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Odorant receptor 75 is essential for attractive response to plant volatile *p*-anisaldehyde in Western flower thrips

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ABSTRACT

The Western flower thrip (WFT), *Frankliniella occidentalis,* is a major pest of many vegetable crops and also a vector for the tomato spotted wilt virus, causing devastating damage worldwide. Odorant receptors (ORs) play an important role in host plant searching, however, specific functions of those ORs in WFT remain unclear. In this study, the attractive activity of four plant volatiles ((*S*)-(−)-verbenone, *p*-anisaldehyde, methyl isonicotinate, and benzaldehyde) to WFT was confirmed using a Y-tube olfactometer. Then, the specific receptor, OR75, was screened out as the candidate OR for these odorants, as its expression was significantly upregulated upon exposure to these odorants. Further *in vitro* functional assays with *Xenopus* oocyte expression system confirmed sensitivity of OR75 to *p*-anisaldehyde and three other odorants (*β*-ionone, undecanal and cinnamaldehyde). Of the three odorants, *β*-ionone was also attractive to WFT. Further, *in vivo* RNA interference experiments showed that the dsOR75 treated thrips lost their attractive response to *p*-anisaldehyde, but retained response to *β*-ionone. Finally, 3-D structures prediction and molecular docking showed that OR75 formed a hydrogen bond with *p*-anisaldehyde at His150 residue, while no hydrogen bond formed with *β*-ionone, undecanal or cinnamaldehyde. Taken together, OR75 plays a crucial role in perception of *p*-anisaldehyde, which helps us understand the host-seeking mechanisms of WFT, and provides a basis for development of olfactory based pest control strategies. This is the first report of an OR playing roles in sensing *p*-anisaldehyde in thrips.

1. Introduction

Insect olfaction plays crucial roles in feeding, predator defense, and mating, all of which are essential for the survival (Hansson and Stensmyr, 2011). The olfactory detection process is mediated by multiple proteins. Among which, odorant receptors (ORs) are essential for the sensitivity and specificity of insect olfaction. The ORs are responsible for converting chemical signals into electrical signals that are further transmitted to the central nervous system for decoding and regulation of behavior or physiology (Kaupp, 2010). Typically, each insect genome encodes a canonical odorant receptor-coreceptor (Orco) and a variable number of conventional ORs (Sato et al., 2008; Suh et al., 2014). Insect ORs can be activated by multiple odorants, and a single odorant can activate several ORs, allowing insects to detect a broad array of odorants (Fleischer et al., 2018). Recent structural studies have revealed that insect ORs operate through insect specific heteromeric ligand-gated ion channels, composed of one OR subunit and three Orco subunits, working in concert to mediate olfactory signal processing (Zhao et al., 2024; Wang et al., 2024). While a large number of ORs have been found in many insect species by taking advantages of DNA and RNA sequencing techniques (Sheng et al., 2017), the detailed functions of most ORs remain unknown. Currently, the techniques for functional study of insect ORs are generally of two types - *in vitro* and *in vivo*. The *in vitro* techniques typically involve expression systems such as human embryonic kidney 293 cells (HEK293 cells), *Spodoptera frugiperda* cells (*Sf9* cells), and *Xenopus* oocytes, whereas *in vivo* techniques are commonly

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based on the *Drosophila* empty neuron system, RNA interference (RNAi), and clustered regularly interspaced short palindromic repeats (CRISPR) approaches. Among these, the *Xenopus* oocyte system and RNAi methods are the two most commonly used (Peterlin et al., 2014; Zhang et al., 2022; Guo et al., 2020). Clarifying the function of insect ORs is of great importance for understanding the mechanisms determining the sensitivity and specificity of insect olfaction, and for developing new strategies used in pest control.

The western flower thrips (WFT), *Frankliniella occidentalis* (Thysanoptera), is a polyphagous pest, damaging over 200 plant species in 66 families (Liang et al., 2010). It damages crops not only by direct feeding, but also by transmitting of various viral diseases caused by Tospoviruses (Rotenberg et al., 2015). Due to the minuteness and high pesticide resistance, WFT is difficult to control, while plant-derived attractants emerge to be one of the prosperous alternatives for the control. At present, the most-used compounds are those based on pyridines, which show a high attractiveness to WFT (Díaz et al., 2023). In addition, *p*-anisaldehyde (Davidsonz et al., 2008), benzaldehyde (Teulon et al., 1993; Koschier et al., 2000), methyl isonicotinate (Teulon et al., 2017; Teulon et al., 2011), and (*S*)-(−)-verbenone (Abdullah et al., 2015) have demonstrated to be highly effective in trapping WFT. Further study is needed to elucidate the molecular mechanisms for better use of these bioactive compounds, and for development of new control techniques targeting the key genes in the WFT olfaction.

