

# Comparison of electrophysiological and behavioral responses of tephritid (Diptera: Tephritidae) pests to cucurbit host odors

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## ABSTRACT

Developing species-specific lures for female tephritid fruit flies is crucial for sustainable pest management, yet remains a significant challenge. This study aimed to identify potential attractants for *Zeugodacus cucurbitae*, a major pest on cucurbit crops, by comparing the olfactory responses of females to host plant volatiles with those of two related fruit-infesting species, *Bactrocera dorsalis* and *Ceratitis capitata*. Using gas chromatography coupled electroantennogram detection (GC-EAD) and GC-mass spectrometry (GC-MS), we identified 28 antenna-active compounds from flowers and fruits of *Cucumis sativus* L., *Cucurbita pepo* L., and *Cucurbita mixta* L. While eight compounds elicited responses in all three tephritid species, indicating shared olfactory sensitivity, *B. dorsalis* and *C. capitata* detected a broader range of volatiles than *Z. cucurbitae*. Behavioral assays in a six-choice olfactometer demonstrated that synthetic blends based on cucurbit host odors attracted females of all three species. These findings suggest that, while there is some overlap in host odor perception, subtle differences exist that could be exploited for species-specific lure development. Further optimization and field testing of these promising blends are recommended to enhance targeted pest management strategies for *Z. cucurbitae*.

## 1. Introduction

Olfaction plays a crucial role in mediating interactions between herbivorous insects and their environment, influencing behaviors essential for survival and reproduction, such as host plant location and mate choice (Hansson and Stensmyr, 2011). Host plant selection, mediated by olfactory cues, is a key driver of insect ecology and evolution, shaping patterns of specialization and resource utilization (Schoonhoven et al., 2005). The peripheral olfactory system, primarily the antennae, filters ecologically relevant volatile cues amidst background noise (Hansson and Stensmyr, 2011). The evolution of this system is fundamental to adaptive behaviors in response to environmental changes or the occupation of new ecological niches (Couto et al., 2020; Oteiza and Baldwin, 2021).

The link between peripheral olfactory circuits and ecological adaptations is well-documented in insects (Zhao and McBride, 2020). For example, drosophilids exhibit species-specific adaptations in their olfactory systems to match ecological needs (Dekker et al., 2006; Keesey et al., 2022; Linz et al., 2013; Stensmyr et al., 2003). *Drosophila sechellia* and *Drosophila erecta* show heightened sensitivity to volatiles from their

respective host plants (*Morinda citrifolia* L. (Noni) and *Pandanus* spp.) compared to generalist relatives, illustrating how receptor tuning and neural circuitry enable specialized host preference (Hallem and Carlson, 2006; Linz et al., 2013; Stensmyr et al., 2003). Similar patterns occur in other insect groups, including tephritid fruit flies (Biasazin et al., 2019; Larsson-Herrera et al., 2024). While ecology often plays a dominant role, phylogeny can contribute to the olfactory sensitivities, as observed in some midges (Molnár et al., 2018) and distantly related tephritids (Fennine et al., 2025; Jacob et al., 2017).

The Tephritidae family encompasses approximately 5,000 described species, with around 1500 species known to infest fruits and vegetables, causing significant agricultural damage and impacting global trade, an issue exacerbated by invasive species (Clarke, 2019; Gutierrez et al., 2021; White and Elson-Harris, 1992). Recent taxonomic advances have clarified the relationships within tephritids (San Jose et al., 2018; Virgilio et al., 2015; Zhang et al., 2023). For example, *Zeugodacus cucurbitae* (Coquillett) and *Bactrocera dorsalis* (Hendel) are closely related at the subgenus level, while *Ceratitis capitata* (Wiedemann) is more distantly related at the tribe level. Diet breadth in tephritids varies from specialists infesting a single/few hosts to generalists utilizing a wide range

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of hosts (Clarke, 2017). However, the extent to which olfaction contributes to these dietary preferences remains poorly understood. While *B. dorsalis* and *C. capitata* are polyphagous, infesting numerous hosts, *Z. cucurbitae* exhibits a strong preference for cucurbitaceous plants (Charlery De La Masselière et al., 2017). Comparing the peripheral olfactory tuning of *Z. cucurbitae* with these related species offers a valuable opportunity to elucidate how ecology is reflected in olfactory system adaptations.

Studies on volatile odors that mediate the attraction of female tephritid fruit flies to their host plants have primarily focused on their potential for synthetic lure development (Biasazin et al., 2014; Royer et al., 2014; Siderhurst and Jang, 2010, 2006). However, the ecological and evolutionary roles of these volatile odors remain largely unexplored. While some studies such as Biasazin et al. (2019), and Ramiaranjatovo et al. (2024) have begun addressing this gap by characterizing antennal responses to ripe fruit volatiles, cucurbit-specific volatiles, particularly across phenological stages, remain unstudied. Incorporating these volatiles would provide critical insights into the ecological drivers of olfactory specialization in tephritid pests. Comparative approaches using closely related taxa with divergent ecologies (e.g., generalists vs specialists) can reveal how neural circuits mediate niche-specific behaviors (Dekker et al., 2006; Keeseey et al., 2022). Furthermore, elucidating the physiological and ecological basis of olfaction is essential for refining behavior-based pest management tools (Biasazin et al., 2019).

In this study, we compared antennal responses of female *Z. cucurbitae*, *B. dorsalis* and *C. capitata*, to cucurbit volatiles using gas chromatography-coupled electroantennographic detection (GC-EAD). We tested whether the distinct host preferences of these tephritids are reflected in their antennal olfactory response profiles. Specifically, we asked: (1) Does *Z. cucurbitae* exhibit stronger antennal responses to cucurbit volatiles than its generalist relatives *B. dorsalis* and *C. capitata*? (2) Can synthetic blends of antenna-active volatiles (derived from cucurbits or ripe fruits) elicit host-preference behaviors in olfactometer assays? By addressing these questions, we provide insights into olfactory adaptation in an evolutionary-ecological context while offering practical implications for targeted pest management strategies.

