SEMIOCHEMICALS MEDIATING OVIPOSITION AND MATING BEHAVIOURS OF MANGO INFESTING FRUIT FLY, *BACTROCERA INVADENS*

By
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A Thesis Submitted for the Degree of Doctor of Philosophy (Chemistry) of the University of Dar es Salaam

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CERTIFICATION

The undersigned certify that we have read and hereby recommend for acceptance by the University of Dar es Salaam, a Thesis entitled: ***Semiochemicals Mediating Oviposition and Mating Behaviour of Mango Infesting Fruit Fly, Bactrocera invadens,*** in fulfillment of the requirements for the degree of Doctor of Philosophy (Chemistry) of the University of Dar es Salaam.

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DECLARATION

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I, **Fikira Kimbokota,** declare that this thesis is my own original work and it has not been presented and it will not be presented to any other University for a similar and/or any other degree award.

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DEDICATION

This Thesis is dedicated to my lovely husband

Salehe,

Our Sons, Idrisa and Said

for their love, patience, encouragement and sharing with me moments of cheerfulness and difficulty during the whole period of my study

and

my late parents

Said Ally Kimbokota and Zubeda Abdala Sinda

for bringing me up until I became who I'm today

The difference between a successful person and others is not a lack of strength, not a lack of knowledge, but rather a lack of will.

By Vince Lambardi

It is a rough road that leads to the heights of greatness.

By Seneca

ABSTRACT

This thesis reports on characterisation of semiochemicals that mediate oviposition and mating behaviour of the fruit fly *Bactrocera invadens* from three host plants namely mango, *Mangifera indica*; the Indian almond, *Terminalia catappa*; and the marula, *Sclerocarya birrea*. Attractive volatile components from a non-host plant, *Gynandropsis gynandra* that only attracted male flies as well as those in volatile emissions collected from both male and female flies were also characterized. Volatile constituents collected from both mature unripe and mature ripe fruits of *Mangifera indica*, *Sclerocarya birrea* and *Terminalia catappa* were identified to constitute terpenes, esters, hydrocarbons, carbonyls, alcohols and others, with terpene, alcohols and esters respectively being the most abundant. In GC-EAD analyses of these volatiles, a number of compounds in each host were found to be electrophysiologically active. *Gynandropsis gynandra* attracted male fruit flies in the field, and two EAG-active peaks were identified by GC-MS to represent 4-methyl-3-penten-2-one and 4-hydroxy-4-methyl-2-pentanone. Volatiles from male flies were significantly more attractive to both male and female flies. Furthermore, in GC-MS analyses these volatiles were found to constitute various groups of compounds in which esters and spiroacetals were the main constituents. This study characterized semiochemicals from host plants, one non-host plant and volatiles from male and female flies and some constituents are candidate attractants to both male and female *Bactrocera invadens*. Thus, the results have laid down significant groundwork in the development of effective attractant(s) from both, host plants (kairomones) and conspecifics (pheromones).

TABLE OF CONTENTS

LIST OF ABREVIATIONS

DDT	Dichlorodiphenyltrichloroethane
IPM	Integrated Pest Management
icipe	International Centre of Insect Physiology and Ecology
AI	Attractancy Index
HPLC	High Performance Liquid Chromatography
SPME	Solid Phase Microextraction
GC	Gas Chromatograph
MS	Mass Spectroscopy/Spectrum
GC-MS	Gas Chromatography-Mass Spectrometry
m/z	Mass to Charge Ratio
GC-EAD	Gas chromatography-Electroantenographic Detection
EAG	Electroantennogram
FID	Flame Ionization Detector
EI	Electron Ionization
RK	Raspberry Ketone
ME	methyl eugenol
MIBK	methyl–isobutyl ketone

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 General Introduction

Natural products play important roles in the life of organisms. They are essential in biological interactions such as defense, signal transduction and other specific functions vital for their life processes.[1] They are grouped into different categories depending on the life processes they are involved in. An important group of natural products, referred to as semiochemicals (which include pheromones, kairomones, allomones and synomones) mediate intra- and inter-specific interactions in nature such as insect-insect, insect-plant, and insect-mammal interactions.[1]

Diverse research work has demonstrated chains of biochemical events through which insects orientate to their hosts, habitats, food, mates and suitable oviposition sites.[2] Biochemical attractants that mediate these processes have demonstrated great potential in the detection, survey and monitoring of potential pest species. They are highly species-specific; they are normally active at very low concentrations and have been successfully deployed in agricultural systems worldwide, either in monitoring or direct control applications.[2,3]

Horticulture is now growing rapidly in the developing world including East African countries. This is coupled with increased capability of exporting crops such as fruits

and vegetables in relatively fresh conditions to distant foreign markets. However, there is an increasing problem with pests, including exotic ones, which are a threat to agricultural production[4]. For example, production of fruits such as mangoes in Africa is highly affected by tephritid fruit flies. Field surveys have revealed that 40-80% of the 1.9 million tonnes of mangoes produced in Africa annually is lost due to attack by fruit flies.[5]

Equatorial Africa is the home of about 915 fruit fly species from 148 genera, out of which 299 species develop in either wild or cultivated fruits. They belong to the family Tephritidae and mainly fall into four genera: *Dacus, Ceratitis, Trirhithrum* and *Bactrocera.*[6] From current records, the major pests of mango across Africa include the Marula fruit fly, *Ceratitis cosyra*[7], the Natal fruit fly, *Ceratitis rosa* and its closely related species *viz. Ceratitis fasciventris*[6], and the Mediterranean fruit fly, *Ceratitis capitata.* These flies cause enormous damage and results in losses ranging from 20% to 100%.[7]

Plate 1.1 Fallen Mangoes Mainly Due to Infestation by Fruit Fly Larvae

(By Fikira Kimbokota, 2009)

Female flies lay batches of eggs just beneath the skin of fruit of suitable host plants after puncturing the fruit skin using their long and sharp telescopic ovipositors.[6] Physiologically, mature unripe or ripe fruits are usually preferred, depending on the fruit fly species.[8] After hatching, larvae feed on fruit pulp which cause rotting of the fruit due to deposition of waste products and attack by bacteria. Larvae develop through to the fifth instar after which they leave the fruits and burrow into the soil to pupate. The adult flies eclose thereafter and start another life cycle.[6] Larval feeding is the most destructive period of the flies and leads to either decay of infested fruits on the tree or their premature drop. This results in both quantitative and qualitative losses, thus making fruit flies one of the major production drawbacks for fruit and vegetable growers.[9]

The number of fruit fly species in Africa is growing rapidly through new introductions from other continents. Recently, a new species, *Bactrocera invadens* (Diptera: Tephritidae), was introduced from Asia, which causes a serious threat to fruit production in East Africa and in Africa as a whole[6]. These flies are polyphagous, although they prefer mangoes. It is now apparent that the species is competing strongly with the indigenous mango fruit fly in eastern and southern part of Africa, *Ceratitis cosyra*, since it is found to be abundant in the infested mangoes.[10] *Bactrocera invadens* causes extensive loss of fruits in the field as it rapidly broadens its host range.[11] Generally, losses are due to the direct damage arising from feeding that lead to loss of export market opportunities through quarantine imposed by importing countries to avoid establishment of unwanted fruit flies. For example,

because of the threat posed by invasive fruit flies, Mauritius and South Africa have recently banned the importation of mango and avocado from Kenya.[12]

Over the last three decades research efforts have been directed towards the search for effective pest control agents to address agricultural problems. The major method of control of fruit flies has been the traditional use of food attractants consisting of hydrolyzed protein or ammonium based mimics and host plant-derived kairomones combined with a toxicant.[13-16] Very little information is available with regard to responsiveness of *B. invadens* to protein and plant-based attractants. However, commercial attractants denoted as parapheromones such as methyl eugenol are available.[17] Nonetheless, these agents are only moderately attractive to these species for their effective management. They are expensive and only attractive to male flies.

Thus, there is need to develop more sustainable and cheaply available control agents for protecting crops through the use of agents such as kairomones or pheromones. This could help meet the requirements of international and national markets, leading to improvement of economic well-being of farmers.

The present research was conceptualized with the aim of providing an understanding on the olfactory cues that are involved in host fruit and mate location by *B. invadens*. Identification and characterization of these chemical cues may provide additional control tools for both male and female fruit flies, either used alone or in combination with the existing ones. Specifically, the objective of the study was to characterize the highly attractive compounds from host plants that are highly susceptible to *B.*

invadens and characterize pheromonal constituents for the development of more effective, cheaply available agents that can be used in the detection, monitoring and control of the pest as part of a comprehensive IPM programme.

1.1 Literature Review

1.1.1 *Bactrocera invadens* (Diptera: Tephritidae)

Plate 1.2 Female Fruit Fly, *Bactrocera invadens* (By, Dr. Sunday Ekesi, 2006)

The invasive tephritid fruit fly, *Bactrocera invadens* Drew Tsuruta & White is probably of Asian origin and it was reported for the first time in Kenya (East Africa)[18,19] in March 2003. Since then, it has rapidly spread across the African continent[5] (hence its name) and it has now been reported in Benin, Cameron, Comoros Island, DR Congo, Equatorial Guinea, Ghana, Guinea, Mali, Nigeria, Senegal, Sudan, Tanzania, Togo and Uganda.[20]

Worldwide, *B. invadens* is regarded as one of the most destructive insects of fruits and vegetables.[5] The insect is closely related to *Bactrocera dorsalis* (Diptera: Tephritidae) complex of tropical fruit flies that comprise of more than 75 species that

are largely endemic to South-East Asia.[18] The group is arguably one of the most important pest species complexes in world agriculture.[5]

Recently, field studies on the host range of this fly showed that, it is capable of attacking both cultivated and wild hosts.[11] However, mango, *Mangifera indica* L., (Anarcadiaceae) is the primary cultivated host while Marula, *Sclerocarya birrea* H., (Anarcadiaceae) and the Indian almond, *Terminalia catappa* L. (Combretaceae) are the major wild hosts.[11] Little information is available on the behaviour of *B. invadens*. However, due to its close relatedness to the oriental fruit fly, *Bactrocera dorsalis* and other species of phytophagous insects, it has over time acquired specific behavioural characteristics in its feeding, oviposition, and mating that are mediated by chemical and/or visual cues.[20,21]

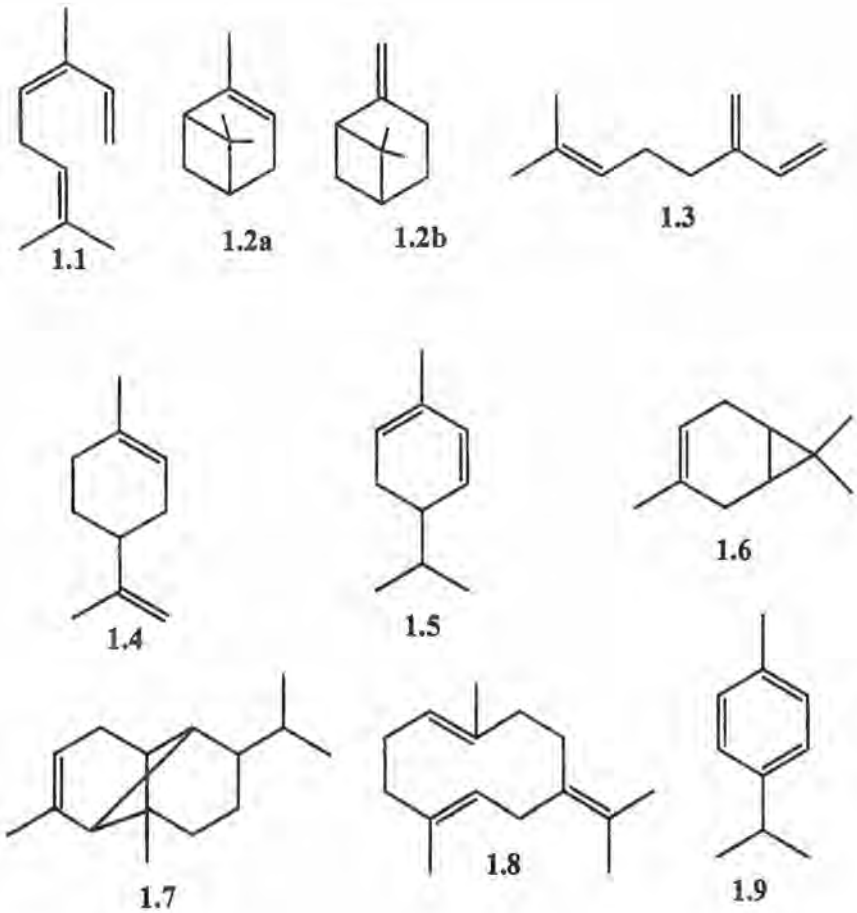
1.1.2 The Mango, *Mangifera indica* (Anarcadiaceae)

Mango, *Mangifera indica* is one of the preferred fruits by *B. invadens* in the tropical and subtropical regions. The mango fruit varieties have excellent flavours, attractive fragrances, delicious taste and high nutritional value to humans that have made it one of the best fruits.[22] A wide range of compounds have been identified from this plant including esters, lactones, mono- and sesquiterpenes and furanones[23], and monoterperne hydrocarbons, including ocimene (**1.1**), α– and β-pinene (**1.2a** and **1.2b**), myrcene (**1.3**) and limonene (**1.4**), which seem to be particularly important contributors to the flavour of the fresh mango.[24] Ocimene (**1.1**) and myrcene (**1.3**)

have been reported to be the characteristic aroma compounds of green (mature unripe) mango depending on the variety.[24]

Most insects use chemical cues to locate food, oviposition sites and their mates. The indigenous mango fruit fly in eastern and southern parts of Africa, *Ceratitis cosyra*, has been reported to be attracted to and oviposit on immature and mature green mangoes rather than ripe yellow fruits in the field.[25] The candidate volatiles on the immature green fruit were identified as terpenoids, which include the monoterpenes ocimene (**1.1**), myrcene (**1.3**), α-phellandrene (**1.5**), 3-Carene (**1.6**) and limonene (**1.4**), and the sesquierpenes α-copaene (**1.7**) and germacrene-B (**1.8**).[25]

The airbone volatiles of the Tommy Atkin's cultivar of mango is reported to emit terpenes that attract Mediterranean fruit fly, *Ceratitis capitata*, while *p*-cymene (**1.9**) and limonene (**1.4**) were found to be the most potent terpene attractants for both males and females.[26] 3-carene (**1.6**) is another monoterpene from mango fruit that has been described as having an aroma of mango leaves.[24]

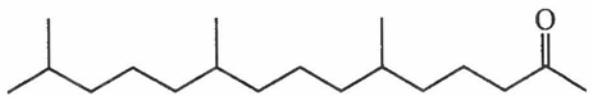


1.1.3 The Indian Almond, *Terminalia catappa* L. (Combretaceae)

Terminalia catappa, L. belongs to the family Combretaceae and originated in Malaysia. This tree is generally confined to mesic and wet coastal habitats and is distributed throughout the Old World tropics and tropical America.[27] It produces fruits (5-10 cm long) with a thin flesh surrounding a large fibrous nut. Natural products reported from *T. catappa* include terpenoids,[28] flavonoids,[29] tannins[30] and esters.[31] The leaf extract from *T. catappa* has been reported to preferentially attract female oriental fruit flies, *B. dorsalis*.[32]

Plate 1.3 Twigs of *Terminalia catappa* Tree with *Bactrocera invadens* on a Ripe Fruit (By Fikira Kimbokota, 2008)

