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GENOMICS, TRANSCRIPTOMICS, PROTEOMICS

RNA sequencing of *Beauveria bassiana* JEF-350-infected *Thrips palmi* reveals change of host defense and homeostasis

Yu Jin Jeong[1] · Jong-Cheol Kim[1] · Gahyeon Song[1] · Mi Rong Lee[1] · Jae Su Kim[1]

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Abstract

Melon thrips, *Thrips palmi*, represent a significant threat to plants, inducing necrosis and acting as vectors for numerous plant viruses. Entomopathogenic fungi present a promising avenue for the management of melon thrips populations resistant to conventional chemical treatments. In this work, an adult colony of melon thrips was exposed to *Beauveria bassiana* strain JEF-350, and the ensuing transcriptional response of the infected thrips was scrutinized to elucidate their reactions during fungal pathogenesis. Utilizing Illumina sequencing, RNA samples were extracted from untreated thrips as well as from thrips continuously infected for 2 and 4 days, each with three biological replicates. While no notable alterations in gene expression were observed between the untreated control and thrips infected for 2 days, those infected for 4 days exhibited a plethora of differentially expressed genes. Specifically, in the thrips infected for the extended period, pathways associated with lysosomal function and insect hormone biosynthesis were notably repressed, while others such as serine and glycine metabolism, Toll and Imd, and circadian rhythm pathways displayed heightened activity. Noteworthy downregulation was observed in numerous lysosomal hydrolase genes encoding glycosidases, sulfatases, and lipases, particularly glycosidases. Furthermore, certain genes related to hydrolase precursors within the Golgi apparatus exhibited heightened expression levels but failed to progress toward hydrolase biosynthesis. Upstream regulation of juvenile hormone biosynthesis was augmented, yet downstream genes were significantly downregulated, leading to a disruption in juvenile hormone production. Similarly, while cytochrome P450 genes in the downstream of ecdysone biosynthesis were upregulated, expressions of cholesterol desaturase and cytochrome P450 genes in the upstream were inhibited, consequently dampening ecdysone biosynthesis. The observed differential targeting of organs or pathways by *B. bassiana* JEF-350, in contrast to conventional chemicals primarily affecting neurotransmission and energy production, suggests its potential efficacy in managing resistant thrips populations. Consequently, integrating JEF-350 into the chemical spray regimen or incorporating it into tank-mix formulations with chemical insecticides emerges as a pragmatic approach within the realm of integrated pest management strategies.

Key points

- *Beauveria treatment inhibited lysosomal function and hormone synthesis in thrips.*
- *Thrips serine/glycine metabolism, Toll and Imd, and circadian rhythm pathways were activated.*
- *Upstream functions of thrips hormone biosynthesis increased, while downstream functions were suppressed.*
- *Regarding biosynthesis of metabolites, this fungus targets other pathways with resistance management.*

Keywords *Thrips palmi* · *Beauveria bassiana* · RNA sequencing · Lysosome · Insect hormone

Introduction

The global distribution of the melon thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae), encompasses diverse regions, including Asia, North America, and Europe (Cannon et al. 2007; Stuart et al. 2011). This pest inflicts substantial economic losses upon agricultural sectors by puncturing and feeding on the tissues of various crops and ornamental plants (Kakkar et al. 2016; North et al. 2006). Furthermore,

Yu Jin Jeong and Jong-Cheol Kim contributed equally to this work.

✉ Jae Su Kim
jskim10@jbnu.ac.kr

[1] Department of Agricultural Biology, College of Agriculture & Life Sciences, Jeonbuk National University, Jeonju, South Korea

T. palmi serves as a vector for several plant viruses, including watermelon silver mottle virus (WMSMoV), groundnut bud necrosis virus (GBNV), and tomato spotted wilt virus (TSWV), thereby exacerbating crop damage through secondary transmission (Daimei et al. 2017; Iwaki et al. 1984; Riley et al. 2011; Yeh et al. 1992). Current management strategies for *T. palmi* predominantly rely on chemical interventions, with synthetic insecticides being widely employed. However, the pervasive use of chemical insecticides precipitated concerns regarding environmental contamination and the emergence of resistance. Consequently, stringent regulatory measures are being enforced in various jurisdictions, with the eventual prohibition of chemical insecticides anticipated in the foreseeable future. This impending regulatory landscape underscores the imperative for alternative pest management approaches, with environmentally benign microbials, such as entomopathogenic fungi, emerging as a promising candidate due to their mode of action and compatibility with prevailing feeding behaviors.

In contrast to conventional chemical pesticides, entomopathogenic fungi exert distinct modes of action and pathogenicity against a broad spectrum of insect pests, thereby constituting environmentally sustainable alternatives for managing resistant insect populations, including thrips. Among these fungi, *Beauveria bassiana* stands out as a prominent species and has been harnessed in the development of various biopesticides such as BotaniGard® (Laverlam International Co., South Parkmont Butte, MT, USA), ChongchaeSak (Farmhannong, Seoul, Korea), Naturalis L (Troy Biosciences Inc., Phoenix, AZ, USA), Broadband, and Velifer (BASF, Ludwigshafen, Germany). These formulations serve as efficacious pest management agents targeting a diverse array of insect pests, including aphids, thrips, whiteflies, cutworms, and beetles. Notably, *B. bassiana* has demonstrated remarkable control efficacy against significant greenhouse and field crop pests such as the western flower thrips (*Frankliniella occidentalis*) and melon thrips (*T. palmi*) (Kim et al. 2020). In our preceding investigations, *B. bassiana* strain JEF 350 emerged as a potent fungal strain exhibiting heightened insecticidal activity against *T. palmi*, thus positing itself as a promising candidate for melon thrips management (Li et al. 2021).

The typical course of *B. bassiana* infection commences with the adherence of fungal conidia to insect cuticles, facilitated by specialized proteins known as adhesins (Altinok et al. 2019). Upon attachment, the fungi secrete an array of hydrolytic enzymes, including lipase, proteases, chitinases, and aminopeptidases, to degrade the cuticular barrier. Subsequently, fungal hyphae infiltrate the insect hemocoel and proliferate in hemolymph. Throughout this progression, the activation of fungal pathogenicity related genes assumes paramount significance, orchestrating strategies to circumvent host insect physiological and immune responses, ultimately culminating in accelerated fungal infection, as underscored by Qu and Wang (2018). Moreover, *B. bassiana* synthesizes toxins to disrupt insect development, growth, and behaviors, thereby fostering a conducive milieu for fungal proliferation and colonization (Wang et al. 2021). Concomitantly, host insects mobilize diverse defense mechanisms to counteract hyphal invasion and mitigate the effects of fungal toxins (Lu and St Leger 2016). Physical defenses commence at the cuticular interface, serving as the primary barrier against fungal intrusion. Following penetration, the insect immune system triggers melanin synthesis via phenol oxidase activation, thereby impeding fungal propagation (OrtizUrquiza and Keyhani 2013). Furthermore, insects deploy detoxifying enzymes to neutralize fungal toxins and synthesize immune related proteins to fortify their defenses against fungal assault (Butt et al. 2016).

Investigating the intricate interplay between host insects and pathogenic microorganisms is imperative for comprehending host defense mechanisms against microbial assaults and delineating the breakdown of this delicate equilibrium conducive to successful pathogenesis by infecting fungi. Such investigations serve as pivotal determinants in augmenting the efficacy, safety, and sustainability of fungal biopesticides, thereby furnishing insights essential for the effective management of insect pests resistant to conventional chemical interventions. In the present study, RNA sequencing emerges as a judicious analytical modality for unraveling the dynamic interactions between host thrips and fungal pathogens. Throughout the course of entomopathogenic fungal infection, this analytical approach holds promise for discerning critical insights into host defense mechanisms and the maintenance of host homeostasis in response to fungal invasion. Moreover, it affords elucidation regarding the molecular events transpiring within host cells during fungal pathogenesis, a facet indispensable for comprehending the intricacies underlying successful fungal infection. Consequently, this study stands poised to furnish invaluable elucidation regarding the heightened virulence of entomopathogenic fungi against target pests vis-à-vis conventional chemical agents, thereby offering pragmatic solutions to mitigate resistance challenges encountered with chemical interventions.

In this study, we conducted experimental investigations into the interactions between melon thrips and the entomopathogenic fungus *B. bassiana* JEF 350, employing RNA sequencing techniques to dissect the early stage responses of infected adult thrips, followed by comprehensive differentially expressed gene (DEG) and pathway analysis. The selection of this isolate from a fungal library was motivated by its demonstrated high insecticidal activity against melon thrips and other insect pests, such as the silverleaf whitefly, in comparison to numerous other *B. bassiana* isolates (Li et al. 2021). Thrips suck on fluids of host

plants after damaging on leaves. So, it would not be realistic for thrips to ingest treated blastospores on the host plants. Instead, active moving adult thrips would have the opportunity to contact blastospores or blastospore originated hyphae on the treated host plants. The analytical outcomes yielded invaluable insights into the mechanisms through which the fungus effectively disrupts the defense mechanisms and homeostatic balance of melon thrips.

