

# Impact of opsin gene knockdown via RNA interference on phototactic behavior in *Megalurothrips usitatus*

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**Abstract:** The developmental duration and compound eye characteristics of *Megalurothrips usitatus* across its life stages were systematically investigated to elucidate the functional roles of opsin genes in *M. usitatus*. RNA interference (RNAi) was employed to suppress the expression of adult opsin genes *Rhodopsin-1* (*Rh-1*) and *Rhodopsin-2* (*Rh-2*), and the phototactic behavioral responses to 520 nm green light following gene knockdown were quantitatively assessed, along with potential mutual regulatory interactions between *Rh-1* and *Rh-2*. Key findings revealed that compound eyes attained full development exclusively during the adult stage. No significant differences in survival curves ( $P > 0.05$ ) were observed among adults fed on *dsEGFP*, *dsRh-1*, or *dsRh-2* at a mass concentration of  $100 \mu\text{g} \cdot \text{mL}^{-1}$  over 24–96 hours. Notably, phototactic choice rates toward green light decreased to 26.67% and 32.00%, respectively at 72 hours after treatment with *dsRh-1* and *dsRh-2*. Gene expression analyses demonstrated no compensatory regulation between *Rh-1* and *Rh-2*, as evidenced by unaltered relative expression levels of *Rh-2* following *Rh-1* knockdown ( $P > 0.05$ ), and vice versa. This investigation provides critical empirical data for advancing visual system-based pest management strategies.

**Keywords:** *Megalurothrips usitatus*; opsin; RNAi; developmental stages

*Megalurothrips usitatus* is a globally significant pest posing severe threats to cowpea quality and safety<sup>[1]</sup>. It has been officially classified as a pest in Category I Crop Disease and Pest according to Document No.654 issued by the Ministry of Agriculture and Rural Affairs of China in 2023. This thrips species exhibits a short developmental cycle with multiple generations per growing season, enabling continuous infestation throughout all the growth stages of cowpea<sup>[2]</sup>. Its rasping-sucking mouthparts and ovipositor cause direct damage through feeding and egg-laying activities on floral organs, fruits, and tender meristems, resulting in characteristic symptoms including apical and basal necrosis of pods, growth retardation, leaf curling and wrinkling, and premature floral abscission, ultimately leading to substantial yield losses and quality degradation<sup>[3]</sup>. The pest's diminutive size (1–2 mm body length, with females typically larger than

males), slender morphology, and cryptic behavior within floral structures collectively contribute to significant challenges in effective pest management.

Vision-based physical control methods have been demonstrated as an efficient, eco-friendly, and cost-effective strategy for pest management, achieving notable success in controlling aphids, *Laodelphax striatellus*, *Bemisia tabaci*, and *Bactrocera dorsalis*<sup>[4–7]</sup>. Regarding thrips control, Tang et al. verified blue sticky traps as highly effective against the common blossom thrips (*Megalurothrips usitatus*)<sup>[8]</sup>. Further studies by Wang et al. and Jin et al. revealed that ultraviolet (UV)-blocking greenhouse films significantly reduced populations of *Thrips palmi* and *M. usitatus* on netted melon (*Cucumis melo* var. *reticulatus*) and cowpea (*Vigna unguiculata*)<sup>[9–10]</sup>. Field trials by Wu et al. confirmed the superior efficacy of blue traps against the western flower thrips (*Frankliniella occidentalis*)<sup>[11]</sup>.

Moreover, Mi et al. reported that 450 nm blue light and blue sticky traps exhibited optimal control effects on *Thrips tabaci*. Recent findings by Ning et al. highlighted strong phototactic behavior of *M. usitatus* toward UV-A and white light under laboratory conditions<sup>[12-13]</sup>.

The rapid advancement of RNA interference (RNAi) technology in pest management has provided innovative solutions for agricultural production and ecological balance. Insect RNAi operates through exogenous double-stranded RNA (dsRNA)-triggered sequence-specific degradation of homologous mRNA, leading to targeted gene silencing that disrupts normal insect growth, development, reproduction, and behavioral patterns. In 1998, Fire et al. first demonstrated this phenomenon by successfully interfering with endogenous gene expression in *Caenorhabditis elegans* through microinjection of exogenous dsRNA<sup>[14]</sup>. Subsequently, RNAi technology has been progressively applied to agricultural pest control. Singh et al. achieved effective downregulation of SNF7 and AQP gene expression in *T. tabaci* through feeding delivery of target-specific *dsSNF7* and *dsAQP*<sup>[15]</sup>. Similarly, Wang et al. successfully suppressed GABA receptor expression in *F. occidentalis* using oral administration<sup>[16]</sup>. More recently, Huang et al. employed dsRNA injection to silence visual perception-related genes in *Zeugodacus cucurbitae*, specifically targeting long-wavelength-sensitive opsins (*dsZcRh1*, *dsZcRh2*, *dsZcRh6*) and ultraviolet-sensitive opsins (*dsZcRh3*, *dsZcRh4*)<sup>[17]</sup>. Nevertheless, current scientific literature reveals a notable research gap regarding RNAi-mediated manipulation of visual genes in *M. usitatus*, with no RNAi-based studies being reported to date.

Based on the application of RNA interference (RNAi) technology in studying visual mechanisms of *M. usitatus*, previous studies have identified two long-wavelength-sensitive opsin genes (*Rh-1* and *Rh-2*) associated with visual perception in this pest species<sup>[18-20]</sup>. To elucidate the potential behavioral

regulation mechanisms mediated by these opsin genes, we conducted RNAi-mediated silencing targeting the opsin genes and subsequently validated their spectral sensitivity responses to green light wavelengths. This investigation aims to establish fundamental evidence for developing visual disruption strategies targeting *M. usitatus*, thereby providing critical theoretical support for the innovative development of phototactic behavior-regulating agents in pest management systems.

