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EDITED BY  
Michael Wink,  
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REVIEWED BY  
Antonino Cusumano,  
University of Palermo, Italy  
Salvatore Guarino,  
Institute of Bioscience  
and Bioresources (CNR), Italy

\*CORRESPONDENCE  
Raphael Njurai Miano  
rmiano@icipe.org;  
mianorn@gmail.com  
Samira A. Mohamed  
sfaris@icipe.org

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# Differential responses of *Bactrocera dorsalis* and its parasitoids to headspaces of different varieties of tree-attached mango fruits and the associated chemical profiles

Raphael Njurai Miano <sup>1,2\*</sup>, Samira A. Mohamed <sup>1\*</sup>,  
Xavier Cheseto <sup>1</sup>, Shepard Ndlela <sup>1</sup>,  
Tibebe Dejene Biasazin <sup>3</sup>, Abdullahi Ahmed Yusuf <sup>4</sup>,  
Egmont Rohwer <sup>2</sup> and Teun Dekker <sup>3</sup>

<sup>1</sup>International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya, <sup>2</sup>Department of Chemistry, Faculty of Natural and Agricultural Science, University of Pretoria, Pretoria, South Africa, <sup>3</sup>Unit of Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden, <sup>4</sup>Department of Zoology and Entomology, Faculty of Natural and Agricultural Science, University of Pretoria, Pretoria, South Africa

*Bactrocera dorsalis* (Hendel) is a major pest of fruits and vegetables worldwide with documented losses of up to 100%. Various management techniques including the use of parasitoids, such as *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) within the context of the Integrated Pest Management (IPM) approach have been deployed for its control. The effectiveness of parasitoids is well understood, but knowledge of the semiochemicals that mediate their behavior, as well as that of the host fruit fly to tree-attached mangoes, is lacking. Here, we first compared the attractiveness of the above-mentioned fruit fly and its parasitoids to volatiles of different treatments (non-infested physiologically mature unripe and ripe mangoes, mangoes newly exposed to ovipositing *B. dorsalis*, and mangoes on day 7 and day 9 post-oviposition) of tree-attached Kent, Apple, and Haden mango varieties relative to control (clean air). The fruit fly was significantly more attracted to the mango volatiles (up to 93% of responsive insects) compared to the control (clean air). *Fopius arisanus* was significantly more attracted to mangoes with ovipositing fruit flies (68–76%) while *D. longicaudata* was significantly more attracted to day 9 post-oviposited mangoes (64–72%) compared to the control. Secondly, we elucidated the headspace volatile profiles of the non-infested and infested tree-attached mangoes using gas chromatography linked to mass spectrometry (GC-MS). The volatiles revealed various types of organic compounds with qualitative and quantitative differences. The majority of the compounds were esters making 33.8% of the total number, followed

by sesquiterpenes-16.4%, and monoterpenes-15.4% among others. Most compounds had higher release rates in headspace volatiles of fruit fly-infested mangoes. Lastly, we harvested the infested mangoes and incubated them for puparia recovery. The number of puparia recovered varied according to the mango variety with Apple mango registering 81.7% of the total, while none was recovered from Kent. These results represent the first report of the changes in the headspace components of non-infested and infested tree-attached mangoes and the associated differential responses of the mentioned insects. A follow-up study can reveal whether there is a convergence in olfactomes which is significant when developing baits that selectively attract the fruit fly and not its natural enemies and fill the knowledge gap from an evolutionary ecological perspective.

#### KEYWORDS

tree-attached mango, *Bactrocera dorsalis*, *Fopius arisanus*, *Diachasmimorpha longicaudata*, headspace, GC-MS

## Introduction

Mango (*Mangifera indica* L.) is one of the most widely grown fruits, ranking fifth among major fruit crops in terms of production, with global production of over 55.9 million metric tons in 2019 (Shahbandeh, 2021). In sub-Saharan Africa, mango is an important commodity as it has considerable socioeconomic importance, as a source of food and income for millions of mango growers and other actors along the mango value chain. However, its production and utilization have been hampered by a plethora of biotic and abiotic constraints key among them being infestation by tephritid fruit flies. *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is one of the most destructive fruit flies (Boinahadjji et al., 2020) causing losses of up to 100% if control measures are not implemented (Nankinga et al., 2014; Ekesi et al., 2016, and reference therein). Integrated pest management (IPM) strategies used in its control include the use of chemicals (Akotsen-Mensah et al., 2017; Díaz-Fleischer et al., 2017), lure and kill traps (Doorenweerd et al., 2018; Stringer et al., 2019), early fruit harvesting, bagging and netting (Ndlela et al., 2016), orchard sanitation (Vergheze et al., 2004), use of sterilized males [Sterile Insect Technique (SIT)] (Enkerlin et al., 2017; Cai et al., 2020), use of semiochemicals (Biasazin et al., 2018, 2019; Cai et al., 2020; Scolari et al., 2021), and the use of fruit fly natural enemies which include pathogens, predators, and parasitoids (Mohamed et al., 2010; Cai et al., 2020). The understanding of the ecological features that influence the interactions between phytophagous insects and their host plant/fruit is of crucial importance in developing sustainable fruit defense strategies. Several studies on herbivore-plant interactions have elucidated the central role of volatile organic compounds that act as host location kairomones for herbivores (Metcalfe and Kogan, 1987; Carrasco et al., 2015;

Guarino et al., 2018). The importance of these secondary plant substances as cues for host plant selection was emphasized several decades ago by Fraenkel (1969).

Volatile organic compounds emitted by plants and fruits play major roles in attracting or repelling insect pests (Benelli et al., 2014; Binyameen and Anderson, 2014), as well as in attracting their natural enemies including parasitoids (Segura et al., 2012; Harbi et al., 2019). Previous studies have highlighted some semiochemical-mediated interactions between fruits, fruit flies, and parasitoids (Carrasco et al., 2005; Harbi et al., 2019). For example, volatiles from three mango varieties (Amate, Coche, and Ataulfo) were found to be attractive to *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) (Malo et al., 2012), and a total of 22 compounds from 'Chausa' and 'Alphonso' were EAG active in female *B. dorsalis* antennae (Kamala et al., 2012). Furthermore,  $\gamma$ -octalactone, ethyl tiglate, benzothiazole, and 1-octen-3-ol either singly or as a blend elicited oviposition response in *B. dorsalis* (Kamala et al., 2014). A blend of common EAG active volatiles from diverse fruits (guava, banana, mango, and orange) increased the attractiveness of a majority of polyphagous fruit fly species in laboratory experiments (Biasazin et al., 2014, 2019).

The Opiinae subfamily of the Braconidae family is made up of over 1,500 koinobiont endoparasitoid species (Copeland et al., 2006; Badii et al., 2016). *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) (both Hymenoptera: Braconidae) are solitary egg-prepupal endoparasitoids that have been used extensively for biological control of *B. dorsalis* with outstanding success in Hawaii (Flávio et al., 2020) and French Polynesia (Roger et al., 2012). Recently, the two parasitoids have been introduced into Kenya (Mohamed et al., 2008, 2010) and subsequently released in several African countries for classical biological control of *B.*

*dorsalis* (Mohamed et al., 2016; Ndlela et al., 2020). Gravid *F. arisanus* females are attracted to their hosts either using volatiles emanating from the fruits during or after oviposition (Cai et al., 2020). Also, female *D. longicaudata* is known to exploit semiochemicals from the hosts' fruits and fruit fly larvae and is more attracted to host-infested fruits than non-infested or mechanically damaged fruits (Carrasco et al., 2005; Segura et al., 2012; Harbi et al., 2019).

In most studies on fruit-fruit fly-parasitoid interactions, little effort, if any, has been made to unravel the changes in volatile composition that occur before and after fruit fly infestation, specifically on tree-attached fruits under field conditions, and how these changes affect the behavior of the fruit fly and its natural enemies. Therefore, the current study aimed at investigating the behavioral responses of *B. dorsalis*, and the parasitoids *F. arisanus* and *D. longicaudata* to volatiles of three tree-attached mango varieties (Kent, Apple, and Haden) that were either non-infested or at different stages of infestation by the fruit fly and then elucidating the chemicals profiles of the mentioned mango varieties headspaces under the non-infested and the infested treatments.

## Materials and methods

### Mango fruits

During the flowering season, in July 2020, three varieties of mango trees (Kent, Apple, and Haden) were identified, in their growing habitat in Gathigiriri (00°41'39.8"S, 037°24'26.7"E,

1,158 m ASL), Mwea East Sub-county, Kirinyaga County, Kenya. The orchard contained 85 mature mango trees comprising the following varieties Kent (13), Apple (36), Haden (6), Van Dyke (4), Ngowe (8), Tommy Atkin (4), Mukarati (4), and 10 local varieties. In this area, Haden mangoes usually ripen in late December, Apples in January, and Kent ripens in April. Two mango trees of each of the three varieties were randomly selected from the orchard. The trees were kept free of insecticides and fungicides during the entire period of the trials. To prevent crawling insects like ants and termites from damaging the flowers and young fruits, duduthrin 1.75 EC (Twiga Chemical Industries Ltd., Nairobi, Kenya) was applied at the base of each tree according to the manufacturer's guidelines. The mango fruits were allowed to develop for 4 months, from the time of flowering, after which they were secured *in situ* (Figure 1) using fine white nets that were mounted on 20 cm × 20 cm × 20 cm of 2.5 mm galvanized metallic wire cube frames sourced from the local market.

Depending on the mango variety and fruit size, each net cage could hold at least four mangoes. From each mango tree, at least 32 mango fruits were secured. The caged mango fruits were inspected every week until they were physiologically mature and ready for use in the trials.

### Fruit flies

*Bactrocera dorsalis* was reared at the International Centre of Insect Physiology and Ecology (*icipe*) Duduville campus (01°13'25.3"S, 36°53'49.2"E; 1,600 m ASL) Nairobi Kenya



FIGURE 1

A mango tree with mango fruits some of which were secured using white netted cages to safeguard them from insect attack.

following already established protocols (Ekesi and Mohamed, 2011; Gordello, 2013), where the fruit fly colony was maintained at  $26 \pm 2^\circ\text{C}$ , 50–60% RH, and a photoperiod of 12:12 h (L: D). Ripe Apple mangoes were purchased from the local market in Nairobi, Kenya, and thoroughly cleaned using liquid soap and tap water to remove surface dirt, rinsed with distilled water which was then wiped out using paper towels. The mangoes were then stored at  $4^\circ\text{C}$  for 48 h to kill any residual fruit fly egg/larvae, followed by thawing for 2 h in a laminar flow hood. The six fruits were offered to 12–16 day old *B. dorsalis* [ $n = 100$  ( $\sigma: \text{♀} = 1:1$ )] held in a Perspex rearing cages ( $30 \text{ cm} \times 30 \text{ cm} \times 30 \text{ cm}$ ) as oviposition substrate for 3 h. The rearing cages had a fine net mounted on two opposite sides to allow for air circulation and a netted window for the provision of food and water to the fruit flies. The adult fruit flies were fed on an artificial diet containing a mixture of finely ground sugar (Mumias Sugar Company, Nairobi Kenya) and enzymatic yeast hydrolyzate (USB Corporation, Cleveland, OH, USA) in a ratio of 3:1. Water was provided *ad libitum* in glass Petri-dishes ( $90 \text{ mm} \times 15 \text{ mm}$ ) with pumice granules to prevent drowning. The infested fruits were then transferred into plastic containers ( $21 \text{ cm} \times 14 \text{ cm} \times 8 \text{ cm}$ ; Kenpoly manufacturers limited, Nairobi, Kenya) for eggs to hatch and larvae to develop. The plastic containers were perforated on their bottom side and a sheet of paper towel followed by a fine net laid on the inside. This was done to allow soaking and drainage of any sap that was produced as the larvae developed and the fruit rot and to prevent larvae from escaping. Each plastic container was covered with a fine net and a perforated plastic lid to allow for air circulation. On the onset of pupation, the infested mangoes were put in plastic basins ( $32 \text{ cm}$  diameter  $\times$   $14 \text{ cm}$  depth, Kenpoly manufacturers limited) that were quarter filled with dry, fine ( $>1.18 \text{ mm}$ ), and sterilized sand for larvae to pupate. The basins were also perforated at the bottom and a fine net was laid covering the perforations before the sand was added to allow sap drainage. The basins were then covered with a white net to prevent third instar larvae from jumping out. After pupation, the content of the basin was soaked in excess water to separate the puparium from the sand and the remains of the mangoes. The floating puparia were then recovered through sieving (Cheseto et al., 2017), put in petri-dishes, and then transferred into the Perspex rearing cages ( $30 \text{ cm} \times 30 \text{ cm} \times 30 \text{ cm}$ ) for eclosion. The adult fruit flies were maintained as aforementioned but at room conditions of temperature (day =  $23 \pm 4^\circ\text{C}$ , night =  $20 \pm 4^\circ\text{C}$ ), humidity (38–68% RH), and natural photoperiod.

