Weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae) headspace volatiles deter oviposition in female Oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae)

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Natural predator–prey interactions in the insect world provide interesting insights into how female herbivores avoid ovipositing in places where a predator's presence can be perceived. Several insects show such innate behavioural traits that can be harnessed to formulate safe pest management strategies in agriculture. Using customized oviposition assays, we studied the innate oviposition avoidance behaviour of the oriental fruit fly, *Bactrocera dorsalis*, a frugivorous pest. Fruit flies preferred to lay eggs in a test region smeared with γ-octalactone (an oviposition stimulant used as a positive control) over one smeared with a mix of γ-octalactone and headspace volatiles of the weaver ant, *Oecophylla smaragdina,* a generalist predator in orchard ecosystems. A combination of the electrophysiologically active odour cues *n*-undecane and *n*-tridecane from the headspace volatiles of weaver ants was found to deter female fruit flies from ovipositing. Using these behaviour-modifying chemicals in a blend as a pre-harvest spray could potentially prevent egg-laying by the oriental fruit flies in ready-to-harvest fruits.

Keywords: Fruit fly, headspace volatiles, oviposition deterrent, predator–prey interactions, weaver ant.

THE body odour of a predator is known to elicit a range of innate behaviours in prey that allows predation risk assessment and taking necessary actions to avoid potential hazards. Such predation risk avoidance strategies employed by the prey are usually based on the detection of predator body odour and have been well documented across the animal kingdom[1–5]. Progeny of phytophagous insects undergoes a metamorphic journey involving egg, larva, pupa and adult phases. Consequently, an innate instinct in the gravid females to pass on the gene pool forms the basis for choosing an ideal oviposition site with minimal predation risk. Thus, effective prioritization of their egg-laying sites includes the availability of food and enemy-free space to improve the survival prospects of their offspring[6,7].

Tephritid fruit flies cause huge losses to fruit and vegetable crops worldwide. The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a major pest of fruit crops in Southeast Asia, with the notorious reputation of being a high-risk quarantine pest across the globe[8,9]. While the oviposition behaviour and host–plant interactions for tephritids have been widely researched, their interactions with predators (=vertical interactions) remain largely unexplored. Moreover, information on the anti-predatory strategies of tephritids that help them escape predation is also limited.

Among several predators like spiders[10,11], rove beetles[12], wasps[13], birds[14] and small mammals[15], ants are listed as the major threat not only to fruit-fly pupae in the soil but also to gravid female flies that frequent oviposition sites[16,17]. Studies indicate that fruit flies can detect ant body odours and assess the predatory threat while laying their eggs. A study performed by Van Mele *et al.*[18,19] showed that the fruit flies, *Ceratitis cosyra* (Walker) and *Bactrocera invadens* (Drew, Tsurata & White) [= *Bactrocera dorsalis*] avoid landing on ant-exposed mangoes and prefer not to oviposit on them. These authors provided evidence on the ability of the fruit flies to differentiate fruits from ant-colonized and ant-free trees. Additionally, the fruit flies spent less time on ant-colonized trees, suggesting that female fruit flies can detect pheromone cues from weaver ants, *Oecophylla longinoda* (Latreille), and that the intensity of these pheromone cues significantly alters their oviposition behaviour[20]. Fruits run over by weaver ants are less likely to be selected by gravid fruit flies as oviposition sites. Thus, we exploited the predator–prey interaction between the weaver ant *Oecophylla smaragdina* (Fab.) and *B. dorsalis* to identify chemical cues associated with the headspace volatiles of the weaver ant, which the gravid female *B. dorsalis* use to assess predation risk during oviposition.

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Materials and methods

Insects

Guava fruits infested by *B. dorsalis* were collected from the experimental field at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India and placed on sterile dry sand to aid pupal development. For adult emergence the collected pupae were transferred to netted cages of dimensions 30 × 30 × 30 cm. Newly emerged adults were fed a diet of yeast extract, sugar and honey solution. Mature gravid females (>15 days old) were used for all bioassays and starved for 2 h before conducting the experiments. Weaver ants (n = ~400) were collected in a sterile polyethylene bag (ca. 30 × 15 cm) from the IIHR mango fields and transferred to a 1 l Shott Duran (Borosil) bottle. Fruit fly and ant colonies were maintained in the laboratory at 27° ± 1°C, 75% ± 2% RH and 14L:10D h photoperiod.

Weaver ant body volatile collection

A customized air entrainment system was used to collect weaver ant body volatiles (OBV) according to the procedure described by Kamala Jayanthi *et al.*[21], with minor modifications. All glassware were washed with liquid soap, rinsed in distilled water followed by acetone and baked in an oven at 200°C for 2 h before use. The Porapak Q tubes (50 mg, 60/80 mesh; Supelco, Sigma Aldrich, India; length = 5 cm, ID = 5 mm) were washed with redistilled diethyl ether (Merck 99.7%) and heated at 120°C for 2 h under a stream of nitrogen gas (99.999% pure) to remove contaminants before activation. Weaver ants were used for volatile collection. Air purified by passage through an activated charcoal filter was pumped into the vessel through the inlet port (500 ml/min). Volatiles were collected on Porapak Q in a glass tube inserted into the collection ports on top of the bottle. Further, pumps drew air (400 ml/min) through these tubes. Airflow rates were controlled so that more purified air was pumped in than was drawn out, ensuring that unfiltered air was not drawn into the bottle from the outside. All connections were made with PTFE tubing fitted with brass ferrules and fittings (Swagelok, India) and sealed with PTFE tape. The headspace volatiles were collected for 24 h and eluted with 750 µl of redistilled diethyl ether and served as OBV for all bioassays. Collected samples were stored in glass vials in a freezer (–20°C) until use.