## 1 Materials and Methods

### 1.1 Insects

The adults of *M. usitatus* were collected from cowpea (*Vigna unguiculata*) crops at Batou Experimental Base (18°23' N, 109°10' E) in Yazhou District, Sanya City, Hainan Province, China, during November to December 2023. Test insects were maintained under controlled laboratory conditions at the temperature of (26±1)°C and the relative humidity of (70±5)%, with a photoperiod of 14 h light: 10 h dark (14L:10D) hours. After 24-hour acclimatization the adults were fed on fresh cowpea pods, and healthy and active adults exhibiting normal locomotor behavior were selected for subsequent experimental procedures.

### 1.2 Reagents and Apparatus

The reagents included TRIzol reagent (Thermo Fisher Scientific, USA), PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Japan), ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech, Nanjing, China), T7 High Yield RNA Transcription Kit (Vazyme Biotech, Nanjing, China), eosin (Servicebio, Wuhan, China), hematoxylin (Servicebio, Wuhan, China), hydrochloric acid-ethanol solution (Servicebio, Wuhan, China), universal tissue fixative (Servicebio, Wuhan, China), and eco-friendly dewaxing clearing agent (Servicebio, Wuhan, China), with all other chemicals being of domestic analytical grade. The instruments and equipment employed in this study comprised an SZ61 stereomicroscope (Olympus, Japan), AriaMx Real-Time PCR System (Agilent Technologies, USA), fluorescence

microscope (Agilent Technologies, USA), rotary microtome (LEICA, Germany), DK320S constant-temperature water bath (Shanghai Jinghong Experimental Equipment, China), SPX-160H biochemical incubator (Xiamen Guoyi Scientific Instruments, China), Micro Drop UV-Vis spectrophotometer (BIO-DL, USA), along with ancillary materials including Parafilm sealing membrane (Thermo Fisher Scientific, USA), rice paper (Shanghai M&G Stationery, China), glass tubes (50 mm height, 38 mm OD, 35 mm ID; Donghai Shixuan Quartz, China), entomological pins (Shenzhen Huayang Biotechnology, China), and a green LED monochromatic light source (520 nm wavelength, 3 W power; Zhongshan Guzhen Youweigu Lighting, China).

The behavioral assay apparatus was custom-built. The entire setup was constructed using transparent and opaque acrylic panels (6 mm thickness), with the dark-colored components in the diagram representing opaque acrylic panels and the light-colored sections denoting transparent ones. The apparatus consisted of five integrated components: a phototactic chamber (A), a response chamber (B), an insect collector (C), a base plate (D), and a light source (E). Detailed parameters of the experimental setup are illustrated in Fig. 1. The dark box featured a cubic structure (30 cm edge length) with 10 mm-thick walls and a hollowed-out base. It was assembled from black polystyrene foam panels to eliminate interference from external light sources.

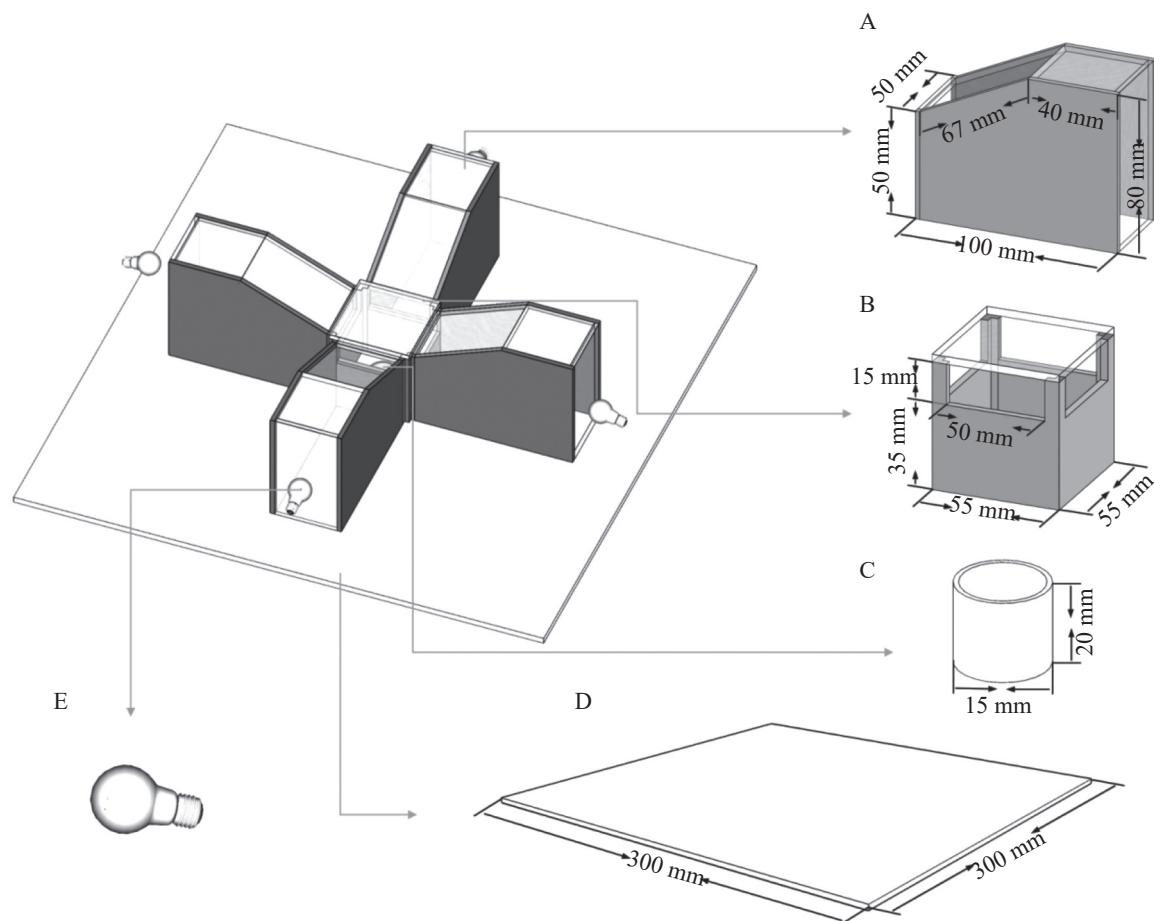
**1.3 Observation on the Developmental Duration of *M. usitatus*** Select 10–15 individuals each of first-instar nymphs, second-instar nymphs, prepupae, pseudopupae, and female/male adults of *M. usitatus*, and place them into pre-prepared 1.5 mL centrifuge tubes. Subsequently, position the tubes in a –20°C freezer for 2–3 minutes to immobilize the test insects. Lay a cowpea leaf flat on the stage of an SZ61 stereomicroscope (Japan), placing insects from one developmental stage at a time above the leaf surface. During photography, use insect pins to adjust specimen orientation and positioning. For