Materials and methods

B. bassiana JEF-350 and melon *thrips*

The fungal strain *B. bassiana* JEF 350 (deposited at KFCC of Korean Culture Center of Microorganisms with no. of KFCC11820P), noted for its substantial insecticidal efficacy against *T. palmi* (Li et al. 2021), underwent culture on quarter strength Sabouraud dextrose agar (1/4 SDA) medium for a duration of 7 days at a temperature of 25 °C. The resultant conidia of strain JEF 350 were then suspended in a 0.03% siloxane solution (Silwet; Farm Hannong, Seoul, Korea), yielding a conidial concentration of 1.0×10^7 conidia/mL. Subsequently, this conidial suspension was introduced at a 1% (v/v) ratio into 50 mL of SSYP medium (comprising 2% soluble starch, 1% yeast extract, and 1% peptone) contained within a 250 mL Erlenmeyer flask (Kim et al. 2011), followed by a 7 day incubation period at 25 °C with agitation set at 180 rpm. Thereafter, the blastospores of strain JEF 350 were harvested for subsequent bioassay and RNA seq sample preparation. A colony of *T. palmi* was sourced from the National Institute of Agricultural Science in Korea and maintained in ventilated acrylic cages (20 × 20 × 30 cm) under controlled conditions of 25 ± 2 °C temperature, a relative humidity (RH) of 40 ± 10%, and a photoperiod of 14:10 (L:D). Cucumber plants (Green Heart Bio, Co., Yeoju, Korea) possessing a minimum of four leaves were provided as sustenance for the thrips.

Bioassay against melon *thrips*

To assess the insecticidal efficacy of *B. bassiana* JEF 350 blastospores against melon thrips, the hyphae were eliminated from the liquid culture medium via filtration using sterilized gauze (Kim et al. 2008 and 2013). Subsequently, the culture medium underwent centrifugation at 10,000 rpm for 5 min within a 50 mL conical tube (SPL, Pocheon, Korea), and the resultant supernatant was discarded. The resulting pellet was then reconstituted in a 0.03% siloxane solution to generate a blastospore suspension with a concentration of 1.0×10^7 blastospores/mL. A cucumber leaf disc, measuring 60 mm in diameter, was immersed in the blastospore suspension for a duration of 10 s within a 50 mL conical tube, with this procedure repeated four times to ensure comprehensive coverage by the suspension. Each treatment was replicated thrice, with three leaf discs per treatment. Following a 20 min air drying period at ambient temperature, the leaf discs were arranged on nitrocellulose membranes within Petri dishes containing 10 mL of solidified 1.5% agar. Leaves treated solely with the siloxane solution were employed as untreated controls. Subsequently, each leaf disc was infested with approximately 20 adult thrips and maintained at a temperature of 25 °C. The survival and mortality of adult thrips were recorded at 24-h intervals over a span of 7 days.

RNA extraction and Illumina sequencing

For RNA extraction, samples of infected melon thrips adults were obtained 2 and 4 days post-fungal treatment, respectively, while adults treated with a 0.03% siloxane solution served as experimental control. The thrips were continuously exposed to the fungus treated leaf discs for 2 or 4 days. Employing a similar bioassay methodology, infected adult samples were acquired for RNA extraction. However, for RNA seq sample preparation, 90 mm cucumber leaves treated with the fungal suspension were positioned onto a nitrocellulose membrane atop 1.5% agar within a 90 × 15 mm Petri dish, following which a total of 400 melon thrips adults were subsequently transferred onto the leaves. RNA extraction was performed on adults treated with *B. bassiana* JEF 350 at both 2 and 4 days post treatment. For the untreated control in RNA seq analysis, RNA was extracted from non fungus treated adults 2 days post treatment. Each treatment was replicated three times during RNA seq sample preparation. Subsequently, for sequencing purposes, the 400 melon thrips adults from each treatment were transferred into a 1.5 mL microcentrifuge tube (SPL Life Sciences, Pocheon, Korea) filled with 400 µL Trizol reagent (Molecular Research Center Inc., Cincinnati, OH, USA) and homogenized using an Ultra grinder BTM (Taeshin Bio Science, Namyangju, Korea). Following the manufacturer's protocol, total RNA was extracted from the Trizol reagent for both fungus treated (at 2 and 4 days post treatment) and non treated adults. The integrity of the extracted RNA was evaluated using gel running and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Sequencing libraries were constructed using the TruSeq RNA kit (Illumina, San Diego, CA, USA) in accordance with the provided instructions. Illumina sequencing and cDNA library preparation were executed at Macrogen Corporation (Seoul, Korea). Initially, mRNA molecules containing poly-A were isolated using poly-T oligo attached magnetic beads and subsequently fragmented under elevated temperatures in the presence of divalent cations. These RNA fragments were converted into first-strand cDNA via reverse transcription

using random primers, followed by second-strand cDNA synthesis with DNA polymerase I and RNase H. Following an end-repair process with a single dNTP, sequencing adapters were ligated. The final cDNA library was generated through product purification and PCR enrichment. All samples were sequenced using an Illumina HiSeq2000 sequencer (Illumina, San Diego, CA, USA), thereby yielding high-throughput transcriptome sequence data with an average read length of 151 bp.

In silico *cDNA library and DEG analysis*

The quality assessment of sequenced short reads obtained from both non-infected and infected thrips was conducted using FastQC ver.0.12.0, following which low-quality sequences were filtered out utilizing the fastp program ver.0.23.4 (Chen et al. 2018). Subsequently, the filtered reads underwent processing with the Trinity program ver.2.15.1 (Haas et al. 2013) for de novo assembly, with a minimum sequence size threshold set at 150. The assembled contigs were then employed for predicting coding sequences utilizing the TransDecoder program ver.5.7.1 (https://github.com/TransDecoder/TransDecoder), employing the single-best-only option. In each treatment group, the trimmed reads obtained from melon thrips adults were aligned to the assembled contigs through an alignment procedure utilizing Bowtie2 ver.2.5.3 (Langmead and Salzberg 2012). Read counts, FPKM (fragments per kilobase million) and TPM (transcripts per million) values were determined using the RSEM program ver.1.1.11 and Kallisto (Li and Dewey 2011). Subsequently, a differential gene expression analysis was conducted based on the expression levels of thrips contigs. The EdgeR package ver.4.0.16 (Robinson et al. 2010) was employed for analyzing DEGs, considering false discovery rate (FDR) values < 0.05 and fold change ($|FC| > 2$ in each treatment.

Functional annotation

The functions of DEGs that displayed significant alterations in gene expression were examined by comparing non-treated thrips with infected thrips at 2 and 4 days post-fungal treatment. The analysis of DEGs encompassed Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and GO enrichment analytical tools. For GO analysis, the Blast2GO program (v.6.0.2) was utilized with the Interpro feature to annotate the DEG set, leveraging the EMBL-EBI database. Annotated genes were then classified into GO terms associated with biological processes, cellular components, and molecular functions. Regarding KEGG pathway annotations of the DEGs, the KAAS analysis platform (https://www.genome.jp/tools/kaas/) was employed. GO and KEGG groups exhibiting variations in fold change were subjected to GO enrichment analysis via the g:profiler (http://biit.cs.ut.ee/gprofiler/). Subsequently, the expression patterns of enriched GO and KEGG groups were analyzed using the edgeR package, and genes belonging to enriched pathways underwent heatmap analysis.

Results

Insecticidal activity of B. bassiana JEF-350 blastospores

Upon exposure to cucumber leaves treated with *B. bassiana* JEF-350, the mortality rate of melon thrips adults increased progressively over time. Specifically, mortality was recorded at 6.25% after 2 days post-fungal treatment, which escalated to 43.8% after 4 days, ultimately reaching 88.75% after 5 days (Fig. 1a). Median lethal time (LT_{50}) of JEF-350 was 3.78 days (95% confidence level: 3.28 ~ 4.39) from a probit analysis. Notably, fungal pathogenesis induced mycosis in treated melon thrips adults by 6 days post-treatment (Fig. 1b). Observations revealed no significant morphological alterations in treated adults up to day 2. However, by day 4 post-treatment, deceased insects exhibited a reddish tone in their bodies. By day 6, white fungal mycelium and conidia enveloped the surface of cadavers, conclusively confirming mortality attributed to *B. bassiana* JEF-350. In contrast, melon thrips adults in the non-treated control group exhibited a mortality rate of less than 20% by day 5 post-treatment.