## Fruit fly parasitoids

The egg parasitoid *Fopius arisanus* and the larval parasitoids *Diachasmimorpha longicaudata* used in this study were also reared at *icipe*, Duduville campus (Nairobi, Kenya). The host

fruit flies were the newly established colony of *B. dorsalis* explained in section “Fruit flies”.

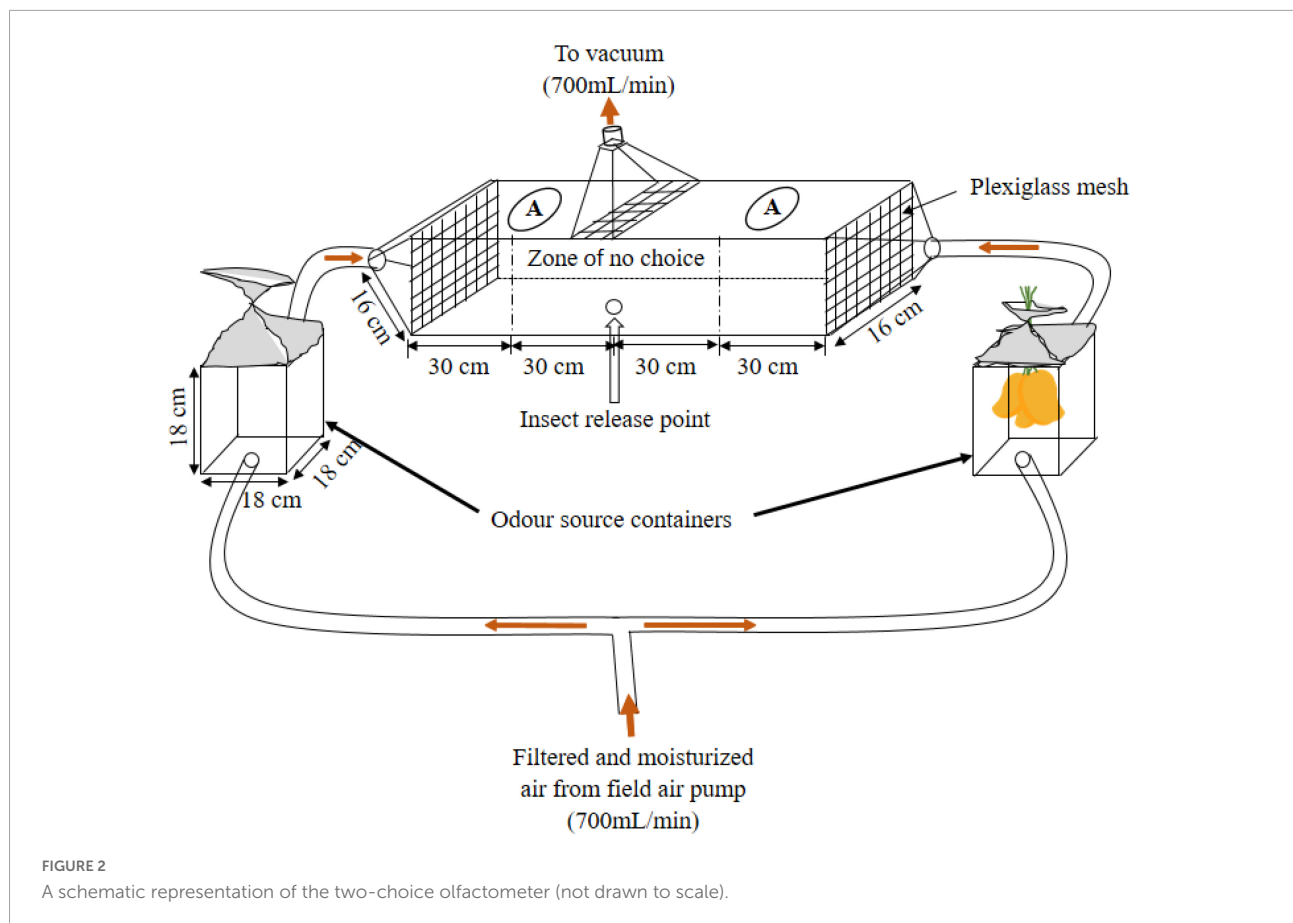
*Fopius arisanus* colony was initiated by exposing six Apple mangoes to a colony of 100 adults of *B. dorsalis* (ratio  $\sigma: \text{♀} = 1:1$ ) for 3 h (8.00 a.m.–11.00 a.m.). Two sets of three mangoes were then put in cages each containing 100 adults of 8–15 days-old *F. arisanus* ( $\sigma: \text{♀}$  ratio = 1:1) for 19 h. For *D. longicaudata*, mangoes were exposed to *B. dorsalis* as aforementioned. The infested mangoes were then incubated for 6 days to allow the larvae to develop to the second instar and then transferred into cages containing 100, 8–15 days-old *D. longicaudata* adults ( $\sigma: \text{♀} =$  ratio-1:1) for 3 days to maximize parasitization. After eclosion, the parasitoids were separated from *B. dorsalis* and transferred into their respective cages. Adult parasitoids were fed on 80% honey (*Eco Honey, icipe*, Nairobi, Kenya) that was spotted on the inside upper surface of the rearing cage, and water was provided *ad libitum* in glass Petri-dishes with gravel granules and rolled cotton wool (Supplementary Figure 1) after every 4 days (Manoukis et al., 2011). The new parasitoid colony was maintained under room conditions.

## Behavioral responses of female fruit flies and parasitoids to tree-attached mango volatiles

Dual-choice olfactometer assays were carried out under the field conditions at Mwea East Sub-county, Kirinyaga County, Kenya ( $00^\circ41'39.8''\text{S}$ ,  $037^\circ24'26.7''\text{E}$ , 1,158 m ASL) to evaluate the responses of fruit flies and parasitoids to mango fruit volatiles, *in situ*, following the methods described by Nyasembe et al. (2012) and Miano et al. (2022) with some modifications. The dual-choice olfactometer and the mango holders were made of Perspex glass (Figure 2).

The temperatures and the humidity during the assays were not regulated since the experiments were conducted in the field. In all the bioassays, the airflow through each of the olfactometer arms was maintained at  $350 \text{ mL min}^{-1}$  and evacuated at the center ( $700 \text{ mL min}^{-1}$ ) using air-flow meters connected to a portable vacuum field air pump (Analytical Research System Inc., Gainesville, FL, USA). For each bioassay, 10 mated adult females (10–15 days old for *B. dorsalis* or 8–14 days for parasitoids) were placed in a releasing vial and kept for 10 min for acclimatization. Thereafter, the group of insects was released through a hole at the center of the bottom of the wind tunnel (Figure 2) using a black coated falcon tube and they were allowed 20 min to choose, following the procedure of group release described with modifications by Nyasembe et al. (2012), Biasazin et al. (2014), Njuguna et al. (2018), Binyameen et al. (2021), and Miano et al. (2022) with modifications. The base of the dual-choice olfactometer was marked from 0 to 60 cm on either side of the insect release point to allow scoring. The insects that moved beyond 30 cm from the center on either





side of the olfactometer were considered to have made a choice, while those that were in the range of 0–30 cm from the release point were non-responsive. Non-responsive insects were not included in the statistical analysis. For the fruit flies and each species of parasitoids, seven replicates were done (as the day would allow) per choice test. The tested insects were removed through windows marked A (Figure 2) and put in a separate cage. Between two runs, clean air was passed through the apparatus for 20 mins to blow out the odor of the previous test, the air inlets were then changed to avoid positional bias, and air from odor sources was allowed to pass through the apparatus for 10 mins to stabilize the airflow. For fruit fly infestation, 15 females were randomly selected from a cage containing a 10–15 day old mixture of males and females ( $\sigma^7$ :  $\varphi$  = 1:1) and then released into the mango holder cages (Figure 2) containing four mangoes as an oviposition substrate. Before the assays, the fruit flies and mangoes were kept together for 20 min for fruit flies to probe (the fruit flies remained with the mangoes until the last replicate of that day was done). To ascertain the activity of the fruit flies, mangoes were assessed before and after exposure for punctures and oozing sap using a hand lens ( $\times 10$ ). The newly infested mangoes were secured in nets and used for subsequent infested mango assays. After the day tests, the odor containers

and the olfactometer were cleaned using hot water and allowed to dry overnight.

Behavioral experiments included the responses of (i) *B. dorsalis*, *F. arisanus*, or *D. longicaudata* to control (clean air); (ii) *B. dorsalis* or *F. arisanus* to volatiles of non-infested physiologically mature but unripe mangoes versus control; (iii) *B. dorsalis* or *F. arisanus* to volatiles of mangoes with ovipositing *B. dorsalis* versus control; (iv) responses of *B. dorsalis* or *D. longicaudata* to volatiles of mangoes on day 7 or day 9 *B. dorsalis* post-oviposited mangoes versus control; and (v) responses of *B. dorsalis*, *F. arisanus*, or *D. longicaudata* to volatiles of non-infested ripe mangoes versus control. Each day, the experimental mangoes were secured back into the fine white netted cages to prevent any additional damage. On the tenth day after exposure to fruit flies, the infested mangoes were harvested and incubated for pupation.

### Performance of *Bactrocera dorsalis* in the different mango varieties

To assess the performance of the fruit flies in the three varieties of mangoes, the infested mangoes were harvested on the tenth day and incubated as aforementioned (section “Fruit

flies"). Fruit harvesting was informed by the fact that on the tenth day, most of the infested Apple and Haden mangoes had detached from the tree. Kent mango was observed to take longer before detaching. The puparia that were recovered from the infested mangoes were counted and recorded.

## *In situ* collection of mango headspaces

The collection of mango headspaces was carried out simultaneously during the bioassay experiments. Dynamic headspace trapping (DHT) system (Ormeño et al., 2011; Miano et al., 2022) was used with some modifications (Supplementary Figure 2). Ambient air was drawn into the system using portable vacuum field pumps and passed via air flow meters at a rate of 250 mL/min and drawn out at the same rate. Headspace volatiles were trapped in tree-attached mangoes, which were bagged in a polyacetate oven bag (KitchenCraft Ltd., Birmingham, UK) (Supplementary Figure 2). Volatiles were collected onto HayeSep-Q adsorbents [30 mg, copolymers of polydimethylsiloxane-divinylbenzene (PDMS-DVB)] which were pre-cleaned with GC-grade dichloromethane (DCM). The headspace volatiles were trapped in quadruplets from the same tree for each of the subsequent mango treatments. These treatments include (i) non-infested physiologically mature unripe mango fruits; (ii) mango fruits with ovipositing *B. dorsalis*; (iii) infested mango fruits, a day after oviposition; (iv) infested mango fruits at (a) day 7 and (b) day 9 post-oviposition; (v) non-infested ripe mango fruits; and (vi) clean air as control. Headspace volatiles were collected for 11 h (7.00 a.m. to 6.00 p.m.). For preservation and transportation of the headspace volatiles trapped in HayeSep-Q adsorbents the procedure explained in Miano et al. (2022) was used. The headspace volatiles trapped in HayeSep-Q were then eluted in 200  $\mu$ L DCM into 250  $\mu$ L conical point glass inserts contained in clear 1.5 mL glass vials (Supelco, Bellefonte, PA, USA) using high-purity nitrogen gas as the pressurizing gas and immediately stored in a freezer at  $-81^{\circ}\text{C}$  until use.

