Olfaction in Tephritidae: a balance between detection and discrimination

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Abstract

Phytophagous insects are capable of detecting and locating suitable hosts, which emit volatile compounds. Polyphagous species appear to have a complex olfactory strategy given that their numerous hosts have diverse emission profiles. In particular, their hosts' volatile emissions share some of the same compounds, providing chemical bridges between them. However, the behavioural plasticity observed in insect host selection suggests that other volatiles have a complementary role. Here we explore how polyphagous insect specialization to detect and discriminate between hosts has driven their chemical selectivity. We predicted *in silico* a functional trade-off between these two functions, using a neuronal model of an olfactory system. The volatile emissions of intact or mechanically damaged fruit from 28 different species were fed into the model and analysed using gas chromatography coupled with mass spectrometry. Using triple point electroantennography and a behavioural assay, we studied the olfactory response of Tephritidae fruit fly species that oviposit on fruit. We observed experimentally two predictions of our model for host discriminating olfactory systems: the olfactory responses of eight species were negatively correlated with the compound's degree of sharedness among fruit emissions; female *Bactrocera dorsalis* showed a behavioural preference for non-shared over shared fruit compounds. A dose-dependent switch in the fly's preference confirmed the ecological importance of

shared fruit compounds. Thus, we propose that insect olfactory systems are chemically tuned to detect suitable hosts and accurately discriminate between them.

Keywords: *Bactrocera dorsalis*, polyphagy, host range, GC-EAD, GC-MS, fruit volatile compounds

1. Introduction

Phytophagous insects need to find a suitable host plant to fulfil their primary functions, such as feeding, mating and oviposition. They rely on their olfactory ability to perceive and identify their host by detecting its volatile compounds[1,2]. Their specialized peripheral olfactory system allows them to detect host compounds and integrate corresponding signals in their environment. However, in a natural setting there is a miasma of volatile compounds, which means that identifying a host is a complex task requiring an effective strategy. The volatile signature that an insect identifies as a host could correspond to particular ratios of a set of compounds or to individual compounds that are highly characteristic of the host[3,4]. Understanding the olfactory strategy involves deciphering both the chemical and sensory components of the communication system, which may facilitate the development of novel odour-based pest control strategies[5].

Polyphagous species have a wide host range, which does not necessarily make their olfactory strategy more complex. It is possible that their preferred volatile signatures fit many plants, including numerous suitable hosts. Focusing on this hypothesis led to the discovery that Miridae or Tephritidae insect species are attracted to sets of the same compounds, which are emitted by their many hosts[5–8]. However, this does not fully explain why polyphagous species can change their host preference depending on their past experience[9–11]. This behavioural plasticity suggests that polyphagous species can differentiate between their hosts, which can not rely on the ability to identify host-shared compounds. We have yet to resolve the relative contribution of compounds that are host-shared and non-host-shared (herein species-specific).

There is little experimental data available because few studies have addressed this question. The family Tephritidae, a group of major pests, includes a substantial contingent of polyphagous species[12–14]. Some have an extremely wide host range, for example, more than 250 different host plants have been reported for the Oriental fruit fly *Bactrocera dorsalis*[15]. Biasazin and colleagues showed that four polyphagous fruit fly species from this family are strongly attracted to compounds that are shared between four different host fruit[16]. As could be expected when dealing with highly polyphagous species, host selection plasticity has also been reported[9,10,17,18].

This study focuses on the evolutionary pressure on the peripheral olfactory system that led to the development of a polyphagous strategy in eight Tephritidae fruit fly species with wide and overlapping host ranges: *B. dorsalis*, *Bactrocera zonata*, *Dacus demmerezi*, *Ceratitis capitata*, *Ceratitis catoirii*, *Ceratitis quilicii*, *Neoceratitis cyanescens* and *Zeugodacus cucurbitae*[19,20]. First, we explored headspaces from 28 highly divergent species of host fruit, by analysing the composition of their volatile emissions using gas chromatography coupled with mass spectrometry (GC-MS). Damaged fruit in orchards may emit new and potentially attractive compounds to fruit flies. Therefore, we sampled both intact fruit still on the tree and harvested fruit with mechanical damages. Using a semi-automated workflow for mass-spectrometry, we were able to isolate and identify several hundred compounds, for which we estimated the degree of sharedness among fruit emissions using alpha-diversity index. As a proxy for evolutionary pressure, we constructed a neuronal model of the peripheral olfactory system based on the likely composition of Tephritidae. We fed the fruit headspace data into the model and assessed how much species-specific and shared fruit compounds contribute to detecting and/or discriminating between host fruit species.

We tested the model's predictions, by mapping antennal olfactory responses to a set of synthetic fruit compounds in females from the eight Tephritidae species. These were selected with a varying degree of alpha-diversity, using triple electroantennography (EAG_3)[21] and chopper-modulated GC, coupled with a triple electroantennogram detector (GC-EAD_3). The resulting data allowed us to test whether the chemical tuning of the insect's olfactory systems is correlated to the degree of compound sharedness among its hosts.

Lastly, we tested the behavioural preference of female *B. dorsalis* using a dual choice trap, *ex situ*, with different doses of two sets of volatiles with a contrasting degree of alpha diversity.

2. Materials and methods

2.1. Insects

Female individuals of eight species of Tephritidae were used for the electrophysiological experiments: *Bactrocera dorsalis* (oriental fruit fly), *Bactrocera zonata* (peach fruit fly), *Ceratitis capitata* (medfly), *Ceratitis catoirii* (Mascarene fruit fly), *Ceratitis quilicii* (Cape fruit fly), *Dacus demmerezi* (Indian Ocean cucumber fruit fly), *Neoceratitis cyanescens* (tomato fruit fly) and *Zeugodacus cucurbitae* (melon fly). They originated from laboratory strains, which were reared according to previously published procedures[19,22]. The individuals selected for electrophysiology had lived for respectively 12-15, 28-32, 10-14, 21-26, 13-17, 11-15, 10-16 and 25-31 days after emergence and belonged to the following generations: 62-64th, 179-180th, 107-108th, 311-312th, 77-79th, 59th, 64-65th and 7th.

The behavioural experiments were conducted on female individuals of *B. dorsalis* from the 76-78th generations, 18-25 days after emergence.

2.2. Plant materials and collection of volatile compounds

Fruit volatile compounds were collected using dynamic headspace extraction. We put individual or clusters of fruit into nalophan sample bags (SenseTrading, Gröningen, Netherlands) that were 47 cm wide. The bags were sealed with plastic ties at both ends to create a headspace. The air content of the headspace was sucked out at 100 mL.min^{-1} for two hours using an adsorption tube filled with Tenax GR (Supelco, Bellefonte, USA) and a portable membrane pump (Spectrex PAS-500; Spectrex, Redwood City, CA, USA). For each sample, a negative control sample was performed with an empty nalophan bag.

Table 1: List of the 28 fruit species studied. Asterisks * indicate the 13 species reported to have a *B. dorsalis* infestation rate of over 5%.

Fruit families	Fruit species	Common names
Anacardiaceae	*Mangifera indica* *	Mango
Cactaceae	*Hylocereus undatus* *	Dragon fruit
Caricaceae	*Carica papaya* *	Papaya
Combretaceae	*Terminalia catappa* *	Indian almond
Cucurbitaceae	*Sechium edule*	Chayote
Cucurbitaceae	*Cucurbita maxima*	Pumpkin
Cucurbitaceae	*Cucumis sativus*	Cucumber
Cucurbitaceae	*Cucurbita pepo*	Zucchini
Cucurbitaceae	*Cucumis melo*	Melon
Cucurbitaceae	*Citrullus lanatus*	Watermelon
Lauraceae	*Persea americana* *	Avocado
Lythraceae	*Punica granatum*	Pomegranate
Moraceae	*Ficus carica* *	Fig
Myrtaceae	*Psidium guajava* *	Guava
Myrtaceae	*Psidium cattleianum* *	Strawberry guava
Myrtaceae	*Syzygium samarangense* *	Java apple
Myrtaceae	*Syzygium jambos* *	Rose apple
Oxalidaceae	*Averrhoa carambola*	Star fruit
Rosaceae	*Eriobotrya japonica* *	Loquat
Rosaceae	*Prunus persica* *	Peach
Rosaceae	*Prunus domestica* *	Plum
Rubiaceae	*Coffea arabica*	Coffee
Rutaceae	*Citrus reticulata*	Tangor
Solanaceae	*Solanum melongena*	Aubergine/Eggplant
Solanaceae	*Solanum mauritianum*	Bugweed
Solanaceae	*Capsicum annuum*	Chilli
Solanaceae	*Solanum betaceum*	Tree tomato
Solanaceae	*Solanum lycopersicum*	Tomato

In this study, we selected 28 fruit species considered to be potential hosts of Tephritidae[20] (Table 1). Headspace samples were extracted from intact fruit on the tree or from sliced fruit at a ripening stage

suitable for oviposition. For each fruit species, sliced or unsliced, 3 to 8 (median 7) replicates were performed, with a total of 193 samples for intact fruit and 176 for sliced fruit.

2.3. Gas chromatography

Tenax traps were stored at room temperature and analysed within seven days using an automated thermal desorber (ATD, Turbomatrix 350, Perkin Elmer, USA), coupled with GC-MS (Clarus 580 GC and SQ8T MS, Perkin-Elmer, USA). The parameters of the ATD were as follows: primary desorption 5 min at 250°C, flow 50 mL.min-1; no inlet split; up to an Air Monitoring trap kept at 2°C; secondary desorption 1 mL.min-1 for 5 min, and a constant flow of 3 mL.min-1. An Elite-5MS capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness, Perkin-Elmer, USA), was used for chromatography. The oven was programmed from 50 to 270°C at 8°C.min-1. Helium was used as a carrier gas with a constant flow of 0.21 mL.min-1.

2.4. Neuronal model of olfactory detection

Our modelling approach was designed to formally validate the two following hypotheses. (1) The detection hypothesis: for a given number of olfactory receptors (ORs), the polyphagous species' ecological need to detect hosts selects stronger olfactory responses to shared volatiles compared to species-specific host volatiles. (2) The alternative discrimination hypothesis: the polyphagous species' use of volatiles to discriminate between hosts selects stronger olfactory response to species-specific compounds compared to shared host compounds. The modelling workflow was as follows: we designed a large number of simple neuronal models of insect olfactory systems, each one responded to a random selection of compounds with random sensitivity. This set of models is intended to include all possible combinations that may have been reached through random mutations, although only some are realistic under natural conditions. We then identified the subset of models under hypothetical selection and analysed their specific properties. To do this, we fed samples of intact or sliced fruit volatile emissions into the model (see collection method above). By drawing on these data sets, we estimated how effectively each model detected or discriminated between fruit species.

More specifically, each model included three categories of virtual compartments: compounds (n = 665 or 511, from sliced and intact fruit, respectively), ORs (one OR unit in the model includes virtually all the olfactory sensory neurons that express the same OR), and total antennal activity (equivalent to EAG_3). An odour (input) was defined by a combination of compounds at different doses (in log units). Each OR was activated by a fixed number of compounds whose identity was randomly chosen. Thus, while some compounds did not activate any OR, others activated several ORs. A compound is considered to have been detected if it activates at least one OR. Each compound dose/OR response relationship was defined by two parameters: a sensitivity threshold (drawn from a uniform distribution covering 5 log units) and a dynamic range width (drawn from a log-normal distribution of mean 3 and standard deviation 1). The activation x_{ij} of an OR_i by compound j was 0 below threshold, 1 above threshold + dynamic range width, and increased linearly between the two. The activity of OR_i was calculated by combining all compound activations with the following equation: $1 - \Pi_j(1-x_{ij})$, which ensured that the activity level remains between 0 and 1. The antennal activity (EAG) was the sum of OR activities.

For each model, a fruit detectability index, which estimates the effectiveness of the insect sensory system to detect fruit, was defined as the sum of total antennal response to all fruit odours. In order to estimate the ease with which the sensory system discriminates fruit species, we calculated a fruit species discriminability index as follows. A random forest analysis was applied to classify the patterns of OR responses to volatile samples into the different fruit species (randomForest function, R package randomForest). The index was defined as the opposite of the out-of-bag scores, i.e. the number of correctly classified samples divided by the total number of samples (Fig. 3b). The random forest classifier is thus considered as a proxy of the brain's ability to discriminate fruit on the basis of OR responses.