Sequencing and de novo *assembly*

In Supplementary Table S1, the summary of short read sequences obtained from both non-treated thrips and fungus-treated thrips, sampled at 2 (day-2 thrips) and 4 (day-4 thrips) days post-treatment, was presented. Upon analyzing all treatment replicates, a total of 3.57 ± 0.8 Mb per replicate was sequenced. Following the filtering process, the phred quality score of 30 (Q30) for sequencing reads increased from $94.5 \pm 0.2\%$ to $95.7 \pm 0.3\%$, indicating high accuracy of the original sequencing data. The total number of assembled coding sequences from all treatments amounted to 19,143 genes, encompassing 46,847 isoforms. Contig lengths ranged from 255 to 18,825 bp, with an N50 value of 1307 bp. Additionally, the GC content of the assembled sequences was calculated to be 51.29%.

DEGs analysis

In the comparative analysis of gene expression, a notable shift was observed in day-4 thrips compared to non-treated thrips, while no significant change was detected between

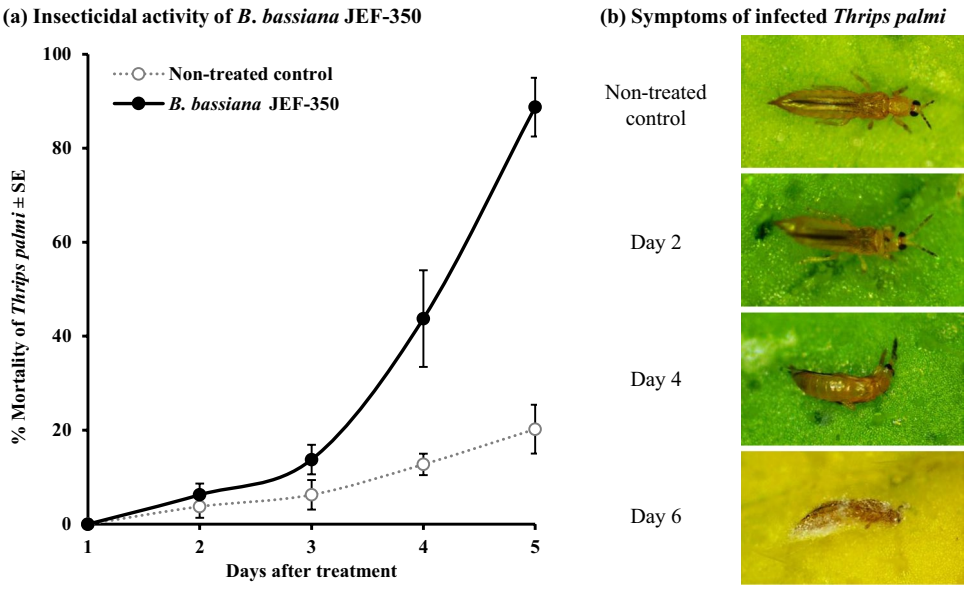


Fig. 1 Insecticidal activity of *Beauveria bassiana* JEF-350 against *Thrips palmi* adults. The insecticidal activity of *B. bassiana* JEF-350 was assessed by treating cucumber leaves with a blastospore suspension of *B. bassiana* JEF-350 using the dipping method and then transferring *T. palmi* adults on the treated leaves. The *T. palmi* adults were maintained at a temperature of 25 ± 2 °C, and the mortality was calculated by counting alive and dead *T. palmi* adults at 24-h intervals for 5 days. Non-treated control *T. palmi* adults were treated with 0.03% siloxane solution. **a** Mortality of *T. palmi* adults by *B. bassiana* JEF-350 treatment and **b** symptoms of *T. palmi* adults induced by a fungal infection

day-2 thrips and non-treated thrips (Fig. 2). Specifically, when evaluating gene expression levels (Log_2FC) of fungus-treated thrips against the non-treated control, only four genes exhibited significant changes in day-2 thrips, whereas day-4 thrips displayed significant alterations in gene expression (Fig. 2a, b). Interestingly, day-2 thrips demonstrated a similar pattern of DEGs to day-4 thrips. In further detail, a total of 1141 genes in day-4 thrips were downregulated compared to the non-treated thrips. Additionally, when compared to day-2 thrips, day-4 thrips exhibited downregulation in 1070 genes. Conversely, a total of 142 genes in day-4 thrips were upregulated in comparison to the non-treated control, with 132 genes showing upregulation compared to day-2 thrips. Furthermore, regarding DEGs from JEF-350-treated thrips compared to the non-treated control, 81 genes were consistently upregulated in both day-2 and day-4 thrips, while 935 genes were consistently downregulated at both time points (Fig. 2c). Hierarchical clustering analysis illustrated that DEGs of day-4 thrips were significantly distinct from those of the non-treated control and day-2 thrips (Fig. 2d).

Functional analysis

The enrichment analysis of DEGs revealed a significantly larger number of downregulated pathways when comparing DEGs of day-4 thrips with those of day-2 thrips and the non-treated control (Fig. 3). Notably, lysosome and metabolic pathways, including tyrosine, retinol, and glycerolipid metabolism pathways, as well as the insect hormone pathway, exhibited notable downregulation when comparing day-4 thrips with both the non-treated control (Fig. 3a) and day-2 thrips (Fig. 3b). Conversely, relatively fewer pathways were upregulated, such as glycine-serine-threonine metabolism, biosynthesis of amino acids, Toll and Imd signaling, circadian rhythm-fly, and sulfur and purine metabolisms (Fig. 3a, b). In day-4 thrips, a larger number of functional GO terms were downregulated compared to day-2 thrips and the non-treated control (Fig. 4). Although the serine metabolic process and response to external stimuli were upregulated, many GO terms were downregulated, including cellular organism development, lipid metabolic process, membrane transport, response to stimuli, and homeostatic process (Fig. 4a, c). Particularly, extracellular region and plasma membrane structures were significantly up and downregulated, respectively (Fig. 4b). Highly enriched pathways were further analyzed in the subsequent heatmaps.

Glycine, serine, threonine (GST) pathway

The enriched pathway of glutathione *S*-transferase (GST) metabolism was meticulously analyzed using a TPM-based heatmap (Fig. 5a). Within this pathway, most of the upregulated genes were associated with serine metabolism. Additionally, several other genes exhibited upregulation in day-2 thrips but displayed a gradual downregulation in day-4 thrips. These temporally dependent up and downregulated