Chemicals, test samples and blends

Authentic chemical standards, γ-octalactone (≤97% pure), *n*-undecane (≥99% pure), *n*-dodecane (≥99% pure) and *n*-tridecane (≥99% pure) were procured from Sigma Aldrich, USA. Headspace volatiles of weaver ant from Porapak extracts (10 µl) were directly used as test samples after elution. The individual authentic standards of electroantennographic detection (EAD) active compounds (10 µl of 100 ng/µl) and a synthetic blend of *n*-undecane and *n*-tridecane in concentrations matching the natural OBV sample (529.0 + 93.0 ng/µl) were prepared with diethyl ether (99.7% pure, Merck).

Oviposition bioassays

Three series of oviposition bioassays were carried out with weaver ant headspace volatiles, EAD active compounds and a synthetic blend to study their oviposition deterrence to female *B. dorsalis*.

Weaver ant body volatiles

Agarose 1% (SeaKem–LE Agarose, Lonza, India) was prepared in distilled water and poured into plates (petriplates, 90 × 14 mm, Tarsons, India) to be used for assays (n = 10). The plates were smeared with γ-octalactone (10 µl of 980 µg/µl) and allowed to dry for a few minutes. A perpendicular line was drawn to divide the plates into two halves. One half was smeared with an OBV test sample (10 µl), while the other half served as a positive control. An untreated agarose plate served as a negative control. The plates were placed in a cage (30 × 30 × 30 cm) containing 100 pairs of *B. dorsalis* and the insects were allowed to oviposit. The egg count was recorded after 24 h.

EAD active compounds

Two types of assays were carried out to identify the relative oviposition deterrence of weaver ant EAD active compounds (*n*-undecane, *n*-dodecane and *n*-tridecane) individually and in various combinations. For these assays, 1.0% agarose plates were prepared as explained earlier and γ-octalactone (10 µl) was smeared uniformly all over the plates. The plates were divided into four quadrants, of which one quadrant served as a positive control (GO). In the first assay, the remaining three quadrants were smeared with *n*-undecane, *n*-dodecane and *n*-tridecane (n = 15) (10 µl of 100 ng/µl) individually. In the second assay, the three quadrants were smeared with a mix of *n*-undecane and *n*-tridecane; *n*-tridecane and *n*-dodecane, and *n*-undecane and *n*-dodecane in the ratio 1 : 1 (10 µl of 100 ng/µl; n = 5) respectively. The insects were allowed to oviposit as described earlier and egg count was recorded after 24 h. Dose–response assays of *B. dorsalis* with authentic EAD active compounds (*n*-undecane and *n*-tridecane) at concentrations ranging from 10^{-1} to 10^{-8} (1000 to 20 ng/µl) were carried out on 1.0% agarose oviposition assay plates. γ-Octalactone served as the positive control (n = 6). The insects were allowed to oviposit and egg count was recorded after 24 h.

Synthetic blend

Two types of oviposition assays were carried out to study the oviposition deterrent activity of the OBV sample and the two-component synthetic blend (SB) prepared with the EAD active compounds (*n*-undecane and *n*-tridecane in natural concentration as observed in OBV sample; 529.0 + 93.0 ng/µl). In the first type, agarose (1.0%) plates were prepared as mentioned earlier. The plates were divided into two halves one half was smeared with OBV (10 µl) and the other half with SB (10 µl). The insects were allowed to oviposit as described earlier ($n = 10$) and egg count was recorded after 24 h. In the second type, mature female *B. dorsalis* ($n = 50$) maintained in a square cage (dimensions: $30 \times 30 \times 30$ cm^2) were exposed to ripe bananas (cv. Yelakki; $n = 6$) smeared with either 100 µl of OBV sample or SB. The fruits were collected 24 h after exposure, placed in plastic containers and observed for the number of pupae formed.