## Chemical analysis of mango headspace volatiles

The headspace volatiles were analyzed (1  $\mu$ L) by GC-MS, a 7890A gas chromatograph linked to a 5975C mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA). The GC-MS instrument was equipped with an HP-5 MS (5% phenylmethylpolysiloxane) column (30 m  $\times$  0.25 mm ID  $\times$  0.25  $\mu$ m film thickness). The oven temperature program was  $35^{\circ}\text{C}$  for 5 min, then increase to  $280^{\circ}\text{C}$  at the rate of  $10^{\circ}\text{C min}^{-1}$ , and then held at this temperature for 10.5 min. The mass selective detector was retained at  $230^{\circ}\text{C}$  ion source temperature and a quadrupole temperature of  $180^{\circ}\text{C}$ . Electron acceleration energy

of 70 eV was used to obtain electron impact (EI) mass spectra while the resulting ions were analyzed over the mass range of 40–550  $m/z$  in the full scan mode. The solvent delay time was set at 3.3 min. High-purity helium gas was used as the carrier gas at a flow rate of  $1.2 \text{ mL min}^{-1}$ .

The qualitative identification of compounds was done by comparing the mass spectrometric data and retention times to those of reference spectra published by the library-MS databases National Institute of Standards and Technology (NIST 05, 08, and 11), Adams and Chemocol, and also based on their retention indices (RI) while some were confirmed using authentic standard retention times (Supplementary Figure 3). The RI (determined using a homologous series of straight-chain alkanes,  $\text{C}_5\text{--C}_{31}$ ) was calculated based on the equation of Van den Dool and Kratz and compared with documented values (van Den Dool and Kratz, 1963; Adams, 1995).

For relative quantification of the release rates of volatiles, a serial dilution (2.25–1,000  $\text{ng } \mu\text{L}^{-1}$ ) of the authentic standards  $\alpha$ -pinene and  $\alpha$ -humulene (98% purity, Sigma-Aldrich® Solutions, St. Luis, MO, USA) were analyzed by GC-MS in full scan mode to generate linear calibration curves (peak area vs. concentration, Supplementary Figure 4; Njuguna et al., 2018; Miano et al., 2022). The linear equations generated were  $y = 2036653.8x - 5127153.0$ ;  $R^2 = 0.9963$  for  $\alpha$ -pinene and  $y = 1127808.7x - 5512234.2$ ;  $R^2 = 0.9991$  for  $\alpha$ -humulene and were used to quantify volatile compounds that had retention times that were either below or above 16 min, respectively.

## Chemicals

All synthetic chemicals used in this study were purchased from Merck, Germany. These compounds included dichloromethane (DCM) for elution, hexanal, (2*E*)-hexenal, *p*-xylene,  $\alpha$ -pinene, camphene, 1-octen-3-ol, myrcene,  $\delta$ -3-carene,  $\delta$ -2-carene, *o*-cymene, limonene, (*Z*)- $\beta$ -ocimene, (*E*)- $\beta$ -ocimene,  $\gamma$ -terpinene, terpinolene, linalool, *n*-nonanal, 1,3,8-*p*-menthatriene, *allo*-ocimene, terpinen-4-ol, *n*-decanal,  $\beta$ -elemene, (*E*)-caryophyllene,  $\alpha$ -humulene, and caryophyllene oxide which had a chemical purity of 90–99.9%,  $\alpha$ -phellandrene and sabinene (purity 85 and 75%, respectively) were used for identification of volatiles.

## Statistical analysis

The data of the numbers of the responsive fruit flies and parasitoids were analyzed using the R software (RStudio Team, 2021) at a significant level of 5%. The choice of fruit flies and parasitoids between host volatiles and clean air was assessed using the Chi-square goodness of test to confirm whether the responsive insects were in the ratio of 1:1.

The numbers of puparia harvested from the three mango varieties and the numbers of compounds detected from

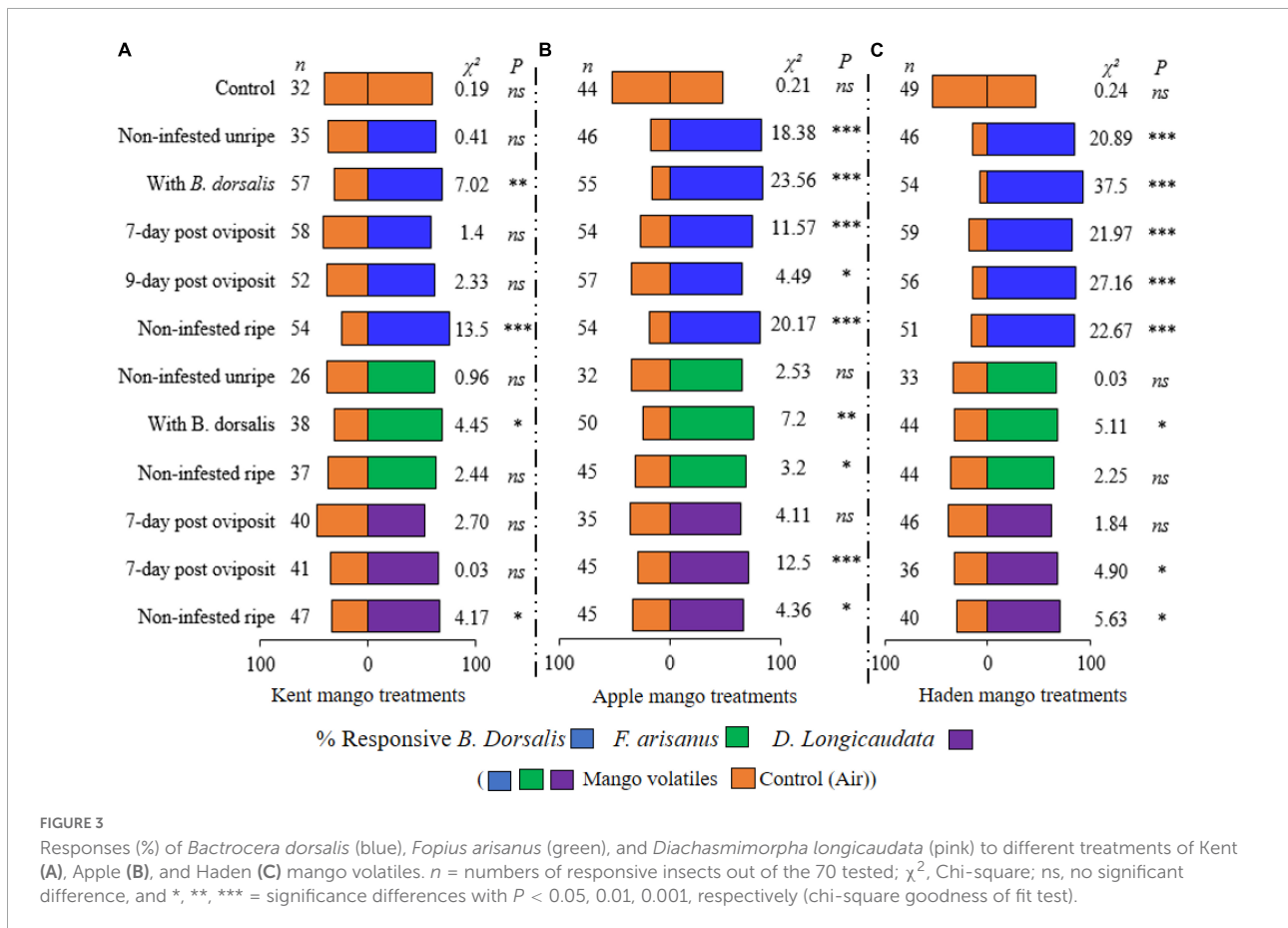
the treatments of each variety of mangoes were compared using Pearson’s Chi-square test followed by Chi-square multi-comparison test in RVAideMemoire (version 0.9-80) (RStudio Team, 2021).

The mango volatile release rates from the three varieties were subjected to the Shapiro-Wilk test and Barlett’s test to check the normality of distribution and homogeneity of variances, respectively. Since the data did not meet these assumptions, non-parametric tests were henceforth performed to analyze the data. The non-parametric Kruskal–Wallis rank-sum test followed by the *post hoc* Dunn test for pairwise comparison was used to test whether the volatile release rates from the three mango varieties under the different treatments were equal (Dinno, 2015) (where volatiles were present in only two treatments, Mann-Whitney *U* test was used). The data were then subjected to the non-metric multidimensional scaling (NMDS), similarity percentages (SIMPER) analysis, and one-way analysis of similarities (ANOSIM) using Bray–Curtis dissimilarity matrix in the *Past 3* software (Hammer et al., 2001).

The volatile release rate data were then analyzed per mango variety where each data was subjected to one-way analysis of similarities (ANOSIM) to determine whether the headspace composition among treatments was significantly different.

Furthermore, the non-metric multidimensional scaling, NMDS and the similarity percentage, SIMPER (Rohart et al., 2017) were performed and the top 30 compounds were visualized graphically. The 30 most discriminant compounds were also used in making NMDS biplots and in the construction of heatmap clusters (Rohart et al., 2017; Ayelo et al., 2021; Miano et al., 2022) using the auto-scaled average of their volatile release rate ( $y = \log_{10} x + 1$ ), where  $x$  = average volatile release rates in ng/mango/h).

The relative release rates of the common compounds present in the headspace of non-infested unripe mangoes or non-infested ripe mangoes were selected from the different treatments of the same mango variety and compared as follows: (i) mango with ovipositing *B. dorsalis* and mango a day after oviposition relative to those of non-infested physiologically mature unripe mango and (ii) day 7 and day 9 post-oviposited mangoes relative to those of non-infested ripe mangoes. A Kruskal-Wallis rank-sum test was performed to test for the difference in headspace volatile release rates in each of the three treatments followed by the Dunn test for pairwise comparison to test where the differences reported originated from. Furthermore, the averages of the compounds that were common in volatiles as selected in (i) and (ii)



were auto-scaled using  $y = 2 + \log_{10} x$  and their number of fold changes in the quantities relative to either those of non-infested unripe mangoes or non-infested ripe mangoes calculated, where the number of fold changes was given by  $y = \frac{\text{Average headspace volatile release rate of a compound in the volatile of interest}}{\text{Average headspace volatile release rate of the same compound in healthy mango}}$  and then visualized using line graphs.

## Results

### Behavioral assays of *Bactrocera dorsalis* and parasitoids to tree-attached non-infested and infested mangoes

In our preliminary assays where control treatments were used, *B. dorsalis*, *F. arisanus*, and *D. longicaudata* showed no significant difference in the number of females that chose either arm of the wind tunnel ( $P > 0.05$ ; **Figure 3**). On the other hand, the attraction of the fruit fly and the wasps to mango headspaces differed in magnitude compared to clean air. *B. dorsalis* was significantly attracted to the volatiles of non-infested ripe and Kent mangoes with ovipositing *B. dorsalis* (respectively,  $\chi^2 = 13.5$ ,  $P < 0.001$ ;  $\chi^2 = 7.02$ ,  $P < 0.01$ ) but not to the non-infested, 7 and 9 days post-oviposited Kent mangoes compared to the control (**Figure 3A**). Apple and Haden mango volatiles were more attractive to *B. dorsalis* ( $P < 0.001$ ) except for 9 days post-oviposited Apple mango ( $\chi^2 = 4.49$ ,  $P < 0.05$ ) compared to the control (**Figures 3B,C**). For the egg parasitoid, *F. arisanus* was not attracted to volatiles from unripe Kent, Apple, or Haden mango, while when ripe only volatiles of ripe Apple mangoes attracted *F. arisanus* ( $\chi^2 = 3.2$ ,  $P < 0.05$ ). In the presence of ovipositing *B. dorsalis*, however, all mango varieties significantly attracted *F. arisanus* ( $\chi^2 = 4.45$ ,  $P < 0.05$ ;  $\chi^2 = 7.2$ ,  $P < 0.01$ ;  $\chi^2 = 5.11$ ,  $P < 0.05$ , respectively, for Kent, Apple, and Haden with ovipositing *B. dorsalis*) (**Figures 3A–C**). *Diachasmimorpha longicaudata* was significantly attracted to ripe mangoes, regardless of variety ( $\chi^2 = 4.17$ ,  $P < 0.05$ ;  $\chi^2 = 4.36$ ,  $P < 0.05$ ;  $\chi^2 = 5.63$ ,  $P < 0.05$ , respectively, for Kent, Apple, and Haden ripe mangoes). Except for the Kent mango, *D. longicaudata* was attracted to day 9 post-oviposited mangoes ( $\chi^2 = 12.5$ ,  $P < 0.001$ ;  $\chi^2 = 4.90$ ,  $P < 0.05$ , respectively, for Apple and Haden) more than the control (**Figures 3A–C**). Curiously, mangoes 7-day post-oviposition did not attract *D. longicaudata* more than the control.

