Transcriptome changes associated with *Tomato spotted wilt virus* infection in various life stages of its thrips vector, *Frankliniella fusca* (Hinds)

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**Abstract**

Persistent propagative viruses maintain intricate interactions with their arthropod vectors. In this study, we investigated the transcriptome-level responses associated with a persistent propagative phytovirus infection in various life stages of its vector using an Illumina HiSeq sequencing platform. The pathosystem components included a Tospovirus, *Tomato spotted wilt virus* (TSWV), its insect vector, *Frankliniella fusca* (Hinds), and a plant host, *Arachis hypogaea* (L.). We assembled (de novo) reads from three developmental stage groups of virus-exposed and non-virus-exposed *F. fusca* into one transcriptome consisting of 72,366 contigs and identified 1161 differentially expressed (DE) contigs. The number of DE contigs was greatest in adults (female) (562) when compared with larvae (first and second instars) (395) and pupae (pre- and pupae) (204). Upregulated contigs in virus-exposed thrips had BLASTX annotations associated with intracellular transport and virus replication. Upregulated contigs were also assigned BLASTX annotations associated with immune responses, including apoptosis and phagocytosis. In virus-exposed larvae, Blast2GO analysis identified functional groups, such as multicellular development with downregulated contigs, while reproduction, embryo development and growth were identified with upregulated contigs in virus-exposed adults. This study provides insights into differences in transcriptome-level responses modulated by TSWV in various life stages of an important vector, *F. fusca*.

**INTRODUCTION**

Persistent and propagative viruses replicate in different tissues of arthropod vectors, resulting in intricate interactions between them. Several studies have investigated the effects of such interactions on arthropod vectors [1–3]. Thrips transmitted *Tomato spotted wilt virus* (TSWV) (Tospovirus, Bunyaviridae) is a propagative and single-stranded RNA virus. TSWV is acquired by thrips during the early larval stages [4]; upon acquisition, the virus replicates in the mid-gut tissues and salivary glands [5–8]. The effects of TSWV replication on the fitness of its vectors *Frankliniella occidentalis* (Pergande) and *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae) have been extensively studied [9–14].

Since TSWV replicates in thrips as well as in host plants, it is difficult to determine whether TSWV-induced effects on thrips fitness are host plant-mediated indirect effects or direct effects of virus replication. Nonetheless, both negative and positive influences of TSWV on thrips biology and behaviour have been observed [12, 13, 15]. TSWV infection negatively affected the survival rate of *F. occidentalis* [16]. Our previous study documented the delayed adult emergence, decreased survival rate and reduced feeding ability of *F. fusca* when exposed to TSWV [12]. Virus-exposed *F. fusca* also developed into smaller adults when compared with non-virus-exposed adults [17]. In contrast, TSWV infection positively influenced the feeding, fecundity, survival and development rate of *F. occidentalis* [10, 13, 15, 18]. Similarly, increased oviposition in virus-exposed *F. fusca* was associated with TSWV infection [12].

Despite the availability of extensive evidence for TSWV-induced effects on thrips preference and fitness, the molecular changes associated with TSWV infection in thrips are just beginning to be explored through transcriptomic and
proteomic approaches. Few studies have characterized transcripts [19–21] and proteins [22] induced by TSWV infection in thrips. All of the studies were conducted exclusively with one thrips species, *F. occidentalis*. *F. fusca* is the primary vector of TSWV in many crops, including peanut (*Arachis hypogaea* L.) in the southeastern United States [23, 24]. Both *F. occidentalis* and *F. fusca* are mainly flower feeders; *F. fusca*, however, seems to adapt much better to foliar feeding and colonizes seedlings more efficiently than *F. occidentalis* of hosts such as peanut [25–27]. Thus far, nine thrips species have been identified as TSWV vectors [28, 29]. However, studies on TSWV-induced molecular changes in thrips have been limited to *F. occidentalis*. Jacobson et al. [30] examined interactions between TSWV isolates and different isofemale lines of *Thrips tabaci* (Lindeman) from several locations, and demonstrated variation in vector competence among *T. tabaci* isofemale lines, as well as variation in transmission by *T. tabaci* among TSWV isolates [30]. These results revealed that even within one thrips species, thrips–TSWV interactions could be different. Therefore, it is likely that TSWV-induced molecular changes in thrips could also vary with thrips species, and they need to be examined.

For successful TSWV transmission, early instar thrips larvae must acquire the virus. If adults acquire TSWV for the first time, they cannot transmit the virus. Such stage-specific acquisition and inoculation of TSWV by thrips [4] could potentially lead to varying molecular interactions at each life stage. Information on TSWV-induced stage-specific changes in thrips at transcript levels is limited and confined to one species. Very recently, Schneweis et al. [20] documented the stage-specific responses of *F. occidentalis* to TSWV [20]. Given the ecological and host utilization differences among various Tospovirus-transmitting thrips species, it is critical to examine the differences in TSWV-induced effects among different vector species.

