The semiochemistry of fruits as seen through the lens of Tephritid fruit flies and their parasitoids

By

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The semiochemistry of fruits as seen through the lens of Tephritid fruit flies and their parasitoids

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Declaration

I declare that the dissertation/thesis, which I hereby submit for the degree of Doctor of Philosophy in Chemistry at the University of Pretoria, is my work and has not been previously submitted by me for a degree at this university or any other tertiary institution.

Ethics Statement

I, the author, whose name appears on the title page of this thesis, did obtain, for the research work herein described, the applicable research ethics approval (Appendix 3).

I also declare that I observed the required ethical standards in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research work.

Raphael Njurai Miano

February 2024

Dedication

I dedicate this work to my late father, Mzee Miano Njogu, who inspired me throughout my entire school life, my mother, Eunice Njoki and my siblings, your prayers and affection counted in every step. To my Late wife Susan Njeri Mung’ere, rest in peace. To my children Gift, Vincent, Cleopas and Crispas, through this thesis, I hope one day you will understand, forgive and embrace me for having been absent the time you needed me most. Your patience, love, prayers and the moral support you accorded me throughout my study period are highly appreciated. Many thanks to my sister-in-law Phyllis, your beloved husband Peter, family and siblings for taking care of my son from the day of birth, it was not an easy task but required brave warriors. Much appreciation to my sister Esther, your beloved husband Ken and your family for accommodating and taking care of my other sons. And to my Friend Jane Maina, for your support, love and care. You consoled and supported me in my time of need. May the Good Almighty God who sees good deeds in secret reward you All in secret. To Prof. Ahmed Hassanali, I honor you.

Thesis Summary

The tephritid fruit fly is a term well-known in fruit and vegetable production. Several techniques including the use of parasitoids have been deployed for fruit fly control. In Sub-Saharan Africa, Fopius arisanus (Sonan) and Diachasmimorpha longicaudata (Ashmead) (Hymenoptera: Braconidae) have been introduced to supplement the existing native parasitoids. Although the effectiveness of parasitoids is known, there is a knowledge gap in the semiochemical-mediated interactions among tree-attached fruits, fruit flies, and parasitoids. Here, I aimed to compare the attraction of fruit flies and parasitoids to different fruits, evaluate fruit fly performances, in terms of recovered puparia, in these fruits and elucidate the fruits' headspace volatile compounds.

First, the attraction of Bactrocera dorsalis (Hendel), F. arisanus and D. longicaudata to the headspace volatiles of different treatments of three varieties of mangoes were compared. B. dorsalis and the two parasitoids were differentially attracted to the mango headspace volatiles compared to the control, clean air. A higher number of B. dorsalis puparia was recovered in the Apple mango variety (81.7%) but none from the Kent variety. Gas chromatography-mass spectrometry revealed several organic compounds with qualitative and quantitative differences. The majority of tentatively identified compounds were esters (33.8%). Most compounds were produced at higher concentrations by fruit fly-infested mangoes than non-infested ones.

A similar approach to B. dorsalis' was followed on Ceratitis cosyra, the two parasitoids and its native Psyttalia cosyrae (Wilkinson). C. cosyra and the parasitoids differentially responded to the treatments of the three mango varieties. Ceratitis cosyra performed better in Kent mango (72.1% of the 287 puparia recovered) compared to Apple and Haden varieties. Esters were the main components of the non-infested ripe and the late post-oviposition larval stages of the three mango varieties. At the same time, monoterpenes and sesquiterpenes were dominant in the other mango treatments.

The performances of B. dorsalis, C. cosyra, Zeugodacus cucurbitae and B. latifrons on different species of fruits (mango cv. Haden, banana cv. Fhia-17, and tomato cv. Improved Nouvelle F1) were investigated and the headspace volatiles of different treatments of the three mango varieties, ripe bananas and tomatoes analyzed using GC-MS and GC-electroantennographic detection (EAD). The fruit fly species performed differentially in the different fruits. There were overlapping detections of most EAD-active compounds across the four fruit fly species and parasitoids with esters being the most prevalent class of compounds.

This study represents the first report of the interactions of different fruit fly species and their parasitoids to in situ headspace volatiles of different treatments of mangoes and the subsequent changes in the headspace components of these mango treatments. Results obtained not only provide a better understanding and add new knowledge to science on the dynamic interactions of the selected tephritid fruit fly species and their parasitoids to a variety of hosts with different physiological states but also show a convergence of fruit fly and parasitoid antennal-active compounds hence presenting an informed foundation for future reference in developing sound Integrated Pest Management (IPM) strategies for managing fruit flies without harming parasitoids.

Preface

When I was growing up in the late 1970s and 80s, getting a balanced diet was not a matter of putting food on a plate but it was a matter of getting out into the forest and gathering different types of fruits and other edibles. A balanced diet was naturally guaranteed to every child. In the late 80s and early 90s, the wild sources of foods started shrinking as a result of population increase, agitation for expansion of agricultural land, increased demand for agricultural products, shelter, and urbanization.

Other than the factors mentioned, in the past few years, there have been negative changes in climatic conditions and the introduction and spread of both native and exotic pests in most parts of Africa. The pests have caused great damage to both wild and agricultural fruits and vegetables. As a result, farmers have resulted to adopting to different intervention measures ranging from the abandonment of agriculture as a source of livelihood, permanent removal of some crops from the farms and introduction of foreign crops, and the uncontrolled application of pesticides among others. These intervention measures have not only affected the producers and the consumers but some have negatively interfered with the human dietary needs, the ecosystem and the general environment. The search for environmentally friendly control measures is inevitable. To complement the existing advocated Integrated Pest Management (IPM) strategies, scientists have to go a notch higher to identify those compounds that attract destructive insects to their host to get eco-friendly fruit fly attractants or repellants.

As a farmer, a teacher, and a chemist, I believe that what is exotic is not part of the local ecosystem and if it is expensive, then concerted efforts must be put in place to eradicate it. Our subject matter, the fruit fly, is a concern to all. The search for green solutions to supplement the IPM packages must continue. The use of semiochemicals has been tested and has proved to be a real-life solution. Although sometimes expensive, we must map, mine and test semiochemicals to manage and if possible eradicate agricultural pests. This project was born from *icipe* fruit fly IPM packages of Africa, aiming at strengthening the packages while at the same time protecting the fruit fly natural enemies. Chemistry in collaboration with other study fields is the option in this endeavor.

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Publications and Presentations

The thesis is based on the following manuscripts:

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Abbreviations

AFFP- Africa Fruit Fly Program

ANOSIM-Analysis of similarities

ANOVA-Analysis of variance

asl-above sea level

BVOCs-Biogenic volatile organic compounds

DCM-Dichloromethane

DDT- Dichlorodiphenyltrichloroethane

DHT-Dynamic headspace trapping

DPO-Day post-oviposition

EAD-Electroantennographic detector

EI-Electron impact

FID-Flame ionization detector

GC-Gas chromatography

HIPVs-Herbivore-induced plant volatiles

HMP-Host marking pheromone

HS–SPME-Headspace-solid-phase microextraction

icipe-The International Centre of Insect Physiology and Ecology

ID-Internal diameter

IPPC- International Plant Protection Convention

L: D-Light, dark

MAT- Male Annihilation Technique

MeSA-Methyl salicylate

MS-Mass spectrometry

NIST-National Institute of Standards and Technology

NMDS-Non-metric multidimensional scaling

P.T.F.E.- Polytetrafluoroethylene

PDMS-DVB-Polydimethylsiloxane-divinylbenzene

RH-Relative humidity

$RI_{(Cal)}$-Calculated retention index

$RI_{(Lit)}$-Literature retention indices

RT-Retention time

SIMPER-Similarity percentage

SIT- Sterile Insect Technique

VHT- Vapor Heat Treatment

VOCs-Volatile organic compounds

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Chapter 1: Introduction

Fruits and vegetables are vital components of the global agricultural production and supply chain (Ravichandra, 2014; Niassy *et al.*, 2022). The increase in human population, decrease in agricultural land, change in climatic conditions and threats by increased invasive pests have negatively affected the global agricultural supply chain. In the year 2020, the total primary food production among 199 producing countries was estimated to be 9.3 billion tons (FAO, 2022). Amongst the continents that supply the global market with agricultural products (i.e. Central America and the Caribbean, Asia, South America, and Africa), Africa contributed the least to fruit and vegetable production (FAO, 2022). Fruits and vegetables are essential diet foods due to their high nutritional contents (Lebaka *et al.*, 2021; Pogonici & Butnariu, 2022) and are major contributors to the economies of producing countries for both domestic and export markets (Macharia *et al.*, 2019; Bekele *et al.*, 2020).

Though agriculture contributes a lot to many economies, its sustainability is being threatened by invasive pests, especially in the subtropical, tropical, and temperate regions of the world. Between the years 2000 and 2020, there has been a global outcry about reduced farm harvests which is compounded by climate change and the globalization of invasive pests such as the South American tomato pinworm, *Phthorimaea absoluta* (formerly *Tuta absoluta* (Meyrick)) (Lepidoptera: Gelechiidae) (Zhang *et al.*, 2021), fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Kassie *et al.*, 2020) and tephritid fruit flies (Reddy *et al.*, 2018; Sultana *et al.*, 2020).

In Africa, the introduction and establishment of exotic fruit flies to an already established native fruit fly population has led to a decline in fruit and vegetable harvests thus affecting the supply chain and the economy in general (Muriithi *et al.*, 2020). The pests have also led to the misuse and overuse of pesticides which are expensive to the farmers and counterproductive to the health of the workers who apply pesticides and the consumers without mentioning the general risks associated with the environment and the ecosystem (Kodandaram *et al.*, 2010; Bon *et al.*, 2014).

Since fruits and vegetables are important components of human nutrition and have high economic value all over the world in particular in Africa, the Africa Fruit Fly Program (AFFP) was established to address the needs of farmers in monitoring and controlling the fruit flies before and after crop harvest. At the International Centre of Insect Physiology and Ecology (*icipe*, Nairobi Kenya), a fruit fly Insect Pest Management (IPM) program package has been developed and rolled out in many countries in Central, Eastern, Western, and Southern Africa (Muriithi *et al.*, 2016; Niassy *et al.*, 2022). One of the components of the fruit fly IPM is the use of parasitoids. To advance this technique, generalized exotic parasitoids, *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) (both Hymenoptera: Braconidae) of Asia origin were imported from Hawaii to *icipe* Nairobi, Kenya where a colony for mass rearing was established (Mohamed *et al.*, 2008; Ekesi *et al.*, 2016) and has been used for local and region releases to supplement the existing native parasitoids.

The native and exotic parasitoids have been shown to coexist and perform in different fruit fly species (Mohamed *et al.*, 2008; Daane *et al.*, 2015; Ndiaye *et al.*, 2015). Mostly, studies involving fruit flies and parasitoid responses, performance, and collection of volatiles are carried out under laboratory set-ups. Findings from these set-ups are vital but the studies that are undertaken under field settings are expected to give a true reflection of what happens in nature, in terms of fruit-fruit fly-parasitoid interaction.

In this thesis, the *in situ* attraction of two fruit fly species (*B. dorsalis* and *C. cosyra*) and three parasitoid species (two exotic, *F. arisanus* and *D. longicaudata* and one native *P. cosyrae*) to the headspace volatiles of different ripening and infestation stages of three mango (Kent, Apple and Haden) varieties and the fruit fly subsequent performance in terms of the number of puparia recovered were investigated. Further, the changes in the volatile chemical composition of headspaces of *in situ* non-infested and infested mangoes of the three varieties, that could have triggered the fruit fly and parasitoid responses, were assessed.

In addition, the performance of *B. dorsalis*, *C. cosyra*, *Z. cucurbitae* and *B. latifrons* in freshly harvested Haden mango variety, ripe banana (Fhia-17 variety) and tomato (Improved Nouvelle F1) under laboratory conditions were assessed. This was followed by mapping out the EAD-active compounds of *B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, *B. latifrons* and the three parasitoid species from the headspaces volatiles of the three mango variety treatments, ripe banana (Fhia-17 variety), and tomato (Improved Nouvelle F1). Other than assessing the performance of different fruit fly species in different fruits, this study was also aimed at revealing whether there were similar responses of fruit fly and parasitoid species to different fruits and whether the antennae of these insects respond to the same compounds. The results obtained are significant as they will open up more research that will provide informed decisions when developing baits that selectively attract the fruit fly and not its natural enemies and also fill the knowledge gap from an evolutionary ecological perspective.

The work in this thesis was performed at three different locations. The behavioral experiments of fruit fly and parasitoid species to different treatments of the three mango varieties and collection of headspace volatiles from mangoes, bananas and tomatoes were carried out at Mwea East Sub County, Kirinyaga County, Kenya. The first analysis of the headspace volatiles of the different treatments of the three mango varieties using GC-MS was performed at *icipe* Nairobi, Kenya while the second analysis, GC-flame ionization detector (GC-FID) and GC-EAD were performed at the Swedish University of Agriculture (SLU, Alnarp, Sweden; Department of Plant Protection Biology).

1.1 Tephritid fruit flies and their distribution

The family Tephritidae has over 4000 documented species out of which more than 35% are pests of fruits and vegetables (White & Elson-Harris, 1992, Qin *et al.*, 2015). Tephritid fruit flies are spread across the tropical, subtropical and temperate regions of the world with each region having its native species (Dyck *et al.*, 2005; Heather & Hallman, 2008).

In Africa, the native fruit flies include *C. anonae* (Graham), *C. capitata* (Wiedemann), *C. cosyra* (Walker), *C. fasciventris* (Bezzi), *C. pedestris* (Bezzi), *C. punctata* (Wiedemann), *C. quinaria* (Bezzi), *C. rosa* (Karsch), *C. rubivora* (Coquillett), amongst others from the genera *Ceratitis* and *Dacus bivittatus* (Bogot), *D. ciliatus* (Loew), *D. frontalis* (Becker), *D. lounsburyii* (Coquillett), *D. punctifrons* (Wiedeman), *D. vertebratus* (Bezzi) amidst others from the genera *Dacus* (Steck, 2000; Copeland *et al.*, 2006; Mohamed *et al.*, 2016). These fruit flies can cause up to 100% loss of fruit and vegetables especially where there are no control interventions (Nankinga *et al.*, 2014; Ekesi *et al.*, 2016).

Over the past few years, exotic fruit flies have invaded Africa and established themselves. These fruit flies include *B. dorsalis*, *Bactrocera latifrons* (Hendel), *B. zonata* (Saunders), and *Zeugodacus curcubitae* (Ekesi & Billah, 2006; Carrillo *et al.*, 2017; Monsia *et al.*, 2019). The introduction of exotic fruit flies has negatively affected fruit and vegetable production in Africa where reports have indicated losses of up to 100% especially where control measures are not available (Nankinga *et al.*, 2014).

1.2 The economic impact of tephritid fruit fly pests and their life cycle

Fruit flies cause up to 100% loss of the expected harvest of fruits and vegetables (Nankinga *et al.*, 2014). These pests are highly adaptive and have short reproductive life cycles (Mze Hassani *et al.*, 2016). The concealed nature of the destructive larval stage has led to overuse and misuse of insecticides which are in most cases expensive and have unintended effects on the people who apply them and agricultural product consumers, the legally tolerated maximum residue levels of pesticide in/on food, and the general ecosystem. The introduction of the larvae gives way to the entry and establishment of bacteria and fungi leading to rot and further degradation which causes contamination-related problems to the consumers (Sarwar, 2015 and references therein). Furthermore, these pests have exacerbated the problems faced by farmers due to phytosanitary trade barriers that have been imposed by major fruit and vegetable importers (Heather & Hallman, 2008; IPPC, 2019).

Tephritid fruit flies have also led to reduced harvests thus affecting the nutrition requirements, the supply chain, and the general economy of the producing countries (Heather and Gay, 2008). With most agricultural parts of Africa providing a conducive environment for the introduction, establishment, and spread of exotic fruit fly species, lack of appropriate cross-border phytosanitary regulations and their enforcement and a good climate have offered refuge to introduced species (Dohino *et al.*, 2016; Musasa *et al.*, 2019).

Generally, different tephritid fruit fly species have similar life cycles. After mating, gravid females pierce through the skin of a fruit using their long sharp ovipositors to a depth of about 2-5mm and lay eggs in batches. Within 1-2 days, the eggs hatch producing larvae that penetrate inside the fruit as it feeds and develops through three instars. In the process the fruit rots and drops to the ground. The third instar larvae fall off the fruit to the soil for pupation followed by the emergence of an adult fruit fly from the puparium (Figure 1-1) (Reddy *et al.*, 2018).

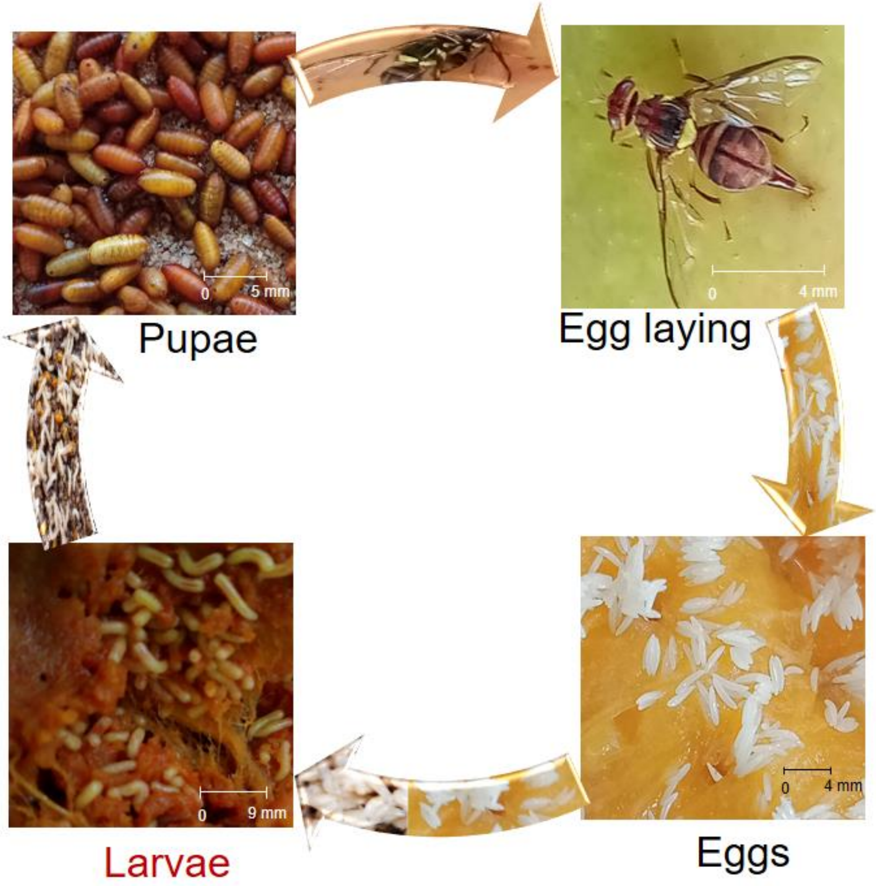

Figure 1-1**:** The life cycle of a fruit fly showing the developmental stages from eggs through to adult which is highly dependent on fruit fly species, temperature, humidity, and nutrition among others

The duration of the life cycle, from egg laying to the emergence of the adult fruit fly, depends on several factors which include the fruit fly species, temperature, humidity, and the host plant fruit/vegetable amongst others (Vargas *et al.*, 2007; Kalia & Yadav, 2015). At a temperature range of 27±1° C, the full life cycle may take 18-26 days and hence they can have up to 10 generations of offspring per year (Shehata *et al.*, 2008; Kalia & Yadav, 2015).

With the prevailing changes in climatic conditions, it is predicted that there will be a widespread fruit fly establishment with multiple pest species distributions (IPPC, 2019; Sultana *et al.,* 2020), where the areas that were considered to be less susceptible will become high-risk areas (Stephens, 2007; Villiers *et al.*, 2016). To manage these pests and their general effects on agriculture, combinations of several strategies have been advocated on an area-wide basis (Suckling *et al.*, 2016).

1.3 Control and management of fruit flies

1.3.1 Cultural methods

These methods include;

Pruning-This is the general removal of unwanted branches from a growing plant thus making harvesting and implementation of fruit fly control methods easier (Bota *et al.*, 2018).

Exclusion-This is where physical barriers such as sleeves, bags and nets are used to prevent and stop gravid female adult fruit flies from reaching and ovipositing on fruits (Heather & Hallman, 2008).

Orchard sanitation-This method involves the collection of all fallen and unwanted fruits from the farm and destroying them either by burying them in the soil or putting them in an augumentorium. Burying infested fruits prevents fruit fly eggs and larvae from developing into adults while the use of augumentorium (which has small holes) prevents adult flies from escaping and multiplying. Orchard sanitation also denies gravid females suitable hosts for egg laying (Adebayo *et al.*, 2021).

Early harvesting-This is harvesting fruits and vegetables before they start attracting gravid fruit fly females. The method denies gravid females the opportunity to lay eggs and is only applicable to fruits and vegetables that are infested after maturing (Grechi *et al.*, 2021).

Host plant removal and crop rotation-This method works well with vegetable and fruit plants that are not perennial such as cucumbers, and tomatoes among others (Lux *et al.*, 2003).

Solarization of soil or infested fruits-This is a process of using solar energy to kill all stages of fruit flies by either exposing bare soil to the sun or putting infested fruits in black plastic bags and exposing the plastic bags to the sun (Lux *et al.*, 2003).

Post-harvest methods include hot water or air treatment, vapor heat treatment (VHT), cold treatment and irradiation which are used for the disinfection of fruits for fruit flies and other pests (Mwando *et al.*, 2021and references in).

1.3.2 Chemical methods

This is the application of an agrochemical to the entire vegetative part of the plant. Since the eggs and the larvae are inside the fruit, systemic insecticides are largely preferred (Kodandaram *et al.*, 2010). Contact and ingestion insecticides such as fenthion, bifenthrin, deltamethrin, mercarptothion, dimethoate (Rogor), lambda-cyhalothrin, clothianidin, trichlorfon, and chlorpyrifos (N'Dépo *et al.*, 2010; Oke and Sinon, 2013) provide quick and responsive control of fruit flies hence farmers have high confidence in their application (Carrillo *et al.*, 2017). However, the use of chemicals has a range of drawbacks. For example, chemicals applied on the surface of fruits and vegetables do not affect the pupating larvae and the pupae in the soil (Heve *et al.*, 2016; Cai *et al.*, 2017). Fruit flies have also shown resistance to some chemicals, for example, the broad-spectrum insecticide Spinosad (Hsu and Feng 2006; Biondi *et al.*, 2012a, Hsu *et al.*, 2012). Furthermore, the wide application of Spinosad in sprays and baited traps is reported to affect fruit fly parasitoids (Biondi *et al.*, 2012b; Biondi *et al.*, 2013) thus disrupting IPM programs. Resistance to chemical control has been attributed to the mutation of the insects, specificity and persistence of insecticides, frequency of applications and the type of insecticides (Talebi *et al.*, 2008).

The high dependence on chemicals to control fruit flies has negatively affected human health, environmental sustainability and the general balance of nature (Campos-Herrera, 2015) and the fruit flies are yet to be controlled (Sarango, 2014; Akotsen-Mensah *et al.*, 2017). This has demanded the removal of some of the most effective systemic-acting and broad-spectrum insecticides like dichlorodiphenyltrichloroethane (DDT) from the market (Turusov *et al.*, 2002; Böckmann *et al.*, 2014). Spot spraying, which uses the 'attract-and-kill' strategy, has been advocated, where attractants of an insect are mixed in an insecticide and sprayed on parts of the foliage (Vayssieres *et al.*, 2009).

Although with no success story on the part of fruit fly control, the search and development of new green chemicals have led to the introduction of biochemicals that are sustainable, environmentally friendly and non-toxic to the end user (Kodandaram *et al.*, 2010). For instance, *Peganum harmala*, a herb originating from the Central Asian desert, extracts have been used in the control of fungi and insect pests (Rehman *et al.*, 2009). Similarly, neem derivatives have been recommended for the control of a majority of insects including tephritid pests (Masood *et al.*, 2009; Elanchezhyana & Vinothkumar 2015).

1.3.3 Lure and kill traps

These are traps that contain strong attractants (like methyl eugenol, terpenyl acetate, isoeugenol, zingerone, yeast, hydrolysed proteins, and fermenting sugars to mention a few) that lure adult fruit flies mixed in an insecticide (like Spinosad or Malathion) (Manuel & Sarango, 2009; Doorenweerd *et al.*, 2018). Methyl eugenol, used in the Male Annihilation Technique (MAT), is specifically used in the suppression of *Bactrocera* species (Haq *et al.*, 2018). Male lure and kill traps suppress the fruit fly population by killing the males thus decreasing mating incidences (Aluja *et al.*, 2014; Stringer *et al.*, 2019). Protein-baited lure and kill traps attract and eliminate both male and female fruit flies (Allwood *et al.*, 2001) though they also attract non-targeted insects. Fruit fly lure and kill traps are used in monitoring and mass trapping whose effectiveness depends on the fruit fly species and the availability and cost of the lure and kill traps (Villalobos *et al.*, 2017).

1.3.4 Biological methods

Biological control (biocontrol) is the reduction of unwanted diseases, pests, or weeds using their natural enemy/enemies such as pathogens (fungi, viruses and bacteria), predators, and parasitoids. Predators such as carabid beetles, spiders, staphylinid beetles, ants, and assassin bugs control fruit flies by feeding on maggots and adults caught in webs while insects such as robber flies and dragonflies feed on flying adults (Mills & Daane, 2005; Hoelmer *et al.*, 2011). Other predators include birds such as Restless flycatchers (*Myiagra inquieta*), Swallows (Pseudochelidoninae and Hirundininae), Willy Wagtails (*Rhipidura leucophrys*) and poultry that feed on exposed larvae, puparia and adult flies (Kumral *et al.*, 2010; Sarwar, 2015). The most advocated biocontrol method for fruit flies is the use of native and exotic parasitoids.

The use of exotic parasitoids in the classical biocontrol of fruit flies started early in the 19th century (Fullaway, 1920) in Hawaii (Deguine *et al.*, 2015). Since then different parasitoid-rearing centers have been established in most parts of the world (Vargas *et al.*, 2012) with over 30 parasitoid species having been introduced from Australia, Africa and Asia (Bokonon-Ganta *et al.*, 2007). The Opiinae subfamily of the Braconidae family which contains over 1500 species, which are koinobiont endoparasitoids, is an important candidate for biological control measures. These endoparastoids fall in the genera *Diachasmimorpha*, *Fopius*, *Diachasma*, *Psyttalia*, *Utetes*, and *Opius*. Most parasitoids are recovered from fruit fly-infested fruits and are generally either egg-pupal or larval-pupal endoparasitoids. Some of the parasitic wasps are host specific while others are generalists (Mohamed *et al.*, 2010; Stuhl *et al.*, 2011). In California, different parasitoids were identified from different regions (Hoelmer *et al.*, 2011) and introduced to counter the rapid establishment and spread of olive fruit flies, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) (Daane *et al.*, 2015).

Two of the most widely reared fruit flies parasitoids are the generalist koinobiont endoparasitoids *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) (both Hymenoptera: Braconidae) of Asian origin. *Fopius arisanus* is a solitary egg-pupal endoparasitoid native to the Indo-Australian region. It has extensively been introduced in most parts of the world as a biological control to a majority of tephritid species (Zenil *et al.*, 2004; Sime *et al.*, 2008) since it has higher efficiency in reducing fruit fly populations and a broad host range of over 40 fruit fly species (Groth *et al.*, 2017; Cai *et al.*, 2020). *Diachasmimorpha longicaudata* is a parasitoid of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) larvae and the most common biological control agent of tephritid fruit flies (Thompson, 2011). This parasitoid can parasitize, spread and compete with native parasitoids (Camargos *et al.*, 2018; Dias *et al*., 2018; Ndlela *et al*., 2020). The host-specific parasitoid *Psyttalia cosyrae* (Wilkinson) (Hymenoptera: Braconidae) of African origin (Badii *et al.*, 2016; Mama Sambo *et al*., 2020) is a larvae-pupal endoparasitoid of *C. cosyra* (Mohamed *et al.*, 2003; 2016; Niassy *et al*., 2022). The diversity, establishment, distribution patterns and success of introducing exotic parasitoids to intermingle with native ones are of paramount importance in biological control programs (Ovruski *et al.*, 2000; Ovruski & Schliserman, 2012) as they will form the basis for better rearing and dispersal strategies.

The life cycles of egg-pupal and larval-pupal endoparasitoids are similar only that the former starts at the egg stage of the host while the latter starts at the larvae stage (Figure 1-2). After the parasitoid egg is laid in the host using the parasitoid ovipositor, it hatches into larvae but remains in the first instar thus allowing the host to feed and develop up to the time of pupation. On the onset of host pupation, the first instar parasitoid larvae kill the host and feed on it until it matures. The adult parasitoid emerges from the host puparia (Figure 1-2) (Lawrence *et al*., 1978; Rocha *et al*., 2004).

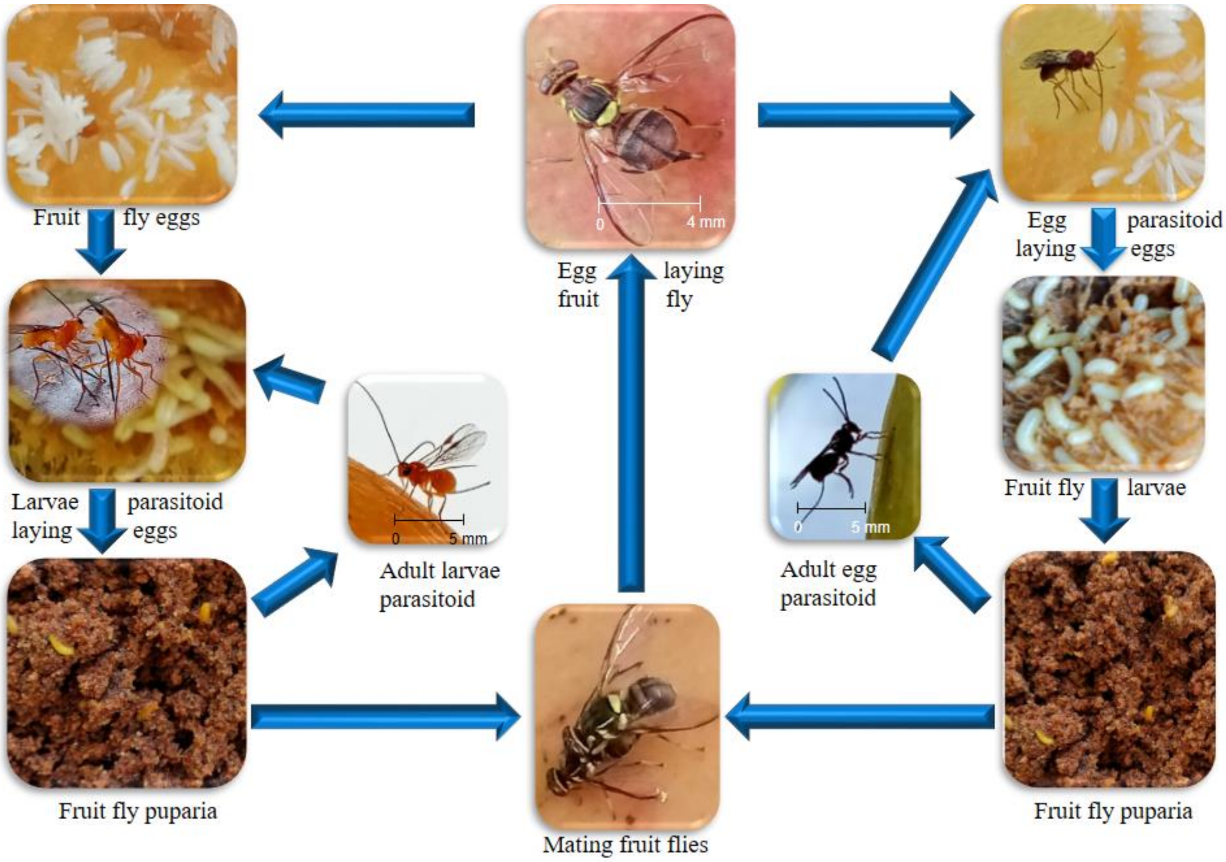

Figure 1-2: The life cycle of parasitoids and how they attack and suppress their fruit fly host either by ovipositing in the hosts egg or larvae

The eggs of the parasitoids hatch in two to five days and the larvae develop through three instars before pupating (Rocha *et al.*, 2004). After successful development, an adult parasitoid emerges from the fruit fly puparia. At 24-27 °C, the egg takes 18-23 days to develop into an adult (Lawrence *et al.*, 1978). *Fopius arisanus* can reduce the number of fruit flies that emerge after fruit fly oviposition either by destroying the host's egg with its ovipositor during the process of piercing to oviposit or by the parasitoid developing to maturity (Rocha *et al.*, 2004). Similarly, the larvae parasitoid can kill the host larvae with its ovipositor, through super parasitism or the parasitoid developing to maturity in its host (Bautista *et al.*, 2004).

The demand for area-wide pest control strategies that are friendly to the environment led to the development of sterile males (Vreysen *et al.*, 2006a). This includes the sterilization of male fruit flies using gamma radiation (Hooper, 1972; Weldon *et al.*, 2010) which are then released to the field to compete with field males for mating hence reducing the number of fertile eggs laid. This method is called the Sterile Insect Technique (SIT) (Vreysen *et al.*, 2006b; Pérez-Staples *et al.*, 2013). This technique requires mass production of sterilized males that will outnumber those in the field (Suckling *et al.*, 2016). Sterile Insect Technique combined with other classical and augmentative biological control programs stands a better chance in the control of invasive pests (Williams *et al.*, 2013). The understanding of the dietary effect on fruit fly sexual communication (Vera *et al*., 2013; Collins *et al*., 2014) and the changes in sexual behavior are important in SIT strategies (Vera *et al*., 2013; Benelli *et al*., 2014) to enhance mating success (Pereira *et al*., 2013; Pérez-Staples *et al*., 2013; Cai *et al*., 2020). Genetically modified sterile males have been evaluated with considerable improvement in SIT strategies (Raphael *et al*., 2014). The use of SIT combined with other biological control strategies has proved to be more effective than a single strategy (Vargas *et al*., 2009; Suárez *et al*., 2019).

1.3.5 Integrated pest management (IPM) strategies

Concerted efforts must be put in place to counter the introduction, spread and control of any exotic fruit fly species using integrated pest management (IPM) strategies (Sarles *et al*., 2015). Hence, agroecological techniques were introduced for the management of invasive pests to address the challenges of chemical-based techniques because they are environment-friendly and more sustainable (Deguine *et al.*, 2015). Other than taking care of the economy and health well-being of the farmers and the consumers, IPM packages, which include the use of biopesticides, field sanitation and augumentoriums, protein baited sprays/spot sprays, SIT, MAT, use of parasitoids, heat and cold treatment technologies among others (Muriithi *et al.*, 2016, 2020; Niassy *et al.*, 2022) also promote natural biological conservation, preserve pollinators and allow the diversity of natural enemies (Deguine *et al.*, 2015). Although the packages have been appreciated and implemented in most parts of Africa and the world in general, there is still room for advancement given that the fruit fly menace has not been eradicated.

1.4 Chemical communication

Chemical communication is as old as the existence of living organisms. Chemical messages are transmitted from one organism to another through organic compounds referred to as semiochemicals (Norin, 2007) which is derived from the Greek word "*semeon*" meaning "signal" (Vandermoten *et al.*, 2012). Semiochemicals are used by different organisms to improve their reproduction, predator avoidance and location of food thus helping them to survive through generations and are divided into allelochemicals and pheromones (El-Shafie & Faleiro, 2017; El-ghany, 2019).

Pheromones are bio-functional molecules that are used for communication within individuals of the same species i.e. they are intraspecific compounds. Pheromones are divided into five groups including (i) marking pheromones – used by insects to mark territorial boundaries); (ii) alarm pheromones – they stimulate insects' tendencies of escaping or defending themselves; (iii) aggregation pheromones – compounds that make insects congregate at the source of pheromones; (iv) trail pheromones – they are mostly used by social insects, especially in search of food; and (v) sex pheromones – they help insects of the same species find their sex mates.

Allelochemicals are used for communication between organisms that belong to different species. They include (i) synomones – compounds that benefit both the emitter and the receiver; (ii) allomones – they benefit the emitter and not the receiver; and (iii) kairomones – they benefit the receiver and are mostly used in host and prey identification (Norin, 2007). Insects use the receptors in their sensilla hairs of the antennae to detect semiochemicals from volatiles that are released by other organisms (Quicke, 2014; Awad *et al.*, 2015).

1.5 Trapping and analysis of headspace volatile organic compounds

Plants' headspace volatile organic compounds are gaseous compounds that are emitted into the atmosphere as a result of abiotic and biotic factors. These compounds play major roles in plant evolution and how it interacts with other surrounding organisms (Ormeño *et al.*, 2011). Different methods are used in trapping and analyzing headspace volatiles released by infested and non-infested plants, fruits and vegetables. Trapping methods include headspace–solid phase microextraction (HS–SPME) (Ormeño *et al.*, 2011) and dynamic headspace trapping (DHT) (Njuguna *et al.*, 2018; Miano *et al.*, 2022). Among the methods used to analyze the headspace volatiles are gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID) for tentative identification. GC-MS tentatively gives the chemical profile of compounds present in the headspace volatiles. Gas chromatography-electroantennographic detection (GC-EAD) is used to determine volatile components (compounds) that elicit activity in the antenna of an insect (Torto *et al.*, 2013; El-Shafie & Faleiro, 2017; Miano *et al.*, 2022). EADs are used in combination with FIDs for tentative identification of active compounds which play major roles in attracting or repelling insect pests. In this study, we collected the *in situ* headspace volatiles of fruits using DHT and analyzed them in GC-MS, GC-FID and GC-EAD.

1.6 Biogenic volatile organic compounds (BVOCs)

The headspace volatile compounds have many benefits in the global arena that can be mapped out and used in controlling, managing, and eradicating destructive insect pests like fruit and vegetable flies (Vandermoten *et al.*, 2012). The practical use of BVOCs is limited or underdeveloped although they have great potential in the formulation of environmentally friendly green chemicals (Suckling, 2015). Several studies have revealed that volatile compounds, emanating from fruits and vegetables, individually or as blends have attractive properties towards tephritid fruit fly species. For example, volatiles emanating from three mango varieties (Amate, Coche and Ataulfo) attracted *Anastrepha obliqua* (Macquart), a West India fruit fly (Malo *et al.*, 2012). Kamala *et al.* (2012; 2014) reported EAG active compounds of *Mangifera indica* cv. ‘Alphonso’ and ‘Chausa’ volatiles some of which attracted *B. dorsalis* females and elicited oviposition. For most polyphagia fruit fly species a blend of shared EAG active compounds from different fruits showed increased attractiveness to the pests (Biasazin *et al.*, 2014).

It has also been reported that when a plant is attacked by a herbivore, the chemistry of its headspace is affected where herbivore-induced plant volatiles (HIPVs) are produced (Dicke & Baldwin, 2010). These HIPVs are mostly specific to the herbivore and they mostly act as plant defenses and are mostly responsible for the attraction of the natural enemies of the pest (Holopainen & Blande, 2013). However, in some cases HIPVs attract conspecifics, for example, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae), an onion thrips, is attracted to conspecific infested onion volatiles (Kumar *et al.*, 2017) thus increasing the herbivore activities. Also, *Scirtothrips dorsalis* (Hood) (Chilli thrips), a pest of *Capsicum annum* (Bell pepper) was more attracted to HIPVs from infested bell pepper than those of the non-infested ones (Shivaramu *et al.*, 2017). On the other hand, *Heterorhabditis megidis* (Poinar), a nematode parasitoid, is attracted by (*E*)-*β*- caryophyllene that is produced when the roots of maize are damaged by *Diabrotica virgifera* (Leconte) rootworm larvae (Rasmann *et al.*, 2005) while at the same time, it attracts conspecifics (Robert *et al.*, 2012). Linalool and farnesenes are HIPVs that are produced by damaged plant leaves and they repel many caterpillar species (Markovic *et al.*, 1996; Rodriguez-Saona *et al.*, 2009) while methyl salicylate

(*MeSA*) is a major ingredient in indirect plant defenses (Dicke *et al.*, 1990; Dicke & Baldwin, 2010) and attracts many insect predators (James, 2003) like the parasitoids.

The parasitoids of fruit flies are attracted to their host fruit flies when the target egg or larvae are inside the fruit. This implies that the parasitoids are attracted to the host using the fruit and/or fruit fly-initiated semiochemicals (Wang & Messing, 2003; Cai *et al.*, 2020). *D. longicaudata* has been shown to positively respond to volatiles of host fruits with a high preference for infested than non-infested fruits in olfactometer and wind-tunnel bioassays (Sime *et al.*, 2006; Segura *et al.*, 2012; Harbi *et al.*, 2019). It has also been reported that *F. arisanus* can detect semiochemicals produced by the fruit fly predator *Oecophylla longinoda* (African weaver ant), which inhibits its ability to parasitize *B. dorsalis* eggs (Appiah *et al.*, 2014).

In most cases, insect studies involving behavioral responses and semiochemicals have been limited to laboratory setups (Siderhurst & Jang, 2006; Cai *et al.*, 2020). Little effort if any has been made to understand the changes that occur before, during and after tephritid fruit flies oviposit on *in situ* tree attached-fruit to unveil the changes in volatile compositions and how these changes affect the behavior of the fruit fly in play and its natural enemies. This study investigated the tri-trophic interactions between fruit flies (*B. dorsalis*, *Z. cucurbitae*, *B. latifrons* and *C. cosyra*), the parasitoids (*F. arisanus*, *D. longicaudata*, and *P. cosyrae*), and different treatments of fruits: mango (*Mangifera indica* L.-Kent, Apple and Haden varieties), banana (*Musa* spp.-Fhia 17 variety), and tomato (*Solanum lycopersicum* L.- Improved Nouvelle F1 variety) using insect behavioral responses and mapping out the headspaces chemical profiles using GC-MS and GC-FID and finally elucidating the antennal active compounds using GC-EAD. The insights produced by this study will be a milestone in developing eco-friendly strategies for managing the menace of fruit flies like the "push-pull" and food-based "lure and kill" that only target the fruit fly but not its enemies. It will also help in providing mineable data for molecular and evolutionary ecological studies.

1.7 Problem statement and justification

Tephritidae fruit flies are pests that cause inconceivable damage to fruits and vegetables in most agricultural parts of the world (Ekesi & Mohamed, 2011; Ekesi, *et al.*, 2016). Over the years, there has been a spread of fruit flies to new geographical regions with new host vegetation regardless of human interventions (Díaz-Fleischer & Aluja, 2001; Rai *et al.*, 2014) which has resulted in the expansion of fruit fly hosts which is compounded by secondary outbreaks (Aluja & Mangan, 2008). Cultural and chemical methods have been used for a long time but they are less effective due to the diverse host range for some of the fruit fly pests, the concealed nature of the larval stage, and the gradual changes in climatic conditions amongst other factors (Nankinga *et al.*, 2014; Reddy *et al.*, 2018; Sultana *et al.*, 2020). The use of synthetic pesticides has not only interfered with IPM but also affected the environment, the health of the producers and consumers, the development of fruit fly resistance to the chemicals, and has generally affected beneficial arthropods hence calling for more eco-friendly management methods (Hegazi *et al.*, 2016; Ndlela *et al.*, 2020; Mwando *et al.*, 2021).

The larval stages of *B. dorsalis, C. cosyra*, *Z. cucurbitae*, and *B. latifrons* cause huge losses to fruit and vegetable farming. *B. dorsalis* and *C. cosyra* are considered polyphagous species, *Z. cucurbitae o*ligophagous while *B. latifrons* is a monophagous species (Allwood *et al.*, 1999; Biasazin *et al.*, 2014; Nanga Nanga *et al.*, 2019). Due to the concealed nature of the egg and the larval stage of the fruit fly, the use of parasitoids is today widely advocated and has been accepted worldwide (Ekesi *et al.*, 2016; Karlsson *et al.*, 2018). Of the Opiinae parasitoid subfamily, *F. arisanus* (Sonan) (egg-prepupal endoparasitoid), *P. cosyrae* (Wilkinson), and *D. longicaudata* (Ashmead) (larval–prepupal endoparasitoids) have proved to be economically viable and environmentally friendly in the fruit fly control strategies. *Fopius arisanus* and *D. longicaudata*, are generalized parasitoids while *P. cosyrae* parasitizes *C. cosyra*.

Generally, in the open field, both fruit flies and parasitoids target their host when the fruit or vegetable is tree-attached. However, whether there is sharedness of olfactory cues among the parasitoids and their host to the fruits or vegetables is not known. Furthermore, there is no comprehensive data that is reliable on the mapping of the *in situ* changes in fruit and vegetable headspace volatiles before and after fruit fly infestation to improve the existing IPM programs.

This study focused on the tri-trophic interactions of four fruit flies (*B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons*), three parasitoids (*F. arisanus*, *P. cosyrae*, and *D. longicaudata*; Figure 1-3) and the fruits mango (*cv*. Kent, Apple, and Haden), banana (*cv*. Fhia 17 variety) and tomato (*cv*. Improved Nouvelle F1 variety).

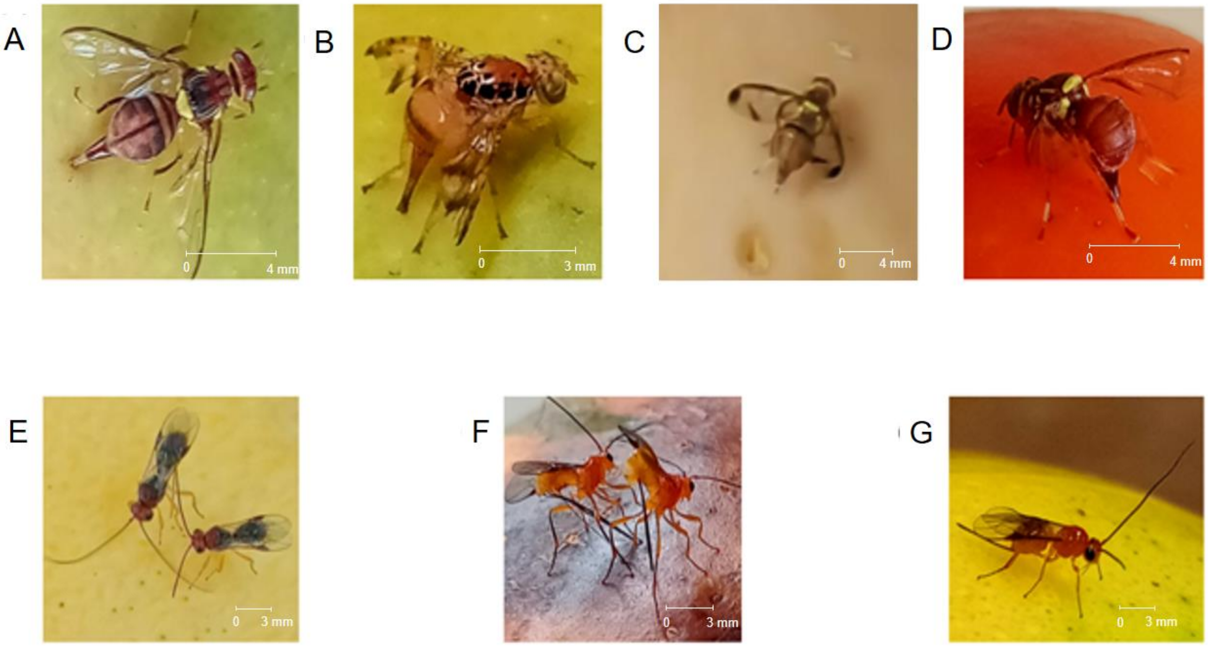

Figure 1-3: The study fruit fly species; *B. dorsalis* (A), *C. cosyra* (B), *Z. cucurbitae* (C), and *B. latifrons* (D); and the parasitoids *F. arisanus* (E), *D. longicaudata* (F), and the Africa native *P. cosyrae* (G)

The study involved (i) the responses of fruit flies (*B. dorsalis* and *C. cosyra*) and their parasitoids to non-infested and infested tree-attached mangoes and the performance of the two fruit fly species in the mango varieties; (ii) a comparison of the chemical profiles of the headspaces of non-infested and infested mango treatments; (iii) assessing the performance of *B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons* in freshly harvested mango (Haden variety), banana (*cv*. Fhia 17 variety) and tomato (*cv*. Improved Nouvelle F1 variety), and (iv) mapping out and comparing the EAD-active compounds of parasitoids and their tephritid fruit fly hosts. Of the fruits selected, *B. dorsalis* and *C. cosyra* generally infest mangoes, *Z. cucurbitae* is associated with attacking cucurbitaceous vegetables but it has been reported to attack tomatoes while *B. latifrons* is a Solanaceae fruit fly. This study is important from an ecological context as it will shed new light on our understanding of the general odor association of fruits, fruit flies and their parasitoids. To the best of our knowledge, this study is very novel as no other group has ever assessed the *in situ* responses of fruit flies and parasitoids and the subsequent performances of fruit flies, followed by elucidation of accompanying headspace volatiles in any cohesive and comprehensive fashion.

1.8 Research hypotheses

There is no convergence in the olfactomes of the parasitoids (*F. arisanus*, *P. cosyrae*, and *D. longicaudata*) with those of fruit flies' (*B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons*) and their host fruit odors (mangoes, banana, and tomato).

1.9 Objectives

1.9.1 Main objective

To characterize and compare the olfactomes of the parasitoids; *F. arisanus*, *P. cosyrae,* and *D. longicaudata* to those of their hosts; *B. dorsalis*, *C. cosyra*, *Z. cucurbitae* and *B. latifrons* niche odors to get insight into general odor circuitry and how it contributes to host specificity, host finding efficiency, host breadth and general suitability of a parasitoid in the host population management.

1.9.2 Specific objectives

i. To determine the responses of fruit flies and parasitoids to non-infested and fruit fly-infested tree-attached mangoes of three different mango varieties (Kent, Apple, and Haden)

ii. To trap and characterize the compounds in the headspace volatiles of different treatments of tree-attached mangoes (Kent, Apple, and Haden), banana and tomato fruits

iii. To compare the olfactomes of *F. arisanus*, *P. cosyrae* and *D. longicaudata* with those of their host *B. dorsalis, C. cosyra*, *Z. cucurbitae* and *B. latifrons* niche odors

iv. To determine the convergence of the olfactomes of the fruit flies and parasitoids with relation to host specificity and host finding efficiency

1.10 Thesis structure

There are three data chapters each of which is presented as a standalone publication. Hence the thesis contains some repetitions and overlaps between the chapters.

Chapter two and three of this thesis mainly dwelt on the responses of fruit flies (*B. dorsalis* and *C. cosyra*) and parasitoids (*F. arisanus*, *D. longicaudata* and *P. cosyrae*) to different treatments of mango headspace volatiles (*cvs*. Kent, Apple and Haden), the performances, in terms of the number of puparia recovered, of the test fruit flies in the mangoes and finally the identification of compounds in the headspace volatiles. The two chapters differ in that each one of them addresses a specific fruit fly and its parasitoids.

Chapter four was on the fruit fly species *B. dorsalis*, *B. latifrons*, *C. cosyra* and *Z. cucurbitae* and the three aforementioned parasitoid species. This chapter addresses the performance, in terms of puparia recovered, of the four fruit fly species in Haden mango variety, banana and tomato in a controlled laboratory setup. It further addresses the headspace volatiles of three treatments of the three mango varieties (which were reported to attract *B. dorsalis*, *C. cosyra* and the three parasitoids used in chapters two and three), the banana and the tomato to identify the compounds that trigger antennal responses of the four fruit flies. Finally, the chapter revealed the compounds of Haden mango that triggered both fruit fly and parasitoid species' antennal responses.

Lastly, the fifth chapter presents the concluding remarks based on each chapter and the whole research project.

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Chapter 2: Differential responses of *Bactrocera dorsalis* and its parasitoids to headspaces of different varieties of tree-attached mango fruits and the associated chemical profile

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2.1 Abstract

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 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, freshly *B. dorsalis* infested mangoes, and mangoes on the 7th-day post-oviposition (DPO) and 9th-DPO) of tree-attached Kent, Apple, and Haden mango varieties relative to control (clean air). *B. dorsalis* was significantly more attracted to the mango volatiles (up to 93% response) compared to the control. *F. arisanus* was significantly more attracted to freshly-infested mangoes (68-76% responses) compared to the control while *D. longicaudata* was significantly more attracted to 9th-DPO mangoes (64-72% responses) compared to the control. Secondly, we elucidated the headspace volatile chemical profiles of the non-infested (mature unripe and ripe) and infested (2nd-, 7th- and 9th-DPO) 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 percentage, followed by sesquiterpenes (16.4%), and monoterpenes (15.4%) among others for both infested and non-infested mangoes of the three varieties. Most compounds had higher concentrations in headspace volatiles of *B. dorsalis*-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 number of puparia, while none was recovered from Kent. These results represent the changes in headspace volatile components of non-infested and *B. dorsalis*-infested tree-attached mangoes and how they affect the responses of the mentioned insects. A follow-up study of the EAD-activity of the headspace compounds is recommended to develop baits that selectively attract the fruit fly and not its natural enemies.

Keywords: *Tree-attached mango. Bactrocera dorsalis. Fopius arisanus. Diachasmimorpha longicaudata. Headspace. GC-MS.*

2.2 Introduction

Mango (*Mangifera indica* L.) is one of the most widely grown fruits, ranking sixth among major fruit crops in terms of production (after bananas, watermelons, apples, oranges and grapes) 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 (Boinahadji *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 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 (Muriithi *et al.*, 2016), SIT (Enkerlin *et al.*, 2017 and references therein), semiochemicals (Biasazin *et al.*, 2019; Scolari *et al.*, 2021), and 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 fruit or vegetable 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 herbivore pests (Metcalf & Kogan, 1987; Carrasco *et al.*, 2015) which 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 & 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, *γ*-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 EAD-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 1500 koinobiont endoparasitoid species (Copeland *et al.,* 2006; Badii *et al.,* 2016). Among these parasitoids are *F. arisanus* and *D. longicaudata* which are solitary egg-prepupal endoparasitoids. The two parasitoid species have been used extensively for the biological control of *B. dorsalis* with outstanding success in Hawaii (Flávio *et al.,* 2020) and French Polynesia (Vargas *et al.,* 2012). Recently the two parasitoids were introduced into Kenya (Mohamed *et al.,* 2008; 2010) and subsequently released in several African countries for classical biological control of *B. dorsalis* and other fruit flies (Mohamed *et al.,* 2016; Ndlela *et al.,* 2020). Gravid females of *F. arisanus* are attracted to their host either using volatiles emanating from the fruit during or after fruit fly 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 to investigate the behavioral responses of the generalist and voracious *B. dorsalis*, and the parasitoids *F. arisanus* and *D. longicaudata* to volatiles of the three tree-attached mango varieties (Kent, Apple, and Haden) that were either non-infested or at different days post-infestation by the fruit fly and then elucidating the chemicals profiles of the aforementioned mango headspaces.

2.3 Materials and Methods

2.3.1 Mango Fruits

During the flowering season, in July 2020, three varieties of mango trees (Kent, Apple, and Haden) were identified, in a two-acre orchard at Gathigiriri (00°41'39.8" S 037°24'26.7" E, 1158m 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), and 14 local varieties. In this area, Haden mangoes usually ripen in late December, Apple mangoes in January, and Kent variety ripens in April. Two mango trees of each of the three varieties were randomly selected from the orchard. Except for duduthrin 1.75 EC (Twiga Chemical Industries Ltd, Nairobi, Kenya) powder that was strewed at the base of each tree (according to the manufacturer's recommendations) to prevent crawling insects like ants and termites from damaging the flowers and young fruits, the trees were kept free of other insecticides and fungicides during the entire period of the trials. The mango fruits were allowed to develop for four months, from the time of flowering, after which they were secured *in situ* (Figure 2-1) using fine white nets that were mounted on 20 × 20 × 20 cm of 2.5 mm galvanized metallic wire cube frames sourced from the local market.

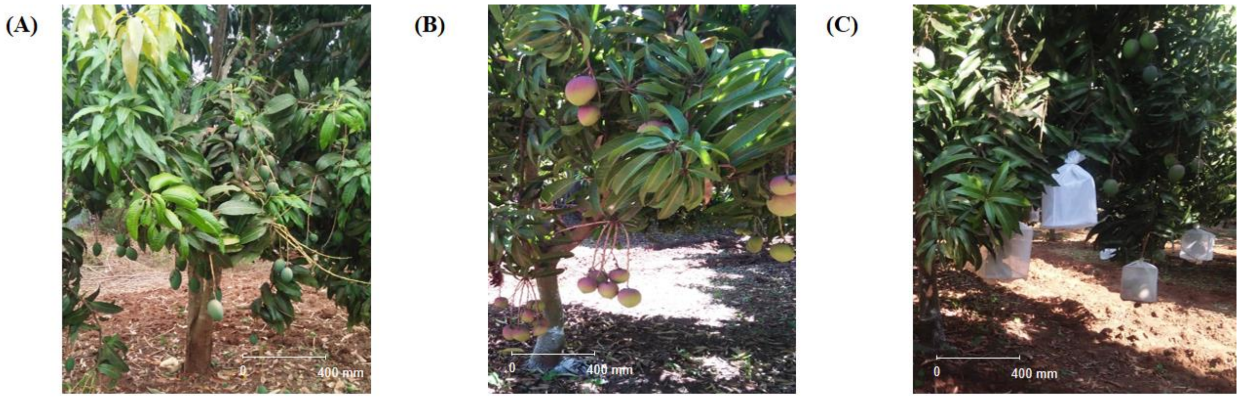

Figure 2-1: Examples of mango trees that were selected for experimental mangoes which were used in this study. (A) Kent variety; (B) Apple variety with duduthrin 1.75 EC dust spread at the base; and (C) Haden variety with some bagged mango fruits

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.

2.3.2 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; 1600 m asl) Nairobi Kenya following already established protocols (Ekesi & Mohamed, 2011; Gordello, 2013), where the fruit fly colony was maintained at 26 ± 2 °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 °C for 48 h and then left to warm to room temperature for two h in a laminar flow hood. Fruits that showed no signs of fruit fly infestation were separated. Six of the fruit fly-free mango fruits were offered as oviposition substrate to 12-16 days old *B. dorsalis* (*n* = 100; ♂: ♀ = 1:1) held in a Perspex rearing cages (30 × 30 × 30 cm) for three 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 hydrolysate (USB Corporation, Cleveland, OH) in a ratio of 3:1. Water was provided *ad libitum* in glass Petri-dishes (90 × 15 mm) with pumice granules to prevent drowning. The infested fruits were then transferred into plastic containers (21 × 14 × 8 cm; Kenpoly Manufacturers Limited, Nairobi, Kenya) for eggs to hatch and larvae to develop. The plastic containers were perforated at their bottom side and a sheet of paper towel followed by a fine net was 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 (10 days after infestation), the infested mangoes were put in plastic basins (32 cm diameter × 14 cm depth, Kenpoly manufacturers limited) that were quarter filled with dry, fine (>1.18 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 tap water (half basin full) to separate the puparium from the sand, the remains of the mango peels and other dirt. The floating puparia were then recovered through sieving (Cheseto *et al.*, 2017a), put on filter paper in a Petri dish, and then transferred into humidified Perspex rearing cages (30 × 30 × 30 cm) for eclosion. The adult fruit flies were maintained as aforementioned but at room conditions of temperature (day = 23 ± 4°C, night = 20 ± 4°C), humidity (38-68% RH), and natural photoperiod.

2.3.3 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 2.3.2.

Fopius arisanus colony was initiated by exposing six Apple mangoes to a colony of 100 adults of *B. dorsalis* (ratio ♂: ♀ =1:1) for 3 h (8.00 am-11.00 am). Two sets of three mangoes were then put in cages each containing 100 adults of 8-15 days-old *F. arisanus* (♂: ♀ 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 (♂: ♀ = ratio-1:1) for three days to maximize parasitism. 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 (Figure 2-2) after every four days (Manoukis *et al*., 2011). The new parasitoid colony was maintained under room conditions.

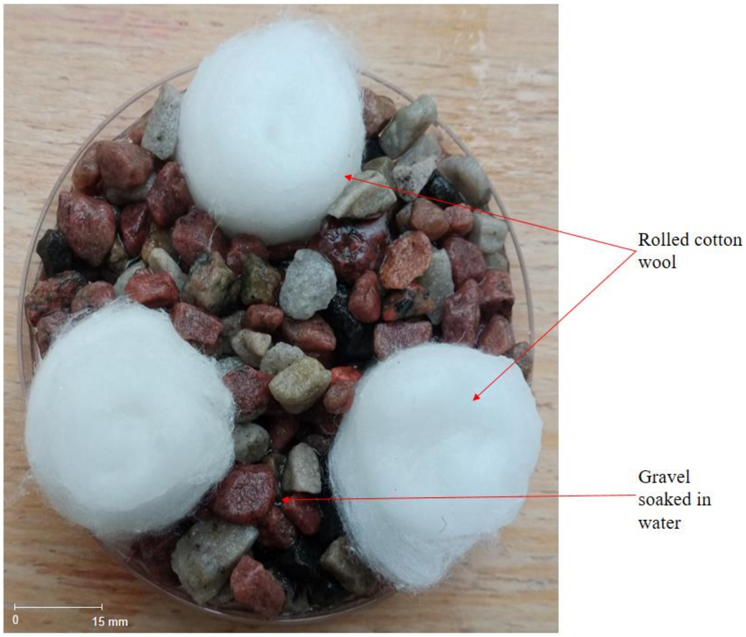

Figure 2-2: Petri dish with water-soaked gravel granules and rolled cotton wool for provision of water to the parasitoids

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

Dual-choice olfactometer assays were carried out in the mango orchard at Mwea East Sub-county, Kenya, 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 2-3).

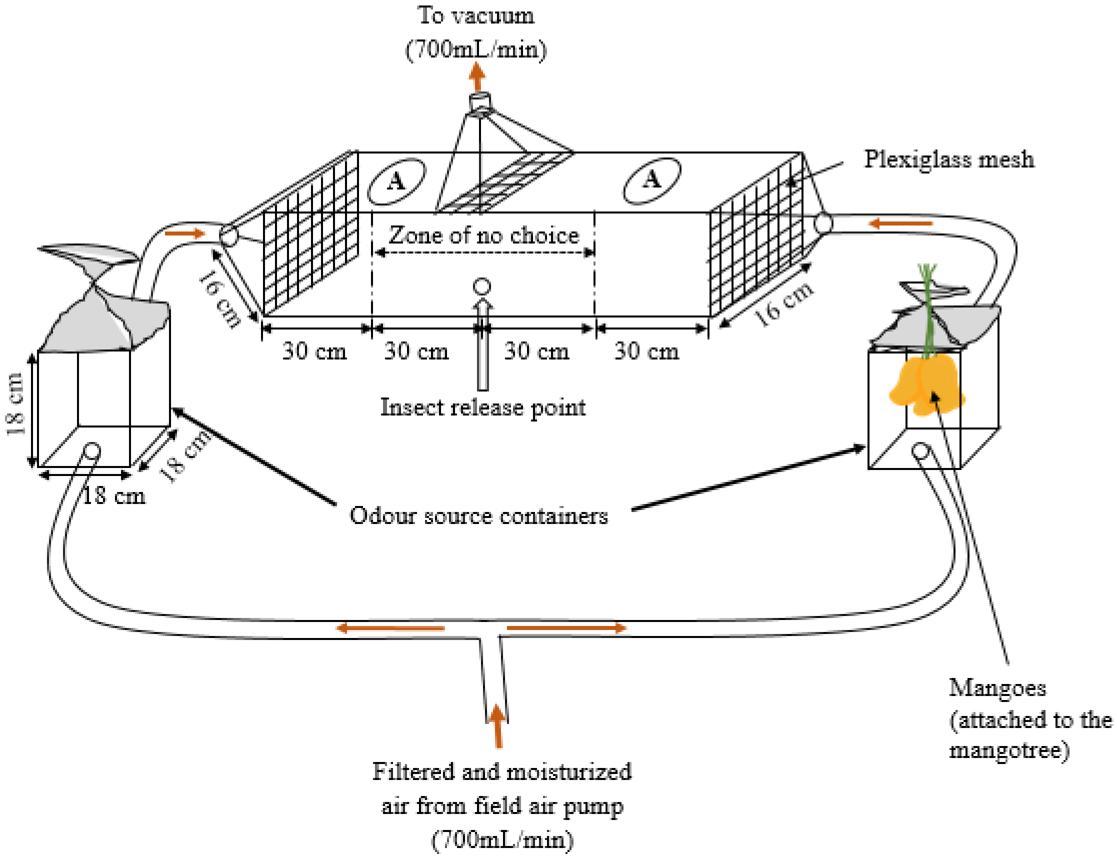

Figure 2-3: A schematic representation of the dual-choice olfactometer (not drawn to scale)

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 mL min $^{-1}$ and evacuated at the center (700 mL min $^{-1}$) using a portable vacuum field air pump connected to air-flow metres (Analytical Research System Inc. Gainesville, Florida 32614 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 (a black masked, using a black tape, falcon tube) and kept for about 10 min for acclimatization. Thereafter, the group of insects was released through the hole at the center of the bottom of the wind tunnel (Figure 2-3) and they were allowed 20 minutes to make a decision, following the procedure of group release described by Nyasembe *et al.* (2012), Njuguna *et al.*, (2018), and Miano *et al.* (2022) with modifications. The base of the dual-choice olfactometer was marked from 0-60 cm on either side of the insect release point to allow scoring. The insects that moved beyond 30 cm from the release point on either side of the olfactometer were considered to have made a choice, while those that were in the range of 0 to 30 cm were non-responsive. Non-responsive insects were not included in the statistical analysis. Seven replicates were done for each experiment using a different batch of insects. The tested insects were removed through widows marked A (Figure 2-3) and put in a separate cage after each replicate. Between two runs, clean air was passed through the apparatus, at the same rate as in the assays, for 20 minutes to blow out odors of the previous test, the air inlets were then changed to avoid positional bias, and air from odor sources allowed to pass through the apparatus for ten minutes 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 (♂: ♀ =1:1) and then released into the mango holder cages (Figure 2-3) which contained four mangoes as an oviposition substrate. The fruit flies and mangoes remained together 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 (x10). The freshly-infested mangoes were secured back into the nets and used for subsequent infested mango assays. After each day’s tests, the odor containers and the olfactometer were cleaned using warm 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 mature but unripe mangoes versus control; (iii) *B. dorsalis* or *F. arisanus* to volatiles of *B. dorsalis*-freshly-infested mangoes by versus control; (iv) responses of *B. dorsalis* or *D. longicaudata* to volatiles of 7th-DPO or 9th-DPO mangoes versus control; and (v) responses of *B. dorsalis*, *F. arisanus*, or *D. longicaudata* to volatiles of non-infested ripe mangoes versus control. On each experimental day, the experimental mangoes were secured back into the fine white netted cages to prevent any additional infestation.

2.3.5 Performance of *B. 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 (since on the 10th-day post-oviposition, most infested Apple and Haden mangoes had detached from the tree) and incubated as aforementioned (section 2.3.2). Pupation took 12-17 days from the day of oviposition. To allow the pupation of all larvae, puparia were recovered from the sand by picking followed by counting and recording.

2.3.6 *In situ* headspace collection of mango volatiles

The headspace collection of mango volatiles 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 (Figure 2-4).

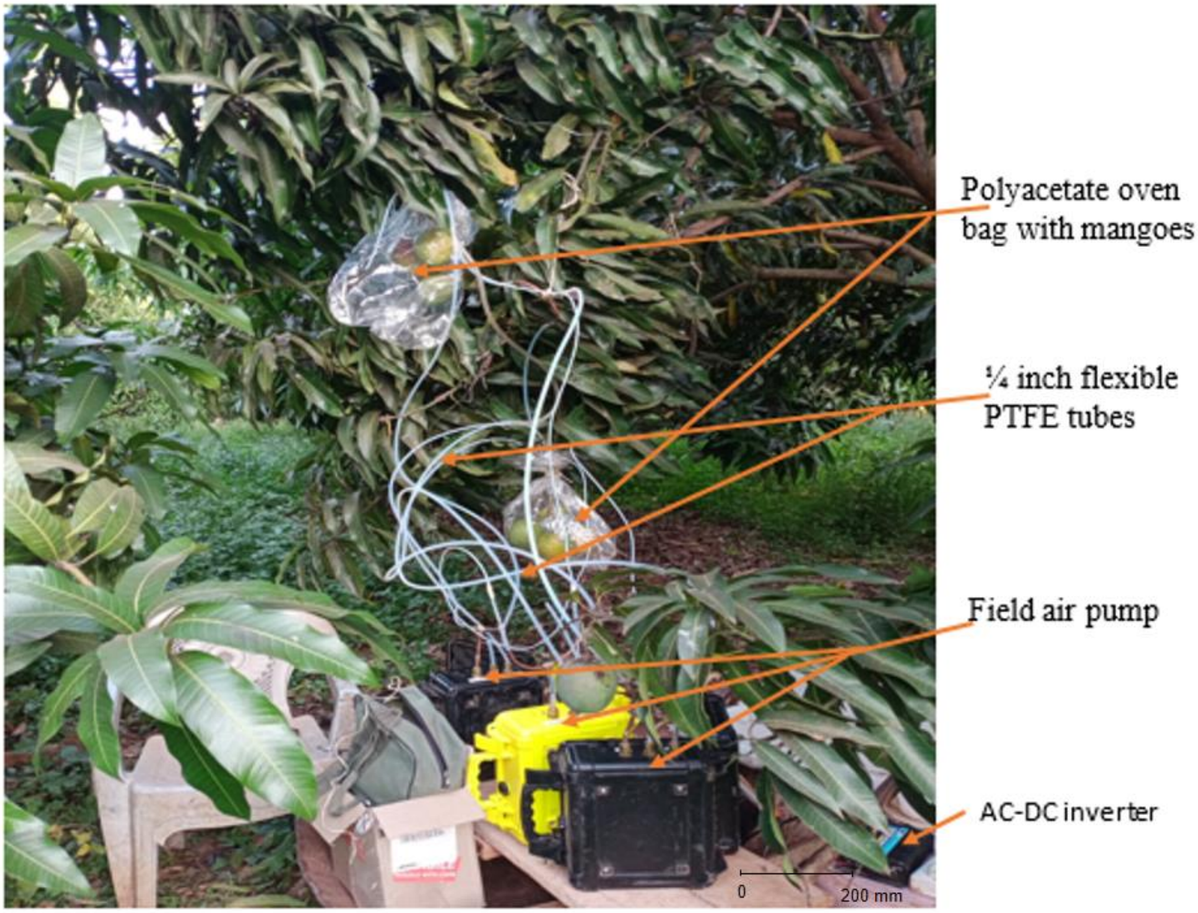

Figure 2-4: Dynamic headspace trapping (DHT) system for the trapping of headspace volatiles of tree-attached mango fruits onto sorbent cartridges. The sorbent cartridges, which were connected to the field air pump using PTFE tube, were held inside the polyacetate oven bag

Clean 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 of four tree-attached mangoes were trapped in polyacetate oven bags (KitchenCraft, Birmingham, B6 7EU Ltd, UK) and volatiles collected onto HayeSep-Q mixed-phase sorbents (30 mg, copolymers of polydimethylsiloxane-divinylbenzene (PDMS-DVB)) which were twice pre-cleaned with 200 µL of GC-grade dichloromethane (DCM). Four replicates of headspace volatiles were collected from each treatment of the mango fruits. The treatments include: (i) Non-infested unripe mango fruits (UR); (ii) *B. dorsalis*-freshly-infested mangoes (BD1); (iii) 2nd-DPO (BD2); (iv) 7th-DPO (BD7); and (v) 9th-DPO (BD9) mangoes; (vi) non-infested ripe mango fruits (HR); and (vii) clean air (an empty oven bag sampled as a method control). Headspace volatiles were collected for 11 hours (7.00 am to 6.00 pm). For preservation and transportation of the headspace volatiles trapped in HayeSep-Q adsorbents, the tips of the adsorbent holder were tightly sealed with a 0.075 mm P.T.F.E. thread seal tape (MAAT, UK), then wrapped in aluminium foil and placed on dry ice (Carbacid (CO_2) Limited) (Carbacid Investment Limited, Nairobi, Kenya) in a cool box (Miano *et al.*, 2022). Then the headspace volatiles were eluted in 200 µL DCM into 250 µL 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 -81 °C freezer until use. The cartridges were also cleaned in DCM and dried using the same pressurizing nitrogen gas.

2.3.7 Chemical analysis of mango headspace volatiles

The headspace volatiles were analyzed (1µl, one minute splitless time, and splitless mode at 270 °C) 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% phenyl- methylpolysiloxane) column (30 m × 0.25 mm ID × 0.25 µm film thickness). The oven temperature program was 35 °C for 5 min, then increased to 280 °C at the rate of 10 °C min^{-1}, and then held at this temperature for 10.5 min. The mass selective detector was retained at 230 °C ion source temperature and a quadruple temperature of 180 °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 constant flow rate of 1.2 mL min^{-1}.

The qualitative identification of compounds was done by comparing the mass spectrometric data to those of reference spectra published by the library–MS databases National Institute of Standards and Technology (NIST 05, 08, and 11), Adams and Chemecol (above 70% match were considered present). The experimental retention indices were calculated and compared to literature values for some compounds while others were authenticated using the retention times of synthetic compounds that were run using the same GC-MS program (Figure 2-5; Table 2-1).

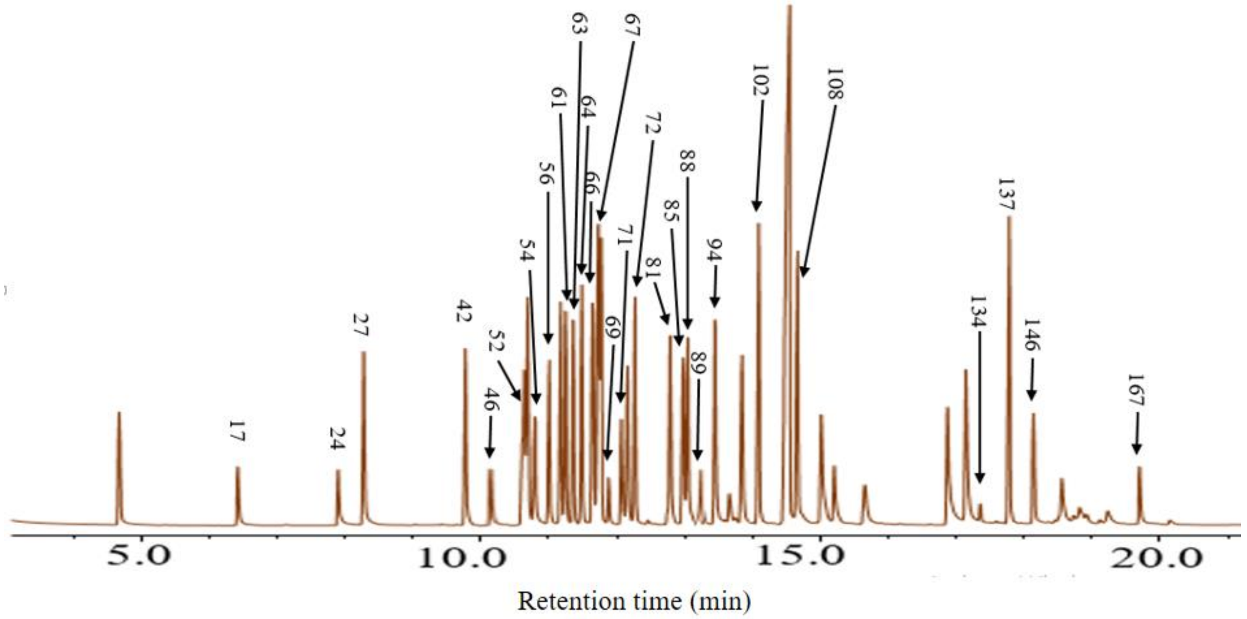

Figure 2-5: A total ion chromatogram (TIC) of analytical standards that were used to authenticate some of the identified compounds. The numbers correspond to those assigned to the compounds in Table 2-1

Further confirmation of compounds was based on their calculated retention indices (RI_{cal}) relative to literature retention indices (RI_{lit}) while some were authenticated using standards. The RI_{cal} was determined using a homologous series of straight-chain alkanes, C_5 – C_{31}) and was calculated based on the equation of Van den Dool and Kratz and compared with documented values (Dool & Kratz, 1963; Adams, 1996; Hérent *et al*.,2007).