imaging the egg stage, position a female specimen at the center of a glass slide, apply 20  $\mu$ L of deionized water to its central area, and carefully dissect the abdominal region using insect pins until eggs become observable.

**1.4 Opsin Gene Primers for *M. usitatus*** Based on the previously obtained opsin genes *Rh-1* (GenBank accession number PP154173), *Rh-2* (GenBank accession number PP154174), and the enhanced green fluorescent protein (EGFP) gene (GenBank accession number AAB02572) from *M. usitatus*, dsRNA targeting fragments were designed. The  *$\beta$ -actin* gene (GenBank accession number KX108734) was selected as the internal reference. Primers were synthesized by Tsingke Biotechnology Co., Ltd., Guangzhou Branch (Table 1).

**1.5 dsRNA Synthesis and Detection** dsRNA was synthesized using the T7 High Yield RNA Transcription Kit (Vazyme Biotech Co., Ltd., Cat# TR101-01, China), and purified via phenol/chloroform extraction. To assess the quality of synthesized *dsEGFP*, *dsRh-1*, and *dsRh-2*, 2  $\mu$ L of purified dsRNA samples were analyzed by gel electrophoresis, while 1  $\mu$ L of each sample was subjected to spectrophotometric absorbance measurement. Purified dsRNA was stored at –80 °C for subsequent experiments.

**1.6 Oral Delivery of dsRNA and Real-time Quantitative PCR (qPCR) Methodology in *M. usitatus*** During the experiment, a double-opening glass tube (height: 50 mm; outer diameter: 38 mm; inner diameter: 35 mm) was prepared as the feeding apparatus. One end of the tube was sealed with a parafilm membrane stretched to its thinnest layer. Fifty adult individuals of *M. usitatus* (female-to-male ratio approximately 3:1 or higher) were introduced into the tube, and the opposite end was covered with tissue rice paper to maintain ventilation, with the paper-covered end oriented downward. A 180  $\mu$ L aliquot of dsRNA solution was pipetted onto the parafilm-sealed end, followed by an additional parafilm layer to prevent contamination and evaporation. The apparatus was



A. Phototactic room; B. Reaction room; C. Insect receiver; D. Bottom plate; E. Light source.

**Fig. 1 Taxis test device of *Megalurothrips usitatus***

Tab. 1 The information of primers

Gene	Primer sequence (5'-3')	Amplicon length/bp	Purpose
<i>Rh-1</i>	F: CTTCGTCTACTTTCTGCCTCTT R: TTCACTCTTGGCTTGCTC	108	
<i>Rh-2</i>	F: CGCTCCGTAACCGTCAAC R: CACCCATAACCCACGTCTCG	120	RT-qPCR
$\beta$ -actin	F: CTTCGTCTACTTTCTGCCTCTT R: TTCACTCTTGGCTTGCTC	125	
<i>dsRh-1</i>	F: TAATACGACTCACTATAGGGACAGTGGTAGACCAGGTGCC R: TAATACGACTCACTATAGGGTGTTCATAGGAGCAGCG	427	
<i>dsRh-2</i>	F: TAATACGACTCACTATAGGGCTACAACGAGACGTGGTT R: TAATACGACTCACTATAGGGAGACGATGACTTCAAGCGGT	363	RNAi
<i>dsEGFP</i>	F: TAATACGACTCACTATAGGGCAGTGTCTCAGCCGCTAC R: TAATACGACTCACTATAGGGTGTCTGTTAGTGGTCG	352	

Note: The underlined part represents the T7 promoter sequence.

incubated in a controlled environment chamber at the temperature of  $(26\pm1)^\circ\text{C}$ , the relative humidity of  $(70\pm5)\%$ , and a 14L: 10D photoperiod. Each dsRNA concentration was tested with three biological replicates, each containing 50 insects. Post-treatment samples were collected, flash-frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$  for subsequent analysis.