A comprehensive analysis of the genome data has identified 84 ORs, 102 gustatory receptors (GRs), and 176 ionotropic receptors (IRs) in WFT, as reviewed in the literature (Rotenberg et al., 2020). However, specific functions of those ORs remain completely unknown. In the present study, to identify the ORs responsible for detecting highly attractive odors, we validated the significant attraction of WFT to the four aforementioned plant volatiles. Subsequently, ORs with significantly altered expression levels were identified following exposure to the odorant mixture, and their functions were characterized using *Xenopus* oocyte expression system and RNAi technique. Finally, the key amino acids interacting with ligands were predicted through 3-D structure modeling and molecular docking.

2. Materials and methods

2.1. Insect rearing

The WFT was provided by Prof. Fa-Jun Chen's laboratory (Nanjing Agricultural University, China) in March 2022, and then reared with fresh sword beans in plastic boxes (19 × 15 × 11 cm) in laboratory. The rearing conditions were 26 ± 1°C, 14/10 h (light/dark) photoperiod and (50 ± 5) % humidity. Tender bean pods deposited with eggs were collected daily and transferred to separate boxes to ensure the same age of thrips in a box. Fresh bean pods were replaced daily for adult feeding and oviposition.

2.2. Behavioral response of thrips to plant volatiles

A glass Y-tube olfactometer (Fig. 1A) was used to perform behavioral assays. A filter paper strip (1 × 2 cm) loaded with 10 μL of volatile solution was placed in one side arm, while the paper strip with an equal volume of solvent (hexane) was placed in the other side arm of the olfactometer. The paper strips were allowed to evaporate for 5 min before being placed in the olfactometer. Ten adult female thrips pretreated by 4 h starvation were introduced into the central arm. Thrip selection behavior was monitored and recorded over a 5-min observation period. If a thrip entered into one side arm halfway and stayed for at least 30 s, it was recorded as choosing that arm; otherwise, the thrip was scored as "no choice". Each volatile was tested for 12–15 times (replicates).

2.3. Odorant exposure, tissue collection, and RNA extraction

To screen the candidate ORs that might be involved in sensing of the four odorants, the approach of deorphanization of receptors based on expression alterations in mRNA levels (DREAM) was used (Zhang et al., 2022). Female thrips were exposed to an odorant mixture in a plastic box (14 × 9 × 5 cm) with a hole (0.25 cm^2) sealed by nylon mesh. The odorant mixture was prepared with hexane as solvent, containing the following components: (*S*)-(−)-verbenone (2.5 %, m/v), *p*-anisaldehyde (2.5 %, m/v), methyl isonicotinate (0.25 %, m/v), and benzaldehyde (2.5 %, m/v). To do the exposure experiment, first, 210 μL odorant

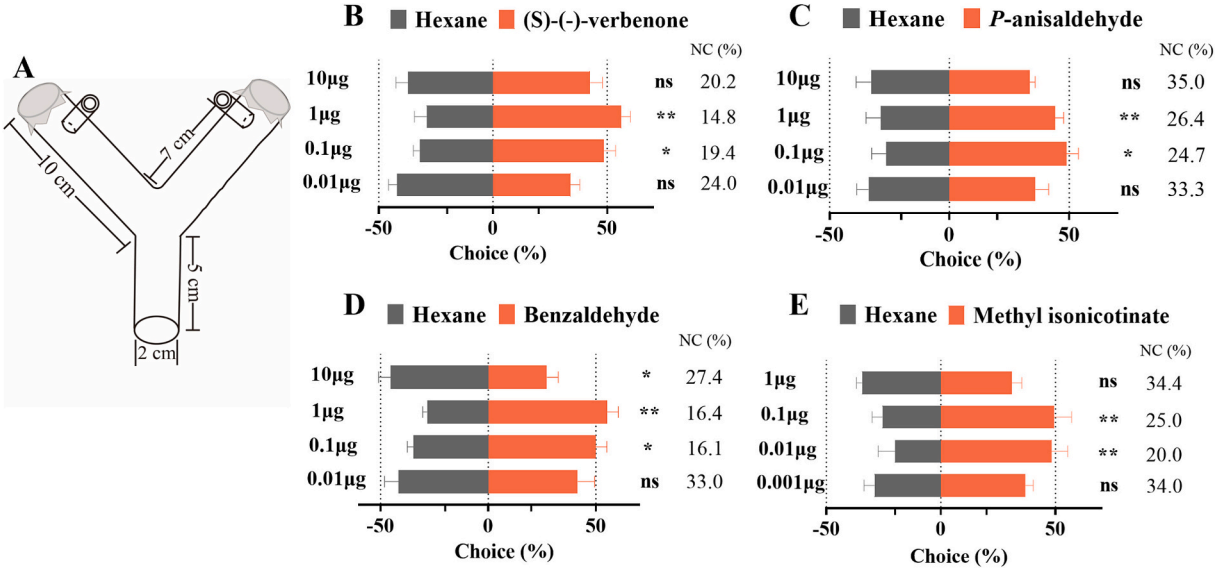

Fig. 1. Behavioral assays of adult *Frankliniella occidentalis* to (*S*)-(−)-verbenone, *p*-anisaldehyde, methyl isonicotinate, and benzaldehyde. (A) Schematic diagram of the Y-tube olfactometer. (B-E) Choice percentages for (s)-(−)-verbenone, *p*-anisaldehyde, benzaldehyde and methyl isonicotinate, respectively (n = 12–15), with hexane as the control. "NC" means percentage of the thrips that did not make a choice. Error bars represent SEM. "*" means significant (*t*-test; * $P < 0.05$, ** $P < 0.01$) and "ns" means no significant difference between the odorant treated and the control.

