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Rearing and ^{60}Co radiation do not affect attractiveness but alter the volatile profiles released by *Anastrepha obliqua* calling males

Frida P. Santiago[1], David Alavez-Rosas[2] and Julio C. Rojas[1]

[1]Grupo de Ecología Química, Departamento de Ecología de Artrópodos y Manejo de Plagas, El Colegio de la Frontera Sur (ECOSUR), Carretera Antiguo Aeropuerto Km. 2.5, Tapachula, Chiapas 30700, Mexico and [2]Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, CDMX 04510, Mexico

Research Paper

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Corresponding author:
Julio C. Rojas;
Email: jrojas@ecosur.mx

Abstract

Calling males of *Anastrepha obliqua* release volatile compounds to attract conspecific males to form leks and females to mate. Male volatiles from Mexican and Brazilian populations of *A. obliqua* have been previously identified. However, there are differences in the number and identity of volatile compounds between the populations. These differences in volatile profiles may be due to male origin (e.g. wild or mass-reared flies) or methodological issues (e.g. sampling techniques). In this study, we evaluated the attractiveness of wild, laboratory non-irradiated, and laboratory-irradiated flies under semi-field conditions. Male volatiles were collected using dynamic headspace sampling (DHS) and solid-phase microextraction (SPME) techniques, and identified using gas chromatography-coupled mass spectrometry. The results showed no difference in the attractiveness of wild, laboratory non-irradiated, and irradiated males to females. However, the number of captured females differed according to the origin; wild and non-irradiated females were captured more frequently than the irradiated flies. A total of 21 compounds were found using SPME, whereas only 12 were collected using DHS, although the relative amounts of these compounds were higher than those obtained using the former sampling technique. In addition, only laboratory non-irradiated males released α-pinene and menthol, which have not been previously reported in this fruit fly species. Additionally, we identified novel compounds in *A. obliqua*; however, certain compounds previously reported were not detected. This study suggests that despite the qualitative and quantitative variations in the volatile profiles of *A. obliqua* males, their attractiveness was unaffected.

Introduction

Male *Anastrepha* fruit flies (Diptera: Tephritidae) release volatile compounds during calling to attract conspecific males to form leks, and females to mate (Aluja *et al.*, 2000). Leks take place mainly on host trees bearing fruits, where two–ten males aggregate, perch, and signal from the undersurface of leaves (Shelly, 2018). The pheromone seems to attract females close to the calling males but not to point sources, and at a close range, females may use acoustic and visual signals to locate males (Tan *et al.*, 2014).

Male volatile compounds have been identified in certain *Anastrepha* spp., particularly those of economic importance such as *Anastrepha suspensa*, *Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha serpentina*, and *Anastrepha fraterculus* (Tan *et al.*, 2014; Scolari *et al.*, 2021). For instance, *A. obliqua* males release esters, terpenes, and alcohols during calling (López-Guillén *et al.*, 2011; Gonçalves *et al.*, 2013; Aluja *et al.*, 2020; De Aquino *et al.*, 2021). Nevertheless, the qualitative volatile profiles of *A. obliqua* varied among studies. López-Guillén *et al.* (2011) identified nine volatiles in the effluvia of mass-reared non-irradiated calling males. The major compounds identified were (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, and (*Z*)-3-nonen-1-ol. Later, Aluja *et al.* (2020) reported up to eight compounds in wild *A. obliqua* males reared in hog plums (*Spondias* spp.), mangoes, guava, and tomatoes. Only four compounds were released by males reared in *Spondias mombin*, the ancestral host of *A. obliqua*, whereas eight compounds were found in the effluvia of males reared in guava, an occasional host of this fruit fly species. They did not find (*Z*)-3-nonen-1-ol in the male effluvia; instead the major compounds identified by Aluja *et al.* were α-bergamotene, (*E*,*E*)-α-farnesene, and (*Z*,*Z*)-3,6-nonadien-1-ol (Aluja *et al.*, 2020). Calling *A. obliqua* males from Brazil reared in mangoes and carambolas released 16 and 21 compounds, respectively (Gonçalves *et al.*, 2013; De Aquino *et al.*, 2021). The major compounds identified in later studies were 1-heptanol and ethylhexanoate. The differences found among these studies may be due to the geographical origin, host plant, or adsorbents used for sampling the volatiles. All studies collected male *A. obliqua* volatiles using the dynamic headspace sampling (DHS) technique with Super Q® (López-Guillén *et al.*, 2011;

Aluja et al., 2020) or Tenax® (Gonçalves et al., 2013; De Aquino et al., 2021) as adsorbents. Adsorbents differ in their affinity and efficiency for trapping certain groups of compounds; therefore, an inadequate selection of the absorbent can result in ample undersampling or even deficiency to collect some volatile compounds (Noushini et al., 2020). For instance, with the solid-phase microextraction (SPME) technique, the detection limit and number of identified compounds depend on the amount and adsorbent type that coated the fibre used for sampling volatile compounds (Pawliszyn, 1997).