The main objective of this study was to examine the transcriptome-level responses of virus-exposed *F. fusca* at various life stages. Total RNA samples from virus-exposed and non-exposed thrips at the larval, pupal and adult stages were sequenced using a HiSeq Illumina pair-end sequencing platform. Reads from all of the life stages were used to perform one de novo assembly and differential expression analysis was conducted between the virus-exposed and non-virus exposed life stages of thrips.

**RESULTS**

**Transcriptome assembly and differential expression analysis**

Quality reads from virus-exposed and non-virus exposed thrips from all life stages were combined and assembled into 72,366 contigs. The de novo assembly consisted of an average contig length of 2161 bases and N50 of 3702 bases (Table 1). Following the assembly, clean reads from each experimental replicate were mapped back to the assembled contigs. The number of reads mapping to each contig was measured and normalized expression values were calculated across experimental replicates. In general, the expression values showed concordance among replicates (Fig. S1, available in the online Supplementary Material). Subsequently, 1161 differentially expressed (DE) contigs with a false discovery rate (FDR)-adjusted *P*-value of <0.001 and a log fold change (FC) ≥1 in expression levels were identified (Fig. 1). The number of DE contigs in larvae (first and second instars), pre- and pupae, and female adults was 395, 204 and 562, respectively (Table S1). Of all the DE contigs, only 8 (0.7 %) were common among three life stages, while 14 (1 %) to 85 (7 %) of the contigs were common between two life stages. The clear majority of contigs were unique to each life stage. Among the three life stages, larvae had the greatest number of unique contigs (90 %), followed by adults (81 %) and pupae (46 %) (Fig. S2).

Validation of differential expression analysis by real-time quantitative reverse transcriptase-PCR (qRT-PCR) demonstrated that the FC direction of 15 randomly selected contigs in 2 experimental replicates was 86 and 73 %, respectively, similar to that of RNA-Seq (Table S2).

**Provisional annotations of the complete list of differentially expressed contigs**

Among 1,161 DE contigs, 967 contigs contained BLASTX matches to the National Center for Biotechnology Information non-redundant (NCBI nr) sequence database. Gene ontology (GO) analysis of the complete list of DE contigs identified a total of 38 GO terms associated with biological process, molecular function and cellular component categories at level 5 (Fig. 2). Under the biological process, the cellular macromolecule metabolic process was the most dominant GO term, consisting of 15 % of contigs, followed by the protein metabolic process (14 %), embryo development (10 %) and gene expression (9 %). In the molecular function category, the annotated contigs were mostly associated with nucleotide binding (30 %), RNA binding (15 %) and kinase activity (11 %). Only two GO terms, namely the plasma membrane and intracellular component, were assigned to the cellular component category.

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis mapped the DE contigs to 61 biochemical pathways.

### Table 1. Total number of quality reads produced by Trimmomatic software in virus-exposed and non-virus-exposed *Frankliniella fusca*, and summary statistics for *F. fusca* transcriptome assembly

<table>
<thead>
<tr>
<th>Life stage</th>
<th>Reads in virus-exposed thrips</th>
<th>Reads in non-virus-exposed thrips</th>
</tr>
</thead>
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<td>Larvae</td>
<td>86 261 736</td>
<td>88 671 587</td>
</tr>
<tr>
<td>Pupae</td>
<td>87 637 511</td>
<td>87 181 255</td>
</tr>
<tr>
<td>Adults</td>
<td>84 420 927</td>
<td>86 862 038</td>
</tr>
<tr>
<td>Assembled contigs</td>
<td>Total assembled contigs</td>
<td>Count of contigs</td>
</tr>
<tr>
<td></td>
<td>72 366</td>
<td></td>
</tr>
<tr>
<td>Total number of genes</td>
<td>41 169</td>
<td></td>
</tr>
<tr>
<td>Total assembled bases</td>
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<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Median contig length (bases)</td>
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<td></td>
</tr>
<tr>
<td>N50 contig length (bases)</td>
<td>3702</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.** Total number of quality reads produced by Trimmomatic software in virus-exposed and non-virus-exposed *Frankliniella fusca*, and summary statistics for *F. fusca* transcriptome assembly.
Differentially expressed contigs at the larval stage

In virus-exposed \textit{F. fusca} larvae, 219 contigs were upregulated and 176 contigs were downregulated. Upregulated contigs were assigned to 13 GO terms, including ion transport, protein and lipid metabolism, and response to stress (Fig. 3a). Downregulated contigs were assigned nine GO terms under the biological process category, including multicellular organismal development, transport and DNA metabolic process (Fig. 3b). In the molecular function category, six and eight GO terms were assigned to upregulated and downregulated contigs, respectively (Fig. 3c). Contigs under the molecular function category included nucleotide binding, hydrolase and transporter activities. Under the cellular component category, three GO terms, such as intracellular membrane-bound organelles [Node score (NS) 6.86], cytoplasm (NS 6.52) and protein complex (NS 8), were assigned to both upregulated and downregulated contigs. The GO-enrichment analysis demonstrated that DE contigs representing GO terms such as cellular amino acid metabolic process, catalytic activity, lipid binding and receptor activity were significantly (\(P<0.05\)) enriched (Table S4).