solution was applied to a filter paper disc (7 cm diameter) in a petri dish (7 cm diameter), and evaporated for 10 min. Then the dish was covered by a parafilm with 20 small holes (1 mm^2), and put into the box. Third, 350 female thrips were placed in the box after 4 h of starvation, starting the exposure for 5 h. Same treatment using 210 μL hexane was used as the control (Fig. S1).

After the exposure, 300 thrips heads were collected and placed into liquid nitrogen immediately. Total RNA was extracted using Trizol Reagent (Invitrogen, USA) following the manufacturer's protocol. The RNA samples were sequenced using Illumina Novaseq 6000 PE50 platform by Novogene Bioinformatics Technology Co. Ltd. (Beijing, China).

2.4. cDNA synthesis, rapid-amplification of cDNA ends (RACE) and gene cloning

First single-strand cDNAs were synthesized from 1 μg of total RNA using HiScript II RT SuperMix (Vazyme, China). RNA and cDNA were stored at − 80 °C and − 20 °C, respectively. As the available Orco in the WFT (accession number: XP_052123992.1) was not a complete sequence, RACE was used to amplify full length of the cDNA. Specific RACE primers for Orco (Table S1) were designed using the platform (appbi.vazyme.com:8085), and the PCR reaction was conducted by following the manufacturer's protocol (Vazyme, Nanjing). The amino acid sequence of OR75 (GenBank number: KAE8747374.1) was confirmed by PCR cloning and sequencing. The reactions were carried out using an Eppendorf Thermal cycler (Eppendorf, Germany) under the following conditions: 95 °C for 3 min; 35 cycles of 95 °C for 15 s, 55 °C for 15 s, 72 °C for 3 min; 72 °C for 10 min. The PCR products were visualized by 1.0 % agarose gel electrophoresis, then purified using the FastPure Gel DNA Extraction Mini Kit (Vazyme, Nanjing). The purified products were ligated into the pEASY-Blunt3 vector (TransGen Biotech, Beijing) and sequenced using single clones.

2.5. Functional study of ORs using Xenopus oocyte expression and two-electrode voltage clamp recording system (XOE-TEVC)

The pGH19 plasmid was digested with the restriction endonucleases of *Xba* I and *EcoR* I. The primers containing the cutting sites and the homologous sequence of pGH19 plasmid (Table S1) were designed, then the PCR products and pGH19 expression vector were recombined by ClonExpress One Step Cloning Kit (ATG Biotechnology, China). The verified recombinant plasmids were linearized by *Not* I. The purified enzyme digestion product was used as a template to synthesize cRNA using the T7 High Yield RNA Transcription Kit (Vazyme, China). After purification, the cRNAs were diluted to 2000 ng/μL with nuclease-free water and stored at − 80 °C until time of use. The treatment, microinjection and culture of *Xenopus* oocytes were the same as the previous study (Liu et al., 2019). All the odor chemicals were prepared into 10^{-1} M stock solutions with dimethyl sulfoxide (DMSO). Odorant mixtures containing odorants of similar structure were made (Table S2). The stock solutions were diluted in standard oocyte saline (SOS) buffer (100 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6) before the experiments. The cell currents induced by odors were recorded with the TEVC System (AutoMate Scientific Inc., USA).

2.6. RNAi assay to knockdown the expression of OR75

The dsRNA primers for OR75 and the control (GFP) gene (Table S1) were designed using SnapDragon (https://www.flyrnai.org/cgi-bin/RNAi_find_primers.pl). The primers were synthesized using the T7 Ribomax Express RNAi system following the manufacturer's protocol (Promega, Madison, USA). Thereafter, RNAi was conducted on the WFT *via* the feeding method according to Han et al. (2019). Leaf discs from 2-week-old bean plants were soaked in 200 μL dsRNA solution for 24 h, and then used to feed 60 three-day-old adults in a centrifuge tube (2.8 cm in diameter and 11.6 cm in length). The bottom end was poured with 1 mL gel solution, and when the gel was polymerized, a leaf disc (0.8 cm^2) was put on the gel. The open end of the tube was sealed with a 200-mesh gauze (Fig. 4A). To determine the optimal concentration of dsRNA solution and treatment time, three dsRNA concentrations (50, 150, and 750 ng/μL) were used, and 20 thrips were sampled every 24 h to assess RNAi efficiency using the quantitative polymerase chain reaction (qPCR) with EF-1α and *β*-actin as reference genes (Zheng et al., 2014). Three biological replicates were conducted. The qPCR primers (Table S1) were designed by Beacon Designer 7 (PRIMER Biosoft International, USA). The qPCR was performed with QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, USA) and the reactions in 20 μL with ChamQ™ Universal SYBR qPCR Master Mix (Vazyme, China) according to the instruction manual. The reaction conditions were 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 60 °C for 34 s, and gene expression levels were analyzed using the $2^{-\Delta\Delta CT}$ method.