Olfactometer bioassay for B. dorsalis

A Perspex four-arm olfactometer (60 mm diameter) was assembled as described by Pettersson[22] to study the behavioural effect of OBV on adult female *B. dorsalis*. The bioassays and consecutive analyses were carried out as described by Kamala Jayanthi *et al.*[21,23]. Three types of bioassays were performed. In type 1, the behavioural response of gravid female *B. dorsalis* to the OBV was recorded in the four-arm olfactometer. In this assay, 10 µl of the test sample was directly pipetted onto a filter paper and the solvent was allowed to evaporate before placing it into the treatment arm. Filter paper strips with solvent (10 µl of diethyl ether) served as a control in the remaining three arms. The type-2 bioassay recorded the response of gravid female *B. dorsalis* to synthetic EAD active compounds (*n*-undecane, *n*-dodecane and *n*-tridecane; 10 µl of 100 ng/µl) individually and type 3 was a choice test between OBV and SB of EAD active compounds (in natural concentration as observed in the OBV sample). In this setup, out of the four arms, two served as a treatment arms (OBV and SB, 10 µl) and the other two as control arms (10 µl solvent blank). Ten ($n = 10$) replicates were carried out and observations were recorded as the amount of time spent by the gravid female in each arm (in all bioassays) and the number of entries made by the female (only in the second bioassay) using Olfa software (F. Nazzi, Udine, Italy). The apparatus was rotated 90° every 2 min to eliminate any directional bias in the bioassay cage.

Coupled gas chromatography-electroantennographic detection studies

The electroantennogram recordings for *B. dorsalis* ($n = 6$) were made as described by Kamala Jayanthi *et al.*[21]. The carefully excised insect head was positioned on a reference electrode glass (Ag–AgCl) filled with saline solution and the tips of both the antennae were placed on the recording electrode. A customized software package (Syntech, Germany) was used to analyse the signal passing through a high impedance amplifier. For coupled gas chromatography-electroantennographic detection (GC-EAD) analysis, effluent from the GC column was split into two parts in the ratio of 1 : 1 and each part was simultaneously directed to the antennal preparation and the GC detector, as described previously[24].

The OBV sample (2 µl) was analysed on an Agilent 7890B GC equipped with an ionizing flame detector (FID) and an Agilent J&W HP-5 (5%-phenyl)-methylpolysiloxane, non-polar, fused, silica capillary tubing column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. The thermal program was initially set at 60°C for 1 min, and later ramped up at 15°C/min up to 240°C and held for 2 min in splitless mode (40 ml/min ratio) with nitrogen as the carrier gas. Data were analysed using Chemstation software (Agilent ChemStation). Quantification of volatiles (*n*-undecane and *n*-tridecane) was done using a single-point external standard method with authentic samples of standards, as described by Skelton *et al.*[25].

Gas chromatography-mass spectrometry

The OBV sample collected in solvent (DEE, diethyl ether, Merck, 99.97%) was analysed using GC-MS (Agilent 7890B GC) system equipped with mass spectrophotometry (MS; Agilent 5977 MSD). An Agilent J&W HP-5ms Ultra Inert (5%-phenyl)-methylpolysiloxane, non-polar, fused silica capillary tubing column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness was used to evaluate the samples. The oven temperature was set as mentioned previously for GC-EAD. MS was set to full scan mode (70 eV) with an AMU range of 40–450. Next, 2 µl of the sample was injected in splitless mode (40 ml/min ratio) with an injection temperature at 270°C. Individual volatile compounds were identified by comparing the GC retention time, mass spectrum and MS spectra with the spectral library, NIST 14. The identified compounds (*n*-undecane, *n*-dodecane and *n*-tridecane) were authenticated by co-injecting standard synthetic compounds along with the OBV sample.

Electrophysiology studies

EAG studies for the OBV sample, SB and individual EAD active compounds (at different log concentrations, log 10^{-1} to 10^{-8}) were carried out as described by Damodaram *et al.*[26] and the normalization was done based on the standards as described previously[27]. For normalization, the antenna was exposed to positive (10% honey) and negative controls (empty air) before and after each test stimulation.

The responses (mV) were averaged and the difference between this value and the corrected EAG response to the test stimuli was calculated. The antennal response to the test samples was further normalized to the solvent. Results obtained for EAG studies were represented as a heat map constructed using GraphPad Prism software ($n = 6$ per treatment).

Statistical analyses

Paired *t*-test was done on the single-choice olfactometer/oviposition bioassay data, whereas ANOVA with Tukey post-test was done to compare means for all other olfactometer/oviposition bioassays with multiple choices. All analyses were carried out using GraphPad Prism (ver. 7) for Windows 10.

Results

Weaver ant headspace volatiles deter fruit flies

Considering the possibility of a change in oviposition preference of *B. dorsalis* in the presence of weaver ant headspace volatiles, a dual-choice bioassay was performed using γ-octalactone, a known oviposition stimulant as a positive control[21,23]. When presented with a second choice, the number of eggs laid by gravid *B. dorsalis* in the zone smeared only with γ-octalactone (GO) was significantly (mean ± SEM; 117.8 ± 19.14) more than in the test zone that contained *O. smaragdina* headspace volatiles (OBV) along with GO (OBV + GO; one-way ANOVA, Tukey's multiple comparison test – mean ± SEM; OBV + GO = 30.5 ± 6.70, $P < 0.0001$, $F_{2,27} = 27.03$). No difference was observed between untreated control and OBV + GO treatment (Figure 1 *a*). Given a choice in the four-arm olfactometer assay, where one arm had the test sample (OBV) while the other three had solvent as control (diethyl ether), the flies spent significantly less time (paired *t*-test, mean ± SEM; OBV = 1.07 ± 0.37; control = 2.72 ± 0.19, $P = 0.006$, $t = 3.52$, d$f = 9$) in the treatment arm containing OBV compared to the control (Figure 1 *b*). These results indicate that female fruit flies not only avoided ovipositing, but also spent less time in the OBV-treated areas.