## 2. Materials and methods

### 2.1. Experimental insects

Electrophysiology was conducted at the Swedish University of Agricultural Sciences (SLU, Alnarp, Sweden) using colonies of *Z. cucurbitae*, *B. dorsalis* and *C. capitata* originating from the International Atomic Energy Agency (IAEA). Behavioral bioassays were conducted at Addis Ababa University, Ethiopia. For these behavioral assays, the *Z. cucurbitae* start-up colony was sourced from Alnarp, while *B. dorsalis* and *C. capitata* were provided by the International Center of Insect Physiology and Ecology (icipe, Nairobi, Kenya; courtesy of Dr Samira Mohammed). Flies were maintained in bugdorm cages (325 × 325 × 325 mm<sup>3</sup>) under controlled conditions at 24–28 °C, 60–65 % relative humidity, and 11:13 L:D photoperiod. Adults were fed on a 1:3 yeast-to-sugar diet, with water provided via water-soaked cotton balls placed in Petri dishes. Oviposition substrates included cucumber for *Z. cucurbitae* and banana for *B. dorsalis* and *C. capitata*.

### 2.2. Plant material and volatile collection

Volatiles were collected from three cucurbit species (*Cucumis sativus* L., *Cucurbita pepo* L., and *Cucurbita mixta* L.) grown at Trädgårdslaboratoriet (Alnarp, Sweden). Sampling was performed at three developmental stages (flowers, immature fruits and mature fruits), from intact plants (Baraki et al., 2025). Adsorption columns (Porapak<sup>TM</sup> Q, 50–80 mesh) were pre-rinsed with 1 mL n-hexane and 1 mL methanol. Plant tissues were enclosed in polyamide bags (Toppits Stekpåsar, Mingen, Germany, 35 × 43 cm), and volatiles were trapped via aeration (150 mL

min<sup>-1</sup>, 4 h) using a KNF pump (KNF NMP830KNDC, KNF, Sursee, Switzerland). Adsorbed compounds were eluted with 0.5 mL n-hexane and stored at –20 °C until analysis. Five independent aeration samples were collected per developmental stage. Samples were analyzed individually using GC–MS and then pooled once their similarity was confirmed. Heptyl acetate (10 ng; Cas 112-06-1) was added as an internal standard for quantification.

### 2.3. Gas chromatography coupled electroantennogram (GC-EAD)

Antennal responses of *Z. cucurbitae*, *B. dorsalis* and *C. capitata* were recorded using gas chromatography coupled electroantennographic detection (GC-EAD) with an Agilent 6890 GC system (Santa Clara, CA, USA) equipped with a DB-Wax column (30 m × 0.25 mm id × 0.25 µm film thickness). The GC was operated with hydrogen carrier gas using a temperature program starting at 40 °C (3 min hold), followed by a 10 °C/min ramp to 240 °C (5 min final hold). The GC effluent was split 1:1 between the flame ionization detector (FID) and the EAD setup, where antennae were exposed to a humidified airflow (1500 mL/min). For antennal preparations, sexually mature female flies (*C. capitata*: ≥10 days; *B. dorsalis* and *Z. cucurbitae*: 14–21 days) were immobilized in 200 µL micropipette tips, with their head and antennae protruding from the tip. Glass capillaries filled with Beadle-Ephrussi ringer solution (7.5 g of NaCl, 0.35 g of KCl, and 0.29 g of CaCl<sub>2</sub> per liter) connected silver electrodes to the medial antennal segment (recording electrode) and head capsule (reference electrode). Signals were amplified through an IDAC-2 interface (Syntech, Kirchzarten, Germany) and analyzed using GC-EAD software (GcEad 2012 v 1.2.4, Syntech, Germany). Three biologically independent replicates per species (selected based on stable baselines) were included in the final analysis.

### 2.4. Gas chromatography-mass spectrometry (GC–MS)

Volatile samples were analyzed using gas Chromatography-mass spectrometry (GC–MS) with an Agilent 6890/5975 system. Complementary separations were achieved using two columns: an Agilent DB-Wax and an HP-5 (both 30 m × 0.25 mm id × 0.25 µm film thickness), which resolved co-eluting compounds that might overlap on a single column. The DB-Wax column used the same temperature program as the GC-EAD analysis (40 °C for 3 min, then 10 °C/min to 240 °C, held for 5 min), with helium as the carrier gas. Samples (2 µL) were injected in splitless mode. Compound identification was achieved through a three-step approach: (1) calculation of Kovats retention indices using C<sub>8</sub>–C<sub>20</sub> alkane standards analyzed under identical chromatographic conditions, (2) comparison of mass spectra with the NIST14 reference library, and (3) confirmation using synthetic standards for target compounds injected with the same analytical parameters.

### 2.5. Synthetic volatile compounds and their formulation

Synthetic blends were prepared by diluting compounds in paraffin oil (Fine Chemical General Trading PLC, Addis Ababa, Ethiopia) at 10<sup>-4</sup> (v/v; 1:10,000) final concentration, using the compound ratios specified in Table 1. All synthetic compounds (90–99.8 % purity) were obtained from Merck (Sigma-Aldrich, Darmstadt, Germany) and first mixed as concentrated stocks before final dilution. The ratios were determined based on their occurrence in host plant volatiles: nine compounds were detected at the flower stage and six at the immature fruit stage, with shared compounds using immature fruit ratios (prioritizing *C. pepo* unless species-specific). Floral-exclusive compounds like (*E*)-non-2-enal and nona-2,6-dien-1-ol retained their flower-stage ratios, consistent with our findings that attraction did not differ significantly between developmental stages (Baraki et al., 2025). The first blend (All<sub>active</sub>), contained all synthetically confirmed antennal-active compounds for *Z. cucurbitae* (10/14 detected compounds), including decanal, 1,4-dimethoxybenzene, nona-2,6-dien-1-ol, benzyl alcohol,

**Table 1**

Purity and ratio of antennae-active compounds used in behavioral assays.

Compound name	Cas-number	Purity	Blend Ratio (relative units)		
			All_active	Zeugo_active	Six_blend
benzyl alcohol	100–51-6	99.8 %	32	32	
benzaldehyde	100–52-7	99 %	2		
ethyl butanoate	105–54-4	>97 %			57
3-methylbutyl butanoate	106–27-4	>98 %			3
2-methylpropyl acetate	110–19-0	>97 %			1
decanal	112–31-2	97 %	152	152	
3-methylbutyl acetate	123–92-2	98 %			9
nonanal	124–19-6	98 %	1	1	
1,4-dimethoxybenzene	150–78-7	99 %	90		
(E)-non-2-enal	18829–56-6	95 %	16	16	
4-ethylbenzaldehyde	4748–78-1	97 %	2		
2-methylpropyl butanoate	539–90-2	>98 %			2
3-methylbutyl-3-methylbutanoate	659–70-1	>98 %			1
nona-2,6-dien-1-ol	7786–44-9	99 %	76	76	
linalool	78–70-6	97 %	30		
propanoic acid	79–09-4	90 %	1		

linalool, (E)-non-2-enal, 4-ethylbenzaldehyde, benzaldehyde, nonanal and propanoic acid. The second blend (Zeugo\_active), represented a simplified version containing five key components from All\_active (decanal, nona-2,6-dien-1-ol, benzyl alcohol, (E)-non-2-enal and nonanal) selected for shared detection patterns and ecological relevance. The third blend (Six\_blend), was based on shared antennal responses of tephritid fruit flies to ripe-fruit volatiles (Biasazin et al., 2019), consisting of 2-methylpropyl acetate, ethyl butanoate, 3-methylbutyl acetate, 2-methylpropyl butanoate, 3-methylbutyl 3-methylbutanoate, and 3-methylbutyl butanoate.