2.7. Structural prediction and molecular docking experiments

The 3-D structures of the ORs were modeled using AlphaFold 3.0 (https://alphafoldserver.com/welcome), and Vina 1.1.2 (Trott and Olson, 2010) was used for docking analysis. The receptor protein and ligand structures were pre-treated using AutoDock 4.2.6 (Morris et al., 2008). The docking parameters were set according to the protein structure and active sites, and the optimal docking model was determined based on energy binding affinities. Docking models were imported into Pymol for analysis and image processing (DeLano, 2002).

3. Results

3.1. The four plant volatiles were all attractive to WFT

Previous studies reported that WFT are attracted to four plant volatiles ((*S*)-(−)-verbenone (Abdullah et al., 2015), *p*-anisaldehyde (Davidsonz et al., 2008), methyl isonicotinate (Teulon et al., 2017), and benzaldehyde (Teulon et al., 1993). Our behavioral assays using a Y-tube olfactometer confirmed this attractive behavior, in which the female thrips significantly preferred the arm with the filter paper loaded with plant volatile dosages (Fig.1A). However, the sensitivity of thrips to the four odorants varied, with methyl isonicotinate displaying significant attraction at a dose of 0.01 μg, while the other three odorants exhibited noticeable attraction at 0.1 μg. In addition, benzaldehyde showed a repellent effect on the thrips at high dosage of 10 μg (Fig.1B-E).

3.2. OR75 showed significant response to p-anisaldehyde

To screen the ORs involved in detecting the four plant volatiles, the DREAM approach was applied (Zhang et al., 2022), and the mRNA expression levels of ORs were analyzed using NanoString nCounter (Geiss et al., 2008). According to the standard of $|Log_2FoldChange| \geqq 2$ and *P*-value <0.05, 187 differentially expressed genes (DEGs) were identified by comparing the odorants-treated and the control thrips, among which 33.69 % (63 genes) were up-regulated and 66.31 % (124 genes) were down-regulated (Fig. S2). However, only one OR (OR75) meet the standard, displaying a significant up-regulation of 4.36 Log_2-FoldChange; and in addition, four more ORs meet the threshold of $|Log_2FoldChange| \geqq 2$, or *P*-value of less than 0.05 (Table S2).

Further, the XOE-TEVC assay was performed to test the response of OR75 to the four plant volatiles with the Orco agonist VUAA1 as the positive control, showing that OR75 was specifically sensitive to *p*-anisaldehyde among the four volatiles (Fig. 2A and B). Dose-response analysis revealed a response current exceeding 100 nA at a concentration of 10^{-2} M, with a calculated EC_{50} value of 2.44×10^{-3} M (Fig. 2C and D). To determine the ligand specificity of OR75, 63 additional odorants (18 alcohols, 20 aldehydes, 9 ketones and 16 esters) were further tested, firstly using odorant mixture (Fig. 2A and B), and then the