In this study, we compared the attractiveness of laboratory-reared (hereafter non-irradiated and irradiated) and wild *A. obliqua* males to conspecific females in field cages. Subsequently, volatiles released by the three types of males were collected using DHS and static SPME techniques. Due to the differences in the reported volatiles from *A. obliqua*, we decided to collect volatiles with two different adsorbents for dynamic aeration and six different SPME fibres. Compounds from the extracts and fibres were identified using gas chromatography coupled with mass spectrometry (GC-MS). We hypothesised that rearing and radiation would affect male attractiveness and the profile of volatiles released during calling activity.

Materials and methods

Insects

Non-irradiated and irradiated *A. obliqua* pupae were provided by the MOSCAFRUT production plant (SADER-IICA) located in Metapa de Domínguez, Chiapas. The flies were reared as previously described (Artiaga-López et al., 2004). Sterile flies were irradiated with ^{60}Co at 80 Gy as pupae 2 days before adult emergence. Pupae were placed in glass cages (35 cm × 35 cm × 35 cm) for adult emergence. Mango (*Mangifera indica*) fruits infested with *A. obliqua* larvae were collected from trees surrounding Tapachula, Chiapas, Mexico to obtain wild flies. Fruits were placed in plastic trays (60 cm × 52 cm × 11 cm) and covered with cloth to allow aeration. After 5–10 days, pupae were collected and placed in glass cages with vermiculite until adult emergence. Upon emergence, the flies were sexed and placed separately in cages (12 cm × 25 cm × 12 cm). Flies were fed with a mixture of sugar and hydrolysed protein (3:1), and water in a plastic container with a slightly moistened cotton swab. The flies were maintained under an artificial photoperiod of 12 h light/12 h dark at 25 ± 2°C and 70 ± 10% relative humidity. Wild flies were 12–17 days old, whereas laboratory-reared flies were 8–15 days old when used in the experiments. This difference in age is because laboratory-reared flies reach sexual maturity earlier than wild flies do (Meza-Hernández et al., 2002).

Male attractiveness trial

The attractiveness of *A. obliqua* males to conspecific females was evaluated using multiple choice tests in cylindrical nylon screen field cages (2.80 m diameter × 2.20 m high). The trials were performed under semi-natural conditions in a shaded area planted with local trees located 5 km outside of Tapachula city, Chiapas, Mexico. Ten males of a given group (i.e. wild, non-irradiated, or irradiated flies) were placed in a container of a multilure trap (Better World Manufacturing, CA, USA), which was lined with mesh to prevent the escape of males. Three traps containing live males from the three treatments were fixed at equal distances in a random manner and hung 10 cm away from the cage roof. A solution of 250 ml propylene glycol (Agrotec, Mexico) and water (1:4) was added to each trap to retain the attracted female flies. Propylene glycol does not affect the behaviour of *A. obliqua* (López-Ley et al., 2016). Twenty wild, non-irradiated, and irradiated females ($n = 60$) were released at 08:00 h into each cage. Females from each treatment group were marked with edible dyes (Bakersfield, Chocolatera de Nayarit, Mexico). The edible dye, one colour for each fly type, was added to the water (150 µl/250 ml of water) consumed by the flies. Coloured water was supplied to flies after emergence. Thus, the abdomen of the flies was fully coloured, which made it possible to distinguish which group they belonged to when the flies were recovered from the traps. The edible dye did not affect the behaviour or survival of flies (Santiago, unpublished observations). The number of trapped flies were recorded 24 h after release, and uncaptured females were taken out from the cages and never used again. After each replication, the traps were rotated counterclockwise to minimise positional bias. In total, 32 replicates were performed.

Volatile sampling

Volatiles from the calling males were collected using DHS and static SPME. For DHS, 20 males from a given group were enclosed in a glass aeration chamber (10 cm long × 8 cm i.d.) 1 h before they began to call. Volatiles were drawn from the aeration chamber using air, previously passed through an activated carbon filter, into a collection trap. Air was drawn through a collection trap at a rate of 2.0 litres min^{-1}. The volatile collection trap contained 50 mg of ORBO Tenax® TA (a porous polymer based on 2,6-diphenyl-*p*-phenylene oxide, 35–60 mesh, 100/50 mg; Merck, Darmstadt, Germany) or Super Q® (a copolymer made of ethylvinylbenzene-divinylbenzene, Porapak Q®, 50–80 mesh, 100/50 mg; Merck, Darmstadt, Germany). Male volatiles were collected during peak calling (07:00–11:00 h) at 25 ± 2°C, 60–80% relative humidity, and 557 lx illumination. The volatiles were eluted from the adsorbent with 1 ml of hexane (high-performance liquid chromatography-grade) and the extracts were concentrated to 100 µl using a gentle N_2 airstream. The extracts were stored in glass vials (1 ml) at −20°C until analysis. Ten replicates were performed for each type of adsorbent.