## 2.6. Six-choice olfactometer bioassay

Behavioral responses to synthetic blends were evaluated using a six-choice olfactometer system (Fig. S1). The setup included a 42 cm × 42 cm × 42 cm glass flight chamber with six top-mounted funnel traps, each connected to double-cylinder units for odor presentation and fly collection (Biasazin et al., 2019). For each replicate, thirty sexually mature female flies, 10- to 21-days old, of either *Z. cucurbitae*, *B. dorsalis*, or *C. capitata* were starved for 18 h (with water provided) before being exposed to three treatments and control (paraffin oil). Each treatment consisted of a 10 µL aliquot dispensed in plastic bottle caps (30 mm diameter, 10.2 mm height) placed in the upper cylinders. Purified air delivered the volatiles into the chamber, and responding flies were captured in the lower cylinders. After 30 min of exposure, the number of flies trapped in each lower cylinder was recorded as a measure of behavioral preference. To control for positional biases, treatment positions were systematically rotated between replicates.

## 2.7. Data analysis

To quantify antennal responses, we used three replicate GC-EAD recordings for each tephritid species. For each recording, we normalized the EAD amplitude by dividing each response by the weighted mean of all responses within that trace. The weighted mean was calculated using the back-transformed (exponential) values of the natural logarithm-transformed depolarization levels. Finally, we averaged the normalized responses across the three replicates for each treatment.

To investigate variation in antennal response spectra (mV) across tephritid species, plant species, and developmental stages, we performed a Principal Component Analysis (PCA) on absolute EAD responses using all antennal active compounds. Numeric response variables were extracted and integrated with metadata (insect species, plant species, and stage). Scatterplots and biplots were generated using the ggplot2 and factoextra packages. Scatterplots included confidence ellipses (95 %) to visualize group clustering, and variance explained by the first two

principal components was annotated on the axes.

The mean likelihood of detection of volatile compounds shared among the three cucurbit plant species was calculated by aggregating detection counts across all three tephritid species and stages. Likelihood was expressed as the ratio of detection events to the total number of plant species associated with each compound. Additionally, the detection likelihood was calculated for each chemical class by expressing it as the ratio of detected compounds to the total number of compounds within each class. Linear regressions were performed separately for each fly species to assess the relationship between sensitivity and amount of the volatile compounds detected.

For the behavior data, a generalized linear model (GLM)-ANOVA fitted with Poisson distribution or negative binomial (in case of over-dispersed data) was carried out to compare the mean response of females to different synthetic blends. Emmeans package was used for pair-wise mean comparison and mean separation. To produce the box plots, radial bar chart, heatmap and PCA plots ggplot2 package in R software version 4.1.2 was used.

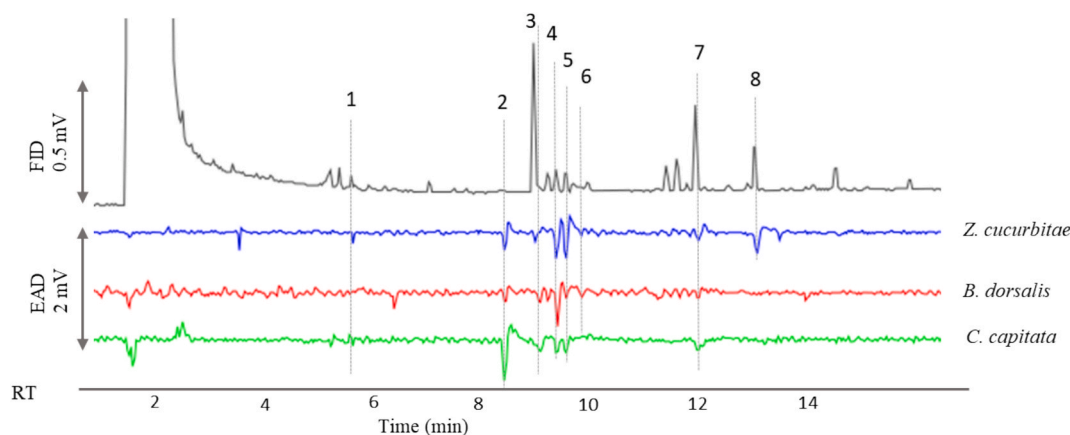
## 3. Results

### 3.1. Tephritid antennal response spectra reveal shared and species-specific sensitivities

Our GC-EAD analysis identified 28 antennal-active volatile organic compounds (VOCs) across three developmental stages (flower, immature and mature fruit) of three cucurbit host species: *C. pepo*, *C. mixta* and *C. sativus* (Table S1). Comparative analysis revealed both shared and species-specific detection patterns among the three tephritid species (*B. dorsalis*, *C. capitata* and *Z. cucurbitae*) (Figs. 1, 2, Fig. S2, 3). Eight VOCs including, decanal, benzaldehyde, nonanal, linalool, propanoic acid, (E)-non-2-enal, nona-2,6-dien-1-ol, and 2-cyclopentylcyclopentan-1-one elicited consistent responses across all three tephritid species. *B. dorsalis* detected the largest number of compounds (19 VOCs), followed by *C. capitata* (17) and *Z. cucurbitae* (14). Interspecific comparisons revealed that 13 compounds (46.4 %) were detected by at least two tephritid species. Species-specific responses were most pronounced in *C. capitata* with eight VOCs undetected by the other species, compared to five for *B. dorsalis* and one for *Z. cucurbitae*.

Principal component analysis (PCA) of antennal responses revealed 33 % of variation explained by the first two principal components (Fig. 3). While responses largely overlapped, distinct clustering was observed for flower-stage VOCs of *C. pepo* and *C. sativus*.