Differentially expressed contigs at the pupal stage

Among the 204 DE contigs in virus-exposed \textit{F. fusca} pupae, 150 were upregulated, while 54 were downregulated. In the biological process category, 14 GO terms, including growth, reproduction, translation and cell death, were assigned to upregulated contigs (Fig. 4a), while downregulated contigs were assigned to one GO term: metabolic processes. Under the molecular function category, downregulated contigs were assigned to ion binding. The upregulated contigs had nine GO terms, including GTPase, ATPase, oxidoreductase and isomerase activities (Fig. 4b). In the cellular component category, five GO terms, viz. mitochondrion (NS 6), endoplasmic reticulum (NS 7), ribosome (NS 13), protein complex (NS 9) and cytoskeleton (NS 5.72), were associated with upregulated contigs. Downregulated contigs were assigned a nucleus GO term (NS 5). Enrichment analysis revealed that GO terms such as vesicle-mediated transport, protein complex organization, hydrolase activity, RNA-binding and endoplasmic reticulum were overrepresented in virus-exposed pupae (Table S4).

Differentially expressed contigs at adult stage

Differential expression analysis identified 478 and 84 contigs upregulated and downregulated, respectively, in virus-exposed \textit{F. fusca} adults. Analysis of GO annotations identified 26, 14 and 3 GO terms under the biological process, molecular function and cellular component categories, respectively. Among the downregulated contigs, three, four and one GO terms were identified under the biological process, molecular function and cellular component categories, respectively. GO terms such as translation, viral process, cytoskeleton organization, protein transport, reproduction and growth were assigned to upregulated contigs under the biological process category (Fig. 5a), while contigs associated with oxidation reduction, organic substance metabolic process and primary metabolic process were assigned to downregulated contigs. In the molecular function category, GO terms like nucleotide binding, transporter activity, protein kinase activity, and peptidase activity were assigned to upregulated contigs. The downregulated contigs were associated with GO terms such as catalytic activity, ATP binding and zinc ion binding (Fig. 5b). Under the cellular component category, most of the upregulated contigs were associated with ribosome (NS 49), protein complex (NS 53), plasma membrane (NS 17), cytoskeleton (NS 22) and microtubule organizing centre (NS 6), while the downregulated contigs were associated with the nucleus (NS 8.08). The contigs associated with developmental process, reproduction, embryo development, structural molecule activity, RNA binding and cytoplasm were significantly overrepresented in virus-exposed adults (Table S4).
Following GO analysis of DE contigs, the contigs were classified into categories that might be associated with virus-vector interaction processes. DE contigs were classified into four categories, including (1) virus entry and movement, (2) virus replication, (3) vector response to virus infection, and (4) the effects of virus infection on vector fitness (Table 3).

**Virus entry and movement**

Virus entry and movement are the initial processes of virus infection. In virus-exposed *F. fusca*, several contigs associated with virus entry and movement were identified. Contigs with the **BLASTX** annotations clathrin and adaptor proteins, which are involved in intracellular transport, were upregulated by 9- and 10-fold, respectively, in virus-exposed adults (Table 3a). Also, one upregulated contig in virus-exposed adults was annotated as spike protein—a viral fusion protein responsible for mediating the entry of coronaviruses. In addition, homologues of aminopeptidase N, a receptor for a plant virus in aphids, were upregulated in virus-exposed larvae and adults.

**Virus replication**

In virus-exposed adults, several contigs known to facilitate the replication of animal- and plant-infecting viruses were upregulated. Six contigs with an annotation associated with the replication of animal-infecting viruses, including 40 s ribosome protein 15, ankyrin repeat domain, histone deacetylase 1 isoform 1 and host cell factor 1 protein, were upregulated by at least sixfold (Table 3b). Also, an upregulated contig was annotated as 60 s acidic ribosomal proteins p0, which is associated with replication of a plant virus, *Potato virus A*.

**Vector response to virus infection**

Following TSWV-exposure, several contigs with functional annotations of transport, lipid and carbohydrate metabolic processes, and response to stress were upregulated in *F. fusca* at all life stages. GO categories under biological process, such as response to biotic and abiotic stimulus, were...
specific to virus-exposed adults. Further, contigs associated with immune pathways – apoptosis, proteolysis and phagocytosis – were upregulated in virus-exposed thrips (Table 3c). The contigs associated with each pathway are described in detail below.

### Apoptosis

A total of 17 homologous contigs that have been known to play a role in apoptosis were identified in virus-exposed *Frankliniella fusca*. Out of 17, 12 homologous contigs were present in virus-exposed adults, 4 were present in virus-exposed pupae and 1 was present in virus-exposed larvae. Some of the contigs were annotated as 40s ribosomal protein s3, calmodulin, deoxyribonuclease I and cathepsin b (Table 3c). Homologues of cathepsin were also present in larvae and pupae.