Real-time quantitative PCR (qPCR) methodology: Total RNA was extracted from samples using TRIzol reagent (Thermo Fisher Scientific, Cat# 15596026, USA). cDNA synthesis was performed following the protocol of the PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Cat# RR047A, Japan), and the synthesized cDNA was

stored at  $-20^{\circ}\text{C}$ . RT-qPCR amplification was conducted using the ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd., Cat# Q711-02, China) according to the manufacturer's instructions. Each sample was analyzed with three technical replicates. The relative expression levels of target genes were calculated using the  $2^{-\Delta\Delta C_t}$  method [21].

**1.7 Silencing Efficiency Assessment at Various dsRNA Concentrations and Time Points** Preparation and administration of dsRNA solutions: Purified *dsEGFP*, *dsRh1*, and *dsRh2* were diluted with RNase-free water to appropriate concentrations and mixed with 20% honey solution (prepared in RNase-free water) at a 1:1 ratio to generate dsRNA solutions at final concentrations of 1, 10, 100, 500, and  $1000 \mu\text{g}\cdot\text{mL}^{-1}$ . These solutions were orally delivered to *M. usitatus* adults for 24 h. All other procedures followed the methodology outlined in Section 2.6.

*dsEGFP*, *dsRh-1*, and *dsRh-2* were mixed with 20% honey solution (prepared in RNase-free water) at a 1:1 ratio to generate dsRNA solutions at a final concentration of  $100 \mu\text{g}\cdot\text{mL}^{-1}$ . Adult *M. usitatus* were fed on the dsRNA solutions (*dsEGFP*, *dsRh-1*, and *dsRh-2*) for 24, 48, 72, and 96 h, with fresh dsRNA solution replenished every 24 h. All other experimental procedures were consistent with the methods described in Section 2.6.

**1.8 Survival Curve Analysis in *M. usitatus*** Adult *M. usitatus* were fed on dsRNA solutions (*dsEGFP*, *dsRh-1*, and *dsRh-2*) at a concentration of  $100 \mu\text{g}\cdot\text{mL}^{-1}$ . Mortality was recorded at 24, 48, 72, and 96 h after treatment, with fresh dsRNA solution replenished every 24 h. The experiment included a treatment group (100 insects) and a control group (100 insects), totaling 400 insects. All other procedures followed the methodology described in Section 2.6.

### 1.9 Phototactic Behavior Assay in *M. usitatus*

The experiment was conducted under dark indoor conditions during daytime. Adult *M. usitatus* were fed on *dsEGFP*, *dsRh-1*, or *dsRh-2* at a concentration of  $100 \mu\text{g}\cdot\text{mL}^{-1}$  along with fresh cowpea leaves for 72 h prior

to evaluating their behavioral response to a green LED light source (wavelength: 520 nm). All ambient light sources in the laboratory were turned off to eliminate interference. Using an aspirator, adult *M. usitatus* were transferred into the insect introduction chamber (C) of the phototactic apparatus, which was then positioned at the center of the base plate (D). The phototactic chamber (A) and response chamber (B) were assembled onto the base plate (D), ensuring the introduction chamber remained centered within the response chamber. A monochromatic light source was affixed to the rear of the phototactic chamber (80 mm length  $\times$  50 mm width) and enclosed within a lightproof box to block external light. The light source (E) was activated for 10 min, after which the number of insects migrating into the illuminated phototactic chamber was recorded. The assay included five biological replicates, each comprising 30 insects. Following each trial, the apparatus was disinfected with 75% ethanol. Phototactic rate was calculated as: (Number of responsive insects/ Total number of introduced insects)  $\times 100\%$ .

**1.10 Cross-regulatory Expression Analysis of Opsin Genes Following RNA Interference in *M. usitatus*** Cross-regulatory expression analysis of opsin genes: Adult *M. usitatus* were fed on *dsEGFP*, *dsRh-1*, or *dsRh-2* solutions at  $100 \mu\text{g}\cdot\text{mL}^{-1}$  for 72 h, with fresh dsRNA solution replenished every 24 h. All other experimental procedures were consistent with the methodology described in Section 2.6.

**1.11 Data Analysis** Statistical analyses were performed using GraphPad Prism version 9.5.1 (GraphPad Software, Inc., USA). Student's *t*-test was employed to evaluate significant differences between two groups, while Tukey's honestly significant difference (HSD) test was applied for comparisons among three or more groups; survival data were analyzed by Kaplan-Meier method with Breslow-Wilcoxon test.