A number of volatile compounds have been identified from leaves of *T. catappa*. In green leaves, 6,10,14-trimethyl-2-pentadecanone (**1.10**) was the most abundant constituent while in yellow leaves, the major compounds were (*E,E*)-2,4-heptadienal, (**1.11**), 6-methyl-5-hepten-2-one (**1.12**) and 1-(2,3,6-trimethylphenyl)-(*E*)-3-buten-2-one (**1.13**).[33] Recently, a range of volatile compounds were reported from ripe fruits, which included esters and terpenes. Twenty two compounds were found to be GC-EAD active peaks were detected by *Bactrocera dorsalis*, in which geranyl acetate (**1.14**) and eugenol (**1.15**) gave the highest responses.[31] Other compounds identified include ethyl acetate (**1.16**), ethyl hexanoate (**1.17**), hexyl acetate (**1.18**), linalyl acetate (**1.19**), ethyl nonanoate (**1.20**), nonyl acetate (**1.21**), ethyl cinnamate (**1.22**) and *(E)*-β-farnesene (**1.23**).[31]



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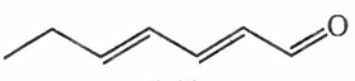

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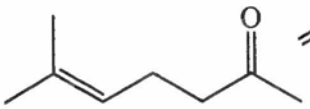

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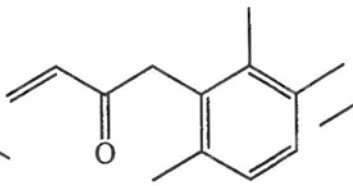

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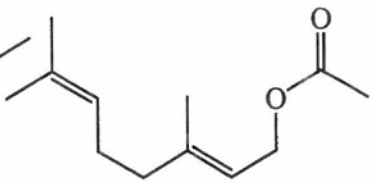

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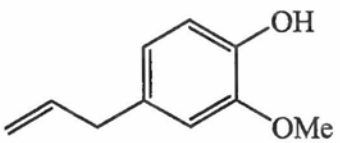

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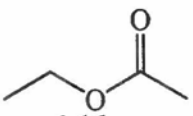

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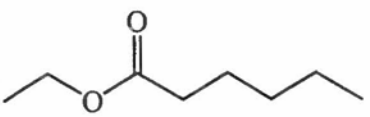

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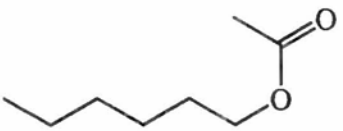

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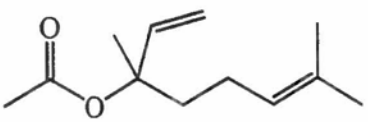

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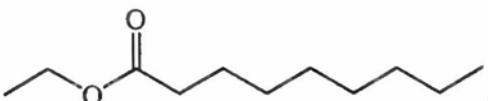

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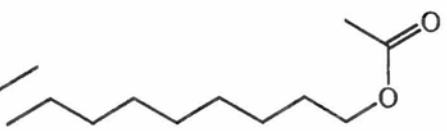

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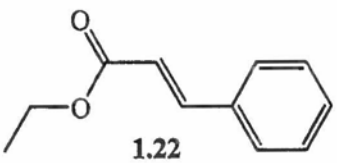

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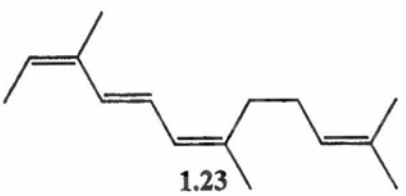

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1.1.4 The Marula, *Sclerocarya birrea* H. (Anarcadiaceae)

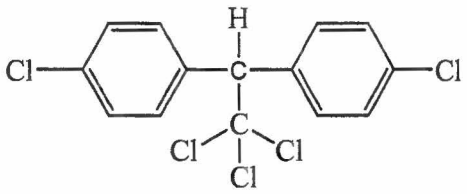
The 'marula' tree, *Sclerocarya birrea* is a medium-sized to large deciduous tree with an erect trunk and rounded crown. It is one of the plants that played a role in feeding people in ancient times. Marula fruit, which is native to sub-Saharan Africa is of growing commercial importance.[34] This tree is distributed throughout Africa with its southern most location being in the lowlands of KwaZulu-Natal (South Africa), from where it extends northwards through tropical Africa into Ethiopia and Sudan.[35] Several products such as beer, juice, jam and jelly have been developed from the mesocarp and successfully marketed, the most recent being a marula liqueur (Amarula®).[36]

Plate 1.4 ***Sclerocarya birrea*** **Tree in the Field (By Fikira Kimbokota, 2010)**

A number of natural products have been reported from this plant.[37] Furthermore, head-space volatiles of the fruit pulp and the whole fruits (skin volatiles) were investigated. The identified compounds included monoterpenes, diterpenes, alcohols, hydrocarbons and esters. The two major compounds in the fruit pulp volatiles have been established to be β-caryophyllene (**1.24**) and α-humulene (**1.25**).[37]

1.2 Fruit Fly Control Approaches

The predominant method to control fruit fly populations is based on the use of conventional pesticides. At the early years of World War II in 1939, pesticide selection was limited to several arsenicals as well as petroleum oils, nicotine, pyrethrum, rotenone, sulphur, hydrogen cyanide gas and cryolite.[38] During the last century, chemical control of insect pests became a vital part of agriculture.[39] This took place with the introduction of a new concept of using synthetic organic insecticides, the first of which was DDT (dichlorodiphenyl-trichloroethane, **1.26**).[40] However, these chemicals pose serious risks to health of millions of farmers and the environment.[41] This is more serious in developing countries where climatic conditions favor the survival of many species of organisms, including those of pests, thus requiring the use of large quantities of pesticides.[42] The situation is compounded by lack of appropriate safe storage and disposal methods for surplus pesticides.[42]



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Thus, the world community is now emphasizing on the use of bio-rational pesticides. These are substances of natural origin that have detrimental or lethal effects on specific target pest(s). They possess a unique mode of action and they are non-toxic to humans and animals. Furthermore, they do not pollute the environment.[38] Semiochemicals such as pheromones and/or kairomones from both pests and beneficial insects are among such chemicals and some are already in commercial use. For example, synthetic sex pheromones are being used for reducing moth population densities through mating disruption.[43] However, successful pest control requires complementary use of different tactics that are compatible, referred to as Integrated Pest Management (IPM). The key component of IPM for insect pest management as far as biorational control is concerned, is to have a greater knowledge of the insect's behavior through the understanding of when and where insects are present and at what stage of their life cycle they are most destructive. This enables those with the responsibility of managing insect infestations to make informed decisions on when to implement a control treatment or a combination of different tactics.[44]

1.2.1 Semiochemicals for Pest Control

Semiochemicals (pheromones, kairomones, allomones, etc.) are invaluable tools in IPM programmes.[31] Olfactory attractants are considered the most important in the recognition of a suitable host plant for food and/or fruit for oviposition. Attractants are useful in the development of effective, nontoxic traps for detection, monitoring and, control of fruit fly pests.[45] Specific behaviours such as feeding, mate location and oviposition site location and selection may be mediated by semiochemicals. These include food odours, conspecific sex and aggregation pheromones, and host fruit odors.[31] Thus, when the semiochemicals are identified, they may be used for either monitoring of pest populations, hence, assisting in timing of treatments, or reduction of populations by mass trapping, lure-and-kill or mating disruption.[46]

1.2.1.1 Pheromones

Pheromones are utilized by a variety of organisms for chemical communication. In insects, several types of pheromones are recognized, based on behaviors produced by the compounds.[47] The most common behavioural traits mediated by pheromones include attraction of the conspecific sex for mating (sex pheromones), aggregation of both sexes to a specific site for feeding and/or mating (aggregation pheromones), and marking sites or forming trails (marking pheromones).[48] In recent years more attention has been focused on the possible use of pheromone-based attractants in fruit fly control.[49] In tephritid pests, except for *Bactrocera aloe,* pheromones are produced by males.[50] The use of synthetic sex pheromones and other intra-specific chemical signals has been established in Lepidopteran species. However, identification of

attractants for other insects has been slow but is now on the rise due to improvements in chemical analytical approaches.[47]

1.2.1.2 Kairomones

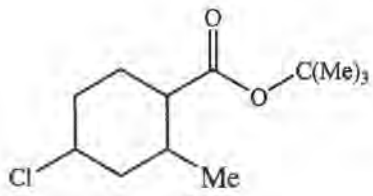
Kairomones are semiochemicals that mediate inter-species interactions. These are chemical cues that are emitted by one organism that benefit individuals of another species that detect them without benefiting (often harming) the emitter(s).[51] There is increasing amount of evidence that fruit volatiles are important in enabling flies not only to locate fruit, but also to discriminate between hosts and non-hosts and among fruits at different stages of ripeness.[52,53] Host plant volatiles have been investigated and have been shown to be potential attractants to some fruit flies. For example, male and female medfly *Ceratitis capitata* tested in laboratory cages were highly attracted to the terpenes *p*-cymene and limonene collected from volatiles of Tommy Atkins mango variety.[54]

Males of several economically important tephritid fruit flies are strongly attracted to specific chemical compounds known as para-pheromones, denoted as male lures. These compounds either occur naturally in plants or have been presumed to be synthetic analogues of plant derived substances.[47] Due to their powerful attractiveness to fruit flies, para-pheromones are frequently used in pest control programs for detecting and monitoring wild populations. Most pest species of *Bactrocera* are attracted to two major natural attractants, namely raspberry ketone (cue-lure) and methyl eugenol.[54,55] Species of fruit flies whose males are attracted to

raspberry ketone include *Bactrocera cucurbitae* and *B. tryoni* which sequester the chemical into their pheromonal system. Ingestion of methyl eugenol by *B. dorsalis* has been studied intensively and revealed that the compound is converted into two major booster components of the sex pheromone in the crop, namely *trans* coniferyl alcohol and 2-allyl-4,5-dimethoxyphenol.[54] The metabolites are stored in the rectal gland and subsequently released in the course of fanning performed during courtship.

The above pheromonal components improve the mating competitiveness of a large number of male flies belongimg to the family Tephritidae by at least three-fold when compared with methyl eugenol-deprived males. On the other hand, the metabolites are related to survival, and one of the components, 2-allyl-4,5-dimethoxyphenol, acts as a very potent allomone, that is a feeding deterrent to vertebrate predators.[56,57]

Parapheromone attractants for male fruit flies that are currently available commercially include, Trimedlure [*tert*-butyl 4- (and 5)-chloro-2-methylcyclohexane-1-carboxylate, **1.27** and **1.28**][58] for the Mediterranean fruit fly, *C. capitata*); cuelure [4-(*p*-acetoxyphenyl)-2-butanone, **1.29**] for the melon fly, *B. cucurbita*[58] and the Queensland fruit fly, *B. tryoni*[59]; and methyl eugenol **(1.30)**[60] for oriental fruit fly, *B. dorsalis* and the peach fruit fly, *B. zonata*. Due to their powerful attractiveness, these lures play an important role in the existing control programmes for tephritid pests, both in the detection and eradication of already established populations. [61]



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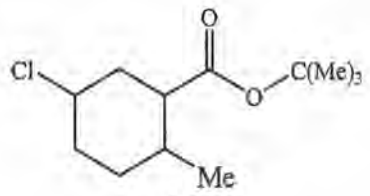

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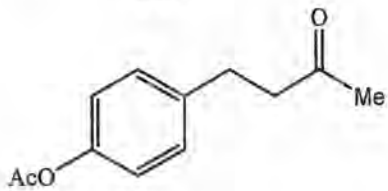

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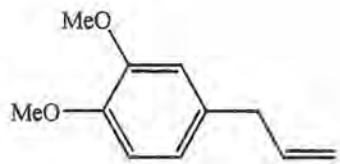


1.30

Although lures of plant origin are vital tools in tephritid pest management, to date the ecological and evolutionary significance of the responses of fruit flies to these lures remain enigmatic.[62] However, there are suggestions that they are used by insects as precursors to pheromones.[63]

These new environment-friendly techniques for pest management are totally different in their action from the presently used pesticide-based control methods. Thus, it is necessary to have a better understanding of the behavior, such as mate finding and host location for oviposition by the target insect.

1.3 Mating Behaviour of Fruit Flies

Mating in most of tephritid fruit flies occurs at dusk in male aggregations that are termed as leks, in which they release pheromones to attract female flies. Males perch singly on the under-surfaces of leaves of both host and non-host (roosting) plants and defend their sites against other intruding males. While perching, males fan their

wings rapidly, thereby producing a high pitched buzzing sound. Wing-fanning also enhances dispersal of a pheromone, produced in the rectal gland that is attractive to females.[64-66] The first mating and egg deposition by *B. dorsalis* females usually takes place from 8 to 12 days after the emergence of adults. However, in the laboratory, flies that feed on a special diet of enzymatic yeast and soy protein hydrolysates may get mature from their fifth day.[67]

1.4 Oviposition Behaviour of Fruit Flies

Oviposition is a very important component of tephritid fruit fly behaviour because it influences the potential survival and development of larvae and the extent of economic losses caused by oviposition punctures and larval feeding.[68,69] Many tropical fruit flies deposit their eggs in punctures just under the skin of mature or ripe fruit, singly or in batches. In some cases, they also deposit eggs through existing cracks or damaged spots made by other insects, rodents or fruit bats.[70]

Several females may successively use the same ovipuncture(s) made by other species, which sometimes result in a large number of eggs being deposited in a single cavity.[71] Females of *B. tryoni* (Froggatt) lay eggs in punctures already present on host fruits much more frequently than *C. capitata.* The shape, size, colour and chemistry of the fruit are also vital considerations for acceptance of host fruit by female flies for oviposition. Females of the olive fruit fly, *Dacus oleae* recognize olive fruits by visual cues including shape, size, and color as well as odours emanating from the fruits.[8]

1.4.1 Oviposition-Deterring Pheromones in Fruit Flies

Oviposition-deterring pheromones (ODPs) are chemicals that are deposited on the oviposition substrate by ovipositing female insect of a given species that deter other conspecific females and/or those of other closely related species from ovipositing on or into the same substrate.[72,73] In tephritid fruit flies, ODPs have been recorded in many species in the genera *Rhagoletis*[72] and *Anastrepha*,[73] and in *Ceratitis capitata*.[74] The host-marking behaviour allows species dispersion within available hosts and reduction of possible intra- and interspecific competition of the immature larvae in fruits.[8]

1.5 Significance of the Study

The invasive mango fruit fly, *B. invadens* is a major threat to a wide variety of maturing fruits in the field due to its rapid broadening of its host range. The situation in Africa is particularly critical because large quantities of fruits are still produced by smallholders who cannot afford expensive monitoring and pest suppression tools that are largely imported from abroad. Volatiles emitted by fruits play a key role in attracting flies meters from a distance to the fruiting trees. Thereafter, shape, colour and size are short-range cues that are involved in host recognition.[8]

The conceptualization of the present study was based on the hypothesis that both host plant volatiles and fruit fly pheromones mediate key behaviours in the chemical ecology of host location by *B. invadens*. Specifically, behavioural assays with

volatiles emanating from host fruits of preferred plants and those released by male flies were initially undertaken. This was followed by a series of experiments to identify the chemical signals. It was envisaged that, the study will establish the groundwork useful in the development of efficient attractants for these insects that can be used as bait(s) for their management.