We then built 12 sets of 10,000 models. Each model had a combination of a fixed number of ORs (10 or 20, a rough estimate of the number of ORs likely to be sensitive to fruit odours in Tephritidae species), and a fixed number of compounds detected per OR (low, medium or high value, i.e. 10, 20 or

60 compounds per OR for models with 10 ORs, 5, 10 or 30 compounds per OR for models with 20 ORs, respectively). Volatile data for sliced or intact fruit was fed into the models.

2.5. Triple electroantennography EAG_3 bioassays

Electrophysiology experiments were conducted using the EAG_3 technique[21] (Fig. 4a). EAG_3 improves the estimation of the insect's olfactory responses by simultaneously recording at three antennal positions. A live fly was immobilized in a 1,000 µL pipette tip, with its head exposed. The base of the head was fixed with dental wax (Enta Periphery Wax, Netherlands). For species of the Dacini tribe with elongated antennae, the first two antennal segments, which do not have olfactory functions, were stabilized with a silicone adhesive elastomer "Kwik-Sil" (World Precision Instruments, Inc., Sarasota, USA). Four electrodes, consisting of a chloride silver wire inserted into a glass micropipette (tip diameter 1-2 µm), filled with an electrolyte solution (120 mM NaCl, 5 mM KCl, 1 mM CaCl2, 4 mM MgCl2, and 10 mM HEPES), were used to perform the EAG_3 recording. The recording electrodes were placed in the latero-proximal, medio-central and latero-distal regions of the right funiculus. The reference electrode was inserted in the middle of the left eye. The recording electrodes were connected to a four-channel differential AC amplifier (Model 1,700, A-M system, Carlsborg, USA) which amplified (gain x 1,000) and filtered the signals between 1 and 1,000 Hz. Data acquisition was fitted by digitalizing the signals at 500 Hz with an acquisition card (NI 9215, National instruments, France), associated with Labview software (National instruments, France). Data were analysed using a current source density model to infer the total antennal response from the EAG signal recorded at three positions[23,24].

Stimuli were delivered through a glass chamber (20 cm x 5 mm i.dFID.). The tube had a small hole 5 cm upstream its outlet into which the odour source was inserted. A continuous humidified and charcoal-filtered airflow (20 $mL.s^{-1}$) across the glass chamber reached the insect, which was 1 cm from the tube outlet. The insect was stimulated using an odour cartridge consisting of a Pasteur pipette with a square of filter paper loaded with 1 µL of a solution of a synthetic compound diluted at 10^{-4} v/v or 10^{-2} v/v in mineral oil. The Pasteur pipette was inserted into the small hole in the glass chamber and exposed

to a puff of air for 200 ms (3 mL.s^{-1}), that was regulated with an electro-valve (LHDA- 1233215-H, Lee Company, France), and controlled by a digital module (NI 9472, National Instruments, Nanterre, France), and Labview software (National Instruments, France). The experiment consisted of passing 30 compounds (listed in Supplementary Table 3) individually in a random order with a time interval of 1 minute between each stimulation. A negative control stimulation (mineral oil) and a positive stimulation (1-octen-3-ol) were performed before and after each experiment.

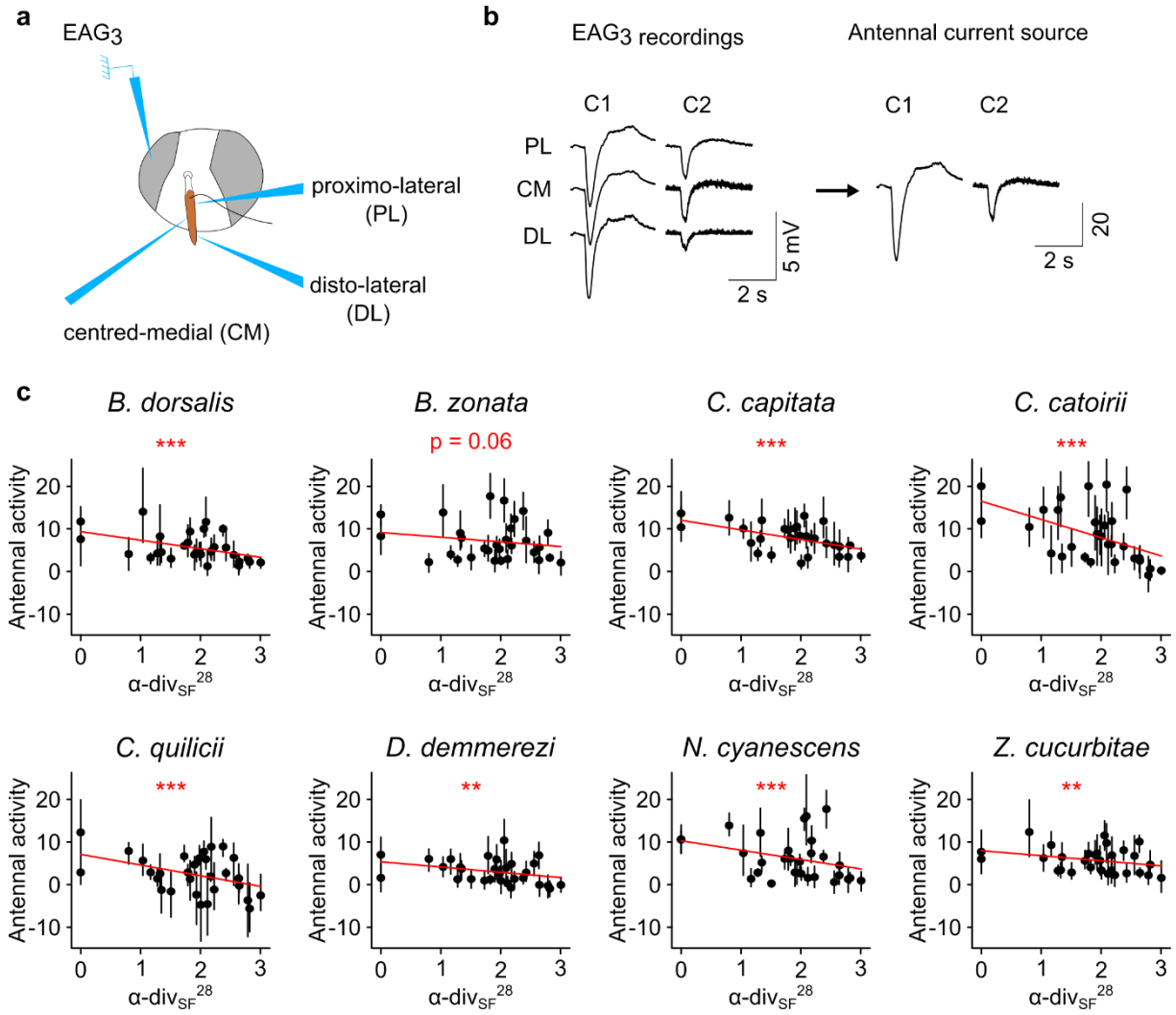

Fig. 4: Antennal responses of eight Tephritidae species measured with EAG_3 correlates with fruit α-diversity. **a** Schematic representation of the EAG_3 mounting. **b** An example of EAG_3 recording on individual female *B. dorsalis* stimulated with two compounds (C1 and C2). Left, the three signals represent the simultaneous recording at the three positions on the antenna. Right, the corresponding antennal current source are inferred from a CSD model and used to estimate the total antennal activity. **c** Antennal responses of females from eight Tephritidae species to synthetic compounds at dilution v/v: 10^{-4} depends on sliced fruit α-diversity. Points: mean value; bars: bootstrapped 95% c.i., n = 3 to 5 individuals per species. Red lines correspond to linear regression curves, whose significance is shown (** $p<0.01$; *** $p<0.001$).

2.6. Coupling chopped gas chromatograph - triple electroantennography detector (GC-EAD_3)

For GC-EAD_3 experiments, EAG_3 antennal preparation was used as a detector in parallel with the flame ionization detector (FID). A fused silica Restek Rxi-5 ms column (30 m x 0.32 mm i.d., 0.25 µm film thickness, Restek, Lisses, France) was used and heated as follows: 50°C for 1 min, then 6.5°C.min^{-1} to 200°C and 40°C.min^{-1} to 250°C, held for 5 min. The temperature of the FID was set to 250°C. Helium N60 was used as the carrier gas. To improve the signal to noise ratio, the GC system was equipped with a D-Swafer Dean's switch (PerkinElmer, Inc., USA) at the column output, as described previously[21]. The device allows for the GC signal to be chopped with the alternate emission of the effluent to both the EAG_3 and FID detectors[21,25]. The chop frequency was regulated to 1 Hz with Labview software (National Instruments, France) and a digital module (NI 9472, National Instruments, Nanterre, France). The signal was demodulated at the chopping frequency (1 Hz) before a similar analysis as for the EAG_3 recordings[21]. The sample was fed into the glass chamber using a 3 m long heated transfer line (Antelia, Dardilly, France). To deliver the stimulations to the insect, a short length of column at the output of the transfer line was inserted into the small hole in the glass chamber used in the EAG_3 experiments.

We manually injected 1µL of solution, which consisted of hexane loaded with a blend of synthetic compounds diluted at 10 ng.µL^{-1} or 1 µg.µL^{-1} each, into the GC. In total, 37 synthetic compounds (listed in Supplementary Table 3) were split into two blends, which were delivered to the same individual in a random order. One run lasted 30 minutes, i.e. one experiment lasted 1 hour. Negative (mineral oil) and positive (hexenyl acetate and 1-octen-3-ol, dilution v/v: 10^{-2} in mineral oil) control stimulations were successively performed before and after each run, following the same procedures as for the EAG_3 experiments.

2.7. Behavioural assay setup

An *ex situ* trapping system was used to study the behavioural response of *B. dorsalis* to different blends of compounds. The system consisted of two handmade traps, which were placed in a rearing cage measuring 30 cm x 30 cm x 30 cm. The traps were made using a 30 mL wide mouth glass vial sealed

with parafilm and lined with yellow coloured tape to guide the flies towards the traps. The lid was then perforated and a sectioned transparent 1,000 µL pipette tip was inserted into the hole, serving as a conduit for the flies. A piece of filter paper loaded with 1 µL of the tested blend was placed at the bottom of the trap and the odour diffused passively. The two traps in the cage were loaded either with different blends or with a negative control (mineral oil only). Six cages were tested simultaneously with the same combination of traps in different positions. In each cage, 30 starved gravid female flies were released simultaneously and the number of flies in each trap was noted every 30 or 60 minutes. Each trap was used only once and the cages were thoroughly cleaned with soap between experiments.

For behavioural assays, two blends of 5 compounds were tested. The first blend consisted of methyl 2-methylbutyrate, butyl butyrate, methyl heptanoate, methyl octanoate and prenyl acetate. The second blend consisted of ethyl acetate, cis-3-hexenyl acetate, hexyl acetate, ethyl propionate and ethyl butyrate. Lastly, we used a blend combining all ten compounds. Preliminary work for calibrating compound ratios was carried out in the SLU laboratory at the Swedish University of Agricultural Sciences in order to homogenize the volatile emission rates in the blend before the behavioural assays. The blends were diluted in mineral oil and tested at two different doses (dilution v/v 10^{-4} and 10^{-3} of total compounds). The percentage of captured flies was analysed using generalized linear mixed-effects models (GLMM) with binomial error distribution (*glmer* function, R package *lme4*). Blends and doses were considered as fixed effects and the experimental date was considered a random effect.

2.8. Data analysis

A pre-processing of GC-MS data was carried out with MZmine2 software[26]. Compound peaks were detected by ADAP algorithm[27] (S/N threshold 3, Wavelet Coeff. 3, minimum feature height 1,000,000, coefficient/area threshold 300, peak duration 0 to 0.2, RT wavelet 0 to 0.05 min), deconvoluted through blind source separation (window width 0.1, minimum number peaks 2, RT tolerance 0.025), RANSAC aligned (m/z tolerance 0.3, RT tolerance 0.1 min, automatic iteration no., minimum points no. 50%, threshold 0.1 min), and gap filled (m/z tolerance 0.3, RT tolerance 0.02 min). Each compound was identified according to its molecular weight and retention index (RI, calculated

from the retention times of C6-C30 n-alkanes), and by comparing its mass spectra with NIST 14 library databases.

Further statistical analyses were conducted using R software[28]. When the quantity of compounds in the control samples was greater than in the samples with fruit, they were excluded from the analysis ($p < 0.05$, Wilcoxon test, *wilcox.test* function, R package *Stats*). Volatilomics data were then normalized by dividing each compound peak by the geometric mean of each corresponding sample, then Box-Cox transformed (*boxcox* function, R package *MASS*, lambda = 0.014) and Pareto scaled (*paretoscale* function, R package *RFmarkerDetector*[29,30]).