Cluster analysis (Euclidean distance) of antennal responses placed *C. capitata* as an outgroup to *B. dorsalis* and *Z. cucurbitae* (Fig. 4). This divergence was reflected in chemical class detection. *Z. cucurbitae*



**Fig. 1.** GC-EAD traces of tephritid females to *C. pepo* flower headspace. The FID (top trace) chromatogram shows the peaks (compounds), and the gray vertical lines indicate the antenna-active compounds. The time base was 2.00 min, FID = 0.5 mV, and EAD = 2 mV. RT stands for retention time of compounds. The EAD in blue, red and green stands for *Z. cucurbitae*, *B. dorsalis* and *C. capitata*, respectively. 1 = (E)-hex-2-enal, 2 = nonanal, 3 = decanal, 4 = linalool, 5 = (E)-non-2-enal, 6 = (2E,6Z)-nona-2,6-dienal, 7 = nona-2,6-dien-1-ol, 8 = benzyl alcohol. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

predominantly detected aldehydes (42.85 %) while *B. dorsalis* responded equally to aldehydes and ketones (31.57 % each). In contrast, *C. capitata* showed stronger alcohol sensitivity (29.41 %) (Fig. 5).

Regression analysis confirmed that EAD response strength was not correlated with compound concentration in the headspace (Fig. 6, Fig. S4, Table S2). The weak coefficients of determination ( $R^2 = 0.003$  for *Z. cucurbitae*  $R^2 = 0.109$  for *B. dorsalis* and  $R^2 < 0.001$  for *C. capitata*) indicate that antennal sensitivity is compound-specific rather than concentration-dependent (Fig. S4).

### 3.2. Shared cucurbit volatiles are preferentially detected

Compounds shared among all three cucurbit hosts were most likely to elicit antennal responses. For *Z. cucurbitae*, 78.5 % (11 out of 14) of detected compounds were shared across hosts, compared to 73.6 % (14 out of 19) for *B. dorsalis*, and 58.8 % (10 out of 17) for *C. capitata* (Fig. 7).

### 3.3. Shared host volatiles attract tephritid females in behavioral assays

Synthetic blends mimicking cucurbit flowers and fruits were tested for attraction. The Zeugo\_active blend consisted of volatiles detected by *Z. cucurbitae* and were shared among the three cucurbit species. Of the five components of the Zeugo\_active blend, decanal, nona-2,6-dien-1-ol, (E)-non-2-enal, and nonanal were detected by all fly species while benzyl alcohol was detected by *Z. cucurbitae* and *B. dorsalis*. All synthetic blends (All\_active, Zeugo\_active and Six\_blend) attracted significantly more *Z. cucurbitae* females than the control, with no differences between blends (Fig. 8). Similarly, all blends were equally attractive to *C. capitata* females (Fig. 8). In contrast, *B. dorsalis* females were more attracted to the Six\_blend than to All\_active or Zeugo\_active at  $P = 0.0033$  and  $P < 0.0001$ , respectively, while all the treatments were more attractive than the control at  $P < 0.05$  (Fig. 8).

## 4. Discussion

The olfactory system of herbivorous insects often exhibits remarkable functional conservation, even among ecologically divergent species. For example, in drosophilids, a group taxonomically distant from tephritids (sharing only a suborder relationship), 86 % of the peripheral olfactory system is conserved (Keesey et al., 2022). Strikingly, 50 % of odorant receptor (OR) genes are shared between these lineages (Jacob et al., 2017; Persyn et al., 2025), suggesting deep-rooted similarities in odor detection mechanisms. This conservation may underpin the overlapping antennal responses we observed across the three tephritid

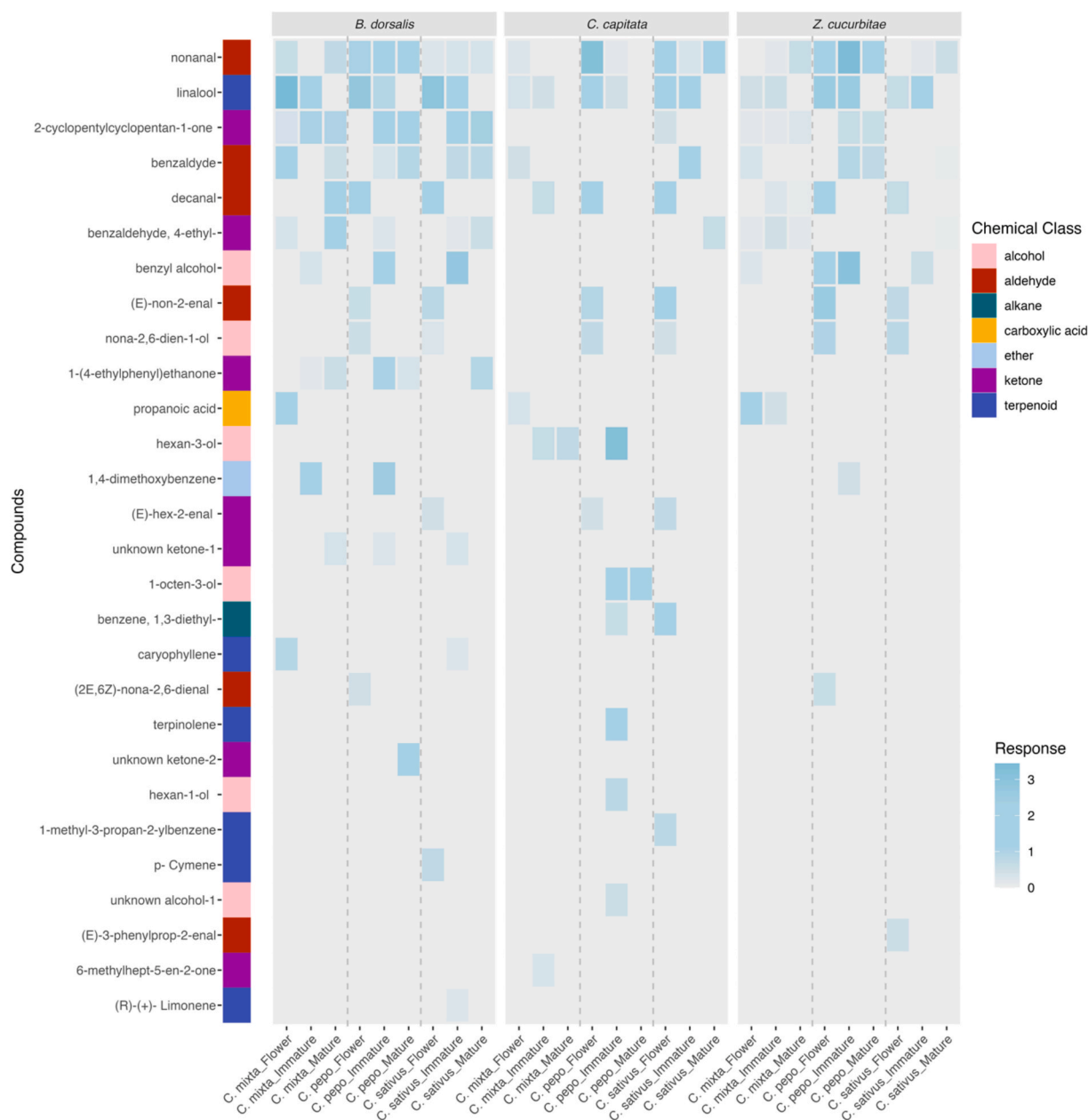
species, despite their niche divergence. Notably, EAD response strength was uncorrelated with compound concentration ( $R^2 < 0.11$  for all species), consistent with findings in moths where insects tolerate volatile ratio variations (Najar-Rodriguez et al., 2010; Tasin et al., 2010). Such dose-independent responses may reflect adaptive plasticity for host location across phenological stages (Baraki et al., 2025; Piñero and Dorn, 2009).