### Performance of *Bactrocera dorsalis* on the different mango varieties

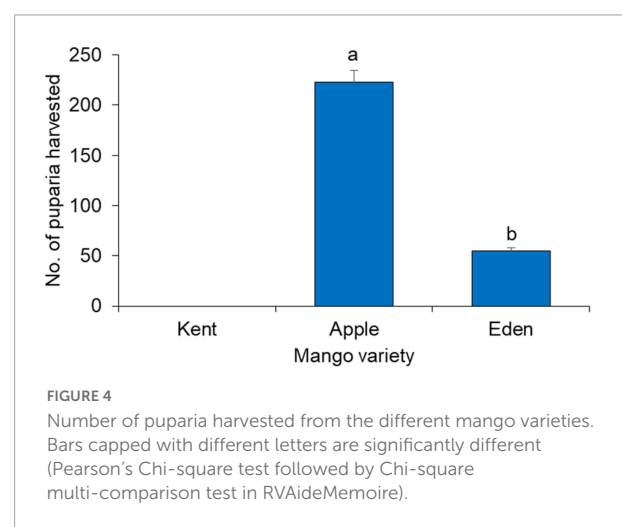
The performance of *B. dorsalis* in the three mango varieties as measured by the number of recovered puparia varied

considerably ( $\chi^2 = 328.39$ ,  $df = 2$ ,  $P < 0.0001$ ) with Apple mango yielding more than 4-fold of the yield from Harden variety (**Figure 4**). Although punctures and fruit sap were observed on the day of oviposition on Kent mangoes, there were no *B. dorsalis* puparia recovered from this variety.

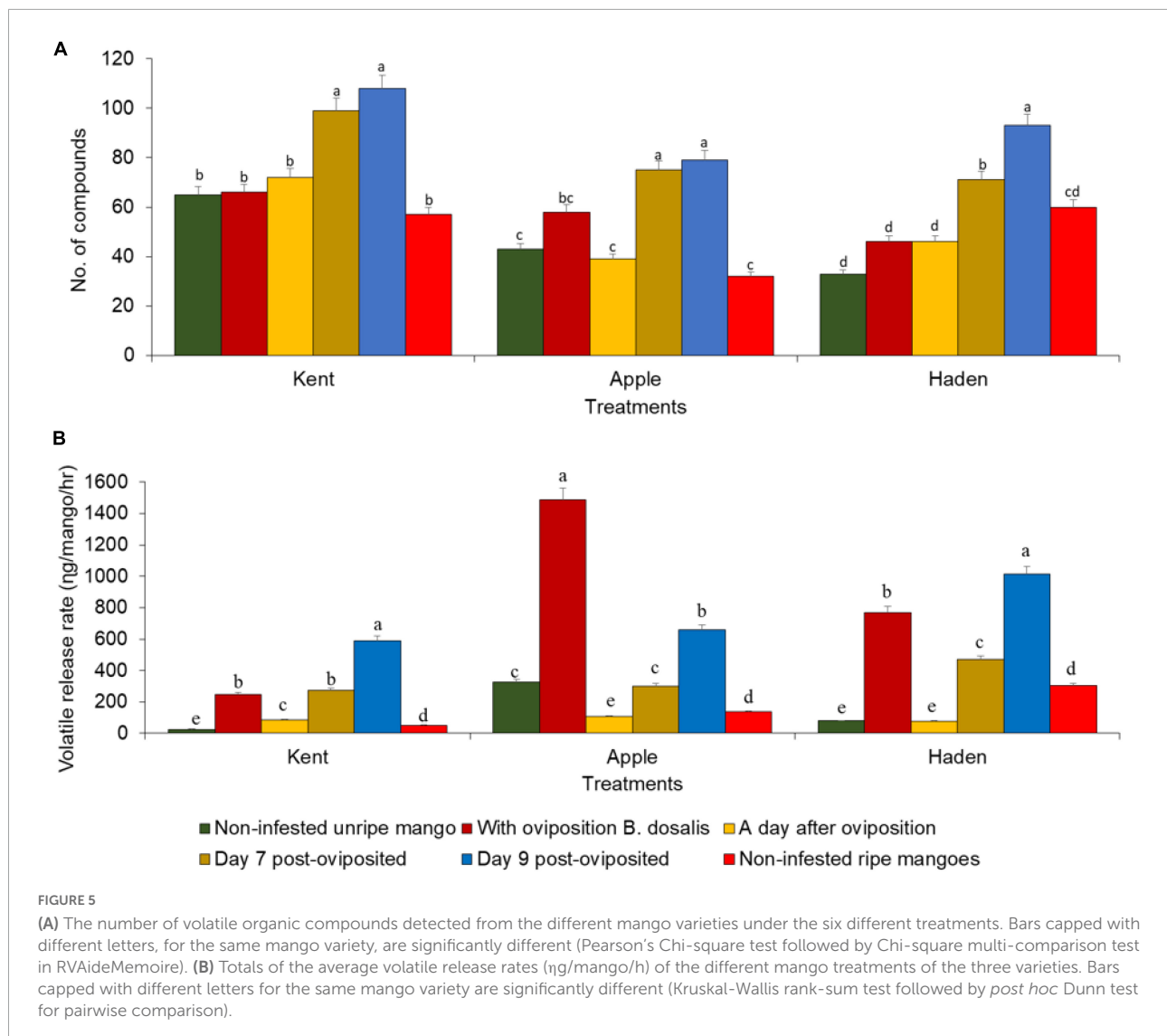
### Headspace volatiles in treatments of the three varieties of mangoes

A total of 195 volatiles were identified in the mango headspaces, the composition of which varied between the treatments and the mango variety (**Supplementary Table 1**). Kent mango registered the highest number of compounds (134) followed by Haden (114) while Apple had the least (102) (**Supplementary Table 1**). Among the compounds detected, 66 were esters, 32 sesquiterpenes, 30 monoterpenes, 14 monoterpeneoids, 12 aldehydes, 9 ketones, 10 alcohols, 6 sesquiterpenoids, 5 benzenoids, 3 organic acids, 3 diterpenes, and 5 others (**Supplementary Table 1**). Among the compounds detected, 9 compounds ( $\alpha$ -pinene,  $\beta$ -pinene, myrcene,  $\delta$ -3-carene,  $\alpha$ -gurjunene, (*E*)-caryophyllene,  $\beta$ -copaene,  $\alpha$ -humulene, and  $\delta$ -cadinene) were present in all treatments of the three varieties of mangoes but with varying release rates (**Supplementary Table 1**). There were significant differences in the volatile release rates between treatments of the three mangoes varieties (**Supplementary Table 1**). Infestation affected the volatile released both qualitatively (**Figure 5A**) and quantitatively (**Figure 5B**), with variations observed between mango varieties. Except for the Apple mango of which the quantitative change was at its peak on the oviposition day, the aspects of qualitative and quantitative increase peaked on day 9 post-oviposition (**Figure 5B** and **Supplementary Table 1**).

The non-metric multidimensional scaling (NMDS) shows a significant difference among the treatments across the three mango varieties ( $k = 2$ , stress = 0.1218; one-way analysis of







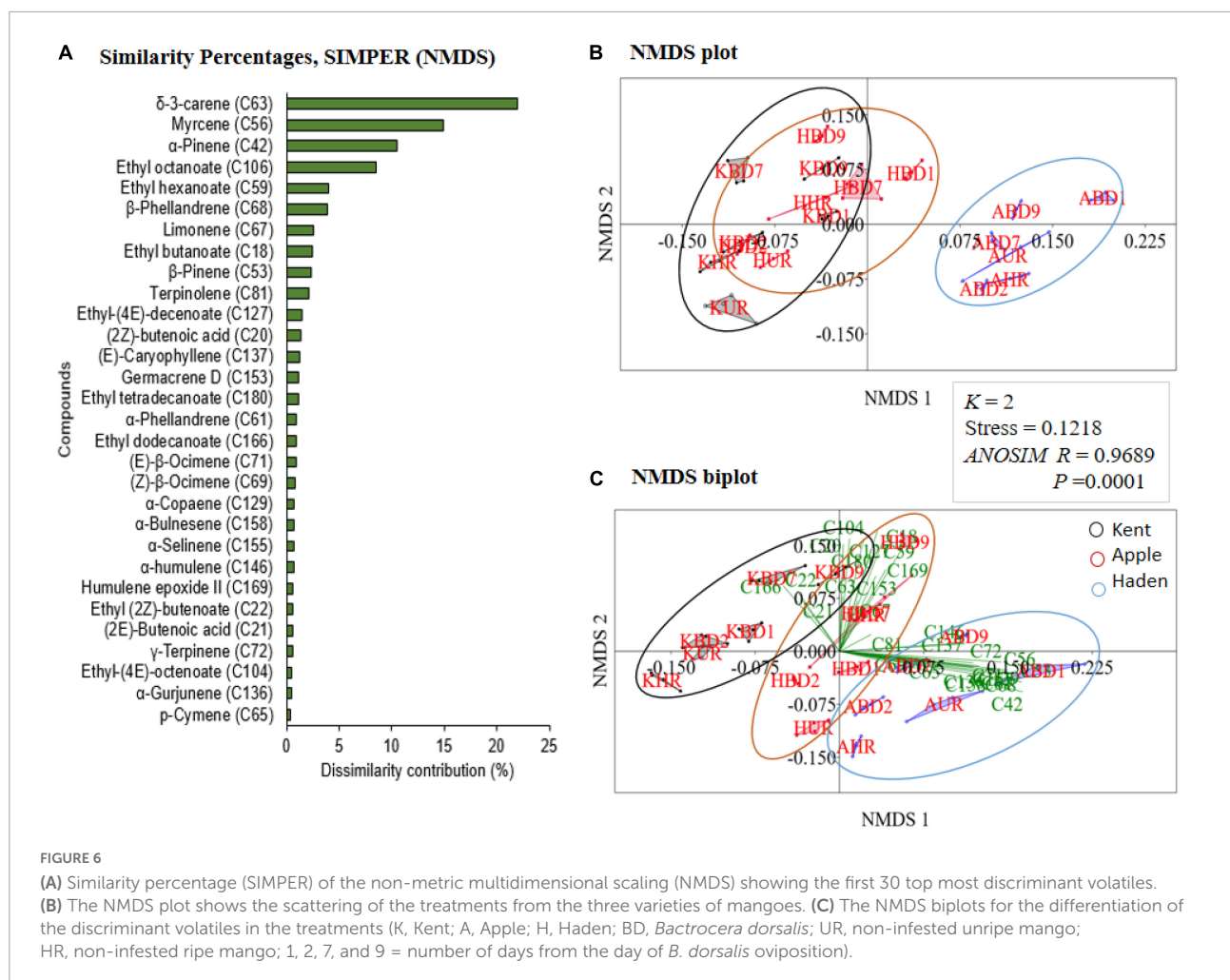
similarity, ANOSIM,  $R = 0.7245$ ,  $P = 0.0001$ ; **Figure 6** and **Supplementary Figure 5A**).