The formula used for retention indices calculation:

$$\text{RIcal} = 100\, n0 + 100 \left[\frac{RTx - RTno}{RTn1 - RTno}\right]$$

x = the target compound

n0 = n-alkane $C_{no}H_{2no+2}$ directly eluting before x

n1 = $C_{n1}H_{2n1+2}$ directly eluting after x

RT= retention time

RI = retention index

n = alkane (C_5-C_{31}) standards.

For relative quantification of the concentrations of volatiles, a serial dilution (eleven calibration standards; 2.25 – 1000 ηg µL^{-1}) of the authentic standards *α*-pinene and *α*-humulene (98% purity, Sigma-Aldrich® Solutions, St. Luis, MO) were analyzed by GC-MS in full scan mode to generate linear calibration curves (peak area vs. concentration, Figure 2-6) (Njuguna *et al.*, 2018; Miano *et al.*, 2022).

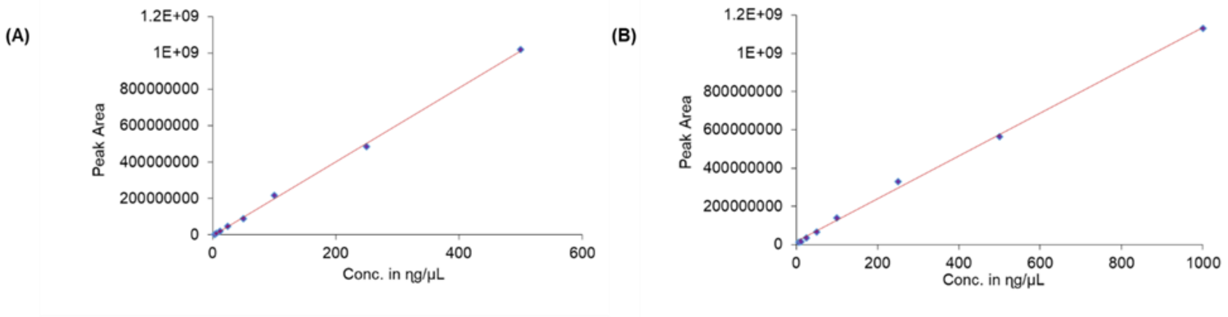

Figure 2-6: Calibration curves of the linear equations of *α*-pinene (42; A) and *α*-humulene (146; B) that were used in the quantification of the identified volatiles (Table 2-1)

The linear equations generated were $y = 2036653.8x - 5127153.0$; R^2 = 0.9963 for *α*-pinene and $y = 1127808.7x - 5512234.2$; R^2 = 0.9991 for *α*-humulene and were used to quantify volatile compounds that had retention times that were either below or above 16 min, respectively.

2.3.8 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, *α*-pinene, camphene, 1-octen-3-ol, myrcene, *δ*-3-carene, *δ*-2-carene, *o*-cymene, limonene, (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene, *γ*-terpinene, terpinolene, linalool, *n*-nonanal, 1,3,8-*p*-menthatriene, *allo*-ocimene, terpinen-4-ol, *n*-decanal, *β*-elemene, (*E*)-caryophyllene, *α*-humulene, and caryophyllene oxide which had a chemical purity of 90-99.9 %, α-phellandrene and sabinene (purity 85 and 75% respectively) were used for identification of volatiles.

2.4 Statistical analyses

The data of the numbers of responsive fruit flies and parasitoids were analyzed using 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 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 volatile concentrations of the three mango 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 posthoc Dunn test for pairwise comparison was used to test whether the volatile concentrations from the three mango varieties under the different treatments were equal but where compounds were present in only two treatments, Mann-Whitney U test was used (Dinno, 2015). The data was 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 *Past 3* software (Hammer *et al.*, 2001)

The volatile concentration data were then analyzed per mango variety where each dataset was subjected to one-way analysis of similarities (ANOSIM) to determine whether the headspace composition among treatments was significantly different. Further, 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 concentration (($y = \ log_{10} x + 1$); where x = average volatile concentrations in ng µL^{-1}).

The relative concentrations 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) *B. dorsalis*-freshly-infested mangoes and 2nd-DPO relative to those of non-infested unripe mango and (ii) 7th-DPO and 9th-DPO relative to those of non-infested ripe mangoes. A Kruskal-Wallis rank-sum test was performed to test for the difference in headspace volatile concentrations 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;

$$y = \frac{Average\ headspace\ volatile\ release\ rate\ of\ a\ compound\ in\ the\ volatile\ of\ interest}{Average\ headspace\ volatile\ release\ rate\ of\ the\ same\ compound\ in\ non-infested\ mango}$$

and then visualized using line graphs.

2.5 Results

2.5.1 Behavioral assays of *B. dorsalis* and parasitoids to tree-attached non-infested and infested mangoes.

In our control (clean air) versus control treatments, there was no significant difference in the number of females of either *B. dorsalis*, *F. arisanus*, or *D. longicaudata* that chose either arm of the wind tunnel ($P>0.05$) (Figure 2-7). On the other hand, the attraction of the fruit fly and the wasps to mango headspaces differed in magnitude compared to the control. *B. dorsalis* were significantly attracted to the volatiles of *B. dorsalis*-freshly-infested and non-infested ripe Kent mangoes (respectively $\chi^2 = 7.02$, $P < 0.01$; $\chi^2 = 13.5$, $P < 0.001$) but not to the non-infested unripe, 7th-DPO, and 9th-DPO Kent mangoes compared to control (Figure 2-7 A). All treatments of Apple and Haden mangoes were attractive to *B. dorsalis* ($P < 0.001$ except for 9th-DPO Apple mango where $P <$ 0. 0.05) compared to the control (Figure 2-7 B and C).

The egg parasitoid, *F. arisanus*, was not attracted to volatiles of unripe mangoes of the three varieties and ripe Kent and Haden but was attracted to volatiles of ripe Apple mangoes ($\chi^2 = 3.2$, $P < 0.05$). However, *B. dorsalis*-freshly-infested mangoes of all 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) (Figure 2-7 A, B, C). *D. 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 9th-DPO mangoes ($\chi^2 = 12.5$, $P < 0.001$; $\chi^2 = 4.90$, $P < 0.05$ respectively for Apple and Haden varieties) and only 7th-DPO Apple mango volatiles ($\chi^2 = 4.11$, $P < 0.05$) compared to control (Figure 2-7 A, B, C).

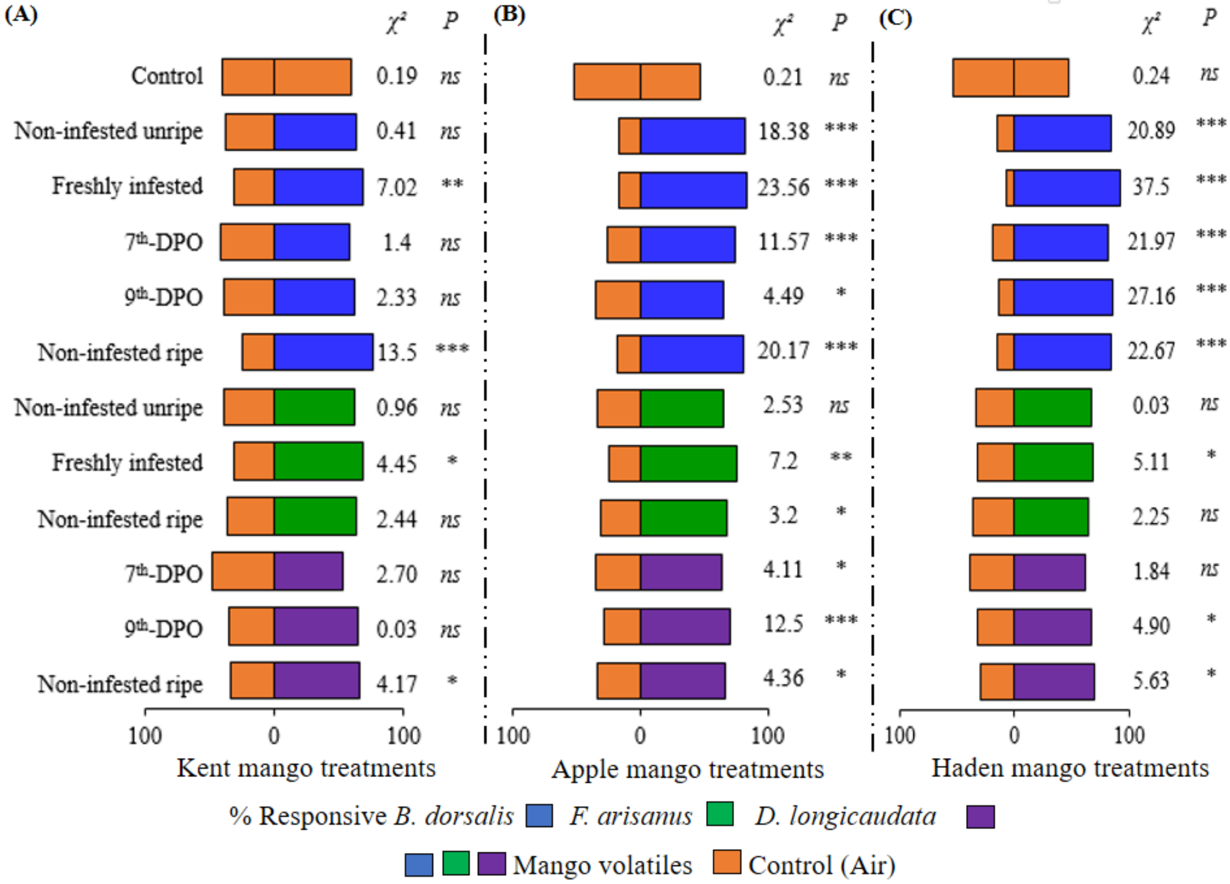

Figure 2-7: Response (%) of *B. dorsalis* (blue), *F. arisanus* (green), and *D. longicaudata* (purple) to different treatments of Kent (A), Apple (B), and Haden (C) mango volatiles. χ^2 = Chi-square, ns = no significant difference, and *, **, *** = significance differences for $P < 0.05, 0.01, 0.001$ respectively, DPO = day post-oviposition, (Chi-square goodness of fit test)

2.5.2 Performance of *B. 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 2-8). Although punctures and fruit sap were observed on the day of infestation on Kent mangoes, there were no *B. dorsalis* puparia recovered from this variety.

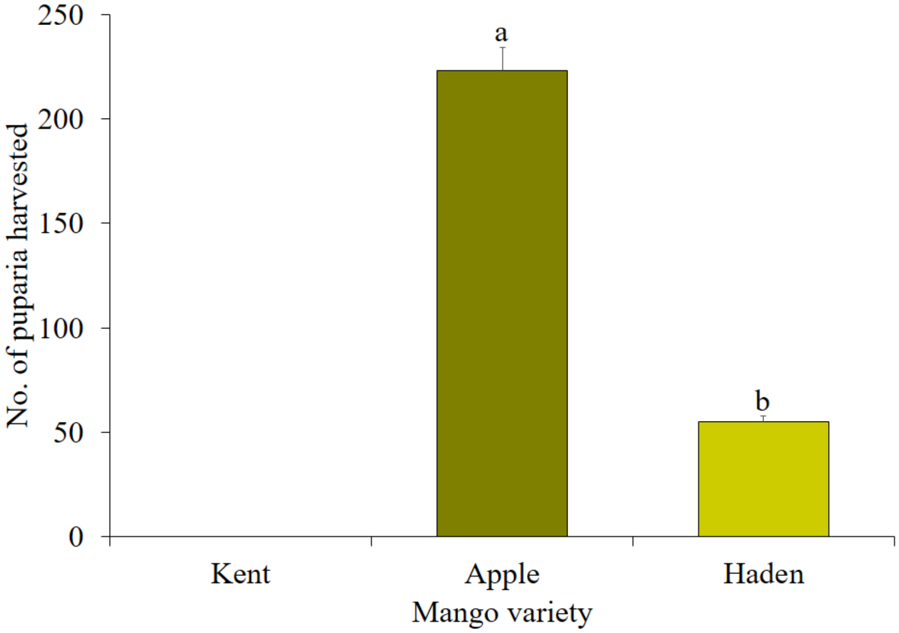

Figure 2-8: Average number of *B. dorsalis* puparia harvested from the different mango varieties. Bars capped with different letters are significantly different (Pearson's Chi-square test followed by Chi-square multi-comparison test in RVAideMemoire)

2.5.3 Headspace volatiles in treatments of the three varieties of mangoes

A total of 194 volatiles were identified in the mango headspaces, the composition of which varied between the treatments and the mango variety (Table 2-1). Kent mango registered the highest number of compounds (134) followed by Haden (114) while Apple had the least (102) (Table 2-1). Amongst the compounds detected, 66 were esters, 32 sesquiterpenes, 30 monoterpenes, 14 monoterpenoids, 12 aldehydes, 9 ketones, 10 alcohols, 6 sesquiterpenoids, 5 benzenoids, 3 organic acids, 3 diterpenes, and 5 others (Table 2-1). Among the compounds detected, 9 compounds (α-pinene, β-pinene, myrcene, δ-3-carene, α-gurjunene, (*E*)-caryophyllene, β-copaene, α-humulene, and δ-cadinene) were present in all treatments of the three varieties of mangoes but with varying concentrations (Table 2-1). There were significant differences in the volatile concentrations between treatments of the three mango varieties (Table 2-1). For example, concentrations of each of the common compounds among the treatments of the three mango varieties were different ($P < 0.001$) (Table 2-1). There was a significant difference among the concentrations of limonene, (Z)-muurola-4(14),5-diene and ethyl hexadecanoate ($P < 0.001$) which was only reported on Kent and Haden varieties; α-cubebene and γ-muurolene of Apple and Haden varieties; and β-phellandrene, linalool, *α*-guaiene and 9-*epi*-(*E*)-caryophyllene detected only in Apple variety (Table 2-1).

Table 2-1: Mean volatile concentration (ng µL^{-1}) of volatile organic compounds identified in the headspace collections of tree-attached mangoes under six different treatments (n = 4). Compounds were identified using their retention times (RT), electron ionization spectrum, and calculated Kovats retention indices (RI (cal)) relative to those of C5-C31 *n*-alkanes run on an HP-5MS, and those obtained from the literature (RI (lit)), as well as comparison of their spectra with the library data and mass spectra from online NIST library database. Compounds marked with ¶ are those that were confirmed using available authentic standards run on an HP-5MS column. Total mean volatile concentrations with different letters are significantly different based on the Kruskal-Wallis ANOVA test at $\alpha = 0.05$. (Rt = retention time; K = Kent; A = Apple; H = Haden; UR = non-infested unripe mango; BD = *B. dorsalis* infested; HR = non-infested ripe mango; 1 = freshly-infested; 2 = 2nd-DPO; 7 = 7th-DPO; and 9 = 9th-DPO; Total mean volatile concentration with different letters are significantly different)

							Mean volatile concentration (ng µL^{-1})																		
Pk No.	Rt	compound	Class	RI $_{cal}$	RI lit	Reference	KUR	KBD1	KBD2	KBD7	KBD9	KHR	AUR	ABD1	ABD2	ABD7	ABD9	AHR	HUR	HBD1	HBD2	HBD7	HBD9	HHR	**P-value**
1	3.12	2-Methylbutan-2-ol	Alcohol				6.6	5.3	5.2	8.7	7.1	2.6	-	-	-	-	-	-	-	-	-	-	-	-	<**0.01**
2	3.29	3-methyl butanal	Aldehyde		656	Xie, et al., 2008	-	-	-	-	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-	--
3	3.75	2-Methyl-3-buten-2-ol	Alcohol	583			-	-	-	3.1	-	1.7	-	-	-	-	-	-	-	-	-	-	-	-	<**0.05**
4	3.95	Pentanal	Aldehyde	666	701	Xie, et al., 2008	-	-	5.9	7.9	9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.05**
5	4.19	Acetoin	Ketone	706	707	Kim and Chung, 2009	-	-	-	49.5	158.7	5.3	-	-	-	206.9	1575.1	-	-	-	-	-	29.0	-	<**0.001**
6	4.27	Ethyl propanoate	Ester	709	709	Zhao, et al., 2008	-	-	-	30.9	48.2	-	-	-	-	23.0	64.6	-	-	-	-	14.0	32.6	-	<**0.01**
7	4.51	*n* -Propyl acetate	Ester	717	715	Isidorov, et al., 2006	-	-	-	-	-	-	-	-	-	-	42.7	-	-	-	-	-	-	-	--
8	4.53	Methyl butanoate	Ester	718	723	Jordán, et al., 2002	-	-	-	7.5	15.1	-	-	-	-	9.7	21.0	-	-	-	-	-	27.5	-	<**0.01**
9	4.8	Isopentyl formate	Ester	728	789	Zhao, et al., 2008	-	-	-	28.7	152.4	2.5	-	-	-	32.2	98.4	-	-	-	-	-	42.9	-	<**0.01**
10	4.91	2-Methyl-1-butanol	Alcohol	732	733	Kim and Chung, 2009	-	-	-	-	50.0	-	-	-	-	28.9	7.4	-	-	-	-	-	46.0	-	<**0.01**
11	5.55	Ethyl 2-methyl propanoate	Ester	755	756	Siegmund, et al., 2001	-	-	-	17.2	37.3	-	-	-	-	32.7	53.2	-	-	-	-	3.0	25.2	5.5	<**0.01**
12	5.64	Toluene	Benzenoid	759	759	Kim and Chung, 2009	16.8	10.3	12.5	15.2	49.3	2.5	-	-	-	10.9	11.3	-	-	-	9.7	19.7	23.3	8.2	<**0.001**
13	5.99	2-Methylpropyl ethanoate	Ester	771	772	Zhao, et al., 2008	-	-	-	-	5.0	-	-	-	-	1.8	10.1	-	-	-	-	-	-	-	<**0.01**
14	6.06	Methyl 2-methyl butanoate	Ester	774	772	Figuérédo, et al., 2005	-	-	-	-	6.3	-	-	-	-	1.7	-	-	-	-	-	-	-	-	<**0.05**
15	6.3	Ethyl 2-methylprop-2-enoate	Ester	783			-	-	-	-	31.7	-	-	-	-	11.8	21.8	-	-	-	-	-	15.2	-	<**0.01**
16	6.47	2,3-Butanediol	Alcohol	789	782	Krist, et al., 2005	-	-	4.2	33.4	266.6	1.9	-	-	-	397.2	654.0	-	-	-	-	-	133.6	-	<**0.001**
17	6.63	Hexanal¶	Aldehyde	795	799	Jarunrattanasri, et al., 2007	10.1	9.3	11.7	12.6	17.4	1.2	-	-	-	-	-	-	-	-	-	-	-	-	<**0.01**
18	6.7	Ethyl butanoate	Ester	797	802	Siegmund, et al., 2001	-	-	5.8	2042.8	3385.9	86.1	-	-	-	2233.6	3927.3	-	12.2	-	145.3	4483.0	15960.2	1976.8	<**0.001**
19	6.79	2-Hexanol	Alcohol	800	803	Jordán, et al., 2002	7.2	14.1	14.4	24.6	29.9	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.01**
20	7.07	(2*Z*)-butenoic acid	Organic acid	812			-	6.0	24.9	3684.9	5197.2	303.7	-	-	-	659.8	1074.1	-	-	-	133.5	1159.9	2239.1	-	<**0.001**
21	7.09	(2*E*)-Butenoic acid	Organic acid	812			-	-	-	4109.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
22	7.83	Ethyl (2*Z*)-butenoate	Ester	841	830	Quijano, *et al.* , 2007	-	-	1.6	3775.8	609.6	36.2	-	-	-	-	-	-	-	-	-	-	-	-	<**0.01**
23	7.97	Ethyl 2-methylbutanoate	Ester	846	846	Martí, *et al.* , 2003	-	-	5.5	67.5	488.6	2.5	-	-	-	379.0	386.9	-	-	-	-	685.4	950.1	412.4	<**0.001**
24	8.05	(2*E*)-hexenal¶	Aldehyde	850	851	Kim, *et al.* , 2000	2.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
25	8.06	Ethyl 3-methylbutanoate	Ester	850	851	Zhao, *et al.* , 2008	-	-	-	31.9	36.5	-	-	-	-	-	-	-	-	-	-	-	-	-	>**0.05**
26	8.17	Ethylbenzene	Benzenoid	854	855	Vichi, *et al.* , 2005	6.8	1.6	15.0	12.9	10.0	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.05**
27	8.36	*p* -Xylene¶	Benzenoid	862	862	Kotowska, *et al.* , 2012	4.0	1.8	3.4	-	-	1.7	-	-	-	-	23.3	-	-	-	-	-	-	-	<**0.01**
28	8.37	Methyl tiglate	Ester	862	868	Isidorov and Jdanova, 2002	-	-	-	6.2	13.3	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.05**
29	8.6	*n* -Hexanol	Alcohol	871	869	Ruther, 2000	-	-	-	6.6	15.8	-	-	-	-	-	-	-	-	-	-	-	40.0	-	<**0.01**
30	8.64	3-methylbutyl ethanoate	Ester	873	876	Forero, *et al.* , 2008	1.9	8.9	-	9.1	-	-	-	-	-	20.3	480.8	-	-	-	-	20.3	127.2	-	<**0.01**
31	8.92	Styrene	Benzenoid	883	886	Ansorena, *et al.* , 2001	25.8	3.4	6.2	12.8	190.4	5.6	-	-	-	58.2	151.3	-	-	-	-	47.3	225.8	131.0	<**0.001**
32	8.94	2-Heptanone	Ketone	884	889	Pino, *et al.* , 2006	-	11.4	-	-	24.4	-	-	-	-	-	132.4	-	-	-	-	-	-	-	<**0.01**
33	9.1	Propyl butanoate	Ester	890	896	Isidorov, *et al.* , 2001	3.7	3.0	3.2	42.0	32.0	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.01**
34	9.15	Heptanal	Aldehyde	892	894	Roussis, *et al.* , 2000	16.6	24.4	24.2	41.7	73.1	-	-	-	-	-	-	-	-	-	-	55.8	-	-	<**0.01**
35	9.21	Ethyl pentanoate	Ester	894	898	Isidorov, *et al.* , 2001	-	-	-	-	-	-	-	-	-	-	145.6	-	-	-	-	40.0	459.4	101.8	<**0.05**
36	9.54	Tricyclene	Monoterpene	909	907	Pitarokili, *et al.* , 2006	-	-	-	-	-	-	36.6	47.6	31.1	41.3	41.1	26.1	-	-	-	-	-	-	>**0.05**
37	9.59	Pentyl ethanoate	Ester	911	911	Larsen and Frisvad, 1995	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27.4	-	-	--
38	9.64	Anisole	Ether	913	918	Isidorov and Jdanova, 2002	-	-	-	-	-	-	-	-	-	7.5	-	-	-	-	-	-	-	-	--
39	9.68	Methyl hexanoate	Ester	915	924	Pino, *et al.* , 2005	-	-	-	54.8	256.5	-	-	-	-	-	-	-	-	-	-	52.0	212.6	-	<**0.05**
40	9.71	*α* -Thujene	Monoterpene	916	919	Özcan, *et al.* , 2003	8.7	-	-	-	-	-	34.0	59.0	10.1	37.4	-	14.6	16.3	26.4	117.5	36.0	-	-	<**0.01**
41	9.75	Ethyl 3-methyl-2-butenoate	Ester	918			-	-	-	-	-	-	-	-	-	22.0	237.9	-	-	-	-	-	-	-	<**0.05**
42	9.82	*α* -Pinene¶	Monoterpene	921	928	Kim, *et al.* , 2000	317.2	2106.8	381.2	435.3	1783.7	359.0	20041.9	20172.1	10581.7	18162.7	30283.4	11163.1	1043.6	3955.2	284.1	2663.3	1213.7	1942.8	<**0.001**
43	9.87	Ethyl, 3-hydroxy-butanoate	Ester	924			-	-	-	55.4	225.3	-	-	-	-	68.4	-	-	-	-	-	-	847.6	-	<**0.001**
44	10.01	Ethyl tiglate	Ester	930	938	Salido, *et al.* , 2004	-	-	-	176.5	1004.2	4.0	-	-	-	98.8	452.1	-	-	-	-	449.6	2374.5	650.2	<**0.001**
45	10.1	*α* -Fenchene	Monoterpene	934	939	Vichi, *et al.* , 2007	-	20.5	-	-	83.3	-	-	-	-	-	-	-	-	272.7	-	189.3	193.9	-	<**0.05**
46	10.15	Camphene¶	Monoterpene	936	936	Couladis, *et al.* , 2003	38.5	33.6	24.7	46.8	52.1	31.3	653.3	637.7	266.5	739.3	1340.9	323.8	44.3	176.7	44.2	-	-	-	<**0.001**
47	10.21	Ethyl (2*E*)-pentenoate	Ester	939			-	-	-	17.9	31.2	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.05**
48	10.34	2-Methylpropyl butanoate	Ester	945			-	-	-	-	30.6	-	-	-	-	28.2	14.3	-	-	-	5.8	26.6	333.0	163.9	<**0.01**
49	10.35	(2*E*)-heptenal	Aldehyde	945	953	Zhao, *et al.* , 2009	10.5	5.4	-	-	15.0	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.05**
50	10.5	Ethyl 2,3-epoxybutyrate	Ester	952			-	-	-	14.6	25.8	-	-	-	-	-	-	-	-	-	-	-	44.8	-	>**0.05**

51	10.54	Verbenene	Monoterpene	953	953	Figuérédo, *et al.* , 2006	-	-	-	-	-	-	15.4	61.1	21.0	29.0	21.3	5.9	-	-	-	-	-	-	<**0.01**
52	10.66	Sabinene[¶]	Monoterpene	959	961	Kartal, *et al.* , 2007	5.4	7.5	6.2	4.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>**0.05**
53	10.72	*β* -Pinene	Monoterpene	962	963	Couladis, *et al.* , 2003	60.6	219.9	43.9	52.2	24.2	45.4	4314.0	7386.6	1619.9	4273.7	8459.5	2115.7	193.6	85.8	48.2	219.6	103.9	486.6	<**0.001**
54	10.84	1-Octen-3-ol	Alcohol	967	969	Tzakou, *et al.* , 2006	10.0	7.6	8.3	6.1	8.9	-	-	-	-	-	-	-	-	-	-	-	-	-	>**0.05**
55	10.97	3-Methyl-3-cyclohexen-1-one	Ketone	973			5.1	7.0	5.9	11.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.05**
56	11.03	Myrcene[¶]	Monoterpene	976	981	Kartal, *et al.* , 2007	79.1	1970.6	287.6	24.1	2685.4	45.6	17945.2	118154.8	5108.6	19118.5	48599.1	9519.8	33.9	10747.1	47.7	3277.5	1747.6	1510.0	<**0.001**
57	11.11	Butyl butanoate	Alcohol	979	993	Jordán, *et al.* , 2002	-	-	-	10.0	-	-	-	-	-	-	-	-	19.4	-	-	-	-	-	>**0.05**
58	11.19	*α* -Terpinene	Monoterpene	983	1008	Basta *et al.* , 2006	-	-	-	-	-	-	-	-	-	-	-	-	-	156.0	32.6	-	-	-	<**0.05**
59	11.2	Ethyl hexanoate	Ester	983	996	Pino, *et al.* , 2005	-	-	40.0	5318.0	11355.8	264.9	-	-	-	583.0	1997.3	-	-	-	315.5	6716.4	28864.5	2720.0	<**0.001**
60	11.26	*n* -Octanal	Aldehyde	986	991	Xian, *et al.* , 2006	36.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
61	11.27	*α* -Phellandrene[¶]	Monoterpene	986	996	Kartal, *et al.* , 2007	25.9	745.5	53.4	-	-	33.1	1202.9	8825.2	238.0	1094.4	2816.4	550.8	51.2	-	22.0	-	-	-	<**0.001**
62	11.31	Ethyl (3*E*)-hexenoate	Ester	988	987	Zhao, *et al.* , 2008	-	-	-	29.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
63	11.39	*δ* -3-Carene[¶]	Monoterpene	992	1003	Bonaiti, *et al.* , 2005	2436.6	28166.3	11879.1	4668.8	35298.1	5189.5	669.4	260.1	101.6	93.0	78.8	195.6	8974.2	96010.7	8207.8	32564.8	39867.9	25189.2	<**0.001**
64	11.51	*δ* -2-Carene[¶]	Monoterpene	997	998	Saroglou, *et al.* , 2006	4.1	250.2	29.4	20.3	162.1	29.1	227.3	1565.1	83.4	291.7	713.3	102.2	-	-	-	-	-	-	<**0.001**
65	11.66	*p* -Cymene	Monoterpene	1005	1018	Saroglou, *et al.* , 2007	131.5	38.9	43.4	36.4	64.3	30.2	57.3	591.1	30.5	255.3	974.0	41.2	639.9	1750.9	-	169.9	196.3	356.7	<**0.001**
66	11.68	*o* -Cymene[¶]	Monoterpene	1006	1020	Tzakou and Couladis, 2001	39.6	-	-	-	-	-	-	-	-	-	-	-	-	29.1	-	-	99.6	226.9	<**0.01**
67	11.73	Limonene[¶]	Monoterpene	1010	1014	Couladis, *et al.* , 2003	58.8	2898.4	550.8	116.8	3641.6	98.7	-	-	-	-	-	-	-	11749.3	683.8	6664.1	3493.0	2928.8	<**0.001**
68	11.75	*β* -Phellandrene	Monoterpene	1010	1012	Song, *et al.* , 2000	-	-	-	-	-	-	6475.6	31005.3	925.0	5178.2	11909.3	2582.4	-	-	-	-	-	-	<**0.001**
69	11.91	(*Z*)-*β* -Ocimene[¶]	Monoterpene	1020	1022	Couladis, *et al.* , 2003	4.4	35.8	11.4	-	28.7	-	48.5	20049.6	-	47.1	82.1	-	-	111.6	-	55.9	-	-	<**0.001**
70	12.01	Ethyl hex-(2E)-enoate	Ester	1027			-	-	-	41.4	191.9	4.1	-	-	-	-	-	-	-	-	-	-	917.8	942.2	<**0.01**
71	12.09	(*E*)-*β* -Ocimene[¶]	Monoterpene	1031	1032	Couladis, *et al.* , 2003	30.2	43.0	29.8	18.3	59.3	7.9	497.3	15326.7	233.1	173.3	1185.7	14.1	27.2	890.4	-	154.1	-	-	<**0.001**
72	12.28	*γ* -Terpinene[¶]	Monoterpene	1043	1042	Couladis, *et al.* , 2003	29.3	63.0	29.5	21.8	138.2	9.7	752.5	5063.4	-	545.3	1112.5	-	48.6	1028.0	-	617.0	-	438.0	<**0.001**
73	12.42	Ethyl sorbate	Ester	1051	1093	Pino, *et al.* , 2005	-	-	-	-	-	-	-	-	-	-	71.9	-	-	-	-	-	-	-	--
74	12.46	(*E*)-Sabinene hydrate	Monoterpenoid	1053	1058	Özcan, *et al.* , 2003	-	-	-	-	-	-	-	109.6	-	37.8	79.9	-	-	-	-	-	-	-	>**0.05**
75	12.5	4-Methoxy-2,5-dimethyl-3(2H)-furanone	Ketone	1056	1057	Siegmund, *et al.* , 2001	-	-	-	-	-	-	-	-	-	155.1	726.9	-	-	-	-	-	26.5	-	<**0.01**
76	12.51	γ-Hexalactone	Lactone	1056	1056	Pino, *et al.* , 2005	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	51.6	-	--
77	12.55	*n* -Octanol	Alcohol	1059	1060	Tzakou, *et al.* , 2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	54.3	-	--
78	12.57	Linalool oxide	Monoterpenoid	1060	1075	Zhao, *et al.*, 2005	-	-	-	-	-	-	-	359.0	-	-	-	-	-	-	-	-	-	-	--
79	12.68	4-Ethyl-1,2-dimethyl-benzene	Benzenoid	1067	1078	Zhao, *et al.* , 2008	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40.5	-	--
80	12.71	*m* -Cymenene	Monoterpene	1068	1084	Andriamaharavo, 2014	-	-	-	-	23.7	-	-	-	-	-	-	-	-	-	-	-	-	-	--
81	12.8	Terpinolene[¶]	Monoterpene	1074	1072	Couladis, *et al.* , 2003	30.9	2749.7	244.9	66.0	2731.9	69.6	46.4	2253.1	38.7	46.3	321.9	-	433.5	14598.6	48.1	3154.8	1297.5	1571.5	<**0.001**
82	12.89	6,7-Epoxymyrcene	Monoterpene	1080			-	-	-	-	-	-	-	502.3	-	-	-	-	-	-	-	-	-	-	--
83	12.96	Propyl hexanoate	Ester	1084	1089	Isidorov, *et al.* , 2001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	198.8	-	--
84	12.98	*p* -Cymenene	Monoterpene	1085	1086	Siani, *et al.* , 2004	-	-	-	-	-	-	-	-	-	-	-	-	-	80.3	40.8	40.4	-	-	>**0.05**
85	13	Linalool[¶]	Monoterpene	1086	1090	Gkinis, *et al.* , 2003	-	-	-	-	-	-	18.1	677.9	22.8	69.4	-	5.3	-	-	-	-	-	-	<**0.01**
86	13.01	Ethyl heptanoate	Ester	1087	1091	Mahattanatawee, *et al.* , 2005	-	-	-	126.5	335.0	12.6	-	-	-	-	49.9	-	-	-	52.5	84.4	728.2	907.2	<**0.01**
87	13.05	Isopropyl hexanoate	Ester	1089			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65.9	--
88	13.06	*n* -Nonanal[¶]	Aldehyde	1090	1092	Tzakou, *et al.* , 2004	53.2	37.7	43.5	195.8	83.2	15.1	-	766.7	-	33.0	156.7	-	-	-	33.9	137.0	698.5	420.2	<**0.001**
89	13.21	1,3,8-p-Menthatriene[¶]	Monoterpene	1099			-	43.6	-	-	33.6	-	-	-	-	-	-	-	-	-	-	-	-	-	>**0.05**
90	13.24	(*Z*)-Methyl 4-octenoate	Ester	1101			-	-	-	29.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
91	13.25	2-Methyl-6-methylene-1,7-octadien-3-one	Monoterpenoid	1102	1117	Liu, *et al.* , 2010	-	-	17.5	-	57.9	-	9.0	551.8	-	-	-	-	-	-	-	-	-	-	<**0.01**
92	13.38	Methyl octanoate	Ester	1109	1120	Pino, *et al.* , 2010	-	-	-	193.4	606.9	20.2	-	-	-	44.0	-	-	-	-	18.7	85.5	920.5	230.9	<**0.01**
93	13.51	Ethyl 3-hydroxy hexanoate	Ester	1117	1130	Jordán, *et al.* , 2002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1965.2	-	--
94	13.52	*allo* -Ocimene[¶]	Monoterpene	1117	1128	Radulovic, *et al.* , 2007	-	-	-	-	-	-	-	107.1	-	-	48.7	-	-	-	-	-	-	-	>**0.05**
95	13.68	(*E*)-Pinocarveol	Monoterpenoid	1126	1130	Basta, *et al.* , 2005	-	-	-	-	-	-	-	-	-	32.4	79.7	-	-	-	-	-	-	-	>**0.05**
96	13.8	2-methylpropyl hexanoate	Ester	1133	1143	Jordán, *et al.* , 2002	-	-	-	7.3	32.9	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.05**
97	13.91	Menthone	Monoterpenoid	1140	1142	Tepe, *et al.* , 2005	-	-	-	-	-	-	-	-	-	-	55.3	-	-	-	-	-	-	-	--
98	13.95	2-Methyl-2-nonen-4-one	Ketone	1142			-	-	-	-	-	-	-	-	-	33.9	-	-	-	-	-	-	-	-	--
99	13.99	p-Mentha-1,5-dien-8-ol	Monoterpenoid	1145	1156	Basta, *et al.* , 2005	-	-	-	-	24.4	-	-	-	-	-	-	-	-	33.4	-	42.4	31.8	437.8	<**0.05**
100	14.05	Pinocarvone	Monoterpenoid	1149	1158	Alma, *et al.* , 2004	-	-	-	-	-	-	-	-	-	18.3	123.2	-	-	-	-	-	43.3	-	<**0.05**
101	14.11	Methyl (2*E*)-octenoate	Ester	1152			-	-	-	33.2	29.3	-	-	-	-	-	-	-	-	-	-	-	-	-	>**0.05**

102	14.28	Terpinen-4-ol¶	Monoterpenoid	1162	1169	Saroglou, *et al.*, 2007	-	-	-	-	-	-	20.2	-	31.2	48.0	343.4	-	-	-	-	-	-	-	<**0.05**
103	14.35	(3*Z*)-Hexenyl isobutanoate	Ester	1166	1142	Figuérédo, *et al.*, 2005	-	-	-	-	-	-	-	-	-	-	22.8	-	-	-	-	-	-	-	--
104	14.4	Ethyl-(4E)-octenoate	Ester	1169			29.9	37.2	23.5	1145.4	2604.9	52.3	-	-	-	25.0	276.6	-	-	187.4	15.8	430.4	2193.7	252.3	<**0.001**
105	14.49	Cryptone	Monoterpenoid	1174	1178	Ferhat, *et al.*, 2007	-	-	-	-	-	-	-	446.8	-	-	-	-	-	-	-	-	-	-	--
106	14.52	Ethyl octanoate	Ester	1176	1195	Tesevic, *et al.*, 2005	-	-	304.4	15216.9	22858.5	1822.7	-	502.3	-	1635.3	5129.3	-	24.1	354.2	1757.7	12524.5	41486.8	8498.3	<**0.001**
107	14.56	Methyl salicylate	Ester	1178	1185	Pérez, *et al.*, 2007	52.0	707.4	197.8	-	-	-	-	1002.7	-	60.0	-	-	-	-	-	-	-	-	<**0.01**
108	14.66	*n*-Decanal¶	Aldehyde	1184	1185	Pavlovic, *et al.*, 2006	38.2	104.8	-	583.7	173.3	44.6	-	-	-	-	-	-	-	-	-	-	581.9	-	<**0.01**
109	14.79	Verbenone	Monoterpenoid	1192	1195	Gkinis, *et al.*, 2003	-	-	-	-	-	-	-	409.5	-	-	610.5	-	-	-	-	-	-	-	>**0.05**
110	14.98	Umbellulone	Monoterpenoid	1203	1184	Zhao, *et al.*, 2006	20.1	41.0	39.8	27.7	88.1	6.3	-	-	-	-	-	-	-	232.6	-	451.8	301.8	517.1	<**0.001**
111	15.26	Ethyl oct-(2*E*)-enoate	Ester	1222	1246	Bicalho, *et al.*, 2000	-	-	-	589.0	1586.7	47.4	-	-	-	-	-	-	-	-	-	-	-	104.8	<**0.01**
112	15.33	2-Methylbutyl hexanoate	Ester	1227	1246	Jordán, *et al.*, 2002	-	-	-	-	40.7	-	-	-	-	-	-	-	-	-	-	-	-	-	--
113	15.42	Car-3-en-2-one	Monoterpenoid	1233	1254	Andriamaharavo, 2014	11.4	24.9	13.4	34.1	225.5	-	-	-	-	-	217.6	-	13.4	199.6	27.2	572.8	4267.7	547.9	<**0.001**
114	15.48	*γ*-Octalactone	Lactone	1237	1256	Isidorov, *et al.*, 2001	-	-	-	41.2	117.2	-	-	-	10.7	-	-	-	-	-	-	-	476.7	-	<**0.01**
115	15.55	*p*-Ethyl acetophenone	Monoterpenoid	1242			22.3	39.0	24.5	-	42.8	-	-	-	-	-	-	-	-	-	-	-	-	-	>**0.05**
116	15.58	3-Caren-10-al	Monoterpenoid	1243			-	-	-	-	-	-	-	-	-	-	-	-	-	45.8	-	-	-	-	--
117	15.86	Propyl octanoate	Ester	1262			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	47.8	77.2	>**0.05**
118	15.9	1-Methylethyl octanoate	Ester	1265			-	-	-	63.3	287.7	9.1	-	-	-	-	-	-	-	-	-	-	-	-	<**0.01**
119	16.15	(*Z*)-Methyl 4-decenoate	Ester	1281			-	-	-	125.7	281.4	15.3	-	-	-	-	-	-	-	-	-	-	131.2	76.4	<**0.05**
120	16.29	Piperitenone	Monoterpenoid	1291	1315	Bertoli, *et al.*, 2011	33.2	70.4	67.4	-	143.8	11.6	-	60.0	-	-	-	-	-	278.9	25.2	358.8	412.9	53.8	<**0.001**
121	16.35	Methyl decanoate	Ester	1294	1322	Zhao, *et al*., 2008	-	-	-	25.4	50.5	-	-	-	-	-	-	-	-	-	-	-	-	-	>**0.05**
122	16.45	Diethyl hydroxy butanedioate	Ester	1302	1271	Andriamaharavo, 2014	-	-	-	79.7	179.6	-	-	-	-	-	-	-	-	-	-	-	258.1	-	<**0.05**
123	16.62	Isobazzanene	Sesquiterpene	1313			-	-	-	-	-	-	-	-	-	-	16.8	-	19.0	97.2	29.2	113.7	76.8	51.8	<**0.001**
124	16.65	Isobutyl caprylate	Ester	1316	1348	Andriamaharavo, 2014	-	-	-	31.4	349.7	-	-	-	-	-	-	-	-	-	-	-	101.6	17.8	<**0.01**
125	16.67	δ-Elemene	Sesquiterpene	1317			-	-	-	-	-	-	11.0	-	23.0	19.1	-	4.0	-	-	-	-	-	-	<**0.05**
126	16.79	*α*-Cubebene	Sesquiterpene	1326	1350	Flach, *et al.*, 2004	-	-	-	-	-	-	63.2	953.5	39.1	32.8	53.4	4.9	17.9	193.2	9.4	104.5	91.2	15.4	<**0.001**
127	17.11	Ethyl-(4*E*)-decenoate	Ester	1349			15.4	15.7	91.8	3360.3	6222.1	502.6	-	-	-	-	-	-	-	-	-	450.1	5869.0	703.6	<**0.001**
128	17.12	*α*-Ylangene	Sesquiterpene	1350	1352	Couladis, *et al.*, 2003	-	-	-	-	-	-	6.7	42.8	7.6	31.4	27.6	3.5	7.5	-	-	-	5440.5	-	<**0.001**
129	17.19	*α*-Copaene	Sesquiterpene	1355	1359	Couladis, *et al.*, 2003	25.9	52.1	21.0	-	-	-	941.7	7106.7	100.7	145.1	366.6	26.2	403.3	1765.4	147.2	885.0	-	355.2	<**0.001**
130	17.21	Butyl caprylate	Ester	1356	1387	Jordan, *et al.*, 2006	-	-	-	136.5	339.5	-	-	-	-	-	-	-	-	-	-	-	1742.6	-	<**0.01**
131	17.29	Ethyl decanoate	Ester	1362	1373	Saroglou, *et al.*, 2006	-	46.2	-	528.7	1058.9	58.1	-	-	-	-	124.9	-	-	-	-	649.6	3495.1	-	<**0.001**
132	17.3	*β*-Bourbonene	Sesquiterpene	1362	1374	Özcan, *et al.*, 2003	-	-	-	-	-	-	-	60.7	-	34.3	57.1	-	10.6	56.8	271.5	645.9	-	122.8	<**0.001**
133	17.32	7-epi-sesquithujene	Sesquiterpene	1364			32.4	-	16.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>**0.05**
134	17.36	*β*-Elemene¶	Sesquiterpene	1367	1373	Couladis, *et al.*, 2003	-	-	-	-	-	-	61.3	310.9	34.8	13.9	-	13.7	102.4	77.7	67.9	-	-	595.8	<**0.001**
135	17.5	(2*E*)-Tetradecen-1-ol	Alcohol	1377			-	-	-	50.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
136	17.64	*α*-Gurjunene	Sesquiterpene	1387	1381	Pavlovic, *et al.*, 2006	98.9	411.9	118.4	50.5	117.5	31.8	341.1	1496.6	25.6	68.8	118.3	21.7	1139.8	775.8	166.0	768.0	305.4	174.4	<**0.001**
137	17.79	(*E*)-Caryophyllene¶	Sesquiterpene	1398	1398	Basta, *et al.*, 2006	191.6	2031.1	685.1	249.6	1195.7	127.5	1070.8	7809.9	133.2	379.5	502.3	65.9	585.2	2767.5	381.8	2252.9	867.9	472.3	<**0.001**
138	17.9	*β*-Copaene	Sesquiterpene	1406	1422	Basta, *et al.*, 2007	18.9	10.7	14.9	34.9	57.7	10.9	23.2	187.7	19.2	66.3	68.6	12.4	56.5	73.8	68.8	241.2	267.5	103.9	<**0.001**
139	17.97	*α*-Guaiene	Sesquiterpene	1412	1413	Basta, *et al.*, 2006	-	-	-	-	-	-	1075.2	6070.6	76.6	38.2	167.9	13.4	-	-	-	-	-	-	<**0.001**
140	18	2-Pentyl octanoate	Ester	1414		Zhao, *et al.*, 2008	-	-	-	9.5	170.8	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.05**
141	18.02	Aromadedrene	Sesquiterpene	1415	1418	Basta, *et al.*, 2006	-	-	-	-	-	-	-	-	16.4	65.2	60.7	9.0	12.3	28.9	21.7	31.9	-	45.4	<**0.001**
142	18.05	(*Z*)-Eudesma-6,11-diene	Sesquiterpene	1418			-	-	-	-	-	-	81.2	333.2	8.8	-	-	-	-	-	-	-	-	-	<**0.01**
143	18.07	Dauca-5,8-diene	Sesquiterpene	1420			-	-	-	-	-	-	-	-	-	-	-	-	17.6	23.6	21.9	105.9	177.4	39.6	<**0.01**
144	18.09	Geranyl ethanone	Ketone	1421			-	-	-	57.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
145	18.15	(*Z*)-Muurola-3,5-diene	Sesquiterpene	1425	1414	Pitarokili, *et al.*, 2006	-	-	-	-	-	-	308.6	2087.0	26.2	35.4	43.8	-	-	-	-	-	-	-	<**0.01**
146	18.23	*α*-Humulene¶	Sesquiterpene	1431	1428	Mahmood, *et al.*, 2004	106.2	1142.0	383.2	169.5	794.3	65.7	763.9	3933.5	79.6	185.0	253.4	21.5	291.3	1517.0	174.6	1181.4	391.0	201.9	<**0.001**
147	18.26	Ethyl-(2*E*,4*Z*)-decadienoate	Ester	1434			-	-	-	82.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
148	18.32	9-*epi*-(*E*)-Caryophyllene	Sesquiterpene	1438	1465	Pino, *et al.*, 2005	-	-	-	-	-	-	57.3	655.7	45.7	254.3	228.7	38.7	-	-	-	-	-	-	<**0.01**
149	18.32	(*Z*)-Muurola-4(14),5-diene	Sesquiterpene	1439	1445	Gkinis, *et al.*, 2003	18.2	37.5	17.0	69.9	70.1	21.1	-	-	-	-	-	-	127.1	132.3	99.2	284.2	289.0	167.5	<**0.001**
150	18.42	4,5-di-epi-aristolochene	Sesquiterpene	1446			11.7	86.4	24.9	-	71.5	-	-	-	-	-	-	-	-	-	-	-	-	-	<**0.01**
151	18.46	*γ*-Muurolene	Sesquiterpene	1449	1457	Couladis, *et al.*, 2003	-	-	-	-	-	-	284.8	608.5	56.8	384.9	334.2	55.1	75.1	244.3	35.4	189.1	157.0	56.1	<**0.001**
152	18.55	*γ*-Gurjunene	Sesquiterpene	1456	1459	Monsef-Esfahani, *et al.*, 2010	-	-	-	-	-	-	856.2	5078.8	218.9	119.8	293.4	19.3	-	-	-	-	-	-	<**0.001**

153	18.56	Germacrene D	Sesquiterpene	1457	1461	Couladis, *et al.* , 2003	16.5	25.4	41.9	369.5	377.5	99.9	-	-	-	-	-	-	802.8	770.0	984.9	3200.8	3693.1	1993.0	**<0.001**
154	18.64	*β* -Selinene	Sesquiterpene	1463	1464	Couladis, *et al.* , 2003	85.4	547.4	139.9	45.1	431.0	19.2	261.8	1294.2	145.7	132.5	40.9	-	-	471.5	218.2	367.3	319.4	96.4	**<0.001**
155	18.72	*α* -Selinene	Sesquiterpene	1469	1470	Vichi, *et al.* , 2005	91.5	138.6	46.2	27.1	70.2	47.5	2004.5	8920.5	-	-	246.5	-	-	841.4	-	-	-	-	**<0.001**
156	18.76	Bicyclogermacrene	Sesquiterpene	1472	1483	Hammami, *et al.* , 2011	-	-	-	32.7	39.2	-	-	-	233.4	144.5	-	41.2	421.7	-	265.8	925.5	647.4	480.5	**<0.001**
157	18.8	Tridecanal	Aldehyde	1475	1506	Lazari, *et al.* , 2000	-	-	-	48.8	56.8	-	-	-	-	-	-	-	-	-	-	156.9	230.6	-	**<0.05**
158	18.85	*α* -Bulnesene	Sesquiterpene	1479	1506	Ogunwande, *et al.* , 2010	-	-	-	-	-	-	2681.0	7508.6	389.9	63.8	230.4	-	-	-	-	-	-	-	**<0.01**
159	18.94	Methyl dodecanoate	Ester	1486	1521	Zhao, *et al.* , 2008	-	51.2	13.6	49.9	65.9	-	-	-	-	-	-	-	-	-	-	-	183.2	-	**<0.01**
160	18.95	*γ* -Cadinene	Sesquiterpene	1487	1493	Couladis, *et al.* , 2003	-	-	-	-	-	-	274.1	337.3	113.6	135.3	231.7	12.3	22.2	46.6	11.1	76.6	-	65.3	**<0.001**
161	19.04	*δ* -Cadinene	Sesquiterpene	1494	1503	Couladis, *et al.* , 2003	31.9	37.8	15.0	21.5	40.7	6.7	808.5	3526.9	329.6	449.0	403.8	32.3	72.6	268.7	64.8	218.9	184.5	128.5	**<0.001**
162	19.16	(*E*)-Cadina-1,4-diene	Sesquiterpene	1504	1527	Hazzit, *et al.* , 2006	-	-	-	-	-	-	515.9	892.8	42.5	125.8	94.2	117.2	-	64.3	-	40.1	35.1	-	**<0.01**
163	19.32	*α* -Calacorene	Sesquiterpene	1517	1522	Couladis, *et al.* , 2003	-	-	-	-	-	-	-	170.9	-	36.5	19.4	-	-	-	-	12.3	37.4	-	**<0.01**
164	19.46	(*E*)-Nerolidol	Sesquiterpenoid	1529	1533	Song, *et al.* , 2000	115.8	282.3	98.1	120.7	65.6	-	-	-	-	-	-	-	-	-	-	-	-	-	**<0.05**
165	19.47	Ethyl 4-decenoate	Ester	1529			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	64.0	316.2	5.9	**<0.01**
166	19.77	Ethyl dodecanoate	Ester	1554	1576	Tesevic, *et al.* , 2005	20.2	3075.9	171.9	2030.4	1971.9	132.1	9.0	258.9	-	8.7	120.7	-	-	-	-	-	-	-	**<0.001**
167	19.85	Caryophyllene oxide¶	Sesquiterpenoid	1561	1561	Couladis, *et al.* , 2003	82.3	-	94.1	-	-	-	-	456.9	-	-	20.0	-	-	-	-	-	-	-	**<0.05**
168	20	Tetradecanal	Aldehyde	1573	1601	Xie, *et al.* , 2008	18.9	-	20.2	26.6	40.7	-	-	-	-	-	-	-	-	-	-	-	-	-	**>0.05**
169	20.15	Humulene epoxide II	Sesquiterpenoid	1585	1605	Mancini, *et al.* , 2009	15.7	54.7	36.1	-	64.9	-	-	112.4	-	-	-	-	-	295.5	54.0	1152.1	7618.9	643.7	**<0.001**
170	20.31	1-epi-Cubenol	Sesquiterpenoid	1598	1619	Feizbakhsh and Naeemy, 2011	-	-	-	-	-	-	41.1	246.0	-	-	-	-	-	-	-	-	-	-	**<0.05**
171	20.43	(*Z*)-Cadina-1(6),4-diene	Sesquiterpene	1609			-	-	-	-	-	-	-	-	-	-	-	-	-	31.8	15.9	122.4	102.9	16.7	**<0.01**
172	20.63	*α* -Cadinol	Sesquiterpenoid	1626	1624	Pavlovic, *et al.* , 2006	124.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65.8	203.5	-	**<0.01**
173	20.64	Pogostol	Sesquiterpenoid	1627			-	-	-	-	-	-	-	220.7	-	-	-	-	-	-	-	-	-	-	**--**
174	20.88	Propyl dodecanoate	Ester	1648	1685	Quijano, *et al.* , 2007	-	26.8	10.7	-	-	-	-	-	-	-	-	-	-	-	-	7.2	140.9	-	**<0.05**
175	20.9	Ethyl tridecanoate	Ester	1650	1687	Pino, *et al.* , 2005	-	-	-	35.7	34.7	-	-	-	-	-	-	-	-	-	-	-	-	-	**>0.05**
176	20.97	2-Pentadecanone	Ketone	1656	1694	Mancini, *et al.* , 2009	39.9	11.4	22.3	28.1	27.1	11.5	-	-	-	-	-	-	-	-	-	115.7	392.5	7.7	**<0.001**
177	21.16	(2*E*)-Tridecenol	Alcohol	1672			58.9	29.9	38.1	126.9	149.5	60.7	-	-	-	-	-	-	-	-	57.3	251.4	395.3	50.5	**<0.01**
178	21.25	Methyl tetradecanoate	Ester	1680	1723	Ansorena, *et al.* , 2001	-	14.9	20.0	61.2	71.5	-	-	-	-	-	-	-	-	-	-	59.1	161.7	-	**<0.01**
179	21.87	*n* -Butyl laurate	Ester	1749	1786	Quijano, *et al.* , 2007	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	125.2	-	**--**
180	21.99	Ethyl tetradecanoate	Ester	**Total n**	1784	Zhao, *et al.* , 2008	18.4	365.8	62.1	2255.6	2167.2	81.7	10.8	66.4	-	7.6	85.6	-	-	32.2	18.3	844.5	9502.5	178.0	**<0.001**
181	22.54	6,10,14-trimethyl-2-pentadecanone	Ketone	1823	1835	Mancini, *et al.* , 2009	18.9	13.5	10.8	24.7	17.0	-	-	-	-	-	-	-	-	-	-	-	-	-	**>0.05**
182	22.74	Lauric anhydride	Anhydride	1841			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	73.4	-	**--**
183	23.37	Methyl hexadecanoate	Ester	1900	1903	Payo, *et al.* , 2011	-	-	-	26.6	57.8	-	-	-	-	-	-	-	-	-	-	-	27.4	-	**>0.05**
184	23.38	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Ketone	1901	1916	Andriamaharavo, 2014	17.3	12.5	11.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**>0.05**
185	23.45	2-Methyl-hexadecanal	Aldehyde	1908			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-	-	**--**
186	23.54	Isobutyl myristate	Ester	1919			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.9	-	**--**
187	23.61	Cyclohexadecanolide	Ester	1925	1928	Lopes, *et al.* , 2004	15.2	2.5	2.7	22.1	12.1	5.1	-	10.8	-	-	-	-	-	-	11.5	10.0	56.8	5.4	**<0.001**
188	23.64	9-Hexadecenoic acid	Organic acid	1929	1942	Zhao, *et al.* , 2008	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.1	-	**--**
189	23.82	Ethyl 9-hexadecenoate	Ester	1949	1978	Andriamaharavo, 2014	-	169.4	53.0	39.1	49.4	-	-	-	-	-	-	-	-	12.4	-	-	125.5	-	**<0.01**
190	23.89	(3*Z*)-Cembrene A	Diterpene	1956	1938	Petrovic, *et al.* , 2006	-	-	-	-	-	-	-	258.8	-	-	-	-	-	-	-	-	-	-	**--**
191	24.13	Ethyl hexadecanoate	Ester	1983	1992	Zhao, *et al.* , 2008	-	66.5	31.2	425.9	1134.7	5.4	-	-	-	-	-	-	-	13.3	12.7	36.3	1663.2	1.3	**<0.001**
192	24.75	Kaurene	Diterpene	2048	2043	Grujic-Jovanovic, *et al.* , 2004	10.4	5.3	4.7	-	4.9	-	-	-	-	-	-	-	-	-	-	-	-	-	**>0.05**
193	24.82	Abietatriene	Diterpene	2056	2056	Lazari, *et al.* , 2000	-	2.1	2.3	3.1	-	2.0	-	-	-	-	-	-	-	-	-	-	-	-	**>0.05**
194	25.91	Ethyl oleate	Ester	2174	2173	Custer, 2009	-	-	-	-	12.2	-	-	-	-	-	-	-	-	-	-	-	5.6	-	**>0.05**
		Total mean volatile concentration (ng µL^{-1})					**5025.6e**	**49342.8b**	**16864.7c**	**54499.5b**	**118179.4a**	**10022.3d**	**65617.9**	**297972.1a**	**21520.7e**	**60211.7c**	**131725.6b**	**27172.7d**	**15760.4e**	**153601.9b**	**15326.8e**	**94024.9c**	**202638a**	**60748.6d**	**<0.001**

Infestation affected the volatile released both qualitatively (Figure 2-9 A) and quantitatively (Figure 2-9 B), 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 2-9 B, Table 1).

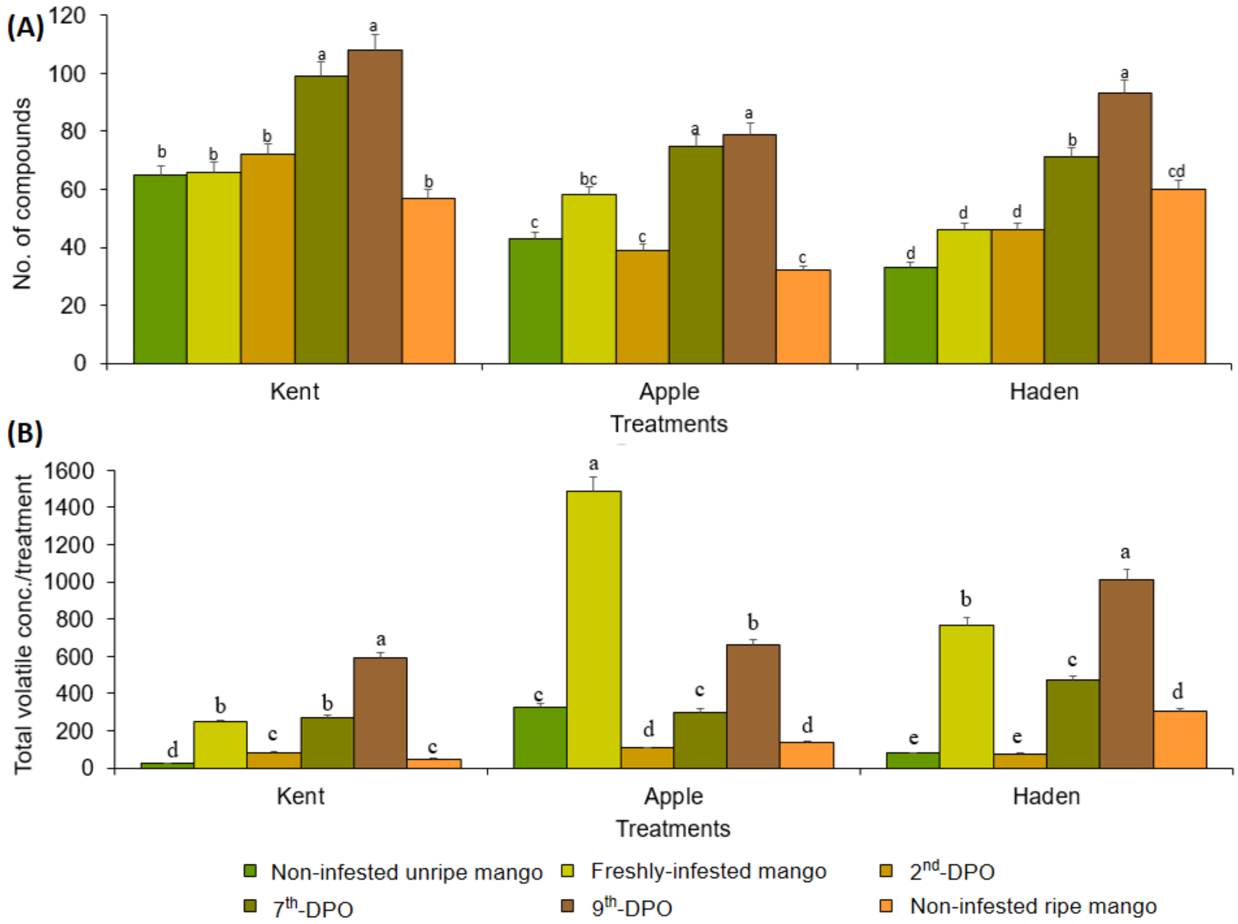

Figure 2-9: The number of volatile organic compounds tentatively identified from the different mango varieties under the six different treatments. Bars capped with different letters, for the same mango variety, are significantly different (Pearson’s Chi-square test followed by Chi-square multi-comparison test in RVAideMemoire) (A). Totals of the average volatile concentrations (ng µL^{-1}) of the different mango treatments of the three varieties (B). Bars capped with different letters for the same mango variety are significantly different (Kruskal-Wallis rank-sum test followed by post hoc Dunn test for pairwise comparison)

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 2-10; Appendix: Figure S2-1).

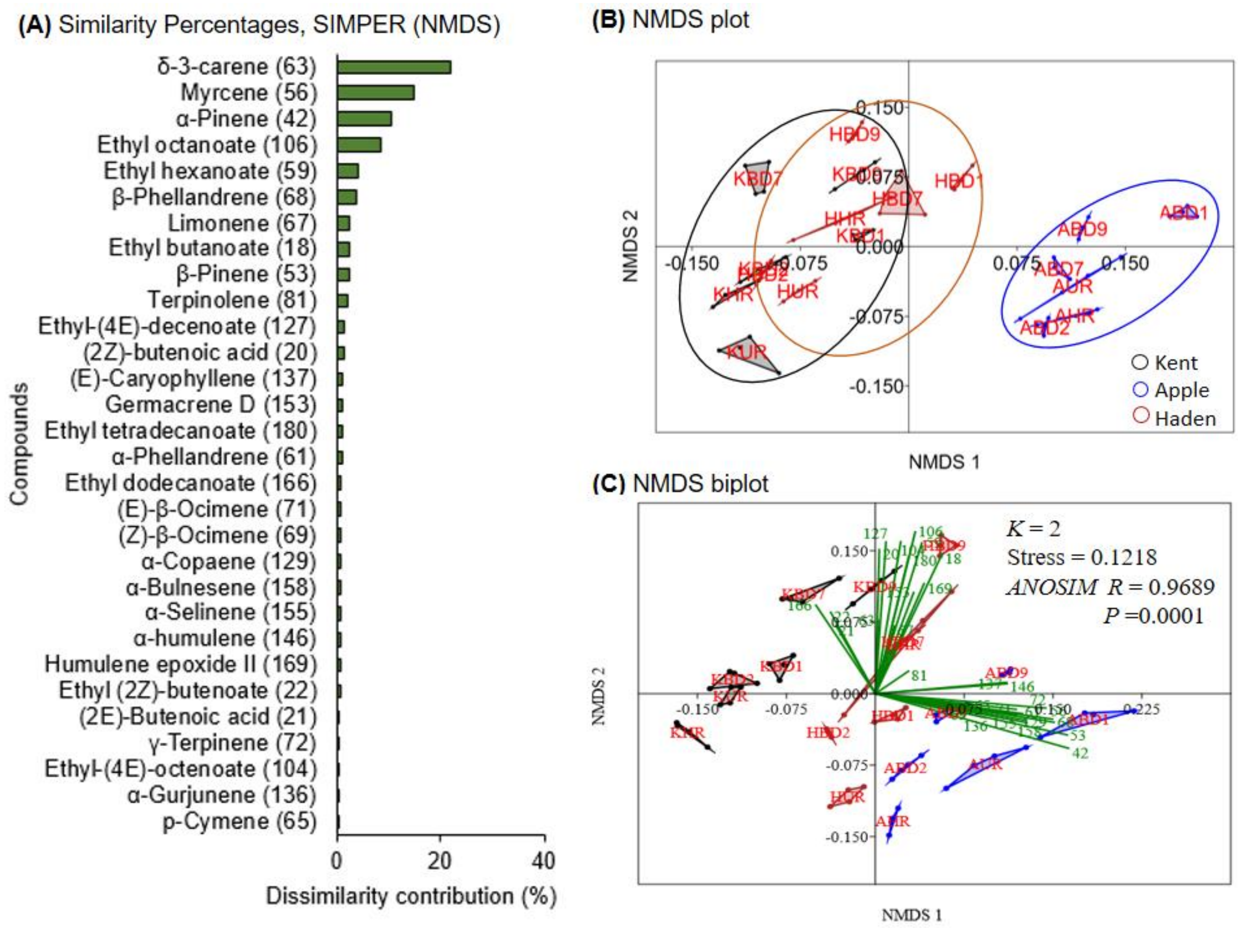

Figure 2-10: (A) Similarity percentage (SIMPER) of the non-metric multidimensional scaling (NMDS) showing the first 30 topmost discriminant volatiles. (B) The NMDS plot shows the scattering of the treatments from the three varieties of mangoes. (C) The NMDS biplots for the differentiation of the discriminant volatiles in the treatments (*k = dimensional number*; K = Kent, A = Apple, H = Haden, BD = *B. dorsalis*, UR = non-infested unripe mango, HR = non-infested ripe mango, 1 = freshly-infested mango, 2 = 2nd-DPO, 7 = 7th-DPO, and 9 = 9th-DPO)

The 30 topmost discriminant volatiles of all treatments of the three mango varieties contributed 89.8% of the total dissimilarity contribution. The highest contributors and their percentage dissimilarity contributions were *δ*-3-carene (21.9), myrcene (14.9), *α*-pinene (10.6), ethyl octanoate (8.5), ethyl hexanoate (4.1), *β*-phellandrene (3.9), and limonene (2.6) (Figure 2-10 A). Volatile compounds of Kent and Haden mango varieties, which overlap, were scattered far from those of Apple mango variety (Figure 2-10 B). Furthermore, the 30 most discriminating volatiles of the three mango varieties were more associated with mango volatiles of freshly-infested mangoes, 7th-DPO, and 9th-DPO mangoes (Figure 2-10 C).

Considering the treatments per mango variety, the multivariate analytical tool showed different discriminants of the volatile organic compounds (VOCs). The 30 topmost discriminant volatiles among Kent mango volatiles as per the non-metric multidimensional scaling's (NMDS) similarity percentages, SIMPER are graphically presented in Figure 2-11 A where δ-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.

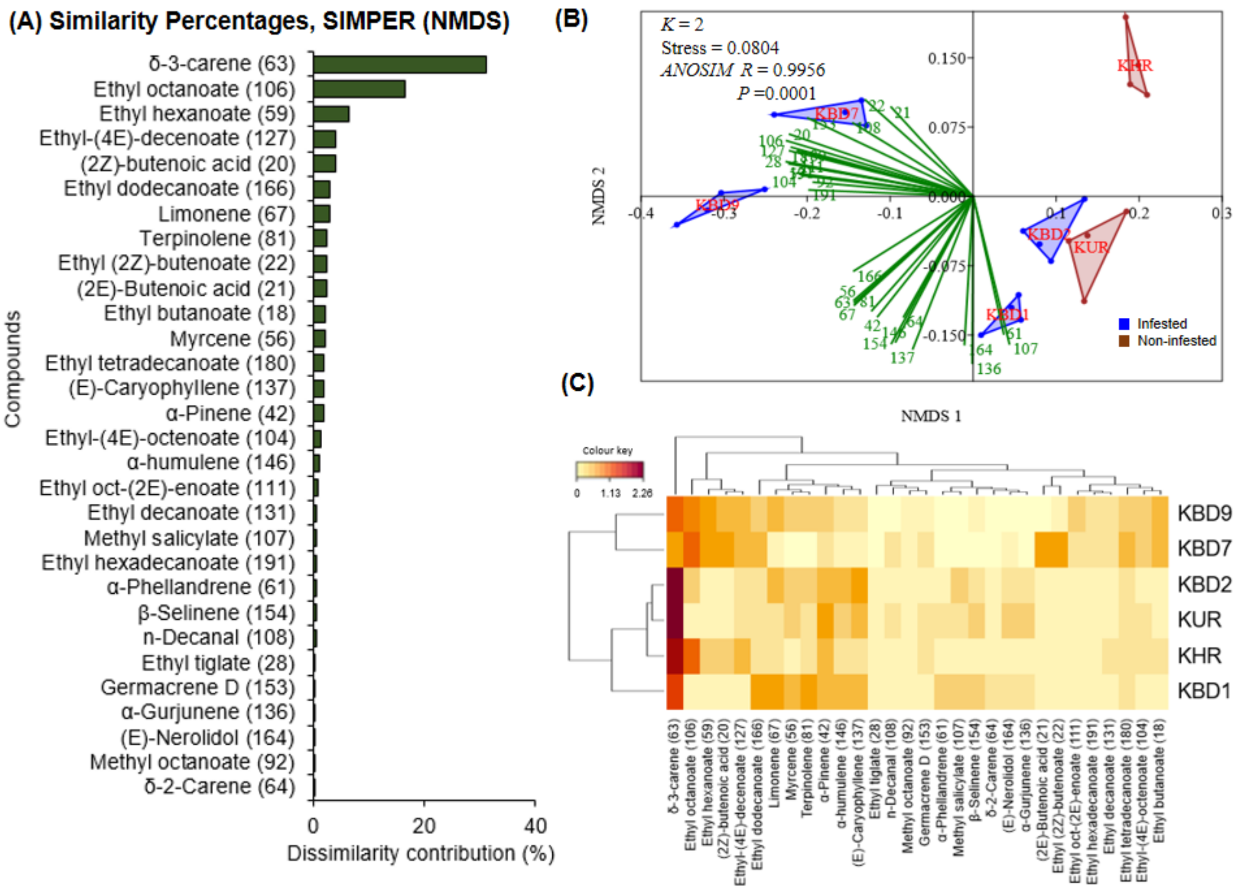

Figure 2-11: (A) The 30 topmost discriminant volatiles of all treatments of Kent mango variety in their decreasing importance based on the non-metric multidimensional scaling's (NMDS) similarity percentage (SIMPER); (B) NMDS biplots for the differentiation of the 30 selected compounds and how they correlate to the mango treatments; (C) Heatmap clustering of the auto-scaled volatile concentration ($y = log_{10} x + 1$) of the 30 compounds. The darker the brown colour the higher the concentration (k = dimensional number; KBD1 = *B. dorsalis*-freshly-infested Kent mangoes; KHR = non-infested ripe Kent mango; KUR-non-infested unripe Kent mango; KBD2 = 2^{nd}-DPO Kent mangoes; KBD7 = 7^{th}-DPO; and KBD9 = 9^{th}-DPO mangoes)

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 2-11 B; Appendix: Figure S2-2). More than 90% of the 30 selected compounds were associated with the volatiles emanating from freshly-infested Kent mangoes (KBD1), 7th-DPO (KBD7), and 9th-DPO (KBD9) post-oviposition mango fruits (Figure 2-11 B). The heatmap clustering (Figure 2-11 C) 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 colour were released at higher rates. For example, δ-3-carene (C63) was released at a higher rate except on the 7th-DPO (Figure 2-11 C).

Furthermore, there was a significant difference in the volatile concentrations of non-infested unripe Kent mango (KUR), freshly-infested Kent mangoes (KBD1), and 2nd-DPO (KBD2) ($\chi^2 = 27.17$, $df = 2$, $P < 0.001$). In pairwise comparison, there was a significant difference between volatile concentrations 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 concentrations of common volatiles on freshly-infested mangoes (KBD1) and 2nd-DPO (KBD2) compared to those of non-infested unripe Kent mangoes (KUR). The following are examples of some compounds that were among the 30 most discriminant compounds (NMDS) together with their number of fold changes i.e. compound (no. of fold change of freshly-infested mangoes (KBD1); no. of fold change of 2nd-DPO (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 2-12 A). On the other hand, there was a significant difference in the volatile concentrations of non-infested ripe Kent mango (KHR), 7th-DPO (KBD7), and 9th-DPO (KBD9) mangoes ($\chi^2 = 121.76$, $df = 2$, $P < 0.001$). The pairwise comparison indicated a significant difference between KBD9 & KHR, KBD7 & KHR ($P < 0.001$), and KBD7 & KBD9 ($P < 0.05$) (Figure 2-12 B). There were changes in the concentrations of common compounds on 7th-DPO and 9th-DPO headspaces compared to those of non-infested ripe mangoes (KHR). Examples of compounds that were among the 30 discriminant compounds (NMDS) with their quantities of fold change on 7th-DPO and 9th-DPO headspaces volatiles respectively compared their counterparts in 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 2-12 B). Other than changes in folds, 47 compounds 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, α-fenchene, and methyl (2*E*)-octenoate.

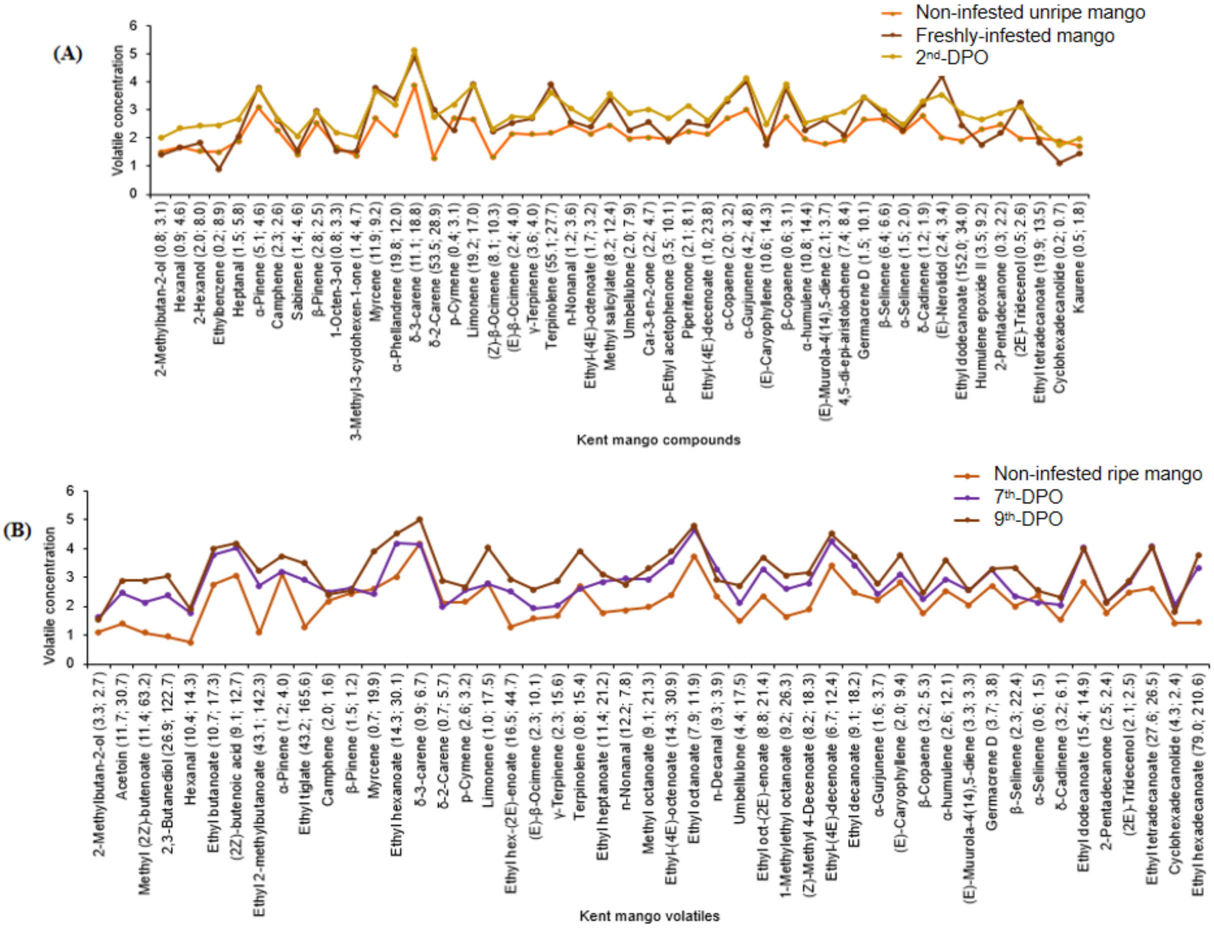

Figure 2-12: Trends in the change of volatile concentrations of the common compounds in headspaces of; (A) non-infested unripe Kent mangoes, *B. dorsalis*-freshly-infested Kent mangoes, and 2nd-DPO Kent mango with the number of fold changes of the common compounds relative to those of non-infested unripe Kent mango; (B) Non-infested ripe Kent mangoes and 7th-DPO, and 9th-DPO Kent mangoes with their number of fold changes relative to those of non-infested ripe Kent mango

For all the treatments of Apple mango variety, the 30 topmost discriminant volatiles as selected by the non-metric multidimensional scaling's (NMDS) similarity percentages, SIMPER are graphically presented in Figure 2-13 A; Appendix: Figure S3. Of these compounds, myrcene, *α*-pinene, *β*-phellandrene, *β*-pinene, (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene, *α*-phellandrene, *α*-bulnesene, *α*-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 2-13 B) and heatmap (Figure 2-13 C).