Specific cues from weaver ant headspace volatiles deter fruit flies

Ants are known to leave odour trails as they move out of their nests while foraging to find their way back, track food sources and provide a trail to conspecifics[28]. We sought to determine the specific body odour cues in weaver ants that allow fruit flies to assess the predation risk. EAG tests carried out on female fruit fly antennae to address this question detected three electrophysiologically active compounds of the higher alkane groups, namely *n*-undecane, *n*-dodecane and *n*-tridecane (Figure 2). To test if the oviposition deterrent activity was mediated by electrophysiologically active compounds, synthetically derived (≥99% pure) *n*-undecane, *n*-dodecane and *n*-tridecane (10 µl of 100 ng/µl) were evaluated against fruit flies in the olfactometer assays. Surprisingly, two of the mentioned synthetics, *n*-undecane and *n*-dodecane, failed to elicit a significant response for the time spent (paired *t*-test, mean ± SEM; *n*-undecane = 1.46 ± 0.90, control (diethyl ether) = 1.74 ± 0.36, $P = 0.81$, $t = 0.24$, d$f = 9$; *n*-dodecane = 2.97 ± 0.57, control (diethyl ether) = 1.80 ± 0.28, $P = 0.15$, $t = 1.56$, d$f = 9$) and the number of entries made (*n*-undecane = 2.50 ± 1.10, control (diethyl ether) = 2.03 ± 0.44, $P = 0.71$, $t = 0.38$, d$f = 9$; *n*-dodecane = 2.60 ± 0.80, control

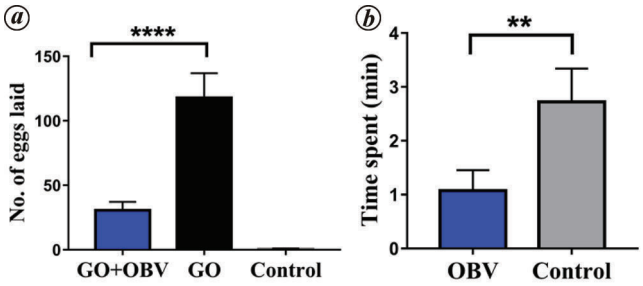

Figure 1. Bioassays for *Bactrocera dorsalis* to test oviposition deterrent effect of weaver ant body volatile (OBV). ***a***, Number of eggs laid (*Y*-axis) plotted for γ-octalactone + weaver ant body volatiles (OBV + GO)-treated region (blue bar) and γ-octalactone alone (GO) (black bar) and control (grey bar). Female flies laid significantly a smaller number of eggs (one-way ANOVA, $P < 0.0001$, $n = 10$) on OBV + GO-treated region (mean ± SEM; 117.80 ± 19.14) compared to the positive control, GO (mean ± SEM; 30.50 ± 6.71) and control (mean ± SEM; 0.60 ± 0.50). ***b***, Time spent (min) plotted against OBV (blue bar) and the control samples (grey bar) in a four-arm olfactometer assay showing the deterrence to gravid females. The flies spent significantly less time (paired *t*-test, $P = 0.006$, $n = 10$) in the treatment arm containing OBV (mean ± SEM; 1.08 ± 0.38 min) compared to the control arm (mean ± SEM; 2.72 ± 0.20).

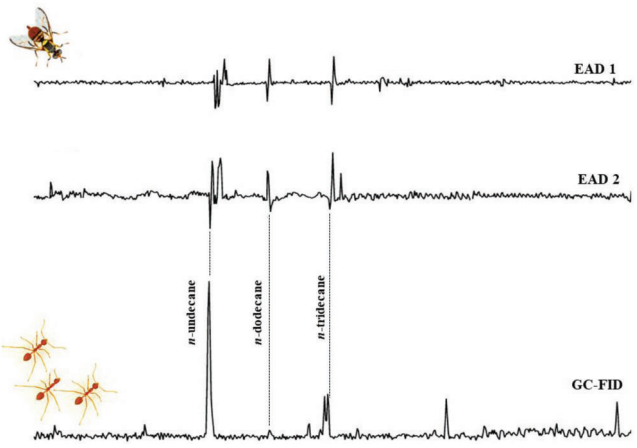

Figure 2. Gas chromatography-electroantennographic detection (GC-EAD) results showing the female *B. dorsalis* antennal response to Porapak-eluted weaver ant headspace volatile.

(diethyl ether); 3.36 ± 0.73, $P = 0.12$, $t = 1.70$, d$f = 9$) by gravid *B. dorsalis* flies, when tested individually compared to control (diethyl ether). However, the time spent and the number of entries made by the fruit flies in the *n*-tridecane-treated arm were significantly reduced compared to the control arm (paired *t*-test, mean ± SEM; time spent: *n*-tridecane = 1.96 ± 0.18, control (diethyl ether) = 3.89 ± 0.13, $P < 0.0001$, $t = 8.71$, d$f = 9$ and entries: *n*-tridecane = 3.80 ± 1.35, control (diethyl ether) = 5.80 ± 1.49, $P = 0.02$, $t = 2.75$, d$f = 9$), confirming its deterrent activity against *B. dorsalis* (Figure 3).