The degree of sharedness among fruit species was calculated for each compound by Shannon α-diversity index (*diversity* function, R package *vegan*)[31]. For the calculation, we used the volatile samples of intact ($\alpha\text{-div}_{IF}^{28}$) and sliced ($\alpha\text{-div}_{SF}^{28}$) fruit of 28 species, as well as volatile samples of intact ($\alpha\text{-div}_{IF}^{13}$) and sliced ($\alpha\text{-div}_{SF}^{13}$) fruit from a subset of 13 species. The subset included species with a *B. dorsalis* infestation rate of above 5%, which were collected in Réunion during a recent campaign to monitor Tephritidae infestation (Table 1) [20]. The indices $\alpha\text{-div}_{IF}^{28}$ and $\alpha\text{-div}_{SF}^{28}$ can vary between a minimal value of 0 (if a single fruit species emits the compound) and a maximal value of 3.33 (when all 28 fruit species emit the compound). Similarly, the indices $\alpha\text{-div}_{IF}^{13}$ and $\alpha\text{-div}_{SF}^{13}$ can vary between a minimal value of 0 (when a single fruit species emits the compound) and a maximal value of 2.57 (when all 13 fruit species emit the compound). To measure the phylogenetic signal, Abouheif's C_{mean} index (*abouheif.moran* function, R package *adephylo*) was computed for each compound based on an autocorrelation approach[32,33]. To calculate the index, the phylogeny of the 28 fruit species was generated by Bayesian phylogenetic inference using Markov Chain Monte Carlo (MCMC) methods[34]. The matrix of phylogenetic proximities was performed with the "oriAbouheif" method (*proxTips* function, R package *adephylo*)[33]. It was calculated for the 28 species using volatile samples of intact ($C_{mean,IF}^{28}$) and sliced ($C_{mean,SF}^{28}$) fruit.

After a preliminary demodulation of the EAD_3 signal at the chopping frequency (1 Hz), we calculated the total antennal activity from EAG_3 and EAD_3 data using the current source density (CSD)

model[23,24]. To apply the linear models, antennal activity data were normalized by adding a constant value before applying a Box-Cox power transformation. The constant value was chosen to ensure that the Box-Cox transformation was log-equivalent. The normality of the resulting distribution was assessed using a Shapiro-Wilk test. For linear models, individual identity was used as an independent variable.

3. Results

3.1. The volatile emissions of many fruit species partially overlap

To explore the diversity of the volatile emissions of 28 species of fruit using ATD-GC-MS, we analysed both intact fruit (still on the tree) and sliced fruit (Fig. 1). For intact fruit, we detected 511 compounds, of which 379 were identified and a further 55 were unidentified, but assigned to a chemical class. For the sliced fruit, we detected 665 compounds, of which 463 were identified and a further 38 were assigned to a chemical class. The main chemical classes are esters (13.31% and 18.35% of the total number of compounds for intact and sliced fruit, respectively) and terpenoids (14.87% and 13.38% of the total number of compounds for intact and sliced fruit, respectively). The remaining compounds are classified as: alcohol, aldehyde, amine, aromatic, carboxylic acid, ether, furanoid, green leaf volatile (GLV), hydrocarbon, ketone, organohalogen, organophosphorus, organosulfur and unknown (Fig. 1e). The proportions of the different chemical families of compounds in intact and sliced fruit were significantly different (χ^2 (15) = 31.084; p = 0.008). Supplementary Table 1 and 2 provides the list of all the identified compounds.

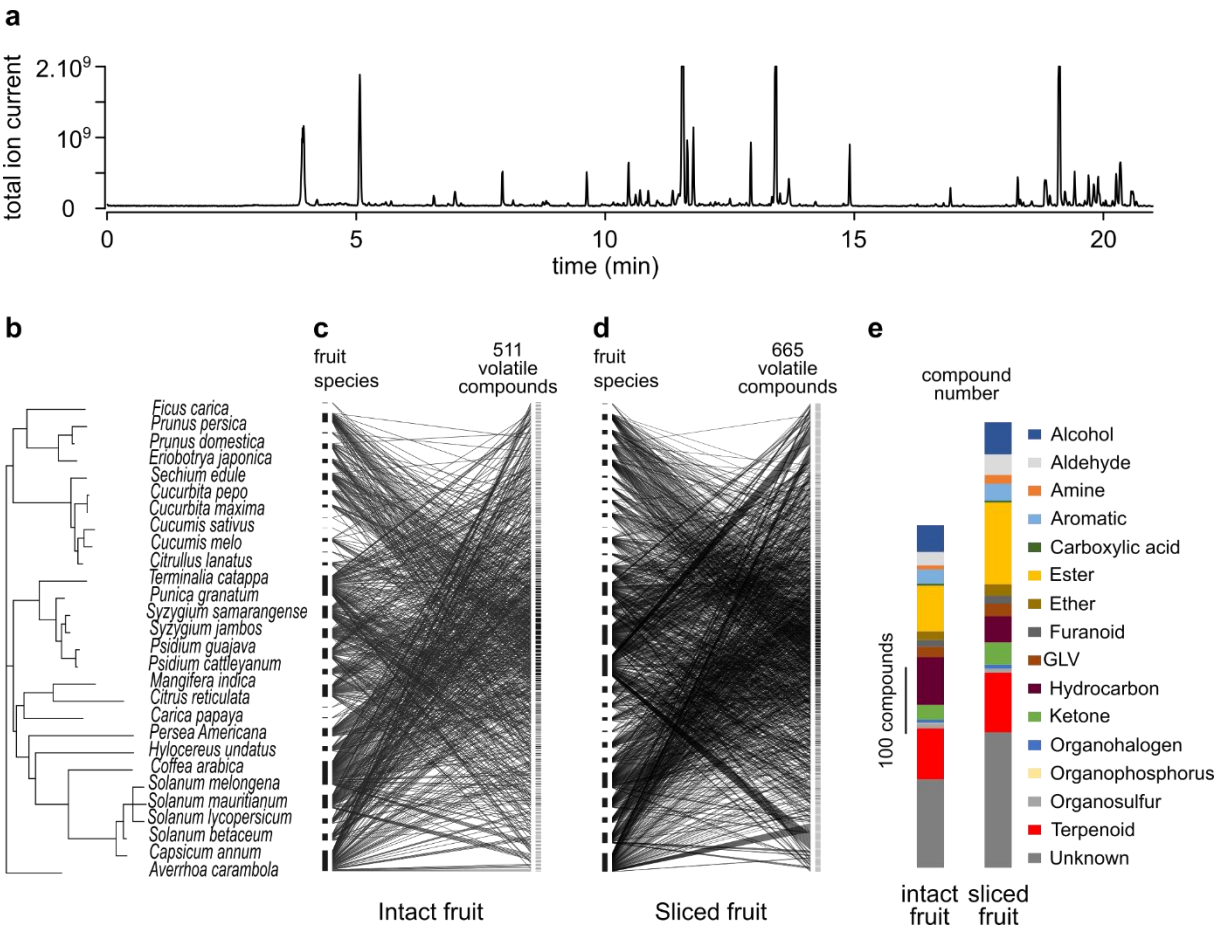

Fig. 1: Diversity of volatile emissions produced by the 28 host fruit species of the Tephritidae studied. **a** Example of a chromatogram collected from the volatile emissions of a guava fruit (*Psidium guajava*). **b** Phylogeny of the 28 fruit species from which volatile emissions were collected. **c** Interaction network between the fruit species (left) and the 511 volatile compounds (right) collected from intact fruit. The fruit species are sorted in the same phylogenetic order as in panel (a), and the compounds are sorted by an index of fruit α-diversity, for which the maximal number is in the middle and the minimal (1 fruit) is at both extremities. **d** Interaction network between the fruit species (left) and the 665 volatile compounds (right) collected from sliced fruit, ordered as in panel (c). **e** Chemical classification of volatile compounds identified for the 28 fruit species. The two bars show the compounds of intact fruit (left) and sliced fruit (right), respectively. Each colour represents the chemical classes of compounds and the corresponding height is proportional to the number of compounds in each chemical class.

Despite the great diversity of fruit species included in this study, fruit emissions overlapped partially, with compounds emitted by 1 to 27 fruit species. We used indices of α-diversity among the volatile emissions of intact ($\alpha\text{-div}_{IF}^{28}$) and sliced ($\alpha\text{-div}_{SF}^{28}$) fruit to quantify the degree of sharedness. If there is a strong phylogenetic signal, closely related fruit species are likely to emit the same compounds. To test if high α-diversity values result from this type of phylogenetic autocorrelation, we computed the phylogenetic signal using the Abouheif's C_{mean} index for intact ($C_{mean,\ IF}^{28}$) and sliced ($C_{mean,\ SF}^{28}$) fruit samples. Indeed, we found a significant correlation between $\alpha\text{-div}_{IF}^{28}$ and $C_{mean,\ IF}^{28}$ ($F(1, 509) = 13.6$;

$p < 0.001$) and between $\alpha\text{-div}_{SF}^{28}$ and $C_{mean,\ SF}^{28}$ ($F\ (1, 663) = 19.3$; $p < 10^{-4}$). However, the variance explained by these models was low ($r^2 = 0.026$ and $r^2 = 0.028$, respectively) (Fig. 2).

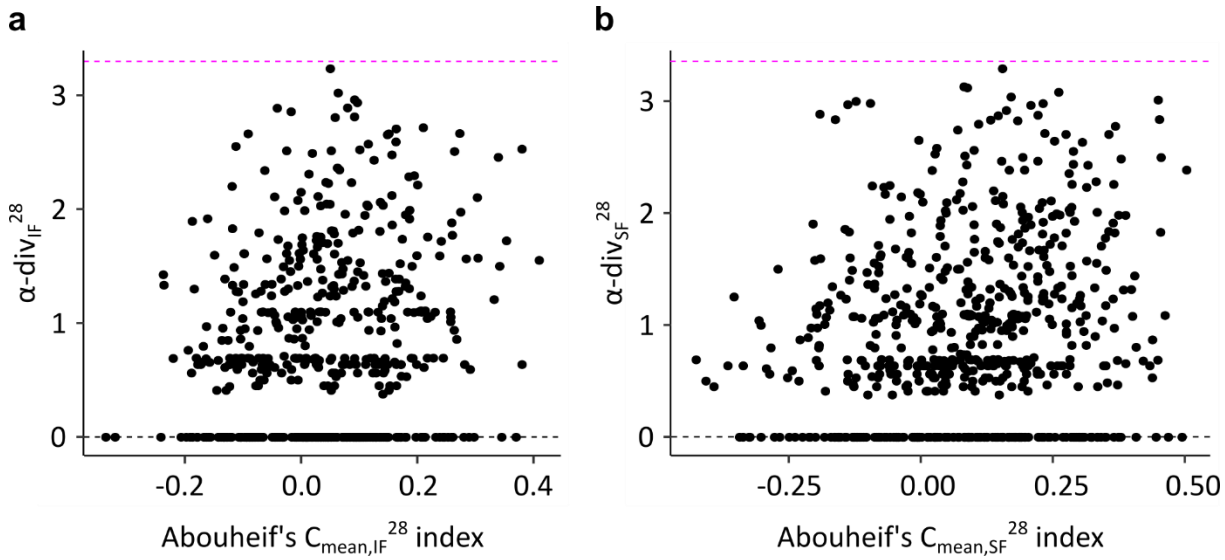

Fig. 2: Relationship between α-diversity indices and phylogenetic signal Aubouheif's C_{mean} for the emissions from intact **a** and sliced **b** fruit. Each point represents a compound. Compounds with an α-diversity of 0 are emitted by only one fruit species. The magenta dashed line indicates the theoretical maximal α-diversity value reached if all fruit species emit the compound. A C_{mean} of 0 means phylogenetic independence, while values above 0.2 are considered phylogenetically correlated.

3.2. A neuronal model predicts a contrasted use of species-specific and shared fruit compounds for odour-based fruit detection and discrimination

Neuronal modelling was used to test the hypothesis that the ecological need to detect a large number of hosts encourages olfactory tuning for host-shared compounds, and the alternative hypothesis that the ecological need to discriminate host species encourages olfactory tuning for species-specific compounds. We built 120,000 random neuronal models of a peripheral olfactory system (Fig. 3a). The GC-MS data for intact or sliced fruit samples were used as input odours for these models in order to consider the natural variability of fruit emissions within and between fruit species, and the natural distribution of the degree of sharedness of volatile compounds in fruit emissions. For each model, we estimated the efficiency of the olfactory system to detect and discriminate fruit using specially designed

indices (Fig. 3b). The performance of the models was heterogeneous, allowing us to explore the typical chemical tuning of the most efficient olfactory systems for either detection or discrimination. Not surprisingly, models with 20 ORs discriminated fruit better than models with 10 ORs (F (1, df > 10^5) = 258,197; $p<10^{-15}$), and the fruit detectability index was correlated with the number of compounds detected per OR (F (2, df > 10^5) = 921,033; $p < 10^{-15}$). For cross-comparison, we standardized the distributions (expressed as z-scores) for each combination of number of ORs, number of compounds detected per OR and input data sets (intact or sliced fruit emissions).