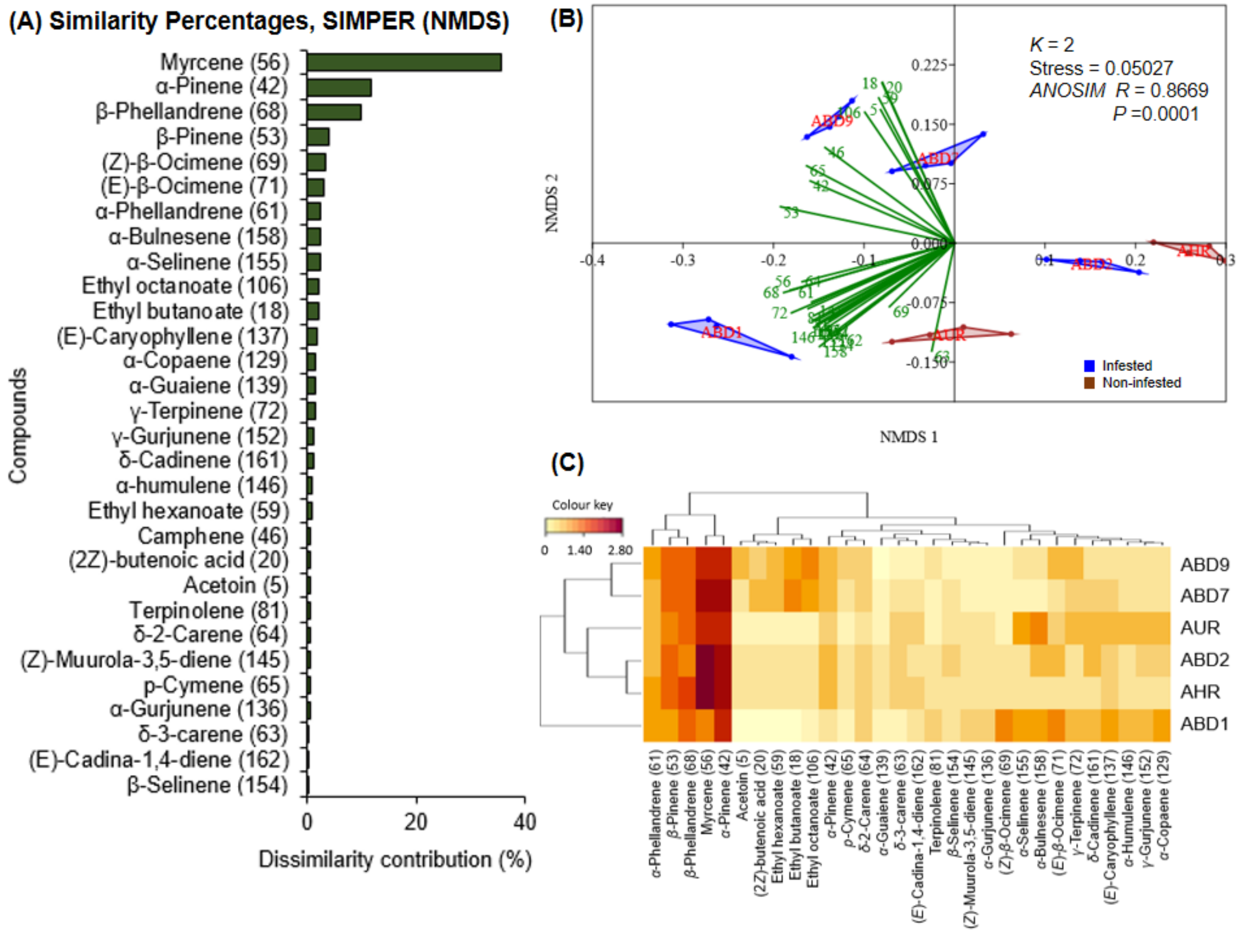

Figure 2-13: (A) The 30 topmost discriminant compounds of all treatments of Apple mango in their decreasing importance based on the non-metric multidimensional scaling's (NMDS) similarity percentage (SIMPER); (B) NMDS biplots for the differentiation of the selected compounds and how they correlate to the mango treatments; (C) Heatmap clustering of the auto-scaled volatile concentration ($y = log_{10} x + 1$) of the 30 discriminant compounds. The darker the brown colour the higher the concentration. (*k* = *dimensional number*; ABD1 = *B. dorsalis*-freshly-infested Apple mangoes; AHR = non-infested ripe Apple mango; ABD2 = 2nd-DPO Apple mangoes; AUR-non-infested unripe Apple mango; ABD7 = 7th-DPO; and ABD9 = 9th-DPO mangoes)

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 concentrations (Figure 2-13 B; Appendix: Figure 2-3). Of the 30 topmost discriminant compounds, over 80% were associated with volatiles of freshly-infested Apple mangoes (ABD1) and 9th-DPO

Apple mangoes (ABD9) (Figure 2-13 B). The heatmap (Figure 2-13 C) shows the distribution of the selected discriminant compounds amongst the Apple mango treatments with their concentrations corresponding to the intensity of brown colour, e.g. the dark brown colour of myrcene (56) and α-pinene (42) indicates that they had the highest concentrations in most treatments (Figure 2-13 C). The dendrograms also show the correlation of the volatiles within and between mango treatments.

There was a significant difference in the volatile concentrations of non-infested unripe Apple mangoes (AUR), freshly-infested Apple mangoes (ABD1), and 2nd-DPO Apple mangoes (ABD2) ($\chi^2 = 44.5$, $df = 2$, $P < 0.001$). On pairwise comparison, there were significant differences between ABD1 & ABD2 ($P < 0.001$), ABD1 & AUR ($P < 0.001$), and ABD2 & AUR ($P < 0.05$). There were changes in the quantities of common compounds in the volatiles of freshly-infested mangoes (ABD1) and 2nd-DPO mangoes (ABD2) relative to those of non-infested unripe mangoes (Figure 2-14 A). Furthermore, there was a significant difference in the volatile concentrations of non-infested ripe (AHR), 7th-DPO (KBD7), and 9th-DPO (KBD9) Apple mangoes ($\chi^2 = 103.77$, $df = 2$, $P < 0.001$). Pairwise comparison indicated significant differences between ABD7 & AHR ($P < 0.001$), ABD9 & AHR ($P < 0.001$), and ABD7 & ABD9 ($P < 0.05$). Most of the common compounds in the volatiles of 7th-DPO and 9th-DPO mangoes showed an increase in the number of folds relative to those of non-infested ripe mangoes (Figure 2-14 B). A total of 52 volatiles including acetoin, ethyl propanoate, methyl butanoate, isopentyl formate, 2,3-butanediol, ethyl butanoate, (2*Z*)-butenoic acid, and ethyl 2-methyl butanoate were detected in headspaces of infested but not in non-infested mangoes.

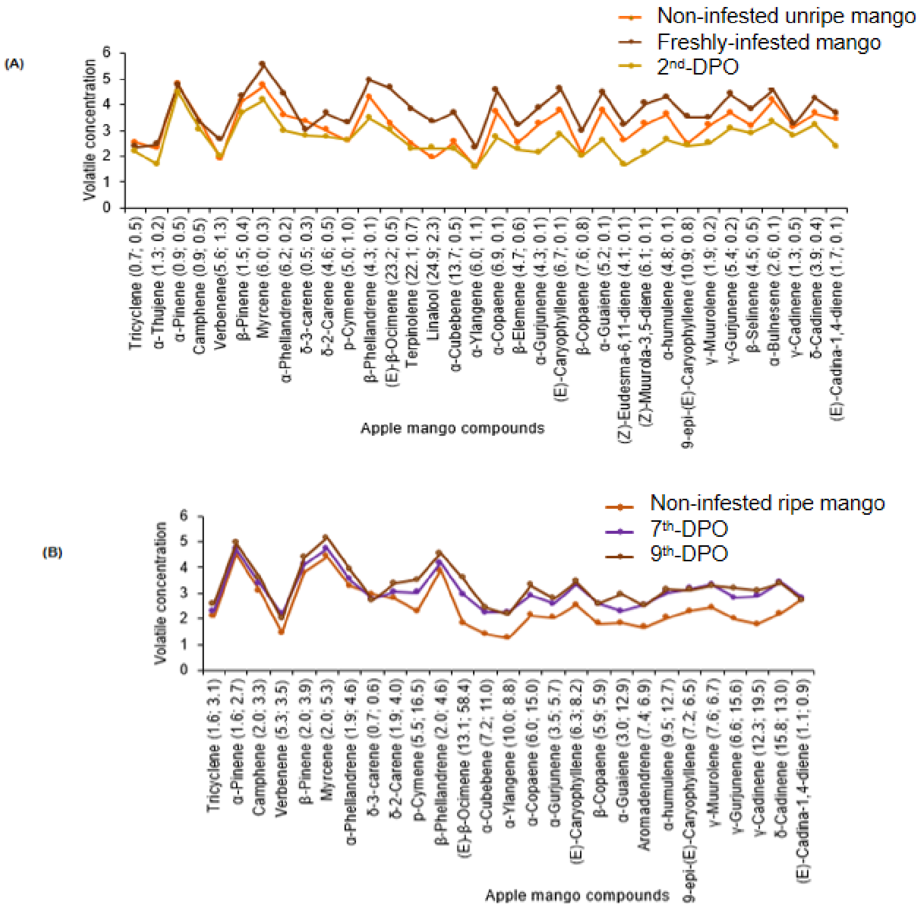

Figure 2-14: Trends in the change of volatile concentrations of the common compounds in headspaces of; (A) non-infested unripe Apple mangoes, *B. dorsalis*-freshly-infested Apple mangoes and 2nd-DPO Apple mangoes with the number of fold changes of the common compounds relative to those of non-infested unripe Apple mango; (B) Non-infested ripe Apple mangoes, 7th-DPO and 9th-DPO Apple mangoes with their number of fold changes relative to those of non-infested ripe Apple mango

For all treatments of 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 2-15 A, Appendix: Figure S2-4. Out of these compounds, δ-3-carene, ethyl octanoate, ethyl hexanoate, limonene, ethyl butanoate, terpinolene, myrcene, ethyl tetradecanoate, α-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 2-15 B) and heatmap (Figure 2-15 C) for visualization of their distributions in the treatment headspaces.

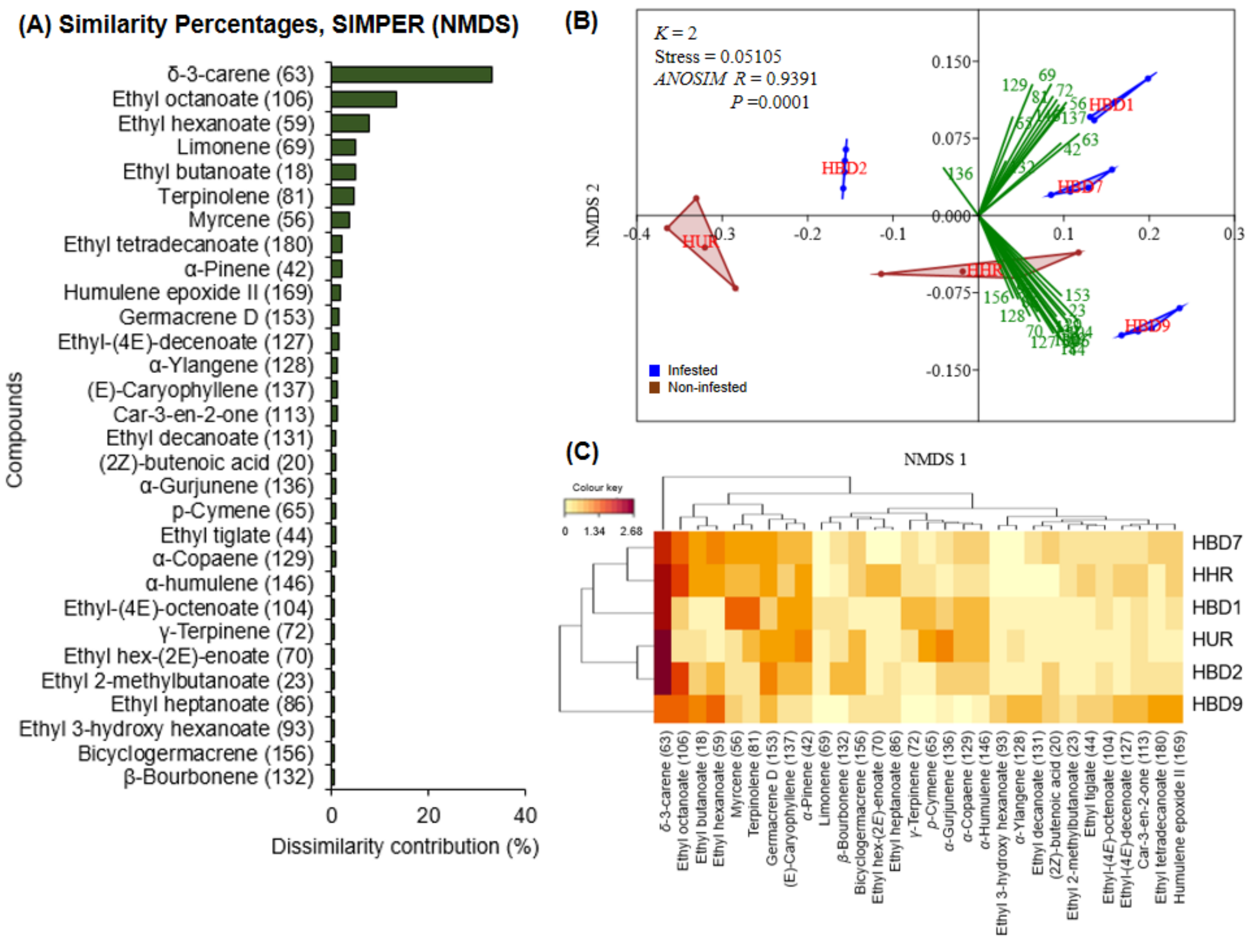

Figure 2-15: (A) The 30 topmost discriminant volatiles of all treatments of Haden mango variety in their decreasing importance based on the non-metric multidimensional scaling's (NMDS) similarity percentage (SIMPER); (B) NMDS biplots for the differentiation of the 30 selected compounds showing how they correlate to the mango treatments; (C) Heatmap clustering of the auto-scaled volatile concentration ($y = log_{10} x + 1$), of the compounds. The darker the brown colour the higher the concentration (*k* = *dimensional number*; HBD9 = 9th-DPO Haden mango; HBD2 = 2nd-DPO Haden mango; HUR-non-infested unripe Haden mango; HBD1 = *B. dorsalis*-freshly-infested Haden mangoes; HHR-non-infested ripe Haden mango, and HBD7 = 7th-DPO Haden mango)

Like in Kent and Apple mangoes, the NMDS indicated a significant difference among the volatile concentrations of the different treatments of Haden mangoes (*k* = 2, stress = 0.05105; one-way analysis of similarity, ANOSIM: *R* = 0.9391, *P* < 0.0001) (Figure 2-15 B). More than 75% of the selected discriminant volatiles were associated with volatiles of *B. dorsalis*-freshly-infested (HBD1), 7th-DPO (HBD7), and 9th-DPO (HBD9) Haden mango (Figure 2-15 B). The heatmap clustering (Figure 2-15 C) shows how the selected compounds were distributed in mango treatments while the dendrograms show how they relate within and between treatments. The volatile whose concentration was high in Haden treatments was *δ*-3-carene hence having an intense brown colour (Figure 2-15 C).

On comparing the volatile concentrations of non-infested unripe Haden mango (HUR), freshly-infested Haden mangoes (HBD1), and 2nd-DPO mangoes (HBD2), there was a significant difference ($\chi^2 = 13.07$, $df = 2$, $P < 0.01$). The pairwise comparison indicated a significant difference between HBD1 & HUR ($P < 0.001$) while there were no differences between HBD1 & HBD2 and HBD2 & HUR ($P > 0.05$). There were notable changes in the volatile concentrations of common compounds of *B. dorsalis* freshly-infested and 2nd-DPO mangoes relative to those of non-infested unripe Haden mangoes (Figure 2-16 A). A significant difference was also found among volatile concentrations of non-infested ripe (HHR), 7th-DPO (HBD7), and 9th-DPO (KBD9) Haden mangoes ($\chi^2 = 21.66$, $df = 2$, $P < 0.001$). 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 2-16 B). Other than changes in the abundance of common compounds, 46 volatiles among them methyl butanoate, isopentyl formate, 2-methyl-1-butanol, 2,3-butanediol, (2*Z*)-butenoic acid, 3-methylbutyl ethanoate, methyl hexanoate, *α*-fenchene, and 3-acetyl-2-octanone were detected only in *B. dorsalis* infested Haden mango treatments.

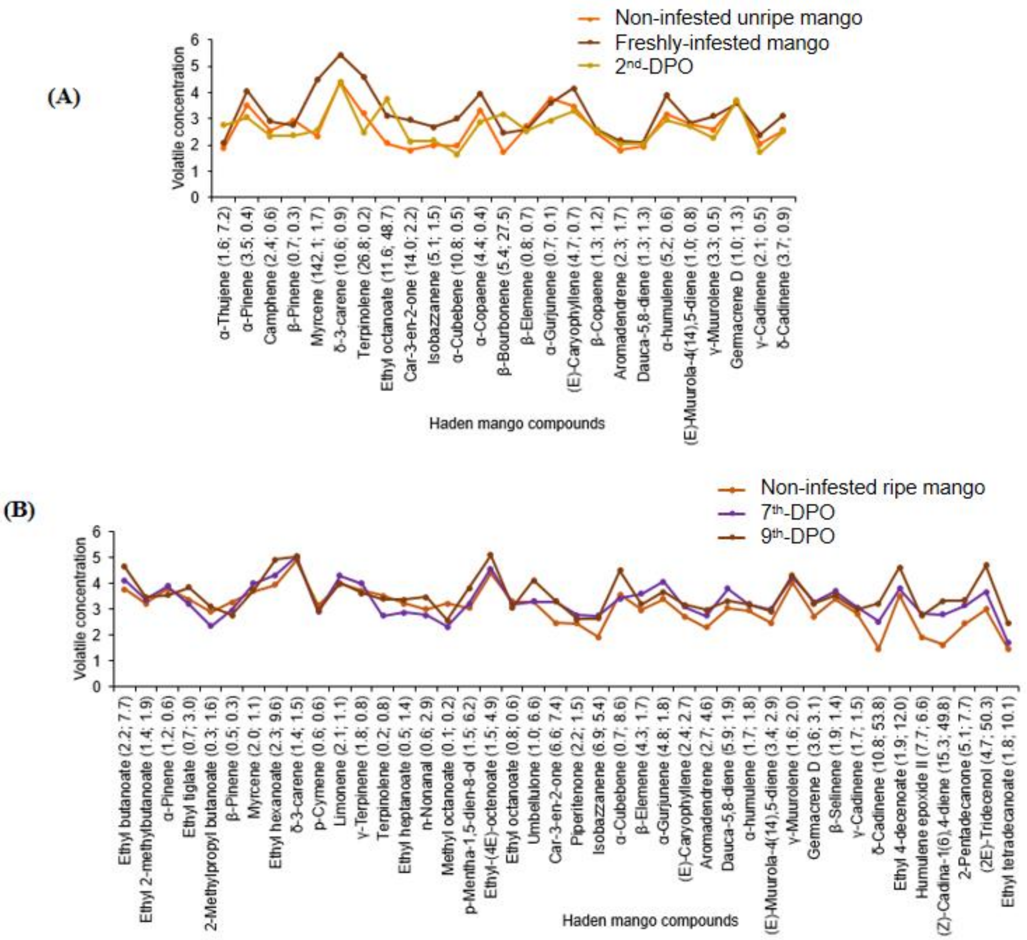

Figure 2-16: Trends in the change of volatile concentrations of the common compounds in headspaces of; (A) non-infested unripe Haden mangoes, *B. dorsalis*-freshly-infested mangoes, and 2nd-DPO mangoes with the number of fold changes of the common compounds relative to those of non-infested unripe Haden mango. (B) Non-infested ripe Haden mangoes, 7th-DPO and 9th-DPO mango compounds with their number of fold changes relative to those of non-infested ripe Haden mango

In the first 10 most discriminant volatiles by the three multivariate analyses tools, (*Z*)-*β*-ocimene and ethyl octanoate were selected as discriminant volatiles in the three mango varieties while *α*-pinene, myrcene, ethyl hexanoate, δ-2-carene, (*E*)-*β*-ocimene, *γ*-terpinene, humulene epoxide II, *δ*-3-carene, limonene, and terpinolene were from 2 mango varieties.

2.6 Discussion

2.6.1 Behavioral assays of *B. dorsalis* and parasitoids to tree-attached non-infested and infested mangoes

Emphasis 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 & Clarke, 2021). In our study, the behaviors of *B. dorsalis* and its parasitoids were conducted using headspace volatiles of tree-attached mangoes in a dual-choice olfactometer *in situ*. In all assays, both *B. dorsalis* and the parasitoids were attracted differentially to the tree-attached mango volatiles compared to 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 towards volatiles of *B. dorsalis*-freshly-infested mangoes and to conspecific-infested mangoes. Possibly, odors from ovipositing conspecific females and/or damaged mangoes signified a suitable host for consequent oviposition as argued by Nishida, (2014) and Masry *et al*. (2018). Similar findings were reported for the congenic *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 & Clarke, 2021). In this study, female *B. dorsalis* were attracted to the headspace volatiles of all treatments of Apple and Haden mango varieties and only to ripe and freshly-infested Kent. Roh *et al.*, (2021) reported that *B. dorsalis* females which were ready to oviposit were highly attracted to host odor. The attraction of *B. dorsalis* to unripe mangoes of the two varieties represents an important finding on the timing of IPM implementation for the control of this fruit fly.

As expected, the egg parasitoid *F. arisanus* was attracted to *B. dorsalis*-freshly-infested mangoes 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 & 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 & Vargas, 2007). We also found that volatiles of non-infested ripe Apple mango 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* may have evolved to utilize volatiles emitted by the preferred ripe fruit of its host fruit fly, hence enhancing tephritid host finding (Nanga Nanga *et al.*, 2019).

The attraction of *D. longicaudata* to volatiles of *B. dorsalis* 7th-DPO and 9th-DPO Apple and 9th-DPO Haden mangoes, and not to infested Kent signify that the fruits with developing larvae produce attractive volatiles compared to those that do not have. *D. longicaudata* was reported to be attracted to volatiles of mango fruits which were infested with larvae of *Anastrepha ludens* (Loew) (Diptera: Tephritidae), but not to mechanically damaged mangoes (Carrasco *et al.*, 2005; García-Medel *et al.*, 2007). Furthermore, we found that the magnitude of *D. longicaudata* attraction to Apple and Haden varieties was higher on the 9th-DPO than the 7th-DPO indicating its preference for the older larval stage. The findings corroborate the report by Harbi *et al.* (2019) on the responses of the same parasitoid to volatiles of *C. capitata*-infested mango fruits tested at different infestation ages. *D. longicaudata* was attracted to non-infested ripe mangoes of the three varieties, indicating that its olfactory circuitry has also evolved sensitivity cues that increase the chances of encountering tephritid hosts (Altuzar *et al.*, 2004; Rousse *et al.*, 2005). It would be interesting to find out how non-infested and infested tree-attached mango headspace compounds contribute to the host-finding efficiency of the parasitoids.

2.6.2 Performance of *B. dorsalis* on the different mango varieties

The discrepancy in the numbers of *B. dorsalis* puparia recovered from the three varieties of mangoes indicates that the fruit fly 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).

Unlike in Apple and Haden mango varieties, there were no *B. dorsalis* puparia that were recovered from Kent mangoes though fresh oviposition punctures were observed on freshly-infested mangoes of the three varieties. This implies that Kent variety is less preferred by *B. dorsalis*. 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, the stage of fruit maturity, the ease of the fruit fly ovipositor penetrating the pericarp, and the chemical composition of the fruit and its ability to sustain the full development of the fruit fly (Diatta *et al.*, 2013; Kamala *et al.*, 2014; Boinahadji *et al.*, 2020) usually affect the performance and survival of insect offspring. Apple mango constituted a better environment (223 puparia) for the fruit fly larvae development. Further studies 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.

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

In this study, *δ*-3-carene, myrcene, *α*-pinene, *β*-pinene, *α*-gurjunene, *(E)*-caryophyllene, *β*-copaene, *α*-humulene, and *δ*-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 of harvested mangoes (Wetungu *et al.*, 2018; Shimizu *et al.*, 2021) and have been associated with the attraction of various insect pests (Benelli, Giunti, *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 concentrations 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 makeup (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 concentrations of monoterpenes and sesquiterpenes identified from the ripe mango of the three varieties were generally less compared to those of unripe mangoes. Monoterpenes are generally associated with the defense mechanisms of plants against herbivorous attack (Singh & Sharma, 2015; Olayemi, 2017), hence their decrease may explain the higher attraction of *B. dorsalis* to non-infested ripe mangoes. A study on the attractiveness of guava to 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 concentrations of most volatiles increased significantly compared to those of non-infested mangoes. An increase in the volatile concentration, 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 concentration of volatiles lead to the 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 7th-DPO and 9th-DPO mangoes of all varieties 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 concentrations 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 microorganisms 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 (Martins *et al.*, 2017; Shivaramu *et al.*, 2017). For example, of the 9th-DPO Apple mango headspace, an increase occurred in most common compounds relative to those of ripe mangoes 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 9th-DPO Apple mango and the increased attraction of *D. longicaudata*. 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 *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) infested and non-infested oranges.

2.7 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 less preferred by *B. dorsalis* hence deterring the fruit fly development while Apple is highly preferred and sought after. 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 and dynamic changes which occur in the headspace volatiles of tree-attached mangoes before, during, and after infestation by *B. dorsalis*, and how this correlates with the 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 not only an evolutionary ecological perspective but also of significance when developing baits that selectively attract the fly and not its natural enemies.

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Chapter 3: Mango headspace volatiles trigger differential responses of the Mango fruit fly *Ceratitis cosyra* and its parasitoids

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3.1 Abstract

Before the introduction of *Bactrocera dorsalis* (Hendel) to sub-Saharan Africa, *Ceratitis cosyra* (Walker) was economically the most important pest in mango production. Among the methods used for its bio-control was its native solitary parasitoid *Psyttalia cosyrae* (Wilkinson), which was later on supplemented by the exotic parasitoids *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) within the Integrated Pest Management systems. To understand the mango-*C. cosyra*-parasitoid tri-trophic interaction, we compared the responses of the fruit fly and the three parasitoids to the headspace volatiles of three mango varieties (Kent, Apple, and Haden), assessed the performance of the fruit fly in the mangoes, and identified the chemical profiles of their headspace volatiles. *Ceratitis cosyra* was attracted to both infested and non-infested mangoes (66-84% of responsive *C. cosyra*) and performed better in Kent mango (72.1% of the 287 puparia recovered from the three mango varieties) than in Apple and Haden varieties. *Fopius arisanus* was more attracted to mangoes volatiles of *C. cosyra*-freshly-infested mangoes (68-70%), while *P. cosyrae* and *D. longicaudata* were more attracted to the 9th-DPO mangoes (68-78%) compared to non-infested unripe and ripe mango volatiles. Gas chromatography-mass spectrometry revealed qualitative and quantitative differences in the volatiles of the treatments. Esters were the main components in the non-infested ripe, 7th-DPO and 9th-DPO mangoes, while monoterpenes and sesquiterpenes were the most dominant in the other treatments. These results improve our understanding of the chemical ecological interactions between the mango fruit fly and its parasitoids and offer prospects for the development of a semiochemical-based management approach for African fruit fly species.

Keywords: *in situ*. *Infested vs. non-infested*. *Parasitoids*. *Psyttalia cosyrae*. *GC-MS. Semiochemicals*

3.2 Introduction

Frugivorous tephritid fruit flies represent a key impediment to the horticultural industry in Africa (Ekesi, *et al.*, 2016). Among the Afrotropical native *Ceratitis* species, mango fruit fly (also known as marula fruit fly), *Ceratitis cosyra* (Walker) (Diptera: Tephritidae) is the most destructive species in sub-Saharan Africa (Steck, 2000 and reference therein; Gikonyo *et al.*, 2005). Although the pest is considered to be polyphagous (Weldon *et al.*, 2016), it has a marked preference for mango, *Mangifera indica* L (Anacardiaceae). Mango yield losses due to the infestation by this pest are estimated to be up to 30% if the pest is left unmanaged (Lux *et al.*, 2003a). Other high-value fruits that are attacked by this pest include common guava, *Psidium guajava* L. (Myrtaceae); custard apples, *Annona reticulata* L., soursop, and *Annona muricata* L. (both Annonaceae) as well as avocado, *Persea americana* Miller (Lauraceae) (Copeland *et al.*, 2006). In addition, to the direct fruit losses, being a quarantine pest, infestation by *C. cosyra* can result in export restrictions to quarantine-sensitive markets (Barnes, 2000).

Following the invasion and widespread of the alien fruit fly, *B. dorsalis* (=*B. invadens*) (Hendel) (Diptera: Tephritidae) (Lux *et al.*, 2003b; Drew *et al.*, 2005), it has been reported that *C. cosyra* had been displaced by the former (Ekesi et al., 2009). Nevertheless, *C. cosyra* remains a formidable challenge to mango production. This is because the pest is adapted to a wide geographical range whereas *B. dorsalis* is largely a low-land resident pest; suggesting that even though the pest has been displaced at low elevations it will remain to be the dominant pest at higher elevations (Copeland *et al.*, 2006). For example, in a study carried out in Kenya, it was reported that *C. cosyra* is distributed across the country at altitudes from 20 to 2,100m asl (Copeland *et al.*, 2006). This wide thermal tolerance makes *C. cosyra* a serious biosecurity risk and an eminent threat to important export mango varieties, such as Kent, a variety with an extremely low preference for *B. dorsalis* (Akol *et al.*, 2013; Miano *et al.*, 2022). *Certitis cosyra* is also considered a key pest of the wild, yet an important and highly cherished fruit, Marula (*Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae) in many African countries (Lux, et al., 2003b; Weldon *et al.*, 2016).

In Africa, traditionally, fruit flies are managed through the use of synthetic chemical insecticides, an approach with far-reaching consequences on One Health (the health of the people, animals and the general ecosystem), in addition to not being neither an effective nor sustainable approach. Efforts have been undertaken to identify biocontrol agents for the management of *C. cosyra*. For example, some isolates of the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) have been identified to be very potent against *C. cosyra* (Dimbi *et al.*, 2013). Also, in a laboratory study, Mohamed *et al.* (2003), found that *Psyttalia cosyrae* (Wilkinson) (Hymenoptera: Braconidae) yielded over 40% parasitism on this pest. However, the field parasitism of *C. cosyra* by this parasitoid has been very low (Copeland *et al.*, 2006).

Following the introduction of the egg-prepupal parasitoid, *F. arisanus* (Sonan) and larval-prepupal parasitoid, *D. longicaudata* (Ashmead) (both Hymenoptera: Braconidae) for classical biological control of *B. dorsalis* in Africa, these parasitoids were able to form a new association with *C. cosyra* with very outstanding performance and certainly complementing the role of indigenous parasitoids. For example, in a choice test involving six fruit fly species, Mohamed *et al.* (2008) demonstrated that *C. cosyra* was the most preferred and most accepted host of *D. longicaudata*. Another bio-based strategy that has been identified and could be explored for the management of this pest as an oviposition deterrent is the host marking pheromone, tripeptide glutathione (GSH) (Cheseto et al., 2017b). Indeed, the application of this compound under field conditions resulted in the reduction of *C. cosyra* infestation by up to 75% (Cheseto *et al.*, 2023). However, unlike the other fruit flies of economic importance in the genera *Bactrocera*, *Anastrepha* and *Rhagoletis*, research on plant semiochemicals for potential use in suppressing fruit flies in the genus *Ceratitis* (except for *C. capitata* (Wiedemann) (Diptera: Tephritidae)) is very scanty.

Semiochemicals play various vital roles in the bi- and tri-trophic, (host plant-herbivores-parasitoid) communication (Vandermoten *et al.*, 2012; Kamala *et al.*, 2014). For instance, in the case of tephritid communities, it has been well-documented that flies use plant semiochemicals to locate suitable host plants as oviposition sites (Siderhurst and Jang, 2006; Biasazin *et al.*, 2014; Cunningham *et al.*, 2016). Likewise, host plant volatiles, herbivore-induced plant volatiles (HIPVs), and herbivores related volatiles are shown to be exploited by fruit fly parasitoids in habitat and host location (Wang and Messing, 2003; Harbi *et al.*, 2019; Cai *et al.*, 2020).

Understanding the bi- and tri-trophic interaction of fruit-fruit flies-parasitoid system as mediated by semiochemicals emitted from infested and non-infested first trophic level (in this case fruits) is among the fundamental premises for the development of sound and sustainable management strategies of these pests. However, most studies involving fruit flies and parasitoid responses, performance, and collection of volatiles are carried out under laboratory set-ups. Without a doubt, the findings from these studies provide vital information; nevertheless, the studies that are undertaken in field settings are expected to give a true reflection of what is happening in nature, in terms of plant-herbivore-parasitoid interaction. In this regard, we have investigated the attraction and subsequent performance (in terms of the number of puparia recovered) of *C. cosyra* on tree-attached mango fruits of different ripening and infestation stages for three mango varieties: Kent, Apple and Haden. Furthermore, we assessed the response of the indigenous parasitoid, *P. cosyrae* and two introduced parasitoid species *F. arisanus* and *D. longicaudata* to infested and non-infested fruits of the three varieties, and identified changes in the volatile chemical composition following *in situ* infestation.

3.3 Materials and methods

3.3.1 Experimental mango fruits

This study was carried out under field conditions in Kirinyaga County, one of the major mango-producing regions in Kenya. The study site was at Mwea-East Sub-County, (00°41'39.8" S 037°24'26.7" E, 1158m asl). In a mango orchard free of insecticide spray, two mango trees each of Apple, Haden and Kent verities, with immature fruits, were used in the trials. The mangoes were safeguarded against insect pests using the protocol described in Miano *et al.* (2022) where mangoes were secured *in situ* with fine white nets mounted on 20 × 20 × 20 cm of 2.5 mm galvanized metallic wire cube frame cages. Each of these cages held a minimum of four mangoes depending on their size and proximity (for each mango variety, at least 32 mangoes were secured on the two trees). The use of a fine net provided a conducive environment, with adequate air circulation, and was easy to handle when assessing the mangoes. Duduthrin 1.75 EC powder, active ingredient Lambda-cyhalothrin (Twiga Chemical Industries Ltd, Nairobi, Kenya), was strewed at the base of each tree monthly to protect them from crawling insects (Figure 2-1). The tree-attached mangoes that reached non-infested physiological maturity were used for *in situ* studies.

3.3.2 *Ceratitis cosyra* and parasitoids colonies

Ceratitis cosyra and the parasitoids, *F. arisanus*, *D. longicaudata*, and *P. cosyrae* were reared at the insectary of the International Centre of Insect Physiology and Ecology (*icipe*), Duduville campus, (01° 13′ 25.3″ S, 36° 53′ 49.2″ E; 1600 m asl, Nairobi, Kenya). The *C. cosyra* colony was initiated from a cohort of flies obtained from field-collected infested mango fruits (Ekesi and Mohamed, 2011) and was infused with wild-collected flies and wasps once every six months to reduce inbreeding depression and laboratory adaptation. The colony was maintained at 26 ± 2 °C temperature, 50-60% RH, and a 12:12 h (L: D) photoperiod cycle. *Ceratitis cosyra* and the parasitoids used in the experiments were reared in Apple mangoes in Perspex cages (30 ×30×30 cm) which had a net-sleeved window (18 cm diameter) for food and water provision, while on the opposite side, a fine white net was mounted to allow air circulation (Miano *et al.*, 2022).

The adult fruit flies were fed on an artificial diet of finely ground sugar (Mumias Sugar Company, Nairobi Kenya) and enzymatic yeast hydrolysate (USB Corporation, Cleveland, OH) in the ratio of 3:1. Water was provided to the insects in Petri-dishes (8.8 cm diameter × 1.5 cm deep) to which pumice granules were added to prevent drowning. The parasitoids were also maintained at similar conditions but provided with water that was in soaked cotton wool, and fed on 70% honey (*Eco Honey*, *icipe*, Nairobi, Kenya). For initiating and maintenance of a mango-reared *C. cosyra* and the parasitoids (*F. arisanus*, *D. longicaudata*, and *P. cosyrae*), the procedure described by Miano *et al.* (2022) was used.

3.3.3 Responses of test insects (*C. cosyra*, *F. arisanus*, *D. longicaudata*, *P. cosyrae*) to volatiles of non-infested and *C. cosyra*-infested mangoes

Physiologically mature and unripe tree-attached mango fruits of the three varieties were used in the assays. A two-choice wind tunnel described in Figure 2-3 was used to assess the response of *C. cosyra* and the parasitoids, *in situ*. The experimental mangoes were put in mango holders made of Perspex glass which had an open oven bag (Lifetime Brands Europe Limited, KitchenCraft, Birmingham, UK) top that allowed securing of the mangoes. The mango holders had an air inlet and outlet. The airflow rate at each arm of the tunnel was maintained at 350 mL min $^{-1}$ and drawn from the center at 700 mL min $^{-1}$ using a portable vacuum field pump (Analytical Research System Inc. Gainesville, Florida, USA).

For each assay purpose, 10 females (8–14-day old *C. cosyra* and 8–12-day old for parasitoids, *F. arisanus*, *D. longicaudata*, and *P. cosyrae*) of each of the test insects (one species at a time) were randomly selected from cages containing a mixture of males and females (♂: ♀ = 1:1), placed in a releasing vial and left to acclimatize for 10 minutes. These insects were then released through the insect release point at the base of the wind tunnel and allowed 20 minutes to make a choice. Those insects that moved beyond the 30 cm mark from the release point were deemed to have chosen while those that remained within the 30 cm mark were considered non-responsive. Seven replicates were conducted for each insect species' choice test. To avoid positional bias, the treatment and control arms were changed between runs, and then clean air was passed through the apparatus for 20 minutes to stabilize its flow and remove the odors of previous experiments.

The two-choice experiment tests were as follows: (i) responses of *C. cosyra*, *F. arisanus*, *D. longicaudata*, or *P. cosyrae* to clean air (blank against blank); (ii) responses of *C. cosyra* and *F. arisanus* to headspace volatiles of non-infested physiologically mature unripe mangoes (UR) against blank; (iii) responses of *C. cosyra* and *F. arisanus* to the headspaces of *C. cosyra*-freshly-infested mature unripe mangoes (CC1) against non-infested unripe mangoes (UR); (iv) responses of *C. cosyra*, *D. longicaudata*, or *P. cosyrae* to the 7th-DPO mangoes (CC7) against non-infested ripening mangoes (NR1); (v) responses of *C. cosyra*, *D. longicaudata*, or *P. cosyrae* to the 9th-DPO mangoes (CC9) against non-infested ripening mangoes (NR1); (vi) responses of *C. cosyra*, *F. arisanus*, *D. longicaudata*, or *P. cosyrae* to non-infested ripe mango volatiles (NR2) against clean air (blank). On the day of oviposition (*C. cosyra*-freshly-infested), which was considered day 1 of infestation, 15 *C. cosyra* females were randomly selected from a mixture of males and females (♂: ♀ = 1:1), released into the mango holder containing four mangoes, and allowed 20 minutes to acclimatize before assays. These insects remained with the mangoes for 11 hours and were removed and placed in a separate cage in the evening.

To ascertain infestation of the mangoes by *C. cosyra*, the mangoes were assessed using a × 10 hand lens for fruit fly oviposition punctures and oozing sap. The experimental mangoes were returned to the netted cage every day to prevent them from any further attack. On the 10th day post-oviposition, the infested mangoes were harvested and incubated to assess the performance of *C. cosyra* in the three varieties of mangoes. Non-infested mangoes ripened several days after harvesting the infested ones i.e. Kent-15 days, Apple-9 days, and Haden-11 days, which made it impossible to compare the attractiveness of the insects among infested and non-infested ripe mangoes.

3.3.4 *In situ* collections of tree-attached mango headspaces

The *in situ* volatile collection was done simultaneously with behavioral experiments using dynamic headspace trapping (DHT) systems. The fine netting and the cages were removed and four tree-attached mangoes were put in an oven bag (Lifetime Brands Europe Limited, KitchenCraft, Birmingham, UK). Clean humidified air was pumped in and drawn out at 250 mL min $^{-1}$ using the field pumps described in section 3.3.3. Volatiles were trapped for 11 hours between 07:00 and 18:00 local time using HayeSep-Q mixed-phase sorbents (30 mg, copolymers of polydimethylsiloxane-divinylbenzene, PDMS-DVB) that were pre-cleaned with GC-grade dichloromethane (DCM). Headspace volatile collections included (i) clean air (an empty oven bag sampled as a method blank); (ii) non-infested mature unripe mangoes (UR); (iii) *C. cosyra*-freshly-infested mature unripe mangoes (CC1); (iv) the 2nd-DPO (CC2); (v) the 7th-DPO (CC7); (vi) the 9th-DPO (CC9); (vii) non-infested ripening mangoes (NR1) and non-infested ripe mangoes (NR2). After collection, the terminals of HayeSep-Q adsorbents holders (with their respective headspace volatile organic compounds) were sealed in Teflon tape (MAAT, UK), wrapped in aluminium foil, and placed on dry ice (Carbacid (CO_2) Limited, Carbacid Investment Limited, Nairobi, Kenya) in a cool box before transporting to *icipe* laboratories, Nairobi. Before analysis, the sorbent cartridges containing the trapped headspace volatiles were eluted using 200 µL of 99.9% dichloromethane (DCM), via high-purity nitrogen gas, into 2 mL glass vials and stored at -80 °C until use. The sorbent cartridges were then purged with nitrogen gas.

3.3.5 Chemical analysis of tree-attached mango fruit headspace volatiles

The chemical analysis of tree-attached mango fruit headspaces was done using gas chromatography linked mass spectrometry (GC-MS), on a 7890A gas chromatograph linked to a 5975C mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA) which was equipped with an HP-5 MS (5% phenyl- methylpolysiloxane) 30 m × 0.25 mm ID × 0.25 µm film thickness column. An aliquot (1 µL) of each headspace was injected into the GC in the splitless mode (270 °C and one minute splitless time) for analysis using helium as the carrier gas at a constant flow rate of 1.2 mL min^{-1}, at the following temperature profile: 5 min at 35 °C, it was then increased at10 °C/min to 280 °C and held for 10.5 min. The mass selective detector and the quadrupled temperature were respectively retained at 230 °C and 180 °C, while the electron impact

(EI) mass spectra were obtained at 70 eV. Furthermore, the mass range of 40–550 *m/z* was used to analyze the fragment ions in the full scan mode, and the filament delay time was set at 3.3 min.

For the qualitative identification of compounds, the mass spectrometric data were compared to those of reference spectra published in the library–MS databases Adams, Chemecol, and the National Institute of Standards and Technology (NIST 05, 08, and 11) at mass spectral fit above 70%. The retention indices (RI) for each compound were also computed using the Van den Dool and Kratz equation of $C_5 - C_{31}$ straight-chain alkanes and comparing them with values from the literature (Dool and Kratz, 1963; Adams, 1996). Some of the compounds were also authenticated using standards (Figure 3-1).

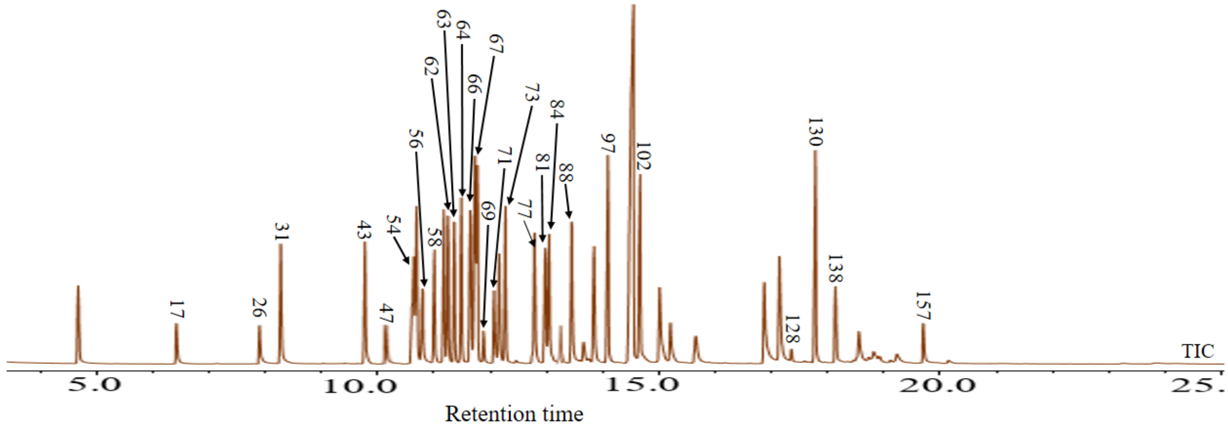

Figure 3-1: A total ion chromatogram (TIC) of analytical standards that were used to authenticate some of the identified compounds. The numbers correspond to those assigned to the compounds in Table 3-1

The quantification of headspace volatiles was achieved using calibration curves of two external standards (α-pinene and α-humulene, both of 98% purity, Sigma-Aldrich® Solutions, St. Luis, MO) prepared in concentrations ranges between 2.25 – 1000 ŋg/µl. The equation $y = 2036653.8x - 5127153.0$; $R^2 = 0.9963$ from α-pinene (43) was used to semi-quantify compounds with retention times below 16.0 min. While the equation $y = 1127808.7x - 5512234.2$; $R^2 = 0.9991$ for α-humulene (138) was used to semi-quantify compounds with retention times above 16.0 min (Chapter 2 of this thesis; Abtew *et al.*, 2015; Cheseto *et al.*, 2017; Njuguna *et al.*, 2018). The volatile concentrations obtained were in ng µL^{-1}.

3.4 Statistical analyses

Data from behavioral assays were analyzed using the Chi-square goodness of fit to determine whether there was a difference in the number of insects that chose either arm of the olfactometer.

The average numbers of puparia recovered from each of the three mango varieties were computed and subjected to one-way ANOVA followed by Tukey's HSD posthoc test for pairwise comparisons in R soft-ware (RStudio Team, 2021).

The numbers of compounds identified from each treatment of the mango variety headspaces were counted and compared using Pearson's Chi-square tests and Chi-square multi-comparison tests in RVAideMemoire (version 0.9-80) in R (RStudio Team, 2021).

The volatile concentration data from the treatments of the three mango varieties were first subjected to the Shapiro-Wilk test and Barlett's test to check for normality and homogeneity of variances. Lacking normal distribution, the data were subjected to the non-parametric Kruskal-Wallis rank-sum test (Dinno, 2015). The volatile concentration data of the mango treatments from each variety of mango were also subjected to the non-parametric Kruskal-Wallis rank-sum test and the post hoc Dunn test for pairwise comparison in R. Furthermore, the non-metric multidimensional scaling (NMDS), the similarity percentages (SIMPER) analysis, and the one-way analysis of similarities (ANOSIM) of Bray–Curtis dissimilarity matrix in *Past 3* software (Hammer *et al.*, 2001) were used to compare the headspace chemical profiles of the three mango varieties.

In addition, to find the variation in the volatile concentration per mango variety, each data was separately analyzed using NMDS and SIMPER (Rohart *et al.*, 2017), and the top 30 most discriminant volatiles were visualized in bar graphs and NMDS biplots. Then the averages of the volatile concentrations of these 30 most discriminant compounds were auto-scaled using the equation $y = log_{10}\,(x + 1,$ where x = Average headspace volatile concentrations in ng µL^{-1}) and used to draw a differentiation heatmap cluster. To further understand the trend in the changes of headspace volatiles per mango variety, the average volatile concentrations of each of the common compounds across the treatments were summed up, and the percentage of each to the total was computed as follows (example):

$$\%\, release\ rate = \frac{volatile\ release\ rate\ of\ X_{NU}}{Volatile\ release\ rates\ (X_{UR} +\ X_{CC1} + X_{CC2} +\ X_{CC7} +\ X_{CC9} +\ X_{NR1} +\ X_{NR2})}\ x\ 100;$$

Where X = the relative concentration of a given compound in a treatment, UR = non-infested unripe, CC1, CC2, CC7, CC9 = *C. cosyra* infested, NR1 = non-infested ripening, NR2 = non-infested ripe.

The results were then visualized in bar graphs. This was done to figure out how these compounds (especially terpenes which are generally associated with plant defense mechanisms) change with time as a result of the treatments.

3.5 Results

3.5.1 Responses of *C. cosyra, F. arisanus, D. longicaudata, and P. cosyrae* to headspace volatiles of non-infested and *C. cosyra*-infested mangoes

In all three mango varieties, *C. cosyra* were significantly attracted to non-infested unripe mango volatiles ($P < 0.01$ for Kent and Apple and $P < 0.05$ for Haden) and ripe mango volatiles ($P < 0.001$) compared to the blank control (air) (Figure 3-2 A, B, and C). *C. cosyra* was also more attracted to headspace volatiles of *C. cosyra*-freshly-infested mango fruits ($P < 0.001$ for Kent and Apple and $P < 0.01$ for Haden) compared to the unripe non-infested mangoes (UR). On the 7th-DPO, volatiles of infested mangoes were more attractive to *C. cosyra* ($P < 0.001$ for Apple and $P < 0.01$ for Kent and Haden). Similarly, the attractiveness of *C. cosyra* was more to the 9th-DPO mango headspace volatiles (CC9; Haden-$P < 0.01$, Apple- $P < 0.01$ and Kent-$P < 0.05$) compared to volatiles of ripening mangoes (NR1; Figure 3-2 A, B, and C).

For the three mango varieties, a significantly greater number of *F. arisanus* were attracted to the headspace volatiles of *C. cosyra*-freshly-infested mangoes (CC1; $P < 0.05$) compared to non-infested unripe ones. Also, *F. arisanus* was attracted to non-infested ripe Apple and Haden mangoes headspace volatiles (NR2; $P < 0.01$ and $P < 0.05$ respectively for Apple and Haden) compared to the blank control. However, non-infested unripe fruit volatiles (UR) of the three varieties, as well as non-infested ripe Kent fruit volatiles (NR2 Kent), were not attractive to *F. arisanus* ($P > 0.05$) compared to the blank control (Figure 3-2 A, B, and C).

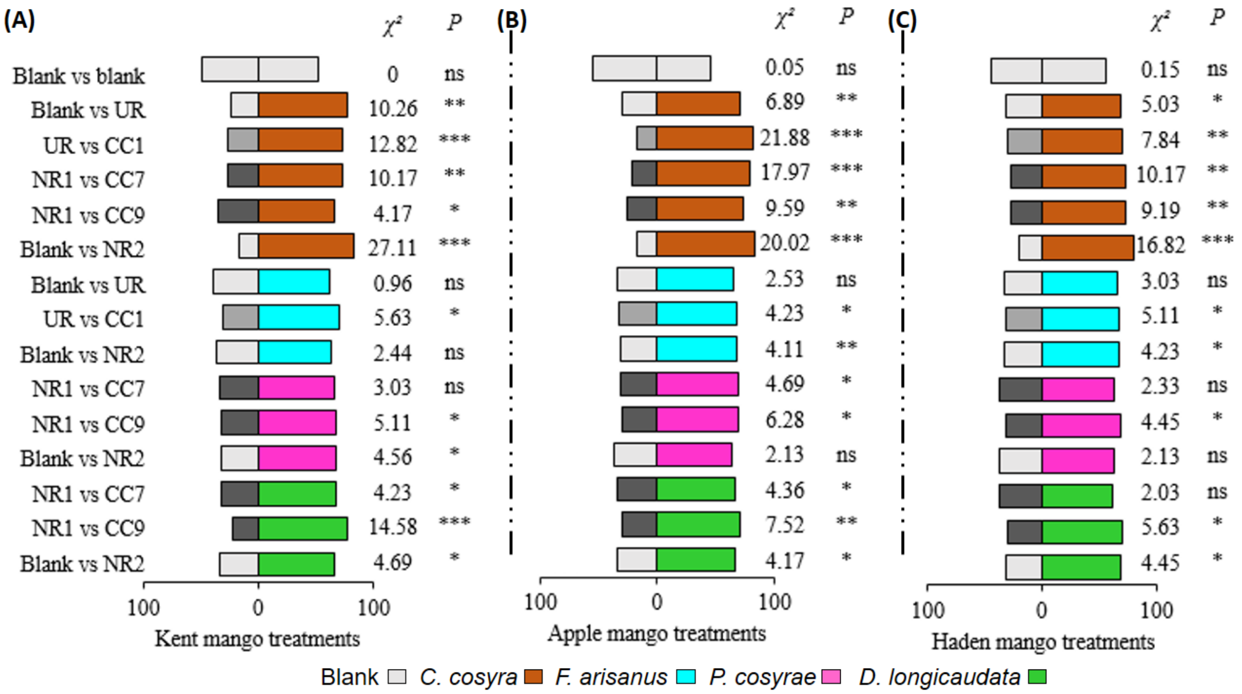

Figure 3-2: Response (%) of *C. cosyra*, *F. arisanus*, *P. cosyrae*, and *D. longicaudata* to headspace volatiles of Kent (A), Apple (B), and Haden (C) mango varieties (UR = non-infested unripe; NR1 = non-infested ripening; NR2 = non-infested ripe mango; CC1 = *C. cosyra*-freshly-infested mangoes; CC7 = 7th-DPO: CC9 = 9th-DPO mangoes; CC = *C. cosyra*; n = numbers of responsive insects; *P* = level of significant difference with *ns* = no significant difference, and *, **, *** = significance differences of $P < 0.05$, 0.01, 0.001, respectively, Chi-square goodness of test at $\alpha = 0.05$)

For the indigenous parasitoid, *P. cosyrae*, significantly more numbers of females were attracted to the 7th-DPO Apple mango volatiles (CC7; $P < 0.05$) when compared to non-infested ripening ones; the 9th-DPO (CC9) of the three mango varieties ($P < 0.05$) compared to the volatiles of their counterpart non-infested ripening mangoes (NR1); and to non-infested Kent ripe mangoes (NR2; $P < 0.05$) compared to the blank. There was no significant attraction of the parasitoid to the 7th-DPO Kent or Haden mango volatiles ($P > 0.05$) when compared to non-infested ripening ones, the non-infested ripe Apple and Haden mangoes (NR2; $P > 0.05$) when compared to blank (Figure 3-2 A, B, and C).

The response of *D. longicaudata* to headspace volatiles of the 7th-DPO mango was similar to those of *P. cosyrae* in the case of Apple and Haden varieties when compared to the non-infested ripening ones (NR1). On the other hand, significantly more female wasps of *D. longicaudata* were attracted to headspace volatiles of the 7th-DPO post-oviposition Kent mangoes (CC7; $P < 0.05$) compared to the non-infested ripening ones (NR1). Additionally, *D. longicaudata* was attracted to 9th-DPO mangoes (CC9; Kent-$P < 0.001$, Apple-$P < 0.01$, and Haden-$P < 0.05$) relative to the non-infested ripening (NR1). More females of *D. longicaudata* were also attracted to non-infested ripe mango headspaces for the three varieties (NR2; $P < 0.05$) compared to the blank control (Figure 3-2 A, B, and C).

3.5.2 Performances of *C. cosyra* in the three varieties of mangoes

The performance of *C. cosyra*, as indicated by the number of recovered puparia exhibited significant variation among the mango varieties (F =260.1, df = 2, P<0.0001) (Figure **2)**. Among the 287 recovered puparia, Kent mango showed the highest yield (72.1%), whereas Haden had the lowest (10.8%), although this was not significantly different from the yield of the Apple mango variety (Figure 3-3).

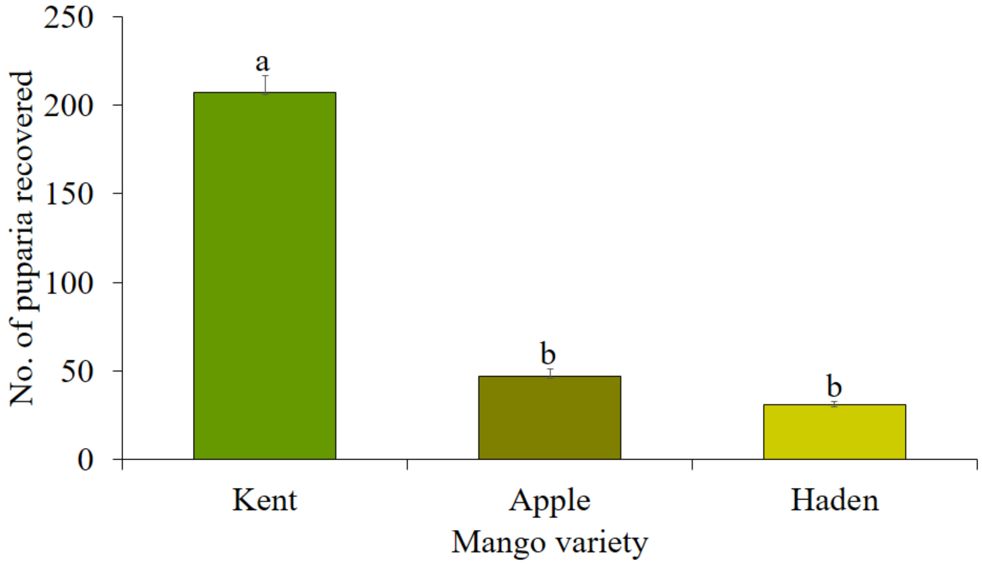

Figure 3-3: Performance of *Ceratitis cosyra* on three mango varieties. Different letters on the bars indicate a significant difference (One-way ANOVA test followed by Tukey's HSD posthoc test)

3.5.3 Chemical profiles of headspace volatiles of the three mango varieties

A total of 184 compounds were tentatively identified from the different treatments of the three mango varieties. Of these, 69 were esters, 34 sesquiterpenes, 25 monoterpenes, 13 alcohols, 11 monoterpenoids, 11 aldehydes, 9 ketones, 4 organic acids, 2 Benzenoids, 2 sesquiterpenoids, 2 diterpenoids, 1 lactone and 1 furanone (Table 3-1). *α*-Pinene, *β*-pinene, myrcene, *δ*-3-carene, αgurjunene, (*E*)-caryophyllene, *β*-copaene, *α*-humulene, and *δ*-cadinene are compounds that were detected in all the treatments of the three mango varieties (Table 3-1). Furthermore, ethyl propanoate, methyl butanoate, 2-methyl-1-butanol, 2-methyl propyl ethanoate, ethyl 2-methyl prop-2-enoate, and ethyl 3-hydroxy butanoate were common compounds detected in the headspaces at 7th-DPO and/or 9th-DPO of all mango varieties.

Table 3-1: The mean volatile concentrations (ng µL^{-1}) of compounds of the headspaces of tree-attached mangoes (n = 4). The tentative identification of compounds was based on their retention times (RT), electron ionization spectrum, and calculated Kovats retention indices (RI$_{cal}$) relative to those obtained from the literature (RI$_{lit}$), and comparing their mass spectra with those from online NIST library database. Compound names with ‡ were additionally confirmed using available authentic standards run on an HP-5MS column. The total mean volatile concentrations of the same mango variety with different letters are significantly different based on the Kruskal-Wallis ANOVA test (α = 0.05). (K = Kent; A = Apple; H = Haden; UR = non-infested unripe mango; NR1 = non-infested ripening mango; NR2 = non-infested ripe mango; CC = *C. cosyra* infested; 1 = freshly-infested, 2 = 2nd-DPO, 7 = 7th-DPO, and 9 = 9th-DPO; Total mean volatile concentration with different letters are significantly different)

							Mean volatile concentation (ng μL^{-1})																					
No.	R.T.	Compound	Class	RI$_{(Cal)}$	RI$_{(Lit)}$	Reference	KNU	KCC1	KCC2	KCC7	KCC9	KNR1	KNR2	ANU	ACC1	ACC2	ACC7	ACC9	ANR1	ANR2	HNU	HCC1	HCC2	HCC7	HCC9	HNR1	HNR2
1	3.1	2-Methyl butan-2-ol	Alcohol		--		7.0	15.3	15.1	18.5	11.3	11.4	2.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	3.3	3-Methyl butanal	Aldehyde		656	Xie, et al., 2008	-	-	-	-	1.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	3.7	2-Methyl-3-buten-2-ol	Alcohol	583.2			-	-	3.8	-	-	-	1.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	3.9	Pentanal	Aldehyde	666	701	Xie, et al., 2008	-	-	9.2	9.8	9.9	3.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	4	3-Pentanone	Ketone	673.3	--		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.7	9.8	-	-
6	4.2	Acetoin	Ketone	706	707	Kim and Chung, 2009	3.4	-	-	93.1	171.0	-	5.6	-	-	-	-	-	-	-	-	-	-	212.3	600.4	-	-
7	4.3	Ethyl propanoate	Ester	709	709	Zhao, et al., 2008	-	-	-	19.3	50.5	-	-	-	-	-	17.5	30.4	-	-	-	-	-	197.1	75.5	-	-
8	4.5	n-Propyl acetate	Ester	717	715	Isidorov, et al., 2006	-	-	-	-	-	-	-	-	-	-	18.5	39.0	-	-	-	-	-	58.4	-	-	-
9	4.5	Methyl butanoate	Ester	718	723	Jordán, et al., 2002	-	-	-	10.3	15.8	-	-	-	-	-	6.7	-	-	-	-	-	-	86.9	35.7	-	-
10	4.8	Isopentyl formate	Ester	728	789	Zhao, et al., 2008	-	-	2.4	27.7	152.4	2.2	2.6	-	-	-	28.2	56.7	-	-	-	-	-	29.8	108.8	-	-
11	4.9	2-Methyl-1-butanol	Alcohol	731.9	733	Kim and Chung, 2009	-	-	-	9.1	52.4	-	-	-	-	-	26.5	43.9	-	-	-	-	-	30.3	90.9	-	-
12	5.6	Ethyl 2-methyl propanoate	Ester	755	756	Siegmund, et al., 2001	-	-	-	14.3	72.1	-	-	-	-	-	18.5	17.5	-	-	-	-	-	17.7	12.1	-	5.8
13	6	2-Methylpropyl ethanoate	Ester	771	772	Zhao, et al., 2008	-	-	-	2.3	5.3	-	-	-	-	-	15.7	10.0	-	-	-	-	-	-	8.6	-	-
14	6.1	Methyl, 2-methyl butanoate	Ester	774	772	Figuérédo, et al., 2005	-	-	-	-	6.6	3.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	6.3	Ethyl 2-methylprop-2-enoate	Ester	782.7	--		-	-	-	5.9	33.3	-	-	-	-	-	4.6	-	-	-	-	-	-	-	1261.5	-	-
16	6.5	2,3-Butanediol	Alcohol	789	782	Krist, et al., 2005	-	-	-	28.0	131.1	-	-	-	-	-	8.2	60.8	-	-	-	-	-	-	-	-	-
17	6.6	Hexanal‡	Aldehyde	794.8	799	Jarunrattanasri, et al., 2007	10.6	7.8	8.3	21.7	18.3	15.8	1.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	6.7	Ethyl butanoate	Ester	797.1	802	Siegmund, et al., 2001	-	-	-	1163.4	2181.7	-	125.8	-	-	-	1195.7	1415.0	-	-	12.7	-	-	6927.6	11134.7	-	1236.8
19	6.8	2-Hexanol	Alcohol	800.3	803	Jordán, et al., 2002	7.6	16.4	15.3	25.5	31.4	14.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	7.1	(*Z*)-But-2-enoic acid	Organic acid	811.5	--		-	-	4.6	2488.5	3310.3	-	261.3	-	-	-	-	-	-	-	-	-	-	577.0	161.4	19.3	-
21	7.1	(*E*)-2-Butenoic acid	Organic acid	812	--		-	-	-	-	-	-	-	-	-	-	-	135.4	-	-	-	-	-	-	-	-	-
22	7.2	4-Hydroxy-2-pentanone	Ketone	814.9	849.4	ell and Alford, 2005	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	237.0	-	-
23	7.6	Butanoic acid	Organic acid	833.3	831	Mahattanatawee, et al., 2005	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	674.4	-	-
24	7.8	(*Z*)-Ethyl but-2-enoate	Ester	840.8	830	Quijano, et al., 2007	-	-	-	553.3	1696.3	-	38.0	-	-	-	222.6	79.6	-	-	-	-	-	-	1982.1	-	-
25	8	Ethyl 2-methylbutanoate	Ester	846.4	846	Martí, et al., 2003	-	-	-	93.6	376.5	-	2.6	-	-	-	201.9	57.2	-	-	-	-	-	123.8	956.9	-	390.0
26	8.1	(2E)-hexenal‡	Aldehyde	849.5	851	Kim, et al., 2000	2.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	8.1	Ethyl 3-methylbutanoate	Ester	849.8	851	Zhao, et al., 2008	-	-	-	16.4	82.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	8.1	(3*Z*)-Hexenol	Alcohol	852.2	853	Shellie and Marriott, 2003	-	-	-	-	4.9	-	-	-	-	-	-	-	-	-	-	-	-	72.7	148.1	-	-
29	8.2	(*E*)-Ethyl 2-butenoate	Ester	853.3	855	Jordán, et al., 2003	-	-	-	-	-	-	-	-	-	-	-	52.4	-	-	-	-	-	-	-	-	-
30	8.2	Ethylbenzene	Benzenoid	854.1	855	Vichi, et al., 2005	7.1	9.9	5.0	6.7	10.5	14.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	8.4	*p*-Xylene‡	Benzenoid	861.5	862	Kotowska, et al., 2012	4.2	-	-	-	-	3.6	1.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	8.4	Methyl tiglate	Ester	861.8	868	Isidorov and Jdanova, 2002	-	-	-	9.5	14.0	-	-	-	-	-	-	-	-	-	-	-	-	183.9	47.6	-	-
33	8.6	*n*-Hexanol	Alcohol	870.8	869	Ruther, 2000	-	-	-	9.5	16.6	-	-	-	-	-	-	-	-	-	-	-	-	-	372.7	-	-
34	8.6	3-Methylbutyl ethanoate	Ester	872.5	876	Forero, et al., 2008	1.9	-	-	3.8	77.7	-	-	-	-	-	1282.1	559.3	-	-	-	-	-	-	892.2	-	-
35	8.9	2-Heptanone	Ketone	884.1	889	Pino, et al., 2006	-	-	-	13.8	25.6	-	-	-	-	-	-	-	-	-	-	-	-	-	190.4	-	-
36	9.1	Propyl butanoate	Ester	890.3	896	Isidorov, et al., 2001	3.9	-	4.6	22.9	33.5	18.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9.1	Heptanal	Aldehyde	892.1	894	Roussis, et al., 2000	17.4	17.9	24.1	96.2	106.3	30.6	-	-	-	-	28.1	-	-	-	-	-	-	-	-	-	-
38	9.2	Ethyl pentanoate	Ester	894.4	898	Isidorov, et al., 2001	-	-	-	-	-	-	-	-	-	-	-	13.8	-	-	-	-	-	31.3	158.1	-	148.5
39	9.5	Tricyclene	Monoterpene	908.7	907	Pitarokili, et al., 2006	-	-	-	-	-	-	-	77.7	51.9	43.0	41.9	36.3	30.2	27.3	-	-	-	-	-	-	-
40	9.6	Bicyclo[3.3.1]nonan-1-ol	Alcohol	911.4	--		-	3.3	3.4	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9.7	Methyl hexanoate	Ester	915	924	Pino, et al., 2005	-	-	-	113.6	232.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9.7	*α*-Thujene	Monoterpene	916	919	Özcan, et al., 2003	9.1	22.1	12.0	-	-	-	-	42.2	162.8	48.8	86.9	49.1	9.6	15.3	17.1	33.2	-	-	30.3	-	-
43	9.8	*α*-Pinene‡	Monoterpene	921.2	928	Kim, et al., 2000	269.8	2201.2	1079.2	996.8	1183.4	150.3	295.8	14580.4	18865.5	11285.7	11167.3	10415.2	5553.4	7027.3	722.4	1161.3	414.9	159.8	957.1	640.9	1527.2
44	9.9	Ethyl, 3-hydroxy-butanoate	Ester	923.5	931	Jordán, et al., 2002	-	-	-	60.1	212.5	-	-	-	-	-	-	32.0	-	-	-	-	-	-	1317.3	-	-
45	10	Ethyl tiglate	Ester	929.5	938	Salido, et al., 2004	-	-	-	210.0	697.8	-	4.2	-	-	-	72.9	62.4	-	-	-	-	-	59.8	1122.6	-	579.5
46	10.1	*α*-Fenchene	Monoterpene	933.7	939	Vichi, et al., 2007	-	232.8	85.8	84.4	124.0	-	-	-	-	-	-	-	-	-	-	73.4	51.6	-	272.8	63.3	-
47	10.1	Camphene‡	Monoterpene	935.8	936	Couladis, et al., 2003	40.4	-	-	-	54.6	37.9	32.8	565.9	706.0	403.0	534.1	412.6	200.5	273.9	76.9	47.5	-	-	-	-	-
48	10.2	Ethyl (2*E*)-pentenoate	Ester	938.8	--		-	-	-	-	32.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	10.3	2-Methylpropyl butanoate	Ester	944.5	--		-	-	-	12.0	32.1	-	-	-	-	-	34.2	16.5	-	-	-	-	-	-	139.4	-	198.1
50	10.4	(2*E*)-Heptenal	Aldehyde	945.1	953	Zhao, et al., 2009	11.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	10.4	(3*Z*)-Hepten-1-ol	Alcohol	948.3	959	Radulovic, et al., 2010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	96.1	-	-
52	10.5	Ethyl 2,3-epoxybutyrate	Ester	951.5	--		-	-	-	-	27.0	-	-	-	-	-	-	-	-	-	-	-	-	-	68.5	-	-
53	10.5	Verbenene	Monoterpene	953.4	953	Figuérédo, et al., 2006	-	-	-	-	-	-	-	18.2	26.3	30.3	35.7	133.2	30.2	6.2	-	-	-	-	-	-	-
54	10.7	Sabinene‡	Monoterpene	959	961	Kartal, et al., 2007	5.7	332.7	83.1	-	-	5.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	10.7	*β*-Pinene	Monoterpene	961.5	963	Couladis, et al., 2003	73.4	178.2	131.0	96.2	73.8	34.6	60.9	3323.0	6019.3	2880.0	3136.8	2823.9	848.1	1390.3	192.8	51.1	26.0	12.2	88.0	43.4	452.4
56	10.8	1-Octen-3-ol	Alcohol	966.8	969	Tzakou, et al., 2006	10.5	-	-	13.0	9.3	-	-	-	-	-	12.5	-	-	-	-	-	-	-	-	-	-
57	11	6-Methyl-5-hepten-2-one	Ketone	972.9	984	Velickovic, et al., 2003	5.3	19.5	25.3	14.5	13.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	11	Myrcene‡	Monoterpene	975.6	981	Kartal, et al., 2007	109.4	15337.1	3828.3	874.4	1745.3	25.1	87.9	13340.6	42504.4	18686.0	21835.6	35801.2	2564.0	6003.5	47.6	2019.4	205.1	44.5	1092.5	160.7	1238.1
59	11.2	*α*-Terpinene	Monoterpene	982.6	1008	Basta et al., 2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20.3	167.4	29.2	-	-	61.1	-
60	11.2	Ethyl hexanoate	Ester	983.3	996	Pino, et al., 2005	-	-	-	4087.1	7147.3	-	237.2	-	-	-	756.3	1034.1	-	-	-	-	-	1033.4	7809.4	-	2101.4