A significant reduction (one-way ANOVA, $F_{3,56} = 5.71$; $P = 0.001$) in the number of eggs laid was observed for the treatments *n*-undecane (mean ± SEM; 7.86 ± 1.83) and *n*-tridecane (15.60 ± 1.88) compared to the positive control (GO-smeared zone; 145.1 ± 40.3), whereas egg count for *n*-dodecane (mean ± SEM; 71.33 ± 34.38; $P = 0.21$) was not significantly different from control (Figure 4 *a*). This experiment demonstrated that the higher alkanes *n*-undecane and *n*-tridecane in weaver ant headspace volatiles probably elicit an oviposition-deterrent behaviour in fruit flies.

Despite being identified previously by Attygalle and Morgan[29], specific studies showing the relative ability of the three compounds [*n*-undecane (U), *n*-tridecane (T), *n*-dodecane (D)] in deterring the fruit flies from ovipositing have not been reported. We further examined whether there is a synergistic effect of these three alkanes on limiting oviposition in fruit flies. The three compounds mixed in various combinations (*n*-undecane and *n*-tridecane; *n*-tridecane and *n*-dodecane; *n*-dodecane and *n*-undecane) were tested in oviposition assays. All dual combinations of synthetic compounds resulted in significantly reduced oviposition numbers compared to the positive control (one-way ANOVA, mean ± SEM; U + T = 0.00 ± 0.00; T + D = 3.60 ± 2.54; D + U = 3.00 ± 2.00, control (GO-smeared zone) = 23.40 ± 6.82, $P = 0.001$, $F_{3,16} = 8.05$, $n = 5$) (Figure 4 *b*), but were not significantly different from each other. The two electrophysiologically active compounds tested, *n*-undecane and *n*-tridecane (individually and in combination), when presented to gravid female *B. dorsalis*, consistently deterred the fruit flies from ovipositing; resulting in fewer eggs. Therefore, a dose–response (10^{-1} to 10^{-8}) study was carried out to identify the minimum dose at which these synthetic cues could deter gravid *B. dorsalis* from ovipositing. With an increase in the dose of synthetic cues, the number of eggs laid by *B. dorsalis*

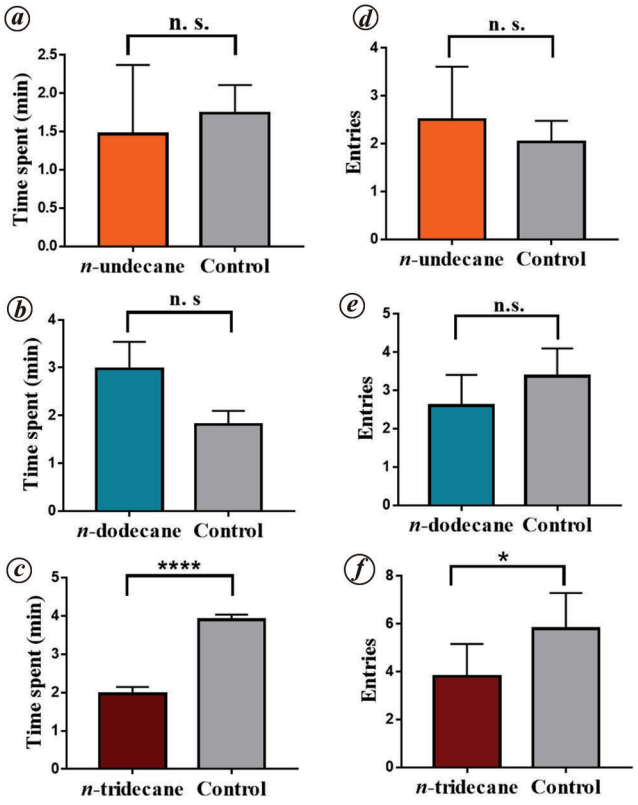

Figure 3. Olfactometer bioassays for *B. dorsalis* to synthetic EAD active compounds. Time spent (min; *Y*-axis) by *B. dorsalis* gravid females in four-arm olfactometer assays plotted for EAD-active synthetic compounds (***a***) *n*-undecane (paired *t*-test, time spent, mean ± SEM, 1.47 ± 0.90; control: 1.74 ± 0.37, $P = 0.71$; orange bar), (***b***) *n*-dodecane (time spent, mean ± SEM, 2.97 ± 0.57; control: 1.81 ± 0.89, $P = 0.15$; blue bar), (***c***) *n*-tridecane (paired *t*-test, time spent, mean ± SEM, 1.96 ± 0.18; control: 3.90 ± 0.14, $P = 0.71$; dark red bar along with the diethyl ether control; grey bar). ***d***, Number of entries made (number, mean ± SEM) (*Y*-axis) plotted for synthetic compounds that elicited EAD response, *n*-undecane (paired *t*-test, entries, mean ± SEM; 2.5 ± 1.11; control: 2.03 ± 0.45, $P = 0.71$, $n = 10$). ***e***, *n*-dodecane (paired *t*-test, entries, mean ± SEM; 2.60 ± 0.81; control: 3.37 ± 0.73, $P = 0.12$, $n = 10$). ***f***, *n*-tridecane (paired *t*-test, entries, mean ± SEM; 3.80 ± 1.36; control: 5.8 ± 1.49, $P < 0.0001$, $n = 10$) along with diethyl ether control (grey bar). The time spent and entries made were found to be non-significant for *n*-undecane and *n*-dodecane, while *n*-tridecane showed significant results for both.