If ecological requirement tunes the peripheral olfactory system to species-specific and shared fruit compounds, responses should be correlated with fruit α-diversity in the efficient models only, resulting in a significant interaction with efficiency indices. Indeed, we found that response parameters depended on a joint effect between compound α-diversity and the fruit detectability index (Fig. 3c). This was driven by the proportion of detected compounds (F (1, df > 10^7) = 285; $p < 10^{-15}$), the sensitivity thresholds of detected compounds (F (1, df > 10^7) = 613; $p < 10^{-15}$) and, to a lesser extent, the dynamic range of detected compounds (F (1, df > 10^7) = 34.3; $p < 10^{-8}$). Specifically, olfactory systems that are efficient at detecting fruit perceive shared compounds better than species-specific ones. We also found that the same response parameters depended on a joint effect between fruit α-diversity and fruit species discriminability index (Fig. 3c), namely the proportion of detected compounds (F (1, df > 10^7) = 453; $p < 10^{-15}$), the sensitivity thresholds of detected compounds (F (1, df > 10^7) = 731; $p < 10^{-15}$) and, to a lesser extent, the dynamic range of detected compounds (F (1, df > 10^7) = 18.6; $p < 10^{-4}$). Olfactory systems capable of discriminating between fruit species efficiently are better at detecting species-specific compounds than shared compounds.

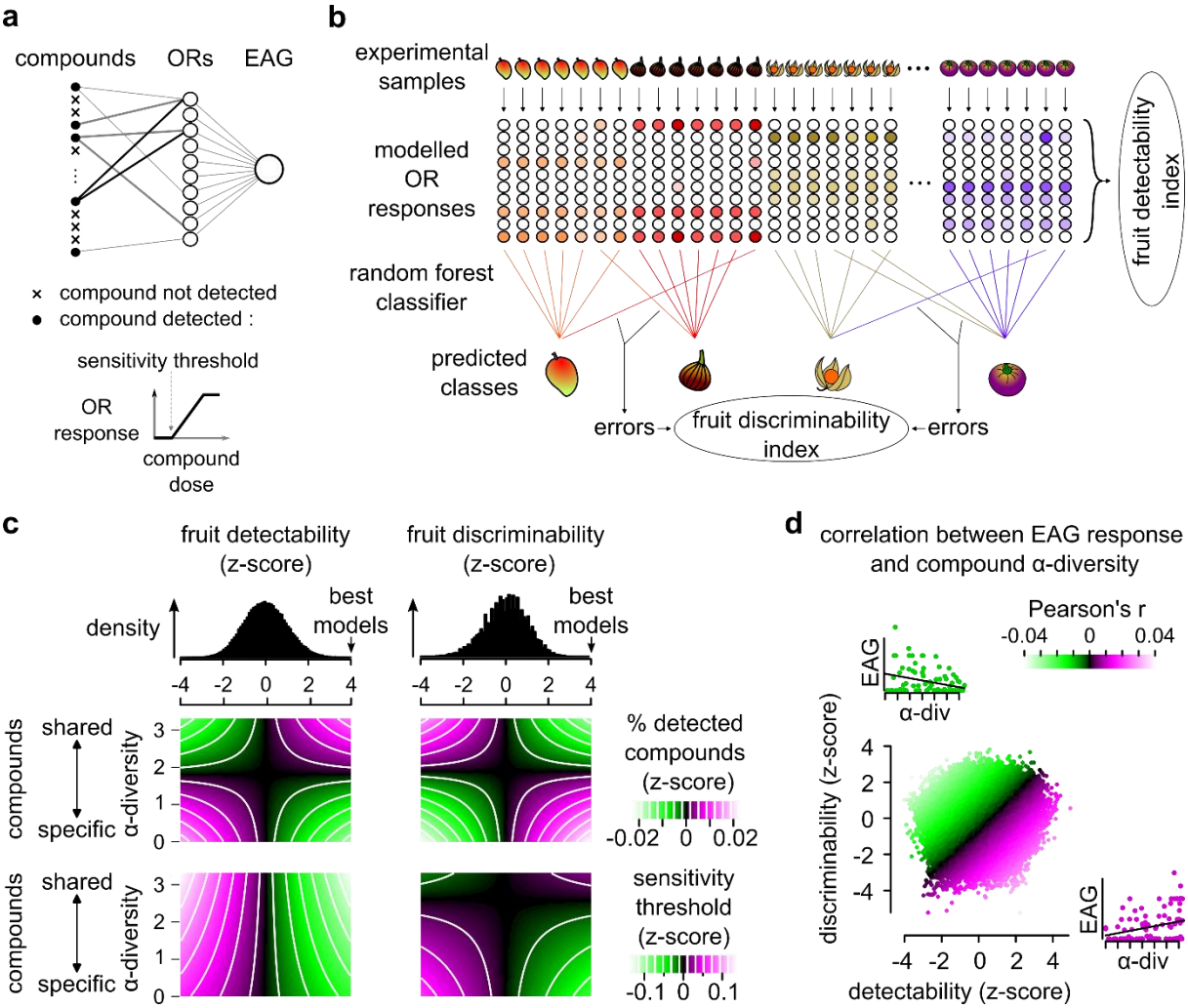

Fig. 3: Neuronal model of olfactory detection reveals a functional trade-off between the effective detection of and discrimination between fruit species. **a** Schematic representation of the parameters included in one random model of an insect's olfactory system. Each olfactory receptor (OR) is sensitive to a random set of compounds, as shown by a simplified dose-response curve, whose threshold and dynamic range have been drawn randomly. **b** Schematic depiction of the algorithms used to calculate the fruit detectability index and the fruit discriminability index for one random model. For this, experimental data corresponding to intact or sliced fruit samples were used as an input (top) and the modelled OR responses were calculated (middle). The fruit detectability index was the sum of all OR responses to all volatile samples (right). The fruit-species discriminability index (bottom) was calculated from the proportion of fruit species misclassified by a random forest algorithm applied on the OR response patterns. **c** Model parameters for 120,000 models with random connectivity. Top: the density distribution of fruit detectability and the fruit-species discriminability index for the 120,000 models. The best models for the two functions are on the upper part of the distribution. The linear projection of the proportion of compounds detected by the olfactory system (upper heatmaps) and that of the sensitivity threshold of detected compounds (lower heatmaps) are drawn on the spaces delineated by the model's fruit detectability indices (left heatmaps) and fruit-species discriminability indices (right heatmaps), as the x-axis, and compound α-diversity as the y-axis (significant interaction in the four cases, $p < 10^{-15}$). **d** The correlation coefficient between EAG responses at four doses and α-diversity was computed for each model. Insets show examples of a negative correlation (green) and a positive correlation (magenta) at a given dose. The scatter plot shows a linear projection of the correlation coefficients on the detectability/discriminability space, where each dot is an individual model.

We then used the model to generate a forecast, which is easy to test experimentally. For each model, we estimated the EAG responses to every detected compound at four different doses spanning four log-units. We calculated how much it correlated with the α-diversity of emitting fruit. The resulting correlation coefficient was positively correlated with the fruit detectability index ($F(1, df > 10^5) = 5{,}373$; $p < 10^{-15}$) and negatively correlated with the fruit species discriminability index ($F(1, df > 10^5) = 4{,}069$; $p < 10^{-15}$), with no interaction between the two ($F(1, df>4.10^5) = 1{,}4$; $p = 0.24$, Fig. 3d). This was the case for all combinations of OR numbers, the numbers of detected compounds per OR and intact or sliced fruit input data (Supplementary Fig. 1). Yet, the correlation with the fruit detectability index was stronger at low compound dose (joint effect $F(3, df > 10^5) = 25$; $p<10^{-156}$), and for a small number of compounds detected per OR (joint effect $F(2, df > 10^5) = 547$; $p<10^{-15}$), but did not depend on the number of ORs (joint effect $F(1, df > 10^5) = 0.33$; $p=0.56$). The correlation with the discriminative index was stronger at low compound dose (joint effect $F(3, df > 10^5) = 10$; $p<10^{-6}$), with 10 ORs than with 20 ORs (joint effect $F(1, df > 10^5) = 61$; $p<10^{-14}$) and for a small number of compounds detected per OR (joint effect $F(2, df > 10^5) = 298$; $p<10^{-15}$).

In short, our modelling study suggests that there is a functional trade-off in terms of the insects' capacity to detect and discriminate between fruit species. Studying the relationship between EAG response and the α-diversity of emitting fruit appears to be a simple measure, which could be compared for the two functional specializations.

3.3. Tephritidae olfactory responses are negatively correlated with the degree of sharedness of volatile compounds among fruit species

A set of 40 commercially available compounds, regularly distributed along the fruit α-diversity indices, was used to test the olfactory system of Tephritidae species (Supplementary Table 3). We chose compounds from two chemical classes only, esters and terpenoids, given that we intended to explore the role of ecological rather than chemical variability. For these compounds, the indices of α-diversity, $\alpha\text{-div}_{IF}^{28}$ and $\alpha\text{-div}_{SF}^{28}$, were significantly correlated ($F(1, 34) = 22.5$; $p<10^{-4}$). Moreover, $\alpha\text{-div}_{SF}^{28}$ was

not correlated with any of the compounds' obvious chemical properties, namely boiling point (F (1, 38) = 2.1; p = 0.16), vapour pressure (F (1, 38) = 1.6; p = 0.21), lipophilicity estimated with log *Poct/wat* (ChemSketch, ACD/Labs) (F (1, 38) = 0.49; p = 0.49), or depression rate[35] (F (1, 38) = 1.2; p = 0.28) (Supplementary Fig. 2).

The antennal responses to 30 of the compounds were measured in females from eight Tephritidae species with EAG_3 at a dose of 10^{-4} (dilution v/v). To rule out possible experimental bias, we tested how this measure correlated with $\alpha\text{-div}_{SF}^{28}$, using the compound depression rate as an independent variable. Antennal response was negatively correlated with $\alpha\text{-div}_{SF}^{28}$ (F (1, 884) = 88.5; p < 10^{-15}), with an interaction with species identity (F (7, 884) = 2.1; p =0.05). More precisely, it was significant in all species tested independently, except *B. zonata*, n = 4, F (1, 104) = 3.59; p = 0.06: *B. dorsalis*, n=4 individuals, F (1, 104) = 12.6; p < 0.001; *C. capitata*, n = 5, F (1, 130) = 24; p < 10^{-5}; *C. catoirii*, n = 5, F (1, 130) = 39; p < 10^{-8}; *C. quilicii*, n = 3, F (1, 78) = 13; p = 0.001; *D. demmerezi*, n = 4, F (1, 104) = 9; p = 0.003; *N. cyanescens*, n = 5, F (1, 130) = 15; p < 0.001; and *Z. cucurbitae*, n = 4, F (1, 104) = 9.8; p = 0.002 (Fig. 4). Antennal response was also negatively correlated with $\alpha\text{-div}_{IF}^{28}$ (F (1, 782) = 56; p <10^{-13}), with an interaction with species identity (F (7, 782) = 2.9; p < 0.01). The effect was similar at a higher dose (10^{-2} dilution v/v) tested in *B. dorsalis*: the antennal response was still negatively correlated with $\alpha\text{-div}_{SF}^{28}$ (n = 7 individuals, F (1, 182) = 23; p < 10^{-5}) and with $\alpha\text{-div}_{IF}^{28}$ (F (1, 161) = 17; p < 10^{-4}, Supplementary Fig. 3).