## 2 Results and Analysis

**2.1 Stage-specific Developmental Duration in *M. usitatus*** *M. usitatus* is a hemimetabolous insect,

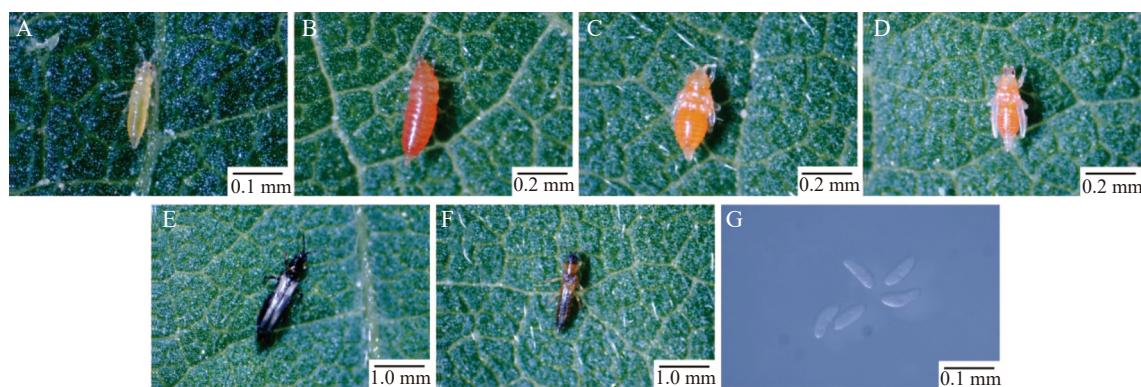
progressing through five developmental stages: egg, first-instar nymph, second-instar nymph, prepupa, pseudopupa, and adult (Fig. 2). The first-instar nymph measures approximately 0.5 mm in length, with a white or pale-yellow body and red punctate compound eyes (Fig. 2-A). The second-instar nymph is approximately 1 mm long, exhibiting an orange-red body and incompletely developed red punctate compound eyes (Fig. 2-B). The prepupa (1 mm in length) displays an orange-yellow body, gradually maturing compound eyes, and short, translucent white wing buds on the dorsum, with stubby, translucent antennae (Fig. 2-C). During the pseudopupal stage (1 mm in length), the orange-yellow body is close to adult morphology, featuring enlarged dorsal wing buds and antennae extending posteriorly (Fig. 2-D). Adult females (1–2 mm) are generally larger than males. Both sexes exhibit dark brown pigmentation along the anterior and posterior margins of the fringed wings and at the abdominal terminus, with creamy-white coloration elsewhere. Females possess a dark brown body, while males display a dark brown head and abdominal transverse bands, with the remaining body regions dark yellow (Fig. 2-E—F). Eggs are minute (0.1–0.2 mm), translucent, and white (Fig. 2-G).

**2.2 Quality Assessment of Synthesized dsRNA in *M. usitatus*** The synthesized *dsEGFP*, *dsRh-1*, and *dsRh-2* exhibited single target bands with correct molecular sizes (*dsEGFP*: 352 bp; *dsRh-1*: 427 bp; *dsRh-2*: 363 bp). The  $OD_{260}/OD_{280}$  ratios ranged

between 1.9 and 2.0, confirming high-quality dsRNA suitable for subsequent experiments in *M. usitatus* (Fig. 3).

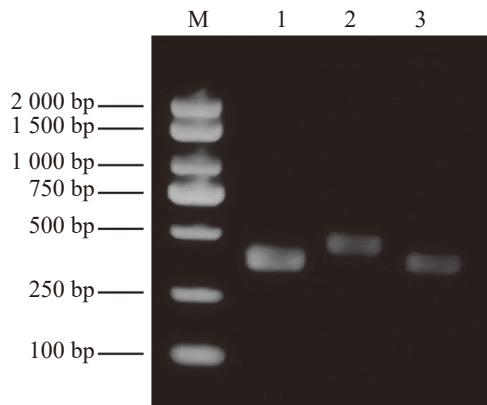
**2.3 Evaluation of Silencing Efficiency in *Rh-1* and *Rh-2* Opsin Genes of *M. usitatus*** After 24 h of feeding on *dsEGFP* and *dsRh-1* at a concentration of 1, 10, 100, 500, or 1000  $\mu\text{g}\cdot\text{mL}^{-1}$ , significant differences ( $P < 0.05$  or  $P < 0.01$ ) were detected in the relative expression of the opsin gene *Rh-1* in *M. usitatus*. The highest silencing efficiency (42.13%) was observed at 100  $\mu\text{g}\cdot\text{mL}^{-1}$ , followed by 26.73% at 500  $\mu\text{g}\cdot\text{mL}^{-1}$ . No significant changes ( $P > 0.05$ ) in *Rh-1* expression were observed in the groups treated with *dsEGFP* or *dsRh-1* at 1, 10, or 1000  $\mu\text{g}\cdot\text{mL}^{-1}$  (Fig. 4-A). When *M. usitatus* was fed on *dsRh-1* at the optimal concentration of 100  $\mu\text{g}\cdot\text{mL}^{-1}$  for 24, 48, 72, or 96 h, significant temporal variations ( $P < 0.05$  or  $P < 0.01$ ) in *Rh-1* expression were observed. Maximum silencing efficiency (53.87%) occurred at 72 h, followed by 43.30% at 24 h and 23.60% at 48 h. No significant difference ( $P > 0.05$ ) in *Rh-1* expression was detected between *dsEGFP* and *dsRh-1* treated groups at 96 h (Fig. 4-B).

After 24 h of feeding on *dsEGFP* and *dsRh-2* at a concentration of 1, 10, 100, 500, or 1000  $\mu\text{g}\cdot\text{mL}^{-1}$ , significant differences ( $P < 0.05$  or  $P < 0.01$ ) were observed in the relative expression of the opsin gene *Rh-2* in *M. usitatus*. The highest silencing efficiencies were 37.70% at 100  $\mu\text{g}\cdot\text{mL}^{-1}$  and 37.54% at 500  $\mu\text{g}\cdot\text{mL}^{-1}$ . In contrast, no significant changes ( $P > 0.05$ ) in *Rh-2* expression were detected in the groups treated with *dsEGFP* or *dsRh-2* at 1, 10, or 1000