The 30 top most discriminant volatiles contributed 89.8% of the total dissimilarity contribution. The highest contributors and their percentage dissimilarity contributions were  $\delta$ -3-carene (C63)-21.9, myrcene (C56)-14.9,  $\alpha$ -pinene (C42)-10.6, ethyl octanoate (C106)-8.5, ethyl hexanoate (C59)-4.1,  $\beta$ -phellandrene (C68)-3.9, and limonene (C67)-2.6 (**Figure 6A**). Volatile compounds from the Apple mangoes were scattered separately unlike those of Kent and Haden mangoes which overlap at some points (**Figure 6B**). Furthermore, the 30 most discriminating volatiles are more associated with mango volatiles on the day of oviposition, day 7, and day 9 post-oviposited mangoes (**Figure 6C**).

Considering the treatments per mango variety, the multivariate analytical tool showed different discriminants of the volatile organic compounds (VOCs). The 30 top most

discriminant volatiles among Kent mango volatiles as per the non-metric multidimensional scaling's (NMDS) similarity percentages, SIMPER are graphically presented in **Figure 7A** where  $\delta$ -3-carene, ethyl octanoate, ethyl hexanoate, ethyl-(4*E*)-decenoate, (2*Z*)-butenoic acid, ethyl dodecanoate, limonene, terpinolene, ethyl (2*Z*)-butenoate, (2*E*)-butenoic acid, ethyl butanoate, and myrcene contributed a total of 80.10% of the total dissimilarity.

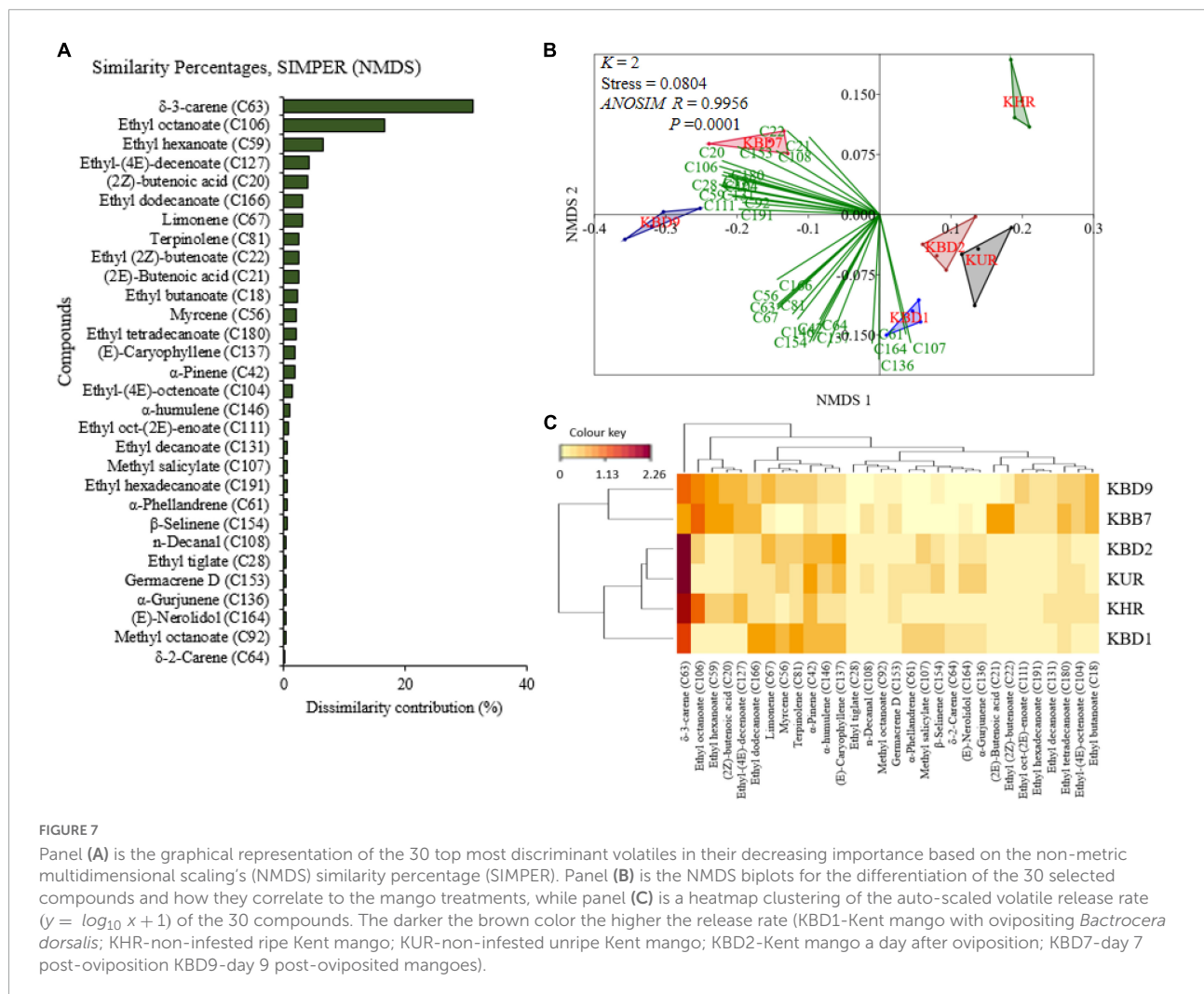
The NMDS biplots of the differentiation of the selected volatiles reveal that there were significant differences between the treatment headspaces ( $k = 2$ , stress = 0.08304; one-way analysis of similarity, ANOSIM,  $R = 0.9956$ ,  $P = 0.0001$ ; **Figure 7B** and **Supplementary Figure 5B**). More than 90% of the 30 selected compounds were associated with the volatiles emanating from the day of oviposition (KBD1), day 7 (KBD7), or day 9 (KBD9) post-oviposited mango fruits (**Figure 7B**). The heatmap clustering (**Figure 7C**) shows how the discriminating



volatiles were spread in the treatments and the dendrograms show how they are correlated. Of the selected compounds, volatiles with dark brown color were released at higher rates. For example, δ-3-carene (C63) was released at a higher rate except on day 9 (Figure 7C).

Furthermore, there was a significant difference in the volatile release rates of non-infested unripe Kent mango (KUR), Kent mangoes on the day of oviposition (KBD1), and Kent mangoes a day after oviposition (KBD2) ( $\chi^2 = 27.17$ ,  $df = 2$ ,  $P < 0.001$ ). In pairwise comparison, there was a significant difference between volatile release rates of KUR and KBD2 as well as KBD1 and KBD2 ( $P < 0.001$ ) while there was no significant difference between KBD1 and KUR ( $P > 0.05$ ). There were several-fold changes in the release rates of common volatiles on the day of oviposition (KBD1) and the day after oviposition (KBD2) compared to those of non-infested unripe Kent mangoes (KUR). The following are examples of some volatiles that were discriminant together with their number of fold changes, i.e., compound [no. of fold change on the day of oviposition (KBD1); no. of fold change a day after

oviposition (KBD2)]: δ-3-carene (11.1; 18.8), limonene (19.2; 17.0), terpinolene (55.1; 27.7), ethyl dodecanoate (152.0; 34.0), and β-selinene (6.4; 6.6) (Figure 8A). On the other hand, there was a significant difference in the volatile release rates of non-infested ripe Kent mango (KHR), Kent mangoes on day 7 (KBD7), and day 9 (KBD9) post-oviposition ( $\chi^2 = 121.76$ ,  $df = 2$ ,  $P < 0.001$ ). The pairwise comparison indicated a significant difference between KBD9 and KHR, KBD7 and KHR ( $P < 0.001$ ), and KBD7 and KBD9 ( $P < 0.05$ ; Figure 8B). There were changes in the release rates of common compounds on day 7 and day 9 post-oviposition headspaces compared to those of non-infested ripe mangoes (KHR). Examples of compounds that were discriminating with their quantities of fold change on day 7 and day 9 post-oviposition headspaces, respectively, compared to non-infested ripe mangoes were δ-3-carene (0.9; 6.7), limonene (1.0; 17.5), terpinolene (0.8; 15.4), β-selinene (2.3; 22.4), ethyl dodecanoate (15.4; 14.9), and ethyl hexadecanoate (79.0; 210.6) (Figure 8B). Other than changes in folds, 47 volatiles were only detected in the headspace of *B. dorsalis*-infested Kent mangoes, among them being pentanal,



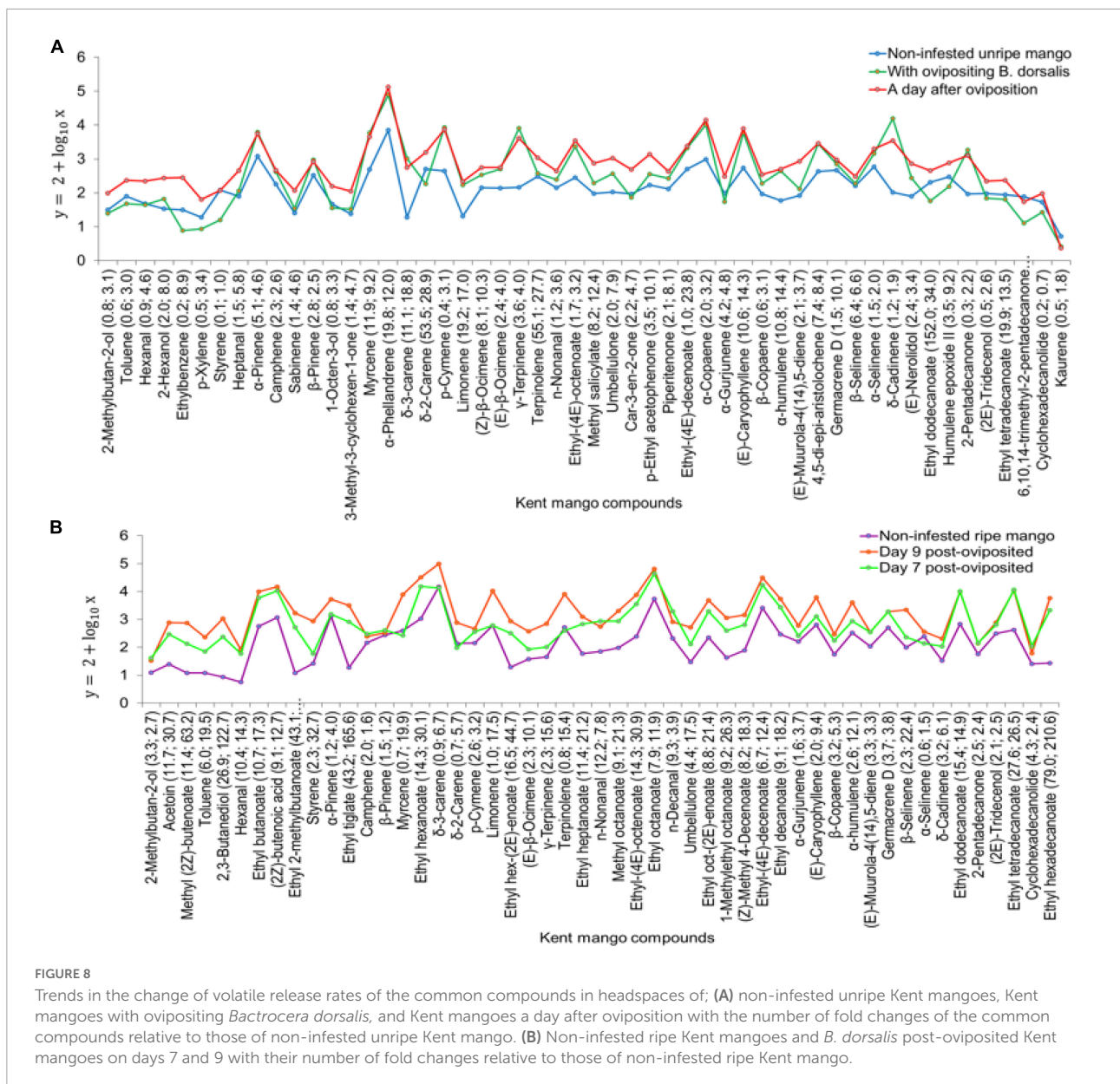
ethyl propanoate, methyl butanoate, ethyl 2-methyl propanoate, methyl tiglate, n-hexanol, methyl hexanoate,  $\alpha$ -fenchene, and methyl (2E)-octenoate.