1.6 Objectives of the Investigations

Insects utilise various stimuli including visual, contact (tactile), acoustic and chemical cues to locate food, mates, and suitable oviposition substrates. Insects detect chemical signals and locate the source by responding differentially to the stimulus depending on their physiological state and the quality of the odour in an environment permeated with a wide range of odours from other sources. Semiochemicals provide humans with excellent opportunities for their application in pest management. An understanding of the nature and role of these chemical cues in the behavioural biology of various insects is already providing solutions for a range of orchard pest problems internationally. More opportunities are likely to be exploited in future with the broadening of research in the understanding of the chemical ecological bases of various behaviours of a wide range of insect species, both beneficials and pests.

Bactrocera invadens is one of the major pests of fruits and vegetables and is currently occurring widely throughout Africa, including Tanzania. Based on the above background, the present research aimed at the characterization of

semiochemicals that mediate location of host fruits for oviposition and mate recognition in the mango-infesting fruit fly *B. invadens*. This knowledge may in turn enable the development of effective attractants using the kairomones and pheromones released by host plants and the insects, respectively. The study involved the use of chromatographic analyses and behavioural bioassays to characterize the volatile constituents from three host fruits and one non-host plant that are attractive to *B. invadens*. It also involved characterization of candidate components of a pheromone that is released by the male fruit flies.

CHAPTER TWO

OLFACTORY ATTRACTION OF *BACTROCERA INVADENS* (DIPTERA: TEPHRITIDAE) TO RIPE FRUITS OF THREE HOST PLANTS

Abstract

In the present study, the behaviour of *Bactrocera invadens* towards different odour blends emitted at different maturity stages of fruits from three hosts, namely mango, *Mangifera indica*; marula, *Sclerocarya birrea*; and the Indian almond, *Terminalia cattapa* from Nguruman and Embu, Kenya were tested using a dual choice olfactometer. Results showed that, both male and female flies were attracted equally to mature unripe and ripe fruits but significantly higher ($P < 0.05$) than to immature fruits, without discriminating between the different host plants. It is concluded that the volatiles emitted by the mature host fruits contained constituent components that are candidate attractants for the fruit flies that when characterized may be useful in detection and monitoring of *B. invadens*.

2.1 Introduction

A number of fruit fly species are economically important agricultural pests because their larval stages are phytophagous and inflict heavy losses on fruit and vegetable crops. Economic effects of pest species include not only direct yield losses and increased control costs, but also the loss of export markets and/or the cost of constructing and maintaining fruit treatment and eradication facilities. Fruit flies are dipteran insects known to cause devastating losses of fruits and vegetables in the tropical and subtropical regions of the world.[6] In Africa, the majority of fruit fly pests are members of the genera *Ceratitis*[75,76] and *Dacus*.[77] However, in March 2003,

Bactrocera invadens was recorded for the first time in Kenya (East Africa), although it was initially thought to be *B. dorsalis* (Hendel).[19] Since this first report, *B. invadens* has been found in other African countries including Angola, Benin, Burkina Faso, Cameroon, Comoro Island, Congo, DR Congo, Equatorial Guinea, Ghana, Guinea, Ivory Coast, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo and Uganda.[20,78,79] *B. invadens* is highly polyphagous and has been reported from over 30 plant species but the most preferred cultivated host plant is mango, *Mangifera indica* (Anacardiaceae). However, Marula, *Sclerocarya birrea* (Anacardiaceae) and the tropical almond, *Terminalia catappa* (Combretaceae) are the most infested non-cultivated hosts.[79,80] It is believed that a number of factors such as semiochemicals, color and shape of fruits mediate the attraction of flies to the host plants.

Semiochemicals play a major role in mediation of fruit fly behaviours, including host-searching, mate recognition, and selection of suitable oviposition sites.[13,69] Adult fruit flies detect odours from host fruits at a distance through olfaction and orientate upwind towards the locality of fruiting host trees.[81] Currently, parapheromones and food baits, such as hydrolyzed protein, fermenting sugars and yeast have been used for the control of fruit flies. However, these liquid lures lack potency, have limited field life and are difficult to handle.[31] One of the reported most powerful attractant of fruit flies is methyl eugenol, which has been used for detection, monitoring, control and management of male flies.[82,83] It is therefore worthwhile to evaluate and develop other attractants including host plant-derived

ones that are more potent but less hazardous, and that unlike the parapheromones could be used to manage both male and female fruit flies.

The objective of the present study was to evaluate the attractiveness of fruit volatiles of three host plants, namely *M. indica, T. catappa* and *S. birrea* to male and female *B. invadens*. In the present study, results on the behavioural responses of both male and female *B. invadens* to volatiles from fruits of the three hosts at different maturity levels that were tested in a dual choice olfactometer are presented.

2.2 Materials and Methods

2.2.1 Insects

Larvae of *B. invadens* were randomly collected from infested mango fruits that were picked from mango orchard at Nguruman (Long: 01° 48' 31 S, Lat: 36 ° 03' 34 E) and Embu (Long: 00° 29' 24 S, Lat: 37° 35' 31 E) in southwestern Kenya. The infested mangoes were then transferred into tyrosine rectangular containers (30 x 30 x 15 cm) with openings at the top that were covered by mosquito net materials to allow for aeration. The containers were kept in a rearing room in the insectary at the International Centre of Insect Physiology and Ecology (*icipe*), in Nairobi, Kenya. The larvae were left to develop in the infested native mango fruits and remained in the cage until they reached the fifth instar. They were then washed out with water and transferred into clean sterile plastic bowls containing sterilized sand that mimicked the soil conditions in the field to enable their pupation. After one week, pupae were washed from the sand, dried and kept in Petri-dishes in a Perplex cage

(50 x 50 x 50 cm) until the emergence of adult flies. The adult fruit flies were fed on an artificial diet (sugar and enzymatic yeast hydrolysate ultrapure, 3:1, USB Corporation, Cleveland, Ohio, USA), and water on pumice granules. The rearing room was maintained at a temperature of 28 ± 2 °C, relative humidity of 60-65 % and 12L:12D photoperiod.

2.2.2 Fruits

Three varieties of mango fruits, namely apple, sensation and Kent, were collected from either Embu or Nguruman. Fruits of marula and the Indian almond were collected from Nguruman. These served as sources of volatiles in the bioassays. The fruits for source of volatiles for analyses were categorized into three maturity stages; fruitlets, mature unripe and mature ripe. These fruits were used for up to a maximum of three days from the day they were collected from the field and were kept at ambient room temperature throughout the period.

2.2.3 Dual Choice Olfactometer

Behavioural observations were made in a glass dual choice flatbed wind tunnel (30 × 30 ×100 cm) equipped with a 4 inch-extractor fan on top of the mid section of the tunnel (Plate 2.1). The extractor fan drew the air from both ends at a speed of 27.6 cm/s. The air flow rate in the working section of the tunnel was maintained at 15 ml/s.

Compressed medical air (BOC gases, Kenya) from a cylinder was passed through activated charcoal and then split into two streams in order to supply air to the

opposite ends (source of volatiles and control) of the wind. The source of volatiles comprised of fruits held in flasks (2 L) that was connected to one end of the wind tunnel. The control was a similar empty (air) 2 L flask that was connected to the other end. Teflon® tubings (5 mm diameter) were used as connectors. The room temperature was maintained at 26 ± 2°C and relative humidity ranging between 59 and 65%.

The flies for bioassays were kept in a Perplex® cage (25 ×25 x 25 cm) and were allowed to acclimatize to the experimental room conditions for four hours prior to the tests. Male and female flies that were 12-15 day old after emerging (DAE) were tested separately with host fruit volatiles. The flies were introduced into the wind tunnel at the central part of the working section through an aperture with a cover. Flies were left for 10 minutes to choose between the side of the tunnel with air enriched with the fruit volatiles or the opposite side which was free of volatiles (control) and the responders recorded thereafter. The flies that flew upwind up to 50% of the distance from the point of release (25 cm of each side of the wind tunnel) into the atmosphere permeated volatiles or to the control were scored as responders. Flies that did not fly beyond 25 cm mark from the point of release were treated as 'non-responders' and were excluded from the statistical analyses. Ten flies were tested in each experiment. Each test with volatiles from a given fruit and from a particular maturity stage was replicated five times. At the end of each test, the wind tunnel was cleaned with acetone and the odour source and control were swapped to either side prior to commencement of another test. All assays were conducted between 10:00 and 16:00 hrs local time.

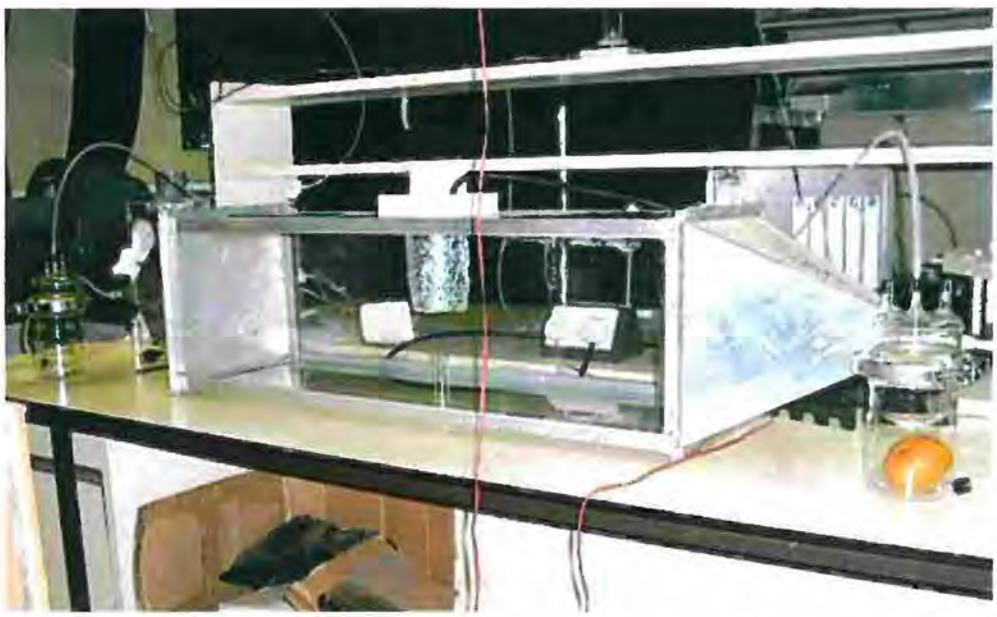


Plate 2.1 Setup of Dual Choice Olfactometer (By Fikira Kimbokota, 2009)

2.3 Data Analyses

For each test, the total numbers of flies responding in the olfactometer were pooled across replicates and then analysed using Chi-square (χ^2, $\alpha = 0.05$) (PROC FREQ, SAS Institute 1999-2000).[84] Flies that did not respond ('No response' group) were excluded from the analyses. ANOVA was used for multiple comparisons of the means of attractancy indices of the different maturity stages as well as for different sexes. Student-Newman-Keuls Test (SNK) was used to separate the means. Attractancy indices were calculated using the following formula: Attractancy Index (A.I.) = (Nt-Nc/Nt+Nc) ×100%, where, Nt = number of flies that flew into the test side and Nc = number of flies that flew into the control section.

2.4 Results

From the above experiments it was apparent that, in most cases both male and female *B. invadens* were attracted significantly more ($P < 0.05$) to the fruit volatiles than to the control. However, in some cases there was no significant different between control and tested volatiles. These include: (i) female flies responding to volatiles from fruitlets of mangoes of the apple variety ($\chi^2 = 2.6552$, $P = 0.7530$) and kent variety ($\chi^2 = 0.012$, $P = 0.9128$); (ii) male flies responding to volatiles from fruitlets of mangoes of the apple variety ($\chi^2 = 0.1006$, $P = 0.7511$), sensation variety ($\chi^2 = 0.1756$, $P = 0.6752$) and kent variety ($\chi^2 = 0.1045$, $P = 0.7465$). Volatiles from mature unripe and mature ripe fruits of the Indian almond, *T. catappa* were significantly more attractive ($P < 0.05$) to both male and female flies than to the control. However, mature unripe fruits of *S. birrea* were not significantly more attractive ($P > 0.05$) to either male or female flies compared to the control. In addition, volatiles from mature ripe fruits of this plant were significantly more attractive ($P < 0.05$) to both sexes than the control.

Dual choice comparisons between volatiles from mangoes of different varieties in olfactometric tests showed no significant differences ($P > 0.05$) in the responses of the flies to apple and sensation volatiles. However, the volatiles of fruits of these two mango varieties attracted significantly more female flies than those from kent mangoes ($\chi^2 = 10.9286$, $P = 0.0274$, $\chi^2 = 3.6000$, $P = 0.0578$, respectively) (Table 2.3). There was no significant difference ($P > 0.05$) when volatiles from fruits of different host plants were compared (Table 2.3).

Responses of fruit flies to volatiles from mature unripe and mature ripe fruits of a given host were significantly different ($F_{(df\ 2,85)} = 8.66$, n = 145, P = 0.003, Student-Newman-Keuls Test) from those of volatiles from the fruitlets, but not between each other, (Tables 2.1 and 2.2). However, there was no significant difference ($F_{(df\ 1,89)} = 2.59$, n =131, P = 0.1098, Student-Newman-Keuls Test) between the responses of male and female flies to the fruit volatiles.