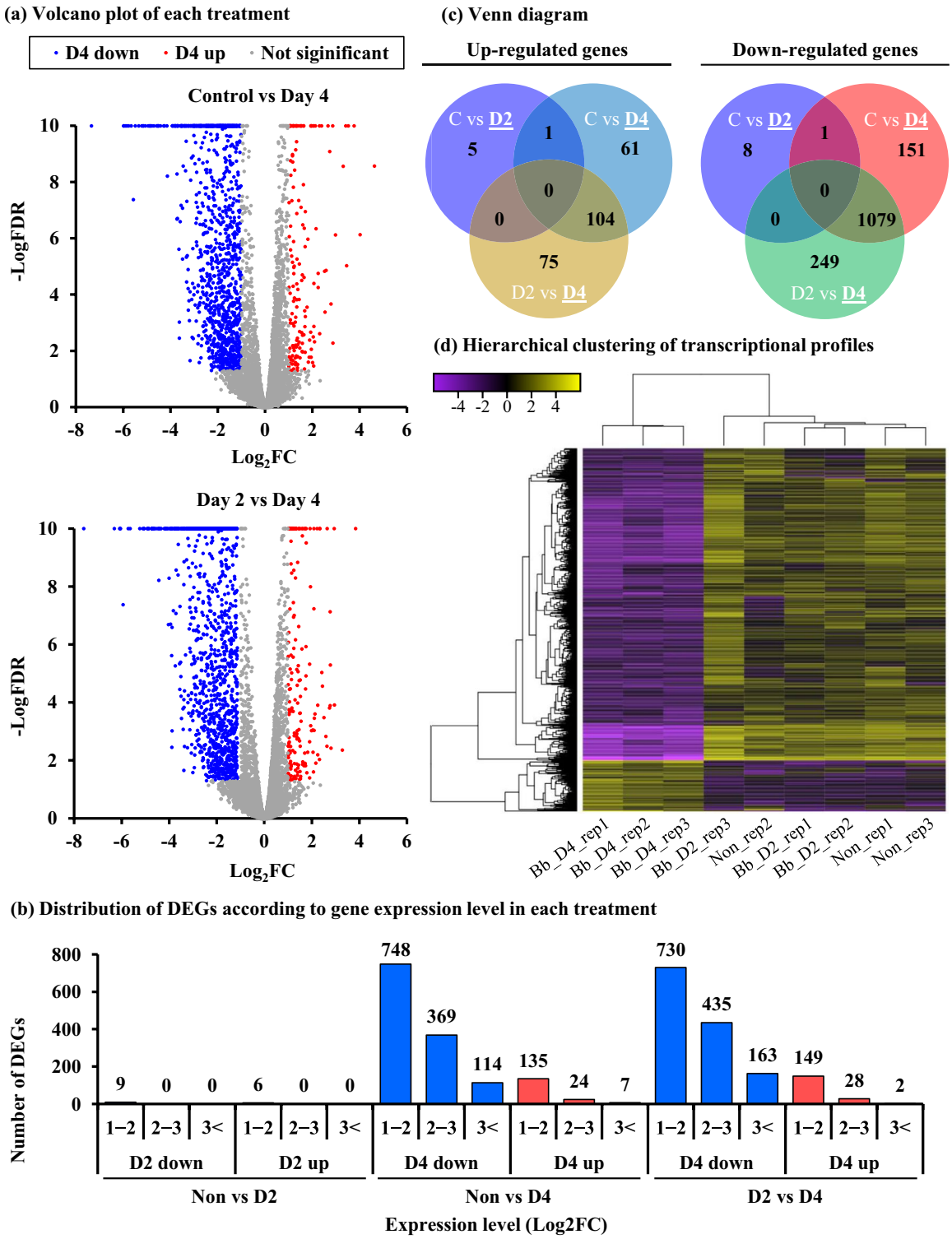


Fig. 2 Distribution of differentially expressed genes in *T. palmi* adults according to *B. bassiana* JEF-350 infection based on fold change values (FDR < 0.05). **a** Volcano plot that analyzed each of the three treatments one-to-one; **b** distribution according to the gene expression level of DEGs in each treatment; **c** Venn diagram of upregulated and downregulated genes in the three treatments; **d** hierarchical clustering of genes from each treatment (fold change > 2 and FDR < 0.05). In the comparison of Non vs D2, Non vs D4, and D2 vs D4, up and downregulation were determined based on D2, D4, and D4, respectively. Non, non-treated control; D2, day 2 after *B. bassiana* JEF-350 treatment; D4, day 4 after *B. bassiana* JEF-350 treatment

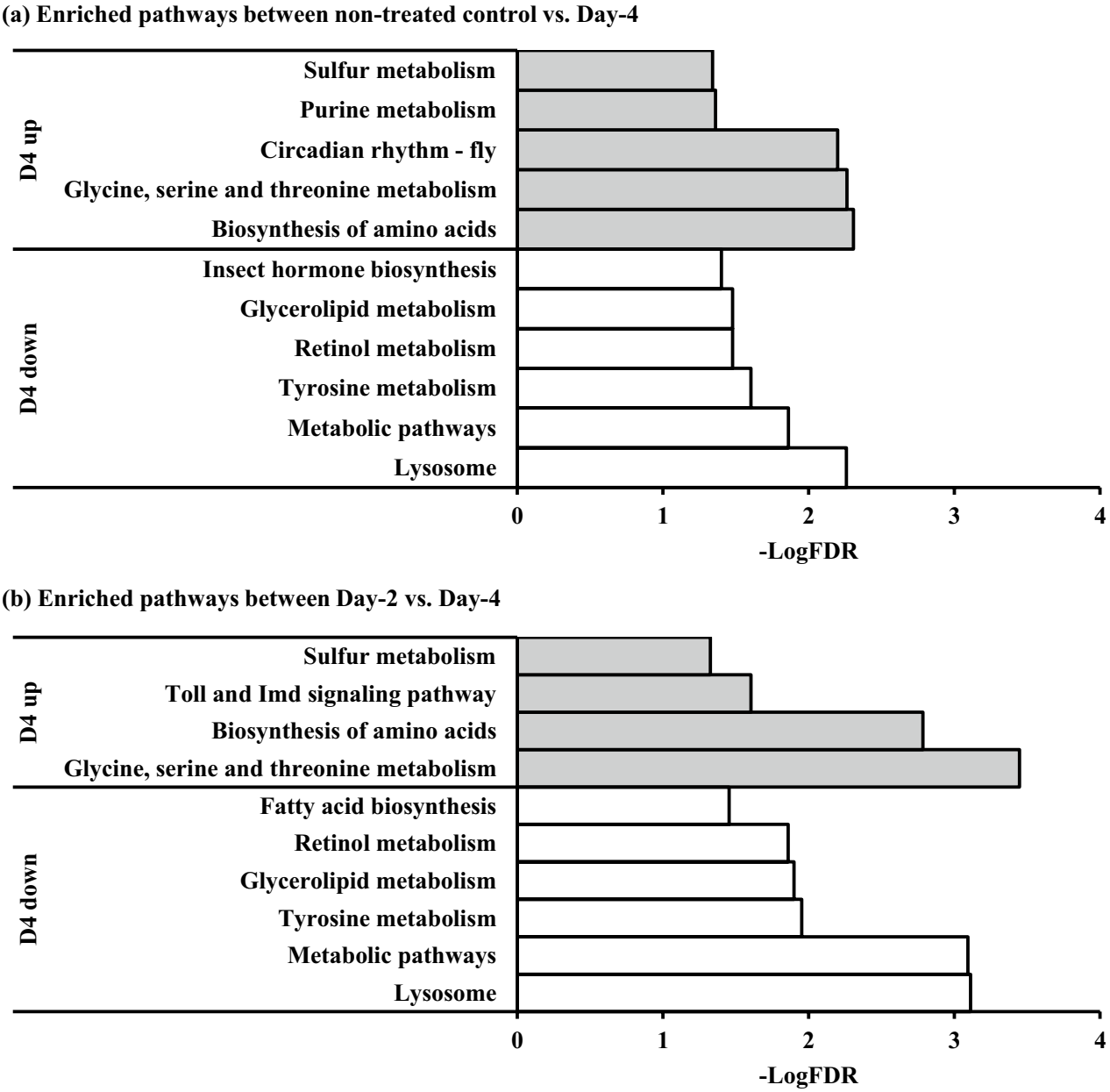


Fig. 3 GO enrichment analysis based on the upregulated and downregulated genes in three treatment groups. **a** KEGG pathways significantly affected by genes in aphids treated with fungi on day 4 compared to those on non-treated control; **b** KEGG pathways significantly affected in aphids treated with fungi on day 4 compared to those on day 2

genes were primarily linked to serine and glycine metabolism. Furthermore, three genes exhibited a gradual downregulation pattern in both day-2 and day-4 thrips, and these genes were implicated in serine and glycine metabolism pathways.

Toll and Imd signaling pathway

The genes associated with the enriched Toll and Imd pathways were analyzed using a heatmap (Fig. 5b). Approximately half of the genes exhibited a consistent upregulation pattern. Among these upregulated genes, some were involved in the Toll signaling pathway, including GNBP1, PSH, Tube, spaetzle, Dl/Dif, and TrCP, while the remaining genes participated in the Imd pathway, encompassing processes such as ubiquitin-mediated proteolysis, apoptosis, and MARK signaling, ultimately leading to the activation of the defense response. Conversely, three genes, namely for caspase, GNBP, and spaetzle, displayed upregulation in day-2 thrips but experienced a