For the SPME sampling, we used six different fibres: bare fused silica (FS), 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 85 µm carboxen/polydimethylsiloxane (CAR/PDMS), 75 µm carboxen/polydimethylsiloxane (CAR/PDMS), 85 µm polyacrylate (PA), and 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB). SPME fibres were obtained from SUPELCO (Deisenhofen, Germany). Fifteen calling males were gently introduced into a 200 ml borosilicate glass Erlenmeyer flask at 09:00 h and the mouth of the flask was covered with aluminium foil and sealed with masking tape. An SPME fibre was then inserted into the flask through aluminium foil and exposed to headspace volatiles. After 30 min, the fibre was withdrawn and inserted into the GC-MS injector. An identical volatile extraction chamber was simultaneously used at the same time to determine the chemical background (emitted by the chamber or in the surrounding air). The flask with flies was maintained at 25 ± 2°C, 60–80% relative humidity, and 227 lx illumination. We used the same SPME fibre for each replicate, with a total of five replicates for each fibre type.

Chemical analysis

To identify the compounds, SPME samples and extracts from the DHS technique were analysed using a GC-MS Shimadzu GC-2010

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plus Triple-Quadrupole TQ8040 (Texas, USA). A DB-5MS non-polar capillary column (30 m × 0.25 mm I.D., Supelco, Bellafont, PA) was used. The injector was operated in split mode. Helium was used as a carrier gas at 1.0 ml min^{-1}. The oven temperature was programmed at 50°C for 2 min, followed by a ramp of 15°C for 1 min until 280°C, and held for 10 min. Ionisation was caused by the electron impact at 70 eV. The compounds were identified using the Kovats retention indices and mass spectra that matched the NIST library. Comparisons with the pure standards were performed when available.

Statistical analysis

Analyses were performed using the statistical software R version 4.3.0 (R Core Team, 2023). A general linear model with Poisson distribution was performed using Tukey's test ($\alpha < 0.05$) (De Mendiburu and Simon, 2015) to test for differences in capture between the males used in traps and the females used as responders. Insect capture was the response variable, whereas trap (male type) and female type were the response factors. The relative amounts of volatile compounds released by males were compared individually among the adsorbents using a one-way analysis of variance. Data were checked for normal distribution using the Kolmogorov–Smirnov test and for homoscedasticity with the Levene test. A heatmap was generated to compare the relative amounts of captured compounds among the different adsorbents.

Results

Male attractiveness

No differences were observed in the number of *A. obliqua* females captured, regardless of the origin of the males placed inside the traps ($X^2 = 1.99$, $df = 2$, $P = 0.37$). However, the number of females attracted by males differed depending on their origin ($X^2 = 12.21$, $df = 2$, $P < 0.01$). It was observed that irradiated females were less attracted to the traps than were non-irradiated and wild females (fig. 1).

Sampling analysis

Twenty-five compounds were collected from the headspace of *A. obliqua* calling males. Qualitative differences were observed between the static and dynamic techniques used. The static technique captured up to 21 compounds, while the dynamic technique detected 12 compounds (fig. 2). Additionally, semi-quantitative differences among the adsorbents used were observed in the relative amounts of the compounds (fig. 3).

Dynamic headspace

Twelve compounds were collected from male *A. obliqua* using Super Q (Supp. table S1). The major compounds identified were (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol (these two compounds co-elute), (*E*,*Z*)-3,6-octadien-1-ol, and *cis*-muurola-3,5-diene (fig. 3). α-Pinene was detected only in non-irradiated males. The relative amounts of seven compounds varied among the three groups. Wild males released higher relative amounts of 2-ethyl-1-hexanol, tetradecane, and (*Z*,*E*)-α-farnesene than non-irradiated and irradiated males. Wild and irradiated males emitted more relative amount of *cis*-muurola-3,5-diene, *trans*-calamenene, unidentified terpene, and farnesene epoxide than non-irradiated males. In contrast, there were no differences in the relative amounts of (*E*,*Z*)-3,6-octadien-1-ol, (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol, ϒ-elemene, and (*E*,*E*)-α-farnesene among wild, non-irradiated, and irradiated males.

Twelve compounds were adsorbed by Tenax (Supp. table S2), similar to those collected by Super Q. The major compounds were (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol and (*E*,*Z*)-3,6-octadien-1-ol (fig. 3). The compound α-pinene was found only in the bouquet of non-irradiated males. The relative amounts of four compounds varied among the three male groups. Wild males emitted higher amounts of *n*-tetradecane, an unidentified terpene, than non-irradiated and irradiated males. Wild and non-irradiated males released more 2-ethyl-1-hexanol than irradiated males, whereas non-irradiated and irradiated males emitted more (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol than wild males. In contrast, no differences were observed for (*E*,*Z*)-3,6-octadien-1-ol, *cis*-muurola-3,5-diene, ϒ-elemene, (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, *trans*-calamenene, and farnesene epoxide.