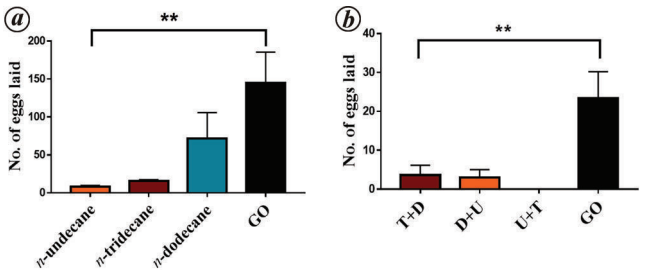

Figure 4. Oviposition agarose plate assay. Histogram representation of the number of eggs laid (*Y*-axis) by gravid female *B. dorsalis* in an agarose plate assay with (***a***) the three electrophysiologically active components identified individually, viz. *n*-undecane (mean ± SEM, 7.87 ± 1.84; orange bar), *n*-dodecane (mean ± SEM, 71.33 ± 34.38; blue bar) and *n*-tridecane (dark red bar; mean ± SEM, 15.60 ± 1.88) compared to γ-octalactone (GO; black bar) (mean ± SEM, 145.10 ± 40.30; one-way ANOVA, $P = 0.002$, $n = 15$). The results were significant for all the three compounds when tested individually. (***b***) Oviposition deterrence of synthetic compounds in dual combinations (*n*-tridecane + *n*-dodecane, T + D: number, mean ± SEM, 3.60 ± 2.54; dark red bar); (*n*-dodecane + *n*-undecane, D + U, mean ± SEM, 3.00 ± 2.00; orange bar); (*n*-undecane + *n*-tridecane, U + T, mean ± SEM, 0.00 ± 0.00); (γ-octalactone (GO), mean ± SEM, 23.40 ± 6.82; black bar) revealed significant difference in the number of eggs laid by *B. dorsalis* (one-way ANOVA, $P = 0.002$, $n = 5$).

decreased (one-way ANOVA, $P < 0.0001$, $F_{9,50} = 11.9$) (Figure 5).

Comparison of synthetically reconstituted ant headspace volatiles to natural ant headspace volatiles

Natural weaver ant headspace volatiles were reconstituted using synthetic forms of the electrophysiologically active chemical cues *n*-undecane (529 µg/µl) and *n*-tridecane (93 ng/µl). The synthetic blend was then tested for its ability to deter the gravid female fruit flies in four-arm olfactometer assays and dual-choice oviposition bioassays. The gravid female flies avoided entering the arms treated with natural OBV and SB (one-way ANOVA, mean ± SEM, OBV = 1.55 ± 0.33, SB = 1.97 ± 0.47, control = 3.46 ± 0.60, $P = 0.02$, $F_{2,27} = 4.28$) in the four-arm olfactometer bioassays (Figure 6 *a*). The antennal response of *B. dorsalis* against OBV, SB and individual electrophysiologically active compounds *n*-undecane, and *n*-tridecane (at different log concentrations, 10^{-1} to 10^{-8}) was quantified using EAG studies and the maximum antennal response (0.08 mV) of fruit flies was recorded against the concentrations 10^{-1} and 10^{-2} (Figure 6 *b*). The number of eggs laid by gravid *B. dorsalis* in multiple-choice assays between OBV (mean ± SEM; 0.00 ± 0.00) and SB (2.30 ± 1.31) was significantly different (one-way ANOVA, $F_{(2,27)} = 19.91$, $P < 0.0001$) from the control (mean ± SEM; 52.75 ± 11.50). However, the gravid fruit flies did not differentiate between the natural OBV and SB ($P > 0.999$; Figure 7 *a*). The above four-arm olfactometer and oviposition assay results together suggest that the synthetically reconstituted blend could successfully mimic the weaver ant headspace volatiles in deterring fruit flies and preventing them from ovipositing.