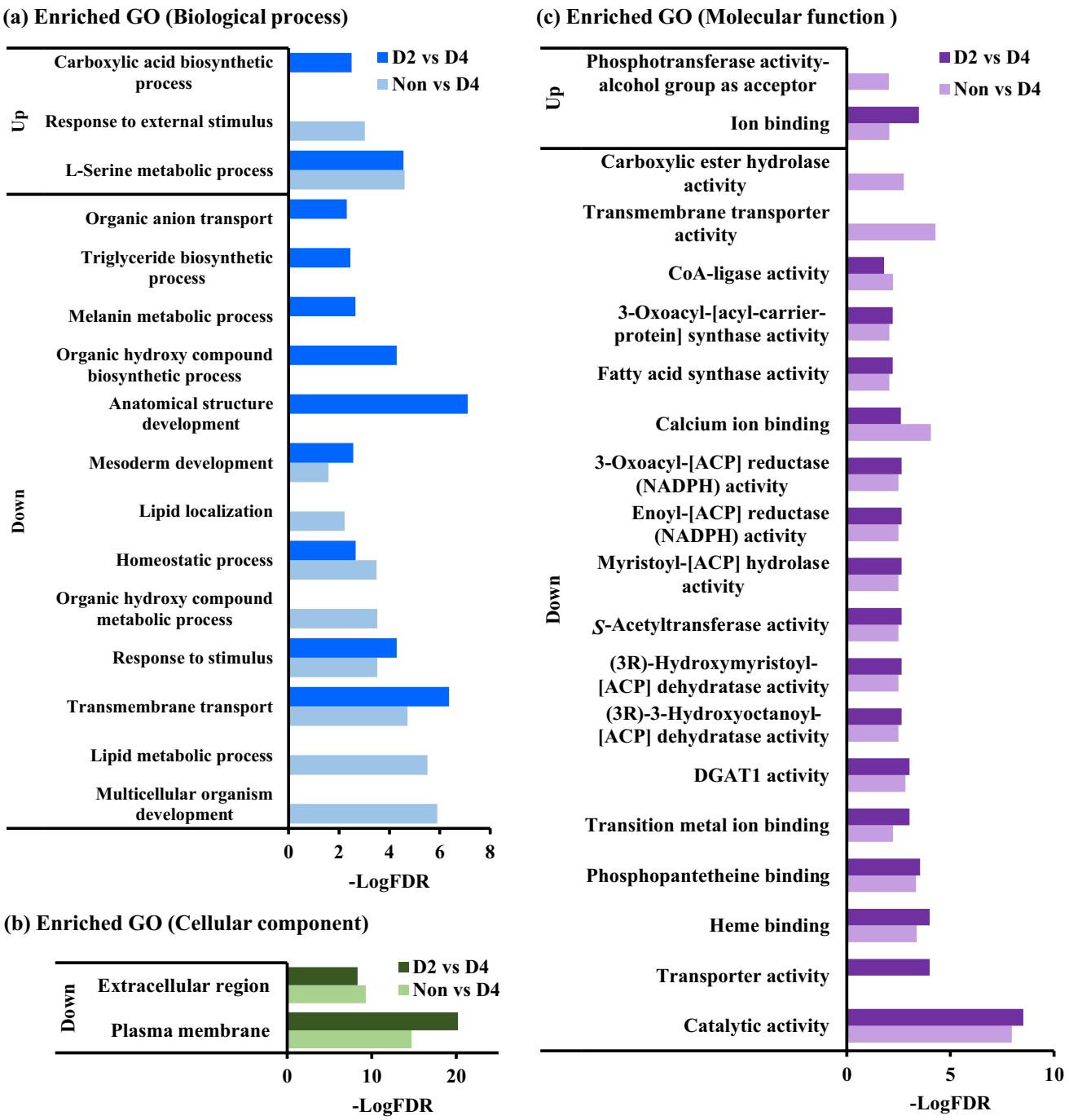


Fig. 4 GO enrichment analysis based on the upregulated and downregulated genes in three treatment groups. **a** GO term annotated to biological process significantly affected by genes in aphids treated with fungi on day 4 compared to those on day 2 of treatment and non-treatment; **b** GO term annotated to cellular component; **c** GO term annotated to molecular function

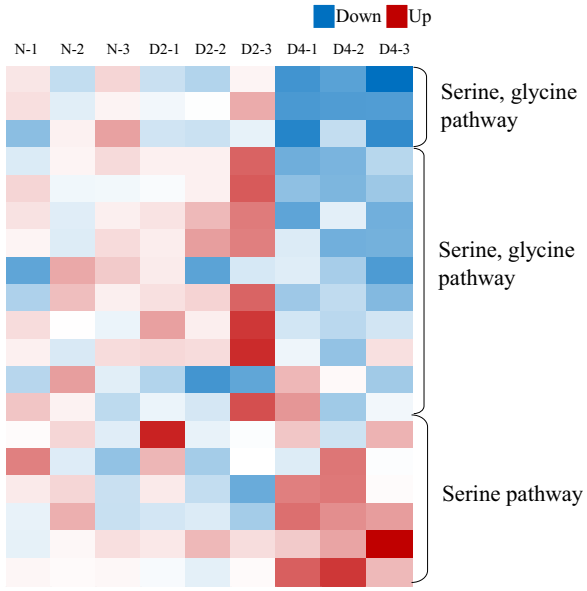
significant downregulation in day-4 thrips. The remaining genes exhibited a gradual downregulation pattern. These downregulated genes encoding ModSP, Toll, and Cactus play roles within the Toll pathway, as well as the genes encoding DUOX, PGRP-LC, P38, JNK, and Jra/Kay in the Imd pathway.

Circadian rhythm-fly pathway

The enriched pathway encompassing the circadian rhythm-fly pathway was depicted in a heatmap (Fig. 5c). Within this pathway, three genes, encoding ataxin (ATX2), period circadian protein (PER), and glycogen synthase kinase (Sgg),

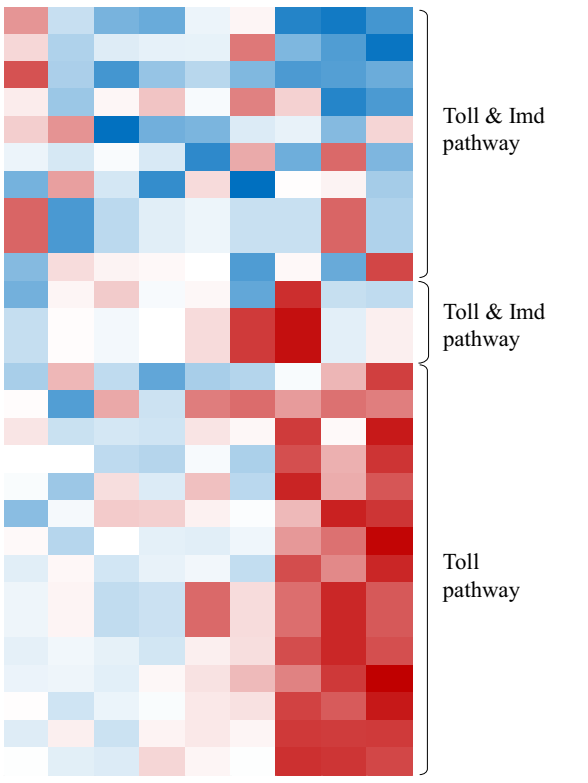
(a) Glycine, serine and threonine pathway

KEEG	Enzyme	Gene function
K01758	4.4.1.1	Cystathionine gamma-lyase
K14085	1.2.1.8	Aldehyde dehydrogenase
K10754	4.3.1.19	Theronine dehydratase
K00827	2.6.1.45	Alanine-glyoxylate transminase
K00552	2.1.1.20	Glycine *N*-methyltransferase
K00281	1.4.4.2	Glycine dehydrogenase
K15789	1.1.1.103	Theronine 3-hydrogenase
K01697	4.2.1.22	Cystathionine beta-synthase
K00830	2.6.1.44	Serine-glyoxylate transaminase
K00605	2.1.2.10	Aminomethyltransferase
K00314	1.5.8.3	Sarcosine dehydrogenase
K00049	1.1.1.81	Glyoxylate/hydroxypyruvate reductase
K00058	1.1.1.95	D-3-Phosphoglycerate dehydrogenase
K00831	2.6.1.52	Phosphoserine aminotransferase
K11529	2.7.1.165	Glycerate 2-kinase
K00600	2.1.2.1	Glycine hydroxymethyltransferase
K01834	5.4.2.11	Phosphoglycerate mutase
K00643	2.3.1.37	5-Aminolevulinate synthase
K01079	3.1.3.3	Phosphoserine phosphate



(b) Toll & IMD pathway

KEEG	Protein	Gene function
K13411	DUOX	Dual oxidase
K24075	FGRP-LC	Peptidoglycan-recognition protein LC
K09031	Kay	Fos-like antigen, invertebrate
K04734	Cactus	NF-kappa-B inhibitor alpha
K04448	Jra	Transcription factor AP-1
K04440	JNK	Mitogen-activated protein kinase
K04441	P38	p38 MAP kinase
K20674	ModSP	Modular serine protease
K18809	Toll	Protein Toll
K04730	Pelle	Interleukin-receptor-associated kinase
K04398	DREDD	Caspase 8
K20697	GNBP1	GNBP 1
K20694	Spz	Protein spaetzle
K04432	MKK3	MAPK kinase 3
K04431	JNKK	MAPK kinase 7
K20700	Kenny	NF-kappa-B essential modulator
K04427	Tak1	MAPK kinase 7
K07209	Ird5	Inhibitor of nuclear factor
K03362	TrCP	F-box and WD-40 domain protein
K04428	MEKK1	MAPK kinase kinase 4
K20703	Caspar	FAS-associated factor 1
K20671	PSH	Serine protease presephone
K04733	Tube	Interleukin-receptor-associated kinase 4
K02580	Relish	Nuclear factor NF-kappa-B p105 subunit
K09254	DI/Dif	c-Rel proto-oncogene protein
K04450	ATF2	cAMP-dependent transcription factor
K16060	Iap2	Baculoviral IAP repeat-containing protein
K06689	Effete	Ubiquitin-conjugating enzyme E2 D



(c) Circadian rhythm-fly pathway

KEEG	Protein	Gene function
K23625	ATX2	Ataxin 2
K02633	PER	Period circadian protein 2
K03083	Sgg	Glycogen synthase kinase 3 beta
K02296	CYC	Cyclic circadian protein 1

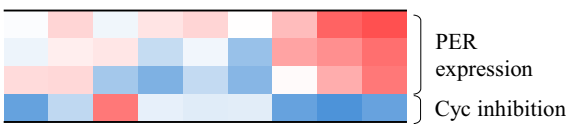


Fig. 5 Heatmap of enriched **a** glycerin-serine-threonine, **b** Toll and Imd, and **c** Circadian rhythm-fly pathways. Compared to the non-treated control, up and downregulated genes were Z-normalized and marked red and blue, respectively

exhibited significant upregulation. Conversely, one gene, for cyclic circadian protein (CYC), was notably downregulated in day-4 thrips.