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61	11.3	*n*-Octanal	Aldehyde	985.7	991	Xian, et al., 2006	27.2	-	-	-	-	36.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62	11.3	α-Phellandrene[‡]	Monoterpene	986.3	996	Kartal, et al., 2007	27.2	7097.0	412.6	238.2	57.1	-	52.1	962.1	2464.6	1280.3	1761.9	2192.6	205.7	415.3	65.3	401.7	19.7	-	-	152.1	-
63	11.4	δ-3-Carene[‡]	Monoterpene	991.7	1003	Bonaiti, et al., 2005	1590.2	48222.4	29646.3	16345.1	22064.6	1028.9	3305.4	524.6	120.8	148.6	135.8	82.0	658.3	194.0	5663.5	43210.7	9791.7	4486.7	26668.7	16161.7	18329.3
64	11.5	δ-2-Carene‡	Monoterpene	997	998	Saroglou, et al., 2006	4.3	-	165.8	119.9	173.1	-	30.5	238.4	548.5	279.8	316.3	431.4	63.2	135.8	-	-	81.6	-	282.6	-	-
65	11.7	*p*-Cymene	Monoterpene	1005.1	1018	Saroglou, et al., 2007	112.2	-	150.5	74.6	101.4	18.2	31.7	96.0	155.7	34.6	139.5	63.2	21.8	43.2	470.8	78.0	-	-	391.5	-	344.1
66	11.7	*o*-Cymene[‡]	Monoterpene	1006.4	1020	Tzakou and Couladis, 2001	41.5	1332.3	986.4	309.1	498.5	-	-	-	-	-	-	-	-	-	-	-	-	-	96.1	-	243.9
67	11.7	Limonene[‡]	Monoterpene	1009.6	1014	Couladis, et al., 2003	97.7	10523.8	4450.9	1344.8	2341.0	41.1	133.6	-	-	-	-	-	-	-	-	4440.8	552.5	253.1	2151.1	1214.2	2277.1
68	11.7	β-Phellandrene	Monoterpene	1010.4	1012	Song, et al., 2000	-	-	-	-	-	-	-	4821.7	11767.1	5411.6	8664.0	11940.2	572.9	1681.1	-	-	-	-	-	-	-
69	11.9	(*Z*)-β-Ocimene[‡]	Monoterpene	1020.1	1022	Couladis, et al., 2003	4.6	122.9	63.4	28.2	30.1	-	-	98.6	195.3	56.4	112.5	175.1	-	-	-	67.2	9.8	-	-	-	-
70	12	Ethyl hex-(2*E*)-enoate	Ester	1026.5	--		-	-	-	79.7	191.7	10.0	4.3	-	-	-	-	-	-	-	-	-	-	-	91.1	-	822.1
71	12.1	(*E*)-β-Ocimene[‡]	Monoterpene	1030.9	1032	Couladis, et al., 2003	31.6	815.4	193.8	51.4	84.0	13.2	8.3	459.2	1015.3	466.9	448.8	2261.7	175.6	14.8	28.5	123.9	1.7	-	-	-	-
72	12.3	2-Ethylbutyl isobutyrate	Ester	1041.5	--		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	174.8	-	-
73	12.3	γ-Terpinene[‡]	Monoterpene	1042.9	1042	Couladis, et al., 2003	30.7	866.2	209.1	89.1	158.2	-	10.1	669.9	1479.1	561.9	684.5	1591.8	-	-	66.6	176.9	91.2	-	118.4	75.2	368.2
74	12.5	4-Methoxy-2,5-dimethyl-3(2H)-furanone	Furanone	1056	1057	Siegmund, et al., 2001	-	-	-	-	-	-	-	-	-	-	145.0	124.1	-	-	-	-	-	-	-	-	-
75	12.7	Octyl methanoate	Ester	1065.2	--		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	102.8	-	-
76	12.7	*m*-Cymenene	Monoterpene	1068.4	1084	Andriamaharavo, 2014	-	-	-	23.0	24.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
77	12.8	Terpinolene[‡]	Monoterpene	1074.3	1072	Couladis, et al., 2003	32.4	12832.5	2793.2	860.3	1774.2	28.9	115.5	68.7	241.2	93.0	187.5	791.4	62.8	-	342.2	3790.1	427.9	35.1	1586.8	521.7	1312.7
78	12.9	6,7-Epoxymyrcene	Monoterpene	1079.8	--		-	-	-	-	-	-	-	-	28.4	4.9	-	120.3	-	-	-	-	-	-	-	-	-
79	12.9	Methyl benzoate	Ester	1081.6	1088	Hammami, et al., 2011	-	313.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	13	Propyl hexanoate	Ester	1083.6	1089	Isidorov, et al., 2001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.4	92.3	-	-
81	13	Linalool[‡]	Monoterpene	1086.3	1090	Gkinis, et al., 2003	-	-	-	-	-	-	-	20.8	-	25.4	77.5	-	14.1	5.5	-	-	-	-	-	-	-
82	13	Ethyl heptanoate	Ester	1086.9	1091	Mahattanatawee, et al., 2005	-	-	-	222.0	280.9	-	13.2	-	-	-	-	-	-	-	-	-	-	290.5	199.6	-	796.8
83	13.1	Isopropyl hexanoate	Ester	1089.4	--		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	104.5
84	13.1	*n*-Nonanal[‡]	Aldehyde	1089.6	1092	Tzakou, et al., 2004	67.4	466.2	292.7	131.6	124.0	134.9	15.9	-	-	-	111.0	-	-	-	-	-	-	-	-	-	398.8
85	13.3	Phenyl ethyl alcohol	Alcohol	1101.5	1116	Alissandrakis, et al., 2007	-	-	-	34.8	78.6	44.8	-	9.0	-	-	-	-	-	-	-	-	-	-	-	-	-
86	13.4	Methyl octanoate	Ester	1108.6	1120	Pino, et al., 2010	-	-	-	552.1	606.9	20.2	21.1	-	-	-	22.3	25.7	-	-	-	-	-	-	386.1	-	230.9
87	13.4	Cyclooctanone	Ketone	1110	--		-	538.2	127.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	13.5	*allo*-Ocimene[‡]	Monoterpene	1117	1128	Radulovic, et al., 2007	-	-	-	-	-	-	-	-	-	-	38.1	50.0	-	-	-	-	-	-	-	-	-
89	13.6	(2-endo,3-exo)-3-Methyl bicyclo[2.2.1]heptane-2-carboxaldehyde	Aldehyde	1121.5	--		-	-	-	210.4	403.3	-	-	-	-	-	-	-	-	-	-	162.9	25.6	339.9	2036.2	226.6	2956.1
90	13.7	(*Z*)-Pinen-3-ol	Monoterpenoid	1128.1	--		-	-	-	-	-	-	-	-	-	-	-	23.1	-	-	-	-	-	-	-	-	-
91	13.8	2-methylpropyl hexanoate	Ester	1133.4	1143	Jordán, et al., 2002	-	-	-	20.5	34.5	-	-	-	-	-	-	-	-	-	-	-	-	-	83.4	-	-
92	13.9	(*Z*)-3-Nonen-1-ol	Alcohol	1141.1	1156	Kim, et al., 2001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	89.0	-	-
93	14	*p*-Mentha-1,5-dien-8-ol	Monoterpenoid	1145	1159	Usubillaga, et al., 2001	-	250.8	142.2	24.5	43.1	-	-	-	-	-	-	-	-	-	-	-	-	-	221.9	-	404.1
94	14.1	Pinocarvone	Monoterpenoid	1148.5	1161	Salido, et al., 2003	-	49.6	35.0	-	-	-	-	-	-	-	47.7	-	-	-	-	-	-	-	-	-	-
95	14.1	6-Camphenol	Monoterpenoid	1149.1	--		-	103.0	93.9	-	-	-	-	-	-	-	71.4	-	-	-	-	-	-	-	-	-	-
96	14.1	(*E*)-Methyl 2-octenoate	Ester	1151.6	--		-	-	-	36.5	30.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
97	14.3	Terpinen-4-ol[‡]	Monoterpenoid	1161.9	1169	Saroglou, et al., 2007	-	-	-	-	-	-	-	26.3	-	25.3	122.6	114.1	-	-	-	-	-	-	-	-	-
98	14.4	*p*-Methyl acetophenone	Monoterpenoid	1168.1	1184	Mancini, et al., 2009	-	1311.6	421.9	-	-	-	-	-	-	-	-	-	-	-	-	9.0	-	-	-	-	-
99	14.4	Ethyl-(4*E*)-octenoate	Ester	1168.7	--		29.9	1989.4	561.3	1620.6	2604.9	52.3	54.8	-	-	-	57.6	34.2	-	-	-	7.0	-	30.1	1001.8	-	252.3
100	14.5	Ethyl octanoate	Ester	1175.7	1195	Tesevic, et al., 2005	-	-	238.9	18784.1	22858.5	1822.7	1207.8	-	-	-	2436.0	923.7	30.7	-	24.1	31.0	-	6786.1	18713.2	66.0	8498.3
101	14.6	Methyl salicylate	Ester	1178	1185	Pérez, et al., 2007	62.8	1223.1	359.4	-	-	60.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	14.7	*n*-Decanal[‡]	Aldehyde	1184.4	1185	Pavlovic, et al., 2006	67.1	-	-	218.7	180.1	209.3	46.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
103	14.8	Verbenone	Monoterpenoid	1191.6	1195	Gkinis, et al., 2003	-	-	-	-	-	-	-	-	29.4	5.6	155.1	101.9	-	-	-	-	-	-	-	-	-
104	15	Eucarvone	Monoterpenoid	1203	1145	Pino, et al., 2005	21.1	185.9	444.7	86.8	53.4	6.3	6.6	-	-	-	-	-	17.7	-	-	43.6	-	77.0	95.9	14.8	474.5
105	15.3	Ethyl oct-(2*E*)-enoate	Ester	1221.7	1246	Bicalho, et al., 2000	-	-	-	1069.1	1586.7	47.4	49.6	-	-	-	-	-	-	-	-	-	-	377.5	87.6	8.9	104.8
106	15.3	2-Methylbutyl hexanoate	Ester	1226.9	1246	Jordán, et al., 2002	-	-	-	-	42.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
107	15.4	Car-3-en-2-one	Monoterpenoid	1232.9	1254	Andriamaharavo, 2014	12.0	474.1	289.5	138.9	212.6	-	-	-	-	-	-	-	-	-	14.0	34.8	15.4	12.4	479.1	23.8	502.9
108	15.5	γ-Octalactone	Lactone	1236.6	1256	Isidorov, et al., 2001	-	-	-	90.5	133.5	-	-	-	-	7.8	-	-	-	-	-	-	-	-	853.2	-	-
109	15.6	*p*-Ethyl acetophenone	Monoterpenoid	1241.5	1281	Singer, et al., 2004	23.4	-	515.1	45.2	44.9	7.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
110	15.6	3-Caren-10-al	Monoterpenoid	1243.2	--		-	232.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
111	15.9	Propyl octanoate	Ester	1261.6	--		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	125.2
112	15.9	1-Methylethyl octanoate	Ester	1264.6	--		-	-	-	184.8	251.4	44.9	9.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
113	16.1	(*Z*)-Methyl 4-Decenoate	Ester	1281.2	--		-	-	-	456.9	318.4	-	17.4	-	-	-	-	-	-	-	-	-	-	-	138.7	-	107.4
114	16.3	Piperitenone	Ester	1290.8	1315	Bertoli, et al., 2011	37.5	1303.2	1074.8	129.2	162.7	-	13.1	-	-	-	-	-	-	-	-	39.0	-	-	113.2	9.5	68.1
115	16.3	Methyl decanoate	Ester	1294.3	1322	Zhao, et al., 2008	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36.6	-	-
116	16.5	Ethyl 3-hydroxy hexanoate	Ester	1301.6	--		-	-	-	168.0	203.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
117	16.6	Isobazzanene	Sesquiterpene	1313.3	--		-	-	-	-	-	-	-	-	-	-	-	-	-	-	21.6	92.3	15.4	18.8	134.1	31.8	64.9
118	16.7	Isobutyl *n*-caprylate	Ester	1315.9	--		-	-	-	228.7	395.7	-	-	-	-	-	-	-	-	-	-	-	-	-	38.2	-	24.0
119	16.7	δ-Elemene	Sesquiterpene	1317	1340	Isidorov, et al., 2004	-	-	-	-	-	-	-	14.3	11.3	17.1	43.3	26.1	31.1	4.5	-	-	-	-	-	-	-
120	16.8	α-Cubebene	Sesquiterpene	1326.2	1350	Flach, et al., 2004	-	-	-	-	-	-	-	85.9	84.6	54.5	86.7	242.3	18.6	5.6	20.3	33.4	10.1	7.6	51.3	10.1	19.5
121	17.1	Ethyl-(4*E*)-decenoate	Ester	1348.9	--		17.4	-	139.5	8143.5	7040.2	-	568.7	-	-	-	-	-	-	-	-	-	-	106.0	3885.8	-	833.7
122	17.1	α-Ylangene	Sesquiterpene	1349.9	1352	Couladis, et al., 2003	-	-	-	-	-	-	-	8.6	16.4	12.0	25.0	21.5	10.1	3.9	8.5	8.2	9.4	5.4	235.1	6.4	-
123	17.2	α-Copaene	Sesquiterpene	1354.5	1359	Couladis, et al., 2003	29.3	205.1	65.7	-	-	8.1	-	1271.9	1086.5	470.4	208.3	2263.0	49.6	29.6	456.3	384.5	76.0	19.2	835.0	108.3	467.3
124	17.2	Butyl caprylate	Ester	1355.8	1387	Jordan, et al., 2006	-	-	-	369.5	384.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
125	17.3	Ethyl decanoate	Ester	1362	1373	Saroglou, et al., 2006	-	-	-	1050.3	1198.1	-	65.7	-	-	-	-	-	-	-	-	-	-	90.7	590.1	-	-
126	17.3	β-Bourbonene	Sesquiterpene	1362.3	1374	Özcan, et al., 2003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.0	68.7	38.4	80.2	768.1	101.7	164.4

127	17.3	Sesquithujene	Sesquiterpene	1363.8	--		36.6	120.2	98.2	-	-	50.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
128	17.4	*β*-Elemene‡	Sesquiterpene	1366.9	1373	Couladis, et al., 2003	-	-	-	-	-	-	-	79.9	52.7	43.4	47.9	99.5	60.7	15.5	115.9	202.9	136.5	-	-	192.9	674.2
129	17.6	*α*-Gurjunene	Sesquiterpene	1387.4	1381	Pavlovic, et al., 2006	111.9	782.5	476.3	164.9	133.0	61.2	35.9	399.8	131.7	85.0	241.1	333.7	35.0	24.5	1289.6	344.6	198.8	124.5	259.5	292.4	215.0
130	17.8	(*E*)-Caryophyllene‡	Sesquiterpene	1397.7	1398	Basta, et al., 2006	216.8	6659.5	4284.4	1338.2	1352.9	98.2	144.3	1420.7	1214.1	577.0	407.7	2412.8	103.9	74.5	662.1	1579.7	492.6	302.4	734.1	885.9	574.8
131	17.9	*β*-Copaene	Sesquiterpene	1406	1422	Basta, et al., 2007	21.4	123.8	167.1	64.9	65.3	33.8	12.3	29.2	29.6	30.1	54.3	68.6	29.8	14.0	64.0	168.0	52.0	121.8	257.9	111.2	121.1
132	18	*α*-Guaiene	Sesquiterpene	1411.6	1413	Basta, et al., 2006	-	-	-	-	-	-	-	1437.8	894.6	404.8	147.4	1903.9	47.9	15.2	-	-	-	-	-	-	-
133	18	2-Methylbutyl octanoate	Ester	1413.9	--		-	-	-	97.1	193.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
134	18	Aromadendrene	Sesquiterpene	1415.2	1418	Basta, et al., 2006	-	-	-	-	-	-	-	-	-	-	-	-	9.6	10.2	14.0	56.1	22.2	86.9	96.9	44.7	51.2
135	18.1	(*Z*)-Eudesma-6,11-diene	Sesquiterpene	1417.9	--		-	-	-	-	-	-	-	92.2	41.7	10.3	-	82.3	-	-	-	-	-	-	-	-	-
136	18.1	*β*-Cubebene	Sesquiterpene	1419.5	1426	Vundac, et al., 2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20.0	50.6	16.4	68.5	149.5	38.0	46.1
137	18.1	(*Z*)-Muurola-3,5-diene	Sesquiterpene	1425.4	1442	Pripdeevech, et al., 2010	-	-	-	-	-	-	-	419.9	195.4	62.5	30.3	584.7	-	-	-	-	-	-	-	-	-
138	18.2	*α*-Humulene‡	Sesquiterpene	1431.3	1442	Özcan, et al., 2003	120.1	3836.8	2388.8	918.1	898.7	60.9	74.3	1001.8	665.8	341.9	231.7	1327.2	55.4	24.3	329.6	861.1	240.9	115.6	499.8	443.4	252.0
139	18.3	9-epi-(*E*)-Caryophyllene	Sesquiterpene	1438.3	1465	Pino, et al., 2005	-	-	-	-	-	-	-	71.5	77.3	93.6	160.3	176.4	50.1	43.8	-	-	-	-	-	-	-
140	18.3	(Z)-Muurola-4(14),5-diene	Sesquiterpene	1439	1458	Pripdeevech, et al., 2010	20.5	86.5	67.1	93.4	79.3	7.9	23.9	-	-	-	-	-	-	-	143.8	245.0	89.0	168.0	436.2	180.5	194.5
141	18.4	4,5-di-epi-aristolochene	Sesquiterpene	1446.1	--		13.3	440.9	221.5	71.7	80.8	10.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
142	18.5	*γ*-Muurolene	Sesquiterpene	1449.2	1457	Couladis, et al., 2003	-	-	-	-	-	-	-	387.4	211.1	148.4	239.1	589.0	46.4	62.4	84.9	153.4	40.3	35.1	122.4	96.2	68.8
143	18.6	*γ*-Gurjunene	Sesquiterpene	1456.4	1459	Monsef-Esfahani, et al., 2010	-	-	-	-	-	-	-	1147.4	606.3	361.1	212.4	1223.7	229.0	21.8	-	-	-	-	-	-	-
144	18.6	Germacrene D	Sesquiterpene	1457.4	1461	Couladis, et al., 2003	18.7	67.1	43.3	440.9	427.1	9.7	113.0	-	-	-	-	-	-	-	908.4	3277.6	616.5	1213.4	6085.3	1954.7	2342.7
145	18.6	*β*-Selinene	Sesquiterpene	1463.3	1464	Couladis, et al., 2003	96.6	3097.9	1649.7	356.2	487.7	49.3	21.7	355.3	154.7	86.7	40.1	350.9	48.3	-	-	438.2	153.1	-	175.7	330.2	126.1
146	18.7	*α*-Selinene	Sesquiterpene	1469.1	1470	Vichi, et al., 2005	103.5	478.9	298.4	-	-	46.0	53.8	2748.1	1005.2	665.4	334.8	2778.1	-	-	-	-	-	-	-	-	-
147	18.8	Bicyclogermacrene	Sesquiterpene	1472.4	1483	Hammami, et al., 2011	-	-	-	367.7	-	-	-	-	-	-	-	-	214.0	46.6	477.2	1165.2	431.4	281.9	1267.8	654.3	580.6
148	18.8	*α*-Bulnesene	Sesquiterpene	1479.2	1506	Ogunwande, et al., 2010	-	-	-	-	-	-	-	3624.5	1556.6	873.4	295.3	3084.6	-	-	-	-	-	-	-	-	-
149	18.9	Methyl dodecanoate	Ester	1486.1	1521	Zhao, et al., 2008	-	-	-	96.6	74.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
150	18.9	*γ*-Cadinene	Sesquiterpene	1487	1493	Couladis, et al., 2003	-	-	-	-	-	-	-	272.5	-	-	160.7	198.3	29.0	13.9	25.1	64.7	12.6	11.3	116.1	34.7	81.4
151	19	*δ*-Cadinene	Sesquiterpene	1493.8	1503	Couladis, et al., 2003	36.1	201.0	113.8	47.0	46.1	9.7	7.6	1136.0	592.5	422.3	441.9	1232.0	120.0	36.6	82.2	209.5	22.0	16.8	343.4	75.7	153.3
152	19.2	(*E*)-Cadina-1,4-diene	Sesquiterpene	1503.8	1527	Hazzit, et al., 2006	-	-	-	-	-	-	-	624.2	137.3	93.8	101.2	492.8	65.2	132.6	-	-	-	-	36.5	-	-
153	19.5	(*E*)-Nerolidol	Sesquiterpenoid	1528.6	1533	Song, et al., 2000	131.0	764.1	480.2	125.4	74.3	220.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
154	19.5	Ethyl 4-decenoate	Ester	1529.3	--		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	52.6	-	7.0
155	19.6	(3*Z*)-Hexenyl benzoate	Ester	1538.7	1554	Pitarokili, et al., 2002	-	99.4	-	189.8	119.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
156	19.8	Ethyl dodecanoate	Ester	1553.6	1576	Tesevic, et al., 2005	22.9	99.3	117.5	2760.9	2231.1	-	149.4	9.8	-	-	21.2	53.0	9.9	-	-	-	-	-	-	-	-
157	19.9	Caryophyllene oxide‡	Sesquiterpene	1560.7	1561	Couladis, et al., 2003	93.1	303.6	306.6	-	-	45.1	-	-	20.0	2.8	32.7	87.0	-	-	-	41.1	8.2	-	-	32.7	-
158	20	Tetradecanal	Aldehyde	1572.9	1601	Xie, et al., 2008	21.4	-	-	89.4	46.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
159	20.1	Humulene epoxide II	Sesquiterpene	1585.1	1605	Mancini, et al., 2009	17.7	133.7	134.4	127.1	-	-	-	-	-	-	-	-	-	-	-	-	-	9.1	3861.9	11.8	760.3
160	20.3	1-*epi*-Cubenol	Sesquiterpene	1598.4	1619	Feizbakhsh and Naeemy, 2011	-	-	-	-	-	-	-	53.4	14.7	3.9	-	-	-	-	-	-	-	-	-	-	-
161	20.4	(*Z*)-Cadina-1(6),4-diene	Sesquiterpene	1608.7	--		-	-	-	-	-	-	-	-	-	-	-	27.9	-	-	-	-	-	-	-	-	18.4
162	20.6	*α*-Cadinol	Sesquiterpenoid	1626.4	1624	Pavlovic, et al., 2006	140.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
163	20.7	(*Z*)-3-Hexenyl salicylate	Ester	1636.2	1670	Pino, et al., 2005	-	25.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
164	20.9	Propyl dodecanoate	Ester	1647.9	1685	Quijano, et al., 2007	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.9	-	-
165	20.9	Ethyl tridecanoate	Ester	1650.2	1687	Pino, et al., 2005	-	-	-	57.4	39.3	-	-	-	-	-	-	-	-	-	-	-	-	-	25.7	-	-
166	21	2-Pentadecanone	Ketone	1656	1694	Mancini, et al., 2009	45.1	52.3	48.4	90.5	49.2	9.5	13.0	-	-	-	-	-	-	-	-	-	-	-	90.4	-	9.5
167	21.2	(2*E*)-Tridecenol	Alcohol	1672.3	--		66.6	172.8	177.7	420.4	169.2	5.1	68.7	-	-	-	-	-	-	-	-	-	-	-	139.2	14.4	67.4
168	21.2	Methyl tetradecanoate	Ester	1680.4	1723	Ansorena, et al., 2001	-	-	-	123.5	80.9	-	-	-	-	-	-	-	-	-	-	-	-	-	78.6	-	-
169	21.7	Ethyl 9-tetradecenoate	Ester	1727.5	--		-	-	-	80.2	96.8	-	-	-	-	-	-	-	-	-	-	-	-	-	18.9	-	-
170	21.8	Benzyl benzoate	Ester	1744.4	1743	Basta, et al., 2007	-	263.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
171	21.9	*n*-Butyl laurate	Ester	1748.5	1786	Quijano, et al., 2007	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22.4	-	-
172	22	Ethyl tetradecanoate	Ester	1762.5	1784	Zhao, et al., 2008	20.8	108.7	66.7	3164.6	2452.2	16.2	92.5	11.0	-	-	22.8	33.7	3.7	-	-	-	-	4.1	4327.6	51.9	240.3
173	22.5	6,10,14-trimethyl-2-pentadecanone	Ketone	1822.6	1835	Mancini, et al., 2009	21.4	31.0	39.6	21.7	19.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
174	23	Benzyl salicylate	Ester	1860.8	1863	Demetzos, et al., 2002	-	412.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
175	23.4	Methyl hexadecanoate	Ester	1899.7	1903	Payo, et al., 2011	-	21.6	19.5	43.2	65.4	-	-	-	-	-	-	-	-	-	-	-	-	-	7.1	-	-
176	23.4	7,9-Di-*tert*-Butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Ketone	1900.7	1916	Andriamaharavo, 2014	19.5	24.0	18.2	-	-	5.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
177	23.5	Isobutyl myristate	Ester	1916.9	--		-	-	-	22.1	10.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
178	23.6	Cyclohexadecanolide	Ester	1925.2	1928	Lopes, et al., 2004	17.2	19.9	13.7	18.7	13.7	-	5.7	-	-	-	-	-	-	-	-	-	-	-	-	-	7.0
179	23.6	9-Hexadecenoic acid	Organic acid	1928.6	1942	Zhao, et al., 2008	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38.1	-	-
180	23.8	Ethyl 9-hexadecenoate	Ester	1948.5	1978	Andriamaharavo, 2014	-	-	-	36.7	55.9	-	-	-	-	-	-	-	-	-	-	-	-	-	13.5	-	-
181	24.1	Ethyl hexadecanoate	Ester	1982.5	1992	Zhao, et al., 2008	-	72.9	29.6	697.6	1283.8	6.7	6.1	-	-	-	-	-	-	-	-	-	-	-	611.8	16.5	1.8
182	24.7	Kaurene	Diterpene	2048.2	2043	Grujic-Jovanovic, et al., 2004	11.8	13.8	15.5	7.6	5.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
183	24.8	Abietatriene	Diterpene	2055.8	2056	Lazari, et al., 2000	-	-	37.9	-	-	3.1	2.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
184	25.9	Ethyl oleate	Ester	2173.6	2173	Custer, 2009	-	-	-	-	13.8	-	-	-	-	-	-	-	-	-	-	-	-	-	30.6	-	-
		Total mean volatile concentration (ng µL-1)					**4392e**	**127365.8a**	**60528.9d**	**77043c**	**97722.2b**	**4756.1e**	**7768.6e**	**56646.7b**	**95181.5a**	**46639.6c**	**60110.5b**	**94127.5a**	**12326.1c**	**17812.9c**	**12500.2e**	**65614.8b**	**14425.9e**	**25453.4d**	**114892.4a**	**25183.6d**	**54767.2bc**

There were qualitative and quantitative differences in the headspace volatile constituents which varied among treatments and time of volatiles collection for each mango variety (Figure 3-4).

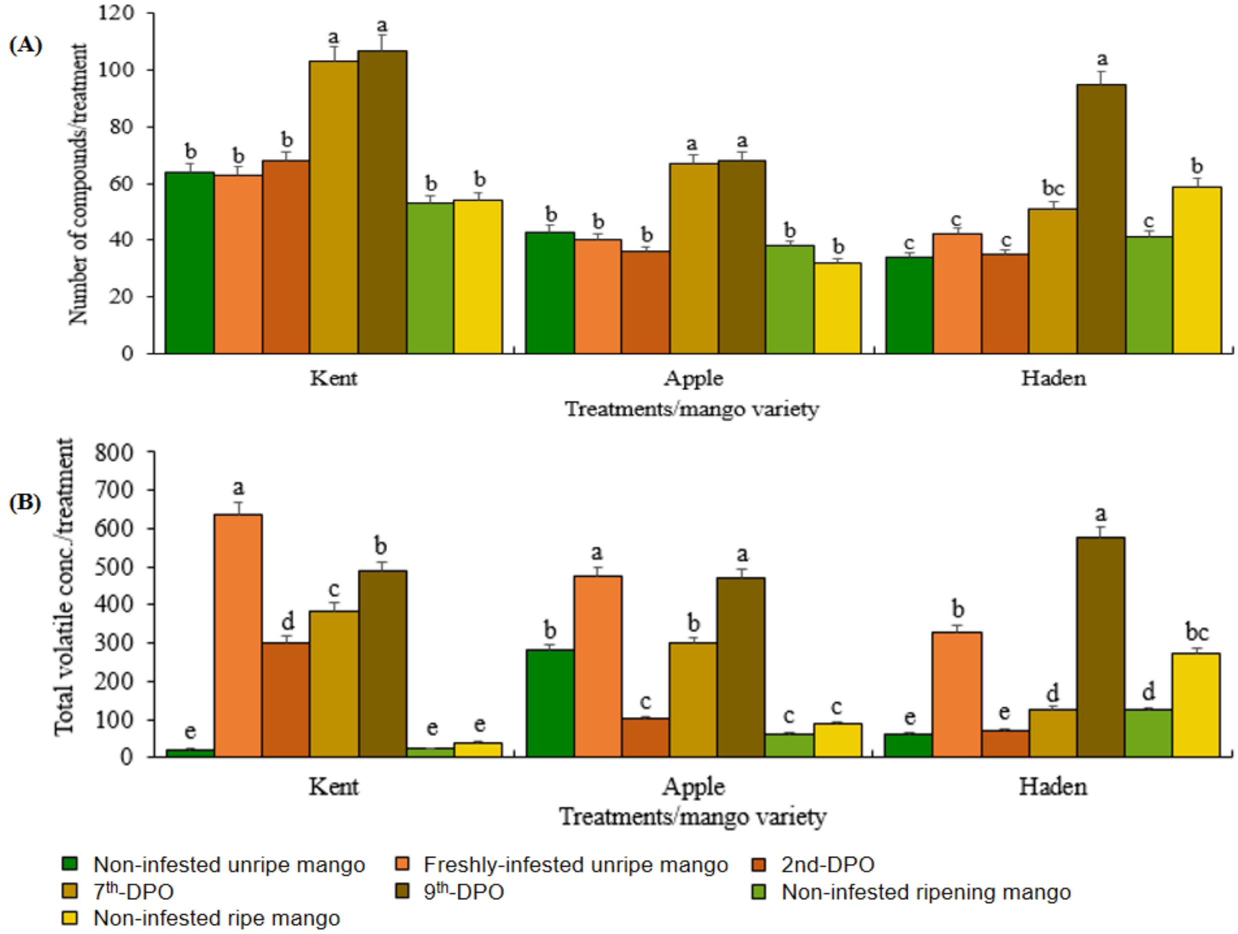
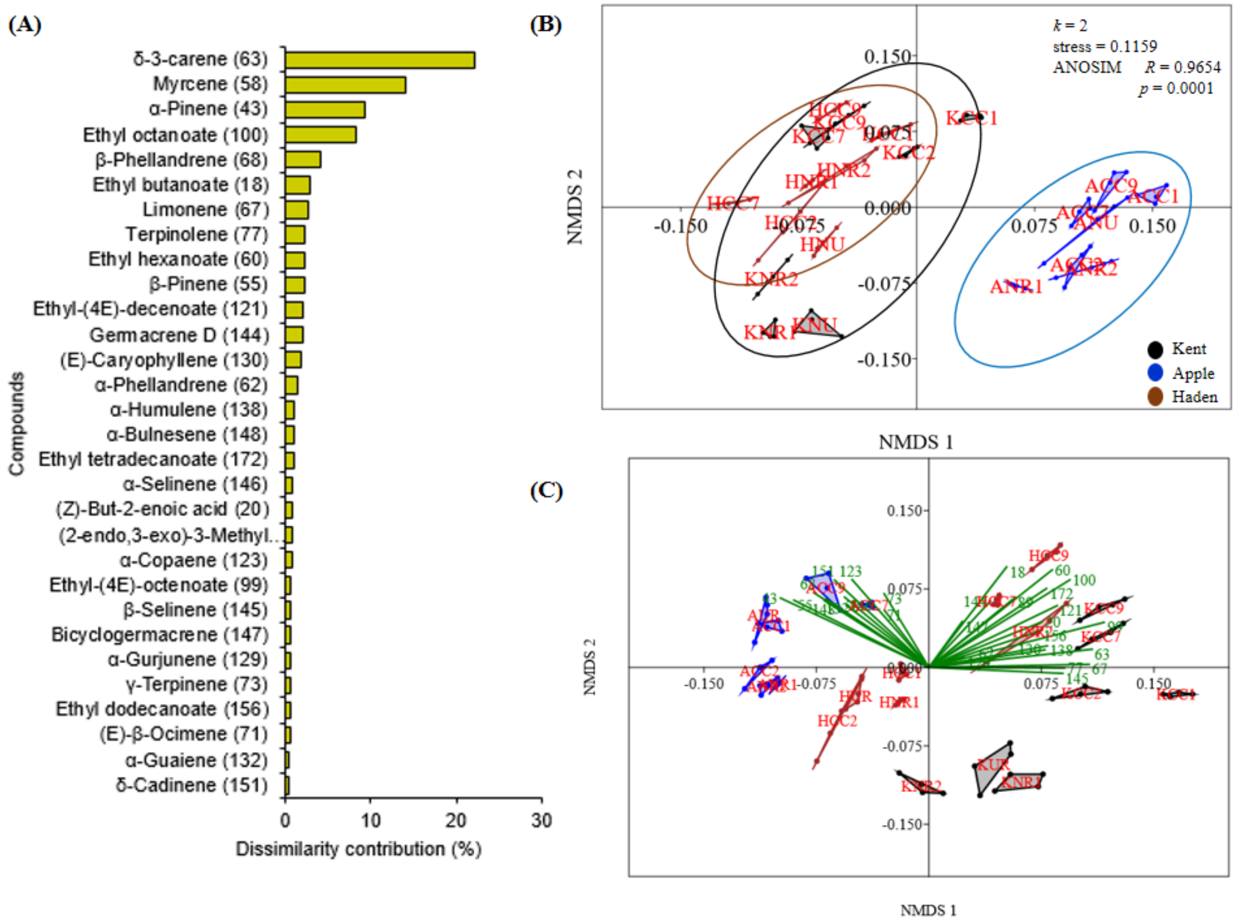


Figure 3-4: The number of tentatively identified compounds of each mango treatment (Pearson's Chi-square test followed by Chi-square multi-comparison test in RVAideMemoire) (A); Total volatile concentrations (ng µL^{-1}) among treatments of each mango variety (Kruskal-Wallis rank-sum test followed by post hoc Dunn test for pairwise comparison at $\alpha = 0.05$) (B). Different letters on bars of the same mango variety indicate significant difference

The number of identified compounds differed significantly among the treatments for the three mango varieties ($\chi^2 = 41.328$, $df = 6$, $P < 0.001$; $\chi^2 = 28.722$, $df = 6$, $P < 0.001$ $\chi^2 = 54.287$, $df = 6$, $P < 0.001$ for Kent, Apple and Haden, respectively), being highest for 7th-DPO and day 9th-DPO for both Kent and Apple varieties, and 9th-DPO for Haden (Figure 3-4 A). Also, the total volatile concentrations varied among treatments of each mango variety ($\chi^2 = 25.012$, $df = 6$, $P < 0.00034$;

$\chi^2 = 22.374$, $df = 6$, $P < 0.001036$; and $\chi^2 = 24.502$, $df = 6$, $P < 0.000422$, for Kent, Apple and Haden, respectively) (Figure 3-4 B), being highest for freshly-infested fruits for Kent while it was highest for 9th-DPO for Haden variety. For the Apple mango variety, freshly-infested fruits and 9th-DPO had the highest concentrations. Generally, the volatile concentrations of non-infested mango fruits were lower than those of infested ones (Figure 3-4 B), especially in the case of the Kent variety.

The 30 topmost discriminant compounds of the volatiles of all treatments of the three mango varieties contributed 88.81% of the total dissimilarities (Bray-Curtis similarity percentage, SIMPER, Figure 3-5 A). The compounds that contributed majorly to the separation and clustering were *δ*-3-carene -22.1%, myrcene-14.1%, *α*-pinene-9.3%, ethyl octanoate-8.3%, and *β*-phellandrene-4.2%. Headspace volatiles from all treatments were successfully grouped into defined clusters, with overlaps between Kent and Haden headspaces (NMDS: k = 2, stress = 0.1159, Figure 3-5 B; Appendix: Figure S3-1).

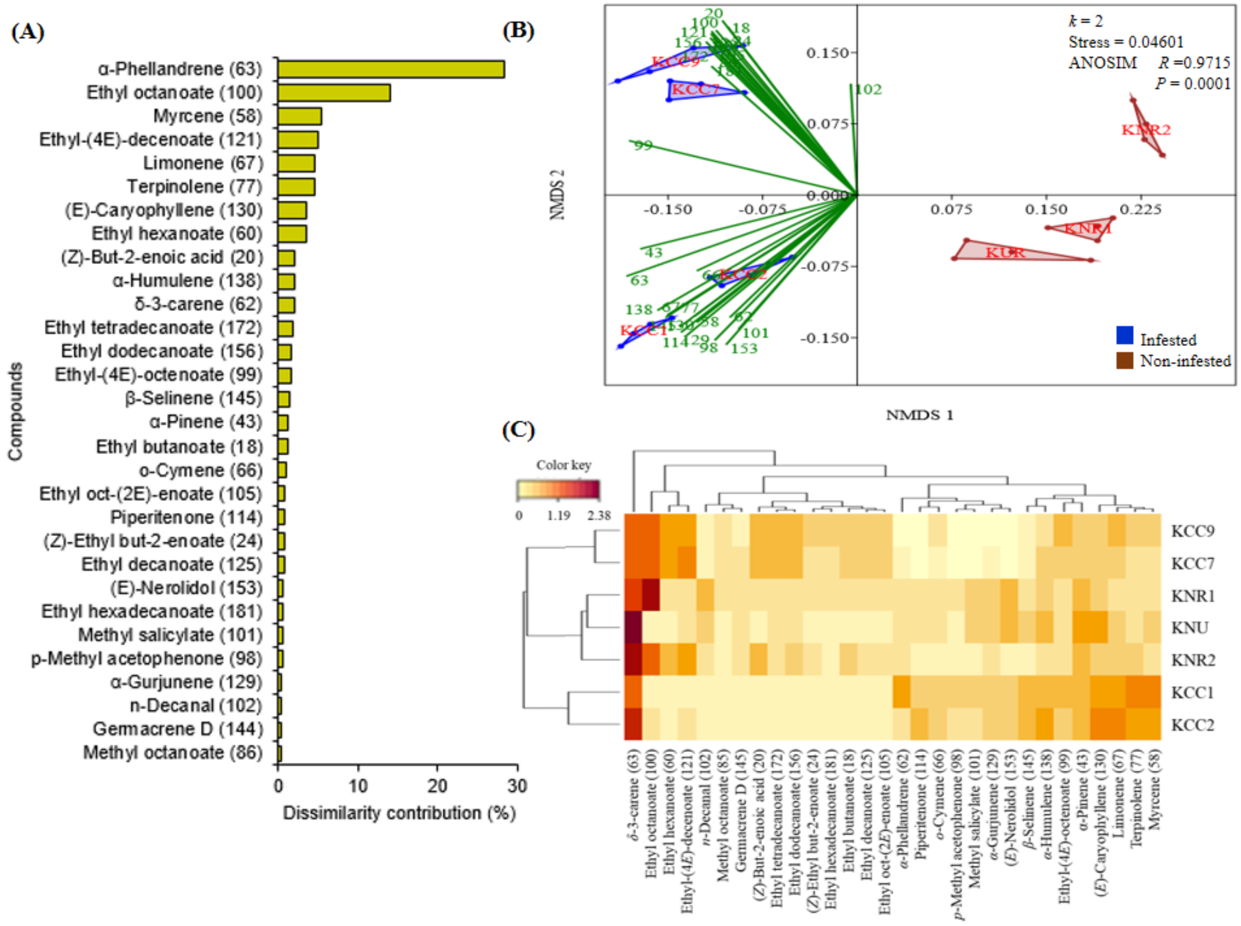


Figures 3-5: The 30 topmost discriminant compounds for all treatments of the three mango varieties (Kent, Apple and Haden) based on similarity percentage (SIMPER) of the non-metric multidimensional scaling (NMDS) (A). The NMDS plot in the Bray–Curtis dissimilarity matrix shows the scattering of the headspace compounds of the treatments from the three mango varieties (k = 2, stress = 0.1159) (B). The NMDS biplots show the spread of the selected 30 most discriminant compounds within the headspaces (C). (K = Kent; A = Apple; H = Haden; CC = *C. cosyra*; UR = non-infested unripe mango; NR1 = non-infested ripening mango; NR2 = non-infested ripe mango; 1 = *C. cosyra*-freshly-infested; 2 = 2nd-DPO, 7 = 7th-DPO, and 9 = 9th-DPO)

There was a significant difference between the headspaces' volatile concentrations (one-way analysis of similarity, ANOSIM, R = 0.9654, P = 0.0001) of all treatments for the three mango varieties. The 30 topmost discriminant compounds were generally associated with the headspace volatiles of *C. cosyra*-freshly-infested, 7th-DPO, and 9th-DPO mangoes (Figure 3-5 C).

Among the treatments of Kent mango, a total of 135 compounds were tentatively identified out of which 23 compounds were shared in all treatments (Table 3-1). Methyl benzoate, cyclooctanone, pinocarvone, 6-camphenol, *p*-methyl acetophenone, 3-caren-10-al, (*Z*)-3-hexenyl salicylate, benzyl benzoate, and benzyl salicylate were present in the headspace of the freshly-infested and/or 2nd-DPO, while ethyl 2-methyl propanoate, 2,3-butanediol, ethyl 3-methyl butanoate, methyl tiglate (methyl 2-methyl-2-butenoate), *n*-hexanol, 2-heptanone, methyl hexanoate, 2-methyl propyl butanoate, *m*-cymenene, and (2-*endo*,3-*exo*)-3-methyl bicycle [2.2.1] heptane-2-carboxaldehyde were among the 36 compounds that were detected only from the 7th-DPO and/or 9th-DPO headspace volatiles. Moreover, 19 compounds were detected in infested and non-infested ripe mangoes which included isopentyl formate, (*Z*)-but-2-enoic acid, (*Z*)-ethyl but-2-enoate, ethyl 2-methyl butanoate, ethyl tiglate (ethyl 2-methyl-2-butenoate), ethyl hexanoate, ethyl hex-(2*E*)-enoate, ethyl heptanoate, phenyl ethyl alcohol, and methyl octanoate.

The 30 topmost discriminant compounds of all treatments of Kent as of the SIMPER of the NMDS (Figure 3-6 A), accounted for 90.9% of the dissimilarity contribution. Of these compounds, *α*-phellandrene, ethyl octanoate, myrcene, ethyl-(4*E*)-decenoate, and limonene contributed 57.4%.

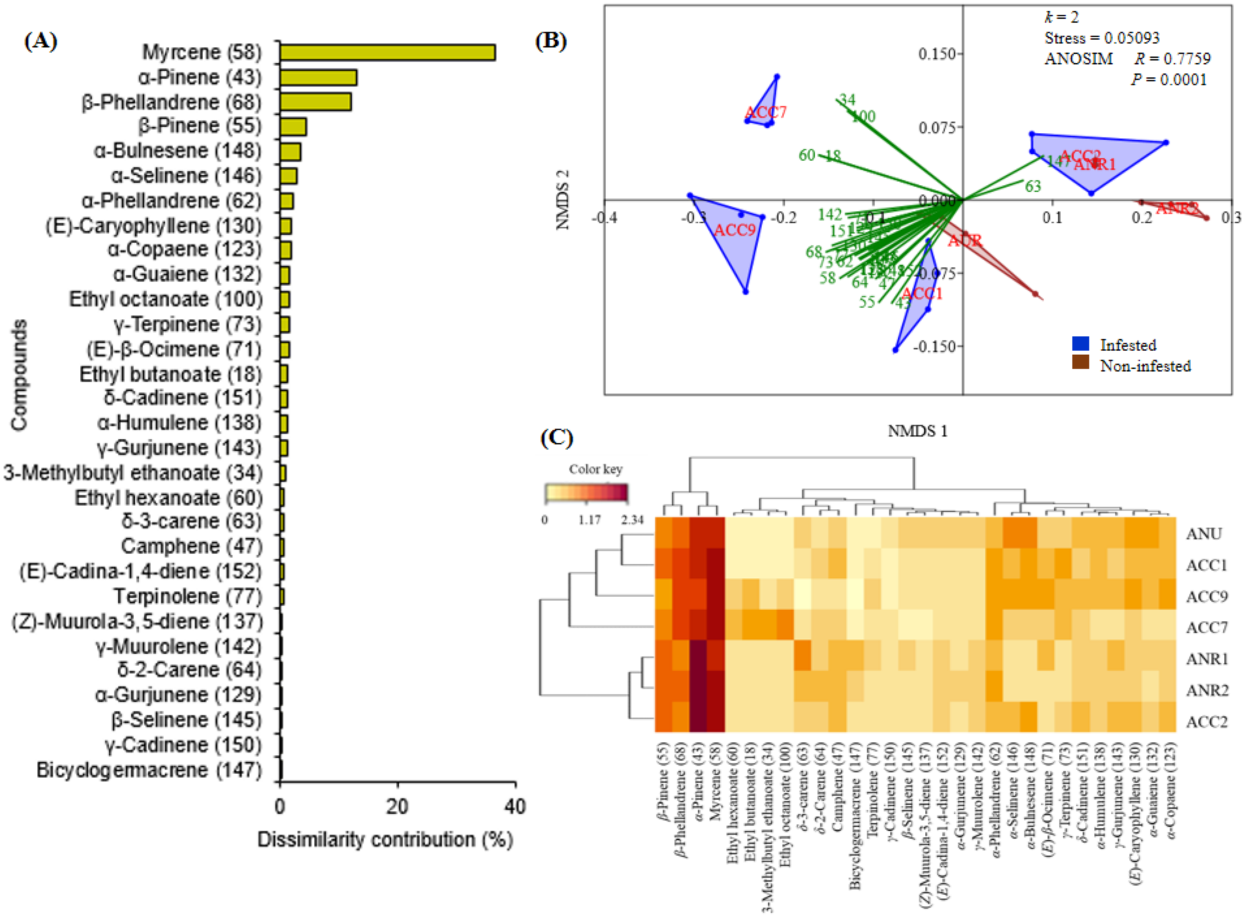


Figures 3-6: The 30 topmost discriminant volatiles of all treatments of Kent mango based on the similarity percentage in decreasing order of importance (A). The NMDS biplots show the differentiation of the 30 compounds in the mango treatments (B). Heatmap clustering of the auto-scaled volatile concentration of the 30 selected compounds. The darker the brown colour intensity, the higher the mean volatile concentration (C). (KCC2 = 2nd-DPO mango; KCC1 = *C. cosyra*-freshly-infested Kent mango; KNR2 = non-infested ripe Kent mango; KUR = non-infested unripe Kent mango; KNR1 = non-infested ripening Kent mango; KCC7 = 7th-DPO, and KCC9 = 9th-DPO mangoes)

There was a significant difference between the headspaces' volatile concentrations among the mango treatments (the one-way analysis of similarity, ANOSIM, *R* = 0.9715, *P* = 0.0001) (Figure 3-6 B; Appendix: Figure S3-2). The most discriminant compounds were associated with headspaces of infested Kent mangoes (Figure 3-6 B). Of the most discriminant compounds, *δ*-3-carene (62) was the most abundant in most of the treatments (Figure 3-6 C). Furthermore, the heatmap shows that the selection of compounds was spread in almost all possible categories (that is compounds that appeared in all treatments and those that did not, compounds with a difference in abundance, and compounds from different classes among others).

A total of 82 compounds were tentatively identified from all treatments of Apple mango headspaces out of which 28 were common in all treatments. Verbenone, 6,7-epoxymyrcene, and caryophyllene oxide were the only compounds that were added in the 2nd-DPO mangoes relative to those of non-infested mango headspace. Thirty-one compounds were identified only from the 7th-DPO and/or 9th-DPO mango headspaces which included ethyl propanoate, *n*-propyl acetate, isopentyl formate, 2-methyl-1-butanol, ethyl 2-methyl propanoate, 2-methyl propyl ethanoate, 2,3-butanediol, ethyl butanoate, (*Z*)-ethyl but-2-enoate, and ethyl 2-methyl butanoate but not in the other treatments. Ethyl octanoate, aromadendrene, and bicyclogermacrene were the only common compounds among the infested and the non-infested ripe Apple mango headspace volatiles.

In addition, for all treatments of Apple mangoes, the 30 topmost discriminant compounds as per SIMPER of NMDS contributed 97.0% of the total dissimilarity contribution (Figure 3-7 A). Myrcene, *α*-pinene, *β*-phellandrene, *β*-pinene, and *α*-bulnesene were the top five discriminant compounds contributing 69.9%.

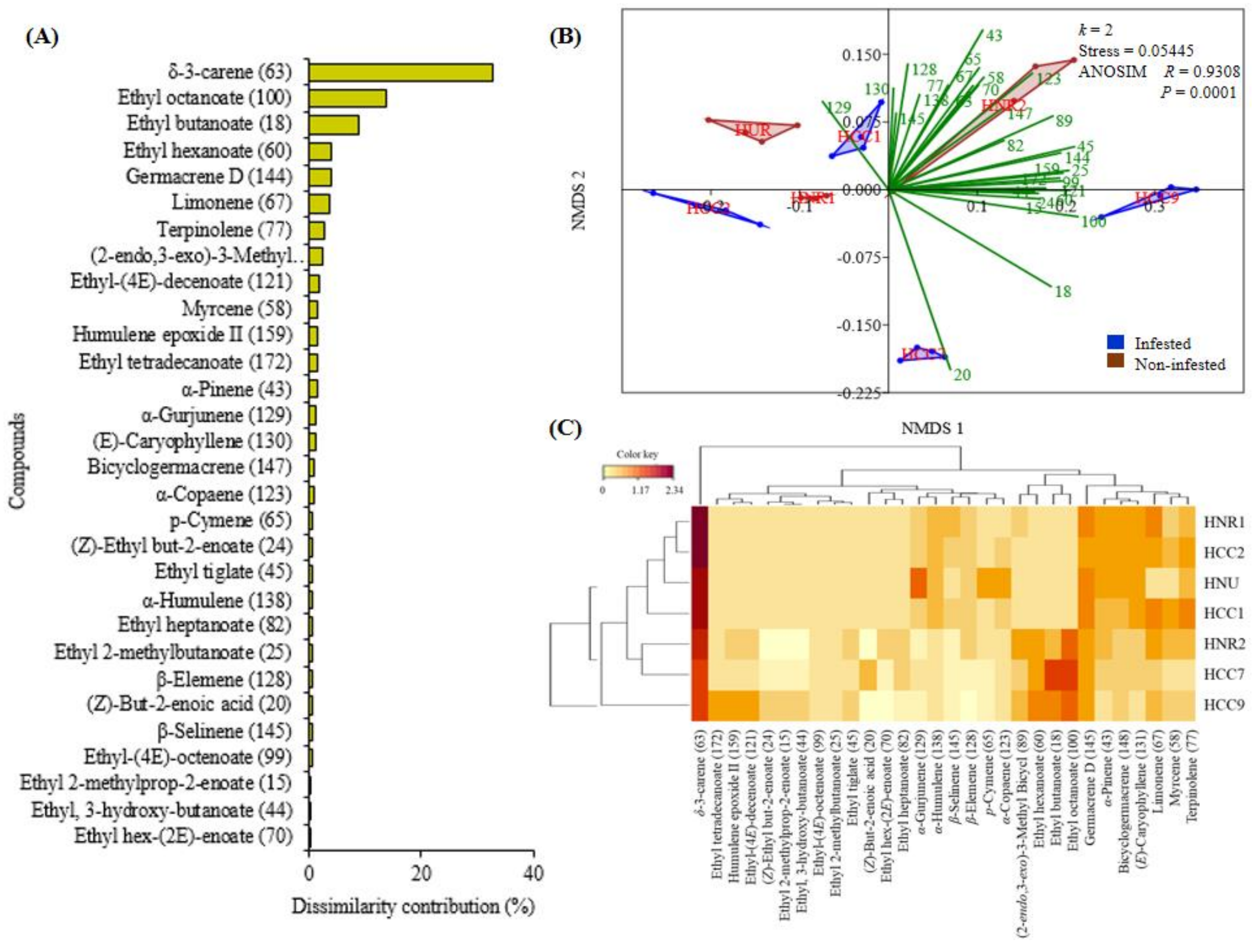


Figures 3-7: The 30 topmost discriminant volatiles of all treatments of Apple mango based on the similarity percentage in decreasing order of importance (A). The NMDS biplots show the differentiation in the mango treatments of the 30 compounds (B). Heatmap clustering of the auto-scaled volatile concentration of the 30 selected compounds, the darker the brown colour intensity, the higher the mean volatile concentration (C). (ACC2 = 2nd-DPO mango; ANR2 = non-infested ripe Apple mango; ANR1 = non-infested ripening Apple mango; ACC7 = 7th-DPO, and ACC9 = 9th-DPO mangoes; ACC1 = *C. cosyra*-freshly-infested Apple mango and AUR = non-infested unripe Apple mango)

Unlike in Kent mango treatments, in Apple mango, there was no clear separation of the 30 most discriminant compounds used in NMDS biplots as either from infested or non-infested headspaces (*k* = 2, stress = 0.05093; Figure 3-7 B; Appendix: Figure S3-3). However, there was a significant difference in the headspaces of the treatments of Apple mango (one-way analysis of similarity, ANOSIM, *R* = 0.6882, *P* = 0.0001; Figure 3-7 B) although there was no distinct separation of infested and non-infested headspace compounds clusters. The most discriminant compounds were selected from most classes of compounds where *α*-pinene and myrcene were the dominant compounds (the darker the colour intensity, the higher the mean volatile concentration of the selected compounds) (Figure 3-7 C).

Haden mango had 109 tentatively identified compounds out of which 22 were common in the headspace volatiles of all treatments. α-Fenchene, (*Z*)-β-cymene, *p*-methyl acetophenone, and caryophyllene oxide were additional compounds identified from the headspaces of freshly *C. cosyra* infested conspecifics compared to those of non-infested unripe mangoes. Moreover, on the 7th-DPO and/or 9th-DPO mango headspaces, 38 more compounds were identified compared to those of non-infested ripe mangoes. Among the additional compounds were 3-pentanone, acetoin, ethyl propanoate, methyl butanoate, isopentyl formate, 2-methyl-1-butanol, 4-hydroxy-2-pentanone, butanoic acid, (*Z*)-ethyl but-2-enoate, (3*Z*)-hexenol, and methyl tiglate (methyl 2-methyl-2-butenoate). Additionally, 25 compounds were common in infested and non-infested ripe mango headspaces. These compounds included ethyl 2-methyl propanoate, ethyl 2-methyl butanoate, ethyl pentanoate, ethyl tiglate (ethyl 2-methyl-2-butenoate), 2-methyl propyl butanoate, ethyl hexanoate, ethyl hex-(2*E*)-enoate, ethyl heptanoate, and methyl octanoate among others.

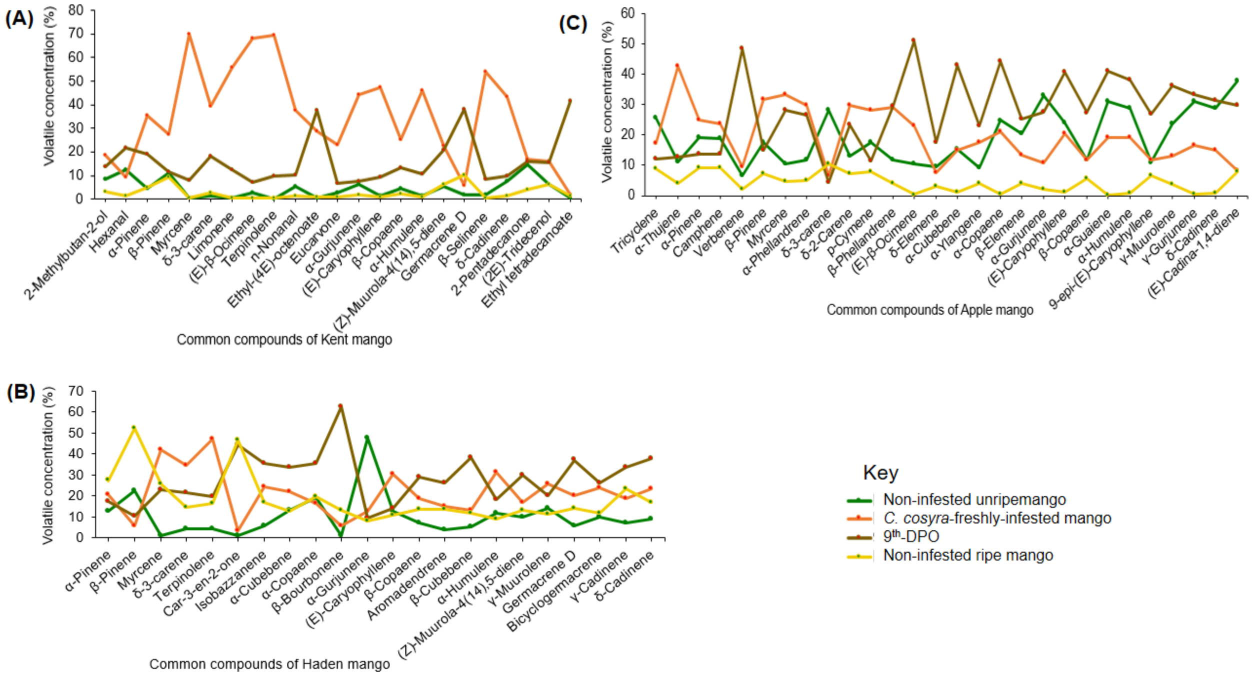
The top 30 compounds, by the SIMPER of NMDS (Figure 3-8 A) for all treatments of Haden mango, accounted for 91.1% of the total dissimilarity. Of these compounds, δ-3-carene, ethyl octanoate, ethyl butanoate, ethyl hexanoate, and germacrene D contributed 63.3%. The volatile concentrations varied significantly among the treatments (one-way analysis of similarity, ANOSIM, $R = 0.9269$, $P = 0.0001$) (Figure 3-8 B; Appendix: Figure S3-4) but like in Apple mango, there was no clear separation of clusters of infested and non-infested mango volatiles.



Figures 3-8: The 30 topmost discriminant volatiles for all treatments of Haden mango based on SIMPER in decreasing order of importance (A). The NMDS biplots show the differentiation in the Haden mango treatments of the 30 compounds (B). Heatmap clustering of the auto-scaled volatile concentration of the 30 selected compounds, the darker the brown colour intensity, the higher the mean volatile concentration (C). (HCC9 = 9th-DPO; HCC7 = 7th-DPO; HNR2 = non-infested ripe; HCC1 = *C. cosyra*-freshly-infested Haden mango; HUR = non-infested unripe mango before infestation; HCC2 = 2nd-DPO mango; and HNR1 = non-infested ripening Haden mango)

However, about 90% of the 30 most discriminant compounds were associated with headspaces of non-infested ripe (HNR2), *C. cosyra*-freshly-infested (HCC1), the 7th-DPO (HCC7), and the 9th-DPO (HCC9) Haden mangoes (Figure 3-8 B; Appendix: Figure S3-4). The selected compounds were spread in almost all categories e.g. classes of compounds, and concentrations amongst others. *δ*-3-Carene had the highest concentration among the selected compounds, except on the 7th-DPO mangoes when ethyl butanoate was the major compound (Figure 3-8 C).

Overall, there was a strong increase in the volatile concentrations of common compounds from Kent mango following infestation (Figure 3-9 A; Appendix 1: Figure S3-5A). However, for this variety, the concentrations of volatiles of non-infested ripening and ripe mangoes were generally less than those of non-infested unripe mangoes. The trend for concentrations of volatiles was different in Apple and Haden mangoes where non-infested unripe and ripe mangoes released substantial amounts of volatiles, although in most cases lower than infested mangoes (Figure 3-9 B and C; Appendix1:Figure S3-5 B and C).



Figures 3-9: Percentages of the average volatile concentrations of each common compound (relative to the total) of non-infested unripe; *C. cosyra*-freshly-infested mangoes; 9th-DPO mangoes; and non-infested ripe mangoes for the three varieties, Kent (A); Apple (B); and Haden (C)

3.6 Discussion

Tephritid fruit flies and their parasitoids are known to use semiochemicals to locate their hosts for food and oviposition (Carrillo *et al.*, 2017). Most of them are generalists with a wide host range (Ekesi & Billah, 2006; Ekesi *et al.*, 2016). *Ceratitis cosyra*, a member of tephritid, is a notorious mango pest (Steck, 2000). The parasitoid *P. cosyra* is naturally associated with this pest (Mohamed 2007), while *F. arisanus* and *D. longicaudata* were found to have formed new associations with

C. cosyra following their introduction and release in Africa (Mohamed *et al.*, 2008; 2010). Here we explored the *in situ* interactions of *C. cosyra* and its parasitoids.

Our results indicate that this fruit fly was differentially attracted to headspace volatiles from unripe and ripe mangoes compared to their respective controls (clean air). Similar results have been reported for *Bactrocera dorsalis* (Chapter 2; Miano *et al.*, 2022). Surprisingly like *B. dorsalis*, *C. cosyra* prefers volatiles from infested over non-infested unripe mangoes, indicating its ability to discriminate between them. Perhaps volatiles emitted as a result of *C. cosyra* infestation masked host marking pheromone (HMP) that have been reported to deter conspecific of this species, as documented by Cheseto *et al.* (2017; 2023). Alternatively, the volatiles released by infested fruit may indicate the presence of an oviposition substrate or the presence of punctures that would facilitate subsequent oviposition by *C. cosyra*. Indeed, congeneric, *C. capitata* was reported to prefer ovipositing in preexisting oviposition punctures instead of making new ones, despite having host-marking pheromones (Papaj *et al.*, 1992). Similarly, *B. dorsalis* was more attracted to mangoes with ovipositing conspecific females in a field set-up (Miano *et al.*, 2022). On the contrary, *B. zonata* (Saunders) was reported to be more attracted to non-infested guava than infested ones (Binyameen *et al.*, 2021). On the other hand, the oviposition choice of *B. tryoni* (Froggatt) was not dependent on the infestation status when offered high-quality guava (Silva and Clarke, 2021).

Our results also demonstrate that the parasitoids used in this study are attracted to headspace volatiles emitted from the host fruit of their host. *Fopius arisanus* was attracted to the headspace of freshly *C. cosyra*-infested mango which agree with earlier findings in which *F. arisanus* is attracted to other fruit fly-freshly-infested hosts (Chapter 2; Wang and Messing, 2003; Pérez *et al.*, 2013; Cai *et al.*, 2020). The attraction of this parasitoid to *C. cosyra*-freshly-infested mango fruits appears to fit with *F. arisanus'* reported preference to parasitize younger eggs of its host compared to older ones (Moretti and Calvitti, 2003; Karlsson *et al.*, 2018). The attraction of *F. arisanus* to the headspace of non-infested ripe Apple mango fruit is in line with the findings of chapter 2.

The attraction of *D. longicaudata* and *P. cosyrae* to the headspace volatiles of advanced-stage infested mangoes (7th-DPO and 9th-DPO), is not unexpected, because *D. longicaudata*, for example, has a marked preference for late larval instar of their host (Harbi *et al.*, 2019). The higher attraction of *D. longicaudata* to *C. cosyra* 9th-DPO mango of Kent variety compared to Apple and

Haden could be because this variety is more preferred by this fruit fly species as indicated by the higher number of puparia recovered from this variety which might have triggered production of a higher number of headspace volatiles compound as shown in Figure (3-4 A). Nunez-Campero *et al.* (2016) reported that the host density highly influences the number of parasitoids that would visit the host fruit of the fruit fly.

Here, we have reported the attraction of *D. longicaudata* to a wider range of mango treatments than *P. cosyrae*. This finding can explain previous laboratory findings in which *D. longicaudata* parasitizes *C. cosyra* better than its native parasitoid *P. cosyrae* (Ndlela *et al.*, 2020).

The differential performance of *C. cosyra* on the mango varieties in terms of the number of recovered puparia, suggests that *C. cosyra* prefers the Kent variety for oviposition. These findings are in agreement with the preference/performance hypothesis (Thompson, 1988; Gripenberg *et al.*, 2010; Carrillo *et al.*, 2017). Diatta *et al.* (2013) reported similar observations on the differential performance of *B. dorsalis* (= *B. invadens*) among mango varieties. Chapter 2 demonstrated that the number of recovered puparia varied with mango varieties where *B. dorsalis* failed to perform in Kent but did better in the Apple variety which requires further investigation.

There were both qualitative and quantitative differences in headspace volatile composition, though with some notable overlaps among the tested varieties and across treatments. These results are in agreement with what has been reported earlier in which the differences in headspace volatile constituents have been linked to the genetic makeup of the mango varieties (Gonçalves *et al.*, 2016; Shimizu *et al.*, 2021) and the treatments for the same fruit variety (Nair *et al.*, 2015; Cunningham *et al.*, 2016; Miano *et al.*, 2022). We found more esters from headspace volatiles of non-infested ripe mangoes compared to those of non-infested unripe mangoes, whereas the number and the concentrations of defense-related monoterpenes and sesquiterpenes were generally reduced in ripe mangoes. Fruit ripening signifies readiness for seed dispersal and is linked to the attraction of predators, insects and different microorganisms and hence mostly characterized by the release of attractive chemical signals (Rodríguez *et al.*, 2013; Jaleel *et al.*, 2021). *Ceratitis cosyra* may therefore be similarly attracted to ripe mangoes due to the increased esters. In earlier reports, *C. capitata* and *B. dorsalis* were attracted to ripened fruits that emitted more esters (Chapter 2; Biasazin *et al.*, 2018; Miano *et al.*, 2022).

War *et al.* (2012) associated changes in volatile content after the herbivorous attack of a plant with defense mechanisms of the plant against the herbivorous but to the contrary, we have reported an increased attractiveness of *C. cosyra*-freshly-infested mango to conspecifics. Similar results were reported for *B. dorsalis* on mangoes with ovipositing *B. dorsalis* (Miano *et al.*, 2022), *C. capitata* on kumquat, *Fortunella japonica* Swingle (Rutaceae) (Papaj *et al.*, 1992), and *Scirtothrips dorsalis* (Hood) (Thysanoptera: Thripidae) on Bell pepper, *Capsicum annuum* L (Solanaceae) (Shivaramu *et al.*, 2017). Females of *C. cosyra* were also attracted to the other treatments regardless of infestation status. Probably, the presence of compounds like *δ*-3-carene, myrcene, *p*-cymene, (*E*)-ocimene, (*Z*)-ocimene, *α*-terpinolene, *allo*-ocimene, ethyl butanoate, *γ*-octalactone, ethyl 2-methylprop-2-enoate, ethyl tiglate, among others which have been associated with other fruit fly attraction (Jayanthi *et al.*, 2012; Biasazin *et al.*, 2019) were responsible for the attraction of *C. cosyra* demonstrated in this study. In addition to the production of the new compounds, the attractiveness of *C. cosyra* to infested mangoes reported in this study could also be attributed to increased concentrations of most of the headspace compounds, especially the terpenes and esters.

The compounds ethyl propanoate, ethyl butanoate, ethyl 2-methyl butanoate, ethyl 3-methyl butanoate, ethyl-(4*E*)-decenoate and *α*-copaene were tentatively identified in this study. These compounds are also produced by marula, *S. birrea* fruit, found in most parts of sub-Sahara Africa and the preferred wild host of *C. cosyra* (Gikonyo *et al.*, 2005). It would be interesting to investigate whether increased concentrations of ethyl propanoate, ethyl butanoate, ethyl 2-methyl butanoate, ethyl 3-methyl butanoate, ethyl-(4*E*)-decenoate and *α*-copaene increase the attraction of *C. cosyra*. Similarly, the increase in the concentration of headspace volatiles by freshly-infested mangoes and the increased number of esters produced by ripe mangoes could be responsible for the increased attraction of *Fopius arisanus*. Miano *et al.* (2022) reported similar results where *F. arisanus* was increasingly attracted to *B.* dorsalis-freshly-infested mango.

On the same note, *D. longicaudata* was attracted to the headspace volatiles of 7th-DPO (Kent and Apple), 9th-DPO (of the three varieties) and ripe mangoes possibly because of the presence of elevated concentrations of compounds like ethyl propanoate, methyl butanoate, 2-methyl-1-butanol, 2-methyl propyl ethanoate, ethyl 2-methyl prop-2-enoate, and ethyl, 3-hydroxy-butanoate. Eben *et al.* (2000) and the references therein demonstrated how infestation levels and the volatiles produced influenced the host-seeking behavior of parasitoids. Female wasps of *D. longicaudata* were more attracted to the *C. cosyra* 9th-DPO Kent mango variety compared to those attracted to Apple and Haden mangoes. It is interesting to note that this variety is also more preferred by *C. cosyra* as indicated by the higher number of puparia recovered and this might have triggered the production of the higher number of headspace volatiles compounds as shown in Figure (3.4 A). Nunez-Campero *et al.* (2016) reported that the host density highly influences the number of parasitoids that would visit the host fruit of the fruit fly.

3.7 Conclusion and further research

For the first time, we have investigated and reported on the in situ responses of *C. cosyra* and its parasitoids to tree-attached mangoes, supported by the performance of the fruit fly and the subsequent changes in headspace volatile composition. The attraction of *C. cosyra* to infested mangoes indicates its readiness to take advantage of existing oviposition punctures or inability to use the host-marking pheromones. While there is a suggestion that *C. cosyra* could be in the process of being displaced by *B. dorsalis*, our study demonstrates that Kent is the most preferred candidate for the former fly's performance (unlike what has been reported for the later), which is important in advising the currently used IPM strategies. Our results also indicate notable differences in the chemical profiles of the headspaces among the mango varieties and treatments which have direct consequences on the responses of *C. cosyra* and its parasitoid. Most compounds were detected in increasing quantities as post-oviposition days progressed where esters were the most prevalent compounds. This was contrary to the decrease in the quantities of monoterpenes as non-infested mangoes ripened, while those of esters increased. This calls for further studies on how individual volatiles may contribute to fruit fly and parasitoid attraction to provide an evolutionary ecological backdrop to olfactory studies and informed leads for developing selective attractants for combatting fruit fly pests and/or enhancing ecosystem services of their parasitoids.

3.8 References

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Chapter 4: Comparative analysis of olfactomes in tephritid fruit flies and their parasitoids: Implications for Pest management and selective Bait development

4.1 Abstract

Tephritid fruit flies are a well-known pest in fruit and vegetable production, affecting the entire market chain. To manage these pests, parasitoids have been incorporated into their management systems, albeit with varying levels of success. Both fruit flies and parasitoids mostly rely on semiochemicals, among other cues, to locate their preferred hosts. However, it remains unclear whether their olfactomes, which encompass their olfactory responses, converged through evolution. In this study, we investigated and compared the reproduction of *B. dorsalis*, *C. cosyra*, *Zeugodacus cucurbitae* and *B. latifrons* on different species of fruits including mango, banana, and tomato (specifically, Haden, Fhia-17, and Improved Nouvelle F1 varieties). Additionally, we extracted the headspace volatiles from different treatments of mango (freshly *B. dorsalis* or *C. cosyra* infested mango, day 9 post-oviposition mango, and non-infested ripe mangoes *in situ* of Apple, Haden, and Kent varieties), ripe banana (Fhia 17) and tomato (Improved Nouvelle F1 varieties) and analyzed these using gas chromatogram-mass spectrometry (GC-MS) and gas chromatography-electroantennographic detection (GC-EAD) to analyze the responses of the four fruit fly species and the parasitoids *F. arisanus*, *D. longicaudata* and *P. cosyrae*. The results revealed differential performance in terms of the number of fruit fly species puparia recovered in the different fruits. *B. dorsalis* and *C. cosyra* performed significantly better in mango and banana compared to *Z. cucurbitae*, while *B. latifrons* did not perform in these two fruit species. *B. dorsalis*, *C. cosyra* and *Z. cucurbitae* reproduced in tomatoes only when the infestation was in the absence of the other fruits, but much less compared to mango and banana. Interestingly, *B. latifrons* reproduced in the tomato only when the tomato was paired with either mango and/or banana. Fruit fly species differed in their antennal responses to the volatiles of the different mango treatments, as well as to the volatiles emitted by the banana and the tomato with an apparent overabundance of responses to volatiles of their preferred hosts. There was much overlap in the detection even though sensitivities across the four fruit flies differed, especially for mango and banana. Similarly, fruit flies and parasitoids shared antennal-active compounds, with esters being the most prevalently shared compounds. These findings shed more light on the evolution of olfactomes among fruit flies and parasitoids which is something interesting that can be explored towards application in the development of selective baits to control fruit flies.

Keywords: *The fruit fly*. *Parasitoid*. *Headspace volatile compounds*. *GC-MS*. *GC-EAD*. *EAD-active compounds*

4.2 Introduction

The tephritid fruit fly is a well-known and problematic pest in fruit and vegetable production, affecting the entire market chain. Classified as quarantine pests, these flies have the potential to cause devastating losses of up to 100% in their target crops, particularly where no control interventions are implemented (Nankinga *et al.*, 2014). The use of parasitoids is among the many methods employed for their control and management. Within the Braconidae family, the Opiinae subfamily stands out with its abundance of koinobiont endoparasitoids. These parasitoids control fruit flies by laying their eggs either in their host's egg or larval stage (Mohamed *et al.*, 2003; Darrouzet *et al.*, 2007; Badii *et al.*, 2016), resulting in the emergence of the parasitoid from the fruit fly cocoon. Parasitoids have been incorporated into Integrated Pest Management (IPM) packages with reasonable success, particularly in Africa (Sarango, 2014; Muriithi *et al.*, 2020; Niassy *et al.*, 2022)

Females of both tephritid fruit flies and parasitoids exhibit high selectivity in the choice of hosts for reproduction. Parasitoids are known to utilize a range of sensory cues to identify suitable hosts while filtering out non-hosts (Bokonon-Ganta *et al.*, 2007; Segura *et al.*, 2007; Quicke, 2014; Cai *et al.*, 2020). These cues encompass various sensory modalities, including environmental, haptic, visual, auditory, and olfactory.

Olfactory cues are bio-functional organic molecules commonly referred to as semiochemicals, which are divided into allelochemicals and pheromones (Norin, 2007; El-Shafie & Faleiro, 2017; El-ghany, 2019). Among allelochemicals are synomones, allomones, and kairomones. Using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-electroantennography detection (GC-EAD) host volatiles that are detected by insects can be determined. For instance, Cossé *et al.*, (1995) reported that (1*S*)-(-)-*β*-pinene, ethyl octanoate, and *β*-caryophyllene derived from mango volatiles induced EAD responses in *C. capitata* (Wiedemann). In another study, Siderhurst and Jang, (2006) identified 21 EAG-active compounds from *Terminalia catappa* L., with a blend of 19 compounds proving attractive to both male and female *B. dorsalis* (Hendel). Similarly, Kamala *et al.* (2012) reported a group of EAD-active compounds from *Mangifera indica* cv. 'Chausa' and 'Alphonso' volatiles that were attractive to female *B. dorsalis*. Additionally, Kamala *et al.* (2014) reported that *γ*-octalactone, ethyl tiglate, benzothiazole, and 1-octen-3-ol either individually or in combination elicited oviposition in the same fruit fly species.

A blend of ethyl acetate, ethyl propionate, and ethyl butyrate identified from the most attractive guava volatiles was found to be attractive to *B. tryoni* (Frogatt) (Cunningham *et al.*, 2016). In a comparative study, Biasazin *et al.* (2014; 2018; 2019) extensively mapped out the olfactomes of tephritid fruit flies in response to fruit and fermentation volatiles. As a result of these studies, a blend of volatiles was formulated and proven to be attractive to the fruit flies in a six-choice olfactometer assay. Interestingly, while fruit compounds play a significant role in attracting fruit flies, they also attract parasitoids as the fruits provide shelter to their preferred host insects (Eben *et al.*, 2000; Segura *et al.*, 2012; Cai *et al.*, 2020).

Plants/fruits which are under herbivore attack produce herbivore-induced volatiles that sometimes may be indicative of certain species of herbivore. Some of these volatiles are used by conspecifics, thereby increasing herbivory (Masry *et al*., 2018; Binyameen *et al.*, 2021; Silva & Clarke, 2021; Miano *et al.*, 2022), but also by natural enemies of the pest which use the volatiles to orient to their host (Hare 2011; Holopainen & Blande, 2013).

Tephritidae fruit fly parasitoids are attracted to both non-infested and fruit fly-infested fruits but have a high preference for infested ones. For example, the egg parasitoid *F. arisanus* exhibits attraction to non-infested ripe fruits and fresh fruit fly-infested fruits (Chapters 1 and 2; Pérez *et al.*, 2013; Cai *et al.*, 2020; Miano *et al.*, 2022). However, *F. arisanus* displays a stronger preference for tree-attached fruits than those collected from the ground (Eitam & Vargas, 2007). Similarly, the larval parasitoids *D. longicaudata* and *P. cosyrae* have shown attraction to ripened fruits as well as fruits harboring developing larvae (Chapters 1 and 2; Sime *et al.*, 2006; Segura *et al.*, 2012; Harbi *et al.*, 2019; Miano *et al.*, 2022). Furthermore, *P. concolor*, (Sze´pligeti), *P. lounsburyi* (Silvestri) and *P. humilis* (Silvestri) (Hymenoptera: Braconidae), which are parasitoids of the olive fruit fly, *B. oleae* (Rossi), detected and responded to volatiles emitted by various olive tree varieties (Billah *et al.*, 2005; Wang *et al.*, 2011). However, the extent to which parasitoids have fine-tuned their ‘olfactomes’ to selectively detect the odors of their prey, and whether their olfactomes have converged onto similar cues as those detected by their hosts, remain yet to be understood.

We hypothesized that there is no convergence among the antennal active compounds of the Tephritidae fruit fly and their parasitoids. Mapping out the olfactory cues of Tephritidae fruit fly parasitoids and comparing them with those of their hosts would provide valuable insights into the selective pressures that have shaped these interactions over evolutionary time. The GC-EAD technique has been particularly valuable in this regard, allowing for the comparison of sensitivities of olfactory responses between different species of fruit flies to volatiles emitted by different fruits (Biasazin *et al.*, 2019).

In this study, mango (*Mangifera indica* L.), banana (*Musa sp*.), and Tomato (*Solanum lycopersicum* L.) were used as the host fruits since they are preferred hosts for different fruit fly species. The Oriental fruit fly *B. dorsalis*, the melon fly *Z. cucurbitae* (Coquillett), and Solanum fruit fly *B. latifrons* (Hendel), and the marula fruit fly *Ceratitis cosyra* (Walker) were used as the Tephritidae fruit fly hosts. Both *B. dorsalis* and *C. cosyra* are polyphagous fruit flies, but are often associated with infesting mangoes; *Z. cucurbitae* is an oligophagous fruit fly often associated with cucurbitaceous, while *B. latifrons* is a monophagous fruit fly associated with Solanaceae (Díaz-Fleischer *et al.*, 1999; De Meyer *et al.*, 2015 and the references therein). These fruit flies lay eggs beneath the skin of their host and as the egg hatch and the larva develop inside the fruit, they cause irreversible damage. The study parasitoids included *F. arisanus*, *D. longicaudata*, and *P. cosyrae*. *F. arisanus* and *D. longicaudata* are generalists and can parasitize *B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons* while *P. cosyrae* is host-specific and only parasitizes *C. cosyra*.