### Proteolysis

Eight and three contigs associated with proteolysis were upregulated in virus-exposed adults and larvae, respectively (Table 3c). Four homologous contigs of 26s proteasome non-ATPase regulatory subunit 1 were upregulated in virus-exposed adults by 11-fold. Further, upregulated contigs were annotated as proteins and enzymes that mediate protein ubiquitination and degradation, including e3 ubiquitin–protein ligase ubr5, serine protease, f-box-only protein 11 and proteasome subunit beta type-7 in virus-exposed adults. In larvae, homologues of serine proteases were upregulated by twofold.

### Phagocytosis

BLASTX annotation identified the upregulation of homologous contigs of tubulin alpha chain and calreticulin family...
proteins in virus-exposed adults and pupae. In virus-
exposed larvae, a contig annotated as beta-mannosidase – a
lysosomal enzyme associated with degradation of glycopro-
tein – was upregulated by twofold (Table 3c).

Further, contigs associated with signal transduction that
trigger immune responses were present in the upregulated
contigs of virus-exposed adults and larvae. In virus-exposed
adults, contigs annotated as ribosomal protein s6 kinase,
serine threonine protein kinases and ras-like GTP-binding
protein were upregulated, while in larvae, contigs annotated
as rho GTPase-activating protein 190-like and zinc finger
protein zpr1-like were upregulated (Table 3c).

**Effects of virus infection on vector fitness**
Exposure to TSWV resulted in the downregulation of con-
tigs associated with multicellular organismal development
in *F. fusca* larvae. The contigs involved in neuron develop-
ment and cell division were also downregulated by several
folds in virus-exposed larvae (Table 3d). In virus-exposed
adults and pupae, reproduction-, reproduction-, embryo development-, cell differen-
tiation- and growth-related contigs were upreg-
ulated. Further, egg production-associated contigs, namely
vitellogenin, were upregulated by ninefold in virus-exposed
pupae. Also, a homologue of lipophorin precursor was
upregulated in virus-exposed adults.

**DISCUSSION**
In this study, we examined the transcriptome-level changes
associated with TSWV infection in *F. fusca* – an important
vector of TSWV in the southeastern United States. This
study also increases our understanding of the stage-specific
responses of *F. fusca* to TSWV infection. The DE contigs
identified in this study provide hypotheses about putative
genes associated with TSWV movement and replication, the
response of thrips to TSWV infection and the effect of
TSWV infection on thrips fitness.

**Differentially expressed contigs associated with
thrips biology and metabolism**
The upregulated contigs in virus-exposed *F. fusca* had GO
annotations such as organization of cytoskeleton, metabolic
process, transport and binding activities. Viruses interact
and reorganize host cytoskeleton components, including
actin filaments and microtubules, for intercellular traffick-
ing and various infection processes, including assembly and
exit [31–34]. Viruses also exploit host resources, including
nucleic acids and proteins, to reproduce viral particles. They
increase the availability of the nucleotide pool by altering
cellular metabolisms and increasing the rate of RNA break-
down [35, 36]. In this study, the KEGG pathway analysis
mostly identified metabolism-associated pathways such as
carbohydrate and lipid metabolisms in the upregulated con-
tigs of virus-exposed thrips at all life stages. TSWV RNA
segments are enclosed by host-derived lipid envelopes, sug-
gest that increased lipid metabolism in thrips could fa-
cilitate envelope formation in replicating virions. Manipula-
tion of host metabolism to facilitate virus replication has
also been observed in other viruses, with an example being
human cytomegalovirus [37].
**Differentially expressed contigs associated with virus movement and replication**

To successfully initiate virus replication, viruses must enter host cells. Several animal-infecting viruses, including members of *Bunyaviridae*, such as nairoviruses and hantaviruses, enter host cells through receptor-mediated endocytosis [38–40]. Clathrin and adaptor proteins are important components of receptor-mediated endocytosis [41].

Contigs with annotations pertaining to clathrin and adaptor proteins were upregulated several-fold in virus-exposed adults. Therefore, it is possible that clathrin-mediated endocytosis could be influencing TSWV infection in thrips. Zhang *et al.* [21] also observed upregulation of vesicle-mediated endocytosis-related contigs in virus-exposed *F. occidentalis*. Badillo-Vargas *et al.* [22] documented increased abundance of vacuolar proton ATP synthase protein in *F. occidentalis* larvae. That protein is associated with clathrin-coated vesicle trafficking. Further, in this study, we identified contigs with BLASTX annotations associated with the replication of animal-infecting viruses, including ankyrin repeat domain-containing proteins, 40 s ribosomal proteins and histone deacetylases in virus-exposed *F. fusca* adults. TSWV is classified in the genus *Tospovirus*, the only genus in the family *Bunyaviridae* that infects plants. The other genera of *Bunyaviridae* are animal-infecting viruses. The identification of animal virus replication-related contigs in thrips suggests that virus replication-related genes are somewhat conserved in diverse vectors.