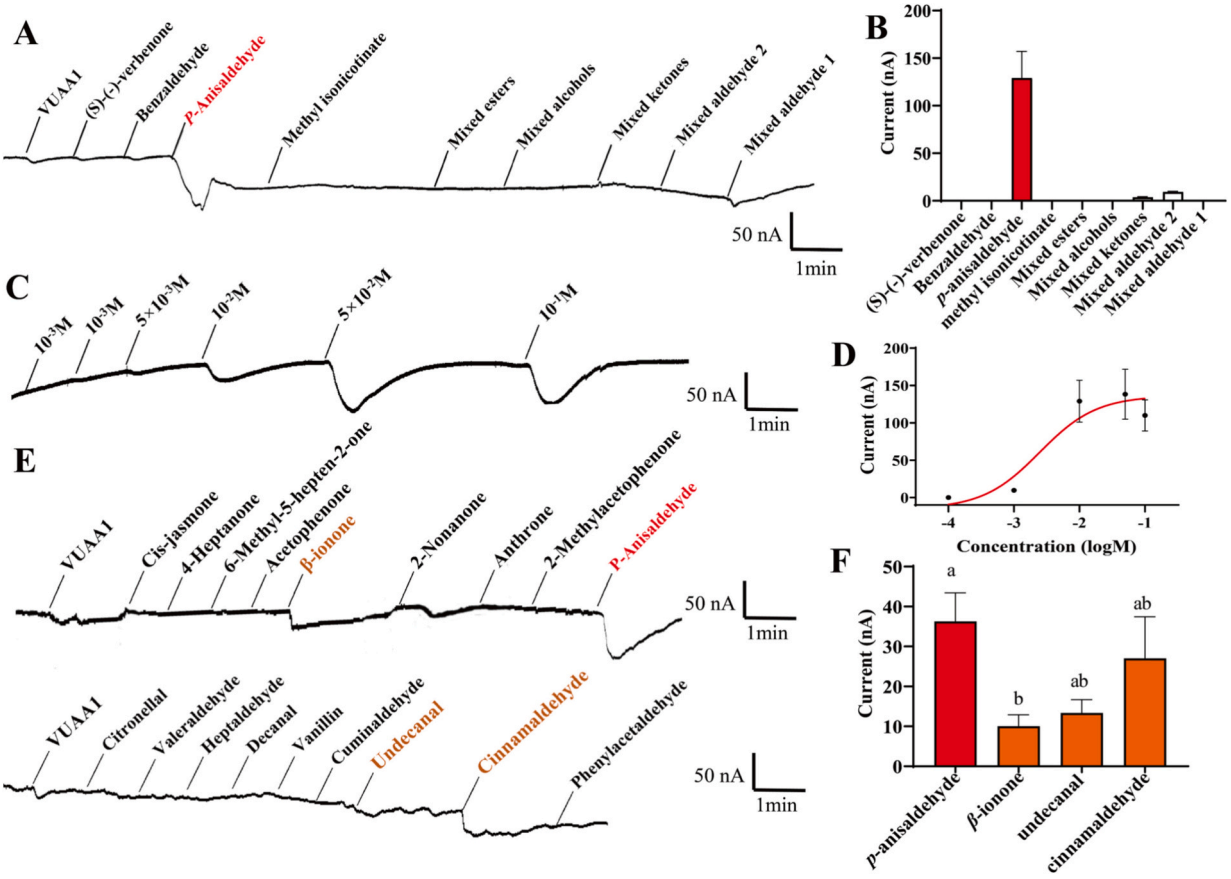

Fig. 2. Two-electrode voltage clamp recording of *Xenopus* oocytes expressing OR75/Orco. (A) A representative recording of the currents induced by four volatiles and five volatile mixtures, with the Orco agonist VUAAI as the positive control ($n = 3$). The test concentration is 10^{-4} M for the mixtures and VUAA1, and 10^{-2} M for the four individual odorants. (B) Responses induced by the four odorants and mixed odorants (n = 3). (C) A representative recording of the currents induced by a range of *p*-anisaldehyde doses ($n = 5$). (D) The dose-response curve to *p*-anisaldehyde. (E) A representative recording of the currents induced by individual odorants of mixed aldehyde 1 and mixed ketones (n = 3), with four volatiles that induced a response being highlighted in red. The test concentration is 10^{-4} M for VUAA1 and 10^{-3} M for the odorants. (F) Currents induced by the four odorants at a 10^{-3} M concentration (n = 3). Different letters indicate significant differences among odorants (one-way ANOVA followed by Fisher's LSD test, $P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

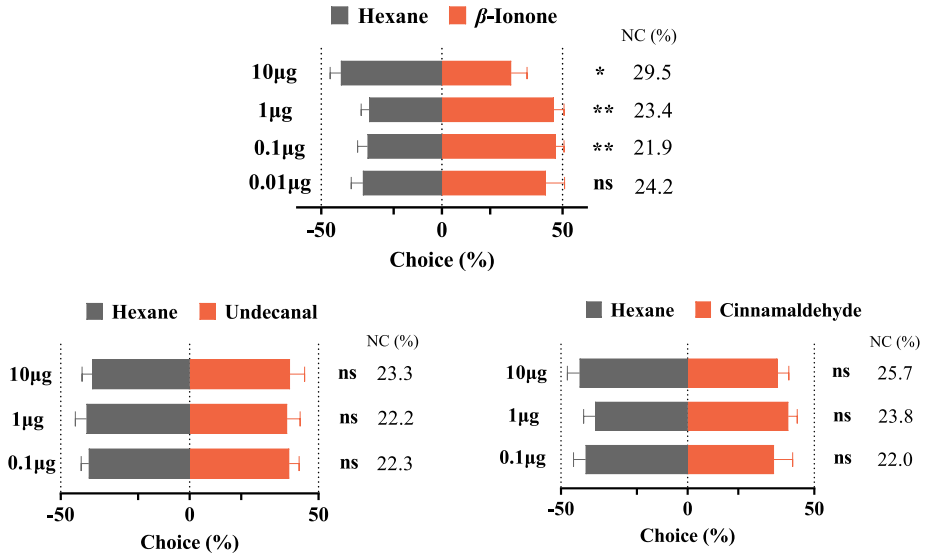

Fig. 3. Behavioral assays of adult *Frankliniella occidentalis* to *β*-ionone (A), undecanal (B), and cinnamaldehyde (C) using Y-tube olfactometer assays (n = 12–15). "NC" means thrips that did not make a choice. Error bars represent SEM. "*" means significant (*t*-test; * $P < 0.05$, ** $P < 0.01$) and "ns" means no significant difference between the odorant treated and the control.

individual odorants if the mixture induced response. Results revealed that OR75 showed sensitivity only to β-ionone, undecanal, and cinnamaldehyde among the odorants of the mixtures, but the responses were lower than that to *p*-anisaldehyde (Fig. 2E and F).