A. First-instar nymph; B. Second-instar nymph; C. Pre-pupae; D. Pseudo-pupae; E. Adult females; F. Adult males; G. Eggs.

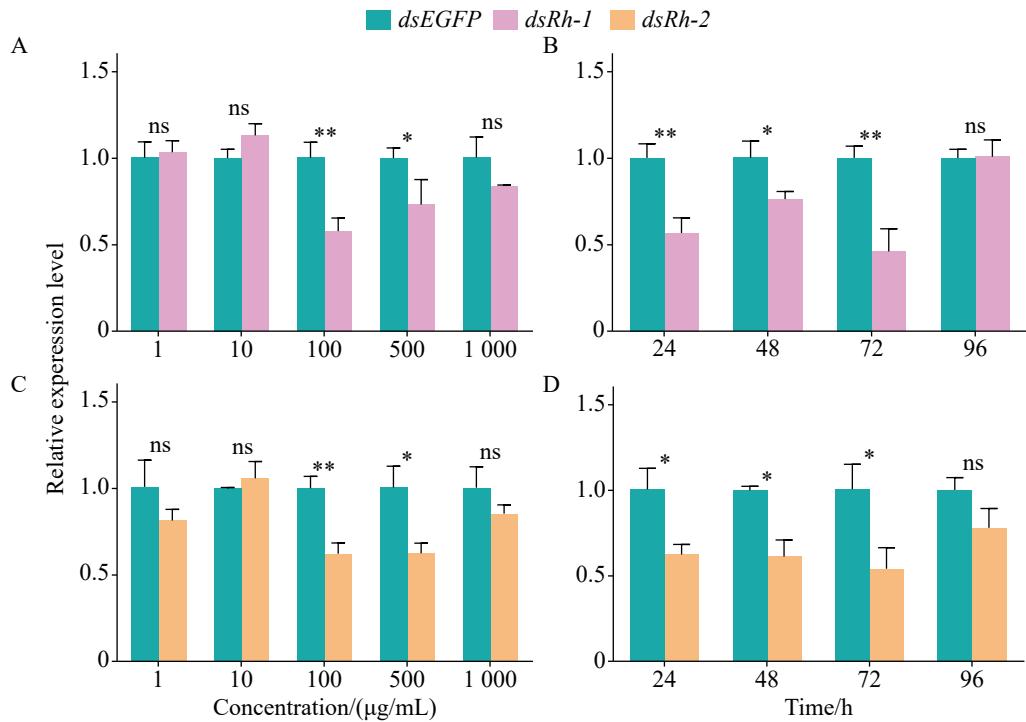
Fig. 2 Developmental stages of *M. usitatus*



**Fig. 3** Electrophoresis results for dsRNA detection M: Marker; 1: *dsEGFP*; 2: *dsRh-1*; 3: *dsRh-2*.

$\mu\text{g}\cdot\text{mL}^{-1}$  (Fig. 4-C). When *M. usitatus* was fed on *dsRh-2* at the optimal concentration of  $100 \mu\text{g}\cdot\text{mL}^{-1}$  for 24, 48, 72, or 96 h, significant temporal differences ( $P < 0.05$ ) in *Rh-2* expression were observed. Maximum silencing efficiency (45.82%) occurred at 72 h, followed by 38.79% at 48 h and 37.54% at 24 h. No significant difference ( $P > 0.05$ ) in *Rh-2* expression was detected between the *dsEGFP* and *dsRh-2* treated groups at 96 h (Fig. 4-D)

**2.4 Life Table Determination of *M. usitatus*** No significant differences in survival rates ( $P = 0.7862$ )



A. Feeding on *dsRh-1* at a concentration of 1, 10, 100, 500, or 1000  $\mu\text{g}\cdot\text{mL}^{-1}$ ; B. Feeding on 100  $\mu\text{g}\cdot\text{mL}^{-1}$  *dsRh-1* at 24, 48, 72 or 96 h; C. Feeding on *dsRh-2* at 1, 10, 100, 500 or 1000  $\mu\text{g}\cdot\text{mL}^{-1}$ ; D. Feeding on 100  $\mu\text{g}\cdot\text{mL}^{-1}$  *dsRh-2* at 24, 48, 72 and 96 h.

**Fig. 4** Knockdown efficiency of opsin genes *dsRh-1* and *dsRh-2* in *M. usitatus*

Note: Columns represent mean  $\pm$  SE; \* or \*\* indicates significant difference by *t* test ( $P < 0.05$  or  $P < 0.01$ ); ns indicates no significant difference.

were observed among *M. usitatus* adults treated with *dsEGFP* or *dsRh-1* at a concentration of 100  $\mu\text{g}\cdot\text{mL}^{-1}$  for 24, 48, 72, or 96 h (Fig. 5-A). Similarly, survival rates showed no significant difference ( $P = 0.5407$ ) between groups fed on *dsEGFP* and *dsRh-2* at a concentration of 100  $\mu\text{g}\cdot\text{mL}^{-1}$  over the same treatment duration (Fig. 5-B).