**Differentially expressed contigs associated with immune response**

Insects rely on innate immunity to fight against invading pathogens [42, 43]. Upon viral infection, several immune pathways are launched in insects, including the RNAi, Toll, JAK/STAT [21], phagocytosis, apoptosis [22], proteolysis [20] and JNK pathways [19]. When compared with *F. fusca* adults, fewer innate immunity-related contigs were upregulated in virus-exposed larvae and pupae. Viruses are known to suppress immune responses regulated by their hosts [44, 45]. In virus-exposed adults, along with antiviral immune responses, contigs known to inhibit immune genes were also upregulated (Table 3c). For instance, cactin is a negative regulator of cactus gene that is associated with the activation of the Toll pathway [46]. We documented the upregulation of a homologue of cactin in virus-exposed *F. fusca* adults. Further, dicer is a member of RNase III family, which cleaves viral particles and activates the RISC of the RNAi pathway, was also upregulated [47]. In virus-exposed adults, homologous contigs of RNase III inhibitor were upregulated by 11-fold. In addition, heat-shock protein 70, which inhibits apoptosis, was also upregulated in virus-exposed adults [48, 49].

![Gene Ontology terms](chart.png)
<table>
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<tr>
<th>Contig ID*</th>
<th>Life stage†</th>
<th>log₂ FC‡</th>
<th>E-value§</th>
<th>Annotation∥</th>
<th>Organism with sequence match¶</th>
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*Table 3a) Virus entry and movement

*Table 3b) Virus replication

*Table 3c) Vector response to virus infection

(i) Apoptosis

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### Table 3. cont.

| Contig ID* | Life stage† | log₂FC‡ | E-value§ | Annotation|| Organism with sequence match¶ | Reference |
|------------|-------------|---------|----------|----------|------------------------------------------------|-----------|
| comp58712  | L           | 2.77    | 2.86E-157| Cathepsin partial | *Frankinella occidentalis* | [88, 89]  |
| comp33849  | P           | 8.77    | 0        | Beta-tubulin isotype 1 | *Haemonchus contortus* | [102]  |
| comp60117  | P           | 8.67    | 0        | Glyceraldehyde-3-phosphate dehydrogenase | *Ascaris suum* | [49]  |
| comp37736  | A           | 11.61   | 8.21E-175| 26 s proteasome non-ATPase regulatory subunit 1 | *Caenorhabditis elegans* | [94]  |
| comp16613  | A           | 11.13   | 0        | 26 s proteasome non-ATPase regulatory subunit 12 | *Ascaris suum* | [103, 104]  |
| comp32606  | A           | 11.16   | 3.9E-133 | 26 s proteasome non-ATPase regulatory subunit 7 | *Wuchereria bancrofti* | [105]  |
| comp60324  | A           | 11.76   | 0        | 26 s proteasome regulatory complex subunit p97 | *Loa loa* | [91]  |
| comp59862  | A           | 10.46   | 1.52E-49 | Serine protease | *Tribolium castaneum* | [90]  |
| comp46002  | A           | 12.66   | 0        | E3 ubiquitin-protein ligase | *Loa loa* | [91]  |
| comp59779  | A           | 11.27   | 0        | F-box only protein 11-like | *Apis florea* | [92]  |
| comp52987  | A           | 12.39   | 9.9E-129 | Proteasome subunit beta type-7-like | *Apis florea* | [93]  |
| comp59296  | L           | 2.14    | 1.45E-71 | Serine protease | *Nasonia vitripennis* | [101]  |
| comp59361  | L           | 2.42    | 2.12E-38 | Serine protease | *Daphnia pulex* | [96, 97]  |
| comp51558  | A           | 8.84    | 0        | Alpha tubulin | *Onchocerca volvulus* | [94]  |
| comp40334  | A           | 10.07   | 0        | Calreticulin family protein | *Loa loa* | [95]  |
| comp51558  | A           | 8.56    | 0        | Tubulin alpha chain | *Haemonchus contortus* | [97]  |
| comp51558  | P           | 10.84   | 8.83E-141| Tubulin alpha chain | *Haemonchus contortus* | [97]  |
| comp58270  | L           | 2.09    | 0        | Beta lysosomal | *Oryzias latipes* | [96, 97]  |
| comp40334  | P           | 10.73   | 0        | Calreticulin family protein | *Loa loa* | [91]  |
| comp60061  | P           | 9.25    | 0        | Calreticulin-like protein | *Ditylenchus destructor* | [95]  |
| comp31036  | A           | 8.93    | 4.51E-109| Ribosomal protein s6 alpha | *Brugia malayi* | [98]  |
| comp58759  | A           | 10.74   | 0        | Ribosomal protein s6 kinase alpha | *Pediculus humanus corporis* | [99]  |
| comp58759  | A           | 11.47   | 0        | Ribosomal protein s6 kinase alpha | *Pediculus humanus corporis* | [100]  |
| comp37730  | A           | 9.17    | 1.27E-157| Serine threonine protein kinase-related domain | *Haemonchus contortus* | [101]  |
| comp15214  | A           | 8.96    | 6.18E-115| Ras-like gtp-binding protein | *Trichinella spiralis* | [102]  |
| comp58908  | L           | 1.82    | 0        | Rho gtpase-activating protein | *Harpegnaothus saltator* | [103]  |
| comp57721  | L           | 1.85    | 0        | Zinc finger protein zpr1 | *Nasonia vitripennis* | [104]  |
| comp22093  | A           | 11.59   | 9.79E-13 | RNase III inhibitor | *Ascaris suum* | [48]  |
| comp57919  | A           | 12.65   | 0        | Cactin | *Bombus terrestris* | [47]  |
| comp17230  | A           | 11.45   | 2.59E-67 | Bax inhibitor 1 | *Haemonchus contortus* | [105]  |
| comp13222  | A           | 10.04   | 0        | Heat shock protein 70 | *Ditylenchus destructor* | [103, 104]  |
| comp15361  | A           | 10.01   | 0        | Heat shock protein 70 b2 | *Ascaris suum* | [49]  |
| comp33168  | A           | 12.02   | 0        | Heat shock protein 70 | *Haemonchus contortus* | [106]  |
### Table 3. cont.