For non-infested and *B. dorsalis*-infested Apple mangoes, the 30 top most discriminant volatiles as selected by the non-metric multidimensional scaling's (NMDS) similarity percentages, SIMPER are graphically presented in **Figure 9A**. Of these compounds, myrcene,  $\alpha$ -pinene,  $\beta$ -phellandrene,  $\beta$ -pinene, (Z)- $\beta$ -ocimene, (E)- $\beta$ -ocimene,  $\alpha$ -phellandrene,  $\alpha$ -bulnesene,  $\alpha$ -selinene, ethyl octanoate, ethyl butanoate, and (E)-caryophyllene contributed 80.81% of the total dissimilarity. The 30 volatiles were used to construct NMDS biplots (**Figure 9B**) and heatmap (**Figure 9C**).

The NMDS ( $k = 2$ , stress = 0.05027) one-way analysis of similarity (ANOSIM,  $R = 0.8669$ ,  $P = 0.0001$ ) indicates there is a significant difference among the mango treatments' volatile release rates (**Figure 9B** and **Supplementary Figure 5C**). Of the 30 discriminant compounds used, over 80% were associated with volatiles of mangoes on the day of oviposition (ABD1)

or day 9 post-oviposited Apple mangoes (ABD9) (**Figure 9B**). The heatmap (**Figure 9C**) shows the distribution of the selected discriminant volatiles among the Apple mango treatments with their release rates corresponding to the intensity of brown color, e.g., the dark brown color of myrcene (C56) and  $\alpha$ -pinene (C42) indicates that they had the highest release rates in most treatments (**Figure 9C**). The dendrograms also show the correlation of the volatiles within and between mango treatments.

There was a significant difference in the volatile release rates of non-infested unripe Apple mango (AUR), Apple mangoes on the day of oviposition (ABD1), and Apple mangoes a day after oviposition (ABD2) ( $\chi^2 = 44.5$ ,  $df = 2$ ,  $P < 0.001$ ). On pairwise comparison, there were significant differences between ABD1 and ABD2 ( $P < 0.001$ ), ABD1 and AUR ( $P < 0.001$ ), and ABD2 and AUR ( $P < 0.05$ ). There were changes in the quantities of common compounds in the volatiles on the day of oviposition (ABD1) and a day after oviposition (ABD2) relative to those of non-infested unripe



mangoes (Figure 10A). Furthermore, there was a significant difference in the volatile release rates of non-infested ripe Apple mango (AHR), Apple mangoes on day 7 (KBD7), and day 9 post-oviposition ( $\chi^2 = 103.77$ ,  $df = 2$ ,  $P < 0.001$ ). Pairwise comparison indicated significant differences between ABD7 and AHR ( $P < 0.001$ ), ABD9 and AHR ( $P < 0.001$ ), and ABD7 and ABD9 ( $P < 0.05$ ). Most of the common compounds in the volatiles of day 7 and day 9 post-oviposited showed an increase in the number of folds relative to those of non-infested ripe mangoes (Figure 10B). A total of 52 volatiles including acetoin, ethyl propanoate, methyl butanoate, isopentyl formate, 2,3-butanediol, ethyl butanoate, (2Z)-butenoic acid, and ethyl 2-methyl butanoate were detected in headspaces of infested mangoes but not in non-infested.

For non-infested and *B. dorsalis*-infested Haden mangoes, the 30 most discriminating volatiles of the headspaces as per NMDS's SIMPER are presented in Figure 11A. Out of these compounds,  $\delta$ -3-carene, ethyl octanoate, ethyl hexanoate, limonene, ethyl butanoate, terpinolene, myrcene, ethyl tetradecanoate,  $\alpha$ -pinene, and humulene epoxide II contributed 78.28% of the total dissimilarity. The 30 most discriminant volatiles were used in plotting the NMDS biplots (Figure 11B) and heatmap (Figure 11C) for visualization of their distributions in the treatment headspaces.

Like in Kent and Apple mangoes, the NMDS indicated a significant difference among the volatile release rates of the different treatments of Haden mangoes ( $k = 2$ , stress = 0.05105; one-way analysis of similarity, ANOSIM:



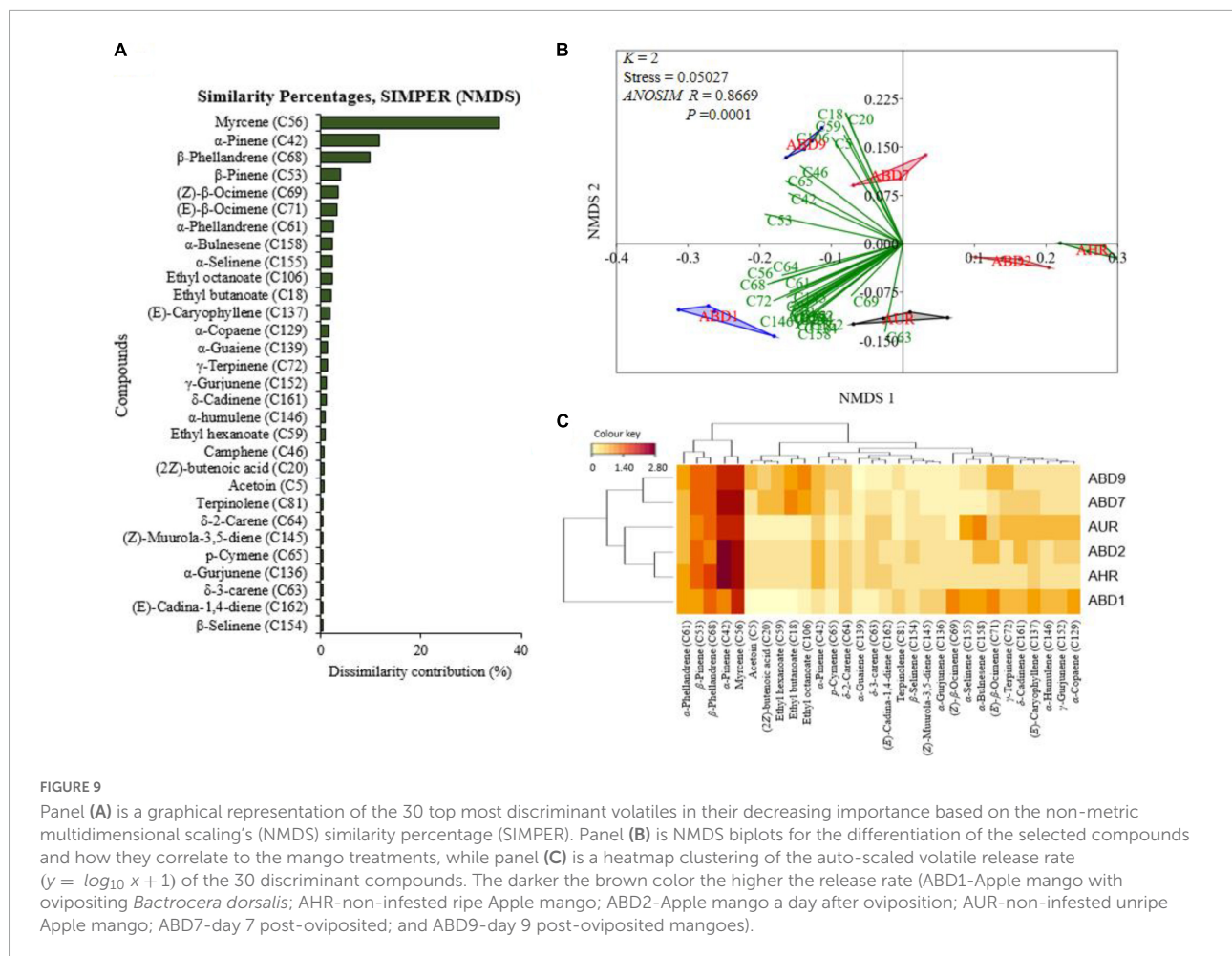


FIGURE 9

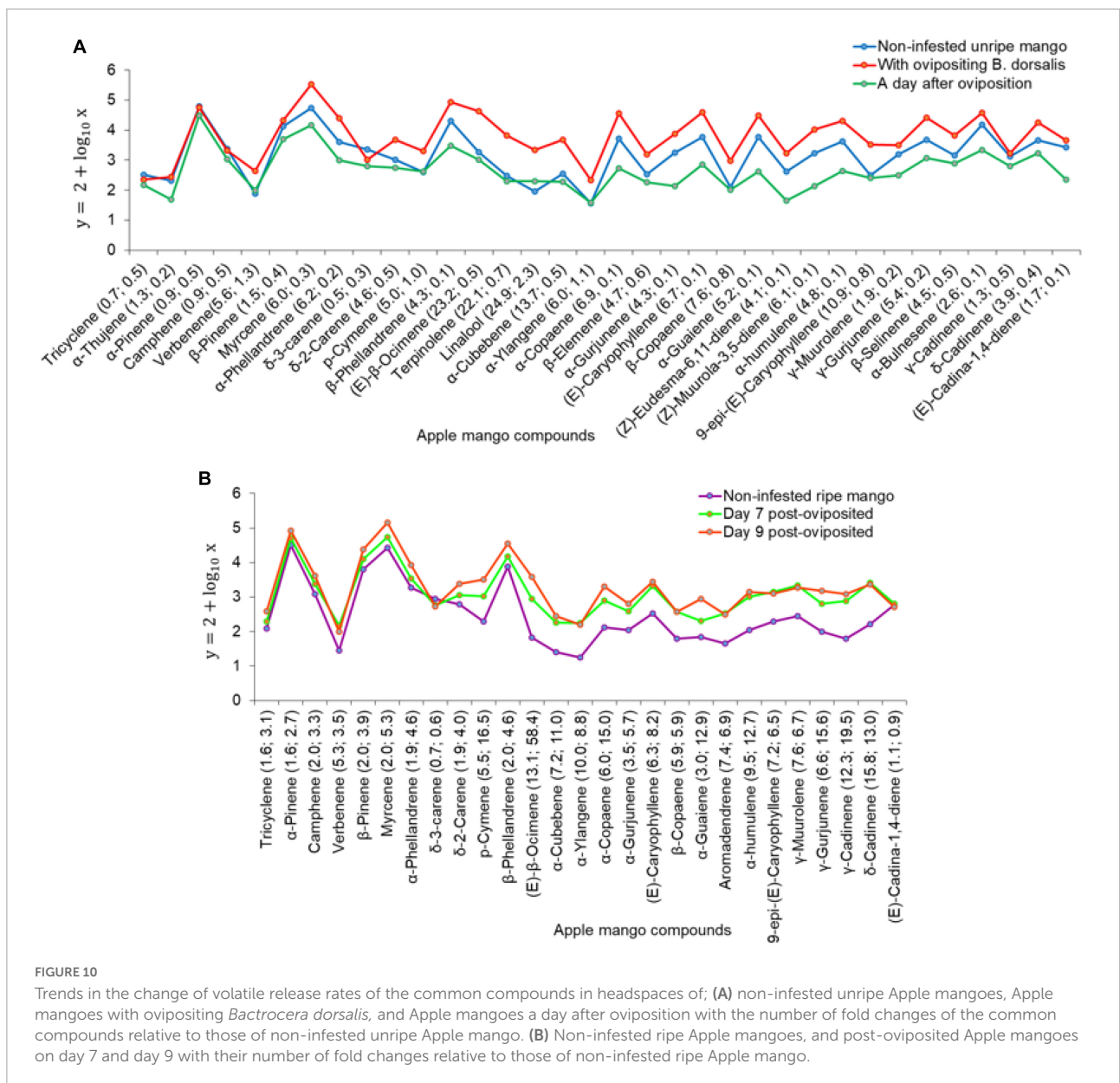
Panel (A) is a graphical representation of the 30 top most discriminant volatiles in their decreasing importance based on the non-metric multidimensional scaling's (NMDS) similarity percentage (SIMPER). Panel (B) is NMDS biplots for the differentiation of the selected compounds and how they correlate to the mango treatments, while panel (C) is a heatmap clustering of the auto-scaled volatile release rate ( $y = \log_{10} x + 1$ ) of the 30 discriminant compounds. The darker the brown color the higher the release rate (ABD1-Apple mango with ovipositing *Bactrocera dorsalis*; AHR-non-infested ripe Apple mango; ABD2-Apple mango a day after oviposition; AUR-non-infested unripe Apple mango; ABD7-day 7 post-oviposited; and ABD9-day 9 post-oviposited mangoes).

