designing effective management strategies for this agricultural pest.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485324000543.

Data. The datasets of this study can be found in the NCBI repository SRA database under the Project PRJNA910932 and BioSamples SRR22675644, SRR22675643, SRR22675642, SRR22675641, SRR22675640, SRR22675639.

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