<table>
<thead>
<tr>
<th>Contig ID*</th>
<th>Life stage</th>
<th>Organism with sequence match</th>
<th>Annotation</th>
<th>Reference</th>
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</thead>
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<tr>
<td>comp5275</td>
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<td>Ditylenchus destructor</td>
<td>Heat shock protein 70</td>
<td>[105]</td>
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<td>comp50016</td>
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<td>Lysophorin precursor</td>
<td>[106]</td>
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<tr>
<td>comp5731</td>
<td>P</td>
<td>Niptanophytoptera lugens</td>
<td>Vitellogenin</td>
<td>[107]</td>
</tr>
<tr>
<td>comp57928</td>
<td>L</td>
<td>Niptanophytoptera lugens</td>
<td>Fasciculin isoform b</td>
<td>[108]</td>
</tr>
<tr>
<td>comp58016</td>
<td>A</td>
<td>Necator americanus</td>
<td>Lipophorin precursor</td>
<td>[109]</td>
</tr>
<tr>
<td>comp59128</td>
<td>L/C0</td>
<td>Aedes aegypti</td>
<td>Cyclin e</td>
<td>[109]</td>
</tr>
</tbody>
</table>

*ID of differentially expressed contigs under each category.
†Life stages of thrips: L, larvae (first and second instars); P, pupae (prepupae and pupae); A, adults (females up to 2 d old).
‡Log 2 FC (ratio of virus-exposed/non-virus-exposed expression levels) of differentially expressed contigs identified in F. fusca/Fe. occidentalis. Log2FC ≥ 1 was used for the study with a false discovery rate of adjusted P-value <0.001.
§E-value assigned to the gene description determined by BLASTX.
¶Gene descriptions determined by BLASTX.
||Organisms with top sequence match for each contig.**

## Differentially expressed contigs associated with thrips fitness

Exposure to TSWV affected the expression of several fitness-related contigs in thrips. We observed upregulation of contigs associated with egg production, embryo development and growth in virus-exposed F. fusca adults and pupae. In our previous study, we demonstrated that virus-exposed F. fusca produced significantly more eggs than non-virus-exposed adults [12]. This study seems to provide evidence at the transcriptome level for upregulation of vitellogenin in F. fusca. In addition, in this study, development-related contigs were downregulated in virus-exposed larvae. In contrast to our results, Badillo-Vargas et al. [22] compared the relative abundances of proteins between virus-exposed and non-virus exposed F. occidentalis, and documented the upregulation of proteins associated with development in virus-exposed F. occidentalis larvae. This discrepancy concerning the effects of TSWV infection on thrips development could be due to differences in approach (proteomics versus transcriptomics), variations in the TSWV accumulation in the larvae used for each study and differences in the TSWV acquisition access time provided (AAP) to the larvae. In this study, F. fusca larvae were reared on TSWV-infected leaflets until they were used for RNA extraction. Badillo-Vargas et al. [22] only provided thrips with a three-hour AAP and moved thrips to their original rearing hosts, healthy green bean pods. This presumably also limited the indirect effects of virus-infected plant material on thrips. Feeding on virus-infected plants alone could alter gene expression in insect vectors. Wang et al. [43] demonstrated that white black planthopper reared on virus-infected plants had 700 genes that were differentially expressed compared to healthy control groups [43]. TSWV infection increased nutrients such as free amino acids in virus-infected plants [12, 50]. TSWV infection also affects host-plant quality by decreasing water storage capacity and photosynthesis [51]. Thus, extended periods of exposure to TSWV-infected leaflets could have negatively affected the development of F. fusca larvae in this study. Our earlier study also demonstrated direct negative effects of TSWV infection on F. fusca survival rate and developmental time [12]. The current study provides evidence at the transcriptome level for some of the observed macro-level fitness effects of TSWV infection on F. fusca.