Solid-phase microextraction

Twenty-one compounds were captured by the coated 65 µm PDMS/DVB fibres (Supp. table S3). The major compounds were (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol and (*Z*,*E*)-α-farnesene (fig. 3). The only qualitative difference was observed for α-pinene, which was only produced by non-irradiated males. Our results showed significant differences in the relative amounts of most volatile compounds released by the different male groups. However, only there were differences among the three groups with phenol and 1-undecene. Overall, there were no significant differences in the relative amounts of these compounds between non-irradiated and irradiated males.

Twenty-one volatile compounds were collected from the coated 75 µm CAR/PDMS fibres (Supp. table S4). The major compounds in the three male groups were (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol (fig. 3). The compounds α-pinene, 1-undecene, 2-ethyl-1-hexanol, and menthol were recovered only from the volatiles of non-irradiated males. Phenol, (*E*,*Z*)-3, 6-octadien-1-ol, α-guaiene, ϒ-amorphene, unidentified terpenes 1, 2, and 3, and farnesene epoxide were not found in the effluvia of wild males. In contrast, the last group of males produced more (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, and β-elemenone than irradiated and non-irradiated males. The relative amount of *n*-tetradecane was higher in irradiated males than in non-irradiated and wild males, whereas non-irradiated males released a higher amount of *n*-tetradecane than wild males. The relative amounts of β-elemene and caryophyllene were higher in irradiated and wild males than in non-irradiated males. In contrast, the relative amounts of (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol, bergamotene, and ϒ-elemene were not significantly different among the three types of males.

Twenty-one compounds were collected from the coated FS fibres (Supp. table S5). The major compounds in the three male groups were (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol, (*Z*,*E*)-α-farnesene, and β-elemenone (fig. 3). The compounds α-pinene and menthol were only found in the volatiles of non-irradiated males. Phenol, (*E*,*Z*)-3,6-octadien-1-ol, 1-undecene, 2-ethyl-1-hexanol, α-guaiene, ϒ-amorphene, unidentified terpenes 1, 2, and 3, and farnesene epoxide were not recovered from the effluvia of wild males. The relative amounts of (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol, *n*-tetradecane, β-elemene, caryophyllene, bergamotene, ϒ-elemene, (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, and β-elemenone did not vary among the male groups.

Twenty-one compounds were collected using the coated 50/30 µm DVB/CAR/PDMS fibres (Supp. table S6). The major compounds