3.3. β-ionone is less attractive to WFT

As mentioned above in the XOE-TEVC assay, OR75 also responded to β-ionone, undecanal and cinnamaldehyde. To test the behavioral response of the WFT to these three volatiles, Y-tube behavioral assay was conducted. It showed that female thrips significantly preferred the arm with β-ionone (0.1 and 1 μg), but showed no preference for undecanal and cinnamaldehyde at the tested dosages (0.1–10 μg) (Fig. 3).

3.4. Knockdown of OR75 significantly decreased preference for p-anisaldehyde and β-ionone

To verify the *in vivo* role of OR75 in the perception of *p*-anisaldehyde and β-ionone, a RNAi experiment was conducted with adult thrips. The qPCR results showed that a significant reduction in expression of OR 75 was observed in dsOR75 treated thrips compared to dsGFP treated control at three days, showing a reduction by 74.90 % and 78.00 % for the dsOR75 concentrations of 150 and 750 ng/μL, respectively (Fig. 4A, B). Therefore, the thrips were used to do the behavioral assay three days after treatment with dsOR75 (750 ng/μL in concentration).

In the behavioral assays, female adults treated with dsOR75 lost the preference to *p*-anisaldehyde, while thrips in the two control groups retained the preference. However, to β-ionone, dsOR75 treated thrips still showed the preference, but the preference index (9.82 %) was much lower than that of the dsGFP treated (23.53 %) and non-dsRNA treated (20.89 %) control thrips (Fig. 4C, D).

3.5. Amino acid His150 of OR75 formed a hydrogen bond with p-anisaldehyde

As the identities between OR75 and published 3-D structures of OR5 in *Acyrthosiphon pisum* (Wang et al., 2024), OR28 in *Anopheles gambiae* and OR10 in *Aedes aegypti* (Zhao et al., 2024) were only 6.09 %, 8.90 % and 10.91 %, respectively, AlphaFold 3.0 was used to predict the heterotetrameric structure of OR75 with Orco, to determine the key amino acid residues in OR75 that bind *p*-anisaldehyde and other three ligands. The accuracy of the predicted structure was assessed using a PROCHECK Ramachandran plot, which showed that 95.1 % of the residues in the predicted structure were located in favored region A, B and L (Fig. S3). Further molecular docking analysis revealed that OR75 displayed a pocket-like cavity that binds *p*-anisaldehyde with a binding energy of −5.3 kcal/mol, and residue His150 formed a hydrogen bond with *p*-anisaldehyde (Fig. 5). In contrast, OR75 did not form hydrogen bond with β-ionone, undecanal or cinnamaldehyde. Other amino acids close to the ligands (<4.0 Å) were also predicted, showing additional differences in number and position of the amino acids among the volatiles (Table S4).

4. Discussion

Plant volatiles are important for insects to locate their host plants. In the present study, we confirmed the attractive effects of four plant volatiles ((*S*)-(−)-verbenone, *p*-anisaldehyde, methyl isonicotinate, benzaldehyde) to WFT, and obtained the candidate odorant receptor (OR75) for perception of these volatiles by the DREAM approach. Further *in vitro* and *in vivo* functional studies showed that OR75 is essential for the perception of *p*-anisaldehyde, and His150 was predicted as a potential key amino acid in binding with *p*-anisaldehyde. Our results provide insights into the molecular mechanisms of host plant finding, and basis for development of olfaction-based pest control techniques in WFT.