Our GC-EAD analyses revealed both shared and species-specific antennal sensitivities. While responses to *C. mixta* volatiles overlapped across all phenological stages, distinct clustering occurred for floral volatiles of *C. sativus* and *C. pepo* (Figs. 1-3). This divergence was associated with the absence of key antennal-active compounds such as (E)-non-2-enal and nona-2,6-dien-1-ol in the *C. mixta* floral headspace. These aldehydes dominated floral volatiles of *C. sativus* and *C. pepo*, with their abundance declining during fruit maturation (Beaulieu and Grimm, 2001; Oh et al., 2011), a pattern that likely contributes to stage-specific sensitivity in tephritid response. Notably, despite its ecological specialization, *Z. cucurbitae* shared 8/14 responses with the generalists *B. dorsalis* and *C. capitata*. This aligns with prior work demonstrating broad overlap in tephritid sensitivity spectra (Biasazin et al., 2019; Ramiaranjatovo et al., 2024), though species-specific tuning persists.

The broader VOC detection range in *B. dorsalis* and *C. capitata* reflects their well-documented polyphagy (Shi et al., 2022; Wang et al., 2022), evidenced by expansive host ranges (*B. dorsalis*: 478 species/76 families, *C. capitata* 361 species/63 families (McQuate and Liqueido, 2017). Recent work links this ecological flexibility to both behavioral adaptations (Wang et al., 2022) and specialized OR repertoires in polyphagous tephritids (Persyn et al., 2025). Although *Z. cucurbitae* was an outgroup to *B. dorsalis* and *C. capitata* in its antennal sensitivity to ripe fruit volatiles (Biasazin et al., 2019) and palpal sensitivity to fruit and food volatiles (Larsson-Herrera et al., 2024), it clustered with *B. dorsalis* in our cucurbit-focused study. This difference likely stems from differences in host volatile profiles (cucurbit-specific vs. broad-spectrum fruit/food volatiles) or sensory organ specialization (antennae vs. maxillary palps), underscoring how trophic niche breadth and peripheral sensory function jointly drive chemosensory adaptation.

Generalist tephritids exhibit antennal tuning to esters, which dominate the volatilomes of ripe fruits, a pattern demonstrated in mango (Miano et al., 2022) and other fruits (Biasazin et al., 2019). In contrast, cucurbit volatilomes are ketone rich (Baraki et al., 2025). In this study, tephritid olfactory responses exhibited slight variations, with *Z. cucurbitae* specifically attuned to aldehydes. This might be an adaptation by *Z. cucurbitae* to volatiles emitted by its preferred stage of cucurbit hosts (flowering and immature fruit stage). In contrast



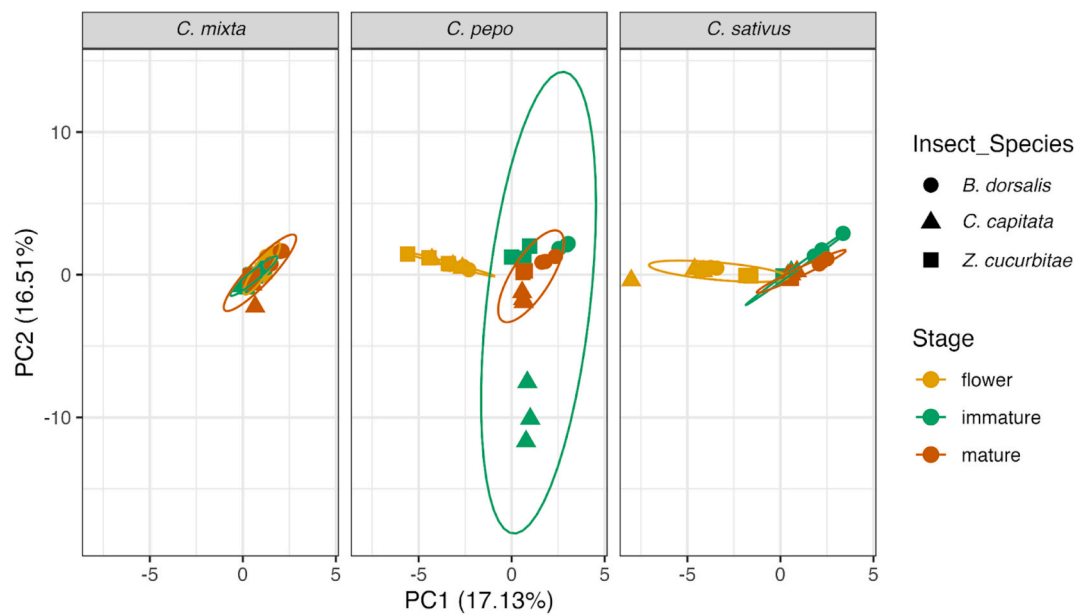


**Fig. 2.** A heatmap that shows antennal sensitivity of female fruit fly species to cucurbit host odor. From left to right shows (1) compound names, (2) chemical classes of the compounds (3) sensitivity of the three fruit fly species (*B. dorsalis*, *C. capitata* and *Z. cucurbitae* respectively) to compounds in the three cucurbit species (*C. mixta*, *C. pepo*, and *C. sativus* at flower, immature and mature fruit stages, respectively). Compounds are arranged in descending order of strength and sharedness of the response. Note that the heatmap is based on normalized data, which means that one cannot directly compare data across columns.