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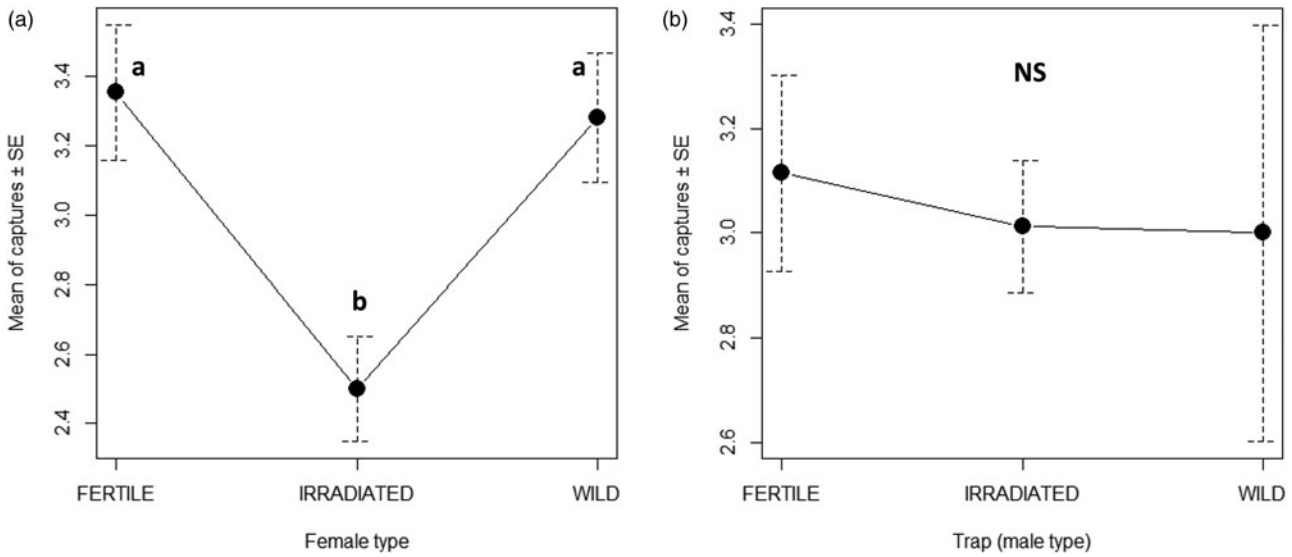
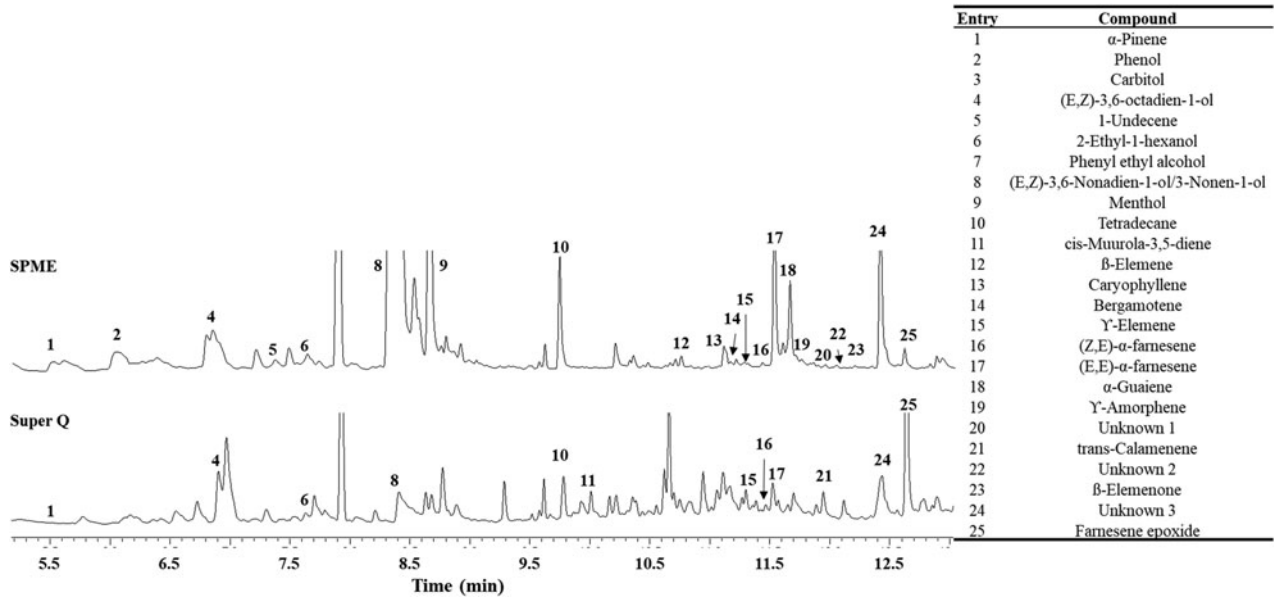


Figure 1. Female capture (mean ± SE) in the semi-field bioassay: (a) female type in traps and (b) male type in traps. Different letters indicate the differences among treatments according to the Tukey's test ($\alpha = 0.05$).

in the three male groups were (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol, (*Z*,*E*)-α-farnesene, and β-elemenone (fig. 3). The compounds α-pinene, phenol, and menthol were recovered only from the volatiles of non-irradiated males. (*E*,*Z*)-3,6-Octadien-1-ol, 1-undecene, α-guaiene, ϒ-amorphene, unidentified terpenes 1, 2, and 3, and farnesene epoxide were not found in the volatiles of wild males. Higher relative amounts of 2-ethyl-1-hexanol, β-elemene, and ϒ-elemene were found in the effluvia of wild males than in those of non-irradiated and irradiated males. The relative amount of caryophyllene was higher in the effluvia of wild and irradiated males than in that of non-irradiated males. In contrast, the relative amounts of (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol, *n*-tetradecane, bergamotene, (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, and β-elemenone were similar among the male groups.

Twelve compounds were collected using the coated 85 µm CAR/PDMS fibres (Supp. table S7). The major compounds in the three male groups were (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol and (*E*,*E*)-α-farnesene (fig. 3). Phenol, (*E*,*Z*)-3,6-octadien-1-ol, 2-ethyl-1-hexanol, unidentified terpene 3, and farnesene epoxide were not found in the effluvia of wild males. In contrast, caryophyllene and β-elemenone were only detected in the volatiles of wild males, whereas α-guaiene was only recovered from non-irradiated males. Wild and non-irradiated males produced more (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol than irradiated males, whereas the opposite was true for *n*-tetradecene. No differences were observed in the relative amounts of β-elemene, (*E*,*E*)-α-farnesene, or farnesene epoxide among the male groups.



Entry	Compound
1	α-Pinene
2	Phenol
3	Carbitol
4	(E,Z)-3,6-octadien-1-ol
5	1-Undecene
6	2-Ethyl-1-hexanol
7	Phenyl ethyl alcohol
8	(E,Z)-3,6-Nonadien-1-ol/3-Nonen-1-ol
9	Menthol
10	Tetradecane
11	cis-Muurola-3,5-diene
12	ß-Elemene
13	Caryophyllene
14	Bergamotene
15	ϒ-Elemene
16	(Z,E)-α-farnesene
17	(E,E)-α-farnesene
18	α-Guaiene
19	ϒ-Amorphene
20	Unknown 1
21	trans-Calamenene
22	Unknown 2
23	ß-Elemenone
24	Unknown 3
25	Farnesene epoxide

Figure 2. Chromatograms from the *A. obliqua* headspace and from two different adsorbents were selected. SPME (PDMS/DVB fibre) from static collection and Super Q from dynamic collection. Compounds **3** (carbitol) and **7** (phenyl ethyl alcohol) were only observed using other SPME fibre types.