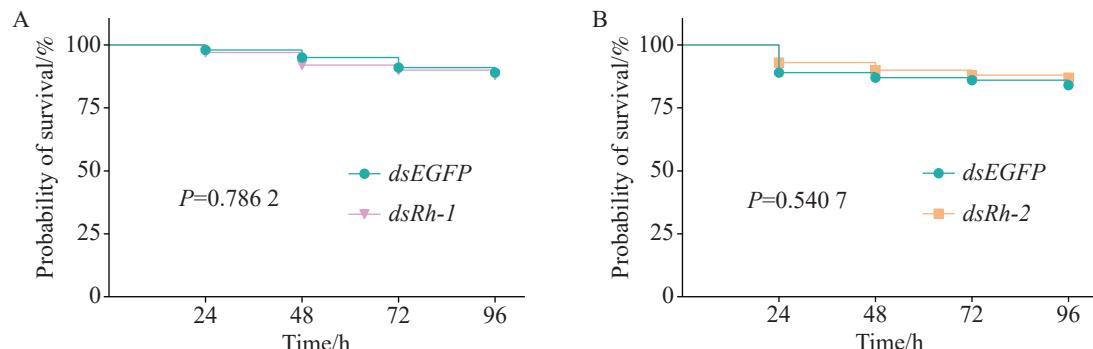
**2.5 Phototactic Behavior Assay and Opsin Gene Expression Analysis in *M. usitatus*** Adult *M. usitatus* were fed on fresh cowpea leaves or *dsEGFP*, *dsRh-1* or *dsRh-2* at a concentration of 100  $\mu\text{g}\cdot\text{mL}^{-1}$  for 72 h and subsequently assayed for phototactic response to a green LED light source (520 nm). No significant difference ( $P > 0.05$ ) in phototactic response rate was observed between the cowpea-fed control group and the *dsEGFP*-treated group (Fig. 6-A). In contrast, the *dsRh-1*-treated group exhibited a phototactic response rate of 26.67%, significantly lower than that of both the control groups ( $P < 0.05$ , Fig. 6-A). Similarly, the *dsRh-2*-treated group showed a rate of 32.00%, significantly lower than that of the cowpea-fed or *dsEGFP*-treated groups ( $P < 0.05$ , Fig. 6-B).

After feeding *M. usitatus* on *dsEGFP* and *dsRh-1* at  $100 \mu\text{g} \cdot \text{mL}^{-1}$  for 72 h (resulting in *Rh-1* knockdown), qRT-PCR analysis revealed no significant difference in the relative expression level of the other opsin gene *Rh-2* compared to the control group ( $P > 0.05$ , Fig. 6-B). Using the same method, feeding on *dsRh-2* at  $100 \mu\text{g} \cdot \text{mL}^{-1}$  (achieving *Rh-2* knockdown) similarly showed no significant difference in *Rh-1* expression as against the controls ( $P > 0.05$ , Fig. 6-C).

### 3 Discussion

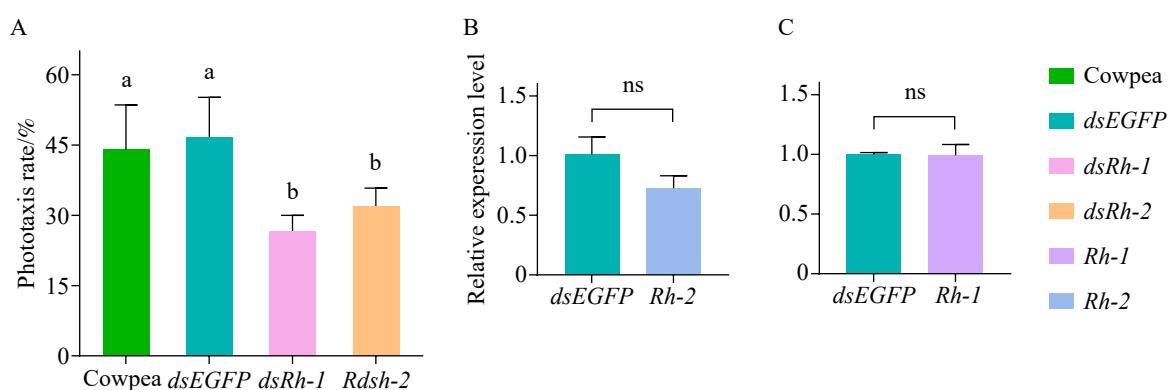
Upon reaching the adult stage, *M. usitatus* develops fully functional compound eyes, enabling acute visual perception. Concurrently, wing buds metamorphose into fringed wings, facilitating short-distance flight. Studies indicate that opsin genes are highly expressed in both female and male adults<sup>[19]</sup>.

Field-based color trapping experiments demonstrated that blue sticky traps captured the highest number of adults, with an average of 337 individuals attracted at between 08:00–10:00<sup>[22]</sup>. Laboratory assays revealed that UV-A light exhibits stronger attraction to adults compared to white, blue, or green light<sup>[23]</sup>. Additionally, exposure to 420 nm light significantly prolongs the nymphal and pseudopupal stages while reducing eclosion rates and adult survival<sup>[23]</sup>. Broad-spectrum illumination (460–730 nm) also markedly suppresses oviposition capacity in females<sup>[24]</sup>. These findings suggest a potential correlation between adult-stage opsin gene overexpression and heightened responsiveness to color-based trapping, as well as light-mediated disruptions to developmental processes likely linked to visual gene regulation. Consequently, visual-targeted control strategies during this critical life stage are essential for



A. *M. usitatus* was fed on  $100 \mu\text{g} \cdot \text{mL}^{-1}$  *dsRh-1* at 24, 48, 72 or 96 h, and its survival rate was calculated;  
B. *M. usitatus* was fed on  $100 \mu\text{g} \cdot \text{mL}^{-1}$  *dsRh-2* at 24, 48, 72 or 96 h, and its survival rate was calculated.

**Fig. 5 Survival rate of *M. usitatus* after feeding on *dsEGFP*, *dsRh-1*, or *dsRh-2***  
Note: Survival curves were plotted by Kaplan-Meier method, and compared using Breslow-Wilcoxon test ( $P < 0.05$ ).



A. Behavioral assay; B. Relative expression level of *Rh-2* after *Rh-1* knockdown; C. Relative expression level of *Rh-1* after *Rh-2* knockdown.