The number of fruit species considered was of little consequence: we calculated indices of α-diversity among the volatile emissions of a subset of 13 fruit species (Table 1), reported to have a *B. dorsalis* infestation rate of above 5% in La Réunion (named $\alpha\text{-div}_{IF}^{13}$ and $\alpha\text{-div}_{SF}^{13}$ for intact and sliced fruit, respectively). We found a significant correlation between $\alpha\text{-div}_{IF}^{13}$ and $\alpha\text{-div}_{IF}^{28}$ (F (1, 34) = 79.8; p < 10^{-9}) and between $\alpha\text{-div}_{SF}^{13}$ and $\alpha\text{-div}_{SF}^{28}$ (F (1, 38) = 128.4; p < 10^{-13}, Supplementary Fig. 4). Again, antennal response was negatively correlated with $\alpha\text{-div}_{SF}^{13}$ (F (1, 884) = 100; p <10^{-15}), with an interaction with species identity (F (7, 884) = 2.1; p =0.05). It was significant in each species tested individually: *B. dorsalis*, F (1, 104) = 13.7; p < 0.001; *B. zonata*, F (1, 104) = 5.1; p = 0.03; *C. capitata*, F (1, 130) = 20; p < 10^{-4}; *C. catoirii*, n = 5, F (1, 130) = 28; p < 10^{-7}; *C. quilicii*, F (1, 78) = 19; p < 10^{-}

^{4}; *D. demmerezi*, F (1, 104) = 14; p < 0.001; *N. cyanescens*, F (1, 130) = 18; p < 10^{-4}; and *Z. cucurbitae*, n = 4, F (1, 104) = 19; p <10^{-4}. Antennal response was also negatively correlated with α-div_{IF}^{13} (F (1, 782) = 55; p <10^{-12}), with an interaction with species identity (F (7, 884) = 2.0; p = 0.05). We then explored the EAD_3 antennal response to 37 compounds. Here, the stimulations were provided through chopping modulated GC. This protocol induced more experimental bias, since antennal responses to GC-stimulations were strongly correlated with compound depletion rate (F (1, 280) = 29; p < 10^{-6}), which might result from the chopping modulation. However, we found that the GC-EAD_3 antennal response of the eight species was negatively correlated with div_{SF}^{28} (F (1, 264) = 10.5; p=0.001) and with div_{IF}^{28} (F (1, 232) = 30.1; p<10^{-7}), with no interaction with species identity (F (7, 264) = 0.4; p=0.9 and F (7, 264) = 0.9; p=0.5, respectively). For each species tested independently, antennal response depended on a joint effect between div_{IF}^{28} and compound depletion rate (Supplementary Fig. 5): *B. dorsalis*, n=4 individuals, F (1, 29) = 8.2; p < 0.01; but *B. zonata*, n = 4, F (1, 29) = 4.1; p = 0.05; *C. capitata*, n = 3, F (1, 29) = 11.8; p = 0.002; *C. catoirii*, n = 3, F (1, 29) = 4.8; p = 0.04; *C. quilicii*, n = 3, F (1, 29) = 9.3; p = 0.005; *D. demmerezi*, n = 3, F (1, 29) = 7.8; p = 0.01; *N. cyanescens*, n = 3, F (1, 29) = 9.2; p =0.005; and *Z. cucurbitae*, n = 4, F (1, 29) = 24.4; p <10^{-4}. The effect was similar at a higher dose (1 μg) tested in *B. dorsalis*: antennal response depended on a joint effect between div_{IF}^{28} and compound depletion rate (n = 5 individuals, F (1, 29) = 8.2; p <0.01). To sum up with regard to GC-EAD_3 data, the main effect was a negative correlation between antennal response and div_{IF}^{13} for compounds with a low depletion rate.

3.4. Female *B. dorsalis* display a dose-dependent switch in behavioural preference between shared and species-specific fruit compounds

Overall, the olfactory tuning of Tephritidae antennae is consistent with the hypothesis that the ability to discriminate fruit species has an ecological role, as predicted by the models. A matching prediction would be that at low doses, flies would only perceive species-specific fruit compounds. We designed a behavioural assay involving a dual choice *ex situ* trap to test this (Fig. 5a). We selected five

species-specific fruit compounds and five shared compounds, all of which were small esters to ensure low chemical variability (Table 2). A behavioural experiment showed that female *B. dorsalis* were more attracted to a blend of the 10 compounds, whose emission rates were homogenized, than to a negative control (GLMM: χ^2 (1, N = 11) = 28.845; $p < 0.001$; mean proportion of flies trapped after 125 min: 0.185 (95% CI 0.118; 0.252) and 0.003 (95% CI -0.003; 0.009), respectively; Fig. 5b). Then, we tested the comparative attractiveness of the two sub-blends of five species-specific fruit compounds and five shared compounds. At the lowest dose tested (dilution v/v: 10^{-4}), insects were more attracted to the species-specific compound blend than to the shared compound blend (GLMM: χ^2 (1, N = 46) = 214.617; $p < 0.001$; mean proportion of flies trapped after 240 min: 0.186 (95% CI 0.118; 0.254) and 0.048 (95% CI 0.027; 0.070), respectively; Fig. 5c). At a higher dose (dilution v/v: 10^{-3}), a reversal of the trend was observed, i.e. females preferred the shared compound blend over the species-specific compound blend (GLMM: χ^2 (1, N = 60) = 38.355; $p < 0.001$; mean proportion of flies trapped after 240 min: 0.133 (95% CI 0.098; 0.168) and 0.109 (95% CI 0.076; 0.142), respectively; Fig. 5d).

Table 2: List of compounds used for behavioural tests. The last column shows a list of fruit species in which the compound was found in sliced fruit samples only and not in intact fruit.

			Found in the emissions of:	
blend	CAS	compound	intact fruits	sliced fruit only
shared compounds	3681-71-8	cis-3-hexenyl acetate	*Indian almond, strawberry guava, peach, plum*	*bugweed, coffee, loquat, mango, melon, star fruit, tomato*
	141-78-6	ethyl acetate	*bugweed, coffee, Indian almond, loquat, mango, peach, strawberry guava, tree tomato*	*chilli, guava, melon, rose apple, star fruit, tomato*
	105-54-4	ethyl butyrate	*strawberry guava, rose apple, mango*	*bugweed, melon, peach, plum, star fruit, tree tomato*
	105-37-3	ethyl propionate	*bugweed, rose apple, tomato, papaya*	*guava, melon, plum, star fruit*
	142-92-7	hexyl acetate	*chilli, eggplant, Indian almond, papaya, peach, pumpkin, star fruit, strawberry guava*	*plum*
species-specific compounds	109-21-7	butyl butyrate	*Plum*	-
	868-57-5	methyl 2-methylbutyrate	*rose apple*	*melon, star fruit*
	106-73-0	methyl heptanoate	*star fruit*	-
	111-11-5	methyl octanoate	*peach, star fruit*	*guava, strawberry guava*
	1191-16-8	prenyl acetate	*Indian almond*	*guava, strawberry guava*

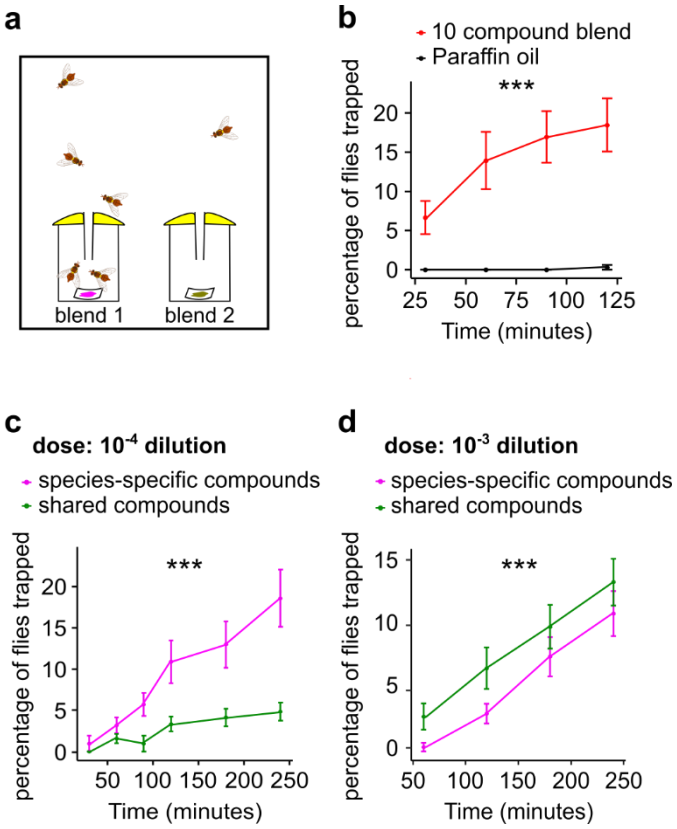

Fig. 5: *B. dorsalis* behavioural preference between species-specific and shared fruit compounds is dose-dependent. **a** Schematic drawing of the *ex situ* trapping bioassay. **b** Dual choice tests between a blend of 10 compounds diluted at 10^{-4} v/v in mineral oil (red) and mineral oil (black); values are mean +- SE. ***: significant difference between the two blends GLMM, $p < 0.001$. **c** Dual choice tests between a blend of five shared compounds (in green) and a blend of five specific compounds (in magenta) tested at the dilution v/v: 10^{-4}. Same convention as in panel (b). **d** Dual choice tests between the same blends as in panel (c) diluted at 10^{-3} v/v. Same conventions as in panel (b).

4. Discussion

In this paper we used chemical analysis and modelling, combined with electrophysiological and behavioural approaches to explore the chemical interactions between Tephritidae species and 28 of their host fruit species. By analysing the chemical composition of volatile emissions from 28 Tephritidae host fruit, either intact (on the tree) or after mechanical damage, we identified a considerable number of compounds. As expected, damaged fruit produced more volatile compounds than intact fruit[36–38]. The volatile emissions were dominated by terpenoids and esters, which are characteristic of volatile fruit emissions and are easily detected by insect olfactory receptors[39,40]. Although each fruit species has its own chemical profile, there is considerable overlap between species. The degree of sharedness among

fruit species (fruit α-diversity) varied, some compounds were specific to a single species, while others were emitted by most of the fruit species considered. We found a significant negative correlation between the antennal responses of several Tephritidae species and volatile α-diversity. The effect was robust, regardless of how the α-diversity index was calculated by considering: all 28 fruit species or a subset of 13 species, with a high infestation rate of generalist species; and intact or mechanically damaged fruit. Lastly, we found that mature female *Bactrocera dorsalis* demonstrated a dose-dependent behavioural preference when we compared a blend of species-specific fruit compounds and a blend of shared compounds. Below, we will discuss how our observations and neuronal modelling support the alternative hypothesis regarding the olfactory strategy used by Tephritidae to explore their wide host range.

The main hypothesis suggests that since Tephritidae females have an ecological need to detect a large number of suitable hosts for oviposition, their olfactory system should be tuned for compounds shared by different fruit species. This type of behaviour would allow females to identify a wide host range with minimum effort in terms of information processing and, therefore, minimum energy consumption[41–43]. Supporting this theory, the addition of three compounds identified in ripe guava considerably increased *Bactrocera tryoni*'s attraction to several non-preferred hosts[7]. The authors suggest that these compounds could be a generic signature for ripe fruit. Furthermore, a blend of volatile compounds shared between four phylogenetically distant fruit species was attractive for *Bactrocera dorsalis* and *Zeugodacus cucurbitae*[16]. The hypothesis raises a number of issues, which we tested. First, do phylogenetically distant fruit species emit the same volatile compounds? Clarke predicted a substantial proportion of shared compounds was an adaptation to generalist seed dispersers[44]. We found that fruit α-diversity was only weakly correlated with fruit phylogeny, i.e. a number of identical compounds were emitted by fruit that did not belong to the same clade. Relying on shared compounds could explain why tephritid host associations do not correlate with fruit phylogeny[19]. Second, do generalist species prefer shared compounds over species-specific fruit compounds? Our observations reveal a preference for shared compounds in *B. dorsalis*, but only for the highest dose tested. Third, do shared compounds induce a stronger antennal response and, therefore, are they overrepresented

compared to species-specific compounds? Our neuronal model confirms that this should be the case and suggests two testable predictions: the proportion of compounds detected by the olfactory systems should correlate positively with the compound's degree of sharedness among fruit species, which was indeed observed in another study on Tephritidae[5]; the response amplitude to detected compounds should also correlate positively with the compound's degree of sharedness among fruit species, but here we observed a negative correlation. Our observation is unlikely to be an experimental artefact because a bias due to the heterogeneous volatility of the tested compounds should not make a difference. Indeed, we found no correlation between fruit α-diversity and boiling point, lipophilicity or depletion rate[35]. In addition, the effect was observed both with a passive and GC-induced vaporization of compounds. The use of triple electroantennography reduces the risk of uneven sampling of the olfactory receptor[23,24]. Artefactual responses to impurities have been reported[45,46], but were refuted here by the GC-EAD analysis of the same compounds.