$R = 0.9391$ ,  $P = 0.0001$ ; **Figure 11B** and **Supplementary Figure 5D**). More than 75% of the selected discriminant volatiles were associated with volatiles of Haden mangoes with ovipositing *B. dorsalis* (HBD1), day 7 (HBD7), or day 9 (HBD9) post-oviposited Haden mango (**Figure 11B**). The heatmap clustering (**Figure 11C**) shows how the selected compounds were distributed in mango treatments while the dendrograms explain their correlation within and between treatments. The volatile whose release rate was high in Haden treatments was  $\delta$ -3-carene hence having an intense brown color (**Figure 11C**).

On comparing the volatile release rates of non-infested unripe Haden mango (HUR), Haden mangoes on the day of oviposition (HBD1), and Haden mangoes a day after oviposition (HBD2), there was a significant difference ( $\chi^2 = 13.07$ ,  $df = 2$ ,  $P < 0.01$ ). The pairwise comparison indicated a significant difference between HBD1 and HUR ( $P < 0.001$ ) whereas there were no differences between HBD1 and HBD2, and HBD2 and HUR ( $P > 0.05$ ). There were notable changes in the volatile release rates of common compounds of Haden mangoes with ovipositing *B. dorsalis* and Haden mangoes on

the second day after oviposition relative to those of non-infested unripe Haden mangoes (**Figure 12A**). A significant difference was also found among volatile release rates of non-infested ripe Haden mango (HHR), Haden mangoes on day 7 (HBD7), and day 9 (KBD9) post-oviposition ( $\chi^2 = 21.66$ ,  $df = 2$ ,  $P < 0.001$ ). The pairwise comparison revealed significant differences between HBD7 and HBD9 ( $P < 0.001$ ), HBD9 and HHR ( $P < 0.001$ ) while there was no significant difference between HBD7 and HHR ( $P > 0.05$ ). There were changes in the quantities of common compounds detected on day 7 and day 9 of Haden mango volatiles relative to those of non-infested mangoes (**Figure 12B**). Other than changes in the abundance of common compounds, 46 volatiles among them methyl butanoate, isopentyl formate, 2-methyl-1-butanol, 2,3-butanediol, (2Z)-butenoic acid, 3-methylbutyl ethanoate, methyl hexanoate,  $\alpha$ -fenchene, and 3-acetyl-2-octanone were detected only in *B. dorsalis* infested Haden mangoes.

In the first 10 most discriminant volatiles by the three multivariate analyses tools, (Z)- $\beta$ -ocimene and ethyl octanoate were selected as discriminant volatiles in the three mango varieties while  $\alpha$ -pinene, myrcene, ethyl hexanoate,  $\delta$ -2-carene,



(E)- $\beta$ -ocimene,  $\gamma$ -terpinene, humulene epoxide II,  $\delta$ -3-carene, limonene, and terpinolene were from two mango varieties.

## Discussion

### Behavioral assays of *Bactrocera dorsalis* and parasitoids to tree-attached non-infested and infested mangoes

A lot of emphases has been given to the investigation of volatiles of harvested fruits when trying to understand

the behavioral dynamics of insects to their hosts (Milonas et al., 2019; Cai et al., 2020; Silva and Clarke, 2021). In our study, the behaviors of *B. dorsalis* and its parasitoids were conducted on headspace volatiles of tree-attached mangoes using a dual-choice olfactometer. In all assays, both *B. dorsalis* and the parasitoids were attracted differentially to the tree-attached mango volatiles compared to the clean air (control). The behavioral responses were highly influenced by the mango variety, the physiological state of the mango fruits, and the infestation status. *Bactrocera dorsalis* was attracted toward volatiles of mangoes with ovipositing *B. dorsalis* females and to conspecific infested mangoes relative to control. Possibly, odors from ovipositing conspecific females and/or mangoes signify suitable hosts, and damage

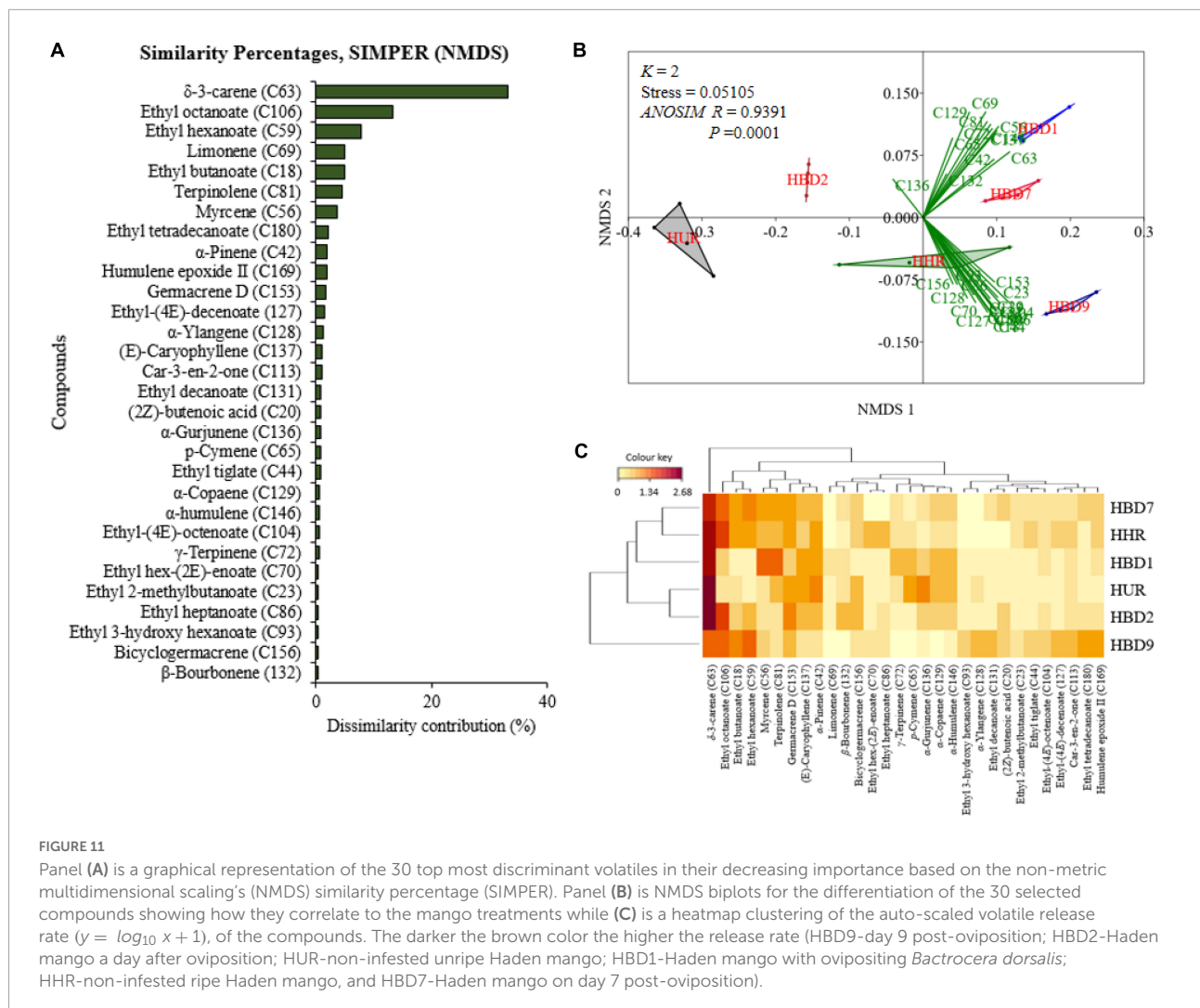
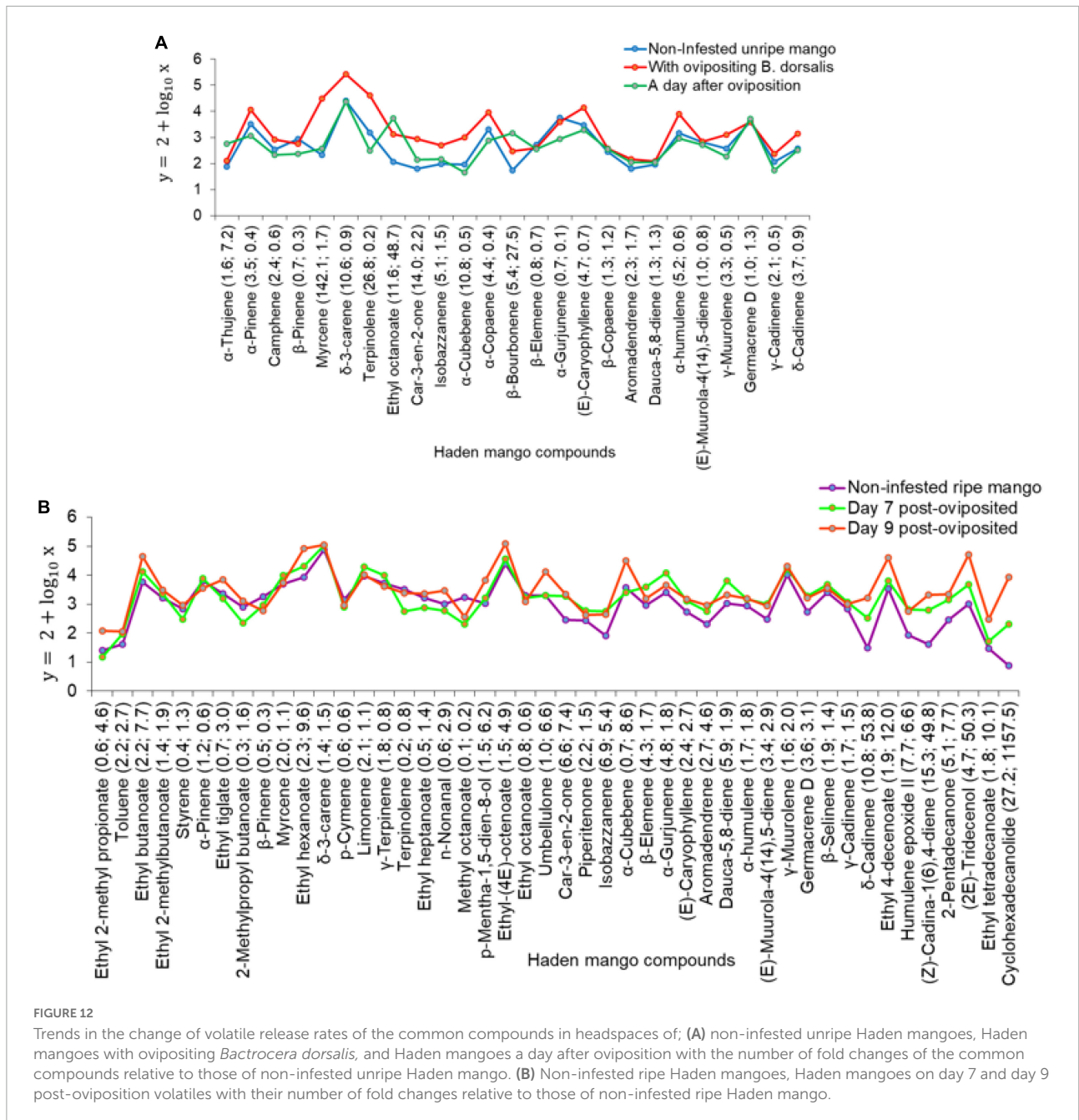


FIGURE 11

Panel (A) is a graphical representation of the 30 top most discriminant volatiles in their decreasing importance based on the non-metric multidimensional scaling's (NMDS) similarity percentage (SIMPER). Panel (B) is NMDS biplots for the differentiation of the 30 selected compounds showing how they correlate to the mango treatments while (C) is a heatmap clustering of the auto-scaled volatile release rate ( $y = \log_{10} x + 1$ ), of the compounds. The darker the brown color the higher the release rate (HBD9-day 9 post-oviposition; HBD2-Haden mango a day after oviposition; HUR-non-infested unripe Haden mango; HBD1-Haden mango with ovipositing *Bactrocera dorsalis*; HHR-non-infested ripe Haden mango, and HBD7-Haden mango on day 7 post-oviposition).

that eases oviposition into the mango, respectively, as argued by Nishida (2014) and Masry et al. (2018). Similar findings were reported for the congeneric *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) females which were found to be highly attracted to volatiles of conspecific-infested guavas compared to a blank (control) (Binyameen et al., 2021). Conversely, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) did not discriminate between conspecific-infested or non-infested fruits (Silva and Clarke, 2021). In this study, except for non-infested unripe Kent mango, female *B. dorsalis* were attracted to the other non-infested unripe and ripe mangoes volatiles compared to the control. Roh et al. (2021) observed that *B. dorsalis* females which were ready to oviposit were highly attracted to the host odor. The attraction of *B. dorsalis* to ripe and unripe mangoes partly disagrees with the findings of Grechi et al. (2021) on *B. zonata*, *Ceratitis capitata* (Wiedemann), and *C. quilicii* (Karsch) (all Diptera: Tephritidae) which were attracted by volatiles of ripe mangoes but not of unripe ones.