Table 2.1 Attractancy Indices (A.I. ± SE %) of *Bactrocera invadens* to Volatiles from Fruits of Three Mango, *Mangifera indica* Varieties at Different Stages of Maturity in a Dual Choice Wind Tunnel

Mango variety	Maturity of the fruits	Attractancy Index (A.I. %)		Responders (%)	
		Females	Males	Females	Males
Apple	Mature ripe	82.74 ± 1.10	50.67 ± 0.80	58.00 ± 4.89	56.00 ± 2.45
	Mature unripe	49.40 ± 0.80	45.08 ± 0.94	75.71 ± 2.97	54.00 ± 7.48
	Fruitlet	34.28 ± 1.17	32.00 ± 1.23	61.67 ± 4.77	68.00 ± 4.89
Sensation	Mature ripe	77.62 ± 0.94	68.57 ± 1.12	40.00 ± 3.16	54.00 ± 4.00
	Mature unripe	68.67 ± 1.10	56.67 ± 1.32	64.00 ± 8.47	64.00 ± 8.12
	Fruitlet	39.33 ± 1.60	4.67 ± 1.47	56.00 ± 8.47	54.00 ± 5.48
Kent	Mature ripe	49.00 ± 1.13	39.52 ± 0.80	42.00 ± 3.74	46.00 ± 5.09
	Mature unripe	44.57 ± 0.78	38.10 ± 0.77	58.00 ± 7.35	62.00 ± 9.69
	Fruitlet	33.33 ± 1.32	0.67 ± 1.13	50.00 ± 7.07	44.00 ± 6.19

Table 2.2 **Attractancy Indices (A.I. ± SE %) of *Bactrocera invadens* to Volatiles from Fruits of *Terminalia catappa* and *Sclerocarya birrea* at Different Stages of Maturity in a Dual Choice Wind Tunnel**

Host	Maturity of fruits	Attractancy Index (A.I. %)		Responders (%)	
		Females	Males	Females	Males
Terminalia	Mature ripe	40.10 ± 0.40	33.20 ± 0.71	56.00 ± 7.68	47.00 ± 6.11
catappa	Mature unripe	14.67 ± 1.12	28.00 ± 1.38	48.00 ± 3.74	44.00 ± 6.00
Sclerocarya	Mature ripe	51.68 ± 0.51	42.29 ± 1.00	76.00 ±10.29	56.00 ± 8.90
birrea	Mature unripe	40.76 ± 0.97	30.19 ± 0.74	58.00 ± 4.89	62.00 ± 8.60

Table 2.3 **Attraction responses (± SE) of Female and Male *Bactrocera invadens* to Volatiles from Fruits of Different Hosts in a Dual Choice Wind Tunnel. Ten Insects Were Used Per Replicate and Each Test had Five Replicates. *Same Letters in the Same Row Indicate Values that are not Significantly Different (Chi-square test, $P < 0.05$)**

Sex	Host pairs	Response (%)	Responders (%)	Statistics*
Female	Apple	47.94 ± 9.12 a	72.00 ± 4.90	χ^2 = 2.2857, P = 0.1306
	Sensation	52.06 ± 9.12 a		
	Sensation	61.71 ± 8.00 a		χ^2 = 10.9286, P = 0.0274
	Kent	38.29 ± 8.00 b	56.00 ± 7.48	
	Apple	60.33 ± 8.33 a		χ^2 = 3.6000, P = 0.0578
	Kent	39.67 ± 8.33 b	56.00 ± 6.00	
	Apple	45.78 ± 6.61 a		χ^2 = 0.3103, P = 0.5775
	Indian almond	54.22 ± 6.61 a	58.00 ± 9.70	
	Apple	46.95 ± 6.67 a		χ^2 = 0.0345, P = 0.1306
	Marula	53.05 ± 6.67 a	58.00 ± 3.74	
	Marula			χ^2 = 0.5775, P = 0.5775
	Indian almond	54.00 ± 2.69 a	58.00 ± 3.74	

Table 2.3 Continued

Sex	Host pairs	Response (%)	Responders (%)	Statistics*
Male	Apple	44.05 ± 7.65 a		$\chi^2 = 0.2195$, P = 0.6394
	Sensation	55.95 ± 7.65 a	58.57 ± 7.04	
	Sensation	56.45 ± 9.18 a		$\chi^2 = 0.1176$, P = 0.7316
	Kent	43.55 ± 9.18a	68.00 ± 8.00	
	Apple	55.90 ± 4.21 a		$\chi^2 = 0.3103$, P = 0.5775
	Kent	44.10 ± 4.21a	58.00 ± 3.74	
	Apple	51.90 ± 5.58 a		$\chi^2 = 0.0286$, P = 0.8658
	Indian almond	48.10 ± 5.58 a	70.00 ± 4.47	
	Apple	57.52 ± 9.70 a		$\chi^2 = 0.7143$, P = 0.3980
	Marula	42.48 ± 9.70 a	70.00 ± 6.32	
	Marula	54.00 ± 7.48 a		$\chi^2 = 0.1538$, P = 0.6949
	Indian almond	46.00 ± 7.48 a	52.00 ± 2.00	

2.5 Discussion

The present study provides results of an evaluation of the attractiveness of odours from fruits of different levels of maturity from three hosts to both male and female fruit flies, *B. invadens* in a dual choice wind tunnel. Both female and male *B. invadens* showed higher responses to odours from mature unripe and ripe mangoes, *M. indica*, marula, *S. birea* and ripe Indian almond, *T. catappa* fruits than to those from fruitlets. This is in agreement with results of earlier studies on other species of fruit flies that select fruits that are at least half-ripe for oviposition.[85] However, some adult females oviposit in mature green fruits in the laboratory and larvae can

successfully develop in mature green and partially ripe fruits.[86] Cornelius *et al.* (2000)[17] have demonstrated that adult female oriental fruit flies, *Bactrocera dorsalis,* are highly attracted to odours from soft and ripe fruits. Preference of flies for mature and ripe fruits could be due to the presence of certain groups of compounds that are produced at these levels of maturity that are detected by the antennal olfactory receptors of the flies, thus facilitating location of their hosts. Furthermore, the presence of these compounds in the fruit volatiles may associatively be an indication to the gravid flies of the soft texture of the mature fruit whose skin can easily be punctured with the ovipositor. The presence of a certain group of compounds may also signal the availability of enough resources for the survival of the larval stages of the insect up to the time of pupation. The major hypothesis of the evolution of oviposition behaviour in insects is that, the females choose host plant species that maximize larval survival and development.[87]

The ability of *B. invadens* flies to be attracted to the volatiles emitted by fruits of three different hosts, *viz.* mango, *M. indica*, Indian almond, *T. catappa* and marula, *S. birrea* demonstrate the polyphagous character of this insect. Oviposition behaviour plays a critical role in the survival of insects and it necessitates some of them to have a broad range of hosts. Selection of suitable oviposition substrates influences the potential survival and development of larvae, although in these flies, it also reflects on the extent of economic losses caused by oviposition punctures and larval feeding. With regard to differences in the responsiveness of flies to fruit volatiles from different varieties of mangoes, these could be due to quantitative and qualitative differences in the composition of their volatiles. Pino and Mesa (2006)[23]

demonstrated quantitative and qualitative differences in the composition of volatiles among 20 different mango varieties. These compositional differences may be due to various factors, such as climate, soil content and other cultivation practices.[88]

Attraction of both male and female flies to crude fruit volatiles may be indicative of the presence of a number of components that are attractive to both sexes. A number of laboratory-cultured male oriental fruit flies, *B. dorsalis*, have been observed on trees with fruits when released in the field at dusk.[89] Host plants have also been reported to influence the sex pheromone biology of phytophagous insects.[90] For example, the search for mates by both sexes of *Rhagoletis* sp. is linked to the search for their host plants. Therefore, the cues used to locate a mate also play a role, either alone or with other cues (visual, tactile, and chemical) in the location of fruits on the host plant.[91,92]

Host plants play an important role in the synthesis of sex pheromones of some phytophagous insects through the acquisition of bioactive chemicals and the necessary chemical precursors for the pheromones via consumption, absorption or inhalation of host plant materials.[90] Many phytophagous insects aggregate at the primary feeding and oviposition sites and any other plant that is preferred by females.[90] What guides them is the chemical stimuli emanating from these plant species.[90] This ensures that there is high chance for mate location and mating and hence propagation of their generations. In other studies, observations have been made on *Ceatitis capitata* where both males and females were strongly attracted to citrus volatiles.[93] Shelly *et al.* (2001)[94] showed that the medfly, *C. capitata* males exposed to oranges performed significantly more copulations than non-exposed

males. Similarly, male medflies exposed to the bark and/or of fruits of guava tree, *Psidium guajava* L. had mating advantage over those that were deprived access to these substrates.[95]

The findings from these investigations indicate that, fruits of the three host release volatiles that have candidate attractants for both male and female *B. invadens.* This is a very significant finding since most attractants already in the market for fruit flies attract only males. One approach in fruit fly control programmes is the use of host-derived compounds. The next step in the study is to identify and characterize components in the volatiles of host fruits of the three plants that are attractive to *B. invadens*. This is the subject matter of the subsequent chapters of this thesis reported hereafter.

CHAPTER THREE

CHARACTERIZATION OF CANDIDATE KAIROMONES FOR *BACTROCERA INVADENS* (DIPTERA: TEPHRITIDAE) FROM *MANGIFERA INDICA* AND *SCLEROCARYA BIRREA* (ANARCADIACEAE)

Abstract

Head space volatiles from ripe and unripe fruits of mango, *Mangifera indica* and marula, *Sclerocarya birrea* were analysed by GC-MS. *S. birrea* represented higher quantities of alcohols (59.48 %) in the ripe fruit volatiles than in the unripe fruit volatiles while quantities of terpenes in volatiles of unripe fruits were higher (69.03 %) than in those of ripe fruits (4.21%). However, in *M. indica*, large quantities of terpenes were found in volatiles from both mature unripe (87.6%) and mature ripe (87.9 %) fruits. Comparison of GC-MS chromatographic profiles of ripe and unripe fruits of mango and marula showed quantitative differences with more constituents present in the ripe fruits.

Responses of the antennal olfactory receptors of male and female *B. invadens* were investigated using coupled gas chromatography-electroantennographic detector (GC-EAD) analysis with the fruit volatiles of the two plants. Eighteen EAG-active constituents were detected by antennae of both male and female flies in fruit volatiles of *S. birrea* while seventeen EAG-active peaks in the volatiles of *M. indica* were identified. The identified EAG-active compounds included terpenes, alcohols, esters and aldehydes.

3.1 Introduction

The mango tree, *Mangifera indica* L., (Anarcadiaceae) is an important source of fruits for inhabitants of the tropics and it is among the oldest cultivated fruit trees. It

originated from south eastern Asia where it had been domesticated for centuries before spreading to other parts of the tropical world.[22,96] The aroma of ripe fruits is one of the important attributes of quality that contributes to their overall flavour and makes them very popular among consumers. Due to this it has been one of the fruit crops of economic importance to both large and small scale growers.[22] However, production of high quality fresh mango fruits for international and domestic markets has been negatively affected by infestation by insect pests of which, fruit flies are very important. This is because gravid female flies puncture fruit skin to oviposit and subsequent feeding by larvae leads to decay of fruit and/or their premature drop.[6,9] Physiologically, mature unripe or ripe fruits are usually preferred, depending on the fruit fly species.[8]

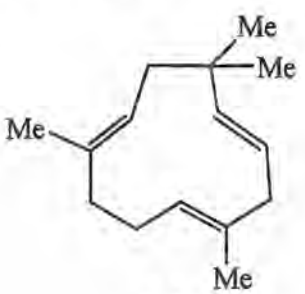
Host location by fruit flies that lead to widespread infestations is mediated by various cues such as visual, size, shape, colour and chemicals. Chemical cues play a major role since insects may respond to them from relatively long distances compared to the other cues. Thus, host-derived chemicals are candidate components for the development of attractant(s) for the control of pests.

A wide range of compounds have previously been identified from volatiles of mango fruits, including esters, lactones, mono- and sesquiterpenes as well as furanones.[23] Ocimene (**1.1**) or myrcene (**1.3**) has been reported to be the characteristic aroma compounds of the green mango depending on the variety.[24] Most of the insects use chemical cues to locate food, oviposition sites and these also facilitate make location.

Ceratitis cosyra has been reported to be probably attracted and oviposit on immature and mature green than ripe yellow mango fruits in the field.[25]

Marula, *Sclerocarya birrea* H. (Anarcadiaceae) is a medium-sized to large deciduous tree with an erect trunk and rounded crown and it is native to sub-Saharan Africa. It is one of the plants that played a major role in feeding people in ancient times and is of the African plants that are of growing commercial importance.[34] The plant is distributed throughout Africa with its southern most location being in the lowlands of KwaZulu-Natal (South Africa), from where it extends northwards through tropical Africa into Ethiopia and Sudan.[35]

Several products such as beer, juice, jam and jelly have been developed from the mesocarp of marula fruits and these have been successfully marketed, the most recent being a marula liqueur.[36] Head-space volatiles of the fruit pulp and the whole fruits (skin volatiles) were investigated and a number of compounds reported therein, including diterpenes, alcohols, hydrocarbons and esters, but not hydrocarbon monoterpenes.[37] The two major compounds in the fruit pulp were α-humulene (**1.24**) and β -caryophyllene (**1.25**).[37]



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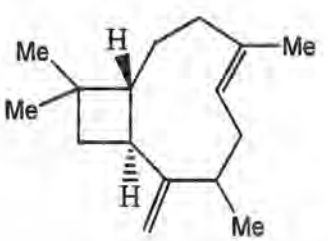

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Fruit production generally is highly threatened due to infestation by *B. invadens* female flies that lay eggs and cause fruit damage by consumption of mesocarp by feeding larvae. Current field control measures for fruit flies are generally based on insecticide cover sprays, bait sprays, physical control (fruit wrapping or bagging), cultural control (crop hygiene, early harvesting, use of resistant varieties), sterile insect technique (SIT), behavioural control (traps with different colours, shapes, odours, male annihilation, protein bait sprays), and biological control.[70] However, none of these approaches focuses directly on reducing oviposition by using deterrents or attractants to alter behavioural responses of females to surfaces of host fruit.[97]

In the study described in this Chapter, investigations were based on the above stated background, with the aim of identifying and characterizing kairomones from *M. indica* and *S. birrea* that are the main hosts of *B. invadens*. Possible development of cost-effective and environmentalfriendly host-derived lures may provide farmers with a nontoxic method for monitoring and controlling populations of the mango-infesting fruit fly,

B. invadens.

3.2 Materials and Methods

3.2.1 Insects and Study Area

The insects and study areas (Nguruman and Embu, in Kenya) were as previously described in Chapter 2.

3.2.2 Collection of Volatiles

An efficient air entrainment system was used. The volatiles were collected from five *Sclerocarya birrea* ripe fruits (ca. 200 g) that were placed in a glass chamber (46 cm long × 19 cm wide, RodaViss Chambers, ARS, Gainesville, FL, USA). For the mangoes, *Mangifera indica* volatiles were trapped overnight from mature unripe and ripe fruits (ca. 400g of each). Briefly, the air entrainment apparatus was set as follows: charcoal-filtered and humidified air was passed over the fruits and then through pre-conditioned Super-Q adsorbent (30 mg, Grace, Alltech, Deerfield, IL, USA) traps for 13 h at room temperature. Then air was pumped out at a flow rate of 360 $mlmin^{-1}$. Each trap was eluted with 150 µl HPLC-grade (Aldrich) forced through the absorbent with filtered nitrogen gas under ice.

3.2.3 Coupled Gas Chromatography-Electroantennographic Detector (GC-EAD) Analysis

Coupled GC-EAD analysis (Plate 3.1) was used to determine the compounds in the trapped complex mixture of host fruit volatiles that stimulated antennal olfactory receptors of female and male flies.

3.2.3.1 Antennal Preparation

The antennal preparation for recording was performed as described by Cosse *et al.*[117] and Du and Millar.[118] Antennae from gravid female flies (12-15 day-old) were used for the analysis. The head of an insect was cut off and a reference electrode was

inserted into its base with a glass capillary tube filled with Beadle Ephrussi Ringer (145 mM NaCl, 1.87 mM KCl, 0.81 mM $CaCl_2$, 2.3 mM. $NaHCO_3$, 0.55 mM $NaHPO_4$). To complete the circuit, the distal end of the antenna was inserted into the tip of the recording glass capillary electrode connected by coaxial cables to a UN 05 amplifier (Syntech) and the recording equipment.

3.2.3.2 GC-EAD Analysis

For coupled GC-EAD tests, aliquots (5-10 µl) of volatile extracts were injected splitless into a HP 5890 Series II GC equipped with an FID detector and an appropriate capillary column (Ultra 1 cross-linked methylsilicone capillary column 25 m × 0.31 mm (i.d.) × 0.025 mm (film thickness)). Nitrogen was used as the carrier gas. The column effluent was split equally with a glass press-fit Y-tube to 2 deactivated fused silica capillaries (50 cm × 0.25 mm i.d.) with one line going to the GC detector and the other through a heated (150 °C) transfer line into a steel stimulus delivery tube delivering moistened air over an antennal preparation of the fruit fly. The injector, in splitless mode, and flame ionization detector (GC-FID) were kept at 250°C and 270°C, respectively. The oven was kept at 40°C for 3 min, and the temperature was then raised by 10°C min^{-1} to a final temperature of 250°C, were maintained for 8 min. The FID and EAG signals were recorded simultaneously on an EAD card (Syntech) in a PC (Dell Optiplex GX280) and the two signals were also viewed on a monitor. Three samples of volatile collections were analysed for each host plant.