First, the reproduction (in terms of puparia recovered) of *B. dorsalis*, *C. cosyra*, *Z. cucurbitae* and *B. latifrons* in natural infestation of Haden mango variety (not a favorite of either *B. dorsalis* or *C. cosyra*; Chapters 2 and 3), banana and tomato under controlled laboratory conditions was assessed. To understand the tri-trophic interactions between the fruit, fruit fly and the parasitoids we mapped out the EAD-active compounds of (i) *B. dorsalis*, *C. cosyra*, *Z. cucurbitae* and *B. latifrons* using *in situ* extracted fruit headspace volatiles of infested and non-infested mangoes (Kent, Apple and Haden varieties), ripe bananas and tomatoes, (ii) the four tephritid fruit flies and the parasitoids (*F. arisanus*, *D. longicaudata*, and *P. cosyrae*) using extracts of 9th-DPO Haden mangoes. Finally, we compared the EAD-active compounds among the fruit flies and parasitoids.

4.3 Materials and methods

4.3.1 Experimental fruits

Fruits used in this study were mango, banana (Fhia 17), and tomato (Improved Nouvelle F1). Two flowering Apple, Haden, and Kent mango trees were selected in a mango orchard at Kirinyaga County (00°41'39.8" S 037°24'26.7" E, 1158m asl), Kenya. The mango orchard contained about 100 trees of different varieties (Chapters 2 and 3). Four months after flowering, the young mango fruits were secured in white nets mounted on locally made galvanized metallic wire cube frames (20 × 20 × 20 cm of 2.5 mm) to prevent them from insect attacks. A minimum of four mangoes (yielding a total of at least 32 mangoes per mango variety) could be accommodated in each net. Weekly inspections were conducted on the enclosed mangoes to inspect and remove any potentially infested or damaged fruits. Except for the application of Duduthrin 1.75 EC (Twiga Chemical Industries Ltd, Nairobi, Kenya) at the base of each tree to deter crawling insects, no other pesticides were applied to the mango trees/mangoes. Physiological mature fruits were used for experimental research.

In the same County (00°45'07.6" S 037°20'00.3" E, 1158m asl), two banana trees (Fhia 17) were chosen, each bearing physiologically mature bananas, but of different ages. Unlike most bananas that are cultivated in the study region, farmers had observed and reported that this exotic banana is infested by fruit flies both when physiologically mature and ripe (Figure 4-1).

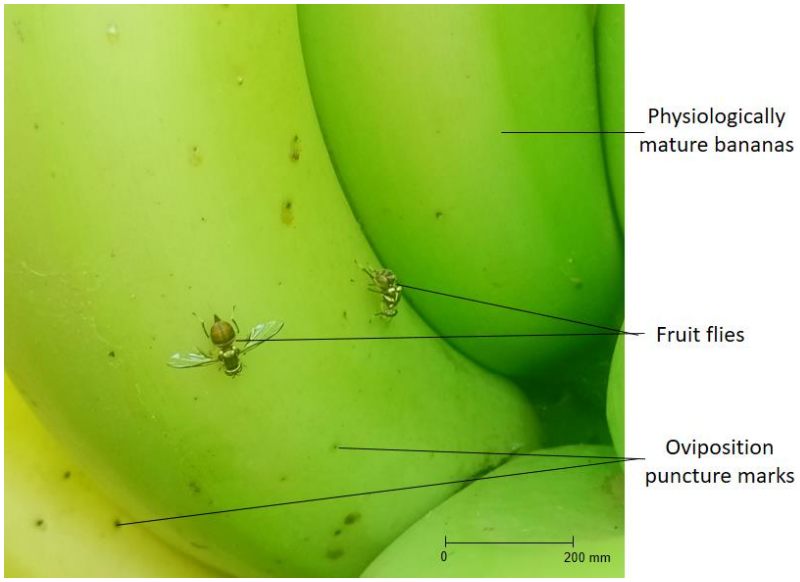


Figure 4-1: Female *B. dorsalis* ovipositing on fruit fly infested bananas (cv. Fhia 17) *in situ*

The lower banana tiers (hands) were removed from the banana heart to remain with two hands each containing at least 16 fruits. The bananas were carefully enclosed in white nets to protect them from insect attack until they naturally ripened.

Tomatoes (Improved Nouvelle F1 tomato seeds (Simlaw Seeds) purchased from an agro vet outlet) were grown in a screened house at Mwea, in the same locality as the bananas, where the tomato seedlings were planted in a mixture of loam soil and goat manure in the ratio of 3:1 and 15 g of diammonium phosphate (DAP) fertilizer. After two months the tomato plants were top-dressed with 15g of calcium ammonium nitrate (CAN). Every week, the tomato plants were watered. No pesticides were applied.

4.3.2 Experimental fruit flies and parasitoids

The fruit fly and parasitoid pupae were sourced from the rearing center at the International Centre of Insect Physiology and Ecology (*icipe)*, (01 ° 13′ 25.3″ S, 36 ° 53′ 49.2″ E; 1600 m asl) Nairobi Kenya. In the center, the fruit fly colony is maintained at 26 ± 2 °C, 50-60% RH, and a natural photoperiod of approximately 12:12 h (L: D). *Bactrocera dorsalis*, *C. cosyra* and the parasitoids were reared following the protocols described by Miano *et al.*, (2022) where ripe Apple mangoes were exposed to the fruit flies as oviposition substrates. After oviposition, some of the freshly-infested mangoes were exposed to the egg parasitoid *F. arisanus* for parasitism and then incubated. The rest of the infested mangoes were incubated and exposed to the larvae parasitoids after the fruit fly larvae had developed to the second instar. On eclosion, fruit flies were separated from the parasitoids. Additionally, *Z. cucurbitae* and *B. latifrons* were respectively reared on cucumber and bitter tomato (*Solanum aethiopicum*). Adult fruit flies were fed on an artificial diet containing a 3:1 mixture of finely ground sugar (Mumias Sugar Company, Nairobi Kenya) and enzymatic yeast hydrolysate (USB Corporation, Cleveland, OH), while adult parasitoids were fed on 80% honey (*Eco Honey*, *icipe*, Nairobi, Kenya). Both parasitoids and fruit flies were provided with water in nine (9) cm glass Petri dishes that had pumice granules to prevent insects from drowning. The adult fruit flies were used *in situ* mango fruit infestations and laboratory experiments. Pupae of the fruit flies and parasitoids were also exported to the Swedish University of Agriculture (SLU, Alnarp, Sweden; Department of Plant Protection Biology) where a colony was set up. The fruit flies and parasitoids were reared using the same protocol as used at *icipe* with the only difference being the use of polyester netting bugdorm-430430 cages (32.5 ×32.5 × 32.5 cm). Fruit flies used in experiments were presumed to be gravid since they were selected from cages that contained a combination of male and female adults (7-14 days for *C. cosyra*, 10-16 days for *B. dorsalis, Z. cucurbitae* and *B. latifrons*, and 6-14 days for parasitoids)

4.3.3 Reproduction of fruit fly species

Ripe non-infested Haden mango, banana, and tomato were harvested from the field sites mentioned in section 4.3.1. The fruits were transported to the *icipe* laboratory for fruit fly reproduction experiments which were conducted at 26 ± 2 °C and relative humidity of 50-60%. Haden mango was chosen to reduce biases in terms of the performance of *B. dorsalis* and *C. cosyra* for Kent and Apple mangoes (Miano *et al.*, 2022, Chapters 2 and 3).

From the cages containing tephritid fruit fly adults (200 adults of each species; ♂: ♀ = ratio-1:1), four sets of 15 egg-laying aged females (12-15 days for *B. dorsalis*, *Z. cucurbitae* and *B. latifrons* and 10-13 days for *C. cosyra*) were selected. Each set was placed in separate cages measuring 30 × 30 × 30 cm. In each cage, a fruit was provided as an oviposition substrate and the fruit flies were given two hours to oviposit (10:00 to 12:00 Kenya local time). The fruits were then removed and incubated individually using the protocol explained in chapters 2 and 3. This procedure was repeated, using different sets of fruit flies of the same cohort and fruits, to get six replicates for each of the three types of fruits (Figure 4-2A$_{1, 2, 3}$). The procedure was repeated using two fruits (Figure 4-2B$_{1, 2, 3}$) and three fruits (Figure 4-2C). After the pupation of all larvae of a given fruit, the puparia were counted and recorded based on the procedure involved.

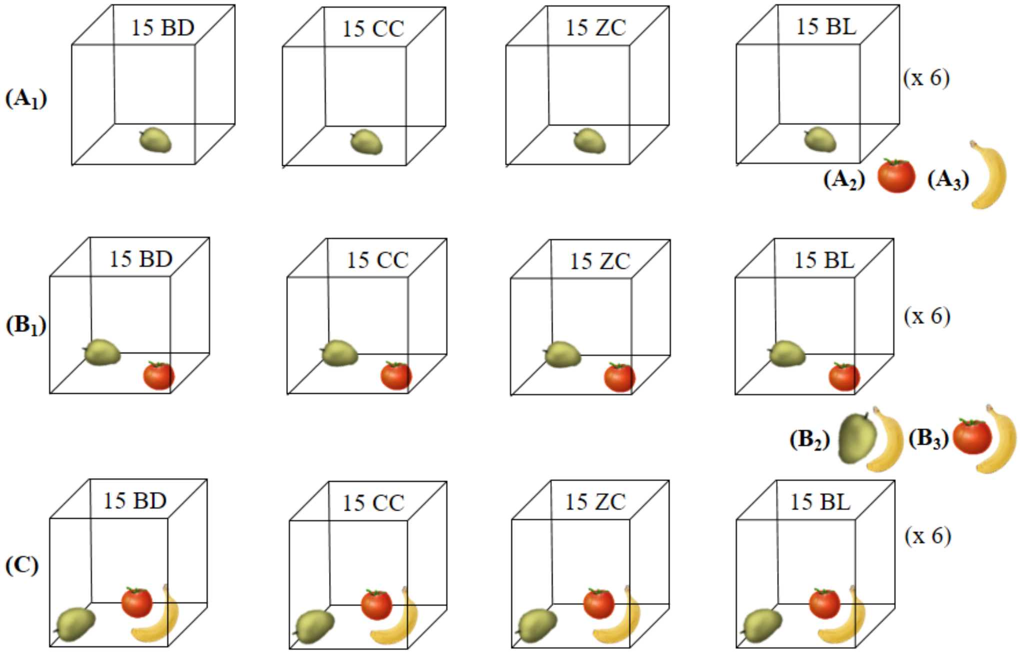


Figure 4-2: Experimental set-ups for infestation with a single fruit species (A), two fruit species (B), and three fruit species (C). The positions of the fruits were randomized in each run to avoid any positional bias. BD, CC, ZC, BL = *B. dorsalis*, *C. cosyra*, *Z. Cucurbitae*, and *B. latifrons* while  denote mango, banana and tomato fruits respectively (Figure not to scale)

4.3.4 Trapping of volatiles from tree-attached fruits

Volatiles were collected *in situ* using HayeSep-Q mixed-phase sorbents which are copolymers of polydimethylsiloxane-divinylbenzene (PDMS-DVB), weighing 30 mg. To ensure cleanliness, the adsorbents secured in delivery glass tubes were initially cleaned with GC-grade dichloromethane (DCM) and dried using a stream of charcoal-purified nitrogen gas. For the collection process, four tree-attached fruits and the sorbent cartridges (attached to $1/4-$inch flexible polytetrafluoroethylene, PTFE, Alltech Associates, Lancashire, UK, tubes) were placed inside clean dry polyacetate oven bags (KitchenCraft, Birmingham, UK) which were tightly sealed with elastic rubber to prevent the entry of non-filtered air. To facilitate sampling, the PTFE tubes were connected to air flow meters and portable vacuum field air pumps (Analytical Research System Inc. Gainesville, Florida 32614 USA). The air pumps were equipped with charcoal air filters, ensuring the purification of the air during the collection process. Clean air was pumped into the dynamic headspace trapping system at a flow rate of 260 mL/min to entrain the volatiles, while the air was drawn out at a rate of 250 mL/min. To prevent contamination excess air was pumped into the trapping chamber to ensure that unclear air did not enter the system.

The headspace volatiles consisted of the following:

i. Headspace volatiles of either *B. dorsalis*- or *C. cosyra*-freshly-infested mature unripe mangoes (Kent, Apple and Haden varieties).
ii. Headspace volatiles of 9th-DPO mangoes (Kent, Apple and Haden varieties) of either *B. dorsalis* or *C. cosyra*.
iii. Headspace volatiles of non-infested ripe mangoes (Kent, Apple and Haden varieties).
iv. Headspace volatiles of fruit fly non-infested ripe bananas.
v. Headspace volatiles fruit fly non-infested tomatoes.
vi. Clean air was used as a control.

The choice of the mango headspace volatiles was informed by the attraction of *B. dorsalis*, *C. cosyra* and their parasitoids as reported in chapters 2 and 3 of this thesis. The volatile collection, elution and storage followed the methods described in the previous study by Miano *et al.* (2022a).

4.3.5 Identification of headspace volatile constituents

The eluents of the headspace volatiles were transported to SLU and kept at -81 °C. Analysis of volatiles was achieved by injecting 1 µL of the sample of extract (obtained by 200 µL elution of the adsorbent cartridge with GC grade dichloromethane) into a GC-MS (Agilent 7890B GC and 5977A MS, Agilent Technologies Inc., Palo Alto, CA, USA). The inlet was in splitless mode with a temperature of 250 °C. The GC-MS used a polar DB-Wax capillary column (60 m × 0.25 mm i.d., 0.25 um film thickness, USD608325H Agilent Technologies Inc.), and helium was used as the carrier gas and at a constant flow rate of 1.2 mL min^{-1}. The GC oven temperature was programmed to start at 40 °C and held for 1 min, after which it increased by 10 °C min^{-1} to 250 ˚C and held for 1 min. Identification of compounds was conducted using Agilent Technologies' Chemstation software. The process involved several steps to ensure accurate identification. First, the mass spectra of the compounds were compared to those available in libraries such as the National Institute of Standards and Technology (NIST) libraries, including NIST 20, as well as Adams and Chemecol libraries (a match above 70% was considered present). Further identification of the compounds was carried out by comparing their calculated retention indices (RI$_{cal}$) with those from the literature (RI$_{lit}$) obtained from published sources. The RI$_{cal}$ values were determined using the Van den Dool and Kratz equation, which involved running a standard mixture of alkanes (C6-C30) under the same experimental conditions as the samples.

4.3.6 Electrophysiology using gas chromatography-electroantennographic detector (GC-EAD)

The samples underwent further analysis using a GC-EAD setup. For this, two µL of the crude sample was manually injected into the GC-EAD setup, which consisted of a DB-Wax column of similar properties and programs as described under the GC-MS setup (section 4.3.5). The GC effluents were split in a 1:1 ratio, with one part flowing through a transfer capillary column to the flame ionization detector (FID), and the other directed to the electroantennography detector (EAD). The EAD transfer capillary column, passing through the GC oven temperature tracking Gerstel olfactory detection port-2, carried the effluents into a 300 mm × 8 mm glass tube. Inside the glass tube, the effluents were mixed with humidified charcoal-filtered air flowing at a rate of 1.5 L min^{-1}. To facilitate the recording of EAD responses an insect antenna was mounted in the system.

For the fruit fly antenna, the fruit fly was immobilized by inserting it into a 200 µL micropipette tip and cutting the tip such that only part of the head and the entire antennae were exposed. Two pulled borosilicate glass capillary tubes (1.5 mm O.D. x 0.86 mm I.D.) filled with Beadle-Ephrussi ringer solution (mixture of 7.5 g NaCl, 0.35 g KCl, and 0.29 g $CaCl_2$ dissolved in 1 L of distilled water) were used. One capillary tube, serving as the reference electrode, was inserted into the head of the fruit fly, while the other capillary tube, connected to the recording electrode, was attached to the tip of the antennae. This arrangement completed the circuit for recording EAD responses (Figure 4-3 A). The recording electrode was connected to a high-impedance GC amplifier interface box (IDAC-2; Syntech, Kirchgarten, Germany) via a pre-amplifier probe.

For the parasitoids, the head with an attached pair of antennae was separated from the body. The head was mounted on a fork using a gel, with the head positioned on the reference electrode and the tips of the antennae placed on the recording electrode. The fork, along with the mounted head, was connected to the pre-amplifier probe, which was further connected to the rest of the system (Figure 4-3 B). All EAD recordings were conducted from the distal position of the antenna as described in Biasazin *et al.*, (2014).

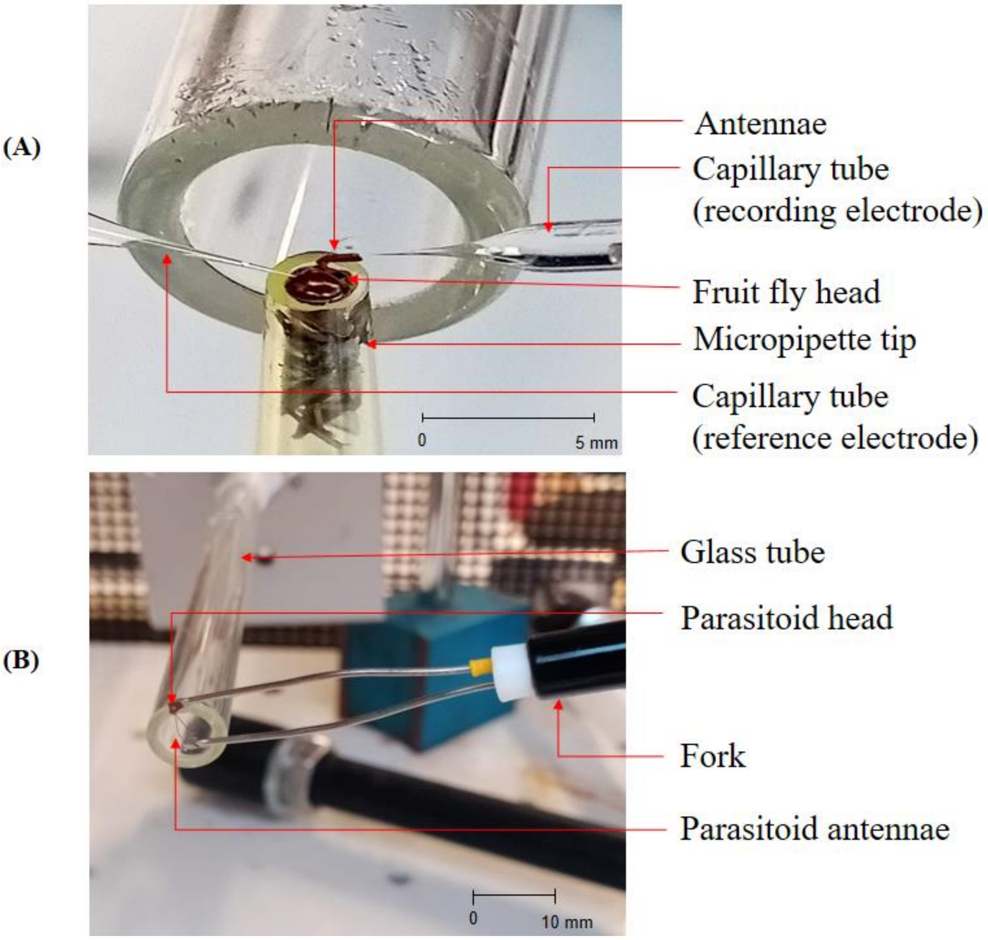


Figure 4-3: Diagrammatic representation of Electroantennography (EAD) setup for recording responses from fruit fly antennae (A) and parasitoid antennae (B). A mixture of volatile components and moisturized air passed through the glass tube to the antennae

GC-EAD tests involved volatile extracts and insect species represented in Table 4-1.

	FRUIT HEADSPACE VOLATILE EXTRACTS															
Antenna	ABD1	ABD9	ACC1	ACC9	ANR	HBD1	HBD9	HCC1	HCC9	HNR	KBD1	KBD9	KCC1	KCC9	KNR	Banana	Tomato
BD	X	X			X	X	X			X	X	X			X	X	X
CC			X	X			X	X	X	X			X	X	X	X	X
ZC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BL							X									X	X
FA							X										
PC							X										
DL							X										

Table 4-1: Volatile extracts and insect species used in GC-EAD. A = Apple; H = Haden; K = Kent; BD = *B. dorsalis*; CC = *C. cosyra*; ZC = *Z. cucurbitae*; BL = *B. latifrons*; FA = *F.*

arisanus; PC = *P. cosyrae*; DL = *D. longicaudata*; NR = non-invested ripe; 1 = freshly-infested; and 9 = 9th-DPO

The EAD recordings of each test were compared and three of the most consistent replicates were considered for antennal active compound screening and further statistical analyses.

4.4 Statistical analyses

The mean numbers of puparia recovered from the reproduction experiment of fruit fly species were computed. To separate the means, one-way analysis of variance (ANOVA) was used followed by post hoc Tukey's honestly significant difference (HSD) test in package Agricolae to identify their differences.

The peak areas of the compounds present in the headspaces of the fruit treatments were used in generating heat maps using the ggplot2 package in conjunction with the geom_tile function in *R* (Wickham, 2016). To obtain these heat-maps, the function pivot longer was utilized to convert multiple columns into two columns: where one column combines the Species (Fruit / Fruit fly) with the Treatment (Day of infestation/Variety) and the second column represents the peak area value. The data frame was filtered to retain only rows whose peak area was greater than zero and then grouped by the headspace volatile compound names and the value of the peak areas. The resulting heat-maps were based on (i) the infestation status of the mango varieties and (ii) the non-infested status of Apple, Haden, Kent, banana and tomato and they were finally saved as a high-resolution image file using the ggsave function. The number of headspace volatile compounds of each chemical class in the various headspace volatiles was visualized in bar graphs.

The number of compounds that elicited antennal responses of the test fruit flies and parasitoids, from each treatment's headspace volatile, were counted and subjected to Pearson's Chi-square test followed by the post hoc Chi-square multi-comparison test in RVAideMemoire to find out whether the fruit treatment and/or species affected the number of EAD active compounds. We also compared the percentages of the number of EAD-active compounds to the headspace volatile compounds of each headspace volatiles. Furthermore, for the 9th-DPO Haden EAD-active compounds of fruit fly and parasitoid species, we compared the number of responses to compounds of each chemical class against the MS tentatively identified headspace volatile compounds of the same class in the treatment to identify the chemical class which had more chances of eliciting antennal activity. Due to the low number of terpenoids detected by the antennae of fruit flies and parasitoids, monoterpenes and monoterpenoids and sesquiterpenes and sesquiterpenoids were respectively combined to monoterpenoid and sesquiterpenoid classes.

The three replicates of antennal responses of each insect species were used to compute a normalized average relative EAD amplitude. Each of the EAD amplitudes was first divided by the weighted mean of the back transformation (exponential, *exp*) of the natural logarithm, *ln*, of all responses in that trace profile. The three normalized amplitude values were then averaged and scaled to the range of 0 to 1 by dividing them with the total sum of averaged normalized responses. Amplitude normalization achieved a relative response which was less sensitive to outliers. The transformation approach allowed us to create tiles with varying colors based on the scaled values of the normalized antennal response (normalized responses). The normalized responses were used to generate heat maps. The normalized response data was also used in similarity percentages (SIMPER) analysis, one-way analysis of similarities (ANOSIM) and in generating NMDS biplots in Bray–Curtis dissimilarity matrix using *PAST 3* software (Hammer *et al.*, 2001).

4.5 Results

4.5.1 Reproduction of fruit fly species

In the no-choice trials, fruit species and varieties (Fhia 17 banana, Haden mango, and Improved Nouvelle F1tomato) differed in their support for oviposition and development of fruit fly species. Whereas a similar number of *B. dorsalis* and *C. cosyra* puparia emerged from bananas, there were much fewer *Z. cucurbitae*, and no *B. latifrons* ($\chi^2 = 196.7$, $df = 3$, $P < 0.001$; Figure 4-4 A). Haden mango supported more *C. cosyra* than *B. dorsalis* and *Z. cucurbitae*, while no *B. latifrons* were recovered ($\chi^2 = 432.9$, $df = 3$, $P < 0.001$; Figure 4-4 B).

Tomato poorly supported fruit fly oviposition and development. Surprisingly, no *B. latifrons* puparia were recovered, whereas those of *B. dorsalis*, *C. cosyra* and *Z. cucurbitae* were statistically equal ($\chi^2 = 19.195$, $df = 3$, $P < 0.001$; Figure 4-4 C).

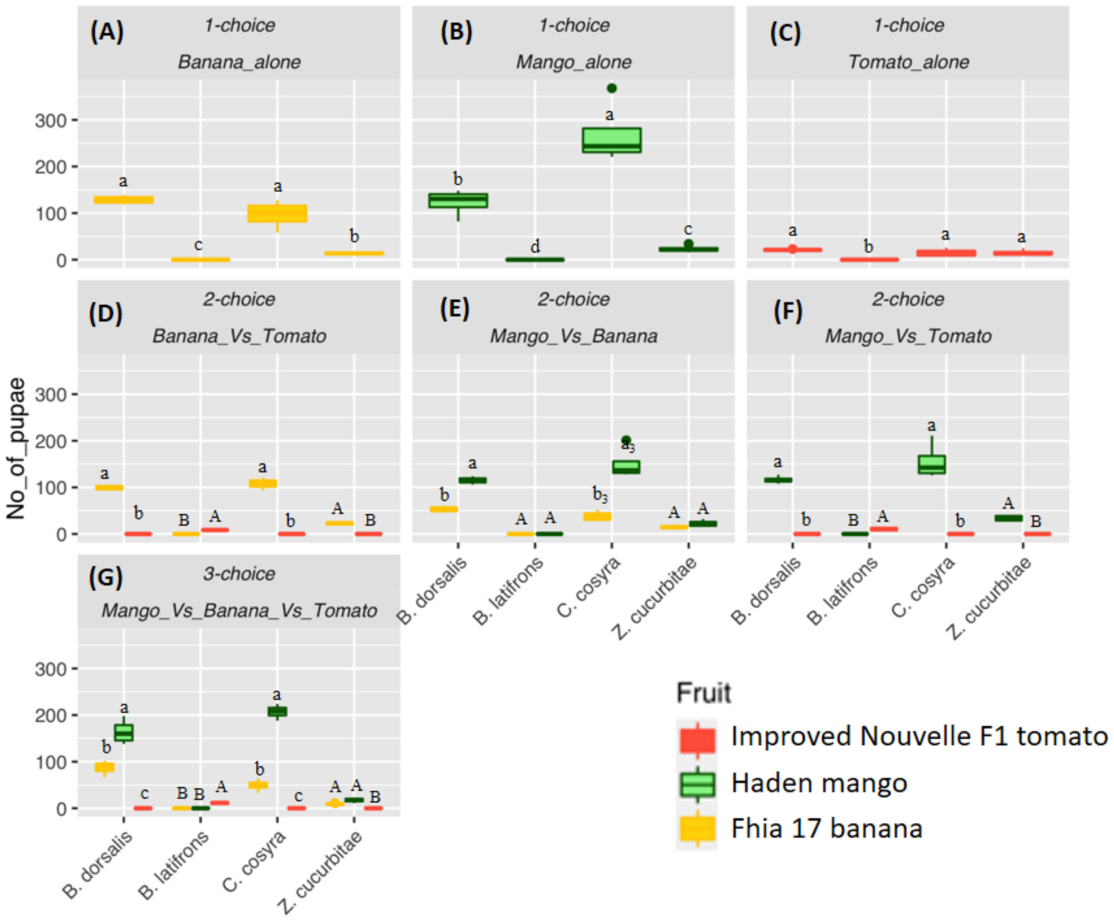


Figure 4-4: The number of puparia observed in the no-choice fruit trials for banana (cv. Fhia 17; A), mango (cv. Haden; B), and tomato (cv. Improved Nouvelle F1; C), 2-choice assays: banana and tomato (D), mango and banana (E), mango and tomato (F), and 3-choice assays (G). Boxplot (interquartile ranges, median and outliers), displays the number of puparia per fruit fly and fruit species. Different letters indicate significant differences

Two-choice experiments reflected the results of no-choice experiments, with tomato not producing any *B. dorsalis*, *C. cosyra*, and *Z. cucurbitae*, when paired with mango or banana ($P < 0.001$, Figure 4-4 D and 4-4 F respectively). *Bactrocera dorsalis* and *C. cosyra* performed better in mango compared to banana ($P < 0.001$), whereas *Z. cucurbitae* performed equally (poor) in both, and none produced *B. latifrons* puparia (Figure 4-4 E). In contrast, *B. latifrons* were recovered only from tomatoes when paired with mango or banana ($P < 0.001$, Figure 4-4 D and 4-4 F respectively).

In the 3-choice experiment, *B. dorsalis* and *C. cosyra* performed better in mango over banana ($P < 0.001$). *Z. cucurbitae* performed equally poorly in both fruits (Figure 4-4G), whereas again no puparia of the three fruit fly species were recovered from tomato. On the other hand, *B. latifrons* puparia were recovered from tomatoes only (Figure 4-4G).

4.5.2 Headspace volatile constituents

Among the different mango varieties and within the treatments of the same variety, the headspace volatiles differed qualitatively and quantitatively. Out of the 238 volatile compounds analyzed in the mango treatments, 233 were tentatively identified. Figure 4-5 is a section of a heatmap of the tentative compounds in decreasing order of sharedness among treatments (Appendix 1: Figure S4-1 for all the compounds; Figure S4-2 for offset chromatograms; Appendix 2: Table T4-1). From the heatmap, volatile compounds of Apple mango are clustered separately from those of Haden and Kent. Among the 238 compounds, 24 were shared among all treatments of the three mango varieties. These compounds included *δ*-3-carene, myrcene, *α*-pinene, *β*-phellandrene, limonene, ethyl octanoate, *β*-pinene, (*E*)-*β*-caryophyllene, terpinolene, *α*-phellandrene, *α*-humulene, ethyl dodecanoate among others (Figure 4-5). Another 24 compounds were absent in only one or two treatments (Figure 4-5). Among the compounds that were absent in all treatments of a given mango variety were sylvestrene and m-cymenene for Apple mango; methyl salicylate, caryophyllene oxide and sabinene for Haden and *γ*-muurolene, camphor, methylethyl tetradecanoate, bicyclogermacrene and *α*-cadinene for Kent (Figure 4-5; Figure S4-1).

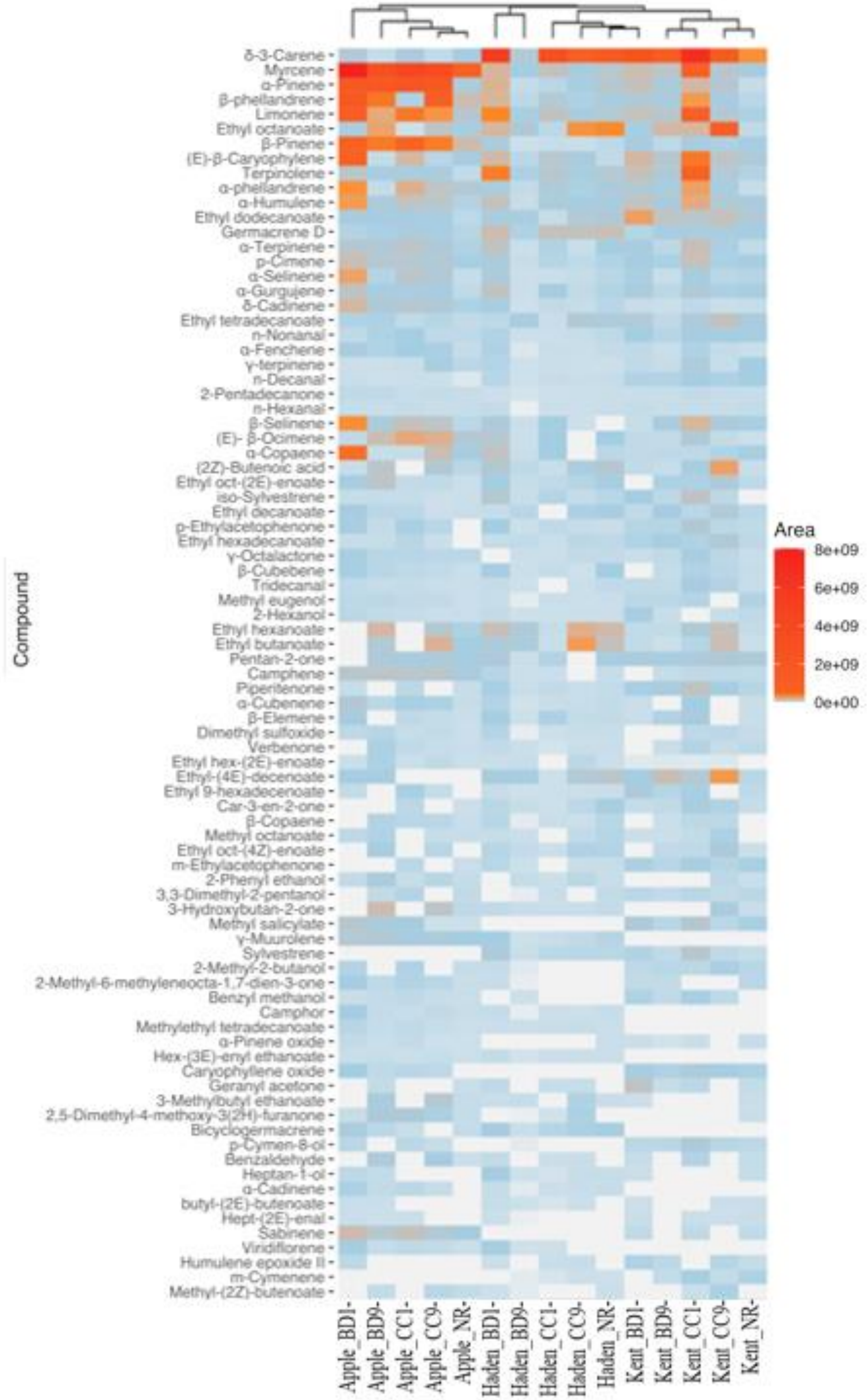


Figure 4-5: Eighty-five of the 238 compounds of mango treatments headspace volatiles in decreasing order of sharedness among treatments. The darker the red colour the larger the compound's peak area and the lighter the blue colour the smaller the peak area in that column.

Corresponding peaks were traced across samples, and the identity of the majority of peaks was tentative. (BD = *B. dorsalis*; CC = *C. cosyra*; 1 = freshly-infested; 9 = 9th-DPO; NR = non-infested ripe)

For the non-infested ripe fruit of banana (cv. Fhia 17), mango (cvs. Apple, Haden and Kent) and Tomato (cv. Improved Nouvelle F1), a total of 239 volatile compounds were reported, out of which 228 were tentatively identified. Figure 4-6 represents the first ninety compounds in their decreasing order of sharedness among fruit headspace volatiles (Appendix 1: Figure S4-3 for all the compounds; Figure S4-2 for offset chromatograms; Appendix 2: Table T4-2).

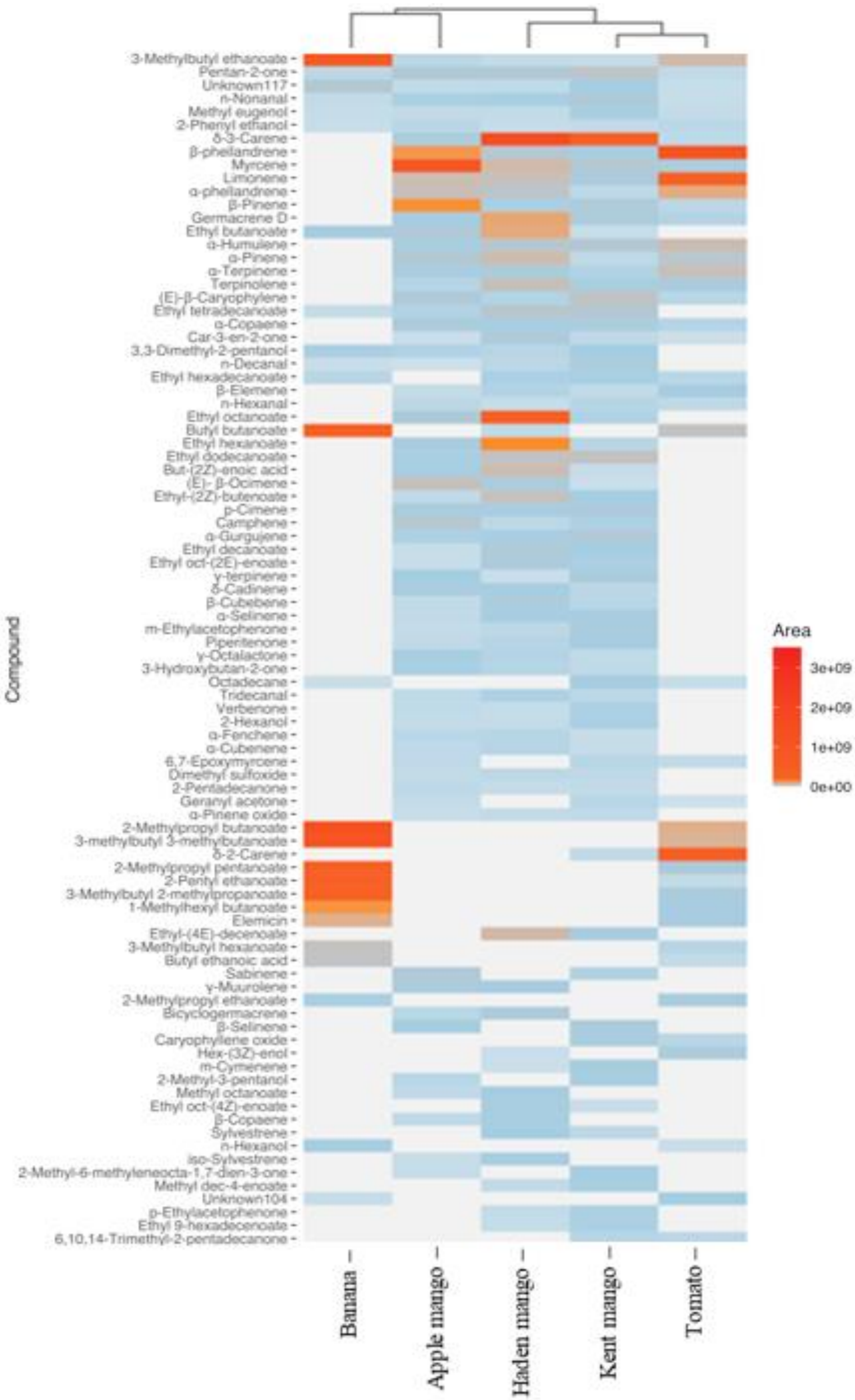


Figure 4-6: Ninety of the 239 tentative compounds of non-infested ripe fruit of banana (cv. Fhia 17), mango (cvs. Apple, Haden and Kent) and Tomato (cv. Improved Nouvelle F1)

headspace volatiles in decreasing order of sharedness. The darker the red colour the larger the compound peak area and the lighter the blue colour the smaller the peak area in that column

Out of these 228 volatile compounds, only six were shared among the fruit species, while four were shared between banana and the three mango varieties, and 16 were shared between tomato and mangoes. The dendrograms indicate some quantitative similarity between pairs of banana and Apple, as well as Kent and tomato headspace, while Haden formed a distinct cluster.

The number of tentatively identified volatile compounds in various chemical classes varied depending on the fruit variety and the corresponding treatment (Figure 4-7).

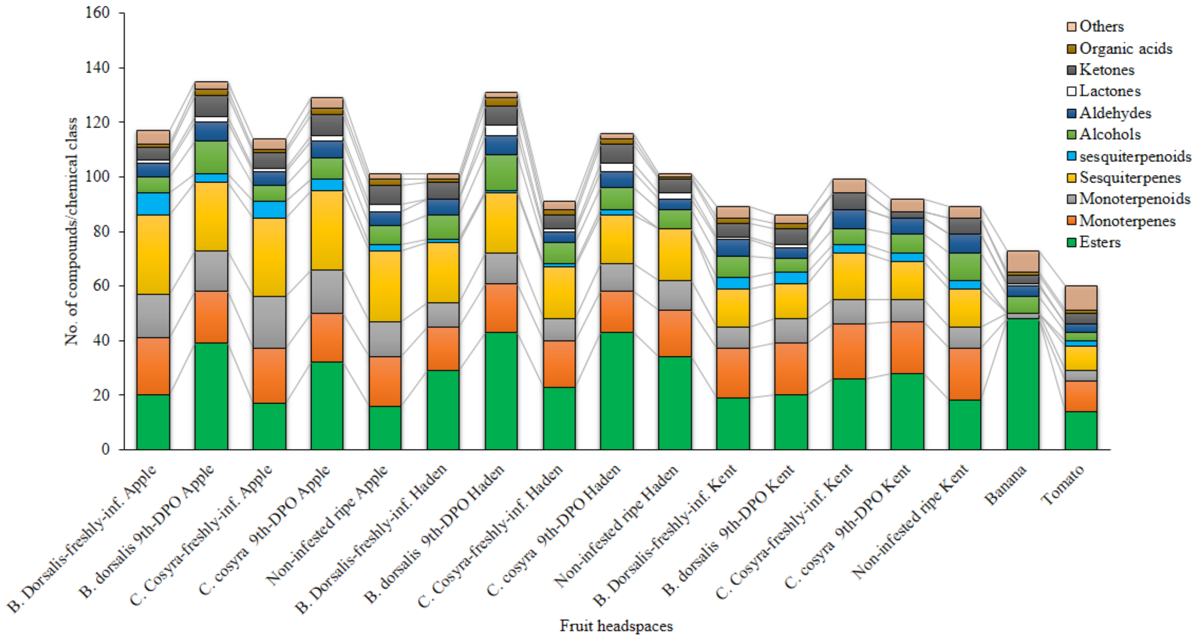
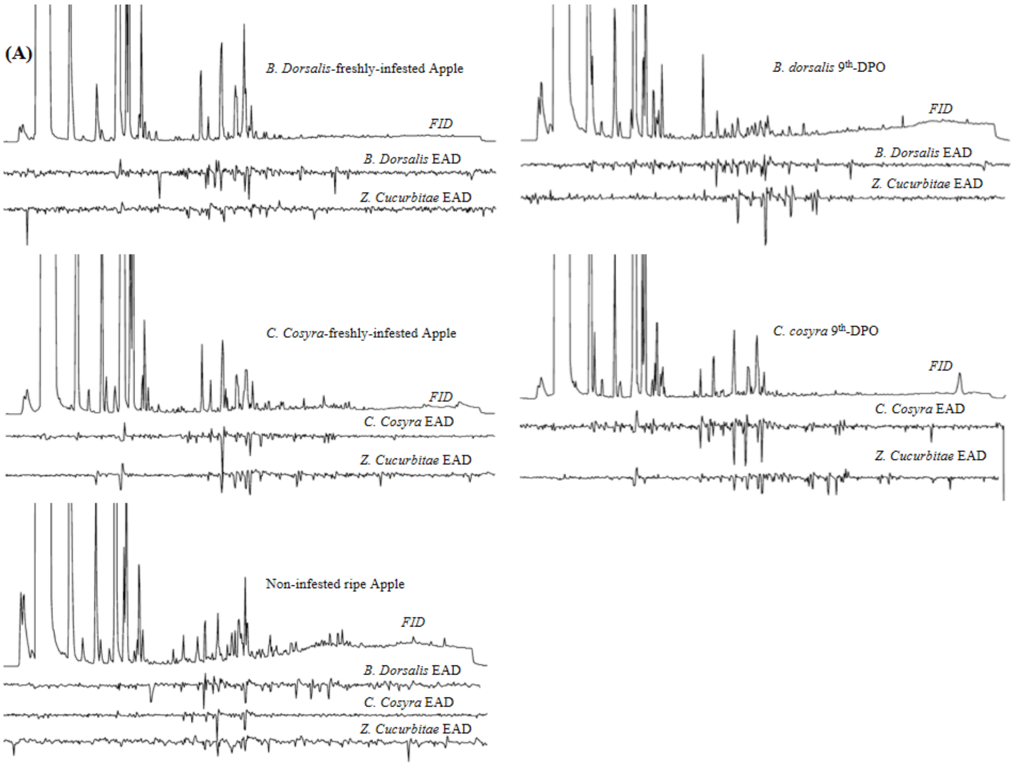


Figure 4-7: The distribution of tentatively identified compounds of all treatments of fruits across the various chemical classes of organic compounds (inf. = infestation; DPO = day post-oviposition)

Sesquiterpenes dominated the headspace of B. *dorsalis*- and *C. cosyra*-freshly-infested and non-infested ripe Apple mangoes (25-26%), whereas in non-infested Kent mango, monoterpenes were more abundant (21%). In all the other headspaces, esters were the majority (22-37% for mangoes, 66% for bananas and 23% for tomatoes). Unlike in mango and tomato, the headspace of banana did not contain monoterpenes, sesquiterpenes, and sesquiterpenoids (Figure 4-7).

4.5.3 Antennal responses of fruit flies and parasitoids

B. dorsalis, *C. cosyra* and *Z. cucurbitae* antennae responded to many of the same compounds (Figure 4-8; Appendix 1: Figure S4-4), with different degrees of depolarization. Some compounds induced responses specific to a species.



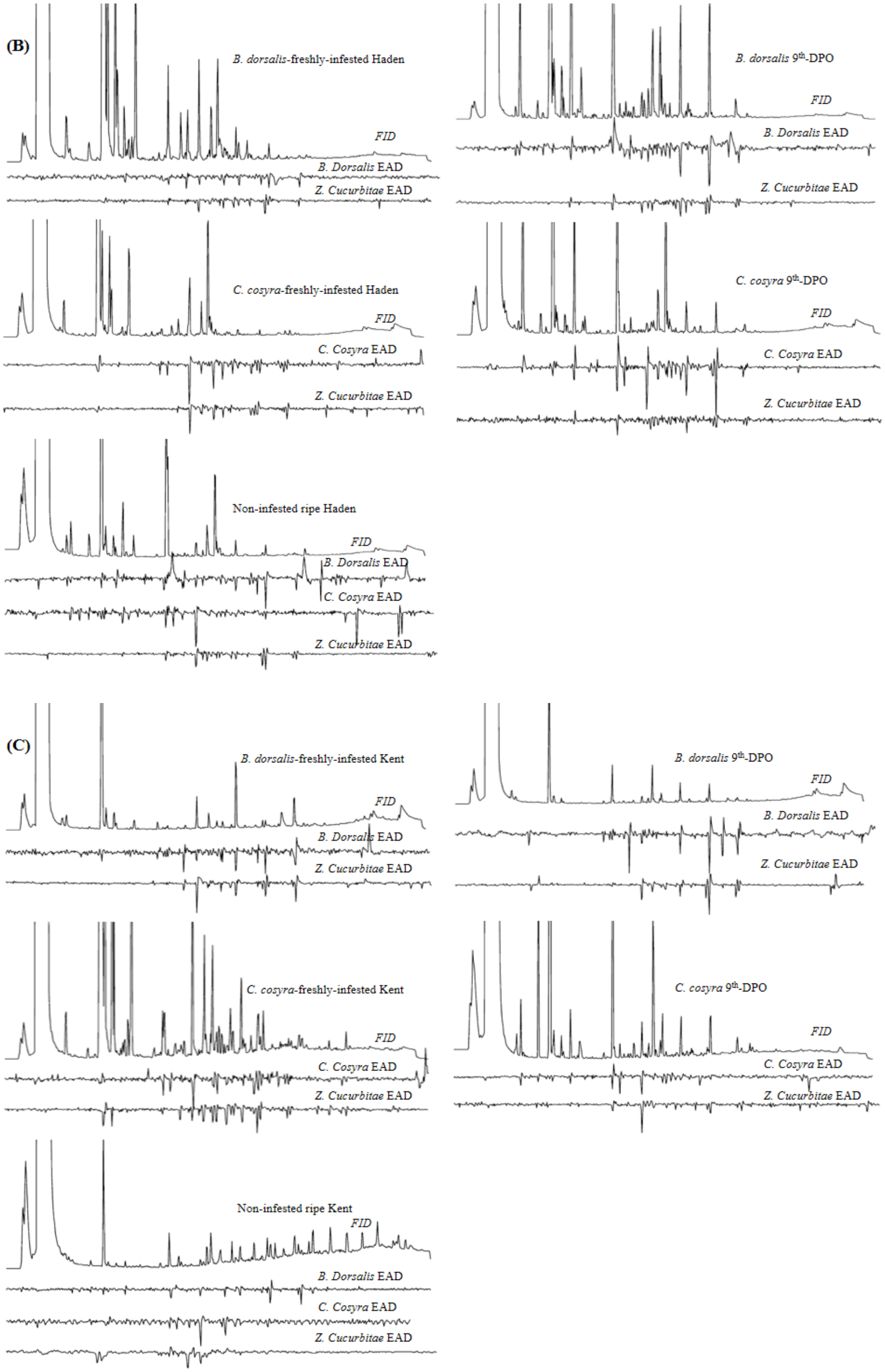


Figure 4-8: Offsets of gas chromatography-electroantennographic detector responses of female *B. dorsalis*, *C. cosyra* and *Z. cucurbitae* to different treatments of mango headspace volatiles of cv. Apple (A); cv. Haden (B); and cv. Kent (C)

For all treatments of the three mango varieties, a total of 129 compounds (Appendix 1: Figure S4-5) elicited antennal responses, and 124 were tentatively aligned with MS spectra using retention indices. Varieties, and treatments within varieties, differed in the number of EAD-active compounds. The 3 treatments of Haden mango differed in the number of antennal-active headspace compounds in *B. dorsalis* ($\chi^2 = 19.6$, $df = 2$, $P < 0.001$) and *C. cosyra* ($\chi^2 = 15.48$, $df = 2$, $P < 0.001$), as did Apple mango for *C. cosyra* ($\chi^2 = 7.34$, $df = 2$, $P < 0.05$) and *Z. cucurbitae* ($\chi^2 = 11.22$, $df = 4$, $P < 0.05$). There were no significant differences in the number of EAD-active compounds of the other treatments in the fruit fly species. Figure 4-9 shows the first sixty-six antennal-active compounds in their decreasing order of sharedness among the fruit fly species.

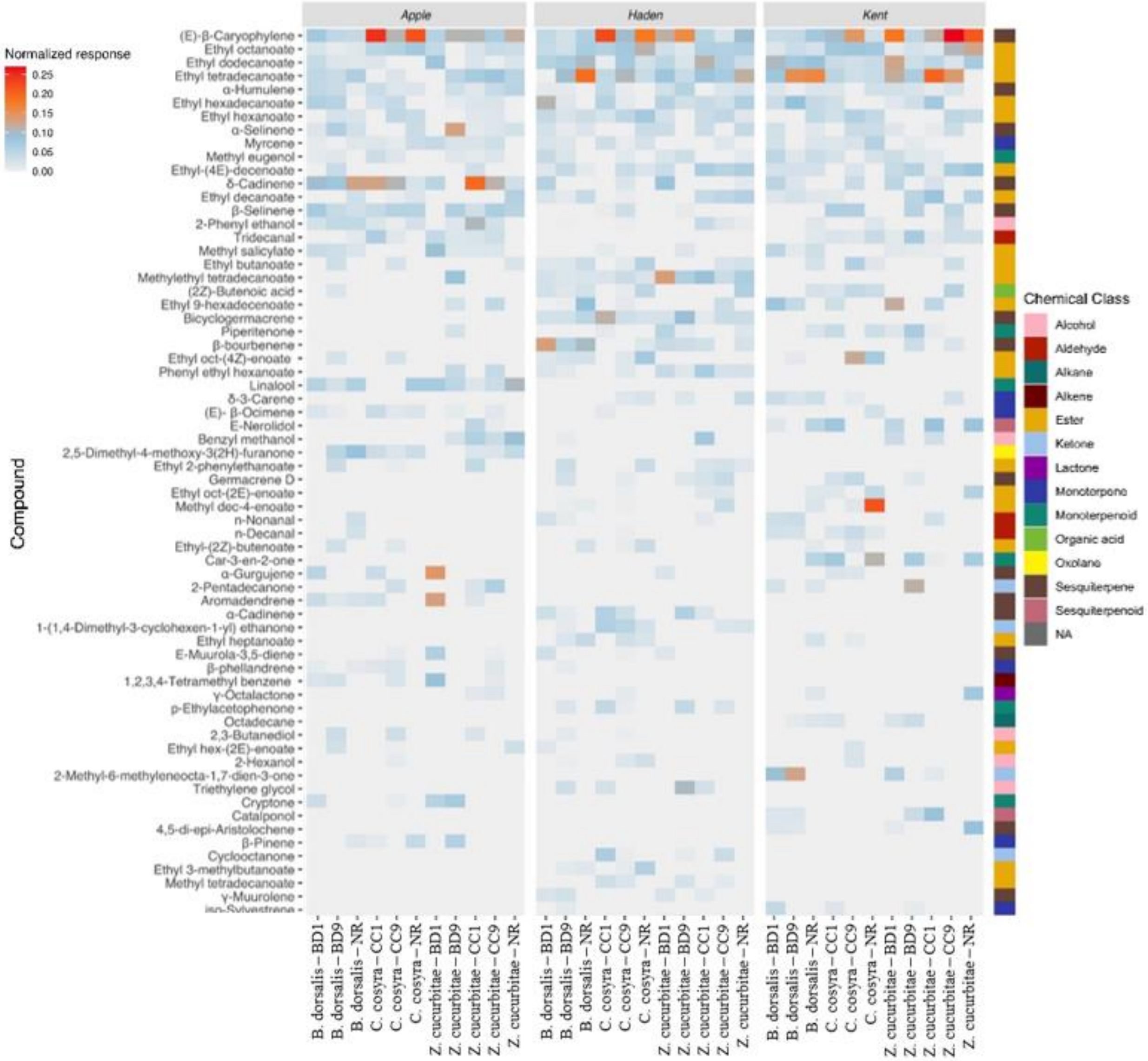


Figure 4-9: The normalized antennal responses of the fruit flies (*B. dorsalis*, *C. cosyra*, and *Z. cucurbitae*) to different treatments of freshly-infested (1), day 9 post-oviposition (9), and non-infested ripe (NR) mango (Apple, Haden, and Kent) headspace volatiles. From left, the columns represent: (a) the tentative names of the compounds in decreasing order of sharedness; (b) heat plot for the antennal normalized responses of the fruit flies per mango variety/treatment (key on the left side of the plot); and (c) a side bar indicating the chemical classes of the compounds (BD = *B. dorsalis*; CC = *C. cosyra*; 1 = freshly-infested; 9 = day 9 post-oviposition; NR = non-infested ripe)

In most treatments and across the mango varieties, myrcene, ethyl hexanoate, ethyl octanoate, (*E*)-*β*-caryophyllene, ethyl decanoate, *α*-humulene, *β*-selinene, ethyl dodecanoate, ethyl tetradecanoate and ethyl hexadecanoate, amongst others, were generally shared among the mango headspace treatments and were consistently detected by the antenna of the three fruit fly species (Figure 4-9). Some EAD-active compounds were unique to a particular mango variety. For instance, linalool was specific to Apple mango, while tridecanal was specific to both Apple and Kent mangoes, and resulted in antennal activity in all three fruit fly species (Figure 4-9).

The number of EAD-active mango compounds from a given mango variety and treatment that were detected by a fruit fly species was expressed as a percentage of the total number of MS compounds from each mango treatment (Figure 4-10).

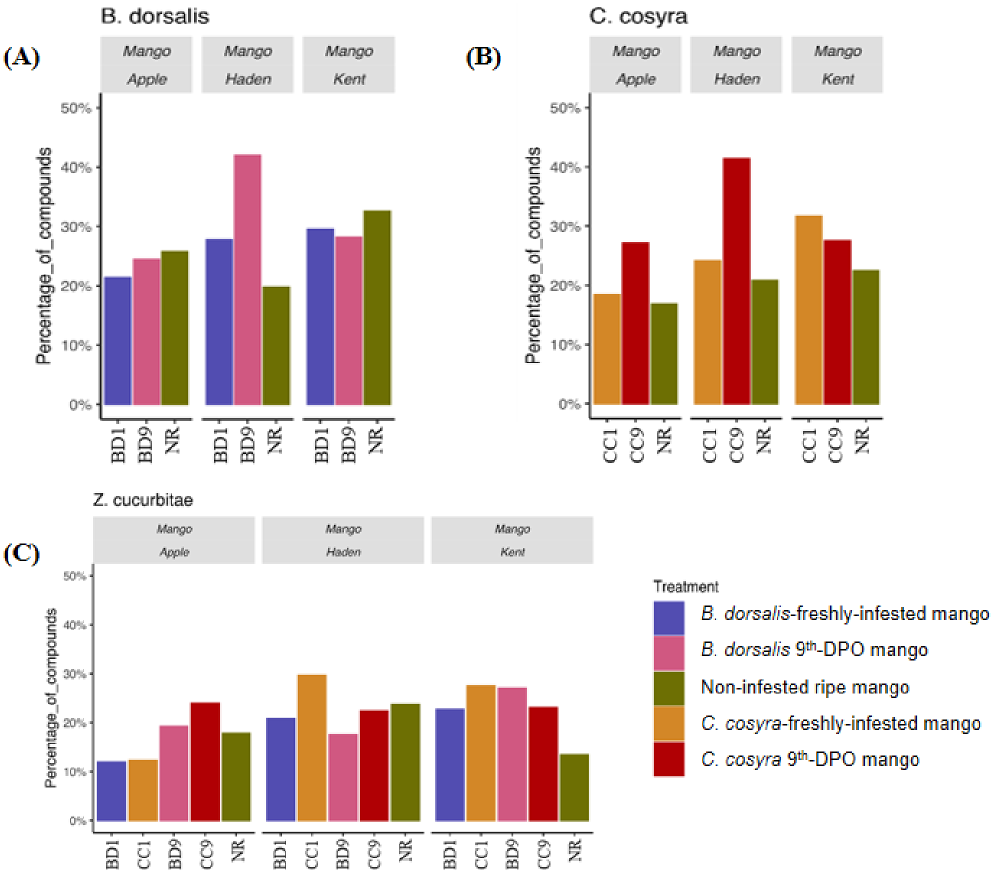


Figure 4-10: The percentages of EAD-active compounds of *B. dorsalis* (A); *C. cosyra* (B); and *Z. cucurbitae* (C) relative to their corresponding MS-volatile compounds of mango treatments (BD = *B. dorsalis*; CC = *C. cosyra*; 1 = freshly-infested; 9 = 9th-DPO; NR = Non-infested ripe)

For *B. dorsalis*, the highest percentage of antennal-active compounds per mango variety, relative to the mango treatment MS tentatively identified compounds, were from Haden *B. dorsalis* 9th-DPO mango volatiles, non-infested ripe Apple and Kent mango volatiles (Figure 4-10 A).

Similarly, the antennae of *C. cosyra* showed the highest EAD-active compounds in *C. cosyra* 9th-DPO mango of Apple and Haden mango volatiles, as well as in *C. cosyra*-freshly-infested Kent headspace volatiles (Figure 4-10 B). *Zeugodacus cucurbitae* antennae detected the highest EAD-active compounds of *C. cosyra* 9th-DPO Apple mango, and both *C. cosyra*-freshly-infested Haden and Kent mango volatiles (Figure 4-10 C).

The similarity percentages (SIMPER) of the EAD-active compounds of the mango headspace volatiles arranged the compounds in a hierarchical order of their dissimilarity contributions. The 30 topmost discriminating compounds accounted for 61.2% of the total dissimilarity contribution (Figure 4-11 A) and their association with the treatments are presented in the NMDS biplots (Figure 4-11 B). One-way analysis of similarity (ANOSIM) revealed a significant difference in the normalized responses of mango treatment headspace volatiles (*R* = 0.5804, *P* = 0.0001, at dimension *k* = 2, *stress value* = 0.293) (Figure 4-11 B; Appendix: Figure S4-6).

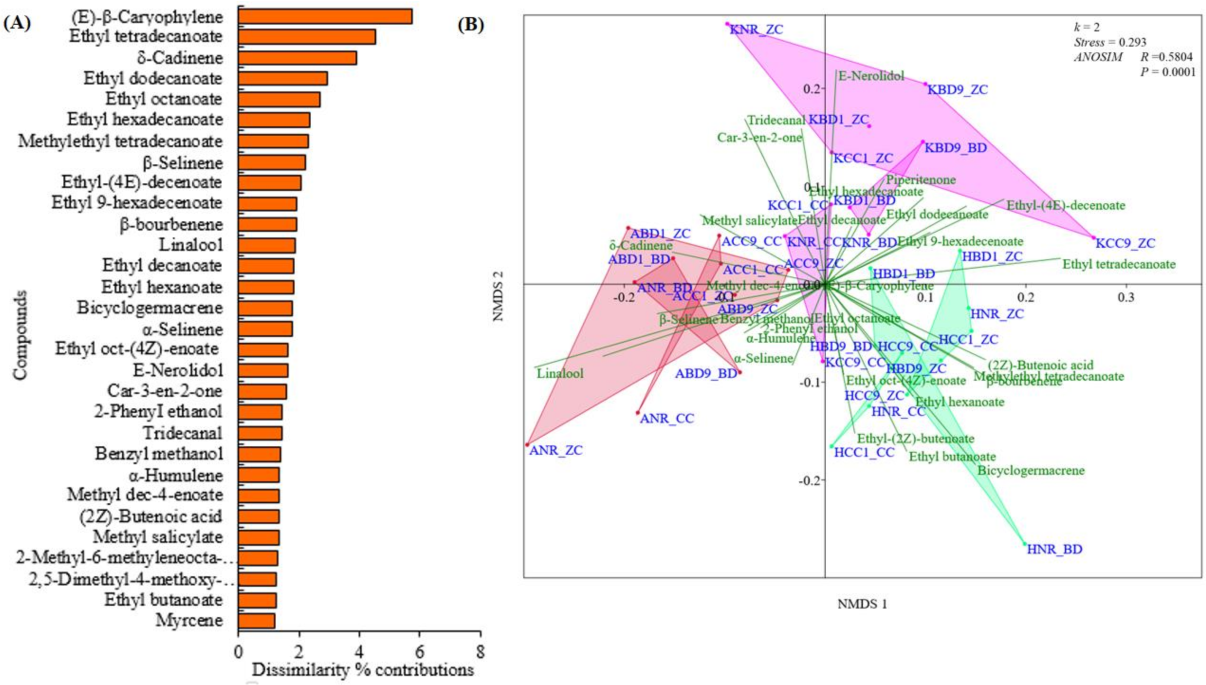


Figure 4-11: (A) The 30 topmost discriminant normalized responses of compounds of mango treatments of the three varieties based on NMDS similarity percentages (SIMPER) in their decreasing order of importance. (B) The NMDS biplots of the 30 topmost discriminant compounds (A = Apple, H = Haden, K = Kent, BD = *B. dorsalis*, CC = *C. cosyra*, ZC = *Z. cucurbitae*, NR = non-infested ripe, 1 = freshly-infested, 9 = 9th-DPO mango)

Out of the 30 topmost discriminant compounds, 40% were esters and 60% elicited antennal responses in *B. dorsalis*, *C. cosyra* and *Z. cucurbitae*. These compounds were identified from almost all mango treatments (Figure 4-11 A). Some compounds were detected from specific mango varieties and triggered antennal activities. For instance, methylethyl tetradecanoate was detected in all Apple and Haden mango headspaces and elicited responses in three species. Other compounds that elicited antennal activities of all fruit flies included methyl dec-4-enoate of Haden and Kent; 2,5-dimethyl-4-methoxy-3(2H)-furanone and linalool of Apple; *E*-nerolidol, tridecanal and methyl salicylate of Apple and Kent treatments; and car-3-en-2-one of Kent. The 30 EAD-active compounds were generally distributed among the antennae of all fruit fly species (Figure 4-11 B).

Bactrocera dorsalis, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons* also shared antennal activity to banana and tomato compounds (Figure 4-12; Appendix 1: Figure S4-7), with the strength of the depolarization consistently differing between species.

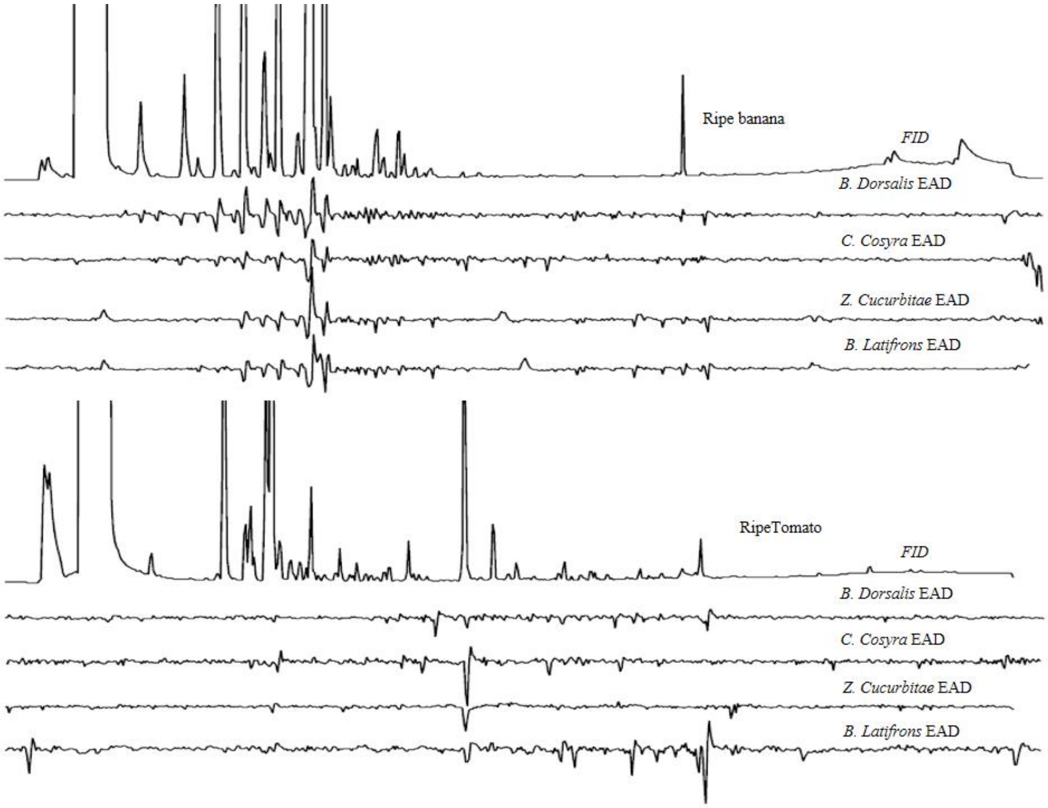


Figure 4-12: Offsets of gas chromatography-electroantennographic detector responses of *B. dorsalis*, *C. cosyra*, *Z. cucurbitae* and *B. latifrons* to ripe banana (cv. Fhia 17) and tomato (cv. Improved Nouvelle F1) volatiles

Ripe bananas and tomatoes had a total of 87 antennal active compounds out of which 75 were tentatively aligned with GC-MS runs and tentatively identified (Figure 4-13). There was a significant difference in the number of EAD-active banana compounds ($\chi^2 = 27.22$, $df = 3$, $P < 0.001$) and tomato compounds ($\chi^2 = 17.2$, $df = 3$, $P < 0.001$) that were detected by the four fruit fly species.

Figure 4-13: The normalized antennal responses of the fruit fly species (*B. dorsalis*, *B. latifrons*, *C. cosyra*, and *Z. cucurbitae*) to banana (cv. Fhia 17) and tomato (cv. Improved Nouvelle F1) volatiles. From left, the columns represent: (a) the tentative names of the compounds in decreasing order of sharedness; (b) a heat plot for the antennal normalized

responses of the fruit flies to banana and tomato volatiles (key on the left side of the plot); and (c) a side bar indicating the chemical classes of the compounds

Of the headspace volatiles, 3-methylbutyl ethanoate, 2-methylpropyl pentanoate, pentyl 2-methylpropanoate, 3-methylbutyl 3-methylbutanoate and 1-methylhexyl butanoate of the banana and (*E*)-*β*-caryophylene of the tomato were shared among the four fruit flies. Fifteen compounds, among them propyl 3-methyl butanoate, 1-methylbutyl butanoate, 3-methyl-2-butyl acetate, 4-methyl-2-pentenoate, 4-heptanone and elemicin of the banana and β-phellandrene, 3-methylbutyl hexanoate, α-humulene, 7-methyl-5-octen-4-one, 2-phenyI ethanol, ethyl tetradecanoate and 6,10,14-Trimethyl-2-pentadecanone of tomato were EAD-active to three of the fruit fly species (Figure 4-13).

The percentages of the EAD-active compounds in each fruit fly species relative to MS volatile compounds of banana and tomato are shown in Figure (4-14). Among the four fruit fly species, *B. dorsalis* and *B. latifrons* antennae detected the highest percentage of banana and tomato headspace volatiles, respectively while *Z. cucurbitae* exhibited the lowest percentage of EAD-active compounds.

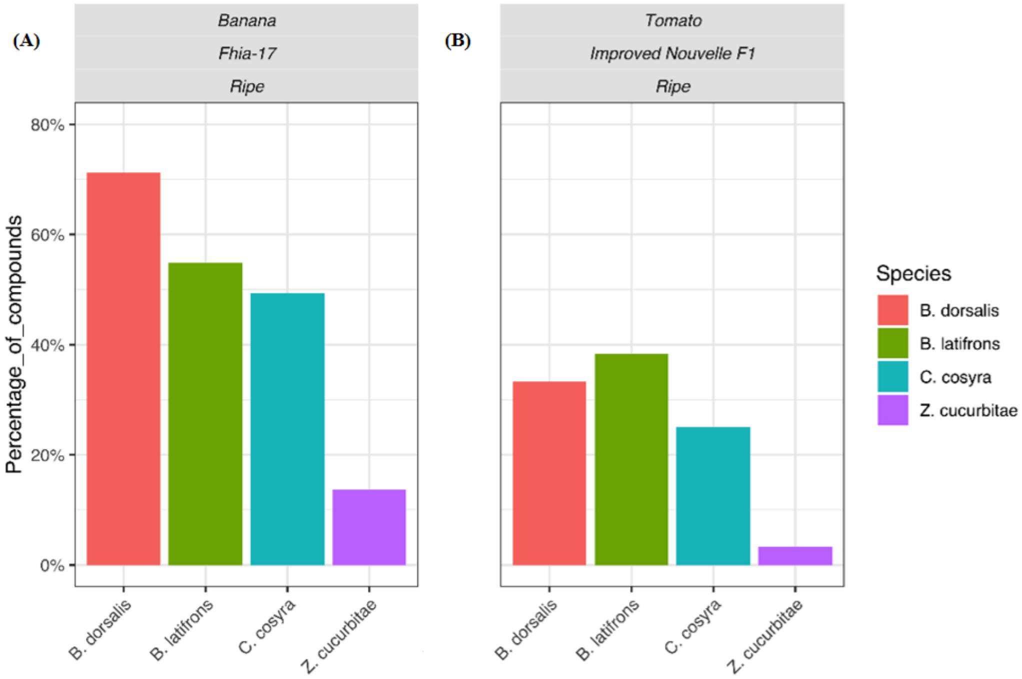


Figure 4-14: The percentages of EAD-active compounds detected by *B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B, latifrons* relative to their corresponding MS-volatile compounds of banana (cv. Fhia 17; A) and tomato (cv. Improved Nouvelle F1; B) volatiles

The 30 topmost discriminant EAD-active compounds of banana and tomato headspace volatiles as per similarity percentage *SIMPER* of the NMDS accounted for 68% of the total dissimilarity contribution (Figure 4-15 A; Appendix 1: Figure 4-8) and their association to the fruit headspace are presented in Figure 4-15 B. There was a significant difference in the normalized antennal responses by the four fruit flies to banana and tomato volatiles compounds (one-way analysis of similarity, *ANOSIM*, $R = 0.5521$, $P = 0.0001$, at dimension $k = 2$, *stress value* = 0.09179) (Figure 4-15 B).

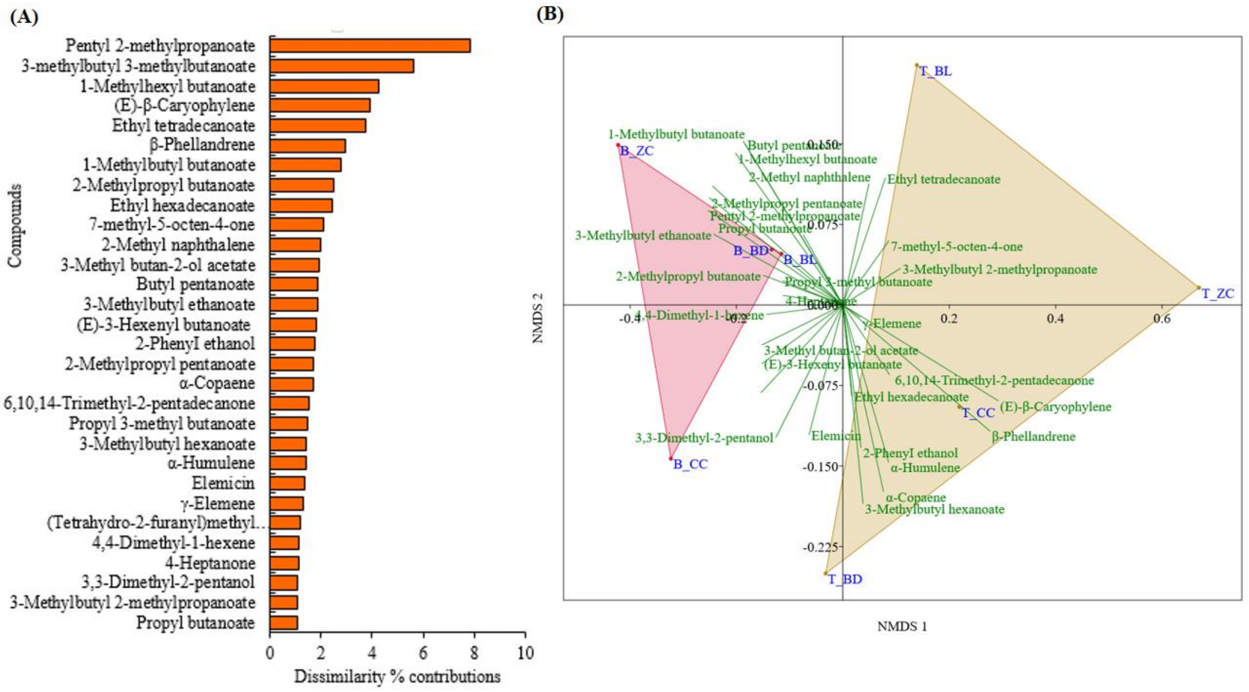


Figure 4-15: (A) The 30 topmost discriminant normalized responses of EAD-active compounds of banana and tomato based on NMDS similarity percentages (SIMPER) in their decreasing order of importance. (B) The NMDS biplots of the 30 most discriminant EAD-active compounds (B = banana, T = tomato, BD = *B. dorsalis*, CC = *C. cosyra*, ZC = *Z. cucurbitae*, BL = *B. latifrons*)

Among the 30 topmost discriminant EAD-active compounds, 50% were esters (Figure 4-15 A). Several compounds, such as 3-methyl butan-2-ol acetate, 3-methylbutyl 3-methylbutanoate, and pentyl 2-methylpropanoate of banana, elicited antennal responses in *B. dorsalis*, *C. cosyra*, *Z. cucurbitae* and *B. latifrons*.

For the antennal responses of the fruit fly and parasitoid species, *B. dorsali*s 9th-DPO Haden mango headspace volatile extracts were used. This is because *B. dorsali*s 9th-DPO Haden mango headspace volatile extracts gave the highest percentage of EAD-active compounds relative to their corresponding MS tentatively identified compounds. Most of the compounds present in this treatment were also there in *C. cosyra* 9th-DPO and non-infested ripe Haden mango treatments although with varying concentrations. This variety of mangoes was also used in the reproduction experiments involving *B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons*. The resulting responses of parasitoids were compared to those of fruit fly species (*B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons*) (Figure 4-16; Appendix 1: Figure S4-9).