Lysosome pathway

The enriched lysosome pathway, characterized by significant downregulation, was analyzed using a heatmap (Fig. 6a). Numerous genes within this pathway displayed downregulation in day-4 thrips, including the functions of GBA, LAMAN, LIPA, MANB, a sialin encoding gene, genes for Cathepsins, LIMP, ATPeV, HYAL1, GLB, ARS, LITAF, NPC, CLN1, CLN7, IDUA, and HGSNAT. These genes are primarily involved in the production of lysosomal hydrolases. Concurrently, approximately half of the pathway genes exhibited upregulation in day-4 thrips, which is involved in the transport of mannose-6-phosphate (M6P) from the Golgi body to late endosomes and lysosomal structure and functioning.

Insect hormone pathway

The regulation of enriched insect hormone pathway genes was analyzed (Fig. 6b). Genes associated with juvenile and ecdysone hormone biosynthesis were primarily downregulated. Additionally, other genes exhibited time-dependent up and downregulations. Furthermore, genes for cytochrome P450 and juvenile hormone-III synthase displayed upregulation in day-2 thrips but experienced a dramatic downregulation in day-4 thrips.

Gene expression flow of enriched pathways

In each enriched pathway, gene expression patterns and flows were separately analyzed in terms of time-based up and downregulation (Fig. 7). The enriched glycerin-serine-threonine pathway map underscores the prominence of serine and glycine biosynthesis as key metabolic processes against fungal infection (Fig. 7a). In the Toll and Imd signaling pathway, overall one-third of genes were downregulated, but half of the genes remained actively transcribed. Particularly on day 4, actively transcribed defense genes were predominantly located inside the cells rather than on the cell surface (Fig. 7b). The lysosomal pathway map shows that enzyme production and transports were highly activated in Golgi body and early and late endosomes, but many lysosomal hydrolase genes were suppressed in day-4 thrips, which is involved in the main lysosomal ability to degrade fungal propagules and fungal-releasing substances (Fig. 7c). Lastly insect hormone pathway map analysis reveals that overall juvenile hormone biosynthesis was suppressed during fungal pathogenesis, as evidenced by the downregulation of key enzymes involved in this process (Fig. 7d).

Discussion

This study offers valuable transcriptional insights into the response of thrips to early-stage infection by *B. bassiana* JEF-350, with a particular focus on the upregulated pathways of glycine/serine/threonine, Toll and Imd, and circadian rhythm, alongside the downregulated pathways of lysosome and insect hormone biosynthesis. Similarly, upregulated immune response was reported in *Ectropis obliqua* moth when infected by *B. bassiana* (Long et al. 2022). This time-based analysis allows for a clearer delineation of the dynamic interplay between the fungal pathogen and the host thrips. However, as widely known, there is a limitation of RNA-seq because this analysis needs to be further connected to proteomics, metabolomics, and specific biological assays.

The enrichment analysis of infected thrips revealed active transcription of pathways such as glycine and serine, Toll and Imd, and circadian rhythm-fly, which are likely mobilized as survival mechanisms against fungal pathogenesis. However, in comparison to the upregulated pathways, a considerable number of pathways were notably suppressed. These include lysosome and metabolic pathways, tyrosine, glycerolipid, and retinol pathways, fatty acid biosynthesis, and insect hormone biosynthesis pathways. These suppressed pathways play crucial roles in various metabolic processes, including cell proliferation, development, growth, and cellular homeostasis. Of particular interest is the potential interaction between tyrosine, retinol, and insect hormone biosynthesis pathways, which could be involved in cuticle structuring and ecdysis, as suggested by previous research (Arakane et al. 2016; Gorman et al. 2007; Němec et al. 1993; Xu et al. 2024). Additionally, it is noteworthy that some genes within the Toll and Imd pathways were also downregulated in day-4 thrips, indicating a complex regulatory response to a fungal infection that involves both up and downregulation of key pathway components. This multifaceted transcriptional response highlights the dynamic interplay between the host thrips and the fungal pathogen, underscoring the complexity of host–pathogen interactions in the context of insect-fungal interactions.

In further detail regarding the downregulated pathways, the lysosome, responsible for the digestion and degradation of toxic substances or hazardous pathogens, is likely inhibited by fungal infection in this study (Rohrer and Kornfeld 2001; Terra et al. 2018). This inhibition suggests a disruption in the thrips' ability to effectively degrade and eliminate harmful materials. The metabolic pathways, encompassing carbohydrate, energy production, lipid, nucleotide, glycan, and cofactors and vitamin metabolisms, were significantly downregulated. This

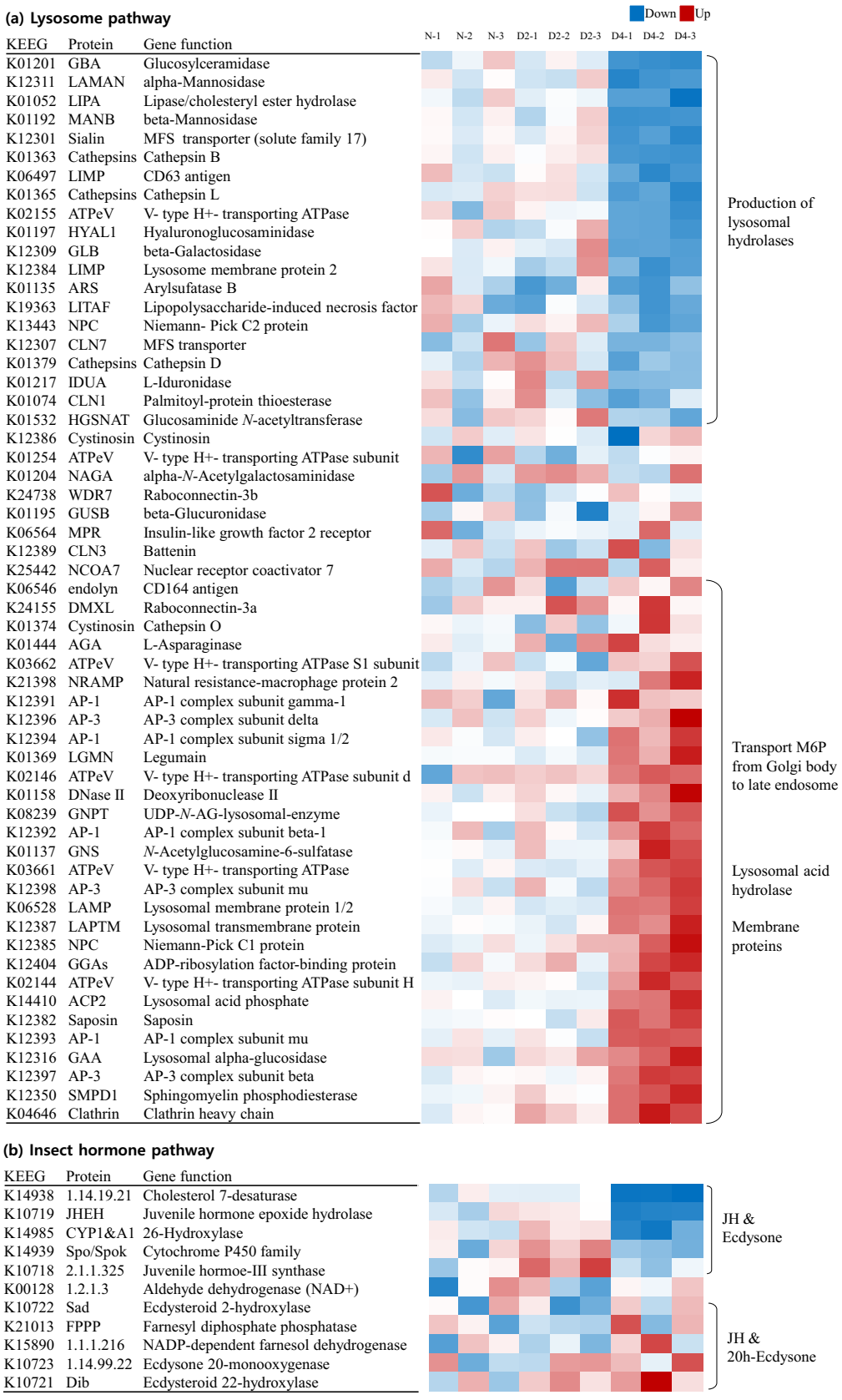


Fig. 6 Heatmap of enriched **a** lysosome and **b** insect hormone pathways. Compared to the non-treated control, up and downregulated genes were *Z*-normalized and marked red and blue, respectively