Plate 3.1 Setup of Coupled Gas Chromatography-Electroantennographic Detector (GC-EAD) (By Fikira Kimbokota, 2009)

3.2.4 Coupled Gas Chromatography-Mass Spectrometric (GC-MS) Analysis

Gas chromatography-mass spectrometric identification of the compounds was carried out on an Agilent Technology 7890A GC coupled to 5975 MSD (Plate 3.2). One µl of each sample of volatiles was used for the analysis. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV and emission current of 34.6 µA. The temperature of the source was held at 230 °C (ion source), 150 °C (Quadropole) and multiplier voltage was 1106 V. The pressure of the ion source was held at 7 $x10^{-6}$ mBar. The spectrometer had a scan cycle of 3 scans per 2 seconds. The instrument was calibrated using heptacosa (perfluorotributylamine) $[CF_3(CF_2)_3]_3N$ (Apollo scientific Ltd. UK). HP-5 GC capillary column, 30 m x 0.25 mm (i.d) x 0.25 µm (film thickness) supplied by J & W Scientific was used. The GC-

MS was linked to a computer with MS library (NIST & WILEY). The compounds were identified by comparing their MS with those of authentic samples or with library data and their fragmentation patterns. For each sample, an aliquot of 1 µl into which an internal standard, methyl salicylate or ethyl nonanoate (equiv. 3 and 4.23 ng respectively; Sigma Aldrich, USA) had been added was analysed.

Plate 3.2 Setup of Coupled Gas Chromatography-Mass Spectrometer (GC-MS) (By Fikira Kimbokota, 2009)

3.3 Results and Discussion

3.3.1 Volatile Constituents Identified from Ripe and Unripe *Mangifera indica* Fruits

In gas chromatographic analyses, the volatiles of both mature unripe and ripe mango fruits were found to constitute of terpenes, esters, hydrocarbons, carbonyls and others. Monoterpene and sesquiterpene hydrocarbons were found to be the most abundant constituents representing 87.6 and 87.9 % of the total volatile content for

mature unripe and mature ripe fruits, respectively. The esters were the next in abundance and constituted 3.14 and 1.99 % in the volatiles from mature ripe and unripe fruits, respectively (Figure 3.3, Table 3.1). These compounds have also been found in volatiles of other mango cultivars.[98,99]

The other compounds in the volatiles from unripe and ripe mature mangoes constituted 2.59 and 4.17% of the headspace, respectively. The major compound in the volatile mixtures from both mature fruits was α-pinene (28.8 % in the unnripe and 28.9 % in ripe fruit volatiles), followed by myrcene (24.9 %) for mature ripe and δ-guaiene (10.2 %) for mature unripe fruits (Table 3.1 and 3.2). With regard to the esters, ethyl octanoate was present at highest proportion at 2.02 % in the volatiles from mature ripe fruits followed by ethyl hexanoate at 0.29 %.

Monoterpene and sesquiterpene hydrocarbons have been reported to be major volatile components representing 70-90 % of total volatiles in all mango cultivars.[100] Differences in profiles of volatile compounds among different cultivars of mango have been reported for a number of mango varieties.[98,99,101,102] Recently, head space volatiles from flowers, as well as green and ripe mango fruits of variety Ataulfo from Soconusco, Chiapas (Mexico), were collected using Solid Phase Microextraction (SPME). Volatiles of these fruits were found to consist of a complex mixture of monoterpenes and sesquiterpenes with the major constituents being 3-carene, α-pinene, myrcene, limonene, terpinolene, β-selinene, and a sesquiterpene tentatively identified as germacrene D.[103]

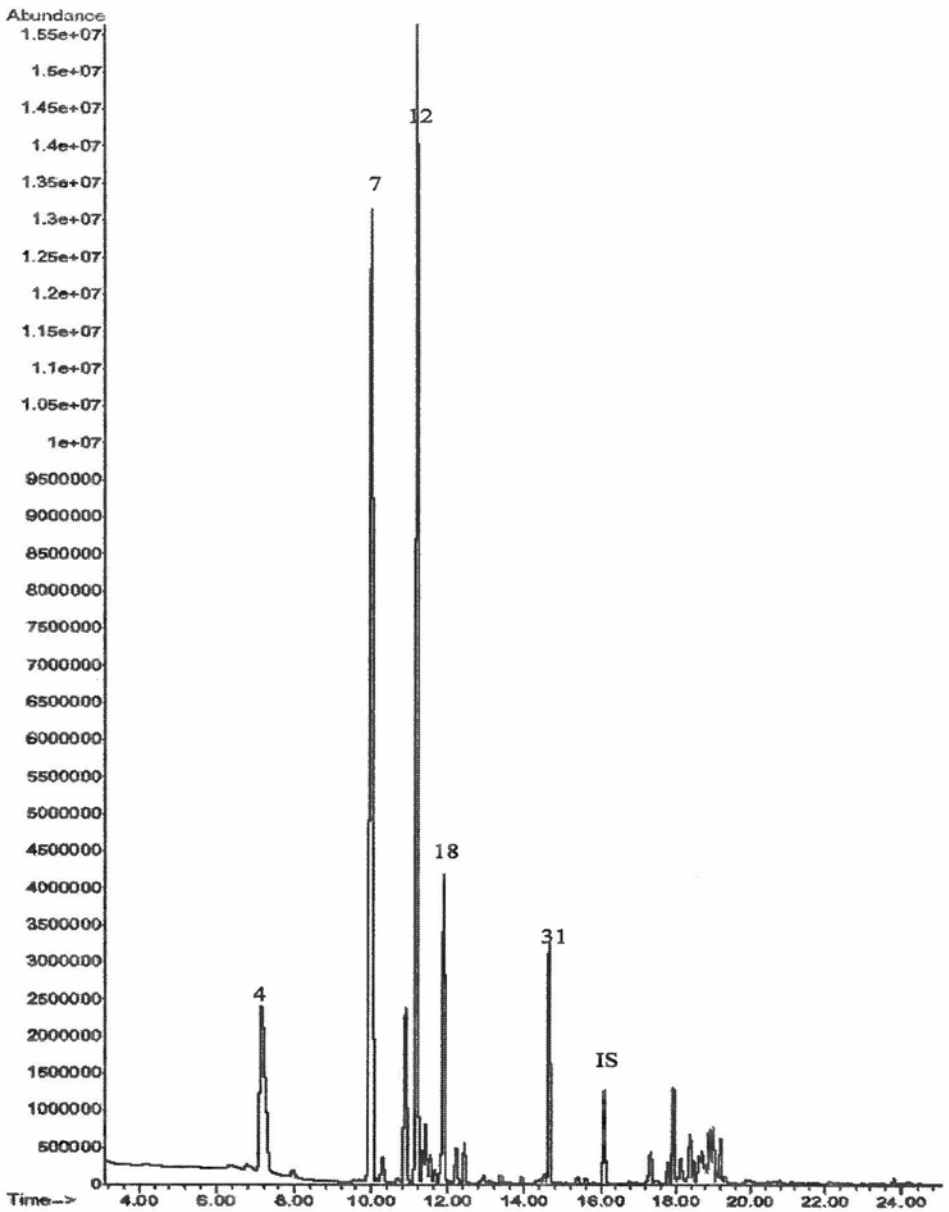


Figure 3.1 **Representative Gas Chromatogram of Volatile Constituents Collected from Mature Ripe Fruits of *M. indica*. Numbers Indicate the Identified Compounds as in Table 3.1.**

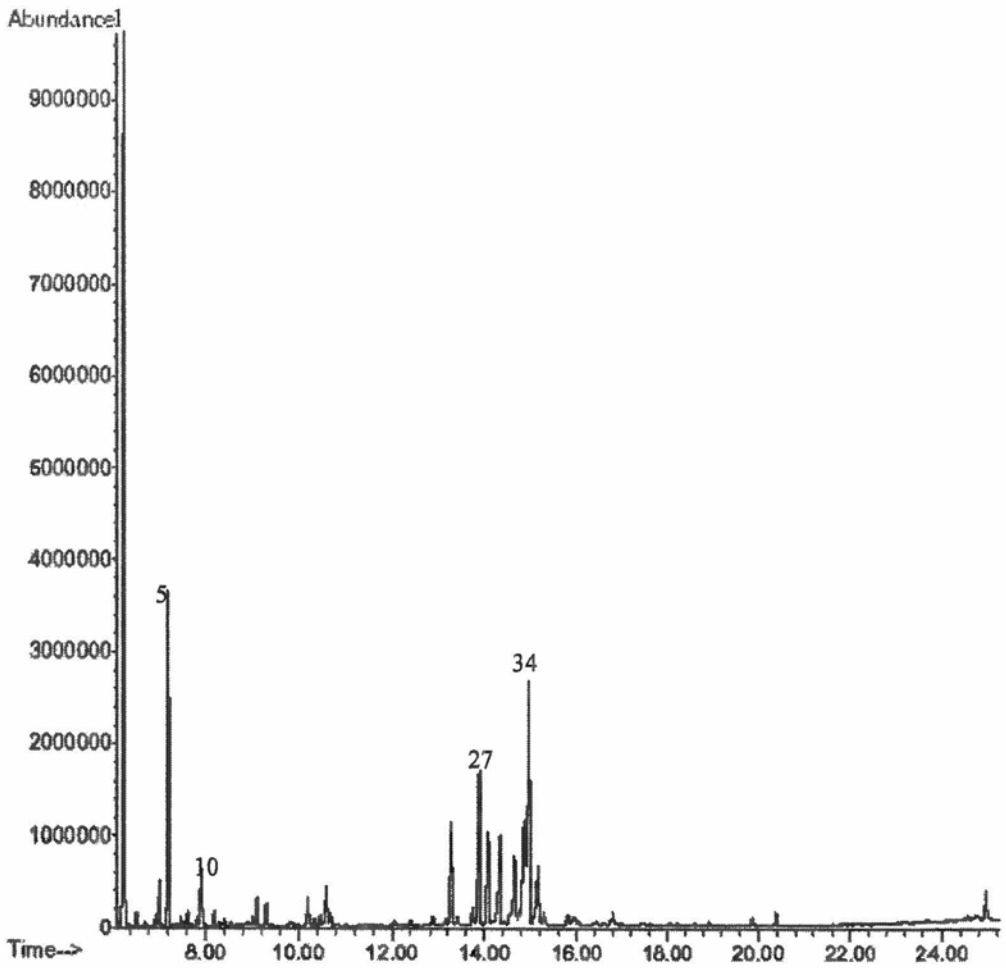


Figure 3.2 **Representative Mas Chromatogram of Volatiles Trapped from Mature Unripe Mango, *M. indica* Fruit. Peaks of Identified Compounds are Numbered as in Table 3.2.**

Table 3.1 Percent Amounts of Compounds in Volatiles of Mature Ripe Mango, *M. indica* Fruits

S/N	Compound	R.T. (min)	% Composition
1	3-hydroxy-2-butanone	4.16	0.04
2	1-methyl-1,4-cyclohexadiene	6.38	0.24
3	ethyl butyrate	6.78	0.23
4	2,3-dihydrothiopyran-4-one	7.16	1.19
5	neryl propionate	9.56	0.02
6	tricyclene	9.69	0.06
7	α-pinene	9.96	28.84
8	ethyl tiglate	10.14	0.07
9	camphene	10.28	0.80
10	*p*-mentha-1,5,8-triene	10.66	0.03
11	β-pinene	10.86	4.57
12	myrcene	11.17	24.35
13	ethyl hexanoate	11.33	0.29
14	α-phellandrene	11.42	1.49
15	δ-3-carene	11.53	2.31
16	α-terpinene	11.64	0.33
17	1-methyl-4-(1-methylethyl)- benzene	11.80	0.12
18	sabinene	11.89	6.65
19	*cis*-ocimene	12.02	0.06
20	*trans*-β-ocimene	12.20	0.82
21	γ-terpinene	12.43	0.70
22	terpinolene	12.94	0.30
23	nonyl aldehyde	13.17	0.01
24	*(E/Z)*-4,8-dimethyl-1,3,7-nonatriene	13.39	0.10
25	methyl octanoate	13.50	0.01
26	*allo*-ocimene	13.59	0.02
27	2-aminoimidazole	13.90	0.19
28	ethyl benzoate	14.29	0.02
29	β-phellandrene	14.40	0.10
30	*(Z)*-ethyl-4-octenoate	14.51	0.08
31	ethyl octanoate	14.62	2.02
32	decanal	14.78	0.01
33	berbenone	14.91	0.03
34	2-methyl-ethyloctanoate	15.07	0.01
35	*(E)*-ethyl-2-octenoate	15.36	0.07
36	γ-octanolactone	15.58	0.06
37	ethyl nonanoate (IS)	16.08	1.75
38	bicycloelemene	16.75	0.06
39	α-cubebene	16.93	0.07
40	α-copaene	17.31	1.15
41	ethyl decanoate	17.42	0.02
42	tetradecane	17.49	0.06

Table 3.1 Continued

S/N	Compound	R.T. (min)	% Composition
43	germacrene-D	17.49	0.06
44	α-gurjunene	17.78	0.71
45	β-caryophyllene	17.91	3.04
46	β-cubebene	18.03	0.18
47	α-guaiene	18.12	1.02
48	α-humulene	18.36	1.87
49	aromadendrene	18.45	0.77
50	α-amorphene	18.61	0.98
51	β-cubebene	18.70	0.95
52	β-selinene	18.76	0.77
53	γ-gurjunene	18.90	1.88
54	δ-guaiene	18.99	1.83
55	γ-cadinene	19.10	0.19
56	δ-cadinene	19.17	0.34
57	1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)- naphthalene	19.30	0.26
58	elemicin	19.46	0.02
59	ethyl dodecanoate	19.88	0.11
60	neoalloocimene	19.97	0.16
61	γ-gurjunene	20.09	0.08
62	cadina-1,4-diene	20.47	0.11
63	α-amorphene	20.62	0.11
64	2-pentadecanone	21.09	0.07
65	2-tridecen-1-ol,	21.27	0.01
66	ethyl tetradecanoate	22.10	0.11
67	farnesol	24.14	0.07
68	1-methylethyl hexadecanoate	24.43	0.04

Table 3.2 Percent Amounts of Compounds in Volatiles of Mature Unripe Mango, *M. indica* fruits

S/N	Compound	R.T. (min)	% Composition
1	α-pinene	6.26	28.86
2	camphene	6.54	0.41
3	sabinene	6.95	0.29
4	β-pinene	7.02	1.19
5	myrcene	7.21	8.31
6	α-phellandrene	7.48	0.25
7	δ-3-carene	7.59	0.17
8	1,4-dichloro-benzene	7.62	0.41
9	2-ethyl hexanol	7.83	0.29
10	sabinene	7.92	2.12
11	*trans*-β-ocimene	8.19	0.39
12	γ-terpinene	8.41	0.25
13	methyl benzoate	9.01	0.33
14	nonanal	9.11	0.83
15	*(E)*-4,8-dimethyl-1,3,7-nonatriene	9.30	0.66
16	ethyl benzoate	10.21	0.93
17	l-4-terpineol	10.35	0.22
18	naphthalene	10.48	0.38
19	methyl salicylate (IS)	10.61	1.64
20	decanal	10.69	0.45
21	tridecane	12.05	0.08
22	α-cubebene	12.87	0.32
23	hexyl isobutyrate	13.16	0.27
24	α-copaene	13.27	4.06
25	tetradecane	13.41	0.36
26	α-gurjunene	13.74	0.70
27	β-caryophyllene	13.88	6.31
28	α-guaiene	14.08	3.76
29	α-humulene	14.33	4.24
30	α-amorphene	14.54	0.49
31	γ-gurjunene	14.65	3.33
32	β-selinene	14.75	0.55
33	α-selinene	14.85	6.02
34	δ-guaiene	14.95	10.18
35	δ-cadinene	15.14	3.03
36	1-heptadecene	15.82	0.50