P-anisaldehyde, also known as 4-methoxybenzenaldehyde, is a

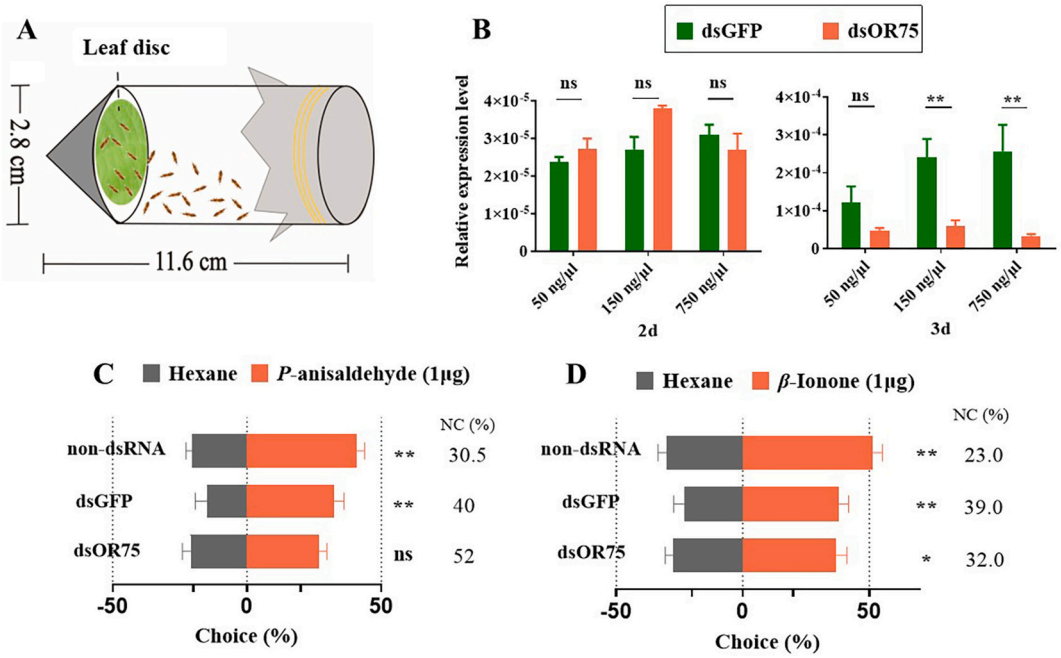

Fig. 4. RNAi of OR75 and the response of *Frankliniella occidentalis* to *p*-anisaldehyde and β-ionone. (A) Schematic of the dsRNA feeding apparatus. (B) RNAi efficiency of thrips treated with dsRNA of different concentrations for two (left) and three (right) days, respectively ($n = 4$). (C—D) Behavioral response of dsOR75-treated adults in a Y-tube olfactometer to *p*-anisaldehyde and β-ionone at 1 μg dosage ($n = 10$), with hexane as the control. "NC" means thrips that did not make a choice. Error bars represent SEM. "*" means significant (*t*-test; * $P < 0.05$, ** $P < 0.01$) and "ns" means no significant difference between the odorant treated and the control.

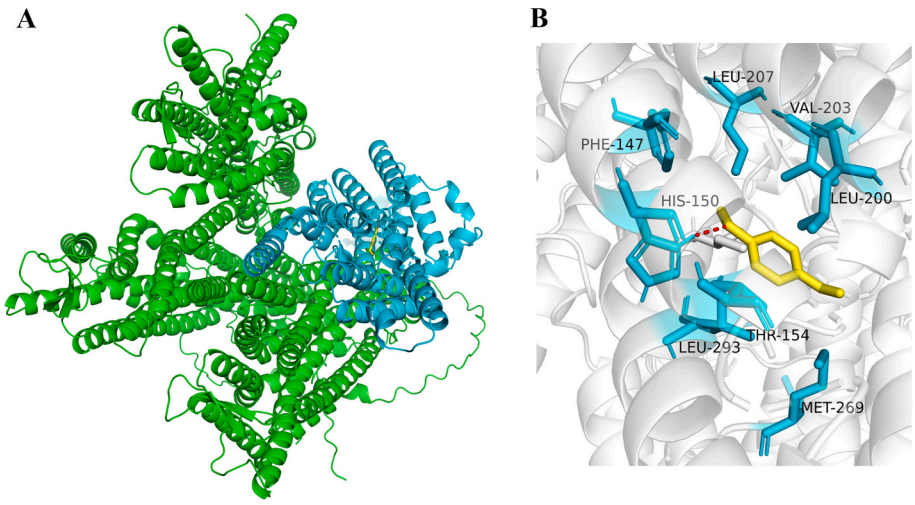

Fig. 5. Molecular docking of OR75/Orco with *p*-anisaldehyde, showing key amino acids interacting with the ligand (within 4 Å). (A) The heterotetrameric structure of OR75 (in blue)/Orco (in green). (B) The residue His150 forms a hydrogen bond (represented by a red dotted line) with *p*-anisaldehyde (in yellow). The other amino acids within 4 Å to *p*-anisaldehyde are shown as blue sticks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

volatile released by various plant species in about 10 families such as Geraniaceae and Liliaceae (Knudsen et al., 2006). *P*-anisaldehyde plays different behavioral roles in different insects, being repellent for mosquitoes (Showler and Harlien, 2019; Hao et al., 2013; Hao and Du, 2008), but attractive to the horn fly (*Haematobia irritans*) (Showler and Harlien, 2018) and the carpet beetle (*Anthrenus verbasci*) (Imai et al., 2002). In Thysanoptera insects, adding *p*-anisaldehyde to water or sticky traps can significantly increase the captures of thrips of six species including *Thrips vulgatissimus*, *T. tabaci, T. major, T. pillichi, T. hawaiiensis* and *frankliniella intonsa* (Kirk, 1985; Murai et al., 2000). In female *Dendrothrips minowai,* the capture numbers on sticky traps baited with *p*-anisaldehyde was significantly higher than the solvent control in tea plantations (Xiu et al., 2022; Xu et al., 2024). *β*-ionone is also a volatile released from flowers, fruits, and vegetables, displaying attractive (Wei et al., 2005; Hammack, 2001) or repellent (Li et al., 2019) effects on insects, as well as antimicrobial and antifungal properties (Paparella et al., 2021). Our study demonstrates that *p*-anisaldehyde and *β*-ionone are significantly attractive to WFT, suggesting their potential use in the pest control. Further studies related to the application are required, such as development of an attractant used either alone or combination with the blue board technique (Sampson and Kirk, 2013; Abdullah et al., 2015) to enhance the trap efficiency.