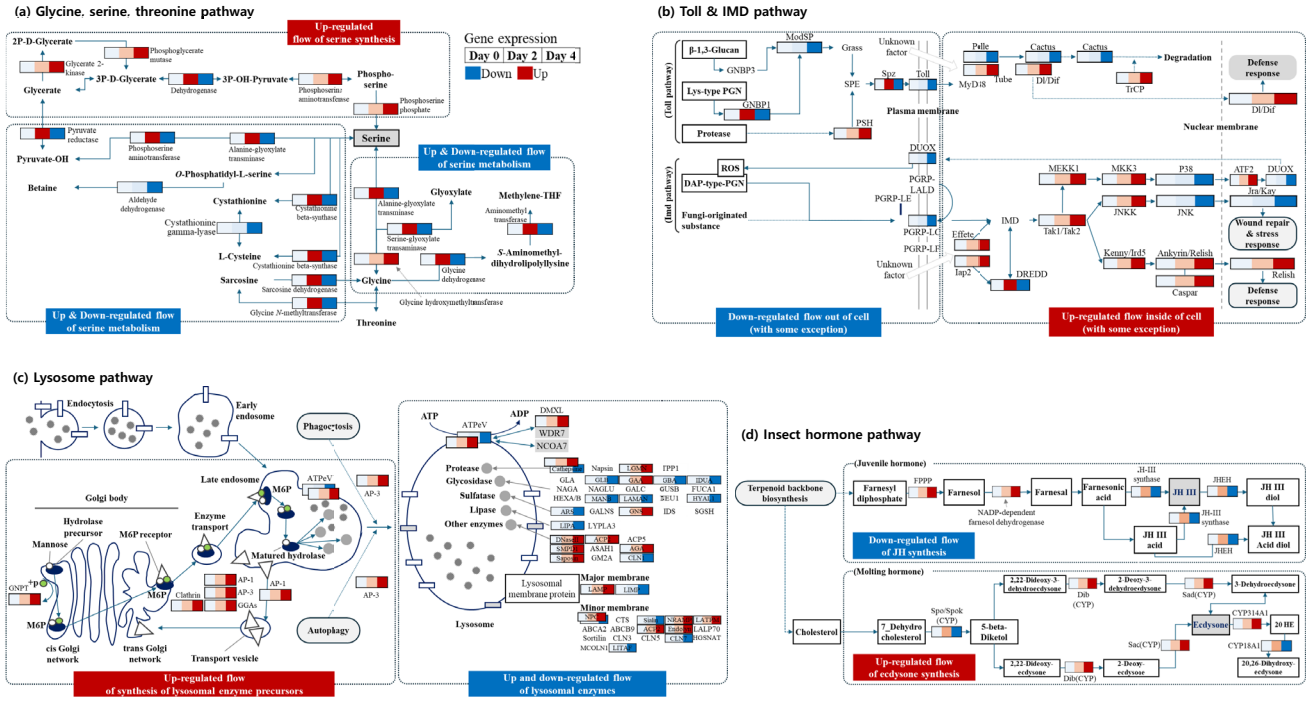


Fig. 7 KEGG maps of enriched **a** glycerin-serine-threonine, **b** Toll and Imd, **c** lysosome, and **d** insect hormone pathways. In each enriched pathway, gene expression patterns were presented in terms of time-based up and downregulation with marking blue (up) and red (down). Time-based gene expression patterns were generated using TPM-based DEG analysis data

broad suppression indicates extensive damage to metabolic processes induced by JEF-350 infection, affecting various essential cellular functions. Tyrosine metabolism, involved in the catabolism and transformation of tyrosine to produce biologically essential products, including dopamine and melanin, crucial for cuticle formation and defense mechanisms in insects, showed downregulation. This suggests a disruption in the homeostatic molting system and melanization-mediated defense mechanisms, potentially weakening the thrips' ability to cope with the fungal infection. Similarly, the downregulation of glycerolipid metabolism, essential for structural and functional roles in insect membranes, indicates the potential breakdown of cellular structures due to JEF-350 infection. This corresponds to the downregulation of plasma membrane-related functions observed in the enrichment analysis. Retinol metabolism, particularly the downregulation of all-trans-retinol, structurally similar to insect juvenile hormone, may disrupt normal thrips molting and metamorphosis processes, further exacerbating the impact of fungal infection on thrips development. Furthermore, the downregulation of fatty acid metabolism, critical for energy production, membrane structuring, and various metabolic and signaling processes, could adversely affect thrips' homeostasis and overall cellular functions (Madariaga et al. 1974; Stanley-Samuelson et al. 1988). Insect hormone pathways, necessary for molting and growth regulation in thrips, appear to be suppressed in response to fungal infection. This suggests a strategic shift in thrips' priorities towards survival mechanisms, such as activating immune-related pathways, rather than investing resources in growth and development under the risky conditions imposed by JEF-350 infection.

The biosynthesis of amino acids, particularly glycine-serine-threonine (GST) metabolism, emerged as a significantly enriched pathway with upregulation in the transcriptional analysis. This pathway exhibited a consistent upregulation pattern, mirrored by the upregulation of serine-related GO terms. While some genes within this pathway were downregulated in day-4 thrips, a notable proportion displayed upregulation in both day-2 and day-4 thrips, as evidenced by the heatmap analysis (Fig. 7a). Serine, a key component of GST metabolism, participates in various cellular functions, including protein biosynthesis, neurotransmission, folate/methionine cycle, phospholipid biosynthesis, and synthesis of sulfur-containing amino acids like methionine (Ducker and Rabinowitz 2017). Additionally, serine can be catalyzed to glycine by serine hydroxyl methyl transferase. In the process of protein activation, phosphate binds to the serine or threonine residues of inactive proteins by protein kinases (Wang et al. 2002; Ahmad et al. 2022). Hence, the active biosynthesis of serine is crucial for the activation

of proteins, which may be essential for the physiological growth and homeostasis of thrips in response to external stresses such as fungal infection. This upregulation of GST metabolism and associated serine biosynthesis suggests a concerted effort by the thrips to bolster their cellular processes, potentially enhancing their resilience and adaptive responses to the challenges posed by fungal infection. By investing resources in the production of serine and its downstream metabolites, thrips may be fortifying their molecular machinery to better cope with the stressors encountered during infection, ultimately contributing to their overall survival and defense mechanisms.

The analysis of the GST pathway revealed intriguing dynamics within the serine metabolism pathway in response to fungal infection in melon thrips. In day-4 thrips, a significant proportion of upregulated genes was associated with serine metabolism, including the genes encoding glycerate 2-kinase, glycine hydroxymethyl transferase, phosphoglycerate mutase, amino levulinate synthase, and phosphoserine phosphate. This upregulation suggests a concerted effort to enhance serine biosynthesis, potentially to support various cellular functions crucial for defense and homeostasis against fungal infection. Interestingly, some genes were upregulated in day-2 thrips but downregulated in day-4 thrips, primarily within the serine and glycine metabolism pathways. These genes, such as for serine-glyoxylate transaminase, glycine methyl transferase, glycine dehydrogenase, and others involved in hydrogenase and reductase activities, exhibit a dynamic regulation pattern that may reflect shifting metabolic priorities or regulatory mechanisms in response to the progression of fungal infection. Notably, genes associated with threonine metabolism were not clearly detected in this analysis, suggesting a potential focus on serine and glycine biosynthesis pathways in the thrips' response to fungal infection. Overall, the active biosynthesis of serine observed in both day-2 and day-4 thrips suggests a crucial role in maintaining thrips homeostasis and defense mechanisms against fungal infection. However, the differential regulation of specific genes within the serine metabolism pathway highlights the dynamic nature of thrips' responses to fungal infection, with potential temporal shifts in metabolic strategies or regulatory mechanisms to optimize survival and resilience in the face of evolving pathogenic challenges.

The Toll and Imd signaling pathway represents a fundamental defense mechanism in insects against fungal infections (Kim et al. 2017). Analysis of the heatmap revealed that half of the genes within this pathway were timely upregulated in both day-2 and day-4 thrips. These upregulated genes include those for several MAPK kinases, NF-kappa-B essential modulator, serine protease Persephone, cAMP-dependent transcription factor, and ubiquitin-conjugating enzyme. Some of these upregulated genes play crucial roles in the Toll signaling pathway, such as genes encoding GNBP1, PSH, Tube, spaetzle, Dl/Dif, and TrCP, while others are involved in the Imd pathway, including ubiquitin-mediated proteolysis, apoptosis, and MARK signaling, ultimately triggering a defense response in thrips. Notably, certain genes exhibited differential regulation between day-2 and day-4 thrips. For instance, genes encoding caspase 8, GNBP 1, and spaetzle were upregulated in day-2 thrips but dramatically downregulated in day-4 thrips. Caspase 8 is involved in the Imd pathway, while GNBP and spaetzle are associated with the Toll pathway (Fukuyama et al. 2013). The gradual downregulation of other genes in both day-2 and day-4 thrips suggests a potential suppression of the Toll and Imd signaling pathway as the fungal pathogenesis progresses. Considering that day-4 thrips exhibited around 30–40% mortality, it is plausible that further progression of the fungal infection may lead to more significant suppression of the Toll and Imd signaling pathway. This defense mechanism against fungal invasion appears to be intricately connected to various metabolic pathways previously described, including tyrosine, glycerolipid, retinol, and fatty acid metabolism. Therefore, a comprehensive understanding of the next-generation sequencing (NGS) data is crucial, integrating biological insights to decipher the complex interplay between thrips defense mechanisms and fungal pathogenesis.