Table 3.2 Continued

S/N	Compound	R.T. (min)	% Composition
37	tetradecene	16.80	0.65
38	1-methylethyl hexadecanoate	20.39	0.47
39	heptadecane	25.74	0.41

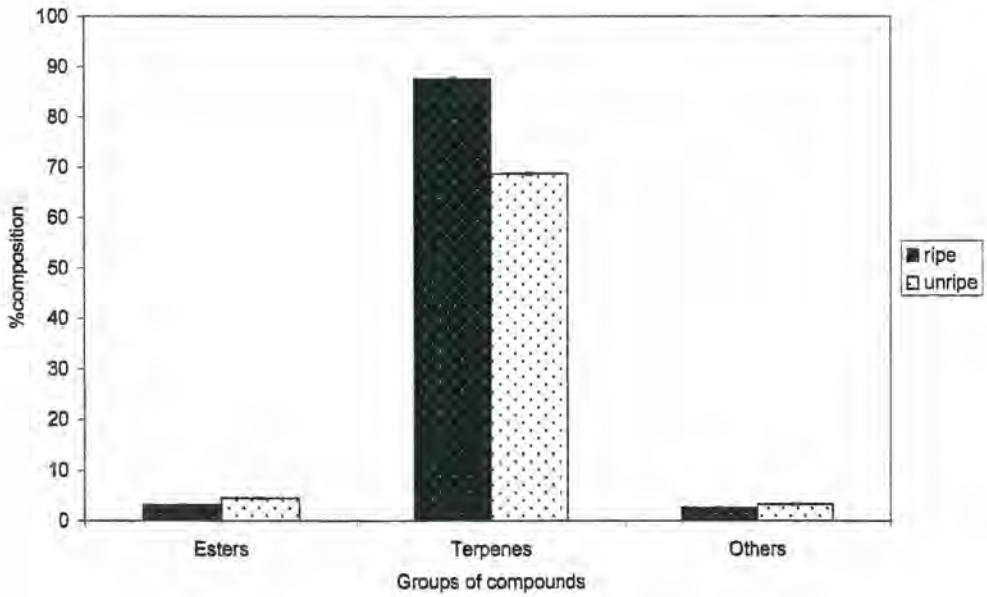


Figure 3.3 **Groups of Compounds Identified in Volatiles from Mature Unripe and Mature Ripe Fruits of Mango, *M. indica*. N = 3 Samples of Volatiles.**

3.2.1 Components Identified in Volatiles from Ripe and Unripe Marula, *Sclerocarya birrea* Fruits

About seventy nine compounds representing 96.95 % of the total composition were identified in the head-space of the ripe fruits in the laboratory trapping (Tables 3.3 and 3.4). Alcohols (59.48 %), esters (8.58 %), hydrocarbons (18.36) and terpenes (4.21 %) were the most dominant compounds in the ripe fruits (Figure 3.4). The

major compounds included *(Z)*-3-decen-1-ol (44.65 %), *(Z)*-cyclododecene, (5.42 %) and ethyl isovalerate (5.09 %). The major alcohol, ester and terpene were *(Z)*-decen-1-ol, (44.65 %), ethyl isovalerate (5.09 %) and camphene (1.21 %), respectively. On the other hand, volatiles of unripe fruits were dominated by terpenes (69.03 %), followed by hydrocarbons (5.36 %) (Figure 3.4). Of these, *trans*–β-ocimene (61.4 %) was the major constituent, followed by linalool (2.76 %) (Table 3.4).

Recently, a number of compounds have been reported from pulp and intact fruits, in which esters and hydrocarbons were found to be the most dominant compounds.[37] The major compounds included heptadecene (16.1%), benzyl-4-methylpentanoate (8.8%), benzyl butyrate (6.7%), *(Z)*-13-octadecenal (6.2%) and cyclopentadecane (5.7%). The major alcohols were *(Z)*-3-decen-1-ol (8.4%) and 6-dodecen-1-ol (3.8%), while the major aldehyde was 11-hexadecanal (4.4%).[37] Some of these compounds were not found in the present investigations; on the other hand, terpenes identified in the present study were not reported in the previous investigations. Some of these compounds may contribute to the attraction of *B. invadens* to M. indicafruits.

Table 3.3 Percent Amounts of Compounds in Volatiles from Ripe Marula, (*S. birrea*) Fruits

S/N	Compound	R.T. (min)	% Composition
1	ethyl propionate	4.25	0.16
2	ethyl isobutyrate	5.60	0.23
3	ethyl isovalerate	8.17	5.09
4	hexanol	8.55	0.01
5	isopropyl 2-methylbutanoate	8.98	0.09
6	isopropyl pentanoate	9.16	1.47
7	α-pinene	9.96	0.07
8	propyl valerate	10.34	0.02
9	heptanol	10.77	0.02
10	myrcene	11.15	0.04
11	octanal	11.40	0.23
12	α-terpinene	11.64	0.03
13	*p*-cymene	11.80	0.04
14	limonene	11.87	0.05
15	*trans*-β-ocimene	12.22	0.67
16	(*Z*)-3-octen-1-ol	12.34	0.28
17	γ-terpinene	12.40	0.05
18	octanol	12.58	1.09
19	octyl formate	12.61	2.18
20	non-1-en-3-ol	12.76	0.11
21	nonan-3-one	12.87	0.03
22	(*E*)-4-undecene	12.99	0.61
23	linalool	13.10	0.61
24	isoamyl-2-methylbutyrate	13.19	0.25
25	(-)-1-methyl-2-norcaranone	13.99	0.05
26	nonanol	14.24	0.09
27	ethyl benzoate	14.26	0.08
28	1,3,5,8-undecatetraene	14.33	0.02
29	*cis*-3-hexenyl butyrate	14.46	0.04
30	methyl salicylate	14.67	0.13
31	decanal	14.78	0.15
32	isopropyl benzoate	14.82	0.13
33	2-methylethyl octanoate	15.07	0.03
34	*(Z)*-3-hexenyl 2-methylbutanoate	15.16	0.34
35	*(E)*-3-decen-1-ol	15.43	0.76

Table 3.3 Continued

S/N	Compound	R.T. (min)	% Composition
36	*(Z)*-3-decen-1-ol	15.54	44.65
37	decanol	15.74	1.67
38	dodecanol	15.92	1.03
39	ethyl nonanoate (IS)	16.08	2.29
40	cyclodecene	16.28	0.02
41	*trans*-dodec-5-enal	17.38	3.38
42	2,4-decadienal	16.41	0.1
43	cyclododecane	16.77	0.02
44	9-decen-1-ol	16.91	0.02
45	(*E*)-4-tetradecenyl acetate	17.00	0.01
46	bicyclo[3.3.0]oct-1-ene	17.06	0.02
47	5-dodecen-1-al	17.38	2.82
48	methyl eugenol	17.58	0.68
49	2-octyne	17.82	0.01
50	*trans*- β-caryophyllene	17.91	0.62
51	hexyl-2-methylbutyrate	17.98	0.03
52	α -bergamotene	18.05	0.14
53	camphene	18.21	1.21
54	5-dodecenol	18.27	5.42
55	bicyclo[5.1.0]octane	18.29	2.65
56	cyclopentadecane	18.54	3.74
57	1-hexadecanol	18.52	2.15
58	pentadecane	18.74	3.45
59	(*E,E*)-α-farnesene	18.90	0.49
60	*cis*-calamenene	19.19	0.11
61	δ-cadinene	19.17	0.04
62	nerolidol	19.57	0.04
63	*cis*-3-hexenyl benzoate	19.71	0.45
64	13-tetradecenal	19.91	0.55
65	delican	20.15	0.48
66	2-methyldecane	20.51	0.03
67	1,13-tetradecadiene	20.78	0.12
68	8-heptadecene	20.87	1.9
69	heptadecane	21.07	0.25
70	isopentyl benzoate	21.27	0.03
71	hexadecane	21.56	0.03
72	13-tetradecenal	22.17	0.46
73	hexadecanal	22.37	0.07

Table 3.3 Continued

S/N	Compound	R.T. (min)	% Composition
74	tetradecanal	22.37	0.12
75	nonadecane	23.20	0.13
76	linoleic acid	24.23	0.11
77	palmitic acid	24.41	0.08
78	heneicosane	26.87	0.08

Table 3.4 Percent Amounts of Compounds Identified in Volatiles from Unripe Marula, *S. birrea* fruits

S/N	Compound	R.T. (min)	% Composition
1	*cis*-ocimene	12.02	0.90
2	*trans*–β-ocimene	12.23	61.40
3	linalool	13.10	2.76
4	*m*-cymene	13.61	1.51
5	*(Z)*-3-hexenyl-2-methylbutanoate	15.18	0.44
6	*(E)*-3-decen-1-ol	15.50	0.72
7	ethyl nonanoate (IS)	16.08	16.40
8	β-caryophyllene	17.91	1.10
9	1-ethyl-2-methylcyclododecane	18.54	1.22
10	pentadecane	18.74	2.03
11	*(E,E)*-α-farnesene	18.90	1.36
12	*cis*-3-hexenylbenzoate	19.71	0.48
13	hexadecane	19.95	0.82
14	8-heptadecene	20.87	0.62
15	heptadecane	21.09	0.67
16	hexadecanoic acid	24.43	0.20

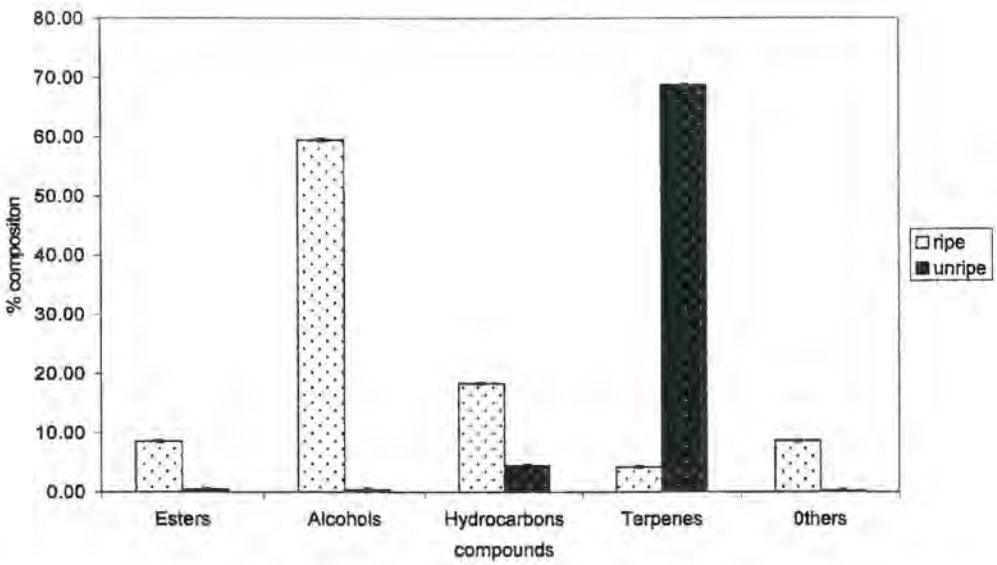
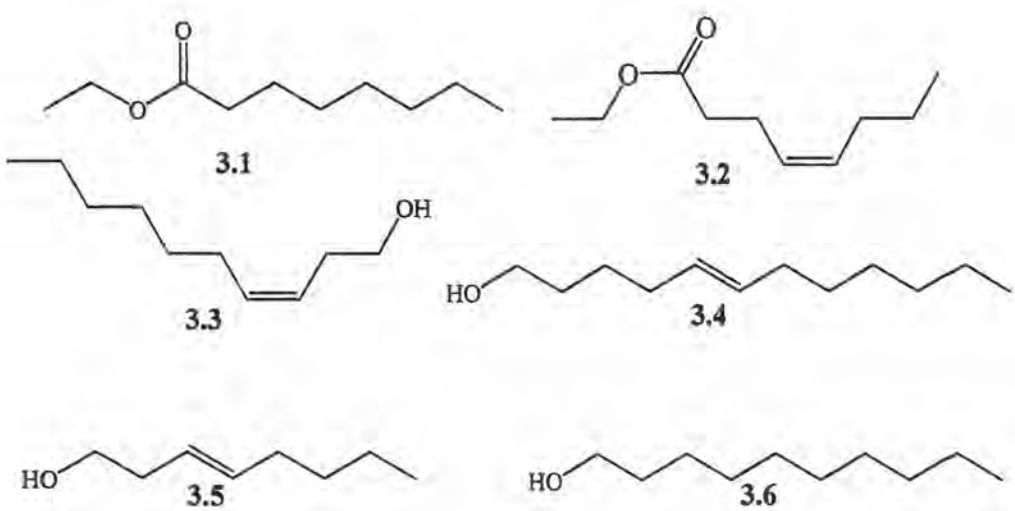


Figure 3.4 **Percent Amounts of Groups of Compounds in Volatiles from Ripe and Unripe Fruits of Marula, *S. birrea*. N = 2 Volatile Samples Analysed.**

3.3.2 Mass Spectral Features of Some Compounds from *M. indica* and *S. birrea*



3.3.2.1 Ethyl octanoate (3.1)

The MS of compound **3.1** showed a molecular ion peak at *m/z* 172.1 corresponding to the formula $C_{10}H_{20}O_2$. The MS exhibited a base peak at *m/z* 88, resulting from McLafferty rearrangement (Figure 3.7). As for most ethyl esters the MS of this compound consisted of fragmentation peaks at *m/z* 101 and 73 due to γ and α cleavages, respectively. A cluster of peaks 14 mass units apart, corresponding to loss of methylene units along the aliphatic part of the compound were observed in the MS.