Comparison of the transcriptome-level responses of TSWV-exposed F. fusca to F. occidentalis identified some similarities and differences between two thrips species. In this study and in Zhang et al. [21], exposure to TSWV induced upregulation of the contigs associated with vesicle-mediated endocytosis, insect development and lipid metabolism. When compared with F. fusca, more immune system-related pathways, such as the Toll pathway, JAK-STAT and RNA interference, were documented in F. occidentalis by Zhang et al. [21]. Unlike in our study, Zhang et al. [21] pooled thrips from all life stages and performed DE analysis between TSWV-exposed and non-exposed F. occidentalis without biological replicates. stage-specific DE analysis of
F. occidentalis by Schneweis et al. [20] allowed for direct comparison of TSWV-induced responses between F. fusca and F. occidentalis at each life stage. Comparison of the findings revealed that in both species only a small percentage (1.6% in F. fusca and <1% in F. occidentalis) of contigs were differentially expressed following TSWV infection. In F. occidentalis, the highest distribution of DE contigs was in pupae (37%), followed by larvae (36%) and adults (26%). In the case of F. fusca, more DE contigs were identified with adults (48%) than larvae (34%) and pupae (17%). Also, while most of the DE contigs were upregulated by TSWV in F. fusca across life stages, in F. occidentalis, there were more downregulated than upregulated contigs in larvae and adults. Further, both studies demonstrated that the majority of TSWV-induced DE contigs were unique to each life stage. Only 0.6% of DE contigs were shared by all life stages in F. fusca, while 0.7% were shared in F. occidentalis. Unlike F. occidentalis, F. fusca larvae had the most unique contigs (90%), followed by adults (81%) and pupae (46%). In F. occidentalis, the pupal stage had 83% unique contigs, while the adult and larval stages had 78 and 68% unique contigs, respectively.

Based on functional annotations, TSWV exposure induced the expression of similar contigs in both F. fusca and F. occidentalis, but the contigs were differentially regulated in each species. For example, the GO term ‘receptor activity’ associated with contigs specific to F. occidentalis was enriched at the pupal and adult stages, but it was only enriched in F. fusca larvae. Proteolysis-associated contigs were downregulated in F. occidentalis larvae in response to TSWV infection, but there was an upregulation of serine proteases in F. fusca larvae. Insect cuticle-related contigs were one of the enriched contigs in F. occidentalis adults, but they were mostly downregulated in TSWV-exposed larvae and pupae. In F. fusca, the contigs associated with the BLASTX annotation for cuticular proteins were upregulated in virus-exposed larvae and adults. The observed differences in the profiles of DE transcripts between F. fusca and F. occidentalis could be due to innate differences in the thrips species, virus isolates, experimental designs and DE analyses used in the studies. Schneweis et al. [20] exposed F. occidentalis larvae to TSWV for only 3 h. In contrast, F. fusca were reared on TSWV-infected leaflets until they were used for RNA extraction. Further, we pooled the first and second instars for the larval stage, the pupae and prepupae for the pupal stage, and female adults up to 2 d old for the DE analysis. However, in F. occidentalis, DE analysis was performed with first instar larvae, prepupae and 24 h-old adults (male and female). Schneweis et al. [20] carried out differential expression analysis using genome-guided assemblies, while we first performed de novo assembly on F. fusca reads by pooling reads from all of the life stages and then conducted differential expression analysis.

The DE analysis in this study identified several contigs with putative roles in viral processes, thrips development, growth and reproduction. The contig functions need to be validated through gene-silencing experiments. Once proven, these candidates could be evaluated as likely targets for pest management. For instance, the contigs associated with egg production, virus movement and virus replication identified in this study could potentially be exploited for RNAi-mediated thrips and TSWV management. Unlike other economically important insect vectors, the genomic resources for thrips are very limited. The development of F. occidentalis-expressed sequence tags by Rotenberg et al. [52] initiated an effort to build sequence resources and annotate putative genes in thrips [53]. The transcriptomic data generated in this study have already been submitted to the NCBI (SUB2623511). The transcriptomic data generated in this study will further enrich the genomic resources got thrips and facilitate functional genomic studies in other thrips species and other insects.

METHODS

Maintenance of non-virus-exposed and virus-exposed thrips colony

Non-virus exposed F. fusca were reared on peanut leaves of the cultivar Georgia Green in Munger cages (0.11×0.89×0.18 m) [54] in a growth chamber (Thermo Scientific) at 25–30°C, 40–50% relative humidity (RH) and L14:D10 photoperiod [52]. Similarly, virus-exposed thrips were reared on TSWV-infected peanut leaflets following the same conditions [55, 56].