When faced with various volatile compound emitters, insects must be able to identify their hosts' volatile compounds from a complex environment, which may include volatile emissions from non-host plants[3,4]. Their olfactory systems may be tuned to host-specific compounds. Some compounds shared between fruit species may also be emitted by other plant parts and non-host species. Therefore, they may be widely distributed in the natural environment where the species evolved and, thus, be neglected by the insect. Cis-3-hexenyl acetate is a case in point. Although emitted by many fruit species, this compound is also emitted by leaves in response to herbivore damage[47]. While plant compounds like this may have high fruit α-diversity, they may have little ecological relevance for insects, if any. In this scenario, a strong antennal response would only be expected for shared compounds that are specifically emitted by fruit. However, the hypothesis would have predicted a low response to shared plant compounds and species-specific host compounds rather than the negative correlation that we observed. Moreover, shared compounds that are specific to fruit should be attractive to insects, irrespective of the dose. We observed a preference to species-specific fruit compounds at low doses, which suggests that another factor may be involved.

Our results led us to consider the alternative hypothesis, namely that polyphagous insects have an ecological need to discriminate between host species. The ability to change host preference to adapt to a dynamic environmental context provides evolutionary advantages, which have been widely discussed[10,48,49]. Host preference in Tephritidae can change, as demonstrated by the comparative analysis of wild and laboratory-reared individuals of the medfly species, *Ceratitis capitata*[50]. A switch in host preference can be induced in *C. capitata* females[9] and adult attraction behaviour can be conditioned[51] or influenced by larval diet[17] in *B. dorsalis*. As an adaptive consequence of learning, host preference of *C. capitata* changes depending on host abundance[18], as predicted by Jaenike's optimal oviposition behaviour theory[48]. A prerequisite to host preference plasticity is the ability to discriminate hosts. We challenged the hypothesis that Tephritidae rely on species-specific fruit compounds to discriminate fruit species using a neuronal model of olfactory detection. The model suggested that ecological pressure to discriminate between fruit species would result in lower sensitivity thresholds for species-specific compounds compared to shared compounds. This phenomenon would generate a negative correlation between antennal response and fruit α-diversity. It is important to note that while our modelling formally validates a hypothesis that is consistent with our experimental observations, it does not constitute proof *per se*.

We do not suggest that Tephritidae rely solely on species-specific fruit compounds. Our hypothesis does not rule out the fact that phytophagous insects may use compounds shared by their hosts. Instead, our observations add an additional level of complexity to their olfactory strategies. Hence, the low antennal response to shared compounds does not mean that insects neglect them, but that high sensitivity is not required to detect them given that they are frequently encountered in the insects' natural environment. Therefore, we expected a dose-dependent switch in preference, which is exactly what we observed in our behavioural assay. It is important to note that blends of species-specific fruit compounds (i.e. with no shared compounds) do not exist in nature. Hence, we consider the effect as a sensory illusion, which stems from an ability to discriminate between hosts. To conclude, we postulate that insects detect hosts through host-shared compounds and choose between hosts through species-specific compounds. More generally, we expect that the three functions, namely, host detection, host vs non-

host selection and discrimination between hosts help shape the chemical tuning of insects' olfactory systems.

Surprisingly, we found a negative correlation between antennal response and fruit α-diversity in five polyphagous species and three oligophagous species of the tribes, Dacini and Ceratitidini. This suggests that the trait is conserved in Tephritidae, regardless of their ecology. This echoes the reported attraction of *Z. cucurbitae* and *B. dorsalis* to a blend of compounds shared by different fruit species[5]. Two hypotheses can explain this. First, the oligophagous species that we studied may not be truly oligophagous. The Cucurbitaceae specialist, *Z. cucurbitae*, is sometimes considered to be polyphagous[13], following reports of infestations in several fruit families including, Anacardiaceae, Caricaceae, Combretaceae, Moraceae, Solanaceae, etc.[19,52–55]. Infestations of other fruit families were also reported for Cucurbitaceae specialists from the Dacus genus[53,55] and for the Solanaceae specialist, *N. cyanescens*[19]. Secondly, phylogenetic analysis of host range diversity within the Dacini tribe revealed multiple episodes of host range switch, which involved broadening or reducing host range[13,56,57]. Since peripheral olfactory systems are generally conserved across Tephritidae[58], oligophagous species might have a polyphagous-type olfactory system due to phylogenetic inertia. To conclude, if the olfactory strategies we observed are related to polyphagy, similar analyses should be conducted on truly monophagous species or on polyphagous frugivores from other insect orders.

Understanding the olfactory strategies adopted by phytophagous insects that allow them to recognize their host plant not only provides fundamental insights into ecology and evolution, but it will also facilitate the effective design of attractants for pest control[59]. When it comes to efficient trapping, our data suggest that tephritids may not have a high sensitivity to a blend of compounds shared by different fruit species. Inversely, a blend of species-specific fruit compounds may not be sufficiently attractive compared to a mixed blend of compounds emitted naturally by various host fruit species. Thus, it would be interesting to take our study further, for example, by combining compounds shared by different fruit species with species-specific fruit compounds chosen from the most attractive fruit. This type of blend would be attractive at both high and low doses and, by extension, at any distance from the

lure. Identifying the optimum ratio of compounds in the blend is also a challenge, given the variation between fruit emissions. The ratio of shared compounds in a blend could either imitate an attractive fruit or correspond to the ratio of compounds most shared by host fruit species.

In conclusion, this study considers two non-exclusive hypotheses regarding the olfactory strategies developed by fruit flies, which allow them to recognize their plant hosts. Our study supports the view that tephritids' olfactory systems are not only designed to detect hosts, but also to discriminate between them, which means they are efficient and flexible in their search for hosts.

Data availability

Data have been deposited in a long term repository service : Jacob, Vincent, 2024, "Raw data and code for Ramiaranjatovo et al. (2024) "Olfaction in Tephritidae: a balance between detection and discrimination"", https://doi.org/10.18167/DVN1/T3XUXW, CIRAD Dataverse, DRAFT VERSION and will be available upon publication.

Code availability

The code used for modelling has been deposited in a long term repository service : Jacob, Vincent, 2024, "Raw data and code for Ramiaranjatovo et al. (2024) "Olfaction in Tephritidae: a balance between detection and discrimination"", https://doi.org/10.18167/DVN1/T3XUXW, CIRAD Dataverse, DRAFT VERSION and will be available upon publication.

References

1. Schoonhoven, L. M., van Loon, J. J. A. & Dicke, M. Insect-plant biology. *2nd ed. Oxford University Press, Oxford* 440 (2005).
2. Cardé, R. T. & Willis, M. A. Navigational strategies used by insects to find distant, wind-borne sources of odor. *J Chem Ecol* **34**, 854–866 (2008).
3. Bruce, T. J. A., Wadhams, L. J. & Woodcock, C. M. Insect host location: a volatile situation. *Trends in Plant Science* **10**, 269–274 (2005).

4. Bruce, T. J. A. & Pickett, J. A. Perception of plant volatile blends by herbivorous insects - Finding the right mix. *Phytochemistry* **72**, 1605–1611 (2011).

5. Biasazin, T. D., Larsson Herrera, S., Kimbokota, F. & Dekker, T. Translating olfactomes into attractants: shared volatiles provide attractive bridges for polyphagy in fruit flies. *Ecol Lett* **22**, 108–118 (2019).

6. Cunningham, J. P. Can mechanism help explain insect host choice? *J Evol Biol* **25**, 244–251 (2012).

7. Cunningham, J. P., Carlsson, M. A., Villa, T. F., Dekker, T. & Clarke, A. R. Do fruit ripening volatiles enable resource specialism in polyphagous fruit flies? *J Chem Ecol* **42**, 931–940 (2016).

8. Pan, H. *et al.* Volatile fragrances associated with flowers mediate host plant alternation of a polyphagous mirid bug. *Sci Rep* **5**, 1–11 (2015).

9. Cooley, S. S., Prokopy, R. J., McDonald, P. T. & Wong, T. T. Y. Learning in oviposition site selection by *Ceratitis capitata* flies. *Entomol Exp Appl* **40**, 47–51 (1986).

10. Cunningham, J. P., West, S. A. & Zalucki, M. P. Host selection in phytophagous Insects: a new explanation for learning in adults. *Oikos* **95**, 537–543 (2001).

11. Cunningham, J. P., Jallow, M. F. . A., Wright, D. J. & Zalucki, M. P. Learning in host selection in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Anim Behav* **55**, 227–234 (1998).

12. He, J., Chen, K., Jiang, F. & Pan, X. Host shifts in economically significant fruit flies (Diptera: Tephritidae) with high degree of polyphagy. *Ecology and Evolution* **11**, 13692–13701 (2021).

13. Starkie, M. L. *et al.* A comprehensive phylogeny helps clarify the evolutionary history of host breadth and lure response in the Australian Dacini fruit flies (Diptera: Tephritidae). *Mol Phylogenet Evol* **172**, 107481 (2022).

14. Clarke, A. R. Why so many polyphagous fruit flies (Diptera: Tephritidae)? A further contribution to the ‘generalism’ debate. *Biological Journal of the Linnean Society* **120**, 245–257 (2016).

15. Schutze, M. K., Virgilio, M., Norrbom, A. & Clarke, A. R. Tephritid integrative taxonomy: where we are now, with a focus on the resolution of three tropical fruit fly species complexes. *Annual Review of Entomology* **62**, 147–164 (2017).

16. Biasazin, T. D., Larsson Herrera, S., Kimbokota, F. & Dekker, T. Translating olfactomes into attractants : shared volatiles provide attractive bridges for polyphagy in fruit flies. *Ecol Lett* **22**, 108–118 (2019).

17. Manoukis, N. C., Cha, D. H., Collignon, R. M. & Shelly, T. E. Terminalia larval host fruit reduces the response of *Bactrocera dorsalis* (Diptera: Tephritidae) adults to the male lure methyl eugenol. *J Econ Entomol* **111**, 1644–1649 (2018).

18. Segura, D., Vera, M. T. & Cladera, J. L. Host utilization by the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *Proceedings of 6th International Fruit Fly Symposium Stellebosch, South Africa* 83–90 (2002).

19. Charlery de la Masselière, M. *et al.* Changes in phytophagous insect host ranges following the invasion of their community: Long-term data for fruit flies. *Ecol Evol* **7**, 5181–5190 (2017).

20. Moquet, L., Payet, J., Glenac, S. & Delatte, H. Niche shift of tephritid species after the Oriental fruit fly (*Bactrocera dorsalis*) invasion in La Réunion. *Divers Distrib* **27**, 109–129 (2021).

21. Ramiaranjatovo, G., Reynaud, B. & Jacob, V. Triple electroantennography captures the range and spatial arrangement of olfactory sensory neuron response on an insect antenna. *J Neurosci Methods* **390**, 11 (2023).

22. Hafsi, A. *et al.* Host plant range of a fruit fly community (Diptera : Tephritidae): does fruit composition influence larval performance ? *BMC Ecol* **16**, (2016).

23. Jacob, V. Current source density analysis of electroantennogram recordings : a tool for mapping the olfactory response in an insect antenna. *Front Cell Neurosci* **12**, 1–19 (2018).

24. Ramiaranjatovo, G., Reynaud, B. & Jacob, V. Triple electroantennography captures the range and spatial arrangement of olfactory sensory neuron response on an insect antenna. *J Neurosci Methods* **390**, 109842 (2023).

25. Myrick, A. J. & Baker, T. C. Increasing signal-to-noise ratio in gas chromatography - electroantennography using a Deans Switch effluent chopper. *Journal of Chemical Ecology* **44**, 111–126 (2018).

26. Pluskal, T., Castillo, S., Villar-Briones, A. & Orešič, M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* **11**, (2010).

27. Ni, Y., Su, M., Qiu, Y., Jia, W. & Du, X. ADAP-GC 3.0: Improved peak detection and deconvolution of co-eluting metabolites from GC/TOF-MS data for metabolomics studies. **88**, 8802–8811 (2016).

28. R Core Team. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Preprint at (2020).

29. van den Berg, R. A., Hoefsloot, H. C. J., Westerhuis, J. A., Smilde, A. K. & van der Werf, M. J. Centering, scaling, and transformations: Improving the biological information content of metabolomics data. *BMC Genomics* **7**, 1–15 (2006).

30. Grace, S. C. & Hudson, D. A. Processing and visualization of metabolomics data - Using R. *Intech* 67–94 (2016) doi:10.1016/j.colsurfa.2011.12.014.

31. Jost, L. Partitioning diversity into independent alpha and beta components. *Ecology* **88**, 2427–2439 (2007).

32. Abouheif, E. A method for testing the assumption of phylogenetic independence in comparative data. *Evolutionary Ecology Research* **1**, 895–909 (1999).