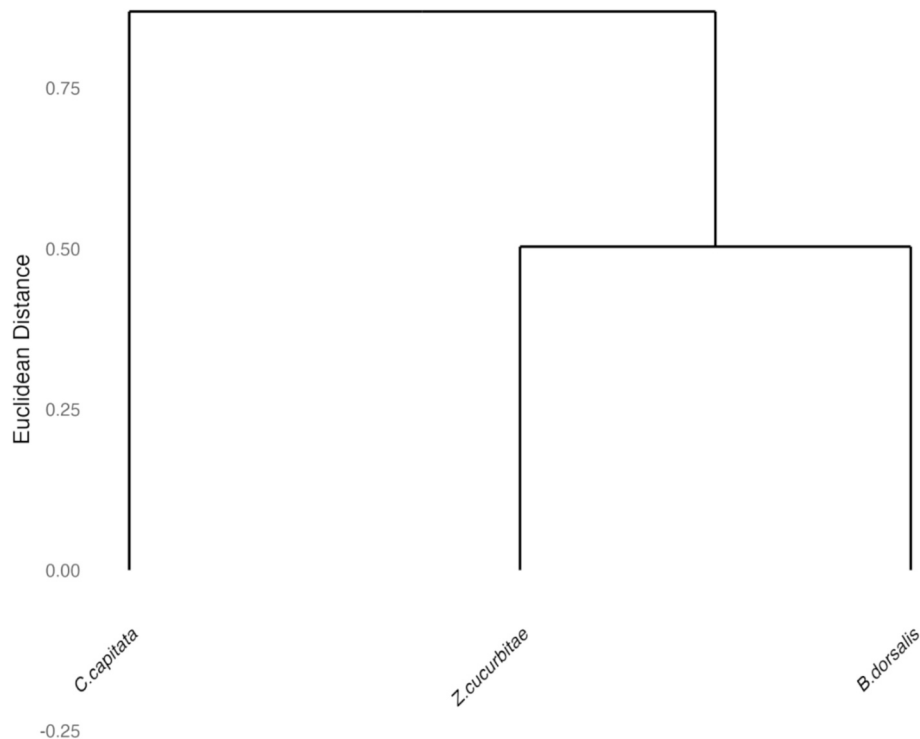
*B. dorsalis* detected comparable numbers of ketones and aldehydes, while *C. capitata* responded primarily to alcohols and aldehydes. Whether this shared sensitivity to overlapping chemical classes (e.g., aldehydes) reflected conserved OR repertoires from ancestral cucurbit-feeding lineages or recent adaptations remains unclear, though Cucurbitaceae are proposed ancestral hosts for Dacini (He et al., 2021), despite recurrent host-range shifts (Virgilio et al., 2009).

The peripheral olfactory circuit is under evolutionary pressure to suit the ecological niche of herbivore insects. For instance *D. suzukii* has expanded olfactory sensory neurons (OSNs) detecting fresh fruit volatiles while reducing those for fermentation odors (Keesey et al., 2022), mirroring niche-driven sensitivity shifts in other drosophilids (Dekker et al., 2006; Linz et al., 2013; Stensmyr et al., 2003). Considering *Z. cucurbitae*'s field preference for cucurbits (Moquet et al., 2021) and its larval performance benefits on these hosts (Charlery De La Masselière

et al., 2017), we expected heightened antennal sensitivity to cucurbit specific volatiles such as (E)-non-2-enal, nona-2,6-dien-1-ol and (2E,6Z)-nona-2,6-dienal, which dominate their characteristic odor profile (Forss and Ramshaw, 1961; Schieberle et al., 1990; Zhang et al., 2022). However, our data revealed no enhanced response to these compounds, paralleling findings in drosophilids where peripheral detection spectra overlap across ecologically divergent species (Depetris-Chauvin et al., 2023). This suggests peripheral tuning may be less predictive of niche specialization than previously assumed with central neural plasticity playing a dominant role. Indeed, *Rhagoletis* races show identical peripheral neuron classes or sensitivities (Olsson et al., 2006), but exhibit reversed valence coding of behaviorally important volatiles (butyl hexanoate and 3-methyl-1-butanol) in the antennal lobe (Tait et al., 2021), demonstrating how higher-order circuits reshape sensory hierarchies (Seeholzer et al., 2018).



**Fig. 3.** PCA plot of antennal response strength of *Z. cucurbitae*, *B. dorsalis* and *C. capitata* at different phenological stages of cucurbits. The plot shows that 33.79% of the variation was explained by PC1 and PC2. The response strength of the three tephritid species were not clearly separated except in the flower stages of *C. pepo* and *C. sativus*.

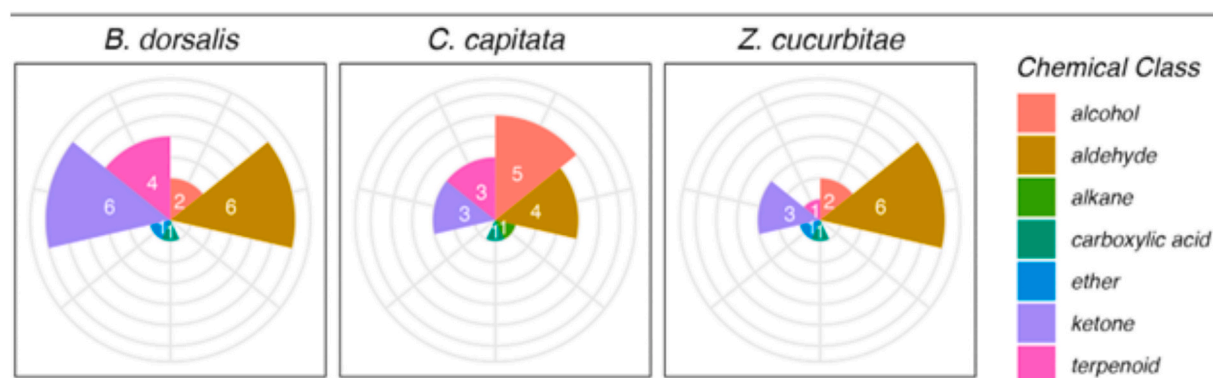


**Fig. 4.** Dendrogram constructed using Euclidean distance to assess the similarity among tephritid female flies in their antennal response spectra. The clustering pattern reflects that *C. capitata* was an outgroup to *Z. cucurbitae* and *B. dorsalis*.