Fruit flies do not prefer fruits smeared with OBV and SB for egg-laying

Among the several predation risks, ants pose the most likely threat that fruit flies encounter in an orchard ecosystem while foraging for oviposition sites, mates and food[16,17]. Previous studies have indicated that fruit flies can differentiate the fruits run over by weaver ants and readily avoid them as oviposition sites[19,20]. To examine whether fruit flies can differentiate between fruits smeared with natural OBV and SB, we examined their oviposition behaviour using ripe bananas in a cage assay. The fruits smeared with OBV and SB yielded significantly fewer pupae (mean ± SEM; OBV = 5.33 ± 2.47, SB = 2.17 ± 1.64) compared to the untreated control (32.67 ± 10.94; one-way ANOVA, $F_{(2,15)} = 6.57$, $P = 0.008$). However, no significant difference in the number of pupae was observed between OBV and SB ($P > 0.999$)-treated banana, indicating the inability of fruit flies to differentiate natural weaver ant headspace volatiles from the synthetic odour (Figure 7 *b*).

Discussion

This study demonstrates that gravid female fruit flies, *B. dorsalis* avoid laying eggs in or even visiting areas with weaver ant body odours. This suggests that female *B. dorsalis* can potentially gauge the immediate predation risk to themselves and their progeny by detecting ant body odours associated with *O. smaragdina*.

Weaver ant nests are common in mango orchards, with guard and worker ants actively foraging across the branches within a tree and between neighbouring trees. The oriental fruit fly, *B. dorsalis*, being a common frugivorous

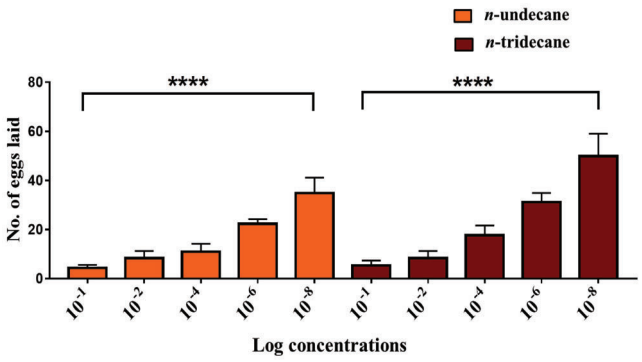

Figure 5. Oviposition plate assays showing dose-dependent oviposition response of female *B. dorsalis* to *n*-undecane and *n*-tridecane. The number of eggs laid (*Y*-axis) for each of the log doses (10^{-1} to 10^{-8}) tested (*X*-axis) was represented as a bar graph for *n*-undecane (orange bar) and *n*-tridecane (dark red bar). Significant difference (one-way ANOVA, $P < 0.0001$, $n = 6$) was observed in the number of eggs laid by *B. dorsalis* across the log doses tested (*n*-undecane: mean ± SEM, 67.33 ± 8.30, 34.83 ± 6.30, 22.33 ± 1.89, 11.0 ± 3.20, 8.33 ± 2.92, 4.33 ± 1.28; *n*-tridecane: mean ± SEM, 94.67 ± 23.78, 49.83 ± 9.23, 31.17 ± 3.77, 17.67 ± 3.98, 8.33 ± 2.92, 5.33 ± 2.02).

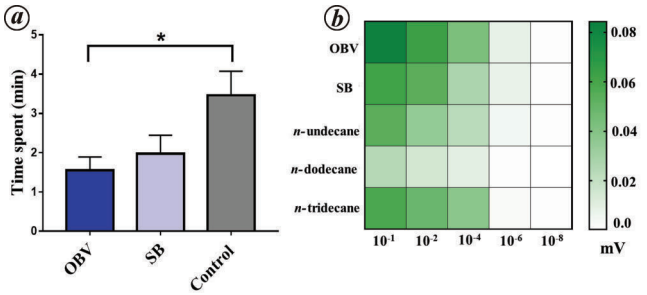

Figure 6. Four-arm olfactometer assay. ***a***, Results of the assay in the time spent (*Y*-axis) by gravid female in each of the treatment arms, OBV, synthetic blend of EAD active components in natural concentration (SB) and diethyl ether (control) is represented as a histogram in blue, faded blue and grey bars respectively. Significant difference was observed (one-way ANOVA, $P = 0.02$, $F_{2,27} = 4.28$) in the time spent (min, mean ± SEM) by gravid female *B. dorsalis* in the treated and control regions for OBV (1.55 ± 0.34 min), SB (1.97 ± 0.47 min) and control (3.46 ± 0.61 min). ***b***, EAG response recorded and depicted as a heat map based on dose–response profile of gravid female *B. dorsalis* antennae towards OBV, SB and EAD active compounds ($n = 6$). The female fly exhibited best response for OBV, SB, *n*-undecane and *n*-tridecane at 10^{-1} and 10^{-2} log concentrations and moderate response for *n*-dodecane.

pest of several perennial tree crops, often encounters with these predatory ants in the orchard ecosystem. The oviposition decision-making by female fruit flies is a dynamic process that balances several factors involving survival and progeny fitness apart from predation risk to the gravid females[23].

During their predator encounters in an orchard ecosystem, fruit flies might have learned to assess the predation risk associated with the body odours of weaver ants. While earlier studies have indicated the preferential avoidance of fruits from weaver ant colonized trees by fruit flies[20], the present study shows that the three aliphatic hydrocarbon components of the headspace volatile of weaver ants, viz. *n*-undecane, *n*-tridecane and *n*-dodecane, identified under laboratory conditions, govern the assessment of predation risk by the female fruit flies. Earlier studies have extensively reported the presence of these compounds in cuticular body extracts of several ant species, either as a component of Dufour's/poison gland and/or alarm pheromones[30–37]. The Dufour's/poison gland, commonly associated with social and solitary hymenopterans, plays a vital role in defence and communication processes.