TSWV infection in subsamples of a virus-exposed thrips colony was tested by qRT-PCR. A total of 30 samples (two thrips pooled for each sample) were used for the study. qRT-PCR was conducted using N-gene-specific primers as previously described [55–57]. All the tested thrips were positive for TSWV, and the TSWV N-gene copy numbers in thrips ranged from 4316 to 6 200 000.

Total RNA extraction and library preparation for sequencing

Total RNA was extracted from virus-exposed or non-virus-exposed larvae (first and second instars), pupae (including pre-pupae) and female adults (up to 2 d old) (six treatments in total). For each treatment, about 35 individual thrips were pooled and tested as one experimental replicate. A total of three experimental replicates were used per treatment (N=105 individual thrips per treatment). Virus-exposed and non-virus-exposed thrips were reared under the same laboratory conditions as previously indicated, and all thrips were collected on the same day for RNA extraction. Total RNA was extracted using the RNeasy plant mini kit (Qiagen) and sent to Georgia Genomic Facility of the University of Georgia for RNA-Seq library preparation. Illumina sequencing libraries were constructed using TruSeq RNA sample preparation kits using at least 1 μg of the total RNA. Briefly, mRNAs (polyadenylated RNAs) were selected using oligo-dT. Subsequently, mRNAs were fragmented and first- and second-strand cDNA were synthesized. An Illumina TruSeqLT adapter was ligated and PCR amplification
was performed to produce final cDNA libraries for sequencing. Sequencing was performed at the University of Texas Health Science Center, at San Antonio, Texas on Illumina HiSeq 2000 platform using v3 paired-end 100 cycle sequencing settings. Six RNA-seq libraries were included in one lane and a total of three lanes were used.

**Transcriptome assembly and differential expression analysis**

Bioinformatics software at Georgia Advanced Computing Resource Center, UGA (https://wiki.gacrc.uga.edu/wiki/Software) were used to process raw reads, perform de novo assembly and conduct differential expression analysis. Trimmomatic was used to trim adapter primers and adapter sequences from the raw reads [38]. Subsequently, quality reads were produced by removing three bases at the beginning and end of each read, setting the minimum read length threshold to 50 bases and discarding reads if the average quality of four bases fell below 20. Quality reads from virus-exposed and non-virus-exposed thrips from all life stages (treatments) and experimental replicates were pooled to perform a single de novo assembly using the Trinity program with the parameters ‘kmer 25–minimum-contig-length 500 bp–Passafy’ [59]. To evaluate the completeness of the assembled contigs (overlapping DNA segments), the Core Eukaryotic Genes Mapping Approach (CEGMA) program was used. CEGMA contains a core set of 248 proteins that are highly conserved within diverse eukaryotes. CEGMA identifies homologues of those conserved proteins in the assembly [60]. With CEGMA, all the 248 core proteins were identified in *F. fusca* transcriptome [61, 62].

For differential expression analysis, cleaned reads from each experimental replicate were first mapped to the de novo assembled transcriptome individually using Bowtie software [63]. The RSEM program then estimated the number of reads corresponding to each contig across the replicates, thereby providing experimental replicate-specific expression values [64]. The expression values were normalized as fragment per kilobase of transcript per million mapped reads and trimmed mean m-values were calculated across the replicates. Also, the concordance in expression values between replicates was estimated by subjecting the data to generalized linear mixed models using Proc GLIMMIX in SAS (SAS Enterprise 4.2; SAS Institute). Subsequently, the DE contigs between treatments were identified using the Benjamin and Hochberg multiple-testing adjustment methodology [55–57]. Each targeted contig included two technical replicates and mean Ct values were calculated. Subsequently, the expression level of each contig was normalized to the expression level of a thrips reference gene (actin). Normalized expression levels of targeted contigs were estimated using the Pfaffl analysis method, which included the PCR efficiency (E) and Ct values of the target contigs and the reference gene, $E^{−1}(\text{target})/E^{−1}(\text{ref})$ [66].

**Provisional annotations of differentially expressed contigs**

DE contigs were characterized by searching for homologous sequences against the NCBI nr database using BLASTX program at an E-value threshold of $10^{−10}$. Further, Java-based Blast2GO software (https://www.blast2go.com) was used to study the functional annotations of DE contigs [67]. Using the GO database, GO terms were assigned to the contigs under biological process, molecular function and cellular components with an E-value hit filter of $10^{−6}$ and an annotation cutoff of 55. To obtain detailed functional information, the GO terms were classified at GO level 5 and a node-score of 5 for the complete list of DE contigs, whereas the GO terms were assigned to DE contigs from each life stage at multilevel with a 5-node score. Further, pathway analysis was performed using the KEGG database with Blast2GO. To identify enriched GO terms in DE contigs, functional enrichment analysis was performed using Fisher’s exact test. The background was set to the complete list of DE contigs and significantly enriched GO terms in each life stage were identified at $P<0.05$.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

No human subjects were included.

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