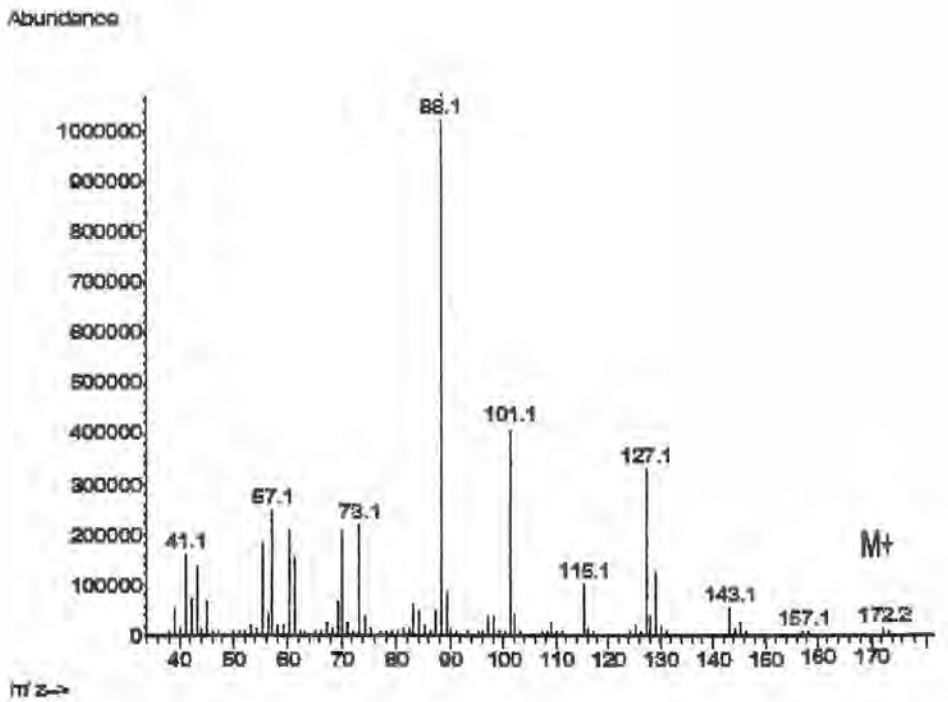
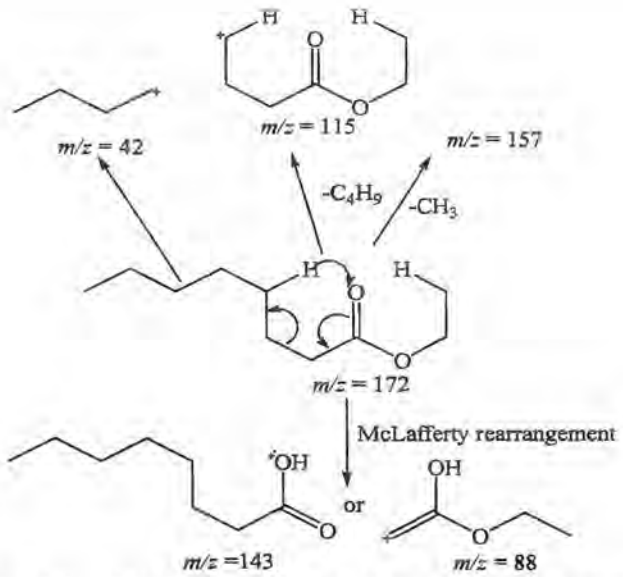
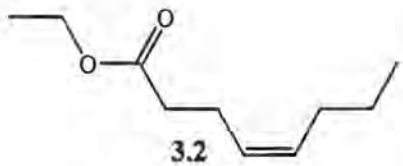


Figure 3.7 Mass Spectrum of ethyl octanoate (3.1)



Scheme 3.1 MS Fragmentation Pattern for ethyl octanoate (3.1)

3.3.2.2 Ethyl *cis*-4-octenoate (3.2)



3.2

The MS of compound **3.2** consisted of a molecular ion peak at *m/z* 170.1 corresponding to the formula $C_{10}H_{18}O_2$. The MS of this compound was very similar to that of **3.1**. Thus, the MS exhibited McLafferty rearrangement, producing an ion peak at *m/z* 88. The MS also indicated fragmentation ion peaks due to α and γ cleavage appearing at *m/z* 73 and 101 respectively. The MS of acyclic alkenes are characterised by clusters of peaks at 14 mass unit intervals. However, the peaks with composition C_nH_{n-1} and C_nH_{2n} are more intense than C_nH_{2n+1}.[104] Such a fragmentation pattern was also indicated in the MS of compound **3.2** (ion peaks at *m/z* 55 and 69

which correspond to ions $C_4H_8^+$ and $C_5H_9^+$, respectively). In the MS fragmentation process, double bonds would normally favour allylic cleavages to give resonance stabilized allylic carbocation.[101] Thus, in the case of compound **3.2** that has a double bond at C-4 such a fragmentation process was indicated in the MS, as shown by the appearance of a peak at *m/z* 96 corresponding to ion $C_7H_{12}^+$. The MS fragmentation pattern is depicted in Scheme 3.2 and is consistent with structure **3.2**

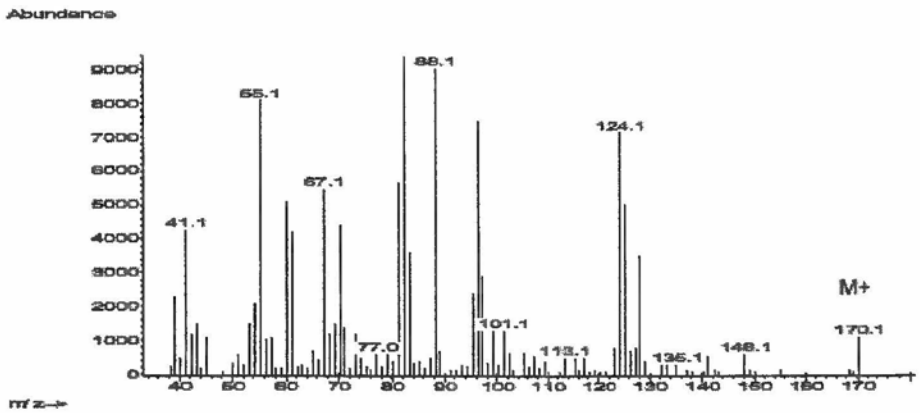
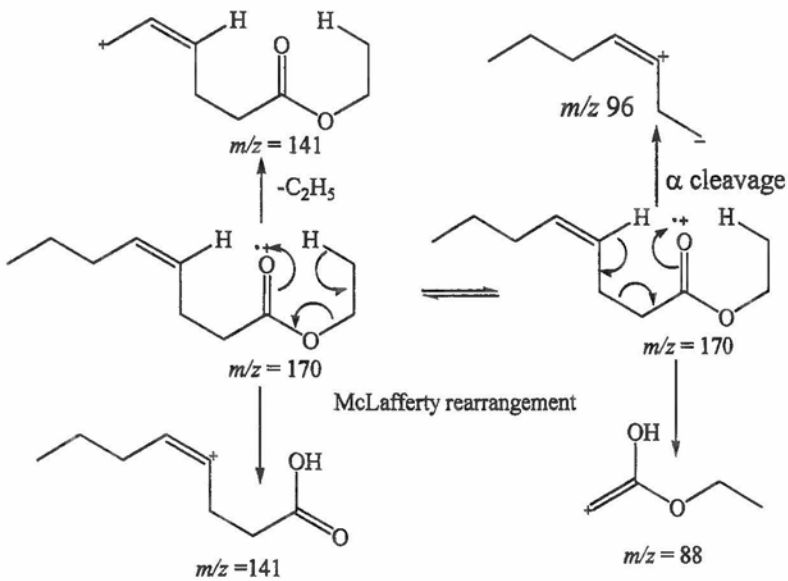
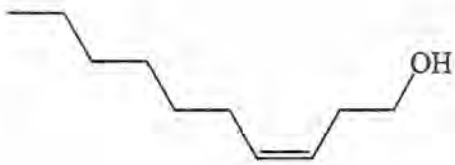


Figure 3.8 Mass Spectrum of ethyl *cis*-4-octenoate (3.2)



Scheme 3.2 MS Fragmentation Pattern for ethyl *cis*-4-octenoate (3.2)

3.3.2.3 *(Z)*-3-Decen-1-ol (3.3)



3.3

The MS of compound **3.3** indicated a very weak molecular ion peak at *m/z* 156.2, corresponding to the formula $C_{10}H_{20}O$. The base peak was observed at *m/z* 81 and this was consistent with the formula $C_{10}H_{18}$. The general characteristic of the MS of alcohols is the loss of a water molecule giving a peak at 18 atomic mass units below the molecular ion. Furthermore, primary alcohols having a four carbon chain or longer are prone to lose of a water molecule from the molecular ion through the formation of a cyclic intermediate.[105] This type of fragmentation was observed for compound 3.3, whose MS indicated a peak at *m/z* 138 corresponding to the fragment $C_{10}H_{18}$ in which the proton lost in this fragmentation would come not from an adjacent C atom, but from a position several C atoms away, removed typically through the formation of a six-membered cyclic intermediate (Scheme 3.3). The subsequent loss of ethene would produce a fragment ion whose peak appeared at *m/z* 110, being due to the formula C_8H_{14}. Furthermore, a subsequent allylic cleavage would lead to the formation of a fragment ion whose peak appeared at *m/z* 70 (C_5H_{10}, Scheme 3.3). The MS fragmentation process as summarized in Scheme 3.3 is consistent with structure **3.3**.

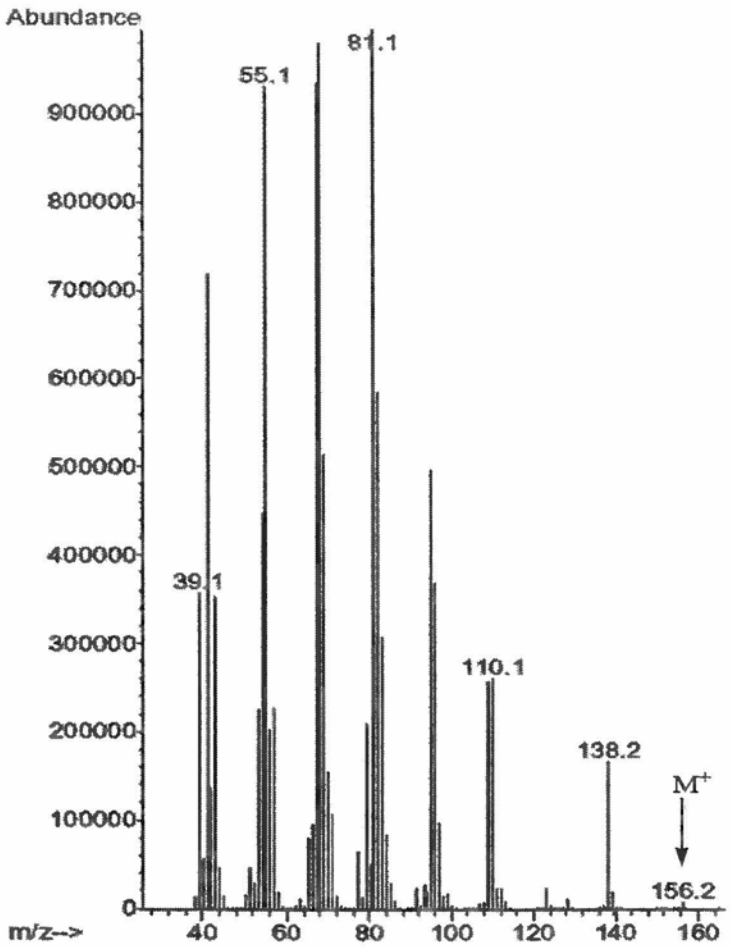
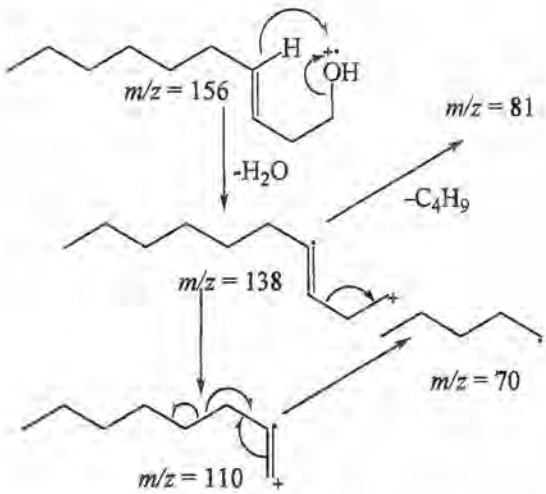


Figure 3.9 **Mass Spectrum of *(Z)*-3-decen-1-ol (3.3)**



Scheme 3.3 MS Fragmentation Pattern for *(Z)*-3-decen-1-ol (3.3)

3.3.2.4 5-Dodecen-1-ol (3.4)

3.4

The MS of compound **3.4** indicated a weak molecular ion peak at *m/z* 184.2 corresponding to the molecular formula $C_{12}H_{24}O$ which upon cleavage of a water molecule would have yielded the fragment ion whose peak appeared at *m/z* 166. Subsequent loss of ethane would have yielded the fragment ion whose peak appeared at *m/z* 138. Another cleavage of ethane would have produced a fragment ion whose peak was observed at *m/z* 110 while loss of C_5H_7 would produce a fragment ion whose peak was observed at *m/z* 67. The detailed fragmentation process is shown in Scheme 3.4 and it is in agreement with structure **3.4**.

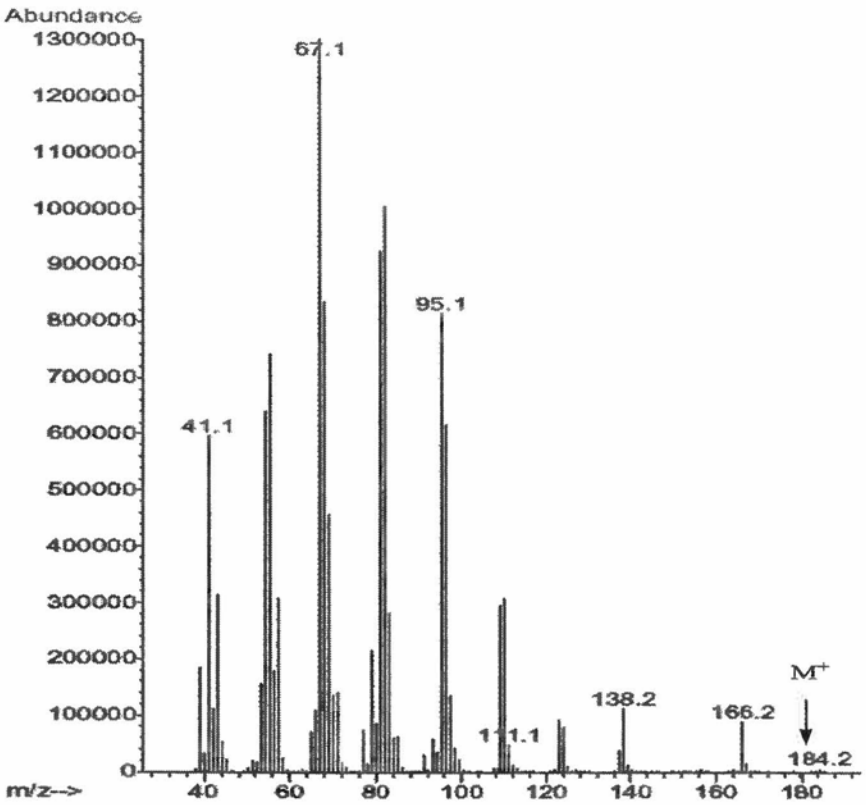
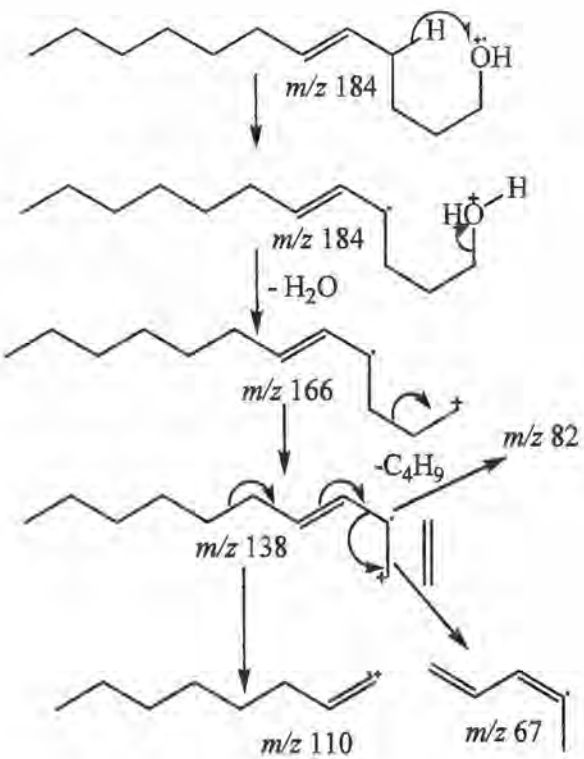
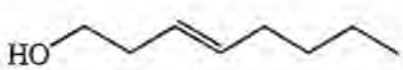