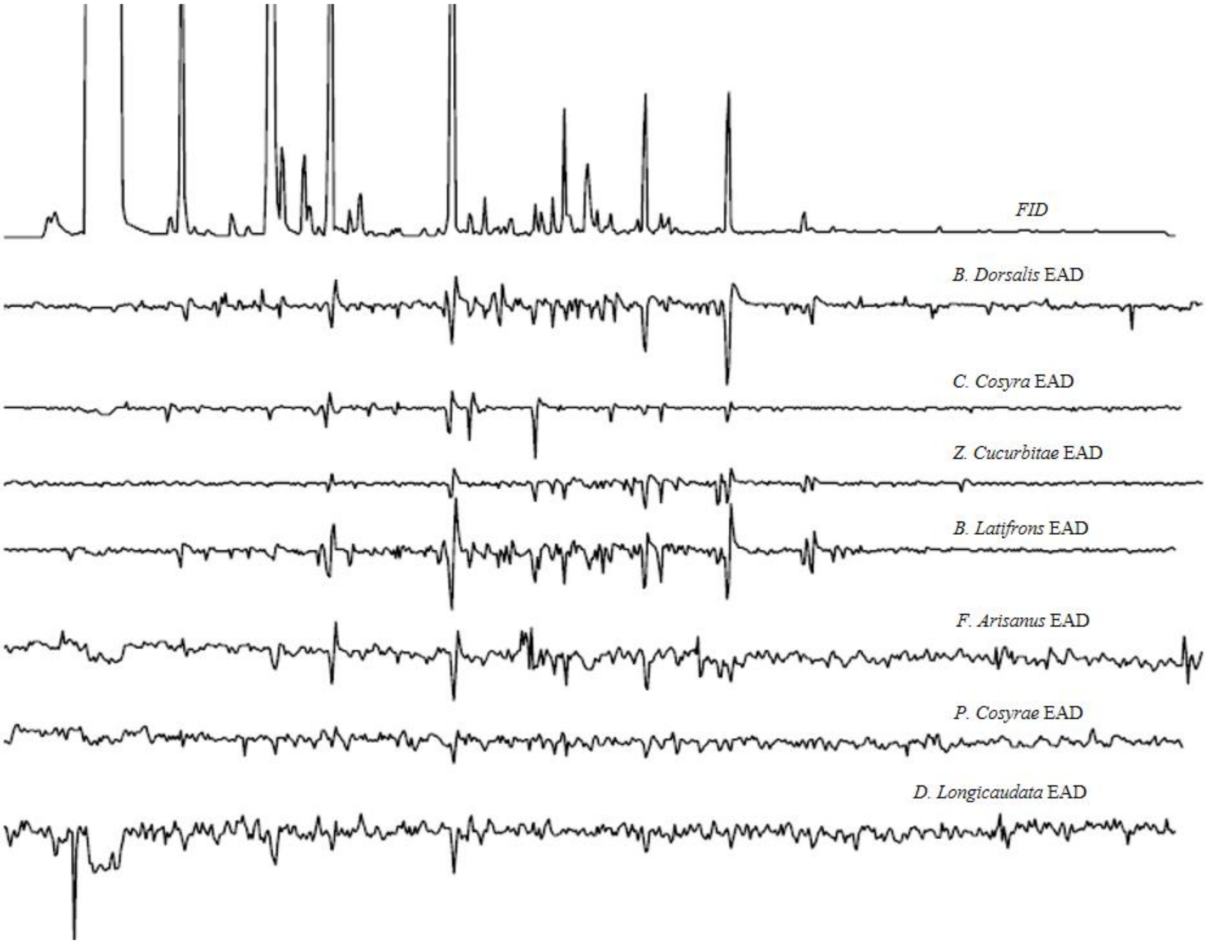


Figure 4-16: Offsets of gas chromatography-electroantennographic detector responses of fruit flies (*B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons*) and parasitoids (*F. arisanus*, *P. cosyrae*, and *D. longicaudata*) to *B. dorsali*s 9th-DPO Haden mango headspace volatiles

A total of 88 compounds elicited antennal activities of fruit flies and parasitoids out of which 86 were tentatively identified (Figure 4-17). There was a significant difference ($\chi^2 = 41.23$, $df = 6$, $P < 0.001$) in the number of EAD-active compounds among the four fruit fly and the three parasitoid species.

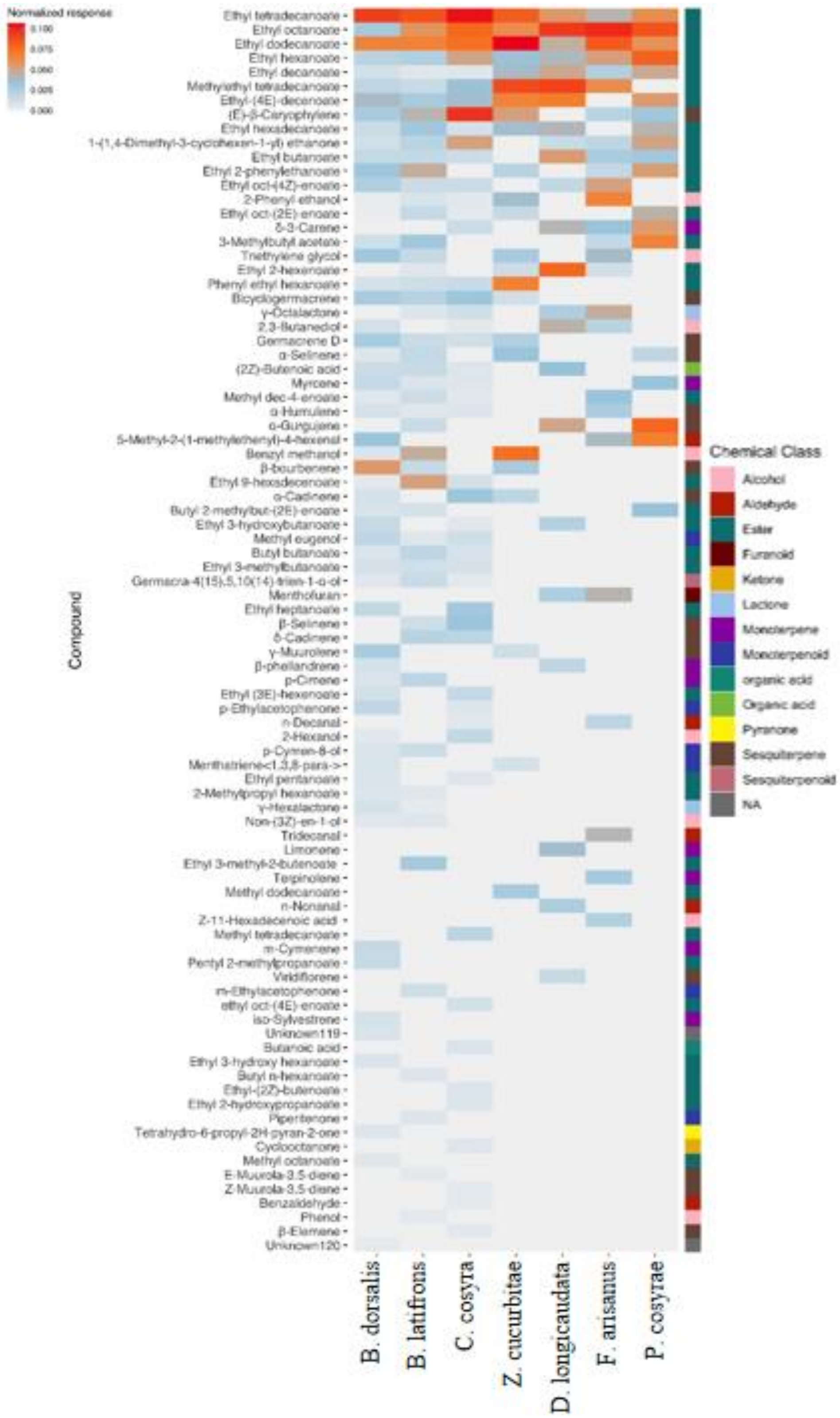


Figure 4-17: The normalized antennal responses of the fruit flies (*B. dorsalis*, *B. latifrons*, *C. cosyra*, and *Z. cucurbitae*) and parasitoids (*D. longicaudata*, *F. arisanus*, and *P. cosyrae*) to

headspace volatiles of *B. dorsalis* 9th-DPO Haden mango. From left, the columns represent: (a) the tentative names of the compounds in decreasing order of sharedness; (b) a heat plot for the antennal normalized responses of fruit flies and parasitoids (key on the left side of the plot); and (c) a side bar indicating the chemical classes of the compound

Of the EAD-active compounds of headspace volatiles of *B. dorsalis* 9th-DPO Haden mango, ethyl tetradecanoate, ethyl octanoate, ethyl dodecanoate, ethyl hexanoate, and ethyl decanoate were detected by all fruit fly and parasitoid species (Figure 4-17).

The percentages of the EAD-active compounds relative to MS compounds of *B. dorsalis* 9th-DPO Haden mango headspace volatile were as shown in Figure 4-20. Of the four fruit fly species, *B. dorsalis's* antennae detected the highest percentage (55%), followed by *B. latifrons* (49%). Among the three parasitoid species, *F. arisanus* detected the highest percentage (26%) of EAD-active compounds (Figure 4-18).

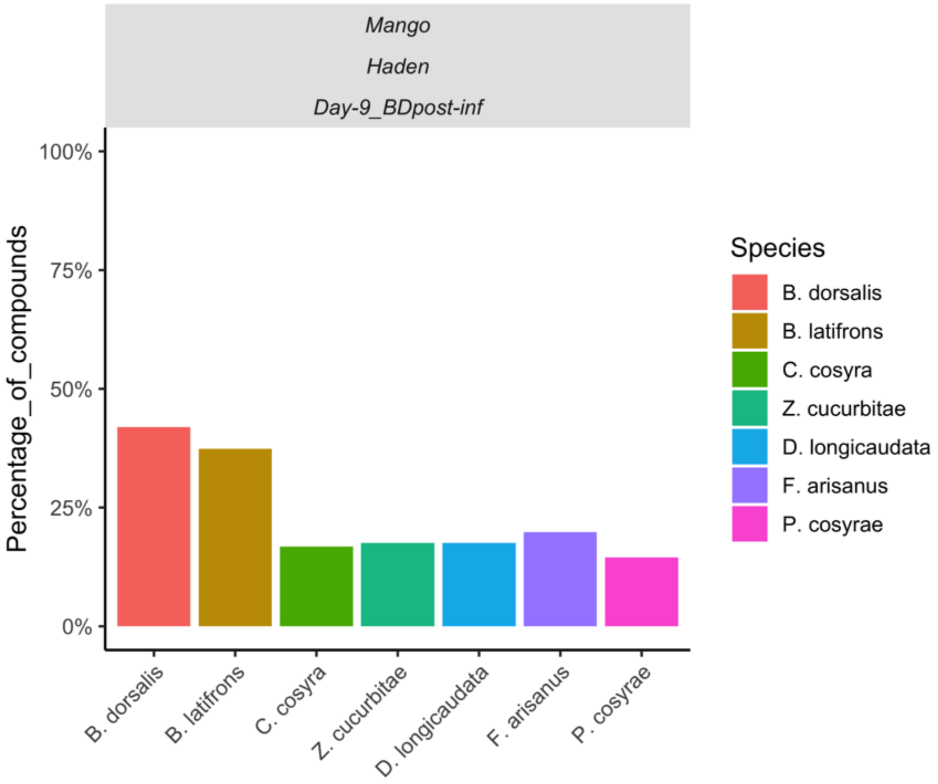


Figure 4-18: The percentages of EAD-active compounds of the fruit flies (*B dorsalis*, *B. latifrons*, *C. cosyra*, and *Z. cucurbitae*) and the parasitoids (*Diachasmimorpha longicaudata*, *Fopius arisanus*, and *Psyttalia cosyrae*) relative to the number of MS compounds of *B. dorsalis* 9th-DPO Haden mango headspace volatiles

The likelihood of an EAD-active compound of a given class being detected by the insects varied considerably among insect species (Figure 4-19)

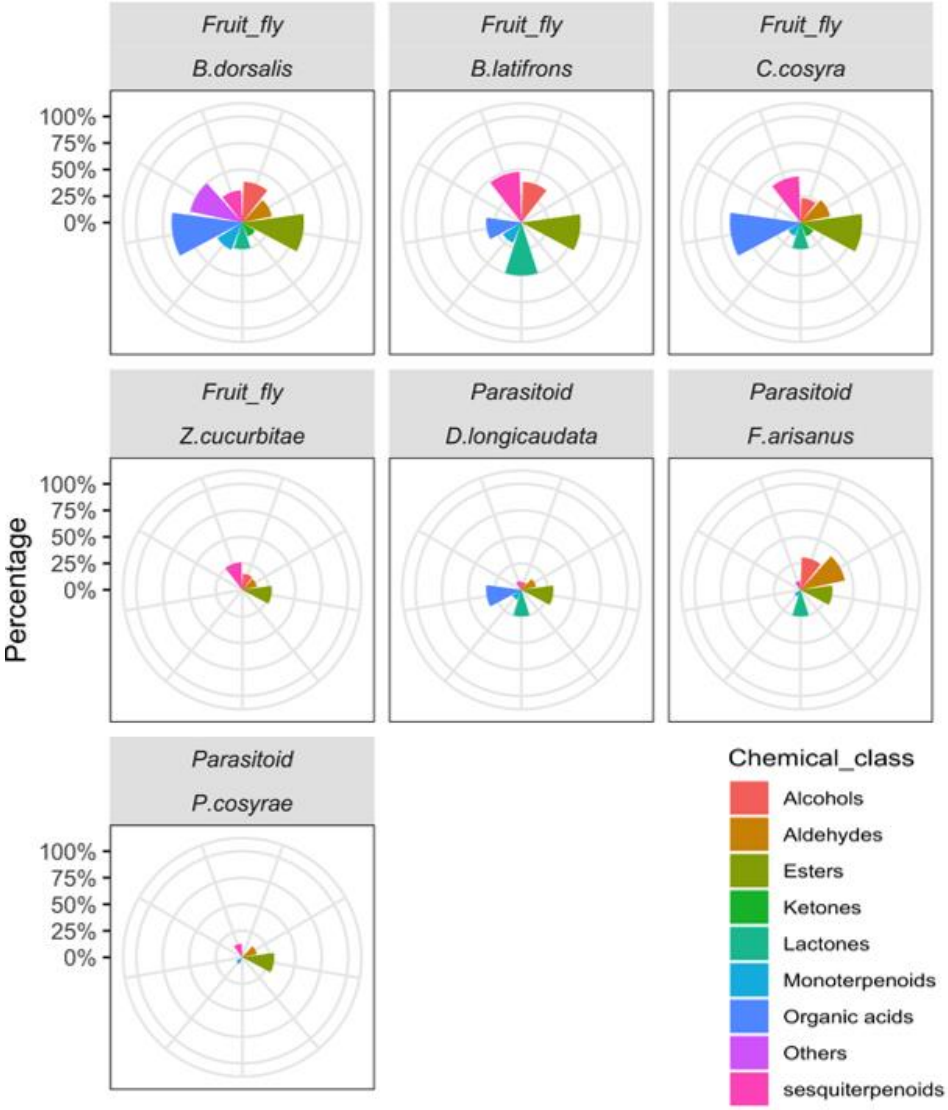


Figure 4-19: The percentages of EAD-active compounds of fruit fly and parasitoid species relative to the number of MS volatile compounds of a given chemical class of *B. dorsalis* 9th-DPO Haden mango

For the fruit flies *B. dorsalis* and *C. cosyra* and the parasitoid *D. longicaudata*, organic acids had a higher likelihood of being detected followed closely by esters and sesquiterpenoids while for *B. latifrons*, *Z. cucurbitae* and *P. cosyra*, esters had a higher likelihood of detection (Figure 4-19). *Fopius arisanus* was likely to detect aldehydes followed closely by esters and sesquiterpenoids.

The similarity percentage (SIMPER) analysis of the NMDS resolved the normalized response data in its hierarchical order of importance of percentage dissimilarity contribution of which the 30 topmost discriminant compounds are presented in Figure 4-20 (A); Appendix: Figure 4-10. There was a significant difference in the normalized response data of the fruit flies and the parasitoids (one-way ANOSIM, R = 0.9444, P = 0.0001, at dimension *k* = 2, *stress value* = 0.1071) (Figure 4-20 B)

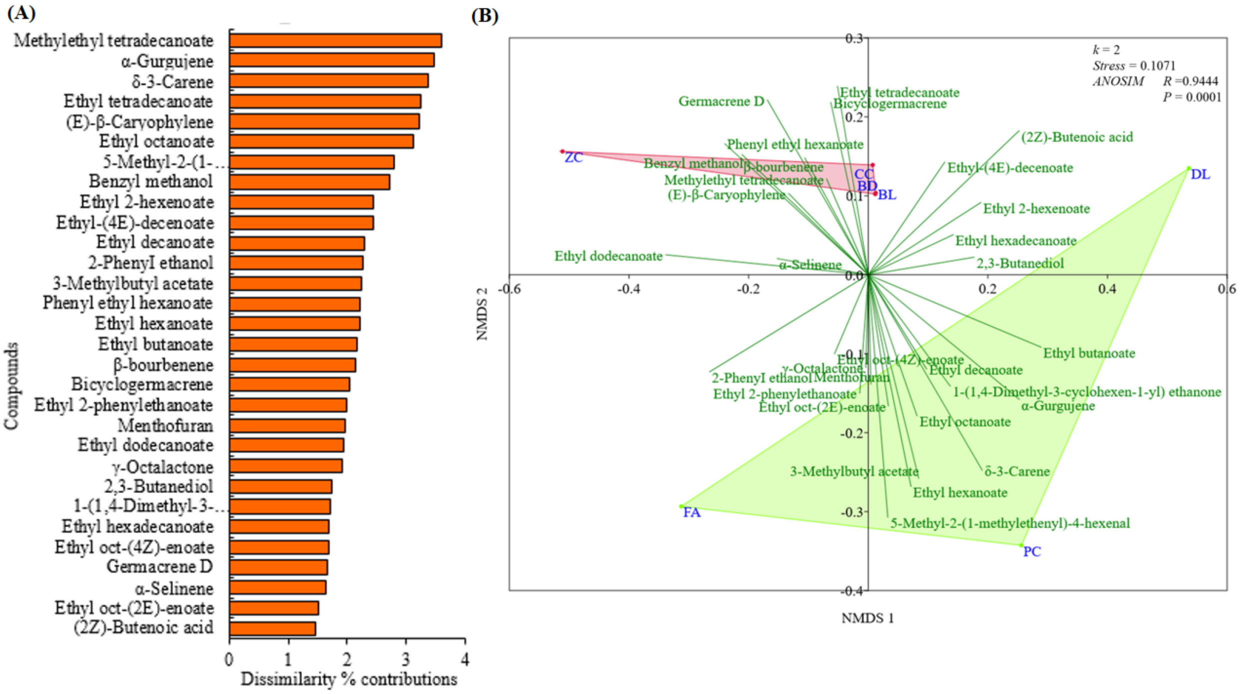
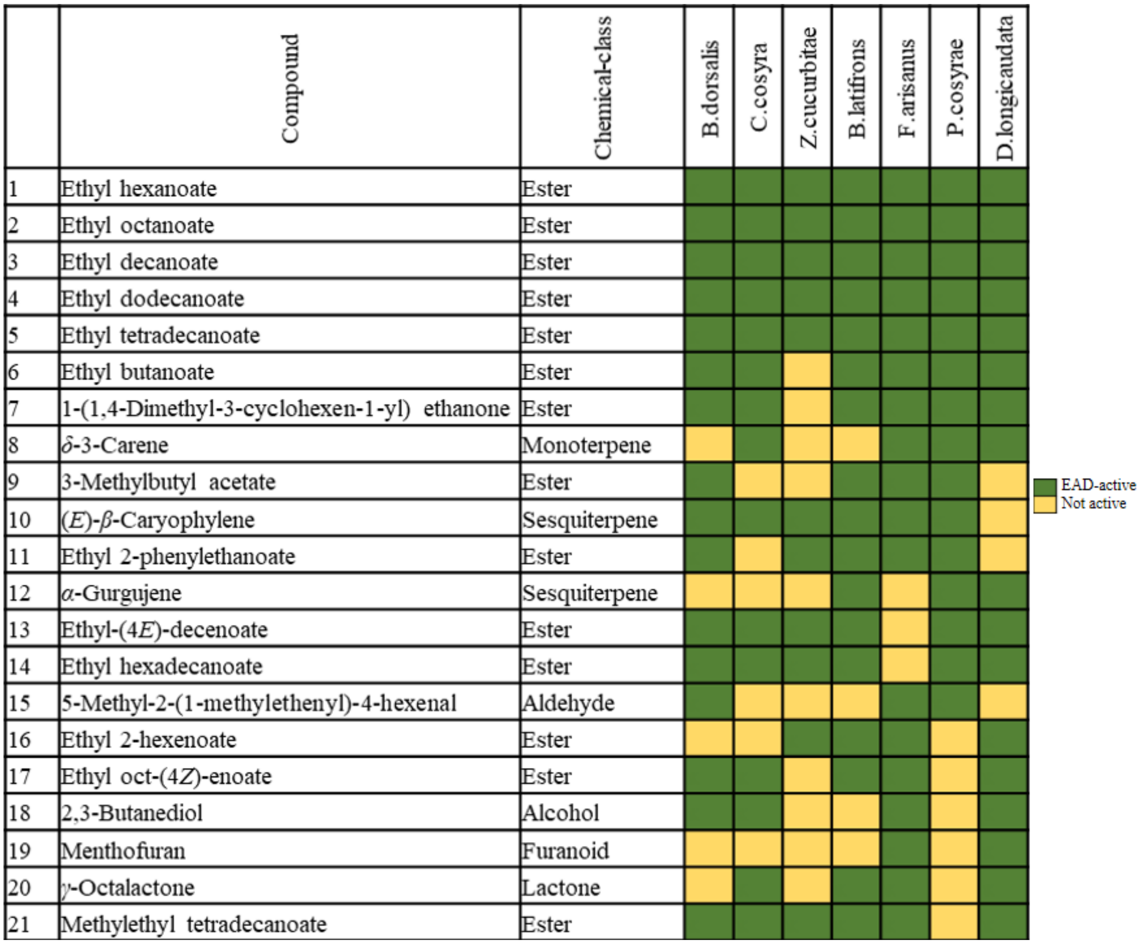


Figure 4-20: (A) The 30 topmost discriminant normalized responses of compounds of *B. dorsalis* 9th-DPO Haden mango headspace volatiles based on NMDS similarity percentages (SIMPER) in their decreasing order of importance. (B) The NMDS biplots of the 30 most discriminant compounds (BD = *B. dorsalis*, CC = *C. cosyra*, ZC = *Z. cucurbitae*, BL = *B. latifrons*, FA = *F. arisanus*, PC = *P. cosyrae*, DL = *D. longicaudata*)

Similar to the SIMPER results obtained from the normalized response data of mango and banana/tomato headspace volatiles, the majority of compounds (50%) selected as most discriminating for fruit fly and parasitoid antennal responses were esters (Figure 4-20 A). The 30 topmost discriminant compounds were distributed among all responsive insects (Figure 4-20 B). Of the selected compounds, ethyl dodecanoate, ethyl hexanoate, ethyl decanoate, ethyl octanoate and ethyl tetradecanoate elicited responses to the antennae of the four fruit flies and the three parasitoids. Table 4-2 show antennal-active compounds that were shared by at least two parasitoid

(*F. arisanus*, *P. cosyrae*, and *D. longicaudata*) species and either non or at least a fruit fly (*B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons*) species. Of these compounds, 67% were esters.



	Compound	Chemical-class	B.dorsalis	C.cosyra	Z.cucurbitae	B.latifrons	F.arisanus	P.cosyrae	D.longicaudata
1	Ethyl hexanoate	Ester	🟩	🟩	🟩	🟩	🟩	🟩	🟩
2	Ethyl octanoate	Ester	🟩	🟩	🟩	🟩	🟩	🟩	🟩
3	Ethyl decanoate	Ester	🟩	🟩	🟩	🟩	🟩	🟩	🟩
4	Ethyl dodecanoate	Ester	🟩	🟩	🟩	🟩	🟩	🟩	🟩
5	Ethyl tetradecanoate	Ester	🟩	🟩	🟩	🟩	🟩	🟩	🟩
6	Ethyl butanoate	Ester	🟩	🟩	🟨	🟩	🟩	🟩	🟩
7	1-(1,4-Dimethyl-3-cyclohexen-1-yl) ethanone	Ester	🟩	🟩	🟨	🟩	🟩	🟩	🟩
8	*δ*-3-Carene	Monoterpene	🟨	🟩	🟨	🟨	🟩	🟩	🟩
9	3-Methylbutyl acetate	Ester	🟩	🟨	🟨	🟩	🟩	🟩	🟨
10	(*E*)-*β*-Caryophylene	Sesquiterpene	🟩	🟩	🟩	🟩	🟩	🟩	🟨
11	Ethyl 2-phenylethanoate	Ester	🟩	🟨	🟩	🟩	🟩	🟩	🟨
12	*α*-Gurgujene	Sesquiterpene	🟨	🟨	🟨	🟩	🟨	🟩	🟩
13	Ethyl-(4*E*)-decenoate	Ester	🟩	🟩	🟩	🟩	🟨	🟩	🟩
14	Ethyl hexadecanoate	Ester	🟩	🟩	🟩	🟩	🟨	🟩	🟩
15	5-Methyl-2-(1-methylethenyl)-4-hexenal	Aldehyde	🟩	🟨	🟨	🟨	🟩	🟩	🟨
16	Ethyl 2-hexenoate	Ester	🟨	🟨	🟩	🟩	🟩	🟨	🟩
17	Ethyl oct-(4*Z*)-enoate	Ester	🟩	🟩	🟨	🟩	🟩	🟨	🟩
18	2,3-Butanediol	Alcohol	🟩	🟩	🟨	🟨	🟩	🟨	🟩
19	Menthofuran	Furanoid	🟨	🟨	🟨	🟨	🟩	🟨	🟩
20	*γ*-Octalactone	Lactone	🟨	🟩	🟨	🟩	🟩	🟨	🟩
21	Methylethyl tetradecanoate	Ester	🟩	🟩	🟩	🟩	🟩	🟨	🟩

🟩 EAD-active
🟨 Not active

Table 4-2: Antennal-active compounds of *B. dorsalis* 9th-DPO Haden headspace volatiles that were shared by at least two parasitoids species (*F. arisanus*, *P. cosyrae*, and *D. longicaudata*) and either none or at least a fruit fly species (*B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons*)

The three parasitoid species also responded to the species' unique compounds. For example, the antenna of *F. arisanus* also responded to terpinolene, *n*-decanal, *α*-humulene and tridecanal, *P. cosyrae* responded to butyl 2-methylbut-(2*E*)-enoate and myrcene while *D. longicaudata* responded to *β*-phellandrene and limonene.

4.6 Discussion

4.6.1 Performance of fruit flies in different varieties of fruits

Previous studies have primarily focused on the preference of fruit flies for the headspace of volatiles of various fruit species/varieties, with limited attention given to their actual reproduction in whole fruits. For example, de la Masselière *et al.* (2017) used pieces of fruits which included peels and pulps in an artificial oviposition substrate to evaluate the reproduction of selected fruit flies. To address this gap and better understand the general importance of visual, tactile and olfaction stimuli, we investigated the reproduction of *B. dorsalis, C. cosyra, Z. cucurbitae,* and *B. latifrons* in specific varieties of different species of fruits in the laboratory set-up.

Bactrocera dorsalis and *C. cosyra* exhibited better reproduction in ripe Haden mangoes and Fhia 17 variety of banana, while *Z. cucurbitae* showed limited reproduction and *B. latifrons* failed to perform at all. The good reproduction, in terms of recovered puparia, of *B. dorsalis* and *C. cosyra* in Haden can be attributed to factors such as the ripe mango used, the controlled laboratory conditions and the potential changes in the physical and chemical properties of the mangoes resulting from harvesting, transportation and storage. These factors may have influenced attractiveness and the subsequent oviposition by the two fruit fly species. In chapters 2 and 3, respectively *B. dorsalis* and *C. cosyra* displayed low performances in terms of the number of puparia recovered from their respective infested Haden mangoes.

Although *B. dorsalis* and *C. cosyra* are polyphagous species (Cruz-López *et al.*, 2006; Biasazin *et al.*, 2019), their reproduction was notably poor in Improved Nouvelle F1 tomato fruits just like that of the oligophagous *Z. cucurbitae*. This implies that these fruit fly species may have potential oviposition substrate-dependent variations in their preference and subsequent reproduction. Or could be this particular tomato variety may not be a preferred host for these fruit flies. In contrast, *Bactrocera latifrons*, a fruit fly highly associated with solanaceous fruits (Bokonon-Ganta *et al.*, 2019), failed to perform in non-choice tomato tests but reproduced, though poorly, when tomato was paired with mango and/or banana.

From the reproduction results, it is evident that the reproduction of a given fruit fly species depended on the degree of specialization in identifying a host followed oviposition hence allowing the pest to choose the best oviposition host for the survival of its offspring (Díaz-Fleischer *et al.*, 1999; de la Masselière *et al.*, 2017). Generalist *B. dorsalis* and *C. cosyra* were able to reproduce in all fruits in no-choice trials but opted for their favorite in choice trials which may be responsible for the species surviving through seasons. Although *B. latifrons* did not reproduce well regardless of the presence of mango and banana, we hypothesize that mango and banana could be producing oviposition-inducing volatiles of *B. latifrons* which can be explored further to promote the problematic protocols of its laboratory rearing.

4.6.2 Headspace volatile constituents

Several factors such as fruit species and variety, non-infestation and infestation status, and the fruit fly species involved in infestation, significantly influence the qualitative and quantitative composition of headspace volatile compounds emitted by a fruit. These compounds have several implications for the chemical environment of the fruit such as the interactions of the fruit with insect pests like fruit flies (Díaz-Fleischer *et al.*, 1999) and their parasitoids (Chapters 2 and 3; Segura *et al.*, 2016; Cai *et al.*, 2020; Miano *et al.*, 2022. Our GC-MS results consistently revealed an increase, qualitatively and quantitatively, of the compounds of 9th-DPO across the three mango varieties, regardless of the fruit fly species responsible for infestation. These increases could be a result of an array of microorganisms enhanced volatile bouquet (Barth *et al.*, 2009; Raza *et al.*, 2020); the conversion of other volatile compounds to more esters (which is associated with ripening or forced ripening as with infestations) (Jaleel *et al.*, 2021); and fruit decay (Engelbrecht *et al.*, 2017) among others. These results align with our earlier findings of *B. dorsalis* and *C. cosyra* (respectively in Chapters 2 and 3) for both non-infested and infested mangoes.

With esters as the majority class, the number of compounds identified from ripe bananas and tomatoes are more than what has been reported in most studies (Biasazin *et al.*, 2014; Jaleel *et al.*, 2021a; 2021b) for bananas and (Silva *et al.*, 2017; *Anastasaki et al.*, 2018) for tomatoes, which we attribute to the fruit variety, the *in situ* volatile collection and the adsorbent used. In most cases, the number of volatile compounds detected from plant headspaces is influenced by the sampling method (Agelopoulos & Pickett, 1998) and in our case, the mixed-phase sorbent used. Similar to other results, in our study, no terpenes or terpenoids were reported from banana headspace volatiles (Jaleel *et al.*, 2021).

4.6.3 Antennal responses of fruit flies and parasitoids

GC-EAD is a standard method that is used in screening active biogenic organic compounds (semiochemicals/ EAD-active compounds) from headspace volatiles of plants and fruits (Scolari *et al.*, 2021 and references therein). Like what was reported in Dicke & Baldwin (2010) and the references therein, it was generally observed that compounds that were most dominant quantitatively in MS of headspace volatiles did not emerge as the antennal-active compounds. Furthermore, the number of EAD-active compounds was generally higher than what has been reported in earlier findings involving the same species of fruits and fruit fly species. The major contributors of the EAD-active compounds among the fruit headspace volatile compounds were esters, similar to earlier findings (Biasazin *et al.*, 2014; 2019). These results also indicated that the variety and status of fruit played an important role in determining the specific EAD-active compounds produced. For example, while linalool is a compound that has been widely reported as a compound present in mango (Andrade *et al.*, 2000; Pino *et al.*, 2005; Quijano *et al.*, 2007), here, it was only identified in headspaces volatiles of Apple mango variety. Other compounds that elicited antennal responses but not from all mango varieties were α-ylangene, 2,5-dimethyl-4-methoxy-3(2H)-furanone, α-guaiaene, aromadendrene, cryptone, and o-cumenol of Apple mango headspaces; ethyl 3-methylbutanoate, ethyl pentanoate, butyl butanoate, m-cymenene, heptan-1-ol, γ-muurolene, bicyclogermacrene, α-cadinene, p-ethylacetophenone, methyl tetradecanoate, and cyclooctanone of Haden; and 4,5-di-epi-aristolochene, car-3-en-2-one, octadecane, catalponol, benzyl phenylmethanoate, and benzyl salicylate of Kent. There is a high probability that these compounds, among others, could have a bearing on the differential responses and the subsequent reproduction of the fruit fly species mentioned in section 4.6.1, chapters 2 and 3 of this thesis.

Some of the EAD-active compounds of mango headspace treatments mentioned here were reported to elicit antennal activity in *B. dorsalis* (Biasazin *et al.*, 2014) out of which a blend containing ethyl butanoate, *β*-myrcene, *β*-pinene, 3-methylbutyl ethanoate, butyl butanoate and ethyl hexanoate among other compounds was attractive to *B. dorsalis* when compared to hexane as the control. The EAD-active compounds α-pinene, ethyl butanoate, ethyl 3-methylbutanoate, 3-methylbutyl ethanoate, ethyl pentanoate, myrcene, butyl butanoate, ethyl 3-methyl-2-butenoate, ethyl hexanoate, (*E*)-*β*-ocimene, ethyl-(2*E*)-pentenoate, methyl octanoate, ethyl octanoate, ethyl oct-(4*Z*)-enoate, ethyl (2*E*,4*E*)-2,4-hexadienoate, ethyl 3-hydroxybutanoate, α-copaene, linalool, butanoic acid, (*E*)-*β*-caryophyllene and *γ*-gurjunene of mango headspaces reported in this study showed antennal response in *B. dorsalis*, *Z. cucurbitae*, *B. zonata* (Saunders) and *C. capitata* (Wiedemann) (Biasazin *et al.*, 2019). Furthermore, some of the antennal-active compounds of this study were reported to individually attract *B. dorsalis* (Kamala *et al.*, 2012; Biasazin *et al.*, 2014) and others like *γ*-octalactone induced oviposition (Kamala *et al.*, 2014).

Generally, infested mangoes produced more EAD-active compounds than non-infested ones. The majority of EAD-active common compounds were produced in higher quantities compared to those of non-infested mangoes (Chapters 2 and 3). When plants are attacked by herbivorous pests, they often release herbivore-induced plant volatiles (HIPVs) some of which are specific to the particular pest species (Hare, 2011; Holopainen & Blande, 2013). Compounds that were only produced by infested mangoes and elicited antennal responses to one or more fruit fly species were, for example, ethyl 3-methyl-2-butenoate, pentyl 2-methylpropanoate, ethyl oct-(4*Z*)-enoate, ethyl (2*E*,4*E*)-2,4-hexadienoate, ethyl 3-hydroxybutanoate, ethyl-(4*E*)-decenoate, ethyl 2-phenylethanoate, *E*-nerolidol, 6,10,14-trimethyl-2-pentadecanone, *o*-cumenol, and ethyl hexadecanoate of Apple mango variety. These compounds may play major roles in attracting conspecifics leading to increased herbivorous activities and/or attracting natural enemies hence serving as a form of indirect defense (War *et al.*, 2012; Miano *et al.*, 2022; Chapter 3). The compounds may also indicate ongoing infestations and signal lazy female flies to easily oviposit. Examples from other studies that also highlight conspecific attraction mediated by herbivorous activities include attraction of *Thrips tabacis* (Lindeman) (Thysanoptera: Thripidae), an onion thrips, to HIPVs emitted by conspecific infested onions compared to non-infested ones (Kumar *et al.*, 2017); and *Leptinotarsa decemlineata* (Say), the potato beetle, which is attracted to a blend of HIPVs, including (±)-linalool, methyl salicylate, and (*Z*)-3-hexenyl acetate, that is produced by *L. decemlineata* infested potatoes (Dickens, 2006).

We recommend screening the EAD-active compounds of infested and non-infested mangoes to figure out their role in the host-searching behavior of the fruit fly and parasitoid species. This may also result in the addition of attractants to the reported compounds or blends.

Our preliminary observations in the banana farms indicated that the banana variety used in this study attracts *B. dorsalis* females both in its physiological mature and ripen status. This banana could be an alternative host of *B. dorsalis*. Interestingly, more than 70% of its MS tentatively identified compounds were *B. dorsalis* EAD-active. On the other hand, *B. latifrons* antennae responded to more than 50% of the tentatively identified MS volatile compounds of the banana yet it did not reproduce in it. Probably in the process of evolution, this *Batrocera* species has evolved to recognize the compounds to signify non-host, unlike *B.dorsalis*. Although *C. cosyra* responded to slightly less than 50% of the compounds, it is a polyphagous fruit fly as supported by its reproduction performance. Zeugodacus cucurbitae antenna responded to lesser compounds of banana which is commensurate with its performance as in Figure 4-4.

Among the 59 identified EAD-active compounds of banana headspace volatiles, only a few, such as methyl eugenol, benzyl methanol, 2,4-dimethyl-1-penten-3-ol, were not in the class of esters (see Figure 4-13). This aligns with previous findings where esters were found to be major contributors to the antennal responses of *B. dorsalis*, *Z. cucurbitae*, *B. zonata* and *C. capitata* against another variety of banana headspace volatiles (Biasazin *et al.*, 2019).

Contrary to the findings of Njuguna *et al.* (2018) where the antenna of *Z. cucurbitae* was active to at least seven compounds, here, it was only elicited by *β*-phellandrene and (*E*)-*β*-caryophyllene of the tomato. Although we can’t fully explain the fewer EAD-active compounds detected by *Z. cucurbitae* antennae in tomatoes, we postulate that the concentration of these compounds in the tomato headspaces may have played a role in the failure to have observable antennal responses.

Previous research on parasitoids revolved around the introduction, adaptation, fitness in parasitism, establishment and competition with native parasitoids (Camargos *et al.*, 2018; Harbi *et al.*, 2018; Yang *et al.*, 2018; Monsia *et al.*, 2019; Ndlela *et al.*, 2020). Although there have been some investigations into the volatiles emitted by host fruits and their influence on parasitoid behavior (Segura *et al.*, 2012; Cai *et al.*, 2020; Miano *et al.*, 2022), a comprehensive understanding of the olfactory responses of parasitoids compared to their host fruit flies remains limited.

The sharedness of antennal-active compounds between *B. dorsalis*, *F. arisanus* and *D. longicaudata* was expected given that these insect species evolved together, just like that of *C. cosyra* and its parasitoid *P. cosyrae*. But the sharing of antennal-active compounds among *B. dorsalis* and the Africa native *P. cosyrae*, and *C. cosyra* and the exotic parasitoids *F. arisanus* and *D. longicaudata* was surprising given that the fruit fly species and parasitoids share no evolution history. Mohamed *et al.* (2008) reported the ability of *D. longicaudata* to successfully parasitize and establish in *C. cosyra* and *C. capitata* hence forming the latest evolution time scale while *P. cosyrae* was unable to be established in *B. dorsalis* (Gwokyalya *et al.*, 2022).

This study has also established that fruit fly and parasitoid species used here share a good number of antennal-active compounds where esters form the majority compared to the other classes of compounds. This implies that the fruit fly and parasitoid could have evolved to detect similar compounds in their respective host searching mission or parasitoids may have evolved the ability to predict the presence of their host based on these compounds. Probably ethyl dodecanoate, ethyl hexanoate, ethyl decanoate, ethyl octanoate and ethyl tetradecanoate, which are shared among the fruit fly and parasitoid species, play general roles during host searching while the other non-shared compounds lead the insect species to the preferred host. Further research on behavioral implications of these compounds is important.

This study also reported that the antennae of the three parasitoids, *F. arisanus*, *D. longicaudata* and *P. cosyrae* responded differentially to volatile compounds of *B. dorsalis* 9th-DPO Haden mango volatiles. The majority of parasitoid EAD-active compounds were also found in non-infested ripe and *C. cosyra* 9th-DPO Haden mango volatiles although they were produced in higher amounts in infested mangoes (also reported in Chapters 2 and 3). There were overlaps of EAD-active compound in parasitoids where esters were the major antennal-active class which could explain their attraction to freshly-infested mango fruits (*F. arisanus*), infested fruits at late stages of larval development (*P. cosyrae* and *D. longicaudata*) and non-infested ripe fruits (for the three parasitoids) as reported in Miano *et al.* (2022) and Chapter 3). As indicated in chapter 2 and 3, the attraction of the parasitoids to non-infested ripe mangoes and the presence of EAD-active compounds in non-infested fruits (extension from *B. dorsalis* 9th-DPO Haden mango) indicate the possibility that the parasitoids have evolved to utilize olfactory cues that would maximize the probability of finding a suitable host. Indeed, a parasitoid may forage on fruits emitting EAD-active compounds and target areas where concentrations are high. Cai *et al.* (2020) reported the attraction of *F. arisanus* to both *B. dorsalis*-infested and non-infested fruits. Additionally, Harbi *et al.* (2019) highlighted the significance of *C. capitata* larval age in the attraction of *D. longicaudata* across different fruit species

Although the number of EAD-active compounds of fruit fly species is generally more than those of parasitoid species, this study has shown some degree of convergence of the EAD-active compounds of fruit fly species and parasitoids. It is puzzling why fruit flies are attracted to an area of heightened risk of parasitism especially where there are ongoing signs of infestation. To resolve this puzzle, we recommend that the role of EAD-active compounds in the fruit fly and parasitoids be investigated with subtleties. The understanding of how fruit flies and parasitoids respond to specific compounds or blends of EAD-active compounds of different fruit species and varieties can shed more light on the complex ecological dynamics of fruit, fruit fly, and parasitoid interactions. Such investigations can have practical applications in the development of sustainable strategies for fruit fly management, including the enhancement of biological control efforts through the manipulation of olfactory cues and attractants

4.7 Conclusion and recommendations

The results obtained in this study can provide practical applications for improving the existing integrated pest management systems of fruit flies. The findings highlight the importance of considering the infestation stage of fruit development in understanding the attraction of conspecific fruit flies. Our results show that infested fruits produce a higher number of EAD active compounds, which may contribute to their attractiveness to fruit flies and parasitoids, as observed in previous studies.

Furthermore, the results suggest a sharedness of EAD-active compounds between parasitoids and their host fruit flies, indicating that they use similar compounds for host location. The concentration of these compounds at the point of fruit release may play a crucial role in parasitoid attraction. It is indispensable to explore the specific role of the compounds that elicited antennal responses in both fruit flies and parasitoids. These findings provide valuable insights for the development of new, sustainable, and environmentally friendly strategies for fruit fly control. Understanding the role of these compounds, individually and/or as blends, and their interactions with fruit flies and parasitoids can inform the formulation of effective green chemicals that target fruit fly populations while minimizing harm to the ecosystem systems, such as biological control. Continued research in this area is essential for the advancement of fruit fly management practices and the promotion of sustainable agricultural practices.

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Chapter 5: General discussion, conclusion and recommendation

5.1 General Discussion

Tephritid fruit flies, both native and exotic, constrain the agricultural sector directly, from the production to the market (Díaz-Fleischer & Aluja, 2001; Doorenweerd *et al.*, 2018). In Africa, the introduction and successful establishment of exotic fruit flies has caused major challenges to fruit and vegetable production (Ekesi *et al.*, 2016; Mohamed *et al.*, 2016; Muriithi *et al.*, 2020; Sultana *et al.*, 2020). Although control methods including cultural, chemical, lure and kill, sterilized males, and biological have been implemented under integrated pest management strategies, the menace caused by fruit flies is still high. In the past, a lot of research has been done on the semiochemical-related interactions among the host fruits, fruit flies, and parasitoids. Recently in a comparative study of the olfactomes of tephritid fruit flies to fruit and fermentation sources, Biasazin *et al.* (2018; 2019; 2022) mapped out EAD-active compounds that revealed both conservancy and convergence as well as divergence. Some of the EAD-active compounds produced blends that were highly attractive to fruit flies from phylogenetically and ecologically distinct taxa. This implies that the olfactory sensitivity of fruit flies across insect taxa can provide important linkages to the development of novel knowledge-intensive agricultural techniques that can be used in sustainable agricultural production. Although this much has been done, there is very little data on the *in situ* interaction of the fruits, fruit flies, and parasitoids.

This thesis presents three stand-alone data chapters written in the form of scientific articles which address the objectives of the research singularly or otherwise. In the first chapter, the *in situ* responses of *B. dorsalis*, *F. arisanus* and *D. longicaudata* to headspace volatiles of different treatments of Kent, Apple and Haden mango varieties were determined and the performances of the fruit fly in the mangoes assessed and the mango headspace volatiles analyzed using gas chromatography-linked to mass spectrometry (GC-MS). In this research, it has been shown that though mango is generally assumed to be the preferred choice as an oviposition substrate for *B. dorsalis*, the variety of the mango determines the preference and the subsequent performance of the offspring which also influences the attraction of the parasitoids. *Bactrocera dorsalis* was found to generally prefer the headspace volatiles of infested and non-infested Apple and Haden mangoes unlike what was observed for the Kent variety where only freshly-infested and ripe mangoes were attractive. The attraction of female *B. dorsalis* to conspecifics freshly-infested mango headspace volatiles of all varieties (including post-oviposited Apple and Haden varieties) and post-oviposition mangoes demonstrated that other than fruit volatiles, this species of fruit fly also uses herbivorous induced volatiles to locate its host. It is very common to find wild female *B. dorsalis* reusing pre-existing oviposition sites on a mango fruit instead of making new ones. For example, figure 5-1 (A) is a photo that was taken *in situ* showing *B. dorsalis* taking advantage of an old oviposition site. After the infested fruit dropped from the tree, it was incubated only to find many larvae of different development stages (Figure 5-1 B) and latter giving forth to four hundred and sixty-two adult *B. dorsalis*. The emergence of adults took a span of fourteen to seventeen days but with many casualties of larvae and puparia which could have been attributed to lack of enough feeds.

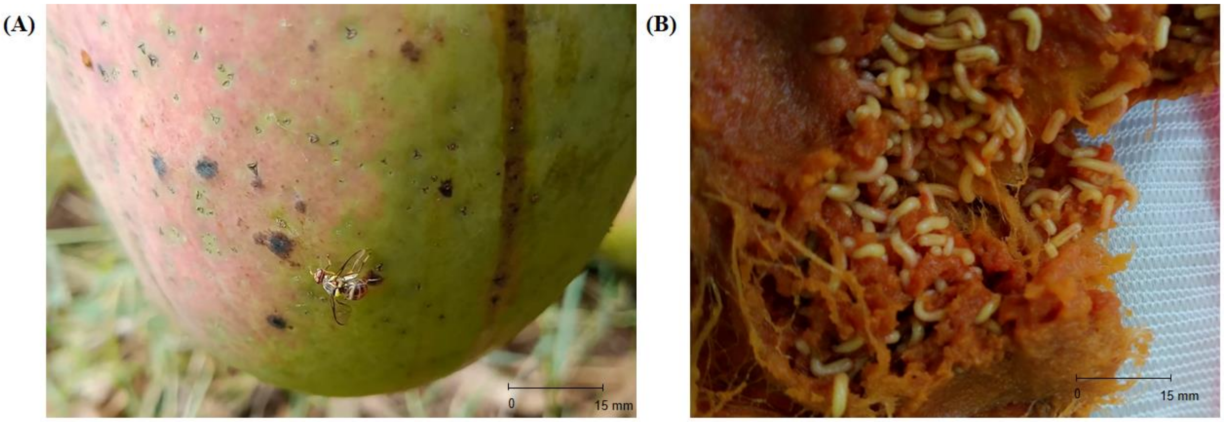

Figure 5-1: An Apple variety of mango with a wild female *B. dorsalis* laying eggs in a preexisting oviposition site (A) and many larvae in the rotting mango on incubation after dropping from the tree (B)

The question that comes from the behavioral responses results of this chapter is whether to pick the infested fruits from the tree or remove them after dropping them. Figure 5-2 shows a mango that was fruit fly infested having attracted the natural enemies of the fruit fly larva yet adjacent to it is a non-infested fruit. It is then easy to conclude that infested fruit should be left hanging on the tree as a protective measure as it attracts more gravid females/natural enemies than the non-infested fruit, and only after it drops from the tree the infested fruit should be removed.

Figure 5-2: Fruit fly-infested Kent mango that has attracted the fruit fly larvae' natural enemy yet adjacent to it is a clean and non-infested fruit

On the other hand, it is also a common experience to find a fruit fly-infested fruit in which only one part of the fruit is damaged while the rest is consumable, an indication that the fruit was attacked from that side. Figure 5-2 explains the possibility that the infested mango had been attacked by the fruit flies from the side that had been eaten out as the natural enemy looked for the larvae.

The headspace volatiles of the ripe mangoes of the three varieties were attractive to *B. dorsalis* which is in agreement with findings by Grechi *et al.* (2021) for *B. zonata* (Saunders), *Ceratitis capitata* (Wiedemann), and *C. quilicii* (Karsch) (all Diptera: Tephritidae) on mango. This observation supports the importance of advocating for the early harvesting of mango fruits.

The attraction of parasitoids was also influenced by fruit status. Apple mango offered the most attractive headspace volatiles compared to Haden and Kent. The better attraction of *F. arisanus* to the headspace volatiles of freshly-infested mangoes of all varieties and *D. longicaudata* to the 9th-DPO volatiles indicated that the insects were able to sense compounds that signified the availability of their host. Figures 5-3 (A) and (B) respectively show *F. arisanus* and *D. longicaudata* parasitizing their host together with standing onlookers who could probably be ready to use the same opportunity for oviposition. The parasitoids could have majorly used volatiles emanating from the same spot of the mango to locate their host.

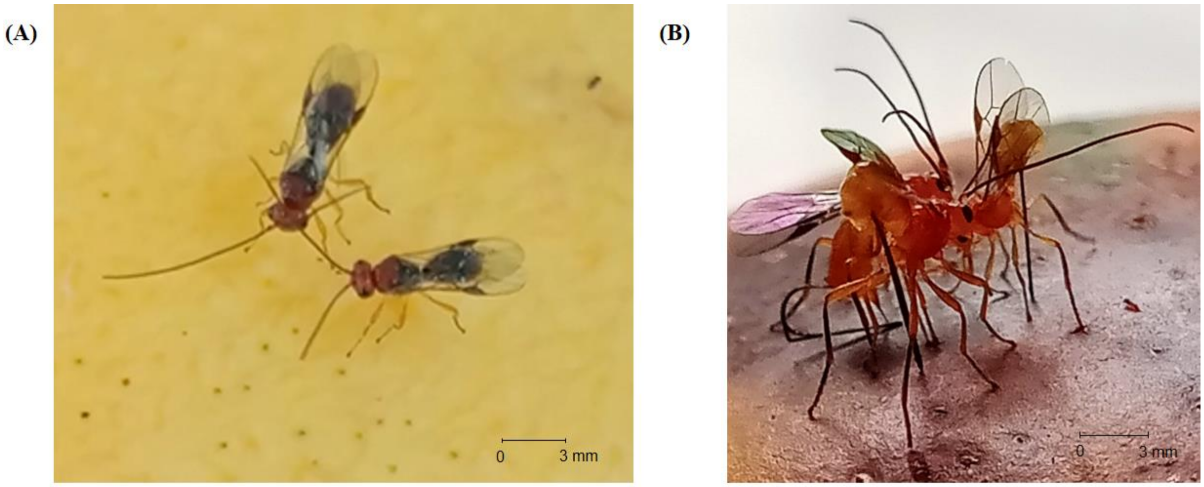

Figure 5-3: Photos of egg parasitoids *F. arisanus* (A) and larval parasitoids *D. longicaudata* (B) each with standby conspecific onlookers

Cai *et al.* (2020) reported that gravid *F. arisanus* is attracted to its host either using volatiles emitted by the fruits during oviposition or by volatiles left by the fruit fly during or after oviposition. Other than the fruit infestation status, the age of the host larvae also determines the attractiveness of *D. longicaudata* to its host (Harbi *et al.*, 2019). Interestingly, *F. arisanus* and *D. longicaudata* were attracted to either one or more headspace volatiles of ripe mangoes which demonstrates that the olfactory circuitry of the parasitoids has evolved to detect volatiles that increase chances of finding their fruit fly host and probably utilizing the same volatile compounds as their host *B. dorsalis*.

Of the three mango varieties used in the test, Apple mango yielded the highest number of *B. dorsalis* puparia while Kent did not yield any puparia. This was an interesting finding given that farmers from the region where this study was done believed that the Kent variety was the mango that was highly destroyed by *B. dorsalis* regardless of its IPM measures. Little did they know that the problem of Kent mango was not infestation by *B. dorsalis* but infestation by *C. cosyra*. This was compounded by the assumption that in many parts of Africa, *B. dorsalis* has displaced the native *C. cosyra* (Ekesi *et al.*, 2009; Rwomushana *et al.*, 2009). From the results obtained, it is evident that the replacement of the indigenous mangoes with new varieties (Apple, Haden, Van Dyke, Ngowe, Tommy Atkin, among others) commonly referred to as ¨commercial mangoes¨, could have greatly influenced the spread and dominance of *B. dorsalis* especially with Apple mango being the most encouraged variety due to its wide domestic and export market. It is worth mentioning that in a parallel study which the results were not given in this thesis, the number of male *B. dorsalis* captured in male annihilation traps in the mango season of 2021, from the mango orchard used in this study, was at its peak during the Apple variety period and significantly low during the Kent mango period. I would imagine a situation where during the suppression of *B. dorsalis* using male-specific attractants, there was a slow resurgence of the native *C. cosyra* which resulted in the invasion and destruction of the rate-maturing Kent mango.

Gas-chromatography-mass spectrometry revealed that the headspace volatile components of the mango varieties and treatments varied qualitatively and quantitatively. The highest total concentrations of compounds of the treatments of the three varieties of mangoes were recorded on freshly-infested mangoes and the 9th-DPO ones. Since the number of compounds of non-infested unripe mangoes and freshly-infested mangoes across the three mango varieties were not significantly different it can be concluded that the increased attraction of the fruit fly species and the egg parasitoid, *F. arisanus* was a result of oviposition induced changes of volatile emission. As the number of post-oviposition days (7th-DPO and 9th-DPO) progressed, the number of compounds and their total concentrations also increased compared to those of non-infested mangoes and there was more production of esters across the mango varieties. Furthermore, unlike in ripe mango headspace volatiles where the volatile concentrations of defense-related compounds (monoterpenes and sesquiterpenes) decreased, on the 7th-DPO and 9th-DPO mangoes, the concentrations of these compounds increased. In collaboration with the behavioral responses results, the conclusion that can be made from the volatile composition changes is that they are responsible for the increased attraction of conspecific *B. dorsalis*, as well as *F. arisanus* and *D. longicaudata* to fruit fly-infested mangoes.

In the third chapter, the *in situ* responses of *C. cosyra*, *F. arisanus*, *D. longicaudata*, and *P. cosyrae* to headspace volatiles of Kent, Apple and Haden mango variety treatments were determined. This was followed by assessing the performances of *C. cosyra* in the mango varieties and lastly analyzing the mango treatment headspace volatiles using gas chromatography linked to mass spectrometry (GC-MS). Interestingly, *C. cosyra* was attracted to headspace volatiles of all treatments of the three mango varieties.

The attraction of *C. cosyra* to conspecific-infested mangoes could imply that the mango headspaces contained more attractive components that could have masked host marking pheromones (HMPs) reported by Cheseto *et al.* (2017). To support this argument, during data correction for this thesis at the field, photos (Figure 5-4) were taken in which two *C. cosyra* females were spotted having identified the same oviposition site (A) but only one managed to make an oviposition puncture (B). A lazy fruit fly smells the newly made oviposition puncture (C) appreciates the effort of the first fruit fly and tries to dislodge it (E). It was later joined by a second conspecific (F) but they did not succeed in the dislodging attempt.

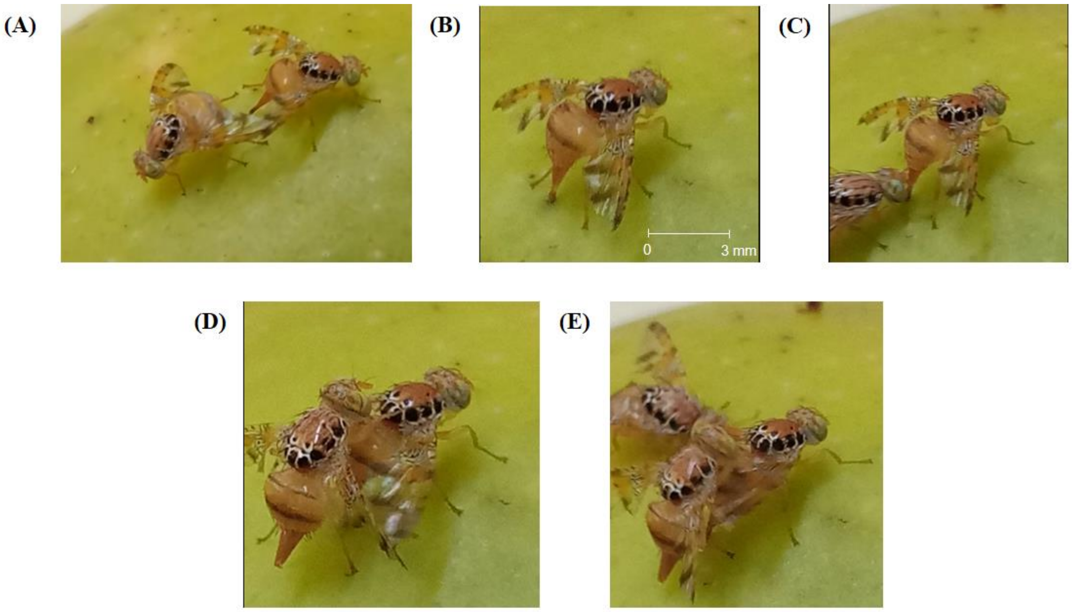

Figure 5-4: Two female *C. cosyra* identify the same oviposition site (A); one of the fruit flies successfully makes an oviposition puncture and proceeds to lay eggs (B); the other fruit fly smells the achievement of newly made puncture (C) but with an ulterior motive of chasing the ovipositing fruit fly (D); It is later joined by another fruit fly (E) but both were unable to dislodge the ovipositing conspecific (Scale of B 5:1)

Figure 5-4 better explain why more *C. cosyra* females were attracted to freshly-infested and possibly post-oviposition mangoes. It also explains the tendency to find many larvae emerging from the incubation of *in situ* infested fruit just like what happens with *B. dorsalis*.

The attraction of *F. arisanus* and *D. longicaudata* to headspace volatiles emanating from *C. cosyra*-infested mangoes provides evidence that the two exotic parasitoids have made new associations with the native fruit fly. It was reported that *P. cosyrae* which co-evolved with *C. cosyra* responded positively to fewer mango treatments than *D. longicaudata* with the latter performing better in *C. cosyra* parasitism (Ndlela *et al.*, 2020).

Unlike what was reported in chapter two about the non-performance of *B. dorsalis* in the Kent mango variety, *C. cosyra* performed exemplary better in this variety compared to Apple and Haden. In the mango season of a majority of mango-producing zones of Kenya, Kent mango matures last compared to other commercial varieties. This implies that due to the more publicity of the highly destructive *B. dorsalis*, farmers take a lot of precautionary measures against it in their mango orchards at the expense of *C. cosyra*. The low performance of *C. cosyra* in the early maturing Apple and Haden mango varieties slowly increases its population and by the time its favorite Kent mango is in season, it invades the Kent mango leaving a lot of damage. A close check of the mango orchard where this study was conducted indicated very low encounters of female *B. dorsalis* during the ripening of Kent mangoes. This finding is very important as it will advise on the IPM measures to put in place where different species of fruit flies are a problem.

Similar to what was reported for *B. dorsalis* (Chapter 2), the headspace volatiles composition of non-infested and *C. cosyra*-infested mangoes of the three varieties depends on the variety and treatment. The highest number of compounds was reported from the 9th-DPO mangoes of the three varieties while the highest concentrations were from freshly-infested mangoes and 9th-DPO ones. These two treatments happened to be the best attractants respectively for the egg parasitoid (*F. arisanus*) and the larvae parasitoids (*P. cosyra* and *D. longicaudata*). The increase in the concentrations of compounds and the number of esters for *C. cosyra*-infested mango headspace volatile treatments may be responsible for the increased attraction of conspecific female *C. cosyra* when compared to those of the non-infested mangoes.

In the fourth chapter, the number of fruits was increased to mango (Apple, Haden and Kent varieties), banana (Fhia 17 variety), and tomato (Improved Nouvelle F1); fruit fly species to four (*B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons*) while parasitoids remained *F. arisanus*, *D. longicaudata*, and *P. cosyrae*. The banana (Fhia 17 variety) was introduced in the study region for commercial purposes due to its good performance. Unfortunately, this variety of bananas (when physiologically mature or ripe) turned out to be a good host for *B. dorsalis* especially in the absence of mango fruits.

The reproduction (in terms of puparia recovered) of the four fruit fly species was assessed in Haden mango, banana and tomato. Haden mango and banana were the most preferred hosts for *B. dorsalis*, *C. cosyra*, and *Z. cucurbitae* though the performance was fruit fly species dependent.

Unlike what was observed in the field (Chapters 2 and 3), *B. dorsalis* and *C. cosyra* performed better in Haden mango, which could be attributed to factors like the condition of the mango, and controlled laboratory conditions, among other factors. The good performance of *C. cosyra* in this banana under laboratory conditions may imply that the fruit fly might also have formed a positive association with it as an alternative host in the banana orchards. I believe that the influx of new varieties of crops and the removal of the local ones in Sub-Saharan Africa play a role in the spread of new species of fruit flies among other pests. Although the three fruit flies performed less in tomato compared to mango and banana, the performance only happened in the no-choice test. This implies that the tomato variety used in the study is a less preferred host for the three fruit fly species. Interestingly, in this study, *B. latifrons* performed in tomato only when the tomato was paired with either mango and/or banana. This finding is important given that this species of fruit fly is problematic when rearing in the laboratory especially if the laboratory-preferred host bitter tomato, *Solanum aethiopicum*, is not available.

The analyses of the *in situ* headspace volatile extracts of the various fruit treatments using DB-Wax GC-MS and GC-EAD revealed remarkable outcomes. The trends of GC-MS profiles of the treatments of interest (non-infested ripe, *B. dorsalis*- or *C. cosyra*-freshly-infested, and 9th-DPO mangoes) of the three mango varieties were similar to those reported in Chapters 2 and 3. A good number of tentatively identified compounds were overlapping among headspace compounds of *B. dorsalis* and *C. cosyra*-infested mangoes of the same variety and treatment stage. For the banana and tomato varieties, the number of headspace volatile compounds that were identified was encouraging, which I attributed to the mixed-phase sorbent used and the *in situ* dynamic headspace collection.

Generally, *B. dorsalis*, *C. cosyra* and *Z. cucurbitae* responded to more compounds of 9th-DPO mango headspaces where esters were the major compounds that elicited antennal activities. The increase in the number of active compounds of infested mangoes could have contributed to the increased attraction of fruit fly and parasitoid species infested mangoes as reported in chapters 2 and 3 of this thesis. For the banana and tomato headspace volatiles, more compounds elicited antennal responses of *B. dorsalis*, *C. cosyra* and *B. latifrons* compared to those of *Z. cucurbitae*. The respondent compounds of banana to *B. dorsalis* and *C. cosyra* were expected since the two fruit fly species performed well in it. For *B. latifrons*, it was captivating given that the fruit fly did not perform in banana but when a banana was paired with a tomato, *B. latifrons* puparia were recovered on tomato (similar to what was observed when mango and/or banana and tomato were put together). This could mean that mango and banana headspaces could be having *B. latifrons* oviposition-inducing volatiles or probably the antennal active compounds acted as repellents hence host discrimination.

The sharedness of EAD-active volatiles of the different fruits among the four tephritid fruit fly species is an important factor in their evolution towards their host range. Although *C. cosyra* and the other three *Bactrocera* fruit flies have distant evolutionary paths, the data provided here show that they have a considerable degree of overlapping EAD-active volatiles. This could explain the adaptive paths and the subsequent fruit fly host breadth. On the other hand, the presence of non-shared EAD-active volatiles facilitates host shifts and host specialization that can explain the distinct preferences or broad host ranges among the *Bactrocera* spp., concepts that have been explained further by Powell *et al.* (2012) and Biasazin *et al.* (2019). Considering *B. dorsalis*, *C. cosyra* and from a distance *Z. cucurbitae*, their performance in tomatoes suggests the importance of shared EAD-active volatiles in locating alternative oviposition substrates to bridge between seasons of preferred hosts that are occasioned by fruit or vegetable seasons.

The results obtained in this chapter provide very resourceful information which can be explored further to explain the tri-trophic interactions observed in chapters 2 and 3 and also the significance of each and/or the blends of antennal active compounds to improve the existing IPM packages. The results promote existing comparative data that provides reliable ecological and evolutionary olfactomes tuning information that will go a long way in advising alternative olfactomes-based strategies of tephritid fruit fly control.

To safeguard the world from global food security which is occasioned by increased human population, climate change, and globalization of agricultural pests, well-thought-out, innovative, sustainable and eco-friendly pest control measures must be advocated. This will only be possible if agriculture is shifted from chemical-based control measures to other alternatives. This study provides the basic framework that can be actualized through further research on EAD-active compounds. For the first time, a study has been conducted to reveal the actual picture of the events that happened *in situ* among the host fruits, the fruit fly, and the parasitoid. The mapping out of the compounds of infested mango headspace volatiles that elicit the antennal activity of parasitoids and comparing them with those of the host fruit flies is remarkable since it will open up a new discussion about the food-based baited traps. Other than the attraction to fruit fly-infested mangoes, where different EAD-active compounds were expected to come into play between the fruit flies and the parasitoids, the results fairly explain the attraction of parasitoids to headspace volatiles of non-infested ripe mangoes. The results have great potential in eco-friendly pest management strategies to specifically target the fruit fly, but not its natural enemies (parasitoids), for example on the formulations of food-based lures that take into consideration fruit fly selective combinations by filtering out compounds that would otherwise end up attracting and harming the parasitoids.

5.2 General conclusion

In summary, the key findings of this study are:

i. *B. dorsalis* and *C. cosyra* and their parasitoids respond differently to different varieties of mangoes and their infestation status.
ii. *B. dorsalis* and *C. cosyra* perform differently in different mango varieties. Kent mango is prone to *C. cosyra* while Apple mango is to *B. dorsalis*.
iii. The headspace volatile composition of mangoes is influenced by mango variety, maturity, and infestation status. The volatiles produced determine the attractiveness of the mango to the fruit flies and the parasitoids.
iv. The presence of mango and/or banana volatiles induces oviposition in *B. latifrons*.
v. There is a substantial degree of EAD-active volatile shared among fruit fly species; fruit fly and parasitoid species; and the parasitoid species.
vi. The majority of EAD-active compounds of fruit fly species and parasitoids are esters followed by sesquiterpenes.
vii. There is a substantial degree of convergence of EAD-active compounds of fruit fly species and parasitoids.

5.3 Recommendations for Future Research Needs

Further studies on:

i. Factors that contribute to the differential performance of *B. dorsalis* and *C. cosyra* in Kent and Apple varieties of mangoes. This will encompass attributes which could be affecting the performance of the said fruit fly, ranging from the nutrition requirements, the ability of the ovipositor to penetrate the pericarp of the fruits, and the chemical nature of the host fruit among others. This study should also be extended to other varieties of mangoes to get new insights that can advise on better ways of managing fruit flies.

ii. The significance of shared and non-shared EAD-active compounds of infested and non-infested fruits in the attraction of individual and combination of fruit fly species to formulate a blend that can be used as a lure for female fruit fly management.

iii. Significance of *B. latifrons* EAD-active volatiles of banana and mango headspace volatiles in inducing its oviposition. Since the compounds have tentatively been identified, their effects either singularly or as blends should be subjected to responses of *B. latifrons*. This might bring about the identification of important semiochemicals relating to its rearing and management.

iv. The significance of the fruit fly and parasitoid shared EAD-active compounds in formulations that filter off attractants of parasitoids and how these can be used to produce fruit fly selective combinations that can be used for the management and control of fruit flies.

v. Selective pressures and evolutionary processes underlying the development of unique EAD-active compounds to provide valuable insight into the evolutionary dynamics between parasitoids, host fruit flies and their host fruits/vegetables.