33. Pavoine, S., Ollier, S., Pontier, D. & Chessel, D. Testing for phylogenetic signal in phenotypic traits: New matrices of phylogenetic proximities. *Theoretical Population Biology* **73**, 79–91 (2008).

34. Ronquist, F. *et al.* Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542 (2012).

35. Andersson, M. N., Schlyter, F., Hill, S. R. & Dekker, T. What reaches the antenna ? How to calibrate odor flux and ligand-receptor affinities. *Chem. Senses* **37**, 403–420 (2012).

36. Geervliet, J. B. F., Vet, L. E. M. & Dicke, M. Volatiles from damaged plants as major cues in long-range host-searching by the specialist parasitoid *Cotesia rubecula*. *Entomol Exp Appl* **73**, 289–297 (1994).

37. Beck, J. J., Higbee, B. S., Merrill, G. B. & Roitman, J. N. Comparison of volatile emissions from undamaged and mechanically damaged almonds. *Journal of the Science ofFood and Agriculture* **88**, 1363–1368 (2008).

38. Meiners, T. Chemical ecology and evolution of plant-insect interactions: a multitrophic perspective. *Curr Opin Insect Sci* **8**, 1–7 (2015).

39. El Hadi, M. A. M., Zhang, F. J., Wu, F. F., Zhou, C. H. & Tao, J. Advances in fruit aroma volatile research. *Molecules* **18**, 8200–8229 (2013).

40. Dweck, H. K. M. *et al.* The olfactory logic behind fruit odor preferences in larval and adult *Drosophila*. *Cell Rep* **23**, 2524–2531 (2018).

41. Bernays, E. A. Neural limitations in phytophagous insects: Implications for diet breadth and evolution of host affiliation. *Annu Rev Entomol* **46**, 703–727 (2001).

42. Niven, J. E. & Laughlin, S. B. Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology* **211**, 1792–1804 (2008).

43. Cunningham, J. P. Can mechanism help explain insect host choice? *J Evol Biol* **25**, 244–251 (2012).

44. Clarke, A. R. Why so many polyphagous fruit flies (Diptera: Tephritidae)? A further contribution to the 'generalism' debate. *Biological Journal of the Linnean Society* **120**, 245–257 (2017).

45. Paoli, M. *et al.* Minute impurities contribute significantly to olfactory receptor ligand studies: tales from testing the vibration theory. *eNeuro* **4**, 1–10 (2017).

46. Schorkopf, D. L. P. *et al.* False positives from impurities result in incorrect functional characterization of receptors in chemosensory studies. *Progress in Neurobiology* **181**, 7 (2019).

47. Scala, A., Allmann, S., Mirabella, R., Haring, M. A. & Schuurink, R. C. Green leaf volatiles: A plant's multifunctional weapon against herbivores and pathogens. *International Journal of Molecular Sciences* **14**, 17781–17811 (2013).

48. Jaenike, J. On optimal oviposition behavior in phytophagous insects. *Theor Popul Biol* **14**, 350–356 (1978).

49. Cunningham, J. P. & West, S. A. How host plant variability influences the advantages to learning: A theoretical model for oviposition behaviour in Lepidoptera. *J Theor Biol* **251**, 404–410 (2008).

50. Joachim-Bravo, I. S., Fernandes, O. A., De Bortoli, S. A. & Zucoloto, F. S. Oviposition behavior of *Ceratitis capitata* wiedemann (Diptera: Tephritidae): association between oviposition preference and larval performance in individual females. *Neotropical Entomology* **30**, 559–564 (2001).

51. Liu, H., Chen, Z. S., Zhang, D. J. & Lu, Y. Y. BdorOR88a modulates the responsiveness to methyl eugenol in mature males of *Bactrocera dorsalis* (Hendel). *Frontiers in Physiology* **9**, 1–17 (2018).

52. Dhillon, M. K., Singh, R., Naresh, J. S. & Sharma, H. C. The melon fruit fly, *Bactrocera cucurbitae*: A review of its biology and management. *Journal of Insect Science* vol. 5 16 Preprint at https://doi.org/10.1093/jis/5.1.40 (2005).

53. Kambura, C. *et al.* Composition, host range and host suitability of vegetable-infesting tephritids on cucurbits cultivated in Kenya. *African Entomology* **26**, 379–397 (2018).

54. Mcquate, G. T., Follett, P. A., Liquido, N. J. & Sylva, C. D. Assessment of navel oranges, clementine tangerines, and Rutaceous fruits as hosts of *Bactrocera cucurbitae* and *Bactrocera latifrons* (Diptera: Tephritidae). *Int J Insect Sci* **7**, IJIS.S20069 (2015).

55. Vayssières, J. F., Rey, J. Y. & Traoré, L. Distribution and host plants of *Bactrocera cucurbitae* in West and Central Africa. *Fruits* **62**, 391–396 (2007).

56. Virgilio, M., De Meyer, M., White, I. M. & Backeljau, T. African Dacus (Diptera: Tephritidae: Molecular data and host plant associations do not corroborate morphology based classifications. *Mol Phylogenet Evol* **51**, 531–539 (2009).

57. He, J., Chen, K., Jiang, F. & Pan, X. Host shifts in economically significant fruit flies (Diptera: Tephritidae) with high degree of polyphagy. *Ecol Evol* **11**, 13692–13701 (2021).

58. Jacob, V. *et al.* Current source density mapping of antennal sensory selectivity reveals conserved olfactory systems between tephritids and *Drosophila*. *Scientific Reports* **7**, 1–13 (2017).

59. Agelopoulos, N. *et al.* Exploiting semiochemicals in insect control. *Pesticide Science* **55**, 225–235 (1999).

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Author contributions

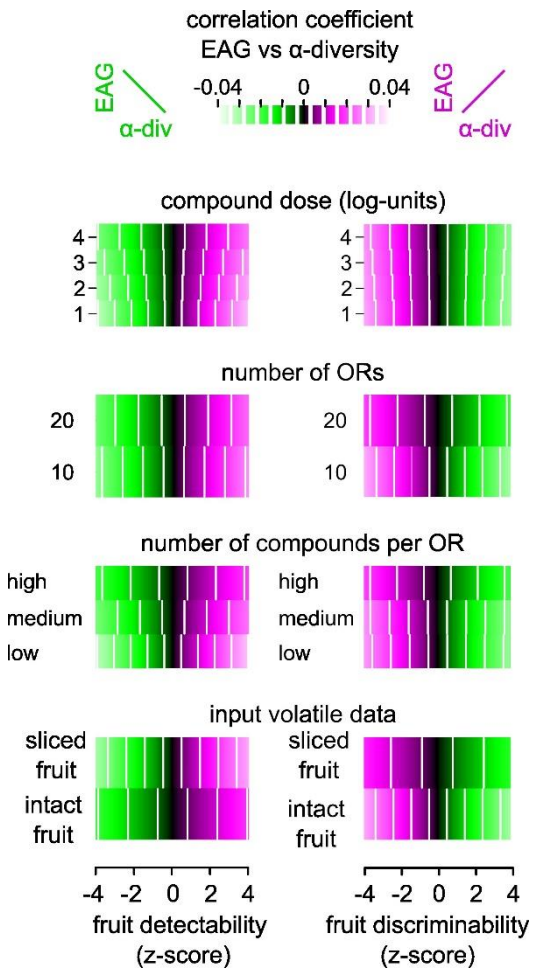
V.J., P.F.D., G.R., S.L.H. and T.D. contributed to the conceptualization; G.R. and M.C.M and V.J. contributed to methodology; G.R. and M.C.M contributed to the data curation: G.R. and V. J. contributed to the formal analysis; V.J. and B.R. contributed to supervision; V.J. contributed to the modelling and to funding acquisition; G.R. and V.J. contributed to writing – original draft. All authors contributed to writing—review and editing.

Supplementary Table 1. List of names, classes, α-div^{28}, α-div^{13} and Cmean values for the 511 compounds from intact fruit samples

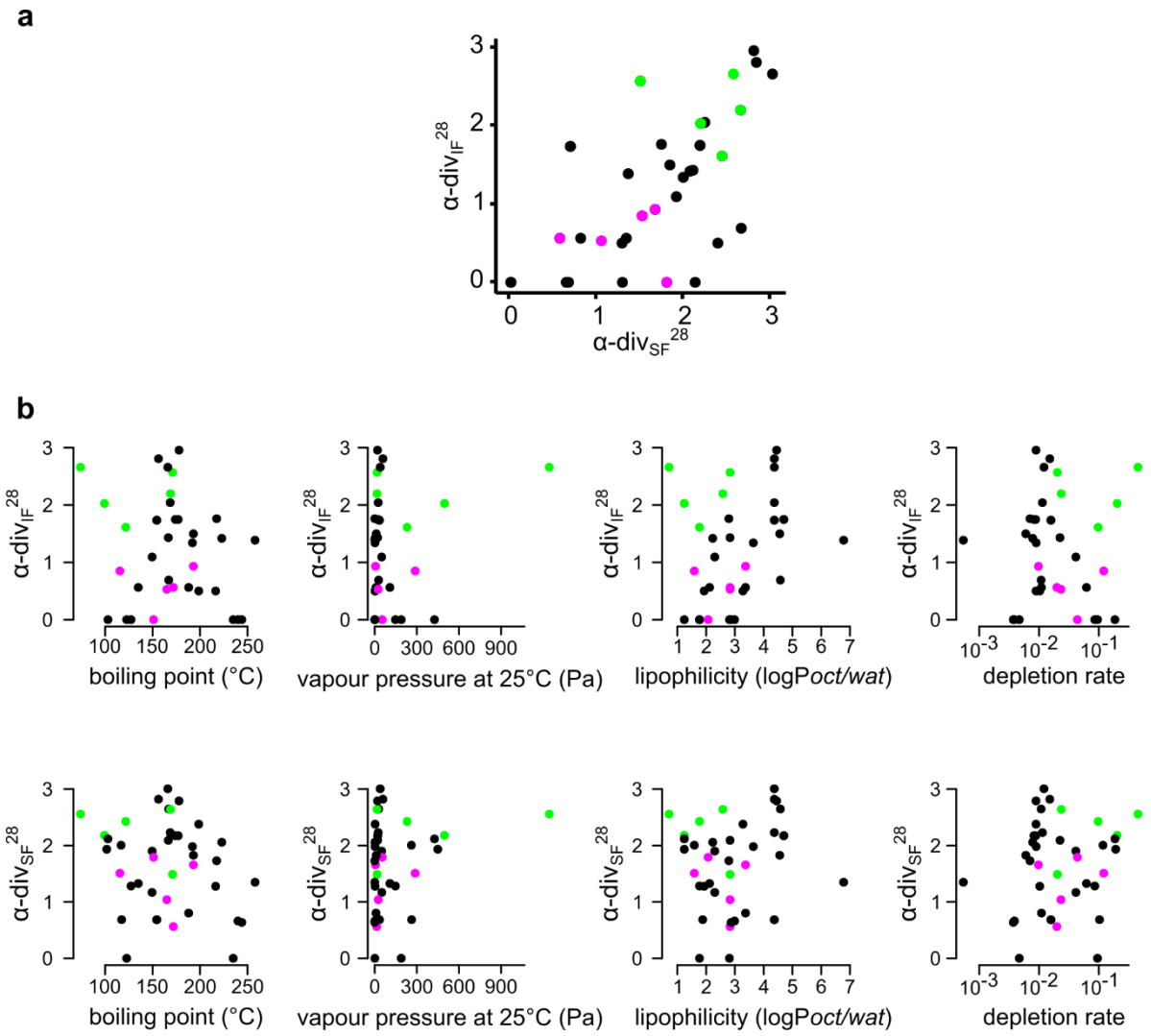
Supplementary Table 2. List of names, classes, α-div^{28}, α-div^{13} and Cmean values for the 665 compounds from sliced fruit samples.

Supplementary Table 3. List of synthetic compounds used for electrophysiological and behavioural tests. Compounds were asigned to two different blends for EAD and behavioural tests.