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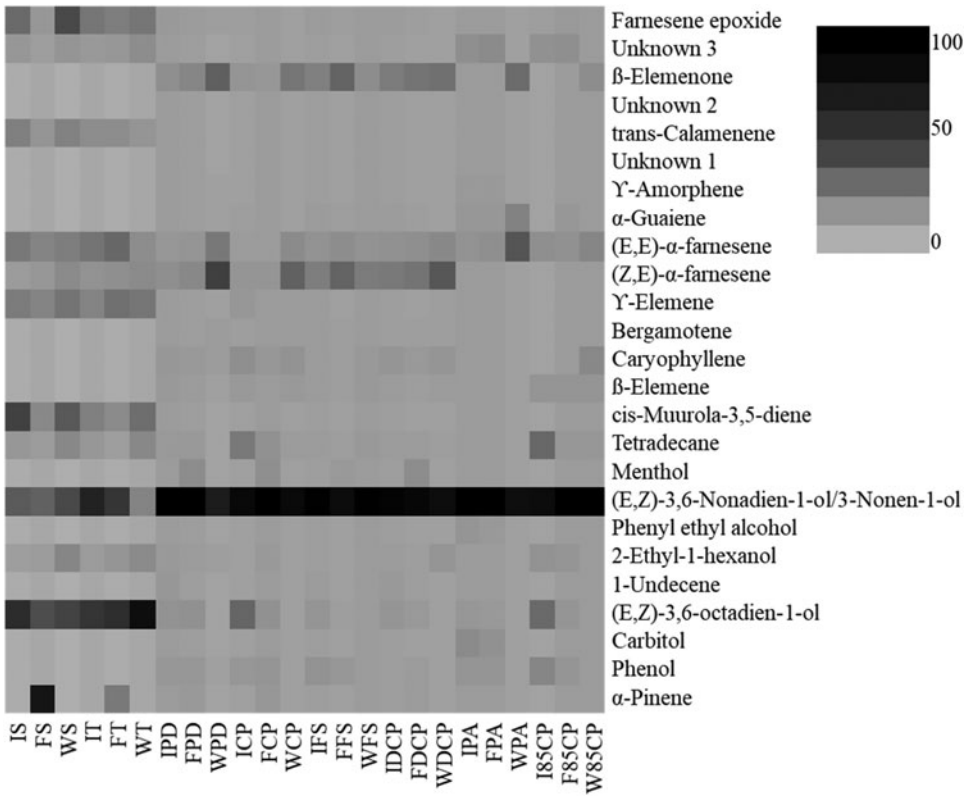

Figure 3. Heatmap representing chemical analysis of volatile compounds emitted by *A. obliqua* males. Compounds were identified by GC-MS. The scale of the heat map represents the relative amounts (percentages) of the compound abundance. The first letter of the acronyms corresponds to male type (I: irradiated, F: fertile, and W: wild), while the second and third letters correspond to the adsorbent used (S: Super Q, T: Tenax, and the following for the SPME fibres: bare FS, 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DCP), 85 µm carboxen/polydimethylsiloxane (85CP), 75 µm carboxen/polydimethylsiloxane (CP), 85 µm PA, and 65 µm polydimethylsiloxane/divinylbenzene (PD).

Ten compounds were collected from the coated 85 µm PA fibres (Supp. table S8). The major compounds in the three male groups were (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol (fig. 3). Phenol, carbitol, (*E*,*Z*)-3,6-octadien-1-ol, phenylethyl alcohol, ϒ-amorphene, and unidentified terpene 3 were not recovered from the effluvia of wild males. β-Elemenone was only found in the volatiles of wild males. Irradiated and non-irradiated males released more (*E*,*Z*)-3,6-nonadien-1-ol/3-nonen-1-ol than wild-type males, whereas the opposite was true for (*E*,*E*)-α-farnesene and α-guaiene.

Discussion

In the present study, we found that wild, non-irradiated, and irradiated *A. obliqua* males were equally attractive to the conspecific females. Our results also showed that the number and relative amounts of volatile compounds released by calling males were influenced by male source and sampling method. Despite the variation in volatile profiles, male attractiveness was not affected, suggesting that rearing and radiation did not influence the synthesis of behaviour-relevant compounds. Thus, the data obtained partially supports our working hypothesis. In addition, we identified several novel compounds in *A. obliqua*; however, we were unable to find some of the previously reported compounds.