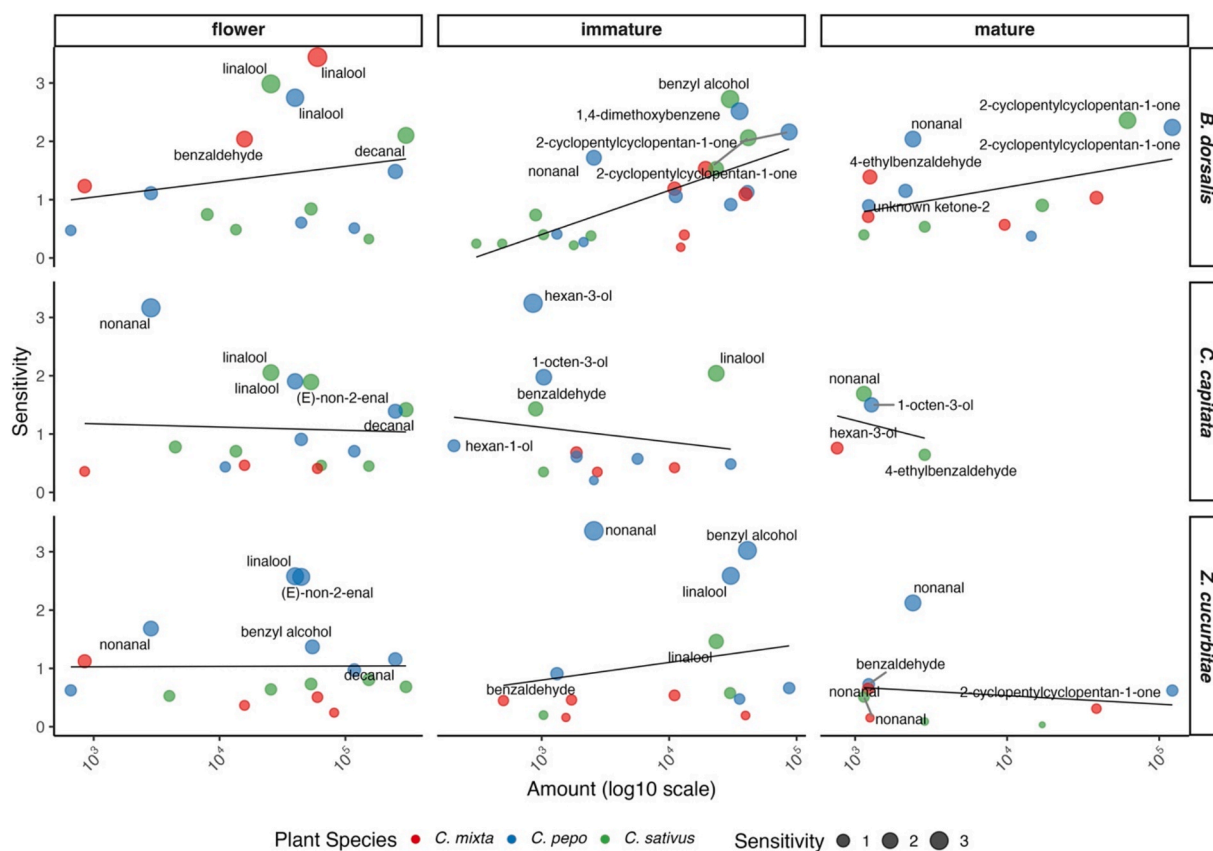
While tephritids are well-documented to respond to ripening fruit volatiles like esters and terpenes (Biasazin et al., 2019; Cortés-Martínez et al., 2021; Cunningham et al., 2016; Kimbokota et al., 2023), our findings reveal they are equally attracted to shared aldehydes and alcohols from cucurbit hosts (decanal, (*E*)-non-2-enal and nonanal, nona-2,6-dien-1-ol and benzyl alcohol). This detection of shared volatiles likely facilitates location of alternative hosts across seasons (Bruce et al., 2005; Pan et al., 2021), supporting recent evidence that tephritids use

common compounds for initial host detection while employing taxa-specific volatiles for finder discrimination (Ramiaranjatovo et al., 2024). The behavioral patterns we observed with *Z. cucurbitae* and *C. capitata* equally attracted to both cucurbit-based and ripe fruit-based blends, whereas *B. dorsalis* preferred the Six\_blend, may reflect distinct ecological strategies.

For *Z. cucurbitae*, this attraction to both host-based and ripe fruit-based blends may stem from several factors. First, the synthetic blends



**Fig. 5.** Radial visualization of volatile detection counts across three tephritid fruit flies (*B. dorsalis*, *C. capitata* and *Z. cucurbitae*). Colored sectors represent chemical classes (legend), with numeric labels indicating the number of compounds detected per class.

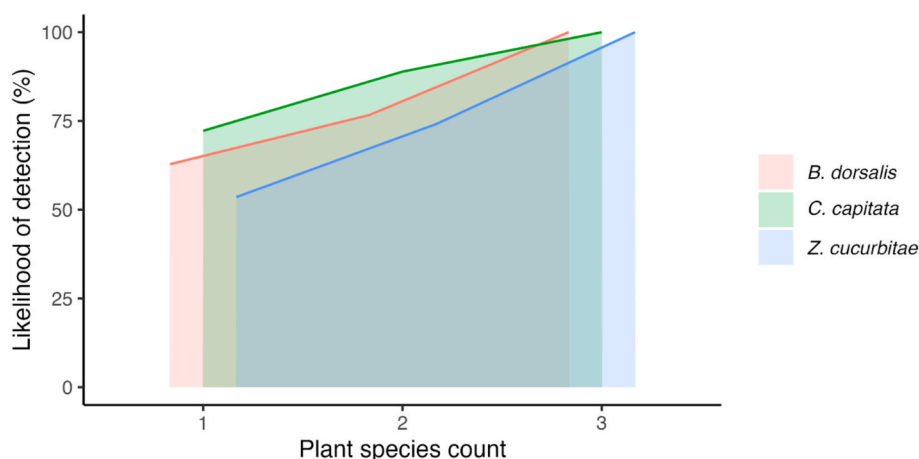


**Fig. 6.** Sensitivity of the top 5 bioactive compounds across plant species, developmental stages and fly species. Points represent individual compounds (colored by plant species; size scales with sensitivity), with labels indicating compound names. Regression lines (black) show the relationship between compound amount (log10-transformed x-axis) and sensitivity for each fly species (*B. dorsalis*, *C. capitata* and *Z. cucurbitae*).  $R^2$  values from linear models are provided in Table S2. Highlighted compounds demonstrate significant bioactivity (sensitivity > 2.0 in *B. dorsalis*).