5.4 References

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APPENDICES

APPENDIX 1: Figures

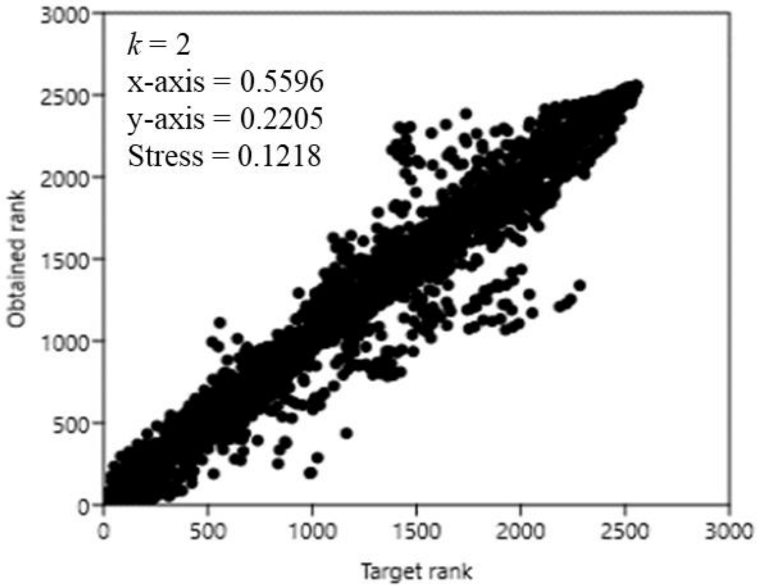


Figure S2-1: The NMDS two-dimensional ($k = 2$) Shepard plots for the volatile concentrations of the treatments of the three mango varieties

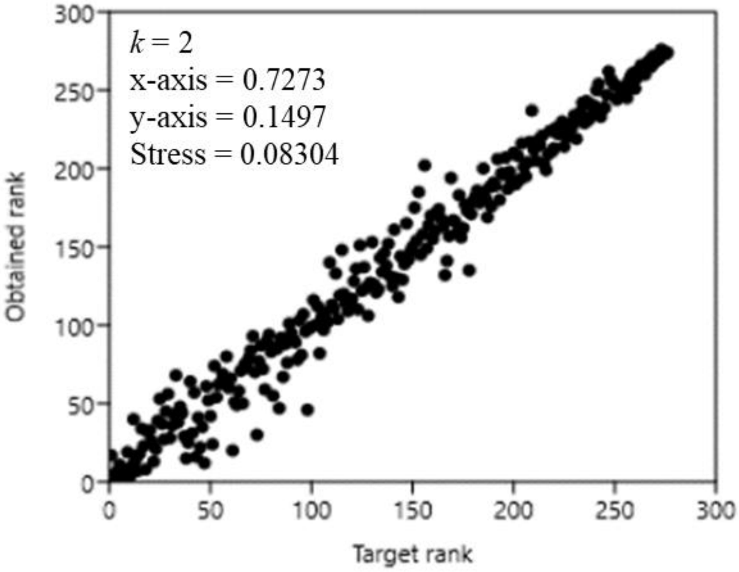


Figure S2-2: The NMDS two-dimensional ($k = 2$) Shepard plots for the volatile concentrations of the treatments of the Kent mango variety

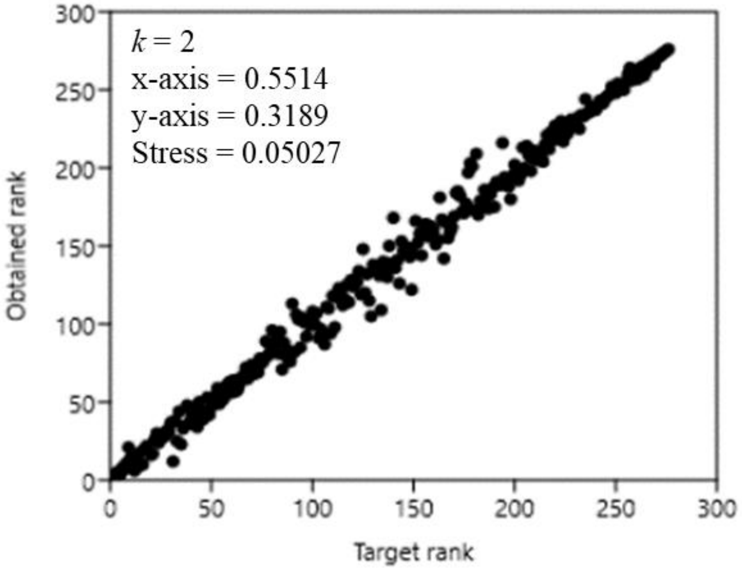


Figure S2-3: The NMDS two-dimensional ($k = 2$) Shepard plots for the volatile concentrations of the treatments of the Apple mango variety

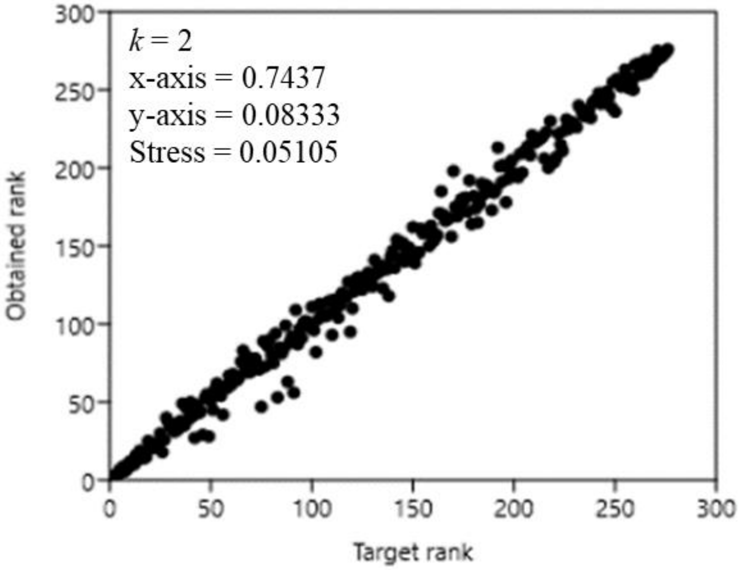


Figure S2-4: The NMDS two-dimensional ($k = 2$) Shepard plots for the volatile concentrations of the treatments of the Haden mango variety

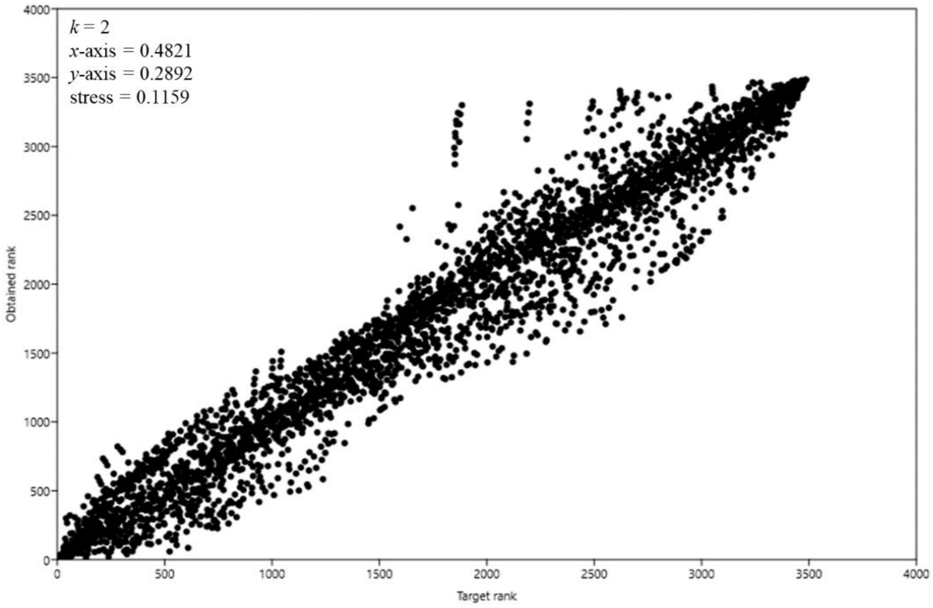


Figure S3-1: The two-dimensional Shepard plot (k = 2) of the volatile concentrations of the three mango variety headspace treatments

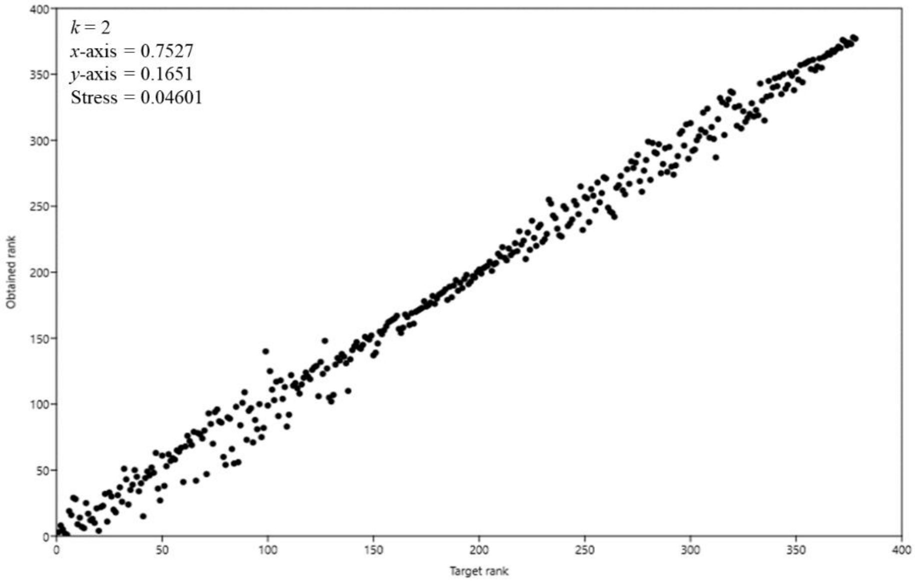


Figure S3-2: The two-dimensional Shepard plot (k = 2) of the volatile concentrations of the Kent mango variety headspace treatments

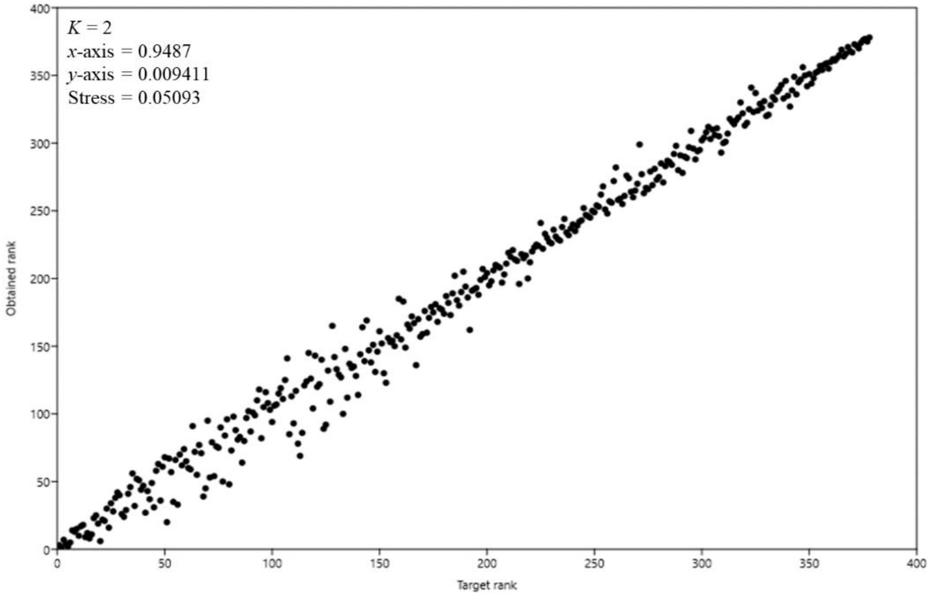


Figure S3-3: The two-dimensional Shepard plots (*k* = 2) of the volatile concentrations of the Apple mango variety headspace treatments

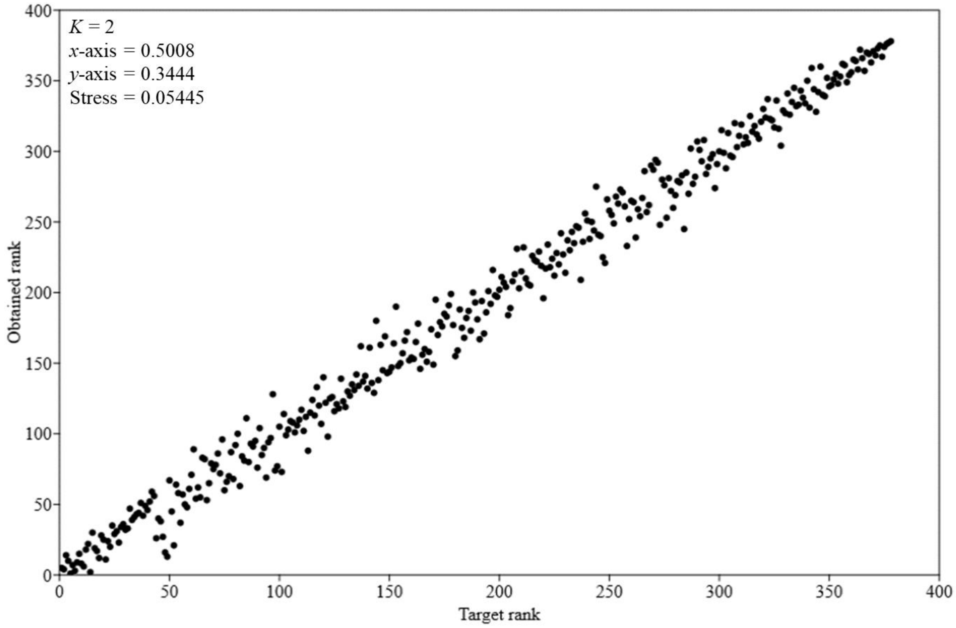


Figure S3-4: The two-dimensional Shepard plots (*k* = 2) of the volatile concentrations of the Haden mango variety headspace treatments

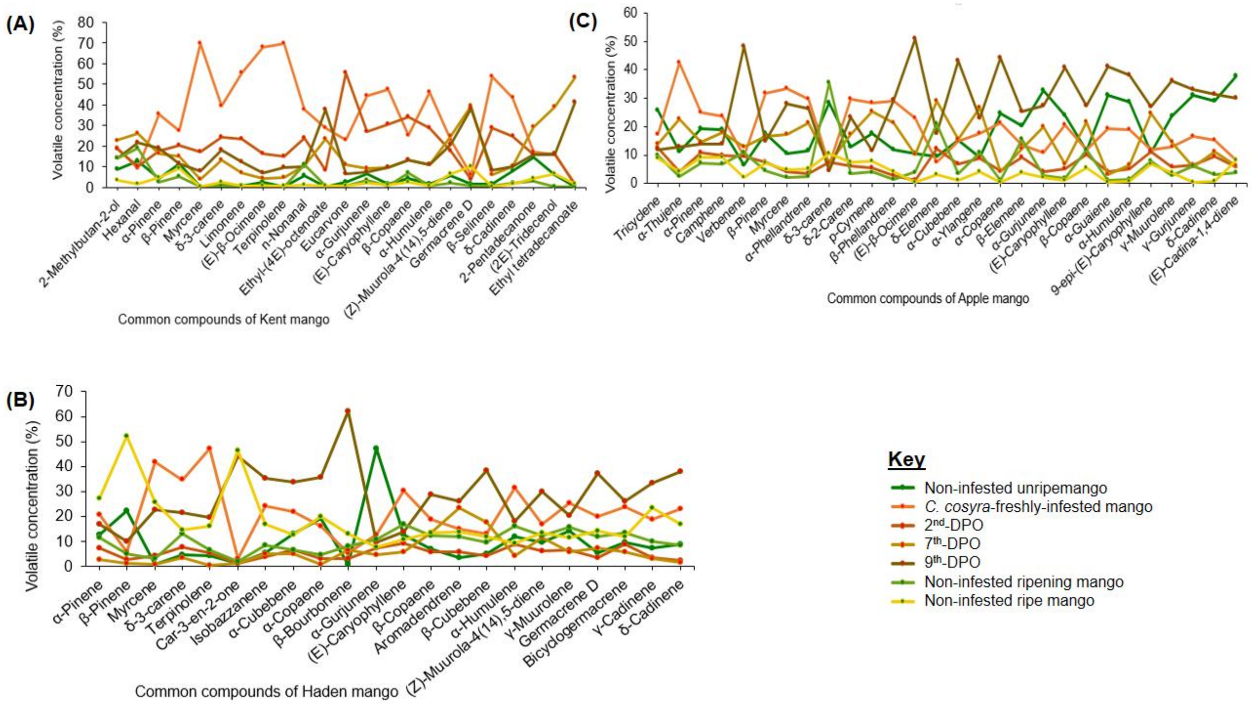
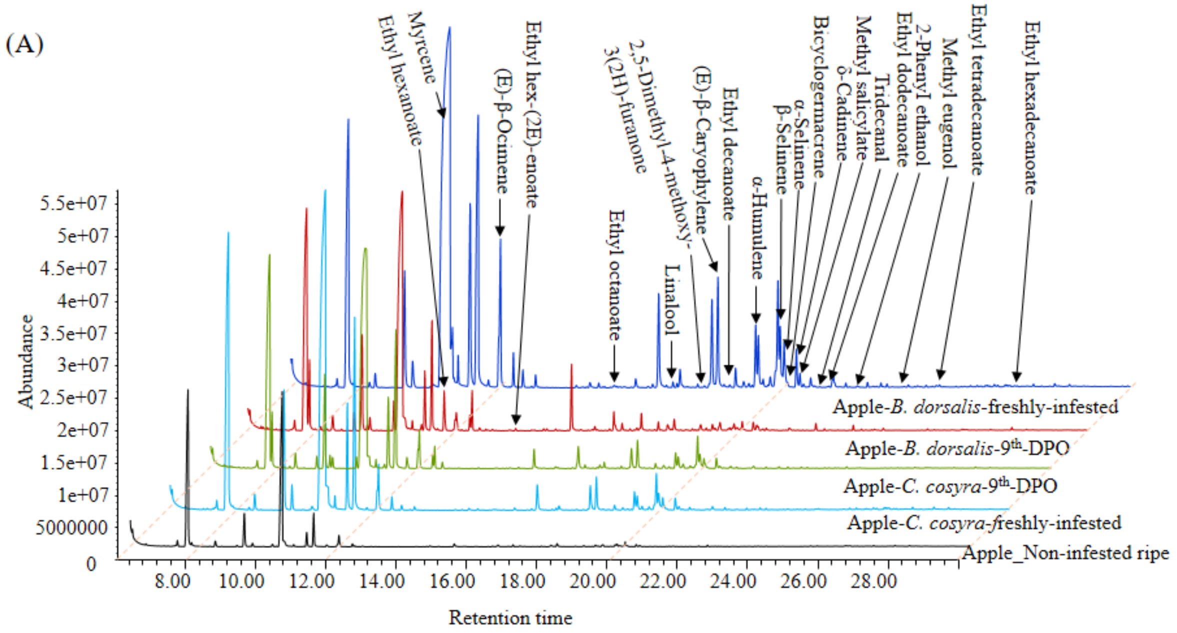


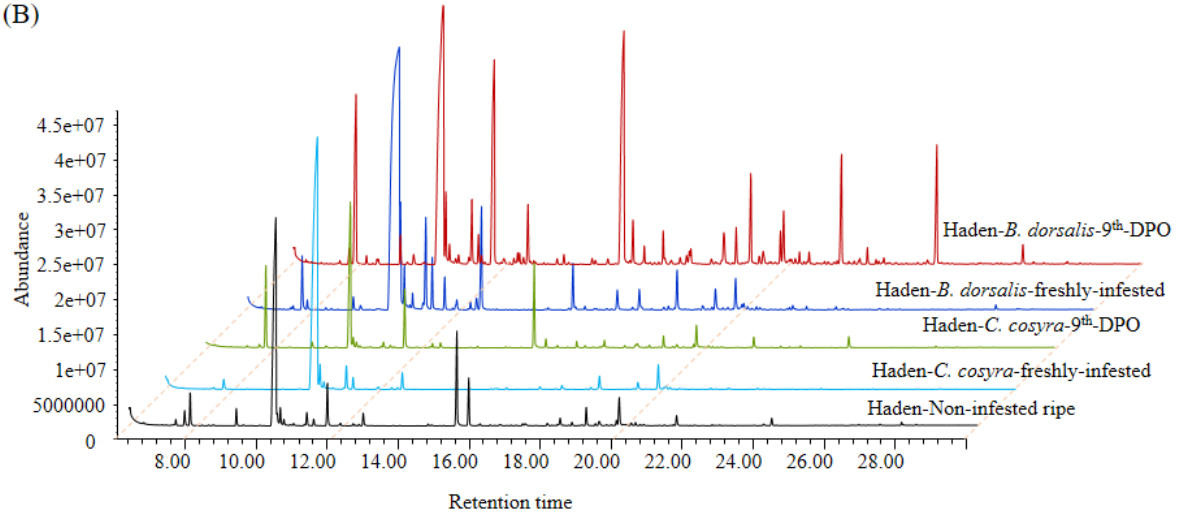
Figure S3-5: Percentages of the volatile concentrations of each common compound (relative to the total) of non-infested unripe; freshly *C. cosyra*-infested mangoes; 2nd-DPO; 7th-DPO; 9th-DPO mangoes; non-infested ripening; and non-infested ripe mangoes of the three varieties, Kent (A); Apple (B); and Haden (C)


A B C D E F G H I J K L M N O
δ-3-Carene
Myrcene
α-Pinene
β-phellandrene
Limonene
Ethyl octanoate
β-Pinene
(E)-β-Caryophylene
Terpinolene
α-phellandrene
α-Humulene
Ethyl dodecanoate
Germacrene D
α-Terpinene
p-Cimene
α-Selinene
α-Gurgujene
δ-Cadinene
Ethyl tetradecanoate
n-Nonanal
α-Fenchene
γ-terpinene
n-Decanal
2-Pentadecanone
n-Hexanal
β-Selinene
(E)- β-Ocimene
α-Copaene
(2Z)-Butenoic acid
Ethyl oct-(2E)-enoate
iso-Sylvestrene
Ethyl decanoate
p-Ethylacetophenone
Ethyl hexadecanoate
γ-Octalactone
β-Cubebene
Tridecanal
Methyl eugenol
2-Hexanol
Ethyl hexanoate
Ethyl butanoate
Pentan-2-one
Camphene
Piperitenone
α-Cubenene
β-Elemene
Dimethyl sulfoxide
Verbenone
Ethyl hex-(2E)-enoate
Ethyl-(4E)-decenoate
Ethyl 9-hexadecenoate
Car-3-en-2-one
β-Copaene
Methyl octanoate
Ethyl oct-(4Z)-enoate
m-Ethylacetophenone
2-Phenyl ethanol
3,3-Dimethyl-2-pentanol
3-Hydroxybutan-2-one
Methyl salicylate
γ-Muurolene
Sylvestrene
2-Methyl-2-butanol
2-Methyl-6-methyleneocta-1,7-dien-3-one
Benzyl methanol
Camphor
Methylethyl tetradecanoate
α-Pinene oxide
Hex-(3E)-enyl ethanoate
Caryophyllene oxide
Geranyl acetone
3-Methylbutyl ethanoate
2,5-Dimethyl-4-methoxy-3(2H)-furanone
Bicyclogermacrene
p-Cymen-8-ol
Benzaldehyde
Heptan-1-ol
α-Cadinene
butyl-(2E)-butenoate
Hept-(2E)-enal
Sabinene
Viridiflorene
Humulene epoxide II
m-Cymenene
Methyl-(2Z)-butenoate
A – Apple_BD1
B – Apple_BD9
C – Apple_CC1
D – Apple_CC9
E – Apple_NR
F – Haden_BD1
G – Haden_BD9
H – Haden_CC1
I – Haden_CC9
J – Haden_NR
K – Kent_BD1
L – Kent_BD9
M – Kent_CC1
N – Kent_CC9
O – Kent_NR


A B C D E F G H I J K L M N O
Compound
β-bourbenene
Ethyl (2E,4E)-2,4-hexadienoate
Methyl butanoate
Methyl dodecanoate
Phenyl ethyl hexanoate
6-Methyl hept-5-en-2-one
Ethyl-(2Z)-butenoate
E-Nerolidol
Butane-2,3-diol
Menthofuran
Ethyl 2-methylbutanoate
Ethyl 3-methyl-2-butenoate
Z-Calamenene
n-Ethyl propanoate
Methyl dec-4-enoate
Z-Muurola-3,5-diene
Non-(3Z)-en-1-ol
Ethyl-2-methylproanoate
6,10,14-Trimethyl-2-pentadecanone
Ethyl heptanoate
Ethyl 2-phenylethanoate
Aromadendrene
Ethyl pentanoate
Phenol
Butyl n-hexanoate
ethyl oct-(4E)-enoate
α-Bulnesene
α-Guaiaene
γ-Gurjunene
1-(1,4-Dimethyl-3-cyclohexen-1-yl) ethanone
α-Ylangene
E-Muurola-3,5-diene
Ethyl 3-hydroxybutanoate
allo-Aromadendrene
(E)-Verbenol
Linalool
6,7-Epoxymyrcene
δ-2-Carene
1-(1,1-Dimethylethyl)-2-methyl-1,3-propanediyl 2-methyl propanoic acid
Cryptone
allo-Ocimene
p-Mentha-1,3,8-triene
Verbenene
Ethyl (3E)-hexenoate
Cyclooctanone
Methyl tetradecanoate
Hex-(3Z)-enol
Hexahydro-1,4-dioxa-cyclopropa[a]pentalen-3-one
1-Oxide 4-methyl-quinoline
Dodecamethyl pentasiloxane
E-Cadina-1,4-diene
4,5-di-epi-Aristolochene
Catalponol
Octadecane
3,3,6-Trimethylhepta-1,5-dien-4-one
1,2,3,4-Tetramethyl benzene
Valencene
4-Hydroxy pentan-2-one
(Z)-α-Bergamotene
Dehydro sabinene ketone
E-Sabinene hydrate
γ-Cadinene
2-Methyl propan-1-ol
Z-Cadina-1(6),4-diene
3,3,5-Trimethyl-1,5-heptadiene
n-Hexadecanol
Pentyl 2-methylpropanoate
Eucarvone
3-(1H-indol-3-yl)-2-Propenoic acid
Tetrahydro-6-propyl-2H-pyran-2-one
Unknown116
n-propyl hexanoate
Aciphyllene
(E,E)-α-Fernesene
2-(Methylenecyclopropyl)prop-2-yl but-(2E)-enoate
3-Methylbutyl methanoate
Ethyl 9-tetradecenoate
4-Ethyl benzaldehyde
2,6,11,15-Tetramethyl hexadeca-2,6,8,10,14-pentaene
7-epi-α-Selinene
Heptan-2-one
Methyl hexadecanoate
1-epi-Cubenol
Pogostol
epi-α-Muurulol
3Z-Octen-2-ol
Dauca-5,8-diene
n-Octadecanol
5-Methyl-2-(1-methylethenyl)-4-hexenal
Butyl 4-methyloctanoate
Area
8e+09
6e+09
4e+09
2e+09
0e+00
A – Apple_BD1
B – Apple_BD9
C – Apple_CC1
D – Apple_CC9
E – Apple_NR
F – Haden_BD1
G – Haden_BD9
H – Haden_CC1
I – Haden_CC9
J – Haden_NR
K – Kent_BD1
L – Kent_BD9
M – Kent_CC1
N – Kent_CC9
O – Kent_NR

Figure S4-1: The 238 compounds of mango treatment headspace volatiles in their decreasing order of sharedness among treatments. The darker the red colour the larger the compound's peak area and the lighter the blue colour the less the peak area in that column. Corresponding peaks were traced across samples, the identity of the majority of peaks was very tentative


(A)
Ethyl hexanoate
Myrcene
(E)-β-Ocimene
Ethyl hex-(2E)-enoate
Ethyl octanoate
2,5-Dimethyl-4-methoxy-3(2H)-furanone
Linalool
(E)-β-Caryophylene
Ethyl decanoate
α-Humulene
β-Selinene
α-Selinene
Bicyclogermacrene
δ-Cadinene
Methyl salicylate
Tridecanal
Ethyl dodecanoate
2-Phenyl ethanol
Methyl eugenol
Ethyl tetradecanoate
Ethyl hexadecanoate
Abundance
5.5e+07
5e+07
4.5e+07
4e+07
3.5e+07
3e+07
2.5e+07
2e+07
1.5e+07
1e+07
5000000
0
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
Retention time
Apple-*B. dorsalis*-freshly-infested
Apple-*B. dorsalis*-9th-DPO
Apple-*C. cosyra*-9th-DPO
Apple-*C. cosyra*-freshly-infested
Apple_Non-infested ripe


(B)
Abundance
4.5e+07
4e+07
3.5e+07
3e+07
2.5e+07
2e+07
1.5e+07
1e+07
5000000
0
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
Retention time
Haden-*B. dorsalis*-9th-DPO
Haden-*B. dorsalis*-freshly-infested
Haden-*C. cosyra*-9th-DPO
Haden-*C. cosyra*-freshly-infested
Haden-Non-infested ripe

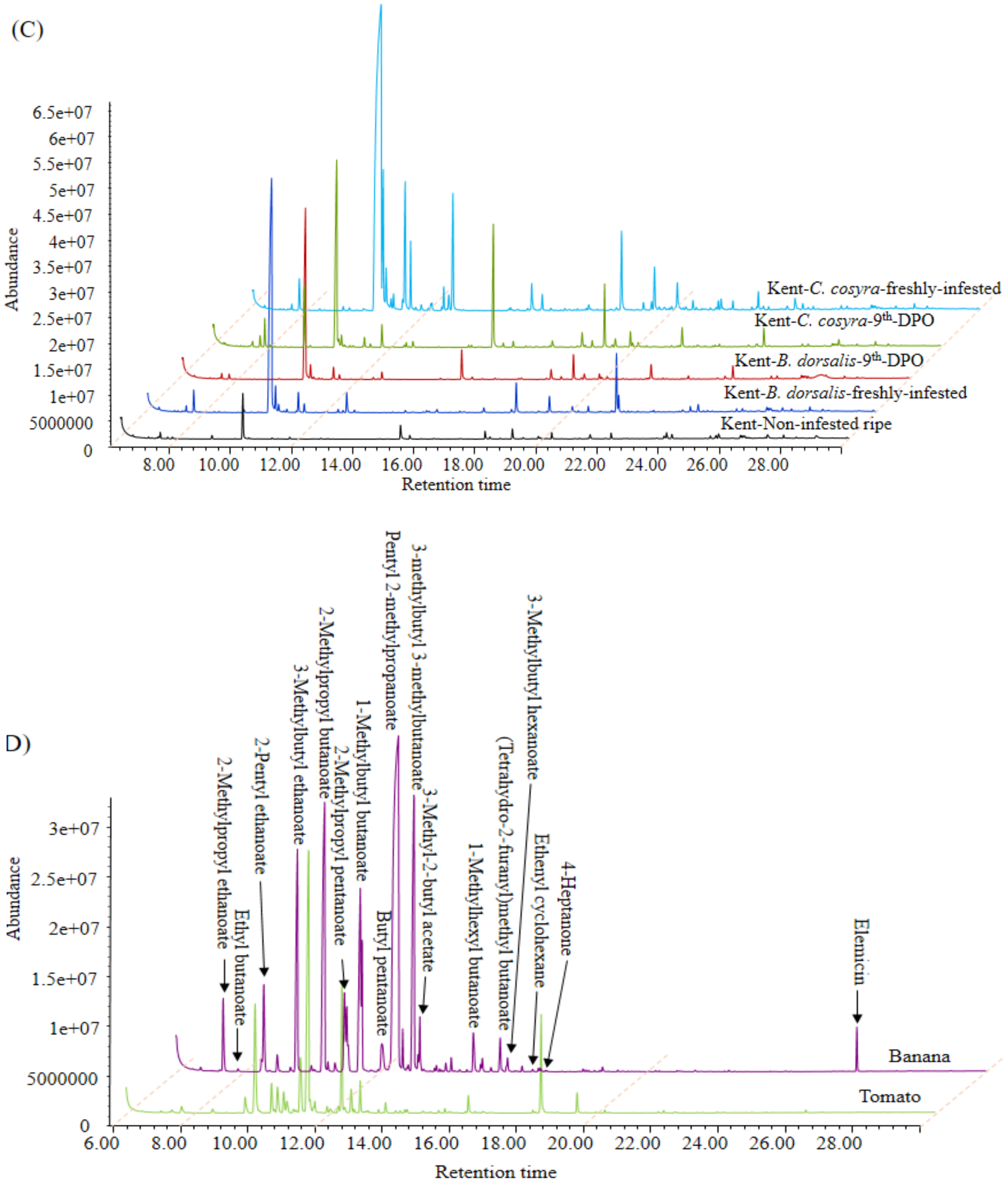
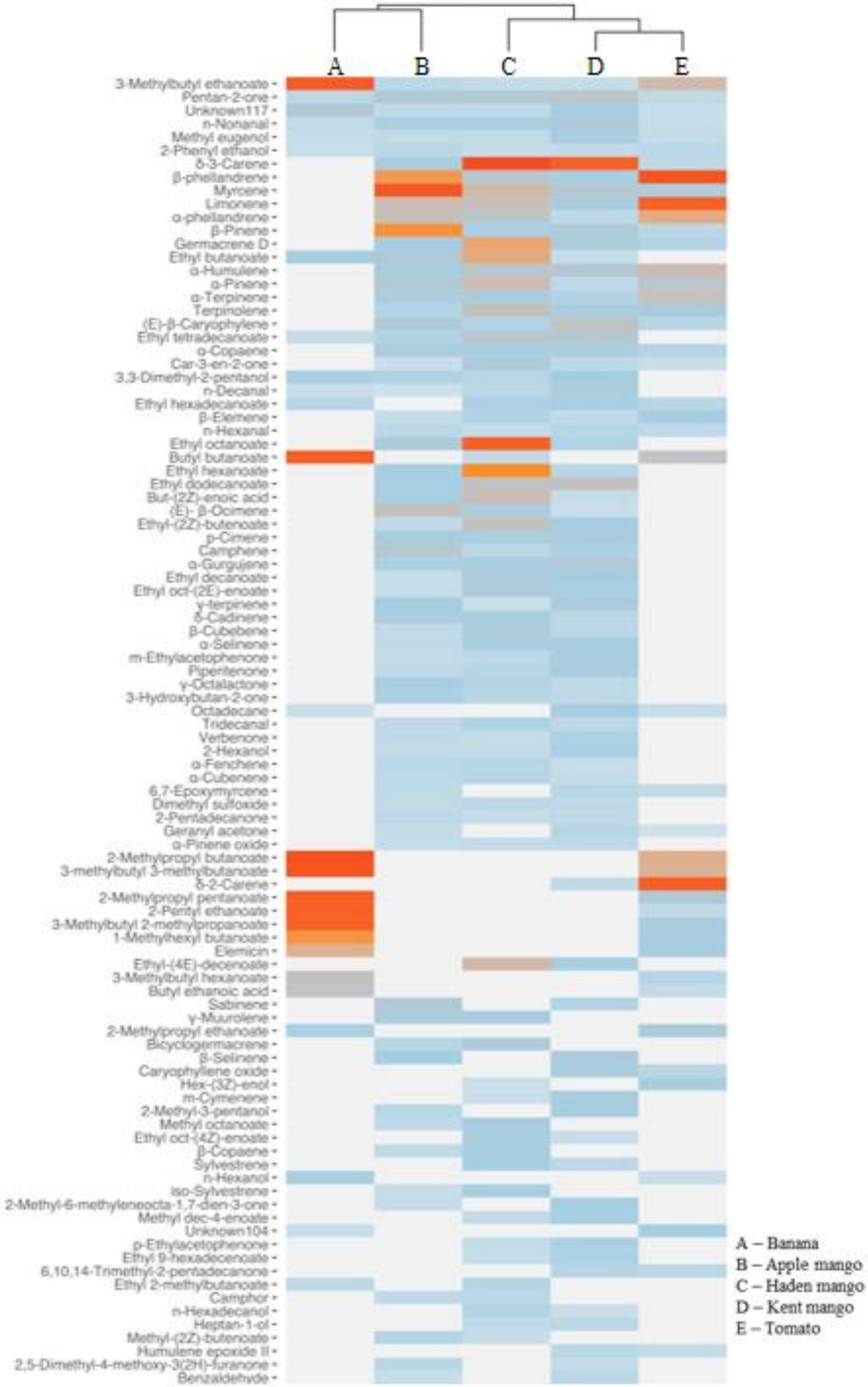
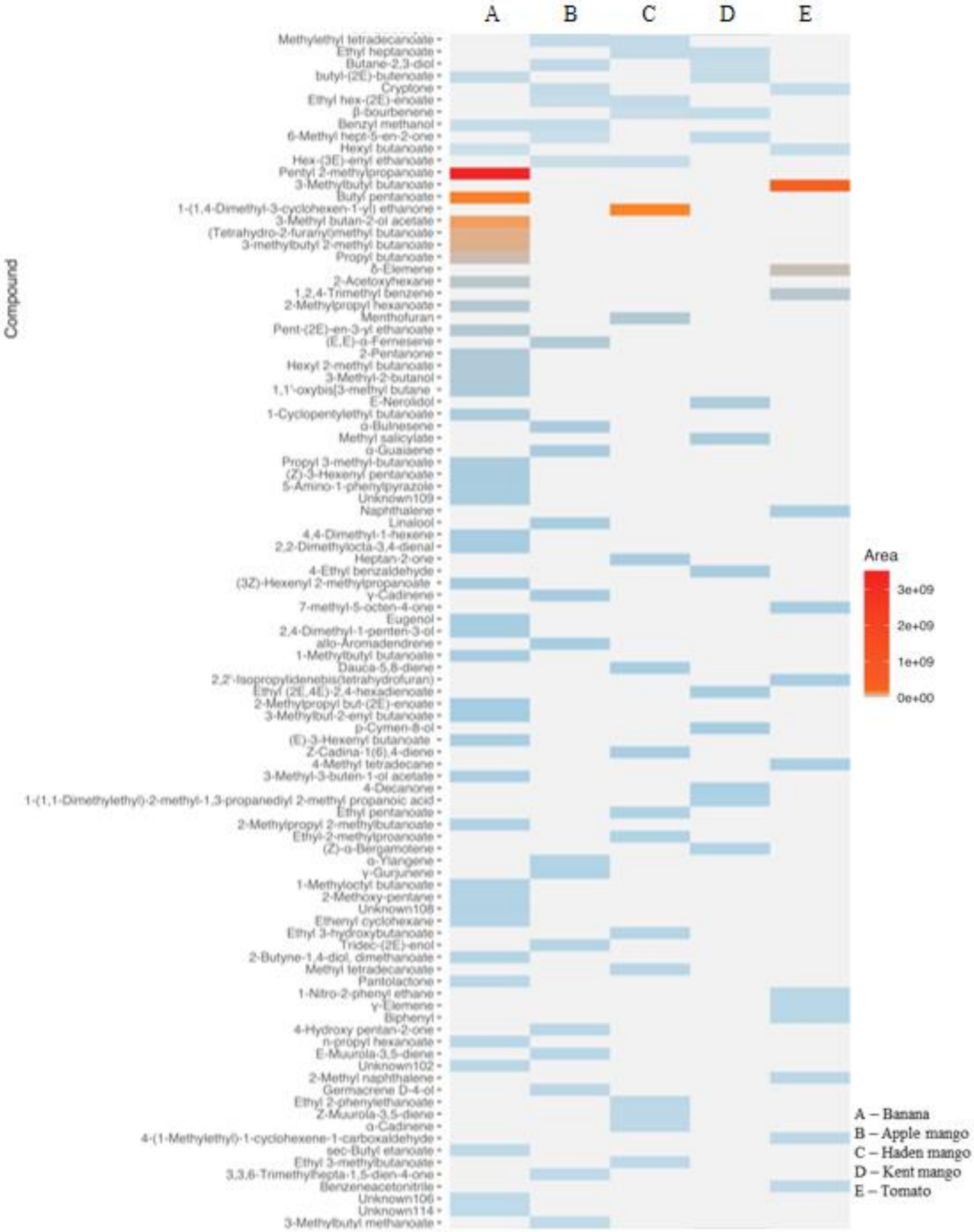


Figure S4-2: Offset chromatograms of Apple (A); Haden (B); Kent (C) mango variety treatments and banana (Fhia 17 variety)/tomato (Improved Nouvelle F1 variety) (D). For the offsets of the Apple mango variety and banana, some of the antennal-active compound peaks are labelled


A
B
C
D
E
3-Methylbutyl ethanoate
Pentan-2-one
Unknown117
n-Nonanal
Methyl eugenol
2-Phenyl ethanol
δ-3-Carene
β-phellandrene
Myrcene
Limonene
α-phellandrene
β-Pinene
Germacrene D
Ethyl butanoate
α-Humulene
α-Pinene
α-Terpinene
Terpinolene
(E)-β-Caryophylene
Ethyl tetradecanoate
α-Copaene
Car-3-en-2-one
3,3-Dimethyl-2-pentanol
n-Decanal
Ethyl hexadecanoate
β-Elemene
n-Hexanal
Ethyl octanoate
Butyl butanoate
Ethyl hexanoate
Ethyl dodecanoate
But-(2Z)-enoic acid
(E)- β-Ocimene
Ethyl-(2Z)-butenoate
p-Cimene
Camphene
α-Gurgujene
Ethyl decanoate
Ethyl oct-(2E)-enoate
γ-terpinene
δ-Cadinene
β-Cubebene
α-Selinene
m-Ethylacetophenone
Piperitenone
γ-Octalactone
3-Hydroxybutan-2-one
Octadecane
Tridecanal
Verbenone
2-Hexanol
α-Fenchene
α-Cubenene
6,7-Epoxymyrcene
Dimethyl sulfoxide
2-Pentadecanone
Geranyl acetone
α-Pinene oxide
2-Methylpropyl butanoate
3-methylbutyl 3-methylbutanoate
δ-2-Carene
2-Methylpropyl pentanoate
2-Pentyl ethanoate
3-Methylbutyl 2-methylpropanoate
1-Methylhexyl butanoate
Elemicin
Ethyl-(4E)-decenoate
3-Methylbutyl hexanoate
Butyl ethanoic acid
Sabinene
γ-Muurolene
2-Methylpropyl ethanoate
Bicyclogermacrene
β-Selinene
Caryophyllene oxide
Hex-(3Z)-enol
m-Cymenene
2-Methyl-3-pentanol
Methyl octanoate
Ethyl oct-(4Z)-enoate
β-Copaene
Sylvestrene
n-Hexanol
iso-Sylvestrene
2-Methyl-6-methyleneocta-1,7-dien-3-one
Methyl dec-4-enoate
Unknown104
p-Ethylacetophenone
Ethyl 9-hexadecenoate
6,10,14-Trimethyl-2-pentadecanone
Ethyl 2-methylbutanoate
Camphor
n-Hexadecanol
Heptan-1-ol
Methyl-(2Z)-butenoate
Humulene epoxide II
2,5-Dimethyl-4-methoxy-3(2H)-furanone
Benzaldehyde
A – Banana
B – Apple mango
C – Haden mango
D – Kent mango
E – Tomato


A
B
C
D
E
Compound
Methylethyl tetradecanoate
Ethyl heptanoate
Butane-2,3-diol
butyl-(2E)-butenoate
Cryptone
Ethyl hex-(2E)-enoate
β-bourbenene
Benzyl methanol
6-Methyl hept-5-en-2-one
Hexyl butanoate
Hex-(3E)-enyl ethanoate
Pentyl 2-methylpropanoate
3-Methylbutyl butanoate
Butyl pentanoate
1-(1,4-Dimethyl-3-cyclohexen-1-yl) ethanone
3-Methyl butan-2-ol acetate
(Tetrahydro-2-furanyl)methyl butanoate
3-methylbutyl 2-methyl butanoate
Propyl butanoate
δ-Elemene
2-Acetoxyhexane
1,2,4-Trimethyl benzene
2-Methylpropyl hexanoate
Menthofuran
Pent-(2E)-en-3-yl ethanoate
(E,E)-α-Fernesene
2-Pentanone
Hexyl 2-methyl butanoate
3-Methyl-2-butanol
1,1'-oxybis[3-methyl butane
E-Nerolidol
1-Cyclopentylethyl butanoate
α-Bulnesene
Methyl salicylate
α-Guaiaene
Propyl 3-methyl-butanoate
(Z)-3-Hexenyl pentanoate
5-Amino-1-phenylpyrazole
Unknown109
Naphthalene
Linalool
4,4-Dimethyl-1-hexene
2,2-Dimethylocta-3,4-dienal
Heptan-2-one
4-Ethyl benzaldehyde
(3Z)-Hexenyl 2-methylpropanoate
γ-Cadinene
7-methyl-5-octen-4-one
Eugenol
2,4-Dimethyl-1-penten-3-ol
allo-Aromadendrene
1-Methylbutyl butanoate
Dauca-5,8-diene
2,2'-Isopropylidenebis(tetrahydrofuran)
Ethyl (2E,4E)-2,4-hexadienoate
2-Methylpropyl but-(2E)-enoate
3-Methylbut-2-enyl butanoate
p-Cymen-8-ol
(E)-3-Hexenyl butanoate
Z-Cadina-1(6),4-diene
4-Methyl tetradecane
3-Methyl-3-buten-1-ol acetate
4-Decanone
1-(1,1-Dimethylethyl)-2-methyl-1,3-propanediyl 2-methyl propanoic acid
Ethyl pentanoate
2-Methylpropyl 2-methylbutanoate
Ethyl-2-methylproanoate
(Z)-α-Bergamotene
α-Ylangene
γ-Gurjunene
1-Methyloctyl butanoate
2-Methoxy-pentane
Unknown108
Ethenyl cyclohexane
Ethyl 3-hydroxybutanoate
Tridec-(2E)-enol
2-Butyne-1,4-diol, dimethanoate
Methyl tetradecanoate
Pantolactone
1-Nitro-2-phenyl ethane
γ-Elemene
Biphenyl
4-Hydroxy pentan-2-one
n-propyl hexanoate
E-Muurola-3,5-diene
Unknown102
2-Methyl naphthalene
Germacrene D-4-ol
Ethyl 2-phenylethanoate
Z-Muurola-3,5-diene
α-Cadinene
4-(1-Methylethyl)-1-cyclohexene-1-carboxaldehyde
sec-Butyl etanoate
Ethyl 3-methylbutanoate
3,3,6-Trimethylhepta-1,5-dien-4-one
Benzeneacetonitrile
Unknown106
Unknown114
3-Methylbutyl methanoate
Area
3e+09
2e+09
1e+09
0e+00
A – Banana
B – Apple mango
C – Haden mango
D – Kent mango
E – Tomato

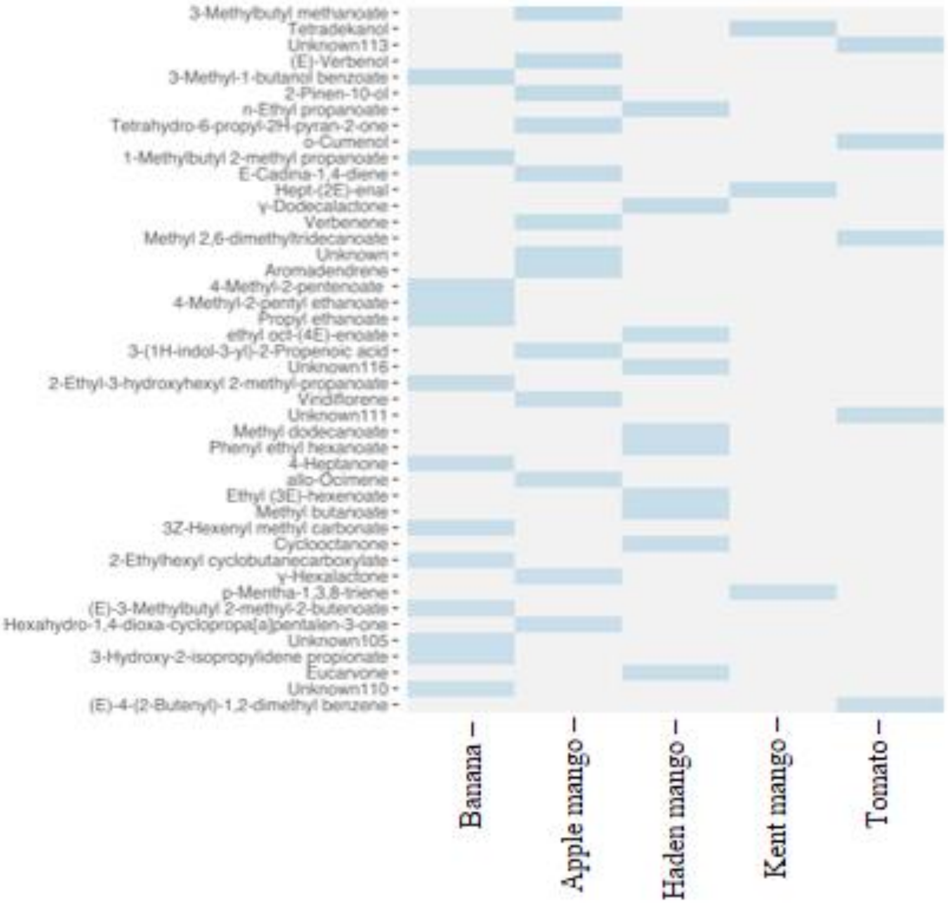
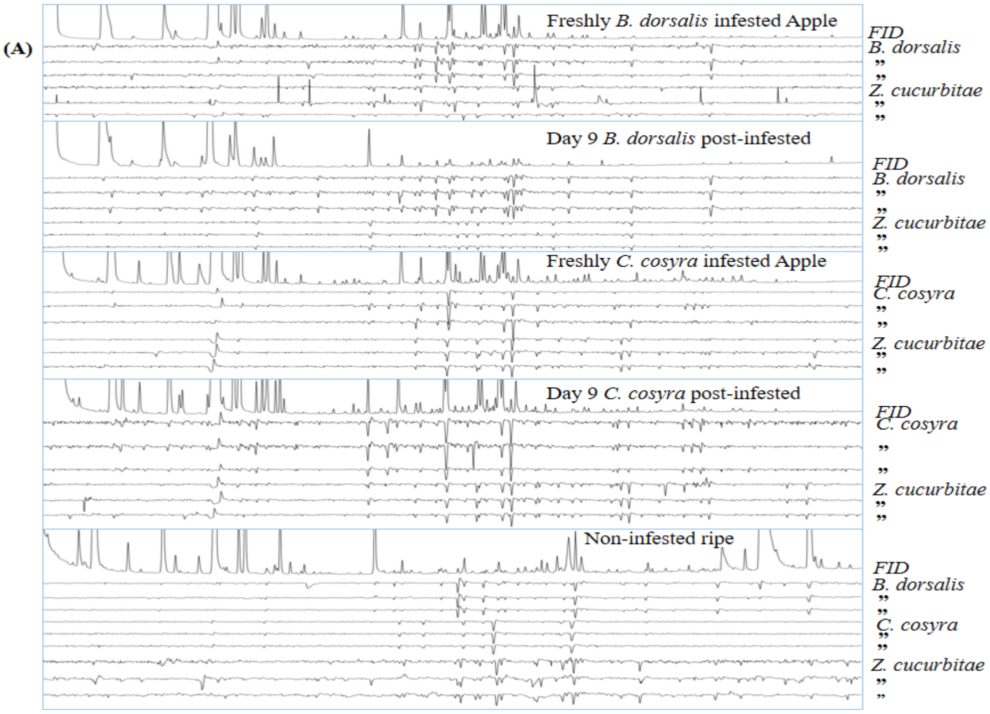
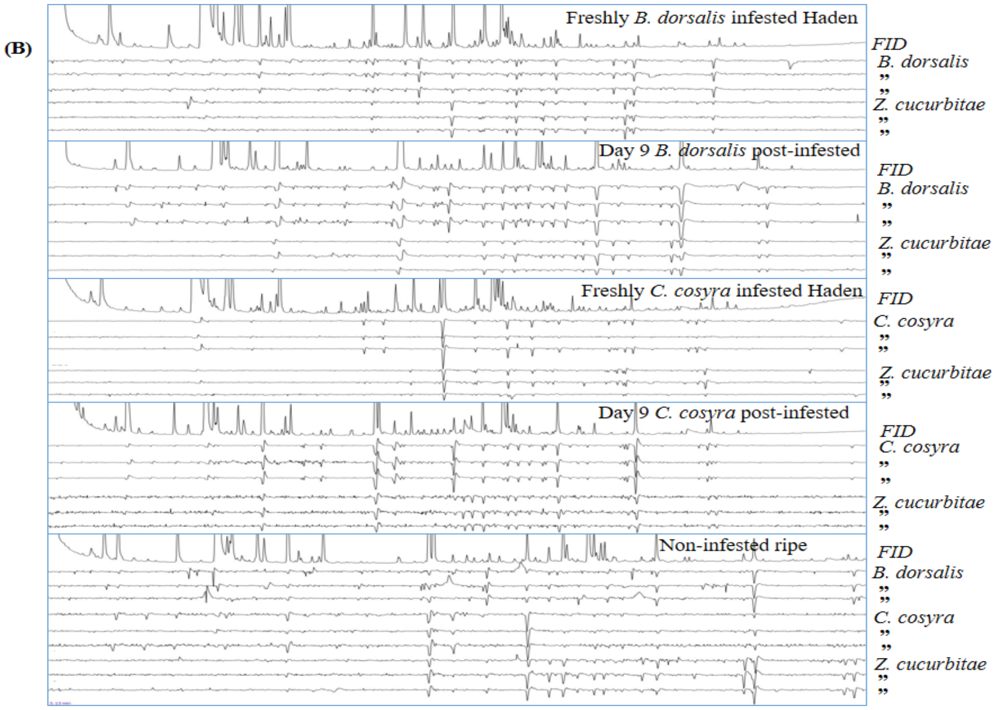


Figure S4-3: The 239 compounds of non-infested ripe fruit headspace volatiles in their decreasing order of sharedness among the fruits. The darker the red colour the larger the compound's peak area and the lighter the blue colour the less the peak area in that column. Corresponding peaks were traced across samples, the identity of the majority of peaks was very tentative


(A)
Freshly B. dorsalis infested Apple
FID
B. dorsalis
”
”
Z. cucurbitae
”
”
Day 9 B. dorsalis post-infested
FID
B. dorsalis
”
”
Z. cucurbitae
”
”
Freshly C. cosyra infested Apple
FID
C. cosyra
”
”
Z. cucurbitae
”
”
Day 9 C. cosyra post-infested
FID
C. cosyra
”
”
Z. cucurbitae
”
”
Non-infested ripe
FID
B. dorsalis
”
C. cosyra
”
”
Z. cucurbitae
”
”


(B)
Freshly B. dorsalis infested Haden
FID
B. dorsalis
”
”
Z. cucurbitae
”
”
Day 9 B. dorsalis post-infested
FID
B. dorsalis
”
”
Z. cucurbitae
”
”
Freshly C. cosyra infested Haden
FID
C. cosyra
”
”
Z. cucurbitae
”
”
Day 9 C. cosyra post-infested
FID
C. cosyra
”
”
Z. cucurbitae
”
”
Non-infested ripe
FID
B. dorsalis
”
”
C. cosyra
”
”
Z. cucurbitae
”
”

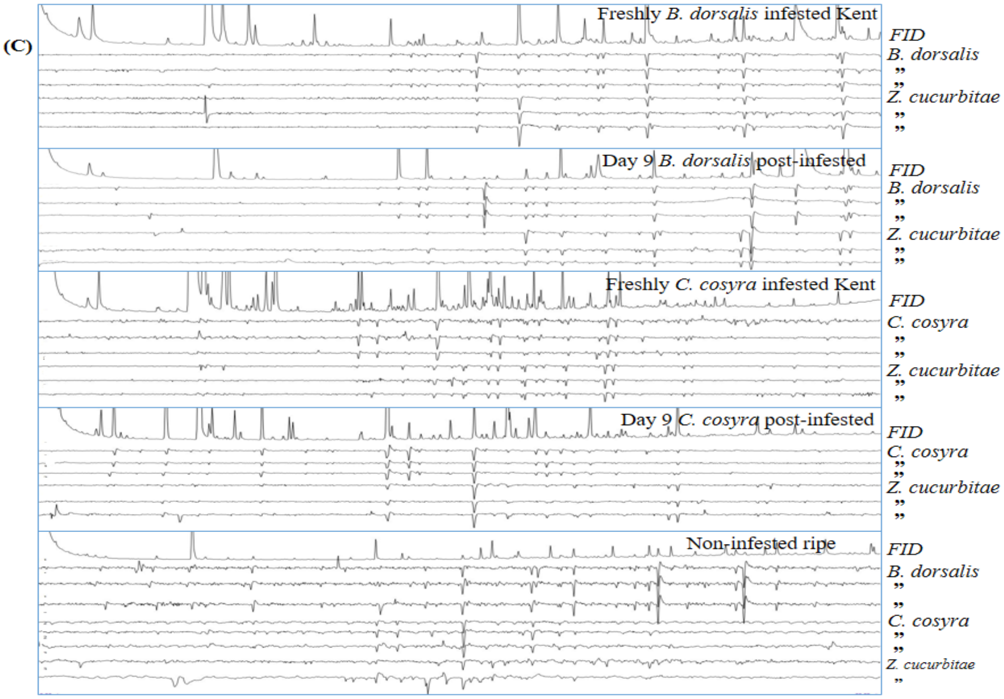


Figure S4-4: Offsets of gas chromatography-linked electroantennography detector responses of female *B. dorsalis*, *C. cosyra* and *Z. cucurbitae* to different treatments of headspace volatiles of Apple (A); Haden (B); and Kent (C). For each frame ionization detection, FID, profile, a triplicate of electroantennography detection, EAD, profiles were done and used in computing the normalized antennal responses


Apple
Haden
Kent
Normalized response
0.25
0.20
0.15
0.10
0.05
0.00
Compound
(E)-β-Caryophylene
Ethyl octanoate
Ethyl dodecanoate
Ethyl tetradecanoate
α-Humulene
Ethyl hexadecanoate
Ethyl hexanoate
α-Selinene
Myrcene
Methyl eugenol
Ethyl-(4E)-decenoate
δ-Cadinene
Ethyl decanoate
β-Selinene
2-Phenyl ethanol
Tridecanal
Methyl salicylate
Ethyl butanoate
Methylethyl tetradecanoate
(2Z)-Butenoic acid
Ethyl 9-hexadecenoate
Bicyclogermacrene
Piperitenone
β-bourbenene
Ethyl oct-(4Z)-enoate
Phenyl ethyl hexanoate
Linalool
δ-3-Carene
(E)- β-Ocimene
E-Nerolidol
Benzyl methanol
2,5-Dimethyl-4-methoxy-3(2H)-furanone
Ethyl 2-phenylethanoate
Germacrene D
Ethyl oct-(2E)-enoate
Methyl dec-4-enoate
n-Nonanal
n-Decanal
Ethyl-(2Z)-butenoate
Car-3-en-2-one
α-Gurgujene
2-Pentadecanone
Aromadendrene
α-Cadinene
1-(1,4-Dimethyl-3-cyclohexen-1-yl) ethanone
Ethyl heptanoate
E-Muurola-3,5-diene
β-phellandrene
1,2,3,4-Tetramethyl benzene
γ-Octalactone
p-Ethylacetophenone
Octadecane
2,3-Butanediol
Ethyl hex-(2E)-enoate
2-Hexanol
2-Methyl-6-methyleneocta-1,7-dien-3-one
Triethylene glycol
Cryptone
Catalponol
4,5-di-epi-Aristolochene
β-Pinene
Cyclooctanone
Ethyl 3-methylbutanoate
Methyl tetradecanoate
γ-Muurolene
iso-Sylvestrene
Heptan-1-ol
Unknown116
n-Hexadecanol
m-Ethylacetophenone
α-Guaiaene
α-Pinene
Ethyl 3-methyl-2-butenoate
6,10,14-Trimethyl-2-pentadecanone
Ethyl 9-tetradecenoate
β-Cubebene
m-Cymenene
Chemical Class
Alcohal
Aldehyde
Alkane
Alkene
Ester
Ketone
Lactone
Monoterpene
Monoterpenoid
Organic acid
Oxolane
Sesquiterpene
Sesquiterpenoid
Others

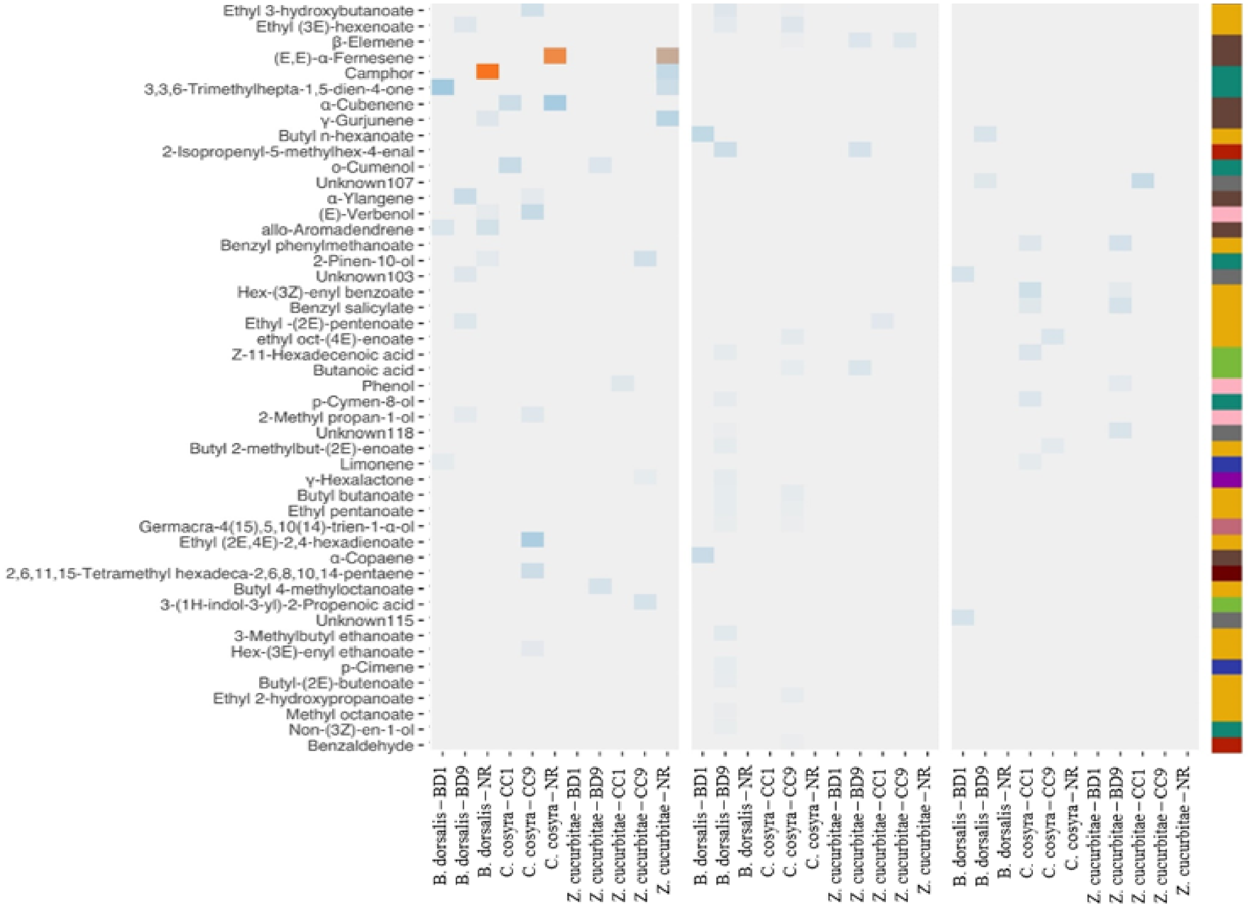


Figure S4-5: The normalized antennal responses of the fruit flies (*B. dorsalis*, *C. cosyra*, and *Z. cucurbitae*) to different treatments of freshly-infested (1), day 9 post-oviposition (9), and non-infested ripe (NR) mango (Apple, Haden, and Kent) headspace volatiles. From left, the columns represent: (a) the tentative names of the compounds in decreasing order of sharedness; (b) heat plot for the antennal normalized responses of the fruit flies per mango variety/treatment (key on the left side of the plot); and (c) a sidebar indicating the chemical classes of the compounds (BD = *B. dorsalis*; CC = *C. cosyra*; 1 = freshly-infested; 9 = day 9 post-oviposition; NR = non-infested ripe)

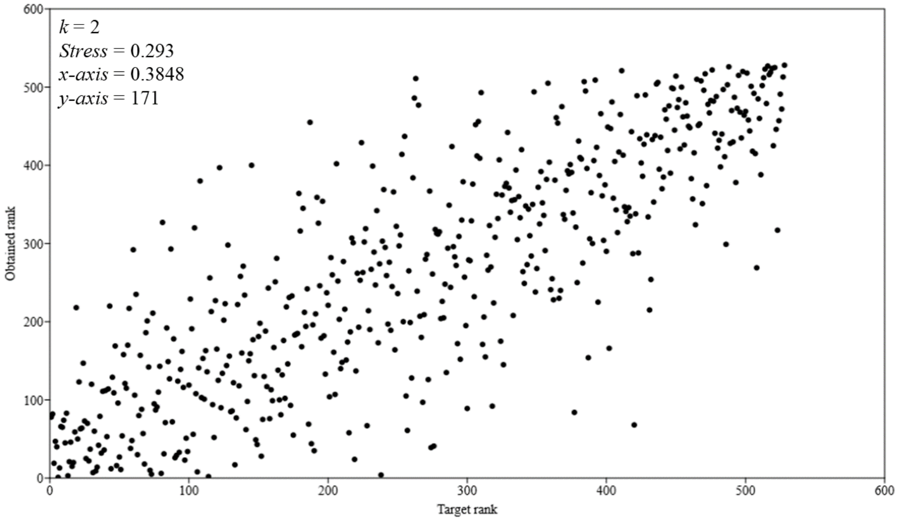


Figure S4-6: The two-dimensional Shepard plots (k = 2) of the normalized responses of compounds of mango treatments

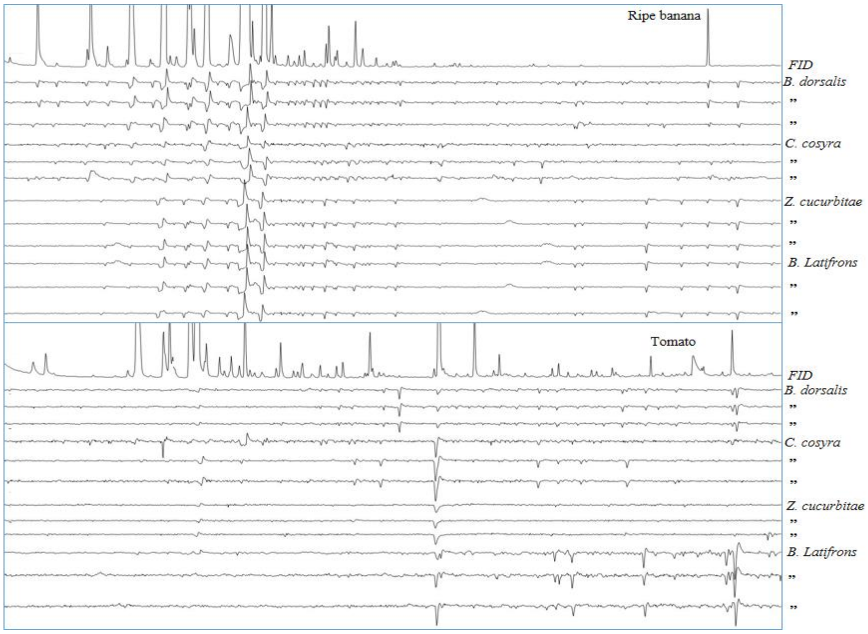


Figure S4-7: Offsets of gas chromatography-linked electroantennography detector responses of female *B. dorsalis*, *C. cosyra*, *Z. cucurbitae* and *B. latifrons* to ripe banana and tomato volatiles. For each frame ionization detection, FID, profile, a triplicate of electroantennography detection, EAD, profiles were done and used in computing the normalized antennal responses

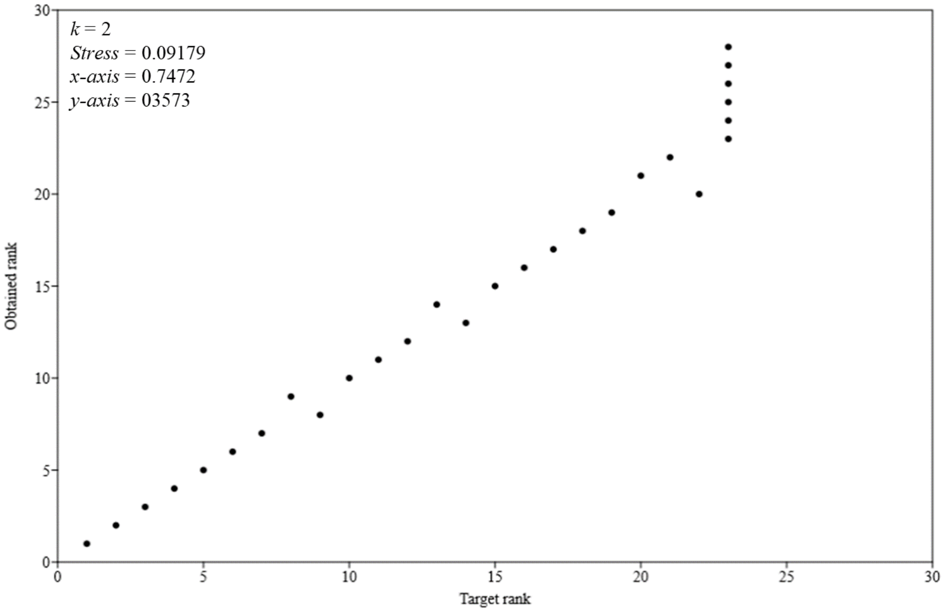


Figure S4-8: The two-dimensional Shepard plots (*k* = 2) of the normalized responses of compounds of banana and tomato

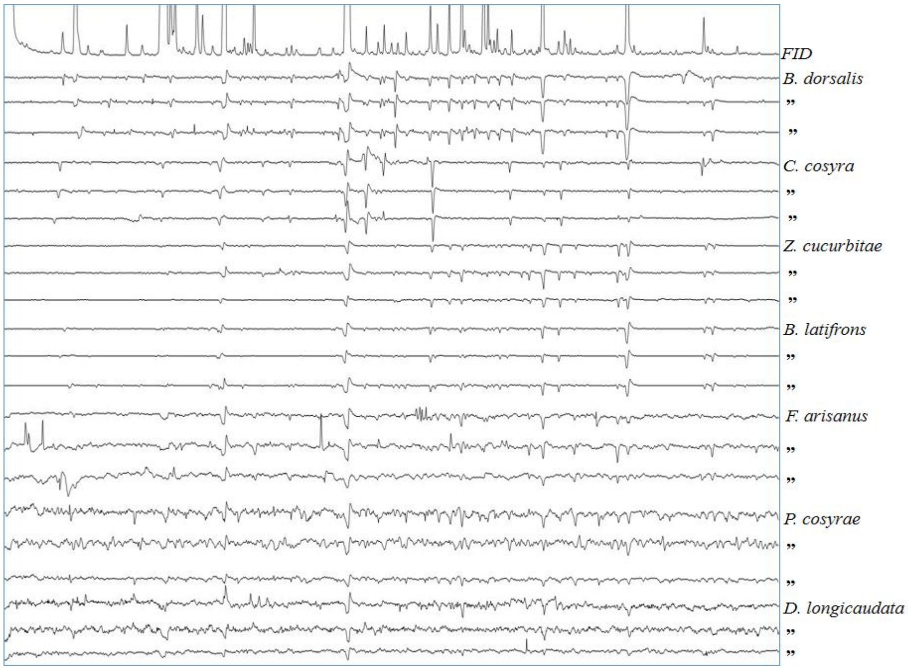


Figure S4-9: Offsets of gas chromatography linked electroantennography detector responses of fruit flies (*B. dorsalis*, *C. cosyra*, *Z. cucurbitae*, and *B. latifrons*) and parasitoids (*F. arisanus*, *P. cosyrae*, and *D. longicaudata*) to the day-9 *B. dorsalis* post-infestation Haden mango headspace volatiles. For each frame ionization detection, FID, profile, a triplicate of electroantennography detection, EAD, profiles were done and used in computing the normalized antennal responses

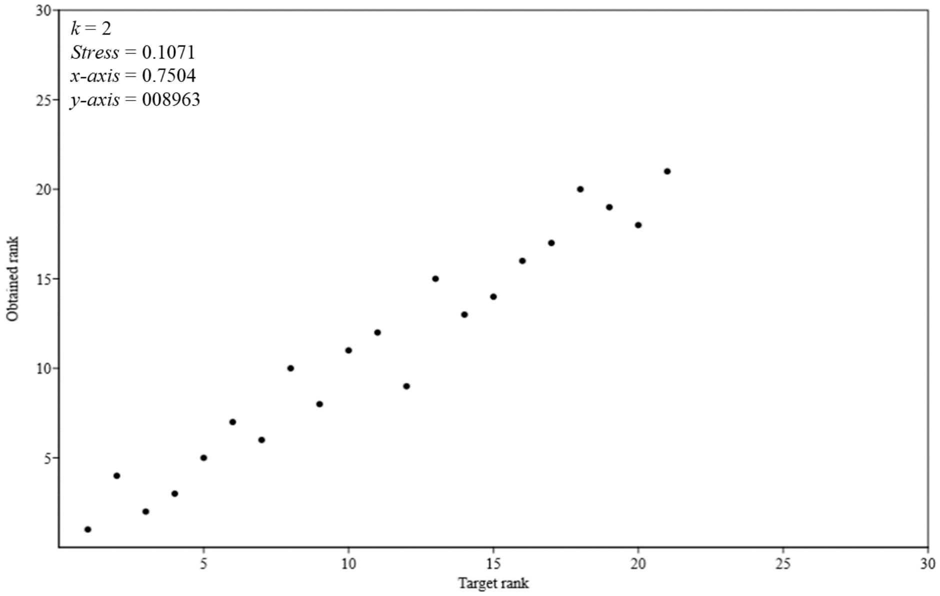


Figure S4-10: The two-dimensional Shepard plots (k = 2) of the normalized responses of compounds of day 9 *Bactrocera dorsalis* post-oviposition Haden mango headspace volatiles

APPENDIX 2: Tables

Table S4-1: The peak areas of the headspace volatile compounds of mango (Apple, Haden, and Kent varieties) treatments. Compounds were tentatively identified based on their retention time (RT), electron ionization spectrum, calculated retention index (RI_{Calc}) in comparison to literature retention index (RI_{Lit}) as well as comparing their mass spectra with those from the *NIST*, Adams and Chemecol libraries database for DB-Wax capillary column (60 m × 0.25 mm i.d., 0.25 um film thickness. (inf = infestation; DPO = day post-oviposition)

No peak	GC-MS_RT	Compound Name	CAS_no	RI$_{(Cal)}$	RI$_{(Lit)}$	Ref	*B.dorsalis* -freshly-inf (Apple)	*B.dorsalis* -9th-DPO (Apple)	*C.cosyra* -freshly-inf (Apple)	*C.cosyra* -9th-DPO (Apple)	Non-infested ripe (Apple)	*B.dorsalis* -freshly-inf (Haden)	*B.dorsalis* -9th-DPO (Haden)	*C.cosyra* -freshly-inf (Haden)	*C.cosyra* -9th-DPO (Haden)	Non-infested ripe (Haden)	*B.dorsalis* -freshly-inf (Kent)	*B.dorsalis* -9th-DPO (Kent)	*C.cosyra* -freshly-inf (Kent)	C.cosyra-9th-DPO (Kent)	Non-infested ripe (Kent)
1	6.2	Pentan-2-one	107-87-9	919.7	938	Fröhlich, Duque, et al., 1989	0	2.7E+07	2.1E+07	2.8E+07	1.5E+07	3.2E+07	1146214	6966467	0	1.7E+07	2.3E+07	1.7E+07	2.7E+07	3.4E+07	3E+07
2	6.5	n-Ethyl propanoate	105-37-3	941	939	Varming, Andersen, et al., 2004	0	4316080	0	3352484	0	1172285	368177	0	1.6E+07	1276144	0	0	0	2120686	0
3	6.7	Ethyl-2-methylproanoate	97-62-1	951.5	954	Wei, Mura, et al., 2001	0	4280312	4569388	2082074	0	3715723	220125	0	2379490	3288932	0	0	0	0	0
4	6.8	Propyl ethanoate	109-60-4	957.6	957	Fröhlich, Duque, et al., 1989	0	3037374	0	1356100	0	0	0	0	0	0	0	0	0	0	0
5	6.9	Methyl butanoate	623-42-7	969	969	Shimoda, Shigematsu, et al., 1995, 2	0	2784635	0	3170334	0	543728	198873	0	2589005	673935	4546798	0	0	2564574	0
6	7.2	3,3-Dimethyl-2-pentanol	19781-24-9	985.5	---	---	0	3138309	6447109	0	3349391	0	91595	971588	0	2452086	4809614	2821874	5254326	3197743	4821606
7	7.4	2-Methyl-3-pentanol	565-67-3	998.5	---	---	6559297	0	7391598	0	2345322	2237008	135960	0	0	0	4159829	5023756	4452618	8806851	5486811
8	7.8	α-Pinene	80-56-8	1019.7	1021	Combariza, Tirado, et al., 1994	1.8E+09	1.8E+09	2.1E+09	1.8E+09	1.8E+07	1.8E+08	2329838	5013021	1.3E+07	5.1E+07	9.4E+07	2.2E+07	1.4E+08	5E+07	1669661
9	7.9	Ethyl butanoate	105-54-4	1023.1	1025	Wei, Mura, et al., 2001	0	4.1E+07	0	1.7E+08	1.4E+07	2.7E+07	2.5E+07	1154625	2.6E+08	9.4E+07	3008588	571523	5950649	1.1E+08	894527
10	8	Methyl-(2Z)-butenoate	4358-59-2	1033.2	---	---	0	2464158	0	5284764	2645148	299597	121447	0	0	840998	0	0	753178	8519567	0
11	8.2	Ethyl 2-methylbutanoate	7452-79-1	1040.9	1042	Umano, Hagi, et al., 2002	0	1.7E+07	0	1.1E+07	0	1020226	1265327	0	3547397	2675276	0	0	0	3579215	0
12	8.4	α-Fenchene	471-84-1	1050.7	1146	Brat, Rega, et al., 2003	1.8E+07	7493944	1E+07	8891998	2254933	7966927	175245	2050117	1164945	2631372	3662599	1257942	7873511	2496392	823322
13	8.5	Ethyl 3-methylbutanoate	108-64-5	1056.1	1055	Varming, Petersen, et al., 2004	0	0	0	0	0	0	763673	0	2617268	1824158	0	0	0	0	0
14	8.6	Camphene	79-92-5	1059.9	1063	Stashenko, Prada, et al., 1996	5.2E+07	6.2E+07	5.9E+07	5.9E+07	2.1E+07	6088815	756649	1183862	0	1949067	0	1768156	3620961	3884169	3654567
15	8.7	n-Hexanal	66-25-1	1068.2	1070	Nielsen and Poll, 2004	2065518	1629136	2249593	1052686	1985394	2612953	51122	892607	612104	969704	2214142	1107960	1781294	1694876	2537642
16	8.8	2-Methyl propan-1-ol	78-83-1	1072.6	1076	Ferrari, Lablanquie, et al., 2004	0	5373747	0	2606915	0	0	60678	0	0	0	0	0	0	775242	0
17	9	3,3,5-Trimethyl-1,5-heptadiene	74630-29-8	1084.4	---	---	3145609	2330757	1737099	0	0	0	70575	0	0	0	0	0	0	0	0
18	9.2	(2Z)-Butenoic acid	503-64-0	1091.1	---	---	1570468	6.1E+07	0	3.9E+07	5024324	4E+07	4161423	1064440	1.8E+07	4.9E+07	2774322	1523671	1.7E+07	2.4E+08	959388
19	9.4	β-Pinene	127-91-3	1102.5	1100	Yu, Kim, et al., 2004	5.3E+08	3.9E+08	4.9E+08	3.8E+08	1.3E+08	1.8E+07	339779	2139197	1121400	3865992	1.5E+07	4062612	1.3E+07	8244413	1.3E+07
20	9.5	3-Methylbutyl ethanoate	123-92-2	1110	1112	Osorio, Alarcon, et al., 2006	0	2.1E+07	0	4.6E+07	2155684	1355502	1464730	0	7539968	1100573	580102	0	0	0	1004769
21	9.6	Sabinene	3387-41-5	1114.3	1114	Yu, Kim, et al., 2004	1.2E+08	4.8E+07	8.8E+07	4.1E+07	1.4E+07	0	0	0	0	0	3382384	0	3277910	0	3218750
22	9.8	Ethyl pentanoate	539-82-2	1124.2	1128	Xu, Fan, et al., 2007	0	1363878	0	0	0	5919621	186741	4029956	3329951	3388048	0	0	0	0	0
23	9.8	δ-2-Carene	554-61-0	1125.5	1122	Nébié, Yaméogo, et al., 2004	0	0	0	0	0	0	0	0	0	0	4087912	742613	1.8E+07	7710117	1477331
24	9.8	1-Butanol	71-36-3	1125.5	1134	Osorio, Alarcon, et al., 2006	1288182	817439	0	0	0	0	341623	0	0	0	0	0	0	0	0
25	10.1	Verbenene	4080-46-0	1140	---	---	3985261	737075	2479862	1737450	1048337	0	0	0	0	0	0	0	0	0	0
26	10.2	δ-3-Carene	13466-78-9	1145.8	1146	Fröhlich, Duque, et al., 1989	3.4E+07	2343339	2.1E+07	3099466	9990725	5.4E+09	3.7E+07	2.9E+09	6.9E+08	1.6E+09	2.4E+09	1.1E+09	6.8E+09	1.3E+09	2.9E+08
27	10.3	Ethyl-(2Z)-butenoate	6776-19-8	1149.4	---	---	0	397866	0	3.6E+07	1557441	0	0	0	3.7E+07	4E+07	0	0	0	4.4E+07	4272994
28	10.4	Myrcene	123-35-3	1154.1	1162	Combariza, Tirado, et al., 1994	8.1E+09	2.9E+09	4.4E+09	3.5E+09	8.6E+08	1.6E+08	1E+07	8.7E+07	2.1E+07	5.9E+07	1.2E+08	6.5E+07	6.7E+08	5.5E+07	2E+07
29	10.5	α-phellandrene	99-83-2	1159.8	1162	Osorio, Alarcon, et al., 2006	2.9E+08	2257471	2E+08	7.4E+07	4.4E+07	1.9E+07	2866448	2.9E+07	8393727	2.7E+07	4E+07	1.7E+07	2.4E+08	1.7E+07	1813486
30	10.7	Heptan-2-one	110-43-0	1169.8	1178	Kim, 2001	0	1.4E+07	0	0	0	0	0	0	1229319	6347163	0	0	0	0	0
31	10.7	α-Terpinene	99-86-5	1171.1	1176	Umano, Hagi, et al., 1994	5.5E+07	3.5E+07	7.1E+07	3.6E+07	7878229	7.5E+07	1379385	7390967	1065769	1.1E+07	1.9E+07	4248796	1.1E+08	8913942	3037642
32	10.7	Methyl hexanoate	106-70-7	1172.3	1176	Varming, Andersen, et al., 2004	0	0	0	0	0	0	0	0	4367726	0	0	0	0	0	0
33	11.1	Sylvestrene	1461-27-4	1189.7	---	---	0	0	0	0	0	2.7E+07	1042714	4779332	5186336	4531086	7321045	3399805	5.2E+07	3836323	1879414
34	11.1	3-Methylbutyl methanoate	110-45-2	1189.7	1070	Wei, Mura, et al., 2001	0	2.5E+07	0	1.8E+07	1608361	0	0	0	0	0	0	0	0	0	0
35	11.2	Limonene	138-86-3	1194.4	1194	Shimoda, Shiratsuchi, et al., 1996	8.9E+08	2.2E+08	4E+08	2.8E+08	5E+07	3.3E+08	9537788	8.1E+07	2E+07	4.3E+07	9.1E+07	5.4E+07	6.5E+08	4.3E+07	8635999
36	11.3	β-phellandrene	555-10-2	1202.9	1210	Kim, Thuy, et al., 2000	1.8E+09	4.1E+08	8087559	5.9E+08	1.2E+08	1.6E+08	4264524	3.8E+07	8194090	2.1E+07	3.8E+07	2E+07	2.6E+08	1.5E+07	5206801
37	11.4	Butyl butanoate	109-21-7	1207	1212	Fröhlich, Duque, et al., 1989	0	0	0	0	0	0	1261728	0	3184939	1568539	0	0	0	0	0
38	11.5	p-Mentha-1,3,8-triene	18368-95-1	1212.8	---	---	0	0	0	0	0	0	0	0	0	0	415459	1212126	1.3E+07	2076368	593717
39	11.5	Ethyl 3-methyl-2-butenoate	638-10-8	1213.9	---	---	0	8622200	8622200	9727718	0	8182915	365062	704420	544668	0	0	0	0	0	0
40	11.7	Ethyl hexanoate	123-66-0	1223.7	1227	Osorio, Alarcon, et al., 2006	0	1.5E+08	0	4.8E+07	8139465	1E+08	2.9E+07	1756648	1.9E+08	1.3E+08	2713492	3009072	3.1E+07	9.7E+07	2496094
41	11.7	(Z)-β-Ocimene	3338-55-4	1224.8	1225	Fröhlich, Duque, et al., 1989	4.3E+07	0	1.4E+07	0	0	0	0	0	0	0	0	0	0	0	0
42	12	γ-terpinene	99-85-4	1240.6	1244	Shiratsuchi, Shimoda, et al., 1994	552491	909453	1638958	1.7E+07	4619579	6281720	293110	1150148	866965	569687	3520019	1844939	2.5E+07	2080857	1E+07
43	12.1	(E)- β-Ocimene	3779-61-1	1243	1243	Zheng, Kim, et al., 2005	3878589	1.2E+08	2.2E+08	1.9E+08	4.1E+07	4.9E+07	857882	1.1E+07	0	1E+07	3659512	3123489	3.6E+07	3149485	533717
44	12.2	Ethyl -(2E)-pentenoate	24410-84-2	1249.1	---	---	0	0	0	0	0	0	87498	0	999282	0	0	0	0	0	0
45	12.2	Ethyl 2-hexenoate	1552-67-6	1250.4	---	---	0	4929847	0	1659280	0	0	115452	0	0	0	0	0	0	0	0
46	12.3	Pentyl 2-methylpropanoate	2445-72-9	1256.7	---	---	0	2181476	891387	0	0	0	854710	0	1533245	0	0	0	0	0	0
47	12.3	3-Methylbutyl butanoate	106-27-4	1257.2	1256	Ferrari, Lablanquie, et al., 2004	2423711	0	0	1308119	0	0	0	0	0	0	0	0	0	0	0
48	12.4	p-Cimene	99-87-6	1263.1	1265	Shiratsuchi, Shimoda, et al., 1994	1.1E+08	4.7E+07	4.7E+07	3.8E+07	9547936	2.4E+07	1677006	5892628	3736681	6902022	1.3E+07	8653933	9.7E+07	8618306	1.2E+07
49	12.5	Acetoin	513-86-0	1266.2	1270	Humpf and Schreier, 1991	0	1.2E+08	0	6.6E+07	3948640	3328240	1620108	1196892	1353293	2705897	0	0	0	1.9E+07	1656921
50	12.6	iso-Sylvestrene	499-03-6	1272	---	---	2140345	2260555	1746322	571821	758462	4.6E+07	995598	6390309	2173558	5344944	1.1E+07	4281184	7.6E+07	4377435	0
51	12.7	Terpinolene	586-62-9	1278.6	1280	Seo and Baek, 2005	6E+07	1.3E+07	1.9E+07	2.2E+07	2831338	3.7E+08	8632982	5.6E+07	1.7E+07	4.1E+07	8.8E+07	3.6E+07	5.5E+08	3.1E+07	6823646
52	12.9	Ethyl (3E)-hexenoate	2396-83-0	1287.6	1290	Ferrari, Lablanquie, et al., 2004	0	5513023	0	1581874	0	0	544752	0	996313	675276	0	0	0	0	0
53	13	2-Hexanol	626-93-7	1290.7	1245	Hayata, Sakamoto, et al., 2002	4509759	2963753	2368587	1327811	1127531	2778568	347074	481442	979511	1114241	1.1E+07	1502466	0	2098011	3885904
54	13.1	2-(Methylenecyclopropyl)prop-2-yl but-(2E)-enoate	1000158-24-3	1297.3	---	---	4.2E+07	6806057	1.2E+07	0	0	0	0	0	0	0	0	0	0	0	0
55	13.2	Hex-(3E)-enyl ethanoate	3681-82-1	1303.1	1308	Xu, Fan, et al., 2007	1042131	1938836	861891	2092686	476638	2560278	259022	731917	909454	697715	0	0	0	0	0
56	13.3	4-Decanone	624-16-8	1306.8	---	---	0	0	0	0	0	0	0	0	0	0	692816	363335	0	0	3737898
57	13.3	n-propyl hexanoate	626-77-7	1309.2	1320	Mattheis, Fan, et al., 2005	0	0	0	0	0	470546	148698	0	700569	0	0	0	0	588178	0
58	13.4	Hept-(2E)-enal	18829-55-5	1313.4	1314	Kim, 2001	1283536	464018	818000	0	0	533641	61047	0	0	0	1154549	0	1420627	624795	1139679
59	13.5	Ethyl 2-hydroxy propanoate	97-64-3	1320.7	---	---	0	0	0	0	0	0	0	0	890557	0	0	0	0	0	0
60	13.5	Ethyl heptanoate	106-30-9	1323.3	1330	Wada and Shibamoto, 1997	0	0	0	0	0	1622001	808482	0	1767218	1394999	0	0	2673585	3231548	1182276
61	13.6	6-Methyl hept-5-en-2-one	110-93-0	1324.3	1329	Lee, Umano, et al., 2005	1345180	3350912	442189	589522	316446	0	0	0	0	0	1157178	431301	2427039	0	0
62	13.7	Ethyl hex-(2E)-enoate	27829-72-7	1334.7	1335	Zhao, Xu, et al., 2009	775340	8108175	606705	3281669	783542	822641	1438384	0	4690483	1248519	156469	186528	152469	185828	0
63	13.8	butyl-(2E)-butenoate	7299-91-4	1335.1	1334	Fröhlich, Duque, et al., 1989	914416	1411545	0	0	0	1975468	315585	224305	521854	0	623882	0	0	1303969	1076775
64	13.9	2-Methylpropyl hexanoate	105-79-3	1344.2	1356	Kumazawa, Itobe, et al., 2008	0	0	0	0	0	0	41569	0	0	0	0	0	0	0	0
65	14.2	3-oxo-2-butyl ethanoate	4906-24-5	1360.1	1361	Fröhlich, Duque, et al., 1989	798509	3325444	0	0	0	0	0	0	0	0	0	0	0	0	0