ORs play a key role in chemosensory signal transduction, significantly contributing to the sensitivity and specificity of insect olfaction (Kaupp, 2010; Andersson et al., 2015). By using the XOE-TEVC assay with 67 plant odorants, we found that OR75 exhibited response only to *p*-anisaldehyde, *β*-ionone, undecanal and cinnamaldehyde, and all these odorants contain a carbonyl group in the chemical structure. However, the response to *p*-anisaldehyde was higher than that to other three odorants. This narrow ligand profile is typical for ORs tuned to ecologically important stimuli (Stensmyr et al., 2012; Carey et al., 2010). It was noted that the current response induced by *p*-anisaldehyde (~150 nA) is relatively small compared to other OR-ligand interactions reported in the literature. This may result from the low expression efficiency of OR75 in the oocyte as the response to the agonist VUAA1 of Orco is also low (Fig. 2). Further RNAi assay using dsRNA feeding confirmed the role of OR75 in perception of the two plant volatiles. Disruption of target RNA by 70–80 %, the dsOR75-treated thrips completely lost their behavioral preference to *p*-anisaldehyde, suggesting that OR75 is essential for mediating the perception of *p*-anisaldehyde. For *β*-ionone however, dsOR75 treatment only resulted in reduction of the preference, suggesting that some other ORs also play roles in *β*-ionone perception. This RNAi result is consistent with the weak response of OR75 to *β*-ionone in the XOE-TEVC assay. Alternatively, the RNAi efficiency in the present study might be not high enough to induce the loss of behavioral preference to *β*-ionone, which needs a further study.

We used the DREAM approach to screen for candidate target ORs, based on the fact that mRNA levels of ORs change post-exposure to high concentrations of corresponding ligands (Koerte et al., 2018). Our present study showed that OR75 is the candidate OR with the highest change in expression relative to the non-exposure control, and the OR is further confirmed to be responsive to *p*-anisaldehyde. The ORs for other three volatiles in the mixture used for exposure experiment remain unclear. In addition to OR75, we also obtained four ORs (Table S2) with $|\mathrm{Log_2FoldChange}| \geqq 2$ or *P*-value <0.05. Whether these ORs are involved in perception of those three volatiles needs further assays. On the other hand, the change rate in expression varies with factors of the exposure experiment, such as the odorant concentration, exposure duration and the specific OR genes (Von der Weid et al., 2015; Koerte et al., 2018; Wan et al., 2015).

Molecular docking has emerged as an efficient method for predicting binding sites between proteins and their ligands (Charlier et al., 2013; Jin et al., 2023), as the accuracy of 3-D structure prediction has been greatly enhanced by AI-assisted techniques in recent years (Abramson et al., 2024). In this study, we employed AlphaFold3 to predict the heterotetrameric structure of OR75/Orco, and further molecular docking experiments suggested that His150 of OR75 formed a stable hydrogen bond with *p*-anisaldehyde but not with *β*-ionone, undecanal and cinnamaldehyde. This result further supports that of XOE-TEVC assay, in which OR75 responded much strongly to *p*-anisaldehyde than to the other three volatiles. To confirm the importance of His150 in OR75-*p*-anisaldehyde interaction, further experiments such as site-directed mutagenesis combined with functional assays (Wang et al., 2023; Zhao et al., 2024), need to be conducted.

CRediT authorship contribution statement

Xuan-Pu Luan: Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation. **Xiao-Tong Zhang:** Writing – review & editing. **Zhi-Qiang Wei:** Writing – review & editing. **Jin-Meng Guo:** Writing – review & editing. **George F.O. Obiero:** Writing – review & editing. **Merid N. Getahun:** Writing – review & editing. **Qi Yan:** Writing – review & editing. **Jin Zhang:** Writing – review & editing.

Shuang-Lin Dong: Writing – review & editing.

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Appendix A. Supplementary data

Primers used in this study (Table S1); Plant volatiles used in the XOE-TEVC assay (Table S2); Candidate differentially expressed odorant receptors (Table S3); Binding energies and amino acid residues interacting between the ligands and OR75 (Table S4); Diagram of the odorant exposure experiment apparatus (Fig. S1); The differential gene volcano plot (Fig. S2). The Ramachandran plot of heterotetrameric structure of OR75/Orco (Fig. S3). Supplementary data to this article can be found online at [https://doi.org/10.1016/j.pestbp.2025.106421].

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