As expected, the egg parasitoid *F. arisanus* was attracted to mangoes with ovipositing *B. dorsalis* which implies that mangoes with ovipositing fruit flies were emitting volatiles that increased attraction. *Fopius arisanus* has been reported to exploit the chemical stimuli emitted by the fruits after fruit fly oviposition (Pérez et al., 2013) and those resulting from the presence of the host fruit fly female (Wang and Messing, 2003) as well as the presence of fertile eggs (Pérez et al., 2013; Cai et al., 2020). Furthermore, *F. arisanus* was reported to prefer parasitizing host eggs that are in tree-attached fruits (Eitam and Vargas, 2007). We also found that volatiles of non-infested ripe mangoes attracted *F. arisanus* implying that the ripe fruits produce volatile that stimulate attraction. Similar observations were made by Altuzar et al. (2004). This indicates that the olfactory tuning of *F. arisanus*, and likely many other parasitoids, utilize volatiles emitted by the host of their host, i.e., environmental cues that enhance finding suitable tephritid hosts (Nanga Nanga et al., 2019; Mama Sambo et al., 2020; Ayelo et al., 2021).



The attraction of *D. longicaudata* to volatiles of *B. dorsalis* infested Apple and Haden mangoes, at both day 7 and 9 post-oviposited mango volatiles and not to volatiles of *B. dorsalis*-infested Kent mango signifies that the fruits with developing larvae produce attractive volatiles compared to those that do not have. *Diachasmimorpha longicaudata* were attracted to volatiles of mango fruits which were infested with larvae of *Anastrepha ludens* (Loew) (Diptera: Tephritidae), but not to mechanically damaged mangoes, suggesting that the presence of maturing larvae is of paramount importance for the parasitoid to be attracted (Carrasco et al., 2005; García-Medel et al., 2007).

Furthermore, we found that *D. longicaudata* is more attracted to volatiles of infested mango at the older larval stage of *B. dorsalis* (day 9), which corroborates the finding by Harbi et al. (2019) on the parasitoid responses to volatiles of medfly, *C. capitata*-infested mango fruits tested at different infestation age, and fits with the ecological niche of the species as a larval parasitoid. Similarly, like *F. arisanus*, *D. longicaudata* was attracted to ripe non-infested fruit, indicating that this species' olfactory circuitry has also evolved a sensitivity to environmental cues, i.e., fruit volatiles, that increases the chances of encountering tephritid hosts (Altuzar et al., 2004; Rouse et al., 2005). It would



be interesting to find out how non-infested and infested tree-attached mango headspaces contribute to parasitoids locating their host larvae inside the fruit.

## Performance of *Bactrocera dorsalis* on the different mango varieties

The discrepancy in the numbers of puparia harvested from the three varieties of mangoes indicates that the fruit fly *B. dorsalis* differs in its performance in the mango varieties as oviposition substrates. This observation is partially in support of the preference-performance hypothesis (PPH) which states, “female insects will evolve to oviposit on hosts on which their offspring fare best” (Gripenberg et al., 2010; Akol et al., 2013). There were no *B. dorsalis* puparia that were recovered from Kent mangoes though fresh oviposition punctures were observed on the day of oviposition which implies that the Kent variety is more tolerant to *B. dorsalis* which is further confirmed by its non-preference to volatiles of several treatments of the mango variety. These results corroborate the findings of Akol et al. (2013) who reported minimal preference and offspring survival of *B. dorsalis* in Kent mangoes compared to other mangoes that included Apple mango. A similar observation was reported for the peach fruit fly, *B. zonata*, which showed differential attraction and survival in different guava varieties (Binyameen et al., 2021). It has been reported that factors like the variety of fruit (Diatta et al., 2013; Kamala et al., 2014), the stage of fruit maturity (Yashoda et al., 2007), the ease of the fruit fly ovipositor penetrating the pericarp (Balagawi et al., 2005), and the chemical composition of the fruit and its ability to sustain the full development of the fruit fly (Boinahadji et al., 2020) affect the performance and survival of insect offspring. Apple mango constituted a better environment (223 puparia) for the fruit fly larvae development. Okoth et al. (2013) reported differences in pH, total titrable acid, and moisture content among other qualities between Kent and Apple mangoes which may play roles in the preference and performance of the *B. dorsalis* offspring in the latter variety. A further study on the chemical factors that are associated with the differential performance of *B. dorsalis* in Kent and apple mango would help in filling the knowledge gap of how the fly assesses the suitability of its hosts.

## Headspace volatiles from all treatments of the three varieties of mangoes

In this study,  $\delta$ -3-carene, myrcene,  $\alpha$ -pinene,  $\beta$ -pinene,  $\alpha$ -gurjunene, (*E*)-caryophyllene,  $\beta$ -copaene,  $\alpha$ -humulene, and  $\delta$ -cadinene, among other volatiles, were differentially released by the three mango varieties which were highly dependent on the status of the mango, i.e., unripe, ripe, non-infested, or infested.

Some of the volatiles have been reported in earlier findings but not from tree-attached mangoes (Wetungu et al., 2018; Maldonado-Celis et al., 2019; Shimizu et al., 2021) and have been associated with the attraction of various insect pests (Benelli et al., 2014; Biasazin et al., 2014; Biasazin et al., 2019) and their natural enemies (Kamala et al., 2012; Segura et al., 2012; Harbi et al., 2019; Cai et al., 2020).

The stress values from all the two-dimensional NMDS plots indicated a good match between ordination fit and real data of the volatile release rates signifying a good fit of solution (Clarke, 1993). The qualitative and quantitative differences in headspace volatiles among the three mango varieties as revealed by the non-metric multidimensional scaling (NMDS) could be a result of differences in the genetic make-up (Lebrun et al., 2008). The qualitative and quantitative variability in headspace volatiles reported in this study corroborates with findings by other authors (e.g., El Hadi et al., 2013; Wetungu et al., 2018). The compounds selected by the multivariate tools were spread out in all categories of VOCs including the most abundant, common, those with significant quantitative changes, and most importantly the compounds emanating from the treatments that could have contributed to the behavioral responses of *B. dorsalis* and the two parasitoids.

Non-infested ripe mangoes produced more volatiles, the majority of which were esters, than non-infested unripe mangoes. These results are in agreement with other results from ripe and unripe mango fruits (Pandit et al., 2009; White et al., 2016). The number and release rates of monoterpenes and sesquiterpenes identified from the ripe mango of the three varieties were generally less compared to those of unripe mango. Monoterpenes are associated with the defense mechanisms of plants against herbivorous attack (Singh and Sharma, 2015; Olayemi, 2017), hence their decrease may explain the higher attraction of *B. dorsalis* to non-infested ripe fruits which appear more suitable for the survival of offspring. A study on the guava attractiveness of the Queensland fruit fly, *B. tryoni* showed that ripe guavas emitted volatiles that were more attractive than unripe ones (Cunningham et al., 2016). Although there was a minimum change in the number of compounds that were produced on the day of infestation on the three mango varieties, the volatile release rates of most volatiles increased significantly compared to those of non-infested mangoes. An increase in the volatile release rate, especially of terpenes, after an attack by herbivorous insects on any part of a plant, has been associated with defense against the herbivorous pest, and in some cases attraction of the pest's natural enemies (parasitoids and predators) (War et al., 2011; Olayemi, 2017), but from our study, the increase in the release rate of volatiles lead to increased attraction of conspecific pests and the egg parasitoid *F. arisanus*. Similar observations for *F. arisanus* were made by Cai et al. (2020). There was an increase in the number of compounds and the volatile total emission on days 7 and 9 of post-oviposited mangoes

relative to those of ripe mangoes. Common knowledge is that fruit ripens in preparation for seed dispersal but the difference in the number of compounds and their release rates of infested mangoes and non-infested ripe mangoes could be attributed to the activities of the mango trying to counter the attacks (Lackus et al., 2018; Sharifi et al., 2018), the activities of the fruit fly larvae in the mangoes, and/or introduction and activity of microbial in the mango (Futagbi et al., 2017). Herbivorous activities may result in the increase or decrease in quantities of compounds produced, formation of new compounds, or disappearance of some compounds as observed from different plant studies (War et al., 2011; Martins et al., 2017; Shivaramu et al., 2017). For example, on day 9 post-oviposited Apple mango headspace, an increase occurred in most common compounds while decreases were only slight for a few compounds. These changes could be responsible for the decrease in the attractiveness of *B. dorsalis* to the day 9 post-oviposited Apple mango and the generally increased attractiveness of the larva parasitoid *D. longicaudata* to the day 9 post-oviposited mangoes. Carrasco et al. (2005) reported that infestation of 'Criollo' (*M. indica*) with *Anastrepha ludens* (Loew) larvae changed the headspace composition and increased the attractiveness of the fruit for *D. longicaudata*. Similar results were reported by Segura et al. (2012), indicating that *D. longicaudata* is attracted to *Ceratitidis capitata* (Wiedemann) (Diptera: Tephritidae) infested and non-infested oranges.

## Conclusion and further research

The responses of the fruit fly *B. dorsalis*, the egg parasitoid *F. arisanus*, and the larval parasitoid *D. longicaudata* are highly influenced by the mango variety, the physiological, and the infestation status of the mango. This is evident from the behavioral response experiments and the number of puparia harvested from each variety of mangoes. The results indicate that Kent mango is highly tolerant to *B. dorsalis* hence deterring the fruit fly development while Apple is highly susceptible. The volatile organic compounds in the headspace of non-infested and *B. dorsalis*-infested mangoes are qualitatively and quantitatively different within and between treatments. This study thus describes the systematic changes which occur in the headspace volatiles of tree-attached mangoes before, during, and after infestation by *B. dorsalis*, and how this correlates with differential responses of the fruit fly *B. dorsalis* and its parasitoids, *F. arisanus* and *D. longicaudata*. Laboratory experiments have shown that parasitoids can distinguish between infested and non-infested harvested fruits, we, therefore, recommend further studies to assess whether the fruit fly and its parasitoids can distinguish between the headspaces of different treatments of infested and non-infested tree-attached mangoes. In addition,

the studies should also determine whether the olfactory convergence of the insects is based on the detection of the same fruit volatile compounds. This is interesting from an evolutionary ecological perspective, but also of significance when developing baits that selectively attract the fly and not its natural enemies.

## Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

## Ethics statement

This research was licensed to be conducted in Kirinyaga County, Kenya, by the National Commission for Science, Technology, and Innovation (NACOSTI) under License No.: NACOSTI/P/20/6447. Consent was also sought from the farm owner to use his plantation of mangoes for the study purpose while other local farmers fully participated in the trials.

## Author contributions

RM, TD, and SM conceptualized the research idea. RM collected and analyzed the data and drafted the preliminary manuscript. SM, XC, and SN supervised the study. All authors participated in providing intellectual inputs, proofreading, and approving the submission of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.1021795/full#supplementary-material>

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