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66	14.2	allo-Ocimene	7216-56-0	1363.2	---	---	1.1E+07	1087010	4251271	2095476	689444	0	0	0	0	0	0	0	0	0	0
67	14.3	Hex-(3Z)-enol	928-96-1	1365.2	1368	1995	0	3027569	0	2038946	0	1182159	0	0	409954	519938	0	0	0	0	0
68	14.4	2,6-Dimethyl-1,3,6-heptatriene	928-67-6	1371.9	---	---	2515271	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	14.4	α-Pinene oxide	1686-14-2	1374	---	---	3063080	1025074	2726765	373457	647014	0	0	0	0	1209364	744596	244864	758809	0	1560633
70	14.5	Methyl octanoate	111-11-5	1379.7	1399	Gurbuz et al., 2006	3882247	7385547	0	2120820	1547042	1857197	886679	0	2905557	6008166	791282	866966	2453613	8642317	0
72	14.6	n-Nonanal	124-19-6	1385.1	1385	Shimoda, Shigematsu, et al., 1995, 2	3453656	8077969	9870152	3984315	3998732	7354998	620178	862522	1897978	4229632	1.1E+07	6235843	1.4E+07	1E+07	1.2E+07
73	14.7	2,6-Dimethyl-(2Z,6Z)-octadiene	2492-22-0	1389.6	---	---	1290997	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	14.9	2-Methyl-3-buten-2-ol	115-18-4	1399.1	---	---	0	0	0	0	0	0	0	0	0	0	0	0	1.2E+07	0	2435892
75	14.9	6,7-Epoxymyrcene	29414-55-9	1399.3	---	---	1.7E+07	6816786	5344626	4957841	1759761	0	0	0	0	0	0	0	0	0	0
76	15	Butyl n-hexanoate	626-82-4	1404.3	1416	Kumazawa et al., 2008	0	0	0	0	0	1669989	823552	623677	0	0	0	633590	1545604	3216133	0
77	15	3-(4-Methyl-3-pentenyl)-furan	539-52-6	1405.5	1421	Mookdasanit, Tamura, et al., 2003	3730878	1421619	0	1188482	0	0	0	0	0	0	0	0	0	0	0
78	15	Ethyl 2-hydroxy-3-methylbutanoate	6/7/2441	1409.1	---	---	0	0	0	0	0	0	0	0	0	0	496377	0	756332	0	0
79	15.1	Butyl 2-methylbut-(2E)-enoate	7785-66-2	1412.5	---	---	0	0	0	0	0	0	51229	0	0	0	0	2993384	0	1642317	0
80	15.2	m-Cymenene	1124-20-5	1417.8	---	---	0	0	0	0	0	0	122447	219938	580492	349938	0	2323876	1.3E+07	2635784	7651293
81	15.3	Ethyl octanoate	106-32-1	1426.6	1427	Lee and Noble, 2003	1.7E+07	2.4E+08	465270	7.1E+07	1.3E+07	1.5E+08	3.5E+07	4474659	2.9E+08	3.2E+08	1.2E+07	1.4E+08	1.4E+08	6E+08	3289256
82	15.4	1,2,3,4-Tetramethyl benzene	488-23-3	1430.5	1430	Shiratsuchi, Shimoda, et al., 1994	5465141	6883725	3042020	6883725	0		0	0	0	0	0	0	0	0	0
83	15.5	Heptan-1-ol	111-70-6	1437.9	1455	2002	3983254	2571489	0	0	0	1.4E+07	365930	242557	2085533	1531781	742541	0	0	0	2034270
84	15.6	4-Hydroxy pentan-2-one	4161-60-8	1441.6	---	---	3461459	7569167	0	2172126	2256528	0	0	0	0	0	0	0	0	0	0
85	15.7	1-(1,4-Dimethyl-3-cyclohexen-1-yl) ethanone	43219-68-7	1446.6	1504	Varming, Petersen, et al., 2004	0	0	0	0	0	1.4E+07	6449697	6347660	2.8E+07	1.5E+08	0	0	0	0	0
86	15.7	Hex-(3Z)-enyl butanoate	16491-36-4	1450.1	1450	Fröhlich, Duque, et al., 1989	604225	822509	0	0	0	0	0	0	0	0	0	0	0	0	0
87	15.8	3-Methylbutyl hexanoate	2198-61-0	1452.2	1450	Ferrari, Lablanquie, et al., 2004	0	0	0	0	0	0	184855	423718	3139851	0	0	0	0	0	0
88	15.8	E-Sabinene hydrate	15826-82-1	1454.1	1456	Fanciullino, Gancel, et al., 2005	3326635	3240300	1243249	1681588	0	0	0	0	0	0	0	0	0	0	0
89	15.9	oct-2-en-4-ol	4798-61-2	1459.2	---	---	0	1883853	0	1464967	0	0	0	0	0	0	0	0	0	0	0
90	16	α-Cubenene	17699-14-8	1463.2	1463	Yu, Kim, et al., 2004	4E+07	4814809	9599974	8149701	1841642	6628111	268783	1109313	2111886	2831682	1102751	0	1E+07	0	953808
91	16	Ethyl oct-(4Z)-enoate	34495-71-1	1466.2	---	---	0	1.6E+07	0	5399322	0	5745883	2692648	553380	7637813	6710019	0	3554424	3880602	2.8E+07	711478
92	16.1	Ethyl (2E,4E)-2,4-hexadienoate	2396-84-1	1471.7	1501	Shiratsuchi, Shimoda, et al., 1993	1173315	1677974	0	1598241	0	0	0	0	0	0	3324591	737419	3409943	773643	5055801
93	16.2	ethyl oct-(4E)-enoate	69668-87-7	1476.2	---	---	1427962	1736827	0	0	0	0	285792	0	700693	873439	0	0	0	2396694	0
94	16.3	δ-Elemene	20307-84-0	1483.6	1476	Ngassoum, Yonkeu, et al., 1999	6801287	242709	1540796	0	0	0	0	0	0	0	0	0	0	0	0
95	16.3	Dehydro sabinene ketone	36262-12-1	1483.7	---	---	7015130	2967427	2126691	1802253	0	0	0	0	0	0	0	0	0	0	0
96	16.4	n-Decanal	112-31-2	1490.9	1498	Zhao, Xu, et al., 2009	944596	544864	758809	458809	160633	5428839	329205	1680938	1153214	1984368	7451640	7278534	1.1E+07	8039274	1.1E+07
97	16.4	α-Ylangene	14912-44-8	1490.9	1482	Christoph, 2001	1.5E+07	7908562	1.1E+08	4504019	3201129	0	0	0	0	0	0	0	0	0	0
98	16.5	Ethyl 3-hydroxybutanoate	5405-41-4	1498.6	1501	Xu, Fan, et al., 2007	0	7.9E+07	0	1790133	0	0	4729119	0	2E+07	2712781	0	0	0	0	0
99	16.6	α-Copaene	3856-25-5	1501.1	1502	Ngassoum, Yonkeu, et al., 1999	4.4E+08	1732251	2358790	1.1E+08	9795474	8.1E+07	931185	1E+07	0	4965672	3741313	3894032	4100272	6875637	9754333
71	16.6	1-(2-Furanyl)-ethanone	1192-62-7	1502.3	1500	Gyawali and Kim, 2012	0	0	0	0	0	0	0	0	0	0	0	0	7054832	0	0
100	16.7	Benzaldehyde	100-52-7	1509.8	1510	Lee and Shibamoto, 2000	0	2.7E+07	0	1.5E+07	647688	3191498	0	759052	1130019	0	1896746	0	7007166	0	2283311
101	16.9	Camphor	76-22-2	1518.9	1519	Gyawali and Kim, 2009	1.3E+07	2645671	1541525	3283466	1720322	2408773	88746	640829	459756	2106067	0	0	0	0	0
102	17	Linalool	78-70-6	1527.4	1528	Zheng, Kim, et al., 2005	2.2E+07	6995203	3712923	3815149	6829297	0	0	0	0	0	0	0	0	0	0
103	17	β-bourbenene	5208-59-3	1528.1	1528	Guo, Wu, et al., 2008	0	0	0	0	0	2569105	122618	3406421	3235493	702141	0	0	3016724	6119954	1105684
104	17.1	2-Methyl-6-methyleneocta-1,7-dien-3-one	41702-60-7	1534.8	---	---	1.5E+07	4184038	6920534	5015179	939492	1443313	72720	0	0	0	3045716	3045716	0	0	4810424
105	17.2	Butane-2,3-diol	513-85-9	1539	1539	Kim, Shin, et al., 2001	0	5672969	0	2.1E+07	1484326	0	2102377	0	2.5E+07	0	1417546	0	0	0	1021627
106	17.2	α-Gurgujene	489-40-7	1540.2	1530	Aromdee and Sriubolmas, 2006	7.5E+07	1.2E+07	1.8E+07	1.4E+07	3618483	7.7E+07	1194126	1.5E+07	3112715	7048365	2.5E+07	4903928	3.2E+07	8509966	1.6E+07
107	17.3	Ethyl oct-(2E)-enoate	2351-90-8	1545.5	---	---	1.2E+07	6.6E+07	2868500	3628309	782422	1E+07	1792198	3280731	6132366	1.1E+07	0	8167031	8101757	3.8E+07	3893021
108	17.4	(E)-Pinocamphone	547-60-4	1553.3	---	---	2690124	0	674208	0	0	0	0	0	0	0	0	0	0	0	0
109	17.4	Eucarvone	503-93-5	1554.4	---	---	0	0	0	0	0	2946760	95531	0	1754948	495219	0	0	0	0	0
110	17.5	Dimethyl sulfoxide	67-68-5	1562.3	1579	Kim. J.H., Ahn, et al., 2004	4218205	5480172	2096487	3229070	1373945	6274093	76607	539377	1702193	1853211	0	0	3891006	4908433	1680218
111	17.7	6-Methyl-triazolo(4,3-b)(1,2,4)-triazine	61139-69-3	1569.8	---	---	0	0	0	0	0	0	0	601619	454756	0	0	0	0	0	0
112	17.7	(Z)-α-Bergamotene	18252-46-5	1570.3	---	---	0	0	0	0	0	0	0	0	0	0	1986266	0	6999135	2387799	3276185
113	17.7	3,3,6-Trimethylhepta-1,5-dien-4-one	546-49-6	1572.1	1346	Umano, Hagi, et al., 2000	1.4E+07	6058650	2998563	0	1768723	0	0	0	0	0	0	0	0	0	0
114	17.8	2,5-Dimethyl-4-methoxy-3(2H)-furanone	4077-47-8	1578.5	1584	Osorio, Alarcon, et al., 2006	1810042	2.8E+07	2.8E+07	8498276	2266023	0	68339	267786	8331654	0	0	0	0	0	1150955
115	17.9	β-Cubebene	13744-15-5	1584.5	1560	Stashenko et al., 1995	9762349	5490831	3848862	3015405	1404532	1.1E+07	751214	2269562	336023	1.1E+07	0	2554019	7094055	5145071	2279407
116	18	β-Elemene	515-13-9	1592.4	1595	2009	1.4E+07	0	3935627	2499076	978374	1.3E+07	338938	8100349	2503319	2961588	1300036	7363973	1.9E+07	0	1201605
117	18.1	α-Guaiaene	12/1/3691	1596.6	---	---	3.6E+08	3.4E+07	1.1E+08	8.8E+07	8827111	0	0	0	0	0	0	0	0	0	0
118	18.2	β-Copaene	18252-44-3	1603.2	---	---	0	6679089	5194261	4784826	1631656	5676051	115452	1140599	641155	5389615	0	823998	4349332	4349332	0
119	18.2	Butanoic acid	107-92-6	1603.6	1628	Rezende and Fraga, 2003	0	0	0	0	0	0	0	2903928	9680289	0	0	0	0	0	0
120	18.3	(E)-β-Caryophylene	87-44-5	1608.5	1610	1994	5.1E+08	2538231	1.4E+08	5485042	1.4E+07	1.4E+08	4529051	4.9E+07	2.2E+07	3068791	1.5E+08	4.9E+07	4E+08	7.4E+07	3.3E+07
121	18.4	Methyl dec-4-enoate	1191-02-2	1614.6	---	---	0	0	0	0	0	0	275542	0	2441586	1246519	0	4064455	5103861	9815395	4153795
122	18.5	Aromadendrene	489-39-4	1620.8	1635	Umano, Hagi, et al., 1994	9420447	3458991	0	5207518	971056	1059621	115452	0	0	0	0	0	0	0	0
123	18.6	Ethyl decanoate	110-38-3	1629.8	1633	Rezende and Fraga, 2003	1.5E+07	5150202	4931772	642536	929536	2038262	5167803	0	8587368	1.3E+07	3394256	1.4E+07	4991343	3.1E+07	4750163
124	18.6	Ethyl 6-methyl-2,4-Heptadienoate	10236-06-3	1632.5	---	---	0	3622194	0	0	0	0	0	0	0	0	0	0	0	0	0
125	18.7	Dec-(2E)-enal	3913-81-3	1637.6	1642	Cha, Kim, et al., 1998	0	0	0	0	0	0	91015	0	0	0	0	0	0	0	0
126	18.8	E-Muurola-3,5-diene	189165-77-3	1647.1	---	---	7.5E+07	7376933	2E+07	7376933	2204474	0	0	0	0	0	0	0	0	0	0
127	18.9	Z-Muurola-3,5-diene	157374-44-2	1647.6	---	---	7786786	4588264	0	4588264	0	3047103	214478	0	4485473	1946801	0	0	0	0	0
128	18.9	Dauca-5,8-diene	142928-08-3	1648.4	---	---	0	0	0	0	0	5680241	269182	0	0	5099954	0	0	0	0	0
129	18.9	3-Methyl butanoic acid	503-74-2	1650.5	1655	2004	0	0	0	0	0	0	232005	0	0	0	0	0	0	0	0
130	19	Ethyl-(4E)-decenoate	76649-16-6	1656.8	1680	Zhao, Xu, et al., 2009	1.3E+07	1.3E+07	0	0	0	2E+07	1.3E+07	424515	3.7E+07	6E+07	1.1E+07	1.1E+08	4.3E+07	2.7E+08	5360582
131	19	allo-Aromadendrene	25246-27-9	1658.4	---	---	3.1E+07	3.3E+07	1.2E+07	1.7E+07	5107930	0	0	0	0	0	0	0	0	0	0
132	19.1	Ethyl 3-hydroxyhexanoate	2305-25-1	1661.7	1664	Osorio, Alarcon, et al., 2006	0	0	0	0	0	0	312144	1453341	360423	0	0	0	0	0	0
133	19.1	Non-(3Z)-en-1-ol	10340-23-5	1666.3	1682	2002	0	0	0	0	0	4714326	177223	963655	865848	0	3087589	8759520	2849337	0	0
134	19.2	(E)-Verbenol	1820-09-3	1668.3	1671	Umano, Hagi, et al., 2002	2.2E+07	1.1E+07	7686200	8504261	1425638	0	0	0	0	0	0	0	0	0	0
135	19.2	Cryptone	500-02-7	1674	1679	Mookdasanit, Tamura, et al., 2003	9820447	3761687	3719167	2675894	1367151	0	0	0	0	0	0	0	0	0	0
136	19.2	Unknown107	107	1674.2	---	---	0	0	0	0	0	0	0	0	0	0	0	623677	0	0	0
137	19.3	5-Methyl-2-(1-methylethenyl)-4-hexenal	35158-25-9	1674.6	---	---	0	0	0	0	0	0	619440	673146	8485473	0	0	0	0	0	0
138	19.3	γ-Muurolene	30021-74-0	1680.9	1655	1995	4.2E+07	4.4E+07	2.1E+07	2.5E+07	1.1E+07	1E+07	359650	1610693	3360423	6022646	0	0	0	0	0
139	19.4	α-Humulene	6753-98-6	1682.8	1682	1996	2.6E+08	2.5E+07	7.3E+07	6.1E+07	6870478	8.9E+07	1847340	2.8E+07	1.5E+07	2.4E+07	8E+07	2.9E+07	2.1E+08	4.3E+07	2E+07
140	19.4	γ-Gurjunene	22567-17-5	1685.8	---	---	2.2E+08	1.2E+07	6.1E+07	4.9E+07	3179868	0	0	0	0	0	0	0	0	0	0
141	19.5	4,5-di-epi-Aristolochene	54868-40-5	1691	---	---	0	0	0	0	0	0	0	0	0	0	4380120	1811204	2.3E+07	3338388	0
142	19.5	γ-Hexalactone	695-06-7	1692.7	1696	1999	0	0	0	2154231	598808	0	255133	0	0	0	0	0	0	0	0
143	19.7	4-Ethyl benzaldehyde	4748-78-1	1702.4	1719	Welke, Manfroi, et al., 2012	0	0	0	0	0	0	0	0	0	0	0	0	1.4E+07	8290221	6149579
144	19.7	α-Phellandren-8-ol	1686-20-0	1703.9	---	---	0	0	0	0	0	0	0	540349	2175025	4934156	0	0	0	0	0
145	19.7	Viridiflorene	21747-46-6	1704.8	1698	Aromdee and Sriubolmas, 2006	1.1E+07	2232768	3994060	2994101	784290	1.2E+07	286807	503652	0	0	0	0	0	0	0

2990

146	19.8	Aciphyllene	87745-31-1	1709.4	---	---	5.5E+07	0	1.8E+07	1.4E+07	0	0	0	0	0	0	0	0	0	0	0
147	19.8	Verbenone	80-57-9	1710.4	1714	Seo, Kim, et al., 2007	0	8335061	3716806	1479552	1641127	2959843	1169440	241167	1033510	1012579	0	942999	6700897	1842254	3727255
148	19.8	Menthofuran	494-90-6	1714.4	---	---	0	1.1E+07	0	4637760	0	5482526	4629913	1027761	1.1E+07	1.7E+07	0	0	0	0	0
149	19.9	Germacrene D	23986-74-5	1720	1726	1996	6528281	9029691	1.9E+07	1.3E+07	6150403	1.2E+08	7637131	8.6E+07	7.9E+07	9.9E+07	5761347	1.1E+07	2.2E+07	2.2E+07	1.1E+07
150	20	α-Bulnesene	3691-11-0	1723.8	1634	Christoph, 2001	5.5E+08	3E+07	1.5E+08	1.4E+08	9165448	0	0	0	0	0	0	0	0	0	0
151	20	Valencene	7/3/4630	1728	1726	Lan Phi N.T., Nishiyama C., et al., 2006	0	0	0	0	0	0	0	0	0	0	2065496	1528247	1.3E+07	2512750	0
152	20.1	Z-Cadina-1(6),4-diene	246522-85-0	1730.4	---	---	0	0	0	0	0	0	440786	945460	2969008	4109433	0	0	0	0	0
153	20.1	β-Selinene	17066-67-0	1732.4	1734	Wei A. and Shibamoto T., 2007	2.9E+08	2.2E+07	8.9E+07	5.8E+07	4800357	2.7E+07	701374	1E+07	303652	0	1562472	1.9E+07	1.5E+08	2.6E+07	9517774
154	20.2	α-Selinene	473-13-2	1736.5	1737	Ngassoum, Yonkeu, et al., 1999	2.4E+08	6416763	6.2E+07	3.8E+07	1470189	2.7E+07	736394	689139	2337712	5053586	8778796	1042463	1.1E+07	5033044	6194475
155	20.2	(E,E)-α-Fernesene	502-61-4	1739.4	1740	2005	0	3.4E+07	0	1.6E+07	1.6E+07	0	0	0	0	0	0	0	0	0	0
156	20.3	Bicyclogermacrene	24703-35-3	1743.7	1707	Brat, Rega, et al., 2003	1.8E+07	4837984	1145869	0	2352474	1.4E+07	806155	5774839	8352408	1.2E+07	0	0	0	0	0
157	20.3	2-Isopropylidene-3-methylhexa-3,5-dienal	1000191-76-5	1745.7	---	---	0	6130683	0	1900427	0	0	0	0	0	0	0	0	0	0	0
158	20.4	Car-3-en-2-one	53585-45-8	1752.1	---	---	0	0	4419827	0	539985	5126087	1709776	420500	4292112	1.2E+07	2236163	3273324	1.7E+07	7470714	1727830
159	20.5	δ-Cadinene	483-76-1	1761.4	1762	Aromdee and Sriubolmas, 2006	1.5E+08	3.3E+07	4.7E+07	4E+07	8081350	1.1E+07	390029	3006539	2358753	5175515	1760786	620819	3950173	1027590	2022606
160	20.6	1-(3-Methylphenyl)-ethanone	585-74-0	1768	---	---	0	0	0	0	0	0	0	0	0	0	0	0	0	9715395	0
161	20.6	γ-Cadinene	39029-41-9	1766.8	1770	Ngassoum, Yonkeu, et al., 1999	0	0	0	1645053	5906554	0	762122	1104839	0	0	0	0	0	0	0
162	20.6	Methyl salicylate	119-36-8	1769.1	1771	Zhao, Xu, et al., 2009	5.4E+07	2.4E+07	1.8E+07	329694	410867	0	0	0	0	0	2.9E+07	5292472	4.8E+07	4970049	9113949
163	20.6	Ethyl 2-phenylethanoate	101-97-3	1771.6	1785	Ferrari, Lablanquie, et al., 2004	0	1.3E+07	0	1.3E+07	0	0	1772122	1388394	1974070	1974070	0	0	0	0	0
164	20.7	7-epi-α-Selinene	123123-37-5	1776.1	1772	2009	1.8E+07	0	5097045	2613287	0	0	0	0	0	0	0	0	0	0	0
165	20.7	2-Ethyl-1,4-dimethyl-benzene	1758-88-9	1776.9	---	---	0	0	0	0	0	3392061	0	1684597	0	0	0	0	0	0	0
166	20.8	2-Pinen-10-ol	515-00-4	1778.8	1787	Christoph, 2001	0	3665759	0	2866877	1299224	0	0	0	0	0	0	0	0	0	0
167	20.9	E-Cadina-1,4-diene	38758-02-0	1789.9	---	---	3.3E+07	0	1.2E+07	8601246	1145374	0	0	0	0	0	0	0	0	0	0
168	21	Methyl dodecanoate	111-82-0	1794	1793	Ferrari, Lablanquie, et al., 2004	0	0	0	0	0	0	141834	1063300	509787	717542	3629412	1258473	6625146	2790625	0
169	21	Octadecane	593-45-3	1798.4	---	---	0	0	0	0	0	0	0	0	0	0	7658408	1620819	1E+07	0	6186443
170	21	α-Cadinene	82468-90-4	1799.2	---	---	8593320	3144290	4827439	1770561	0	2367619	129694	710867	1082723	1903069	0	0	0	0	0
171	21.1	Phenyl ethyl hexanoate	6290-37-5	1802.3	2160	Fan and Qian, 2006	0	2253690	810867	5266853	0	1756535	408714	641202	1729363	715483	0	0	0	0	0
172	21.2	Tridecanal	10486-19-8	1809.7	1821	Shiratsuchi, Shimoda, et al., 1994	3604033	1725748	2454508	1625811	1612692	2683541	340446	0	1412924	3578657	2913203	3074367	1.4E+07	6885539	1909661
173	21.4	m-Ethylacetophenone	22699-70-3	1823.8	---	---	0	0	4883994	0	1173104	5059125	299224	0	1127829	1872266	8242672	5476340	1.6E+07	5786265	9151461
174	21.4	p-Cymen-8-ol	1197-01-9	1828.9	1838	Yu, Kim, et al., 2004	4495045	0	4563162	1765559	0	0	115452	0	0	0	4577681	5077853	3.5E+07	5720701	4344313
175	21.6	Ethyl dodecanoate	106-33-2	1837.3	1835	Ferrari, Lablanquie, et al., 2004	2E+07	1.5E+07	1.5E+07	1.2E+07	4069329	1.6E+07	1.5E+07	1673507	3.7E+07	3.5E+07	2.5E+08	7.3E+07	5.4E+07	9.1E+07	3.6E+07
176	21.6	E-Calamenene	73209-42-4	1837.8	---	---	5E+07	0	1.9E+07	0	0	0	0	0	0	0	0	0	0	0	0
177	21.7	Geranyl acetone	3796-70-1	1846.1	1856	Zhao, Xu, et al., 2009	0	2666400	0	0	1170142	3722346	0	1513572	1875535	0	7.1E+07	1841020	4869028	0	2461720
178	21.8	Benzyl methanol	100-51-6	1852.8	1853	Parada, Duque, et al., 2000	1078665	2887699	4166338	1811850	1065405	0	412111	0	0	0	7897281	3343581	9194359	3744135	0
179	21.9	2-Methyl-3-phenylpropene	3290-53-7	1859	---	---	0	0	6387933	0	0	0	0	0	0	0	0	0	0	0	0
180	21.9	p-Ethylacetophenone	937-30-4	1863.8	1867	Shimoda, Shigematsu, et al., 1995, 2	1.6E+07	2455385	9095042	5109061	0	1E+07	457963	3570963	2084395	1142202	6045099	5788351	3.9E+07	4508758	3722120
181	22	3Z-Octen-2-ol	69668-89-9	1871.3	---	---	1967088	2210855	7504239	0	0	0	0	0	0	0	0	0	0	0	0
182	22	1-(1,1-Dimethylethyl)-2-methyl-1,3-propanediyl 2-methyl propanoic acid	74381-40-1	1872.1	---	---	0	0	0	0	0	0	0	0	0	0	4708861	3457565	4517173	8505223	3464371
183	22.2	Hexahydro-1,4-dioxa-cyclopropa[a]pentalen-3-one	143393-90-2	1881.7	---	---	0	1193847	0	555265	562146	0	824439	0	982442	0	0	0	0	0	0
184	22.2	Dodecamethyl pentasiloxane	141-63-9	1887	---	---	0	0	0	0	0	0	0	0	0	0	6669501	0	4755732	1992541	5.7E+07
185	22.3	2-PhenyI ethanol	60-12-8	1891.2	1896	Ferrari, Lablanquie, et al., 2004	2861271	2.4E+07	1728743	5564786	2238465	0	2336839	441102	4622299	974403	0	0	0	4288398	1284398
186	22.5	γ-Octalactone	104-50-7	1909.8	1923	2002	1.7E+07	5715985	5682269	4713393	4929701	0	668581	1175965	2847907	2896558	967837	3362693	1.2E+07	4706199	1621889
187	22.8	Piperitenone	491-09-8	1926.3	1918	Lee, Umano, et al., 2005	2145646	0	4239088	0	1443855	9350287	911397	3035936	1641554	2621340	8836845	1.8E+07	7.6E+07	1.2E+07	5874227
188	23.2	Tetrahydro-6-propyl-2H-pyran-2-one	698-76-0	1964.6	1985	2002	0	2036515	0	0	1221408	0	109933	0	656771	0	0	0	0	0	0
189	23.4	Phenol	108-95-2	1972.7	1973	Osorio, Duque, et al., 2002	0	883076	0	1943505	0	0	76616	0	497189	0	2034003	0	4458406	0	0
190	23.4	4-Hydroxy-4-methyl-cyclohexanone	17429-02-6	1980.1	---	---	0	0	1736591	0	0	0	0	0	0	0	0	0	0	0	0
191	23.5	2,5-Dimethyl-2-nitro-1,3-cyclohexanediol	114454-79-4	1982	---	---	0	0	0	0	0	1586690	56469	865828	0	0	0	0	0	0	0
192	23.5	7-(1,1-Dimethylethyl)-3,4-dihydro-1(2H)-naphthalenone	22583-68-2	1984	---	---	0	0	0	0	0	0	0	0	0	0	3590248	0	0	0	0
193	23.5	3-(1H-indol-3-yl)-2-Propenoic acid	1204-06-4	1984.6	---	---	0	1014472	2237006	554228	866494	0	0	0	0	0	0	0	0	0	0
194	23.6	Methyl eugenol	93-15-2	1990.2	2007	Lee, Umano, et al., 2005	4363003	4937336	3373686	2896511	1293981	1778087	102858	1370876	663856	1196511	4862717	4719334	6295234	0	7332600
195	23.7	Caryophyllene oxide	1139-30-6	1998.8	2005	1996	1.1E+07	3578290	5076460	3839050	0	0	0	0	0	0	1.6E+07	7729203	2.5E+07	1.2E+07	8252117
196	23.7	Methyl tetradecanoate	124-10-7	1999.4	2008	Shiratsuchi, et al., 1994		0	0	0	0	2514661	252961	1201244	946904	2502358	0	0	0	0	0
197	23.9	2-Pentadecanone	2345-28-0	2013.7	2021	Ott, Fay, et al., 1997	3637647	3292382	2119443	2621931	1869230	549403	229973	634820	835611	1030552	1066580	1646191	2861676	1573774	1451536
198	24	E-Nerolidol	40716-66-3	2019.9	2020	Zheng, Kim, et al., 2005	8190218	6930743	0	2801947	0	0	0	0	0	0	2.7E+07	1.7E+07	6.9E+07	0	1.1E+07
199	24	Tridec-(2E)-enol	74962-98-4	2022	---	---	0	0	0	0	2706984	5350287	0	0	0	0	0	0	0	0	0
200	24.1	Methylethyl tetradecanoate	110-27-0	2031.7	---	---	5424742	2380941	3483102	1983112	1354570	1403721	603021	1004273	2011618	1250227	0	0	0	0	0
201	24.2	Ethyl tetradecanoate	124-06-1	2043.2	2040	Ferrari, Lablanquie, et al., 2004	7273390	8081196	3698376	5883563	3127083	4484054	1.7E+07	1637627	3.8E+07	2.4E+07	3.7E+07	3844511	3.1E+07	8.6E+07	2E+07
202	24.3	Germacrene D-4-ol	74841-87-5	2050.2	2050	Umano, Hagi, et al., 2000	1675115	162730	0	0	2001712	0	0	0	0	0	0	0	0	0	0
203	24.4	Humulene epoxide II	19888-34-7	2057.4	---	---	4204737	0	0	0	0	687601	0	1131675	1484913	0	7786084	4449439	0	5993118	2360738
204	24.5	1,10-di-epi-Cubenol	73365-77-2	2063.8	---	---	8182257	0	2550847	0	0	0	0	0	0	0	0	0	0	0	0
205	24.5	Cyclooctanone	502-49-8	2067.9	---	---	0	0	0	0	0	3468229	157732	3364102	327775	628411	0	0	0	0	0
206	24.6	Ethyl 9-tetradecenoate	24880-50-0	2068.1	---	---	0	0	0	0	0	0	0	0	0	0	1E+07	2833618	1.9E+07	0	0
207	24.6	1-epi-Cubenol	19912-67-5	2070.9	2072	Aromdee and Sriubolmas, 2006	1.1E+07	0	4184124	1711201	0	0	0	0	0	0	0	0	0	0	0
208	24.7	Unknown115	115	2077.7	---	---	0	0	0	0	0	0	0	0	0	0	7507577	0	0	0	0
209	24.7	Guaiol	489-86-1	2083.8	---	---	1517565	0	4385687	0	0	0	0	0	0	0	0	0	0	0	0
210	25.2	6,10,14-Trimethyl-2-pentadecanone	502-69-2	2118.2	---	---	0	0	6398145	924659	0	0	59926	401033	583793	0	0	0	5452508	0	2509669
211	25.2	Hex-(3Z)-enyl benzoate	25152-85-6	2119.2	2155	Wei A. and Shibamoto T., 2007	0	0	0	0	0	0	0	0	0	0	0	0	7615800	0	0
212	25.4	Unknown		2139.2	---	---	0	0	0	0	1010142	0	0	0	0	0	0	0	0	0	0
213	25.4	δ-Nonalactone	3301-94-8	2139.5	---	---	0	0	0	0	0	0	140622	0	1448382	0	0	0	0	0	0
214	25.5	Tetradekanol	112-72-1	2146.6	2145	Ferrari, Lablanquie, et al., 2004	0	0	6029973	0	0	0	0	0	0	0	0	0	0	0	1467573
215	25.9	epi-α-Muurulol	19912-62-0	2183.3	2184	Christoph, 2001	6541749	0	4155211	1871478	0	0	0	0	0	0	0	0	0	0	0
216	26	o-Cumenol	88-69-7	2188.5	---	---	3734531	0	2531537	1389754	0	0	0	0	0	0	0	0	0	0	0
217	26.1	2,6,11,15-Tetramethyl hexadeca-2,6,8,10,14-pentaene	38259-79-9	2200.7	---	---	1.2E+07	0	9180940	5097685	0	0	0	0	0	0	0	0	0	0	0
218	26.2	Methyl hexadecanoate	112-39-0	2207.7	2213	Ferrari, Lablanquie, et al., 2004	0	0	0	0	0	0	64011	0	0	0	0	0	1.6E+07	3472110	0
219	26.2	Pogostol	21698-41-9	2209.4	---	---	6511031	0	4902946	2120999	0	0	0	0	0	0	0	0	0	0	0
220	26.3	Butyl 4-methyloctanoate	1000360-29-6	2217.9	---	---	0	0	8006718	0	0	0	0	1122902	516826	0	0	0	0	0	0
221	26.4	α-Eudesmol	473-16-5	2220.9	2220	Lee, Umano, et al., 2005	0	0	2.1E+07	0	2.1E+07	0	0	0	0	0	0	0	0	0	0

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222	26.5	Unknown116	116	2228.3	---	---	0	0	0	0	0	0	220361	1240924	1340977	846437	0	0	0	0	0
223	26.6	Catalponol	34168-56-4	2237.1	---	---	0	0	0	0	0	0	0	0	0	0	3590248	946426	1.4E+07	9141080	0
224	26.7	Ethyl hexadecanoate	628-97-7	2247.9	2246	Ferrari, Lablanquie, et al., 2004	2002397	2002397	5659518	831545	0	1794642	2794642	1175977	4524216	3794322	7532323	1.2E+07	7764904	3.2E+07	4239113
225	26.8	Z-Calamenene	72937-55-4	2258.4	---	---	0	0	3419116	0	1405282	0	0	0	824874	1636723	6483495	0	1.1E+07	0	4360128
226	26.8	1,2,3,4-Tetrahydro-1,1,6-trimethyl naphthalene	475-03-6	2258.8	1565	Gurbuz O., Rouseff J.M., et al., 2006	0	0	0	0	0	0	0	0	0	0	0	6572569	0	6032363	0
227	27	Ethyl 9-hexadecenoate	54546-22-4	2272.9	2283	Zhao, Xu, et al., 2009	2639625	0	8967851	2274865	0	1863224	359640	824996	716424	985665	2.6E+07	6184382	9382008	0	3705032
228	27.1	3,3,13,13-Tetraethylpentadecane	1000360-42-3	2282.6	---	---	0	0	0	0	0	0	0	0	0	0	0	0	1.3E+07	7880457	0
229	27.3	Triethylene glycol	112-27-6	2297.6	---	---	0	0	0	0	0	0	115452	324462	0	0	0	0	0	0	0
230	27.3	1-Oxide 4-methyl-quinoline	4053-40-1	2298.3	---	---	0	0	0	0	0	0	0	0	0	0	1.7E+07	4E+07	2.4E+07	1.3E+07	0
231	27.9	Germacra-4(15),5,10(14)-trien-1-alpha-ol	81968-62-9	2357.6	---	---	0	0	0	0	0	0	134616	0	114326	0	0	0	0	0	0
232	27.9	n-Hexadecanol	36653-82-4	2358.5	2363	Osorio, Alarcon, et al., 2006	0	0	0	0	0	2150293	0	0	0	3055735	0	59926	0	0	586432
233	28	γ-Dodecalactone	002305-5-7	2369	2365	Umano, Hagi, et al., 1994	0	0	0	0	0	0	0	0	0	1134619	0	0	0	0	0
234	28.3	Z-11-Hexadecenoic acid	2416-20-8	2390.2	---	---	0	0	0	0	0	0	115452	0	0	0	0	0	2.9E+07	0	0
235	29.5	Unknown118	118	2489.6	---	---	0	0	0	0	0	0	0	0	0	0	1.1E+07	0	1.3E+07	0	0
236	30.5	n-Octadecanol	112-92-5	2564.1	---	---	0	0	0	0	0	0	0	0	0	0	4522245	0	1855006	4458515	0
237	31.3	Benzyl phenylmethanoate	120-51-4	2621.2	2636	Zhao, Xu, et al., 2009	0	0	0	0	0	0	0	0	0	0	0	0	5369240	0	0
238	34.1	Benzyl salicylate	118-58-1	2776.7	---	---	0	0	0	0	0	0	0	0	0	0	0	0	8012182	0	0

Table S4-2: The peak areas of the headspace compounds of ripe mangoes (Apple, Haden, and Kent varieties), ripe banana (FHIA 17) and tomato (Improved Nouvelle F1). Compounds were tentatively identified based on their retention time (RT), electron ionization spectrum, calculated retention index (RI_{Calc}) in comparison to literature retention index (RI_{Lit}) as well as comparing their mass spectra with those from the *NIST*, Adams and Chemecol libraries database of DB-Wax capillary column (60 m × 0.25 mm i.d., 0.25 um film thickness

No.	RT	Compound Name	CAS_no	RI $_{Calc}$	RI $_{Lit}$	Ref	Apple	Haden	Kent	Banana	Tomato
1	6.2	Pentan-2-one	107-87-9	919.7	938	Fröhlich, Duque, et al., 1989	15082466	16724379	29538797	14981761	1588001
2	6.5	n-Ethyl propanoate	000105-37-3	941	939	Varming, Andersen, et al., 2004	0	1276144	0	0	0
3	6.7	Ethyl-2-methylproanoate	000097-62-1	951.5	954	Wei, Mura, et al., 2001	0	3288932	0	0	0
4	6.8	Propyl ethanoate	000109-60-4	957.6	957	Fröhlich, Duque, et al., 1989	0	0	0	909728	0
5	6.8	sec-Butyl etanoate	105-46-4	964.1	---	---	0	0	0	1824353	0
6	6.9	Methyl butanoate	623-42-7	969	969	Shimoda, Shigematsu, et al., 1995	0	673935	0	0	0
7	7.2	3,3-Dimethyl-2-pentanol	19781-24-9	985.5	---	---	3349391	2452086	4821606	3840433	0
8	7.3	2-Methylpropyl ethanoate	110-19-0	993.4	1007	Varming, Petersen, et al., 2004	0	0	0	4024350	10503616
9	7.4	2-Methyl-3-pentanol	565-67-3	998.5	---	---	2345322	0	5486811	0	0
10	7.8	α-Pinene	80-56-8	1019.7	1021	Combariza, Tirado, et al., 1994	17744685	50687461	1669661	0	24703962
11	7.9	Ethyl butanoate	105-54-4	1023.1	1025	Wei, Mura, et al., 2001	14057664	94210116	894527	6593919	0
12	7.9	2-Butyne-1,4-diol, dimethanoate	36677-73-3	1025.4	---	---	0	0	0	2586462	0
13	8	Methyl-(2Z)-butenoate	4358-59-2	1033.2	---	---	2645148	840998	0	0	0
14	8.2	Ethyl 2-methylbutanoate	7452-79-1	1040.9	1042	Umano, Hagi, et al., 2002	0	2675276	0	1811903	0
15	8.4	α-Fenchene	471-84-1	1050.7	1146	Brat, Rega, et al., 2003	2254933	2631372	823322	0	0
16	8.4	Butyl ethanoic acid	123-86-4	1054.4	1074	Kumazawa, Itobe, et al., 2008	0	0	0	30675579	1274948
17	8.5	Ethyl 3-methylbutanoate	108-64-5	1056.1	1055	Varming, Petersen, et al., 2004	0	1824158	0	0	0
18	8.5	2-Pentyl ethanoate	626-38-0	1057.8	1075	Iwaoka, Hagi, et al., 1994	0	0	0	256687735	1339682
19	8.6	Camphene	79-92-5	1059.9	1063	Stashenko, Prada, et al., 1996	21114737	1949067	3654567	0	0
20	8.7	n-Hexanal	66-25-1	1068.2	1070	Nielsen and Poll, 2004	1985394	969704	2537642	0	1669816
21	8.9	Propyl butanoate	105-66-8	1078.5	1208	Welke, Manfroi, et al., 2012	0	0	0	49031336	0
22	9.2	But-(2Z)-enoic acid	503-64-0	1091.1	---	---	5024324	49312540	959388	0	0
23	9.2	4-Methyl-2-pentyl ethanoate	108-84-9	1092.1	---	---	0	0	0	931970	0
24	9.3	3-Methyl-2-butanol	598-75-4	1099.4	1089	Parada, Duque, et al., 2000	0	0	0	14113795	0
25	9.4	β-Pinene	127-91-3	1102.5	1100	Yu, Kim, et al., 2004	127000000	3865992	12990605	0	2046035
26	9.5	3-Methylbutyl ethanoate	123-92-2	1110	1112	Osorio, Alarcon, et al., 2006	2155684	1100573	1004769	758369533	57626320
27	9.6	Sabinene	3387-41-5	1114.3	1114	Yu, Kim, et al., 2004	13852972	0	3218750	0	0
28	9.8	Ethyl pentanoate	539-82-2	1124.2	1128	Xu, Fan, et al., 2007	0	3388048	0	0	0
29	9.8	δ-2-Carene	554-61-0	1125.5	1122	Nébié, Yaméogo, et al., 2004	0	0	1477331	0	392774287
30	9.9	1-Methylbutyl 2-methyl propanoate	54340-93-1	1128.7	---	---	0	0	0	1183228	0
31	9.9	Pent-(2E)-en-3-yl ethanoate	1000374-05-0	1131.4	---	---	0	0	0	17060622	0
32	10.1	Verbenene	4080-46-0	1140	1126	Ngassoum, Yonkeu, et al., 1999	1048337	0	0	0	0
33	10.2	Propyl 3-methyl butanoate	557-00-6	1142.9	---	---	0	0	0	8127551	0
34	10.2	δ-3-Carene	13466-78-9	1145.8	1146	Fröhlich, Duque, et al., 1989	9990725	1550000000	291000000	0	1778037
35	10.3	Ethyl-(2Z)-butenoate	6776-19-8	1149.4	---	---	1557441	39735614	4272994	0	0
36	10.3	2-Methylpropyl butanoate	539-90-2	1151.4	---	---	0	0	0	1223337759	84648905
37	10.4	Myrcene	123-35-3	1154.1	1162	Combariza, Tirado, et al., 1994	863000000	58816406	20425128	0	10060573
38	10.4	2-Acetoxyhexane	5953-49-1	1156.7	---	---	0	0	0	25487435	0
39	10.5	α-phellandrene	99-83-2	1159.8	1162	Osorio, Alarcon, et al., 2006	43703904	26683554	1813486	0	91035875
40	10.6	2-Methylpropyl 2-methylbutanoate	2445-67-2	1166.9	---	---	0	0	0	3340433	0
41	10.7	Heptan-2-one	110-43-0	1169.8	1178	Kim, 2001	0	6347163	0	0	0
42	10.7	α-Terpinene	99-86-5	1171.1	1176	Umano, Hagi, et al., 1994	7878229	10535200	3037642	0	42601134
43	10.8	3-Methyl-3-buten-1-ol acetate	7/2/5205	1178.5	---	---	0	0	0	3804687	0
44	10.9	2-Methylpropyl pentanoate	10588-10-0	1181.9	---	---	0	0	0	251655829	12275454
45	11	3-Methylbutyl 2-methylpropanoate	1/3/2050	1185.4	1183	Wei, Mura, et al., 2001	0	0	0	235906400	9579034
46	11.1	Sylvestrene	1461-27-4	1189.7	---	---	0	4531086	1879414	0	0
47	11.1	3-Methylbutyl methanoate	110-45-2	1189.7	1070	Wei, Mura, et al., 2001	1608361	0	0	0	0
48	11.2	Limonene	138-86-3	1194.4	1194	Shimoda, Shiratsuchi, et al., 1996	49591347	42577331	8635999	0	231591736
49	11.3	β-phellandrene	555-10-2	1202.9	1201	Umano and Shibamoto, 1988	116000000	20544719	5206801	0	1039007916
50	11.4	1-Methylbutyl butanoate	60415-61-4	1206.3	1216	Strohalm, Dregus, et al., 2007	0	0	0	5104687	0
51	11.4	Butyl butanoate	109-21-7	1207	1208	Welke, Manfroi, et al., 2012	0	1568539	0	266362573	33523227
52	11.5	p-Mentha-1,3,8-triene	18368-95-1	1212.8	---	---	0	0	593717	0	0
53	11.7	Hexyl 2-methyl butanoate	10032-15-2	1223.3	---	---	0	0	0	14184927	0
54	11.7	Ethyl hexanoate	123-66-0	1223.7	1227	Osorio, Alarcon, et al., 2006	8139465	132000000	2496094	0	0
55	11.9	2,4-Dimethyl-1-penten-3-ol	19781-54-5	1234.7	---	---	0	0	0	5317447	0
56	12	γ-terpinene	99-85-4	1240.6	1244	Shiratsuchi, Shimoda, et al., 1994	4619579	569687	10472642	0	0
57	12	Butyl pentanoate	591-68-4	1240.8	---	---	0	0	0	162809987	0
58	12.1	(E)- β-Ocimene	3779-61-1	1243	1243	Zheng, Kim, et al., 2005	40619579	10030689	533717	0	0
59	12.2	1,2,4-Trimethyl benzene	95-63-6	1251.1	---	---	0	0	0	0	22376452
60	12.3	3-Methylbutyl butanoate	106-27-4	1257.2	1256	Ferrari, Lablanquie, et al., 2004	0	0	0	0	312364960
61	12.4	p-Cimene	99-87-6	1263.1	1265	Shiratsuchi, Shimoda, et al., 1994	9547936	6902022	12042052	0	0
62	12.5	3-Hydroxybutan-2-one	513-86-0	1266.2	1270	Humpf and Schreier, 1991	3948640	2705897	1656921	0	0
63	12.5	Pentyl 2-methylpropanoate	2445-72-9	1267	---	---	0	0	0	3513415806	0
64	12.6	iso-Sylvestrene	499-03-6	1272	---	---	758462	5344944	0	0	0
65	12.6	3-methylbutyl 2-methyl butanoate	27625-35-0	1274.1	1274	Zhao, Xu, et al., 2009	0	0	0	84457348	0

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66	12.7	Terpinolene	586-62-9	1278.6	1280	Seo and Baek, 2005	2831338	40946911	6823646	0	9151741
67	12.8	2-Methylpropyl but-(2E)-enoate	73545-15-0	1280.7	---	---	0	0	0	4437984	0
68	12.9	Ethyl (3E)-hexenoate	2396-83-0	1287.6	1290	Ferrari, Lablanquie, et al., 2004	0	675276	0	0	0
69	13	2-Hexanol	626-93-7	1290.7	1245	Hayata, Sakamoto, et al., 2002	1127531	1114241	3885904	0	0
70	13	3-methylbutyl 3-methylbutanoate	659-70-1	1292.1	1287	Tian, Zhang, et al., 2007	0	0	0	1101002068	74039618
71	13.1	1-Methyloctyl butanoate	69727-42-0	1298.6	---	---	0	0	0	3165759	0
72	13.2	3-Methyl butan-2-ol acetate	5343-96-4	1301.6	---	---	0	0	0	109374486	0
73	13.2	Hex-(3E)-enyl ethanoate	3681-82-1	1303.1	1308	Xu, Fan, et al., 2007	476638	697715	0	0	0
74	13.3	4-Decanone	624-16-8	1306.8	---	---	0	0	3737898	0	0
75	13.3	n-propyl hexanoate	626-77-7	1309.2	1320	Mattheis, Fan, et al., 2005	0	0	0	2243690	0
76	13.4	Hept-(2E)-enal	18829-55-5	1313.4	1314	Kim, 2001	0	0	1139679	0	0
77	13.5	Ethyl heptanoate	106-30-9	1323.3	1330	Wada and Shibamoto, 1997	0	1394999	1182276	0	0
78	13.6	6-Methyl hept-5-en-2-one	110-93-0	1324.3	1329	Lee, Umano, et al., 2005	316446	0	1182276	0	0
79	13.6	(3Z)-Hexenyl 2-methylpropanoate	41519-23-7	1329.4	---	---	0	0	0	6120683	0
80	13.7	Ethyl hex-(2E)-enoate	27829-72-7	1334.7	1335	Zhao, Xu, et al., 2009	783542	1248519	0	0	0
81	13.7	n-Hexanol	111-27-3	1335.2	1340	Canuti, Conversano, et al., 2009	0	0	0	5646209	462590
82	13.8	butyl-(2E)-butenoate	7299-91-4	1335.1	1334	Fröhlich, Duque, et al., 1989	0	0	1076775	1402742	0
83	13.9	2-Methylpropyl hexanoate	105-79-3	1344.2	1347	Cha, Kim, et al., 1998	0	0	0	19012782	0
84	14.1	Unknown102	102	1354.5		---	0	0	0	2186554	0
85	14.2	allo-Ocimene	7216-56-0	1363.2	---	---	689444	0	0	0	0
86	14.3	Hex-(3Z)-enol	928-96-1	1365.2	1368	Stashenko, Torres, et al., 1995	0	519938	0	0	9597109
87	14.3	4,4-Dimethyl-1-hexene	1647-08-1	1368.8	---	---	0	0	0	6426776	0
88	14.4	α-Pinene oxide	1686-14-2	1374	---	---	647014	1209364	1560633	0	0
89	14.5	Methyl octanoate	111-11-5	1379.7	1399	Gurbuz O., Rouseff J.M., et al., 2006	1547042	6008166	0	0	0
90	14.6	3-Methylbut-2-enyl butanoate	1000299-11-8	1380.8	---	---	0	0	0	4349250	0
91	14.6	n-Nonanal	124-19-6	1385.1	1385	Shimoda, Shigematsu, et al., 1995, 2	3998732	4229632	12476067	1195883	868525
92	14.7	1-Methylhexyl butanoate	39026-94-3	1391.9	---	---	0	0	0	121605594	6337586
93	14.9	6,7-Epoxymyrcene	29414-55-9	1399.3	---	---	1759761	0	2435892	0	1264236
94	15	4-Methyl-2-pentenoate	10321-71-8	1404.4	---	---	0	0	0	937681	0
95	15	Hexyl butanoate	2639-63-6	1407.6	1407	Fröhlich, Duque, et al., 1989	0	0	0	359130	967210
96	15.2	m-Cymenene	1124-20-5	1417.8	---	---	0	349938	7651293	0	0
97	15.3	1-Cyclopentylethyl butanoate	1000282-59-8	1422.8	---	---	0	0	0	10201915	0
98	15.3	Ethyl octanoate	106-32-1	1426.6	1427	Lee and Noble, 2003	12568766	317000000	3289256	0	0
99	15.4	Unknown104	104	1431		---	0	0	0	900672	4272115
100	15.5	Heptan-1-ol	111-70-6	1437.9	1455	Hayata, Sakamoto, et al., 2002	0	1531781	2034270	0	0
101	15.5	2,2'-Isopropylidenebis(tetrahydrofuran)	89686-69-1	1438.8	---	---	0	0	0	0	5072735
102	15.5	(Tetrahydro-2-furanyl)methyl butanoate	2217-33-6	1439.3	---	---	0	0	0	87593991	0
103	15.6	4-Hydroxy pentan-2-one	4161-60-8	1441.6	---	---	2256528	0	0	0	0
104	15.7	1-(1,4-Dimethyl-3-cyclohexen-1-yl) ethanone	43219-68-7	1446.6	1504	Varming, Petersen, et al., 2004	0	148000000	0	0	0
105	15.8	3-Methylbutyl hexanoate	2198-61-0	1452.2	1450	Ferrari, Lablanquie, et al., 2004	0	0	0	39644224	2554595
106	15.9	(E)-3-Methylbutyl 2-methyl-2-butenoate	41519-18-0	1462	---	---	0	0	0	567969	0
107	16	α-Cubenene	17699-14-8	1463.2	1463	Yu, Kim, et al., 2004	1841642	2831682	953808	0	0
108	16	Unknown105	105	1466		---	0	0	0	548172	0
109	16	Ethyl oct-(4Z)-enoate	34495-71-1	1466.2	---	---	0	6710019	711478	0	0
110	16.1	Ethyl (2E,4E)-2,4-hexadienoate	2396-84-1	1471.7	1501	Shimoda, Shiratsuchi, et al., 1993	0	0	5055801	0	0
111	16.2	ethyl oct-(4E)-enoate	69668-87-7	1476.2	---	---	0	873439	0	0	0
112	16.2	3Z-Hexenyl methyl carbonate	67633-96-9	1478	---	---	0	0	0	653500	0
113	16.3	δ-Elemene	20307-84-0	1483.6	1476	Ngassoum, Yonkeu, et al., 1999	0	0	0	0	45272115
114	16.3	2-Ethylhexyl cyclobutanecarboxylate	1000282-22-0	1487	---	---	0	0	0	608650	0
115	16.4	n-Decanal	112-31-2	1490.9	1498	Zhao, Xu, et al., 2009	160633	1984368	11018508	700350	0
116	16.4	α-Ylangene	14912-44-8	1490.9	1499	Stashenko, Prada, et al., 1996	3201129	0	0	0	0
117	16.5	Ethenyl cyclohexane	695-12-5	1496.9	---	---	0	0	0	2736827	0
118	16.5	Ethyl 3-hydroxybutanoate	5405-41-4	1498.6	1501	Xu, Fan, et al., 2007	0	2712781	0	0	0
119	16.6	α-Copaene	3856-25-5	1501.1	1502	Ngassoum, Yonkeu, et al., 1999	9795474	4965672	9754333	0	2800462
120	16.7	(Z)-3-Hexenyl pentanoate	35852-46-1	1507.7	1518	Fröhlich, Duque, et al., 1989	0	0	0	7655294	0
121	16.7	Benzaldehyde	100-52-7	1509.8	1510	Lee and Shibamoto, 2000	647688	0	2283311	0	0
122	16.7	4-Heptanone	123-19-3	1511.6	---	---	0	0	0	699523	0
123	16.8	(E)-3-Hexenyl butanoate	53398-84-8	1514.2	---	---	0	0	0	4184038	0
124	16.8	4-(1-Methylethyl)-1-cyclohexene-1-carboxaldehyde	21391-98-0	1514.8	---	---	0	0	0	0	1871253
125	16.9	Camphor	76-22-2	1518.9	1519	Gyawali and Kim, 2009	1720322	2106067	0	0	0
126	17	Linalool	78-70-6	1527.4	1528	Zheng, Kim, et al., 2005	6829297	0	0	0	0
127	17	β-bourbenene	5208-59-3	1528.1	1528	Guo, Wu, et al., 2008	0	702141	1105684	0	0

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128	17.1	2-Methyl-6-methyleneocta-1,7-dien-3-one	41702-60-7	1534.8	---	---	939492	0	4810424	0	0
129	17.2	α-Gurgujene	489-40-7	1540.2	1530	Aromdee and Sriubolmas, 2006	3618483	7048365	15739595	0	0
130	17.3	Ethyl oct-(2E)-enoate	2351-90-8	1545.5	---	---	782422	11165951	3893021	0	0
131	17.3	Butane-2,3-diol	513-85-9	1547.7	1539	Kim, Shin, et al., 2001	1484326	0	1021627	0	0
132	17.4	Eucarvone	503-93-5	1554.4	---	---	0	495219	0	0	0
133	17.5	Dimethyl sulfoxide	67-68-5	1562.3	1579	Kim. J.H., Ahn, et al., 2004	1373945	1853211	1680218	0	0
134	17.7	(Z)-α-Bergamotene	18252-46-5	1570.3	---	---	0	0	3276185	0	0
135	17.7	3,3,6-Trimethylhepta-1,5-dien-4-one	546-49-6	1572.1	1346	Umano, Hagi, et al., 2000	1768723	0	0	0	0
136	17.7	3-Hydroxy-2-isopropylidene propionate	1000153-27-1	1573.5	---	---	0	0	0	524886	0
137	17.8	2,5-Dimethyl-4-methoxy-3(2H)-furanone	4077-47-8	1578.5	1584	Osorio, Alarcon, et al., 2006	2266023	0	1150955	0	0
138	17.9	β-Cubebene	13744-15-5	1584.5	---	---	1404532	10610277	2279407	0	0
139	18	2,2-Dimethylocta-3,4-dienal	590-71-6	1591.8	---	---	0	0	0	6370337	0
140	18	β-Elemene	515-13-9	1592.4	1595	Shimizu, Imayoshi, et al., 2009	978374	2961588	1201605	0	6355215
141	18.1	α-Guaiaene	12/1/3691	1596.6	---	---	8827111	0	0	0	0
142	18.2	Pantolactone	599-04-2	1602.2	---	---	0	0	0	2330757	0
143	18.2	β-Copaene	18252-44-3	1603.2	---	---	1631656	5389615	0	0	0
144	18.3	(E)-β-Caryophylene	87-44-5	1608.5	1610	Combariza, Tirado, et al., 1994	13818726	3068791	33196820	0	2360236
145	18.4	2-Methoxy-pentane	6795-88-6	1614.2	---	---	0	0	0	3065071	0
146	18.4	Methyl dec-4-enoate	1191-02-2	1614.6	---	---	0	1246519	4153795	0	0
147	18.5	Aromadendrene	489-39-4	1620.8	1635	Umano, Hagi, et al., 1994	971056	0	0	0	0
148	18.5	Unknown106	106	1624.9	---	---	0	0	0	1629136	0
149	18.6	4-Methyl tetradecane	25117-24-2	1629.8	---	---	0	0	0	0	3807491
150	18.6	Ethyl decanoate	110-38-3	1629.8	1633	Rezende and Fraga, 2003	929536	12945941	4750163	0	0
151	18.6	1,1'-oxybis[3-methyl butane	544-01-4	1630.3	---	---	0	0	0	11981427	0
152	18.7	γ-Elemene	29873-99-2	1639.9	1641	Wei A. and Shibamoto T., 2007	0	0	0	0	2300317
153	18.8	E-Muurola-3,5-diene	189165-77-3	1647.1	---	---	2204474	0	0	0	0
154	18.9	Z-Muurola-3,5-diene	157374-44-2	1647.6	---	---	0	1946801	0	0	0
155	18.9	Dauca-5,8-diene	142928-08-3	1648.4	---	---	0	5099954	0	0	0
156	19	Ethyl-(4E)-decenoate	76649-16-6	1656.8	1680	Zhao, Xu, et al., 2009	0	59801585	5360582	0	0
157	19	allo-Aromadendrene	25246-27-9	1658.4	---	---	5107930	0	0	0	0
158	19.2	(E)-Verbenol	1820-09-3	1668.3	1671	Umano, Hagi, et al., 2002	1425638	0	0	0	0
159	19.2	Cryptone	500-02-7	1674	1679	Mookdasanit, Tamura, et al., 2003	1367151	0	0	0	1056051
160	19.3	Unknown108	108	1681	---	---	0	0	0	3037374	0
161	19.4	α-Humulene	6753-98-6	1682.8	1682	Stashenko, Prada, et al., 1996	6870478	24110999	19591892	0	49335259
162	19.4	γ-Gurjunene	22567-17-5	1685.8	---	---	3179868	0	0	0	0
163	19.5	γ-Hexalactone	695-06-7	1692.7	1696	Umano, Nakahara, et al., 1999	598808	0	0	0	0
164	19.6	γ-Muurolene	30021-74-0	1695.1	1655	Stashenko, Torres, et al., 1995	10761630	6022646	0	0	0
165	19.7	4-Ethyl benzaldehyde	4748-78-1	1702.4	1719	Welke, Manfroi, et al., 2012	0	0	6149579	0	0
166	19.7	Viridiflorene	21747-46-6	1704.8	1698	Aromdee and Sriubolmas, 2006	784290	0	0	0	0
167	19.8	Verbenone	80-57-9	1710.4	1714	Seo, Kim, et al., 2007	1641127	1012579	3727255	0	0
168	19.8	Menthofuran	494-90-6	1714.4	---	---	0	17341767	0	0	0
169	19.9	Germacrene D	23986-74-5	1720	1726	Stashenko, Prada, et al., 1996	6150403	98893039	11052067	0	2844925
170	20	α-Bulnesene	3691-11-0	1723.8	1634	Christoph, 2001	9165448	0	0	0	0
171	20.1	Z-Cadina-1(6),4-diene	246522-85-0	1730.4	---	---	0	4109433	0	0	0
172	20.1	β-Selinene	17066-67-0	1732.4	1734	Wei A. and Shibamoto T., 2007	4800357	0	9517774	0	0
173	20.2	α-Selinene	473-13-2	1736.5	1737	Ngassoum, Yonkeu, et al., 1999	1470189	5053586	6194475	0	0
174	20.2	Naphthalene	91-20-3	1737.7	1740	Shiratsuchi, Shimoda, et al., 1994	0	0	0	0	7147230
175	20.2	(E,E)-α-Fernesene	502-61-4	1739.4	1740	Gancel, Ollitrault, et al., 2005	16029266	0	0	0	0
176	20.2	Unknown109	109	1741.7	---	---	0	0	0	7493944	0
177	20.3	Bicyclogermacrene	24703-35-3	1743.7	1707	Brat, Rega, et al., 2003	2352474	11990653	0	0	0
178	20.4	Car-3-en-2-one	53585-45-8	1752.1	---	---	539985	12122270	1727830	0	340782
179	20.5	δ-Cadinene	483-76-1	1761.4	1762	Aromdee and Sriubolmas, 2006	8081350	5175515	2022606	0	0
180	20.6	γ-Cadinene	39029-41-9	1768.3	1770	Ngassoum, Yonkeu, et al., 1999	5906554	0	0	0	0
181	20.6	Methyl salicylate	119-36-8	1769.1	1771	Zhao, Xu, et al., 2009	0	0	9113949	0	0
182	20.6	Ethyl 2-phenylethanoate	101-97-3	1771.6	1785	Ferrari, Lablanquie, et al., 2004	0	1974070	0	0	0
183	20.8	2-Pinen-10-ol	515-00-4	1778.8	1787	Christoph, 2001	1299224	0	0	0	0
184	20.9	(E)-4-(2-Butenyl)-1,2-dimethyl benzene	54340-86-2	1790	---	---	0	0	0	0	359099
185	20.9	E-Cadina-1,4-diene	38758-02-0	1789.9	---	---	1145374	0	0	0	0
186	21	Methyl dodecanoate	111-82-0	1794	1793	Ferrari, Lablanquie, et al., 2004	0	717542	0	0	0
187	21	Octadecane	593-45-3	1798.4	1800	---	0	0	6186443	444435	924207
188	21	α-Cadinene	82468-90-4	1799.2	---	---	0	1903069	0	0	0
189	21.1	Phenyl ethyl hexanoate	6290-37-5	1802.3	2160	Fan and Qian, 2006	0	715483	0	0	0
190	21.2	Unknown110	110	1809.2	---	---	0	0	0	363878	0
191	21.2	Tridecanal	10486-19-8	1809.7	1821	Shiratsuchi, Shimoda, et al., 1994	1612692	3578657	1909661	0	0

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192	21.4	m-Ethylacetophenone	22699-70-3	1823.8	---	---	1173104	1872266	9151461	0	0
193	21.4	p-Cymen-8-ol	1197-01-9	1828.9	1838	Yu, Kim, et al., 2004	0	0	4344313	0	0
194	21.6	Ethyl dodecanoate	106-33-2	1837.3	1835	Ferrari, Lablanquie, et al., 2004	4069329	35206360	36409514	0	0
195	21.6	Unknown111	111	1840.1	---	---	0	0	0	0	769354
196	21.7	Geranyl acetone	3796-70-1	1846.1	1856	Zhao, Xu, et al., 2009	1170142	0	2461720	0	156051
197	21.7	2-Ethyl-3-hydroxyhexyl 2-methyl-propanoate	74367-31-0	1848.2	---	---	0	0	0	817439	0
198	21.8	Benzyl methanol	100-51-6	1852.8	1853	Parada, Duque, et al., 2000	1065405	0	0	737075	0
199	21.9	7-methyl-5-octen-4-one	32064-78-1	1864	---	---	0	0	0	0	5483607
200	21.9	p-Ethylacetophenone	937-30-4	1863.8	1867	Shimoda, Shigematsu, et al., 1995, 2	0	1142202	3722120	0	0
201	22	1-(1,1-Dimethylethyl)-2-methyl-1,3-propanediyl 2-methyl propanoic acid	74381-40-1	1872.1	---	---	0	0	3464371	0	0
202	22.2	Hexahydro-1,4-dioxa-cyclopropa[a]pentalen-3-one	143393-90-2	1881.7	---	---	562146	0	0	0	0
203	22.2	2-Methyl naphthalene	91-57-6	1888.3	1852	Peng, Yang, et al., 1991	0	0	0	0	2002276
204	22.3	2-PhenyI ethanol	60-12-8	1891.2	1893	Shimoda, Shiratsuchi, et al., 1996	2238465	974403	1284398	643339	2124233
205	22.5	3-Methyl-1-butanol benzoate	94-46-2	1908.3	1928	Christoph, 2001	0	0	0	1399245	0
206	22.5	γ-Octalactone	104-50-7	1909.8	1923	Kumazawa and Masuda, 2002	4929701	2896558	1621889	0	0
207	22.5	Benzeneacetonitrile	140-29-4	1910.3	---	---	0	0	0	0	1671253
208	22.8	Piperitenone	491-09-8	1926.3	1918	Lee, Umano, et al., 2005	1443855	2621340	5874227	0	0
209	23.1	Unknown113	113	1951.8	---	---	0	0	0	0	1452152
210	23.2	Tetrahydro-6-propyl-2H-pyran-2-one	698-76-0	1964.6	1985	Kumazawa and Masuda, 2002	1221408	0	0	0	0
211	23.5	3-(1H-indol-3-yl)-2-Propenoic acid	1204-06-4	1984.6	---	---	866494	0	0	0	0
212	23.5	Biphenyl	92-52-4	1984.6	1981	Morales, Albarracín, et al., 1996	0	0	0	0	2294058
213	23.6	Methyl eugenol	93-15-2	1990.2	2007	Lee, Umano, et al., 2005	1293981	1196511	7332600	1058258	1109658
214	23.7	Caryophyllene oxide	1139-30-6	1998.7	1998	Mookdasanit, Tamura, et al., 2003	0	0	8252117	0	2272774
215	23.7	Methyl tetradecanoate	124-10-7	1999.4	---	---	0	2502358	0	0	0
216	23.9	2-Pentadecanone	2345-28-0	2013.7	2021	Ott, Fay, et al., 1997	1869230	1030552	1451536	0	0
217	24	E-Nerolidol	40716-66-3	2019.9	2020	Zheng, Kim, et al., 2005	0	0	10942774	0	0
218	24	Tridec-(2E)-enol	74962-98-4	2022	---	---	2706984	0	0	0	0
219	24.1	Methylethyl tetradecanoate	110-27-0	2031.7	---	---	1354570	1250227	0	0	0
220	24.2	Methyl 2,6-dimethyltridecanoate	73105-76-7	2040.1	---	---	0	0	0	0	1020896
221	24.2	Ethyl tetradecanoate	124-06-1	2043.2	2040	Ferrari, Lablanquie, et al., 2004	3127083	24294078	19578571	1060335	0
222	24.3	Germacrene D-4-ol	74841-87-5	2050.2	2050	Umano, Hagi, et al., 2000	2001712	0	0	0	0
223	24.4	Humulene epoxide II	19888-34-7	2057.4	---	---	0	0	2360738	0	1071442
224	24.5	Unknown114	114	2061.9	---	---	0	0	0	1629136	0
225	24.5	Cyclooctanone	502-49-8	2067.9	---	---	0	628411	0	0	0
227	25	1-Nitro-2-phenyl ethane	6125-24-2	2102.8	---	---	0	0	0	0	2304458
228	25.2	6,10,14-Trimethyl-2-pentadecanone	502-69-2	2118.2	2110	Gyawali and Kim, 2012	0	0	2509669	0	2034807
229	25.4	Unknown		2139.2	---	---	1010142	0	0	0	0
230	25.5	Tetradekanol	112-72-1	2146.6	2145	Ferrari, Lablanquie, et al., 2004	0	0	1467573	0	0
226	25.8	Eugenol	97-53-0	2171.85	2171	Kumazawa and Masuda, 2002	0	0	0	5373747	0
231	26	o-Cumenol	88-69-7	2188.5	---	---	0	0	0	0	1220115
232	26.2	Elemicin	487-11-6	2202.6	---	---	0	0	0	81333910	6313466
233	26.5	Unknown116	116	2228.3	---	---	0	846437	0	0	0
234	26.7	Ethyl hexadecanoate	628-97-7	2247.9	2246	Ferrari, Lablanquie, et al., 2004	0	3794322	4239113	2464158	2362355
235	26.8	Unknown117	117	2259.1	---	---	1405282	1636723	4360128	17219509	921305
236	27	Ethyl 9-hexadecenoate	54546-22-4	2272.9	2283	Zhao, Xu, et al., 2009	0	985665	3705032	0	0
237	27.3	5-Amino-1-phenylpyrazole	826-85-7	2297.7	---	---	0	0	0	7493944	0
238	27.9	n-Hexadecanol	36653-82-4	2358.5	2363	Osorio, Alarcon, et al., 2006	0	3055735	586432	0	0
239	28	γ-Dodecalactone	002305-05-7	2369	2365	Umano, Hagi, et al., 1994	0	1134619	0	0	0

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