**Fig. 6 Feedback regulation between phototactic behavior and opsin genes in *M. usitatus***

Note: Columns represent mean  $\pm$  SE; a or b indicates significant difference by Tukey test ( $P < 0.05$ ); ns indicates that there is no significant difference by t test ( $P > 0.05$ ).

effective management of *M. usitatus*. Innovative approaches integrating chromatic traps and photoperiod modulation could enhance eco-friendly control efficacy against this pest species.

Studies utilizing RNA interference (RNAi) to silence insect opsin genes remain relatively limited. Chen et al. successfully employed CRISPR/Cas9 to knockout the *LW-opsin* gene in *Plutella xylostella*, creating a LW-13 mutant line that showed significantly reduced phototactic responses to seven light spectra (white, ultraviolet, blue, yellow, green, red, and infrared) under 2.5 lx illumination<sup>[25]</sup>. Similarly, Li et al. achieved 44.79%, 54.81%, and 43.00% reductions in phototactic behavior towards ultraviolet, blue, and green light, respectively in *Diaphorina citri* through oral delivery of dsRNA targeting opsin genes *Dc-UV*, *Dc-BW*, and *Dc-LW*<sup>[26]</sup>. Wang et al. demonstrated that microinjection of *Rh6* targeting dsRNA in *Bactrocera minax* adults significantly decreased their preference for green substrates and reduced oviposition on immature (green) citrus fruits compared to mature (yellow) controls<sup>[27]</sup>. Complementary findings by Liu et al. showed that injecting 6  $\mu$ g of *Se-lw* specific dsRNA into each compound eye of *Spodoptera exigua* led to a 57.1% decrease in green light phototactic after 15 min exposure to green LEDs<sup>[28]</sup>. Our study corroborates these findings, demonstrating that feeding *M. usitatus* adults on *dsRh-1* or *dsRh-2* at 100  $\mu$ g·mL<sup>-1</sup> for 72 h significantly reduced their phototactic preference for green light (520 nm). This RNAi-mediated suppression of opsin genes effectively alters spectral sensitivity in this pest species. The practical application of this approach could be particularly valuable for protecting cowpea (*Vigna unguiculata*) crop, as the plants maintain green pigmentation throughout their growth cycle, making them potentially less attractive to opsin-silenced *M. usitatus* populations. In follow-up assays, *M. usitatus* adults fed on *dsRh-1* or *dsRh-2* at 100  $\mu$ g·mL<sup>-1</sup> for 24, 48, 72, or 96 h exhibited no significant differences in survival rate compared to the *dsEGFP* treated control group ( $P > 0.05$ ),

confirming that RNAi mediated opsin silencing, unlike chemical pesticides, avoids broad-spectrum toxicity and ensures agricultural safety. After feeding on *dsRh-1* at 100  $\mu$ g·mL<sup>-1</sup> for 72 h, the relative expression level of opsin gene *Rh-2* showed no significant difference compared to the control group ( $P > 0.05$ ). After feeding on *dsRh-2* using the same method, the relative expression level of *Rh-1* also showed no significant difference ( $P > 0.05$ ). These suggest that *Rh-1* and *Rh-2* may possess independent regulatory regions and exhibit autonomous expression regulation mechanisms. However, deeper functional operations of opsin genes and related synergistic effects require further verification. Wang Yaohui reported that after interfering with opsin genes in *Bactrocera minax* and *Bactrocera dorsalis*, the expression levels of the target opsin genes were significantly down-regulated, while other opsin genes showed no significant changes, consistent with the results of this study<sup>[29]</sup>. This indicates that independent expression of opsin genes likely reduces the complexity of visual signal integration, enabling each opsin gene to maintain independent spectral sensitivity, which can be validated by methods such as electrophysiology. qRT-PCR results by Liu et al. demonstrated that after knocking down the opsin gene *Se-lw*, the expression levels of other opsin genes increased, differing from the results of this study<sup>[28]</sup>. The discrepancy may be related to neural feedback compensation mechanisms of opsin *Se-lw*, insect species, and experimental methods.

This study demonstrates that RNA interference (RNAi)-mediated silencing of opsin genes in adult *M. usitatus* alters their spectral sensitivity. These findings provide foundational data for developing visual disruptants and phototactic-based control strategies targeting this pest species.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# 敲低视觉基因对普通大蓟马趋光行为的影响

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**摘要:** 为明确普通大蓟马 *Megalurothrips usitatus* 视蛋白基因的功能, 观察了普通大蓟马各发育历期及复眼特点, 并基于 RNAi 技术敲低了成虫视蛋白基因 *Rhodopsin-1* (*Rh-1*) 和 *Rhodopsin-2* (*Rh-2*), 测定了敲低后成虫对 520 nm 绿光的趋光行为变化, 以及 *Rh-1* 和 *Rh-2* 之间可能的相互调节作用。结果表明, 普通大蓟马复眼在成虫期发育完全; 饲喂质量浓度为 100  $\mu\text{g}\cdot\text{mL}^{-1}$  的 *dsEGFP*、*dsRh-1* 和 *dsRh-2* 在 24、48、72 和 96 h 后, 生命曲线无显著差异 ( $P>0.05$ ); 在饲喂 72 h 时, 普通大蓟马成虫对绿光趋向选择率下降至 26.67% 和 32.00%; 敲低 *Rh-1* 后, *Rh-2* 的相对表达量与对照组比较无显著差异 ( $P>0.05$ ); 敲低 *Rh-2* 后, *Rh-1* 的相对表达量也未见显著差异 ( $P>0.05$ )。研究结果以期为基于昆虫视觉系统的防治策略提供新思路。

**关键词:** 普通大蓟马; 视蛋白; RNAi; 发育历期

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