In ants, the Dufour's gland is an exocrine gland that secretes chemicals into the surroundings that have a communicative function with conspecifics. Therefore, it might have a potential role in coordinating nest-building and oviposition. These glands help produce trail pheromones/territory-marking pheromones, and play an important role in communicating with fellow worker ants while foraging for food[28]. To release these pheromones, ants usually drag their abdominal sting on the substrate as they move, thereby providing a directional signal to their fellow worker ants to track the footprints of preceding foragers[28]. Not surprisingly, along with fellow ants, fruit flies might have also acquired the ability to associate these odours with the presence of ants and consequently avoid such places while foraging and ovipositing.

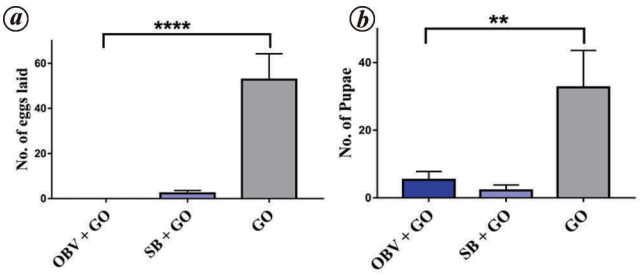

Figure 7. ***a***, Oviposition assay showing the egg-laying response of female *B. dorsalis*. The number of eggs laid (*Y*-axis) by gravid female *B. dorsalis* in agarose plates with *γ*-octalactone + OBV, *γ*-octalactone + SB of EAD active components in natural concentration and *γ*-octalactone alone (control, GO) represented as a bar graph. Female flies laid significantly more eggs (one-way ANOVA, $F_{(2,27)} = 19.91$; $P < 0.0001$) in control, GO-smeared zone (mean ± SEM; 52.75 ± 11.50) compared to OBV (mean ± SEM, 0.00 ± 0.00) and SB (mean ± SEM, 2.30 ± 1.32). ***b***, Cage assay with treated fruits. Banana fruits smeared with OBV, SB of the EAD active components in natural concentration and untreated (control) were examined and the number of pupae (*Y*-axis) was plotted as a histogram against the treatments (*X*-axis). Significant difference was observed (one-way ANOVA, $F_{(2,15)} = 6.57$; $P = 0.008$) in the number of pupae recovered from fruits in different treatments – OBV (mean ± SEM, 5.33 ± 2.47) and SB (mean ± SEM, 2.17 ± 1.64) 577 and control (mean ± SEM, 32.67 ± 10.94).

The female insect has to quickly assess the surrounding during oviposition, considering all possible factors that maximize the survival chance of its progeny and itself. Therefore, such a decision might depend on certain innate recognition cues critical to assessing important criteria like the quality of the host–plant, predation risk and competition[23]. In the present study, we have established that specific chemical components of the headspace volatiles of weaver ants, namely *n*-undecane and *n*-tridecane, can deter female fruit flies from ovipositing. Behavioural analyses revealed that the EAG active compounds *n*-undecane and *n*-dodecane could not independently deter the gravid females. However, *n*-undecane, a liquid alkane hydrocarbon, could significantly reduce the number of eggs laid by gravid females compared to *n*-dodecane. Further, *n*-tridecane could repel the gravid females and serve as an effective oviposition deterrent for female *B. dorsalis*. Similar studies conducted by Kempraj *et al.*[38–57] identified an alcohol kairomone component, 1-octanol as a potent repellent and oviposition deterrent compound for Queensland fruit fly, *Bactrocera tryoni* Froggatt from the headspace volatiles of *O. smaragdina*. However, they also identified alkane hydrocarbons such as *n*-undecane, *n*-dodecane and *n*-tridecane from the headspace volatiles *O. smaragdina*, and found that especially *n*-undecane as a major compound among all other hydrocarbons. Thus, this study identifies and characterizes the predatory ant headspace volatile cues that induce behavioural changes in the fruit pest *B. dorsalis*.

Under laboratory conditions, a standardized recipe of synthetic weaver ant headspace volatiles comprising *n*-undecane and *n*-tridecane when smeared on test fruits performed similarly to natural body volatiles of weaver ants and significantly reduced the number of eggs laid by *B. dorsalis*. These results provide a basis for developing a synthetic blend of weaver ant body cues that can effectively be used to prevent fruit fly oviposition during fruit harvest, when applying synthetic chemical insecticides is forbidden given the pesticide residue issues. The present study also highlights the potential scope of using a blend with *n*-undecane and *n*-tridecane as components for a 'behaviour modifying approach' that effectively discourages *B. dorsalis* egg-laying in ready-to-harvest fruits. Hence these chemical compounds could potentially prevent post-harvest losses in mango and can be an important component in upgrading the current fruit-fly IPM strategy.

Conflict of interest: The authors declare that there is no conflict of interest.

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