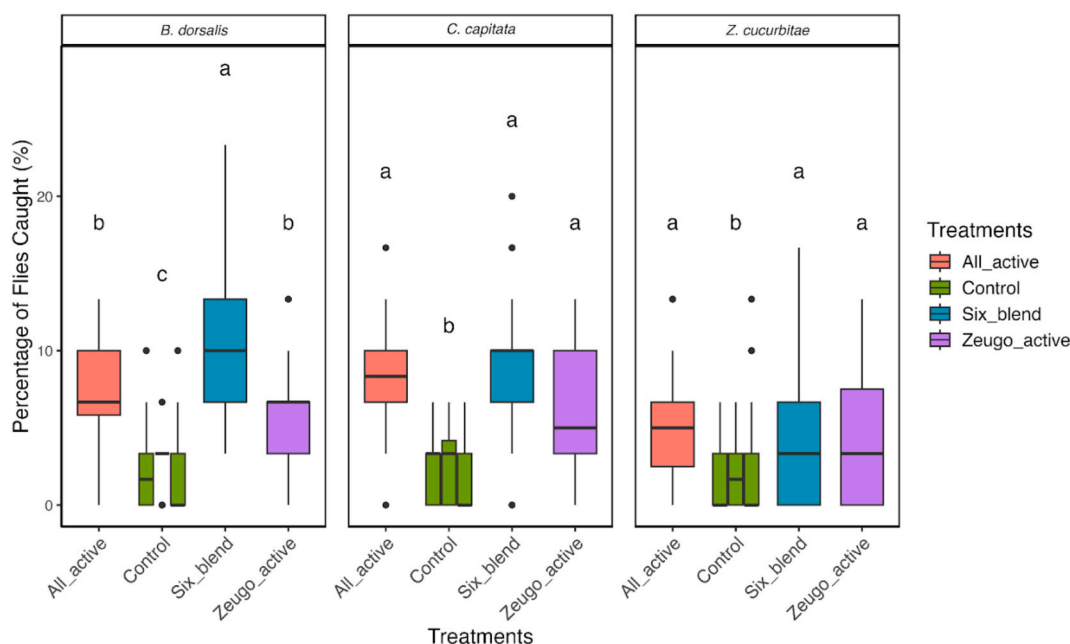
may lack key behaviorally active compounds or fail to fully engage the olfactory system, as optimal attraction requires activation of both antennal and palpal receptors (Biasazin et al., 2025), and underestimates the role of other sensory modalities in close-range host selection (Alyokhin et al., 2000; Finch and Collier, 2000; Visser, 1988), particularly considering the species's strong reliance on visual cues (Piñero et al., 2017). Second, while field observations show cucurbit preference, attraction to the Six blend aligns with documented infestation of non-cucurbit hosts (De Meyer et al., 2015; Kambura et al., 2018; McQuate et al., 2017). Third, the response may reflect adult feeding behavior, given that ripe fruit volatiles are also emitted by fermenting

substrates, which are associated with the evolutionary transition from saprophagy to frugivory in tephritids (Biasazin et al., 2022; Figueroa, 2019). This would parallel *D. suzukii* which orients to rotten fruit odors for feeding, despite preferring ripe fruits for oviposition (Karageorgi et al., 2017), suggesting future oviposition assays could clarify *Z. cucurbitae*'s host selection priorities.

Collectively, our electrophysiological and behavioral data uncover a broader paradox in tephritid olfaction. While antennal response spectra show substantial overlap across species, with shared volatiles consistently detected, the specialist *Z. cucurbitae* displays a distinct profile, predominantly detecting aldehydes, yet showing no enhanced



**Fig. 7.** The likelihood of detection of volatile compounds shared among the three cucurbit host species. The probability of detection by all the three tephritid species increases across sharedness of the volatiles.



**Fig. 8.** Behavioral response of sexually mature *B. dorsalis*, *C. capitata* and *Z. cucurbitae* females to cucurbit host odor based synthetic blends (Zeugo\_active and All\_active) and to synthetic blend of volatiles shared among ripe fruits (Six\_blend). Three paraffin oil controls were placed in between each treatment. Box plots with the median, interquartile ranges and outliers of fly catches of the different blends and control. The experiment was replicated 10 times for *B. dorsalis* and *C. capitata* and 11 times for *Z. cucurbitae*. For each experiment 30 sexually mature female flies were released. Different letters indicate significant differences between means at  $p < 0.05$  means-Kramer, followed by Tukey post hoc tests.

sensitivity to cucurbit-specific volatiles compared to the generalists *B. dorsalis* and *C. capitata*. This paradox aligns with behavioral outcomes, as all three species were attracted to cucurbit-based synthetic blends in six-choice olfactometer assays. The disconnect suggests host selection involves higher-order neural processing and multimodal integration, as seen in other insect systems (Depetris-Chauvin et al., 2023).

Future comparative electrophysiological studies of ecologically divergent species such as *B. dorsalis*, *Bactrocera minax*, and *Bactrocera oleae*, could clarify olfactory niche adaptation, but should integrate higher-order neural mechanisms, as peripheral tuning alone poorly predicts specialization (Depetris-Chauvin et al., 2023). Techniques like single sensillum recording (SSR) and calcium imaging are needed to assess central processing shifts, akin to *Rhagoletis*'s valence rewiring (Tait et al., 2021). Host location also involves multimodal sensing, while tephritids like those studied here use vision and olfaction synergistically

(Aluja and Prokopy, 1993; Díaz-Fleischer et al., 2014; Piñero et al., 2017), trade-offs in sensory investment may occur, as suggested by stronger visual responses in the specialist *B. minax* versus the generalist *B. dorsalis* (Wang et al., 2022), mirroring drosophilid resource allocation patterns (Keese et al., 2020). Future work should test such trade-offs explicitly while linking sensory profiles to oviposition behavior and larval performance.

#### CRediT authorship contribution statement

**Yoseph Baraki:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yitbarek Woldehawariat:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization. **Teun Dekker:** Writing – review &



editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Conceptualization. **Tibebe Dejene Biasazin:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## 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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## Declaration of generative AI in scientific writing

Not applicable.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jinsphys.2025.104865>.

## Data availability

Data will be made available on request.

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