Against the fungal infection, in day-4 thrips, it was observed that actively transcribed defense genes were predominantly located inside the cells rather than on the cell surface. Conversely, genes associated with this pathway that are typically located on the cell surface or outside of cells were significantly downregulated. The infecting fungus, *B. bassiana* JEF-350, appears to have targeted the signaling pathway, particularly affecting the signal reception process and receptors in day-4 thrips. Despite this suppression, the thrips' defense system remains active within the cells. It is plausible that as fungal pathogenesis becomes more active beyond day 4, the majority of genes in this pathway would be further suppressed, potentially leading to a breakdown of the defense system. This observation underscores the sophisticated strategies employed by the fungal pathogen to evade host defenses and highlights the dynamic interplay between the host immune response and fungal virulence factors. Further investigation into the mechanisms underlying this pathway suppression and its impact on thrips survival and fungal pathogenesis is warranted for a comprehensive understanding of host–pathogen interactions in this system.

In the circadian rhythm pathway, RNA sequencing of fungus-infected insects rarely provides information. However, in this analysis, three genes were upregulated. Ataxin 2 (ATX2) binds to twenty-four (TYF) protein and protein LSM12, forming a complex that activates the transcription of period circadian protein 2 (PER) (Lee et al. 2017).

Glycogen synthase kinase activates the transcription of the timeless gene (TIM) (Reynolds et al. 2022). In this upregulated pathway, the *PER* gene is actively transcribed in many steps of cascades, and the expressed PER protein ultimately binds to double-time protein (Dbt) and activates the transcription of cycle and clock genes. The overexpressed *PER* gene product negatively regulates the transcription of the *PER* gene. Against fungal infection, the interactive *ATX2* and *PER* genes were actively upregulated, potentially disrupting the biorhythm of thrips and causing abnormal cell growth and development. This pathway also regulates other cellular pathways, including cell proliferation, gene damage repair, homeostasis, and immune response (Kim and Edery 2006). Cyc is responsible for the negative feedback of overexpression of *CLK* (Hardin 2005). Thus, the downregulation of the *Cyc* gene could adversely influence the circadian rhythm. The acceleration of the circadian rhythm-fly pathway could be beneficial for the infected thrips to escape risky conditions, but conversely, it might induce abnormal behaviors or cell proliferation. This topic warrants further study, potentially utilizing the knockdown of the actively transcribed *PER* gene in the infected thrips for deeper insights.

Lysosomes are crucial organelles responsible for the degradation of hazardous materials and microorganisms within the cell (Jin et al. 2020). In the heatmap analysis, it is observed that several genes were upregulated, although relatively many genes were downregulated. The upregulated genes play essential roles in various processes related to lysosomal function, such as the transport of mannose-6-phosphate (M6P) from the Golgi body to the late endosome, the production of lysosomal acid hydrolases and membrane proteins, and maintaining lysosomal environment homeostasis (Coutinho et al. 2012). Lysosomal hydrolases, which are vital for the breakdown of substances within lysosomes, are synthesized in the Golgi apparatus and then transported to lysosomes in vesicles containing M6P (Terra et al. 2018). Several proteins, including clathrin light chain A, activator protein (AP) complex subunits, and ADP-ribosylation factor-binding protein GGA, are involved in the transport of M6P from the Golgi body to transport vesicles (Eissenberg et al. 2011). Once inside the lysosome, lysosomal hydrolases such as glucosidase, sulfatase, nuclease, phosphatase, esterase, and saposin work to degrade various substrates (Patel 1970). Additionally, lysosomal-associated membrane proteins and other membrane proteins such as Niemann-Pick C1, natural resistance-associated macrophage protein, lysosomal acid phosphatase, and CD164 antigen are essential for the structure and function of the lysosomal membrane (Vasanthakumar and Rubinstein 2020). V-type transporting ATPase generates ATP for lysosomal function, while rabconnectin-3A regulates lysosomal acidification to maintain an optimal pH within the lysosome (Tazeh et al. 2009). Interestingly, the infecting *B. bassiana* JEF-350 appears to suppress the transcription of lysosomal hydrolase genes while still allowing the active upregulation of genes involved in the production and transport of M6P. This suggests a sophisticated mechanism employed by the fungus to evade lysosomal degradation while still allowing essential cellular processes to continue. However, the suppression of lysosomal hydrolase gene transcription could severely impair lysosomal function, potentially compromising the cell's ability to degrade harmful substances and pathogens.

The insect hormone pathway plays a crucial role in regulating insect molting and development by controlling the transcription of genes involved in juvenile hormone and ecdysone biosynthesis (Dhadialla et al. 1998; Riddiford 2012). In the analysis, several genes were upregulated in day-4 thrips, including those for farnesyl diphosphate phosphatase, NADP-dependent farnesol dehydrogenase, ecdysone 20-monooxygenase, and ecdysteroid 22-hydroxylase. These genes are involved in juvenile hormone biosynthesis and the conversion of ecdysone into active forms such as epi-ecdysone and 20-hydroxyecdysone. Farnesyl diphosphate phosphatase and NADP-dependent farnesol dehydrogenase are enzymes responsible for the biosynthesis of juvenile hormone, while ecdysone 20-monooxygenase and ecdysteroid 22-hydroxylase are involved in the conversion of ecdysone into active forms (Riddiford 2012). However, other genes such as for cholesterol desaturase, juvenile hormone epoxide hydrolase, and 26-hydroxylase were highly downregulated in day-4 thrips. On the other hand, the upregulation of genes involved in the ecdysone sub-pathway indicates that 20-hydroxyecdysone synthesis may still be active. This dysregulation of hormone biosynthesis genes could potentially disrupt the normal physiological development or oviposition in infected thrips. However, further studies are needed to fully understand the impact of these gene expression changes on thrips physiology and behavior.

The hypothesis of cross-kingdom RNA interference provides an intriguing explanation for the simultaneous downregulation of multiple pathways in day-4 thrips infected with *B. bassiana* JEF-350. In this scenario, fungal-generated small RNAs could be released from infecting hyphae and subsequently bind to mRNA molecules produced by the thrips. These small RNAs might target genes involved in essential physiological processes, including metabolism, immune response, and stress tolerance, leading to their downregulation (Mahanty et al. 2023). By exploiting the host's RNA interference machinery, the fungus could effectively silence key genes involved in the thrips' response to infection, including those related to immune defense, stress tolerance, and metabolic pathways. This coordinated suppression of gene expression could help the fungus establish itself within the host and evade or overcome the host's defenses. Further research into the molecular mechanisms

underlying cross-kingdom RNA interference in fungal infections of insects could provide valuable insights into the dynamics of host–pathogen interactions and potentially lead to the development of novel strategies for pest control.

Indeed, the transcriptional analysis of *B. bassiana* JEF-350-infected thrips sheds light on the intricate dynamics of host–pathogen interactions at the molecular level. The upregulation of defense pathways in the early stages of infection reflects the thrips' attempts to mount a response against the invading fungus. However, the observed inhibition of gene transcription within these upregulated pathways suggests that the fungus has evolved mechanisms to counteract the host's defense responses. Moreover, the simultaneous downregulation of multiple homeostasis-related pathways in the infected thrips, particularly in day-4 samples, indicates a coordinated effort by the fungus to manipulate host physiology for its own benefit. The suppression of these pathways likely creates more favorable conditions for fungal proliferation and pathogenesis within the host. Further investigation into the specific mechanisms underlying the downregulation of host pathways, such as the role of fungal small RNAs, could provide valuable insights into the strategies employed by the fungus to subvert host defenses and promote infection. Additionally, exploring the functional significance of the downregulated pathways and their interconnections could uncover novel targets for intervention strategies aimed at disrupting fungal pathogenesis and enhancing insect resistance. Overall, RNA sequencing of JEF-350-infected thrips opens up avenues for deeper investigation into the molecular basis of fungal pathogenesis and insect defense, offering opportunities for the development of innovative approaches for pest management and crop protection.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author, Jae Su Kim, upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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