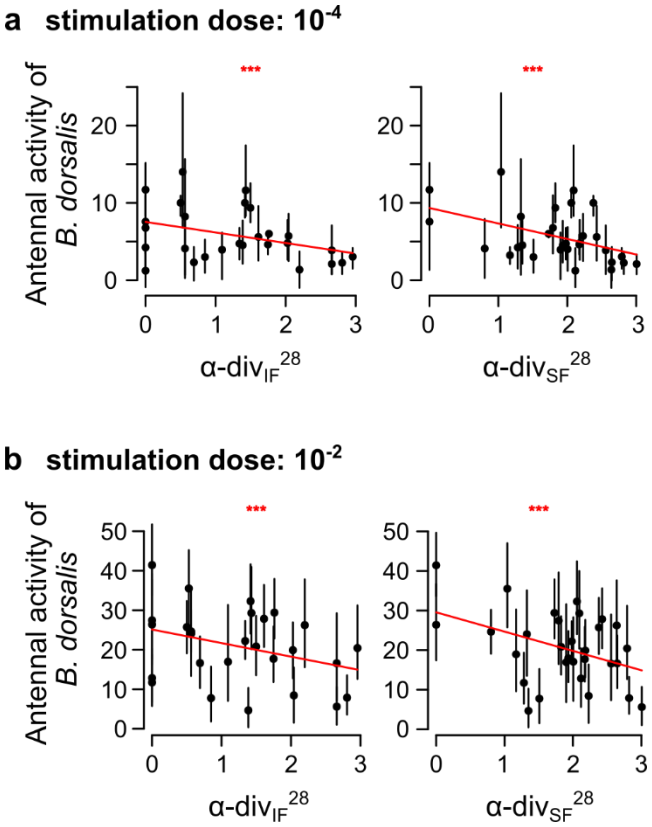
CAS	compound	EAG_3	$GC\text{-}EAD_3$ blend #	behavioural assay blend #
79-92-5	(-)-camphene		A	
127-91-3	(-)-β-pinene	x	B	
5794-03-6	(+)-camphene		A	
3016-19-1	(4E,6E)-allocimene	x	B	
53398-83-7	(E)-2-hexenyl butyrate	x	A	
3779-61-1	(E)-β-Ocimene		A	
3338-55-4	(Z)-β-Ocimene	x	A	
13466-78-9	3-carene	x	B	
140-11-4	benzyl acetate		A	
103-37-7	benzyl butanoate		A	
103-28-6	benzyl isobutyrate	x	B	
109-21-7	butyl butyrate	x		species-specific
3681-71-8	cis-3-hexenyl acetate	x	B	fruit-shared
141-78-6	ethyl acetate	x		fruit-shared
105-54-4	ethyl butyrate	x	A	fruit-shared
106-30-9	ethyl heptanoate	x	A	
123-66-0	ethyl hexanoate	x	B	
108-64-5	ethyl isovalerate	x	B	
97-63-2	ethyl methacrylate		B	
105-37-3	ethyl propionate	x	A	fruit-shared
142-92-7	hexyl acetate		A	fruit-shared
110-19-0	isobutyl acetate	x	B	
138-86-3	limonene	x	B	
78-70-6	linalool	x	B	
868-57-5	methyl 2-methylbutyrate	x	A	species-specific
623-42-7	methyl butyrate	x	A	
106-73-0	methyl heptanoate		A	species-specific
106-70-7	methyl hexanoate	x	A	
111-11-5	methyl octanoate		A	species-specific
119-36-8	methyl salicylate	x	B	
624-24-8	methyl valerate	x	A	
123-35-3	myrcene	x	A	
628-63-7	pentyl acetate	x	A	
122-72-5	phenylpropyl acetate		B	
1191-16-8	prenyl acetate	x	B	species-specific
109-60-4	propyl acetate	x	B	
106-36-5	propyl propionate	x	B	
80-56-8	α-pinene	x	B	
98-55-5	α-terpineol	x		
87-44-5	β-caryophyllene	x	B	



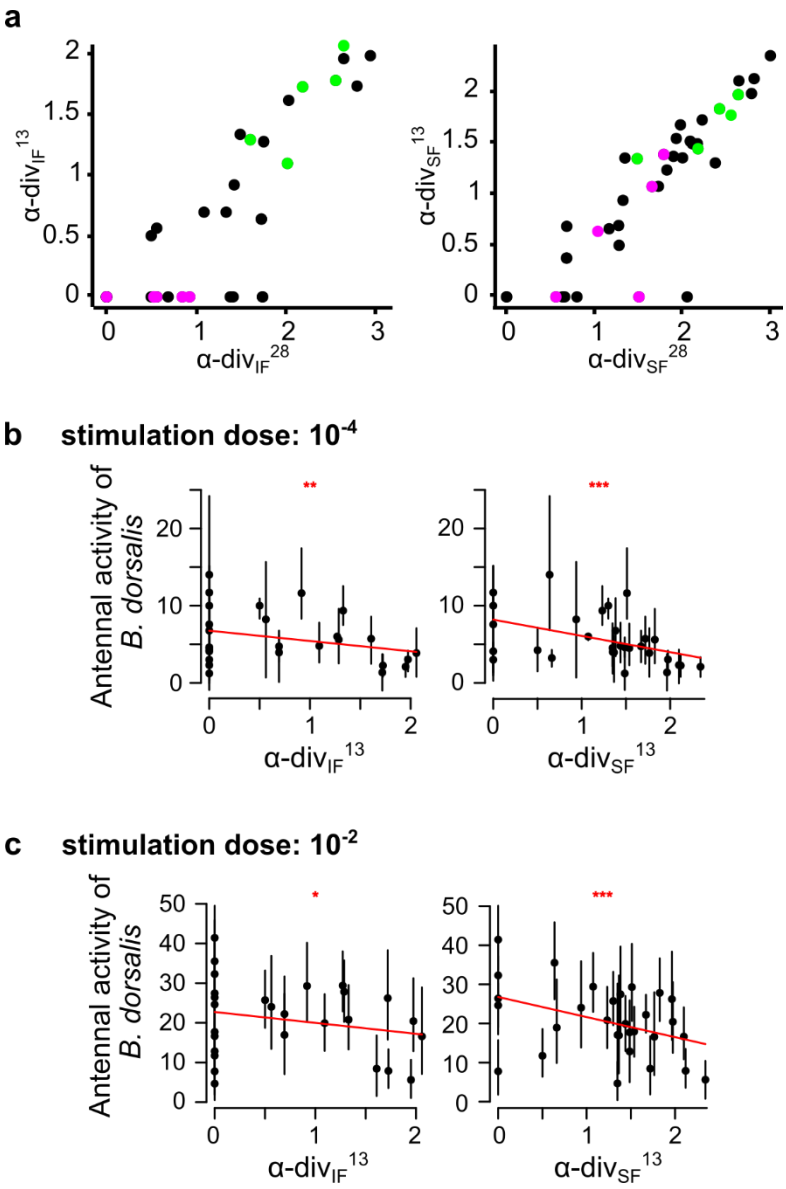
Supplementary Fig. 1. Additional characterization of the models. The figure shows the linear dependence of the coefficient of correlation between EAG response and fruit a-diversity and the following parameters: compound dose, number of ORs, number of compounds per OR, volatile data input (intact or sliced fruit).



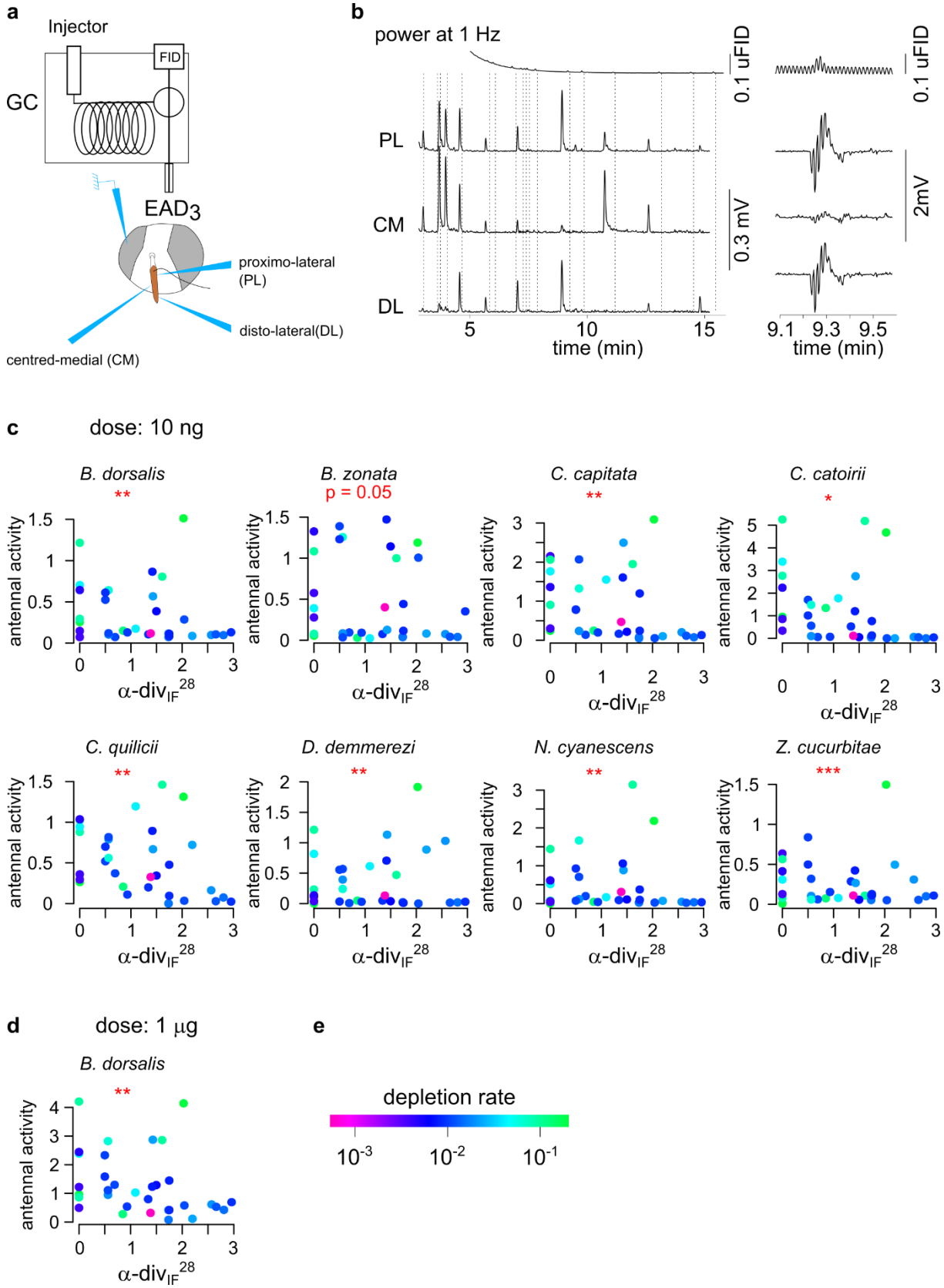
Supplementary Fig. 2. Chemical characteristics of the 40 compounds used for testing the olfactory system (30 of which were used for EAG_3 tests and 37 of which were used for GC-EAD_3 tests). **a** Relationship between the fruit α-diversity indices calculated from intact and sliced fruit. Coloured dots show the compounds used for behavioural tests: the five compounds included in the species-specific compound blend are in magenta and the five compounds included in the shared fruit compound blend are in green. **b** Indices of α-diversity are not correlated with any chemical properties. Same conventions as in panel (a).



Supplementary Fig. 3. Antennal responses of *Bactrocera dorsalis* measured with EAG_3 correlate with fruit α-diversity calculated from intact and sliced fruit emissions. **a-b** Scatterplot at the two doses tested. Same conventions as in Figure 4.



Supplementary Fig. 4. Antennal responses of *Bactrocera dorsalis* measured with EAG_3 correlate with indices of α-diversity calculated from a subset of 13 intact or sliced fruit emissions (Table 1), at the two doses tested. **a** relationship between α-diversity indices calculated from 13 of the 28 fruit species. **b**-c Scatterplots at the two dose tested. Same conventions as in Supplementary Fig. 1 and 2.



Supplementary Fig. 5. Antennal responses of eight Tephritidae species in GC-EAD3. **a** Schematic representation of the chopped GC-EAD3 system. **b** An example of GC-EAD$_3$ recording on individual female *B. dorsalis*. Left:

Signal is demodulated at 1 Hz, the frequency of the chopping modulation. The first line is the FID signal and the three lines below are the EAD signals at the three antennal positions. Right: zoom on a single response, showing the full signal before demodulation. **c**-**d** Antennal responses to synthetic compounds at dose 10 ng (c) and 1 µg (d, n=4), depending on the fruit α-diversity of intact fruit emissions. Each point represents a mean response to a compound (n = 4 for dose 10 ng, n = 5 for dose 1 µg). **e** Compound depletion rate is colour coded. The significance of a correlation between antennal activity and an interaction between $\alpha\text{-div}_{IF}^{28}$ and depletion rate is indicated (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).