Several studies have reported that radiation and artificial rearing can affect the sexual competitiveness of *Anastrepha* males compared with their wild counterparts (Meza-Hernández *et al.*, 2002; Rull *et al.*, 2005; Gallardo-Ortiz *et al.*, 2018). For instance, Meza-Hernández *et al.* (2002) found that mass-reared *A. obliqua* males competed with wild males for wild females. However, wild females prefer wild males to mass-reared ones. The last authors also reported that mass-reared males emitted less pheromone than their wild counterparts. They speculated that this is a disadvantage for mass-reared males, as female attraction depends on the amount of male pheromone. However, our data showed that the attractiveness of laboratory-reared (non-irradiated and irradiated) and wild *A. obliqua* males to conspecific females was similar. Hence, our data do not align with the findings of Meza *et al.* (2002). Thus, our results showed that at a distance, females could not discriminate among the volatiles of the different types of males. This suggests that discrimination is likely performed within a close range using acoustic, visual, or chemical signals. *Anastrepha* males produce two sounds in sexual context: calling sound and precopulatory sound (Sivinski *et al.*, 1984; Briceño *et al.*, 2009). When electing potential partners, *Anastrepha* females reject conspecific smaller males, which generate calling songs with a higher fundamental frequency and longer intervals between bursts (Mankin *et al.*, 1996). The acoustic songs of *A. suspensa* males are affected by radiation from cesium-137 (Webb *et al.*, 1987). To the best of our knowledge, the influence of rearing and radiation on the acoustic songs of *A. obliqua* males has not been investigated.

There were differences in the number of volatile compounds captured using the SPME and DHS techniques. Volatile compounds released by *A. obliqua* males into the atmosphere are perceived by females at a distance from their release point, thus

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mediating sexual behaviour. The identity and relative amounts (ratio or proportion) of such compounds convey specific information about the physiological state of males (Meza-Hernández *et al*., 2002). Thus, it is important to perform a robust chemical analysis of these compounds to reduce biases when interpreting their behavioural importance (Noushini *et al*., 2020). For this purpose, both dynamic and static samplings were used. Using SPME, 21 volatile compounds were collected from wild, non-irradiated, and irradiated male *A. obliqua*. The higher sensitivity of this technique allows the collection of more compounds in shorter periods of time. However, this technique has some limitations, mostly in terms of the quantitation of compounds (Cagliero *et al*., 2021). In terms of sensitivity, the coated 65 µm PDMS/DVB fibre was the most effective for collecting volatiles from *A. obliqua* males. Twelve volatile compounds were collected by DHS. It is possible that this technique collected fewer compounds because it requires an extended sampling time to collect sufficient volatiles (Alborn *et al*., 2021). However, *A. obliqua* males exhibit calling behaviour for a few hours. Additionally, we observed that *A. obliqua* males were under stress during dynamic volatile collection because of the noise caused by the pumps or airflow in the pull system. This stress could affect calling behaviour and the release of compounds. Despite its low sensitivity, it is possible to perform quantitative analysis of the compounds. Both adsorbents used in this study were suitable for sampling volatiles from male *A. obliqua*.

Both methods used to sample the headspace of *A. obliqua* males have advantages and disadvantages that are strongly related to the physical and chemical properties of the adsorbent, concentration of the released volatiles, chemical properties of the compounds, and diffusion of the volatiles in the collection chamber (Angelopoulos and Pickett, 1998; Noushini *et al*., 2020; Alborn *et al*., 2021). Ten compounds were collected in common using both sampling methods. The compounds *cis*-muurola-3,5-diene and *trans*-calamene were only captured by DHS, whereas menthol was only adsorbed by the SPME fibres. In this context, Super Q®, Tenax®, and the 65 µm PDMS/DVB fibre were the most suitable options for capturing volatiles from *A. obliqua* males.

Most of the compounds identified in this study have not been identified previously. For instance, we identified (*E*,*Z*)-3,6-octadien-1-ol, a possible precursor or decomposition product of (*E*,*Z*)-3,6-nonadien-1-ol, in the three types of flies, whereas α-pinene was only found in non-irradiated males. We identified eight compounds that were previously found in the effluent of *A. obliqua* males. For example, males of *A. obliqua* from Veracruz, Mexico reared on mangoes, as in our case, released only three compounds in common with *A. obliqua* males from Chiapas, Mexico: 3,6-nonadien-1-ol, (*Z*,*E*)-α-farnesene, and (*E*,*E*)-α-farnesene. It is possible that (*Z*)-3-nonen-1-ol is released by *A. obliqua* males from Veracruz, but the authors have not been able to identify it because it co-elutes with 3,6-nonadien-1-ol. The compounds 3,6-nonadien-1-ol, (*Z*,*E*)-α-farnesene, and (*E*,*E*)-α-farnesene are also emitted by *A. obliqua* males from Veracruz reared on *Spondias* spp., guava, and *Solanum lycopersicum*, a non-natural host of *A. obliqua* (Aluja *et al*., 2020). Males of *A. obliqua* from Brazil and Chiapas reared in mangoes released six common compounds: 3,6-nonadien-1-ol, (*Z*,*E*)-α-farnesene, (*E*,*E*)-α-farnesene, 2-ethylhexane-1-ol, β-elemene, and caryophyllene (Gonçalves *et al*., 2013).

We do not know the possible functions of most of the compounds identified in this study in the physiology and ecology of *A. obliqua*. Only two studies have evaluated some of the compounds released by *A. obliqua* males (López-Guillén *et al*., 2011; De Aquino *et al*., 2021). In semi-field tests, López-Guillén *et al*. (2011) found that more females and males were captured by traps baited with (*Z*)-3-nonen-1-ol and (*Z*,*E*)-α-farnesene than unbaited traps. Unfortunately, the responses of flies to these compounds were not compared with those of live calling males or calling male extracts to determine whether these compounds explain the attraction of flies to calling males or whether it is necessary to add other compounds. Later, De Aquino *et al*. (2021) reported that 1-heptanol, linalool, (*Z*)-3-nonen-1-ol, (*Z*,*E*)-α-farnesene, and (*E*,*Z*)-3,6-nonadien-1-ol released by *A. obliqua* males from Brazil elicited antennal responses from conspecific females. A quaternary blend composed of the first four compounds attracted more females than male calling extracts in laboratory assays. Interestingly, 1-heptanol and linalool were not emitted by *A. obliqua* males in Mexico (López-Guillén *et al*., 2011; Aluja *et al*., 2020; this study). This suggests that there is geographic variation in the chemical communication of *A. obliqua*. Previous studies have reported that *A. obliqua* from Mexico mate in the morning, whereas *A. obliqua* from Brazil mate in the afternoon (Aluja *et al*., 1993; Henning and Matioli, 2006). Further studies are required to confirm whether there is variation in the chemical communication of this fruit fly species.

Some of the compounds (e.g. α-pinene, caryophyllene, β-elemene, α-guaiene, bergamotene) identified in the effluvia of *A. obliqua* calling males have also been reported in mangoes and other natural hosts of this fruit fly species. At first glance, one might believe that these compounds are contaminants. However, the fact that these compounds were found in the volatiles of mass-reared males that did not have contact with mangoes seems to rule out this possibility. Previous studies on *Anastrepha* fruit flies have reported that some male compounds are emitted by their host plants. De Aquino *et al*. (2021) found compounds such as 1-heptanol, limonene, linalool, and ethyl octanoate in the effluvia of *A. obliqua* calling males, which also have been identified in fruits of mangoes and *Spondias* spp. (Pino *et al*., 2005; de Sousa Galvão *et al*., 2011). The pheromone of *Anastrepha striata* is composed of ethyl hexanoate, linalool, and ethyl octanoate (Cruz-López *et al*., 2015), which are constituents of the host fruit volatiles of *Anastrepha* spp. (Pino *et al*., 2005; de Sousa Galvão *et al*., 2011). Diaz-Santiz *et al*. (2016) reported that both female and male *A. striata* were attracted to a six-component blend isolated from guava consisting of ethyl butyrate, (*Z*)-3-hexen-1-ol, hexanol, ethyl hexanoate, hexyl acetate, and ethyl octanoate. The fact that male pheromones and fruit volatiles of *Anastrepha* spp. share some common attractive compounds suggests that they can use the same sensory mechanisms in both sexual and host plant locations.

One of the limitations of our study is that most compounds were identified using Kovats retention indices and mass spectra that matched the NIST library. Unfortunately, in many cases, these compounds are not commercially available, and their synthesis is complicated. Future studies should confirm the identification of these compounds, particularly those ecologically relevant to *A. obliqua*. For example, Aluja *et al*. (2020) identified (*Z*,*Z*)-3,6-nonadien-1-ol in the effluvia of *A. obliqua* males. However, it was not possible to discern between the (*Z*,*Z*) and (*E*,*Z*) isomers using GC-MS. The retention indices of both isomers are almost the same: 1156 and 1161, respectively. Because we had a retention index of 1161, we were inclined to believe that the isomer was (*E*,*Z*), coupled with the fact that De Aquino *et al*. (2021) reported that this isomer is bioactive. Further studies (e.g. nuclear magnetic resonance analysis) are needed to determine whether the isomer is (*E*,*Z*) or (*Z*,*Z*).

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In summary, we did not find any differences in the attractiveness of wild, sterile, and fertile *A. obliqua* males. In addition, a robust chemical analysis of volatiles from males revealed differences in the chemical profiles of the different types of males. We identified volatile compounds released by *A. obliqua* calling males that have not been reported in previous studies with this species. Further studies are needed to investigate the role of the identified compounds in the behavioural ecology of *A. obliqua*. Ecologically relevant compounds can be used for the monitoring or mass trapping of this fruit fly species.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S000748532400004X.

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