Figure 3.10 Mass Spectrum of 5-dodecen-1-ol (3.4)



Scheme 3.1 MS Fragmentation Pattern for 5-dodecen-1-ol (3.4)

3.3.2.5 *(Z)*-3-Octen-1-ol (3.5)



3.5

The MS of compound **3.5** indicated a very weak peak at *m/z* 128.1 corresponding to the molecular ion of formula C_4H_7, while the base peak was observed at *m/z* 55 corresponding to the formula $C_8H_{16}O$. A peak due to loss of water which is also common to alcohols was observed at *m/z* 110 corresponding to the formula C_8H_{14} **(Scheme 3.5).** Due to the presence of a double bond at carbon 3, fragment ion peaks

that are characteristics of alkenes were also expected. Indeed, peaks were observed at *m/z* 55 and 56 due to vinylic carbon-carbon bond cleavage. Usually alkenes would form fragment ions corresponding to the formulae $C_nH_{2n+1}^+$, $C_nH_{2n}^+$, and $C_nH_{2n-1}^+$ (the latter two fragment ion series being more abundant).[105] and this was observed to be the case in the MS of **3.5**, indicating the fragment ions $C_4H_9^+$, $C_4H_8^{+\cdot}$ and $C_4H_7^+$ represented by peaks at *m/z* 57, 55 and 56 and accounting for the base peak at *m/z* 55 (Figure 3.11). Usually it is difficult to locate the position of the double bond in an alkene because of the easy migration of the double bond by hydride and hydrogen atom shifts. The most important fragmentation events for alkenes involve cleavage of the allylic (favoured) and vinylic (less favoured) carbon-carbon bonds.[105] However, in the case of compound **3.5** the position of the C-3 double bond could be traced by considering the previously stated fragmentation pattern.

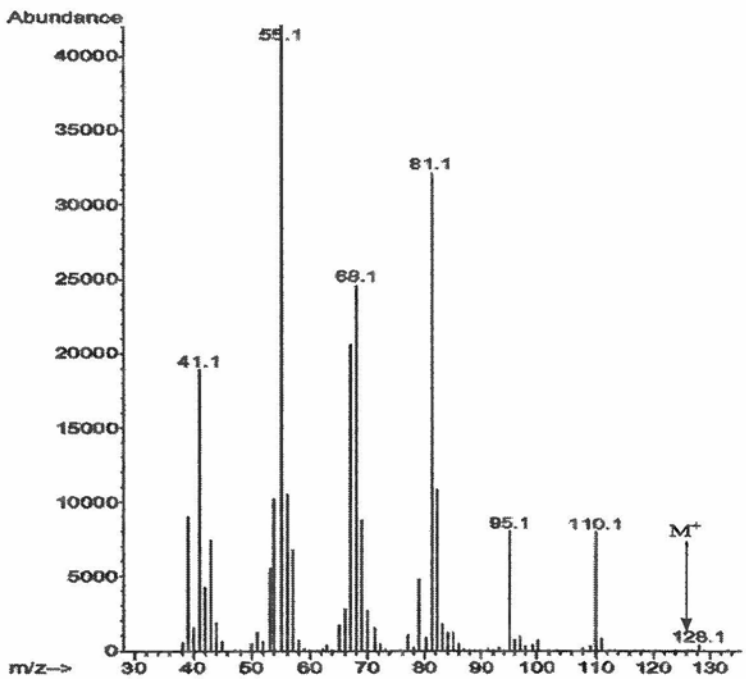
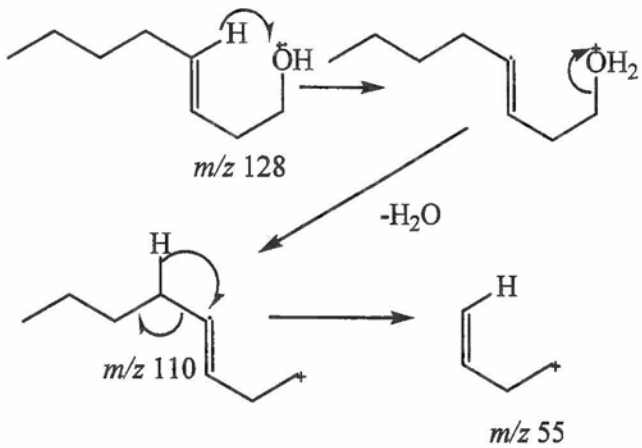
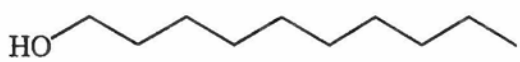


Figure 3.11 Mass Spectrum of *(Z)*-3-octen-1-ol (3.5)



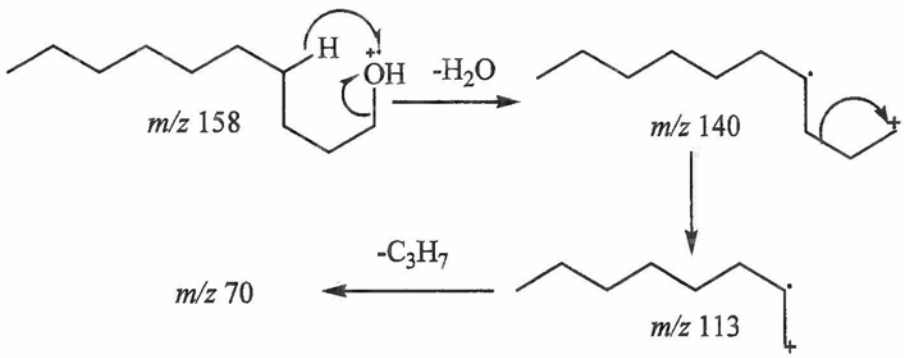
Scheme 3.5 MS Fragmentation Pattern for *(Z)*-3-octen-1-ol (3.5)

3.3.2.6 Decanol (3.6)



3.6

The mass spectrum of compound **3.6** did not indicate a molecular ion peak which was supposed to have appeared at *m/z* 158. Instead, a fragment ion peak was observed at *m/z* 140 due to loss of water from the molecular ion. Subsequent loss of ethene would have led to the formation of a fragment ion with a peak at *m/z* 113 while the base peak was observed at *m/z* 70, resulting from cleavage of a C_3H_7 fragment ion that appeared at *m/z* 113.



Scheme 3.6 MS Fragmentation Pattern for decanol (3.6)

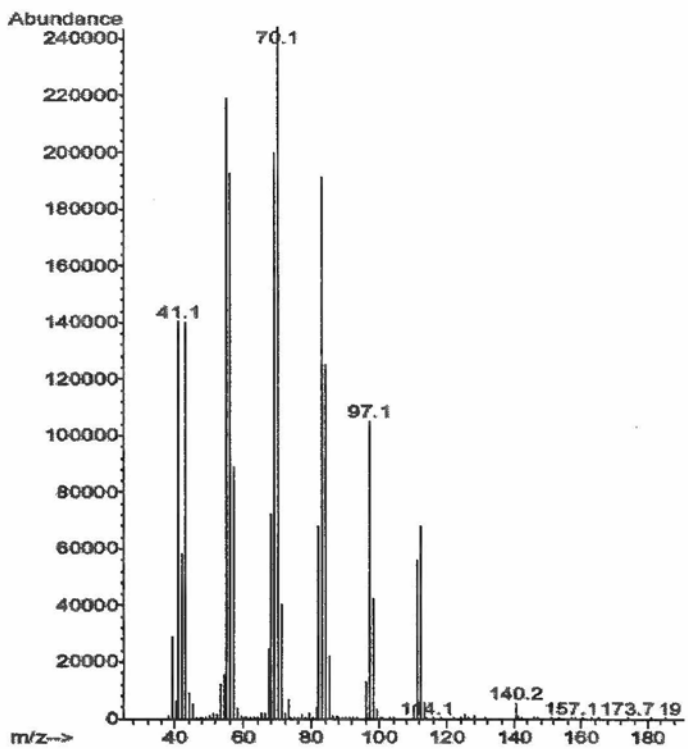


Figure 3.12 Mass Spectrum of decanol (3.6)

3.3.3 Coupled Gas Chromatography-Electroantennogram Detection (GC-EAD) Analysis of Volatiles from *Mangifera indica* and *Sclerocarya birrea* Ripe Fruits

The coupled GC-EAD analysis of volatiles collected from *S. birrea* ripe fruits indicated repeated female and male electroantennographic activity associated with 18 peaks. The peaks that elicited EAD responses were identified by GC-MS and structure assignments were made by mass spectral database matching, fragmentation patterns and subsequent comparison by GC-co-injections with authentic standards. Some of these standards were also run on both GC-EAD to confirm their electrophysiological activity and GC-MS to chemical confirmation of their identities.

The identified compounds included esters, alcohols, terpenes and unsaturated hydrocarbons, which are most widely found in aromas of a variety of fruits and flowers. The compounds detected by EAG were isopropyl acetate, ethyl propionate, ethyl isobutyrate, ethyl butyrate, ethyl 2-methylbutyrate, ethyl isovalerate, isopropyl valerate, propyl isovalerate, *p*-cymene, *trans*-β-ocimene, *(Z)*-3-octen-1-ol, octanol, linalool, *(Z)*-3-decene-1-ol, 5-dodecen-1-ol, *(E,E)*-α-farnesene and 13-tetradecenal. The major EAG-active peak was established to be due to ethyl isovalerate for both male and female flies. This was the first time that *B. invadens'* electrophysiologically active compounds were reported from *S. birrea*.

A number of the EAG-active compounds identified in this study are also known to elicit electroantennographic responses from other fruit fly species, such as *B. dorsalis,*[35] *Ceratitis capitata,* [106,107,108] *R. pomonella*[109,110] and *B. Tryoni.*[111] These

flies have shown electrophysiological activities to at least one of the following compounds identified in this study: isoamyl acetate, prenyl acetate, isopentyl acetate, isopentenyl acetate, ethyl hexanoate, geranyl acetate and the isomers of farnesene (Table 3.1).[35]

On the other hand, GC-EAD analysis with the antenna of both male and female flies led to the detection 17 compounds from volatiles collected from apple mango (*M. indica*) ripe fruits. The detected compounds included 3-ydroxy-2-butanone, crotenoic acid, ethyl-*(cis)*-crotonate, tricyclene, α-pinene, camphene, myrcene, sabenene, *trans*-β-ocimene, γ-terpinene, terpinolene, ethyl *cis*-4-octenoate, ethyl octanoate and α-humulene.

In previous studies, the attractiveness of airborne volatiles of Tommy Atkins cultivar of mango (*Mangifera indica*) was established to be caused by terpenes (*p*-cymene and limonene) as the best attractive constituents for both males and female *Ceratitis capitata* in laboratory cages. [112] Other compounds identified to exhibit attractiveness included α-pinene, 3-carene, and α-terpinolene.[112] Furthermore, in similar previous studies the response of the oriental fruit fly, *Bactrocera dorsalis* Hendel to the odors of different stages and types of fruit presented on potted trees in a field cage were analysed and females were found to be most attracted to odors of soft, ripe fruit. Thus, McPhail traps baited with mango fruits captured more females than for the visual fruit-mimicking sticky traps.[113]

In the present investigations, among the compounds detected by the antennae of fruit flies from both *M. indica* and *S. birrea* only *trans*-β-ocimene was common to both

hosts while the rest of the compounds appeared in either of the hosts. These investigations demonstrated how *B. invadens* was capable of infesting different hosts by using different chemical information provided.

In the present investigations, the EAD analyses of antenna of both male and female flies with volatiles from *M. indica* and *S. birrea* indicated the flies to respond to similar peaks in the EAD plots. This suggested that the volatiles from the two hosts might have been providing information to both sexes on the possible feeding, mating sites, as well as oviposition sites to females.

There are several reports that associate the behaviour of flies to fruits odors, since volatiles have major influence on host searching behaviors of frugivorous tephritid flies. The flies can detect host fruit volatiles from at least several meters away, although after encountering a host, shape, color and size of the fruits become added factors in their choices.[114] There is an increasing amount of evidence that fruit volatiles are important in enabling flies not only to locate host fruits, but also to discriminate between hosts and non-hosts as well as among fruits at different stages of ripeness.[68,115]

Volatile fruit odors have been used to successfully attract apple maggot fly, *Rhagoletis pomonella,* and the volatiles are highly attractive to both males and females.[106,107] Likewise, volatiles have been shown to be potential sources of attractants for male and female *Anastrepha ludens*.[116]

In these studies, the electrophysiological response of *B. invadens* male and female to volatiles from ripe fruits is being reported for the first time, in which both sexes responded to similar components. The results obtained in these investigations suggest that more studies are needed to be conducted on the identified compounds so as to identify the most attractive blend of compounds that could be used to protect farms and improve the market of fruits locally and internationally.

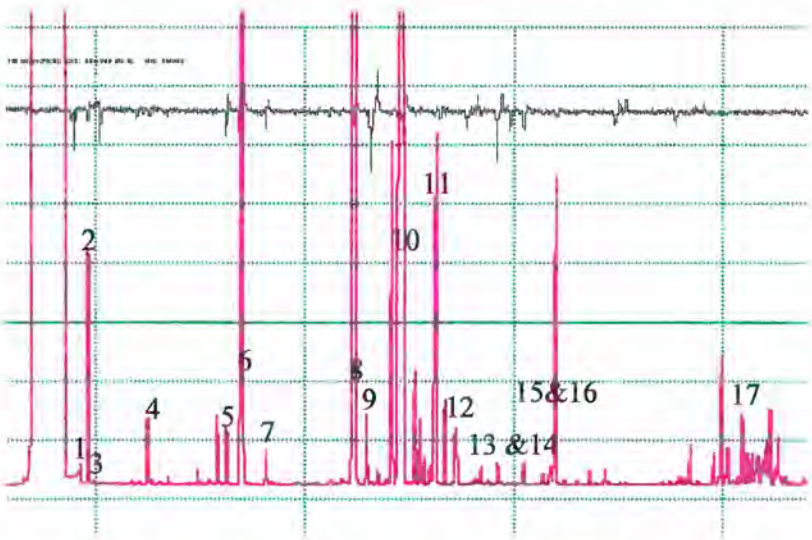


Figure 3.13 GC-EAD Profile of Ripe Mango (*M. indica*) Fruits Volatiles Applied to the Antennae of Female *B. invadens*.

The Numbers Represent Peaks with EAD Responses: 1-3 = (unidentified), 4 = 3-hydroxy-2-butanone, 5 = ethyl butyrate, 6 = *(Z)*-ethyl crotonate, 7 = tricyclene, 8 = α-pinene, 9 = camphene, 10 = β-pinene, 11 = sabinene, 12 = *trans*-β-ocimene, 13 = γ-terpinene, 14 = terpinolene, 15 = *(Z)*-ethyl 4-octenoate, 16 = ethyl octanoate and 17 = α-humulene.

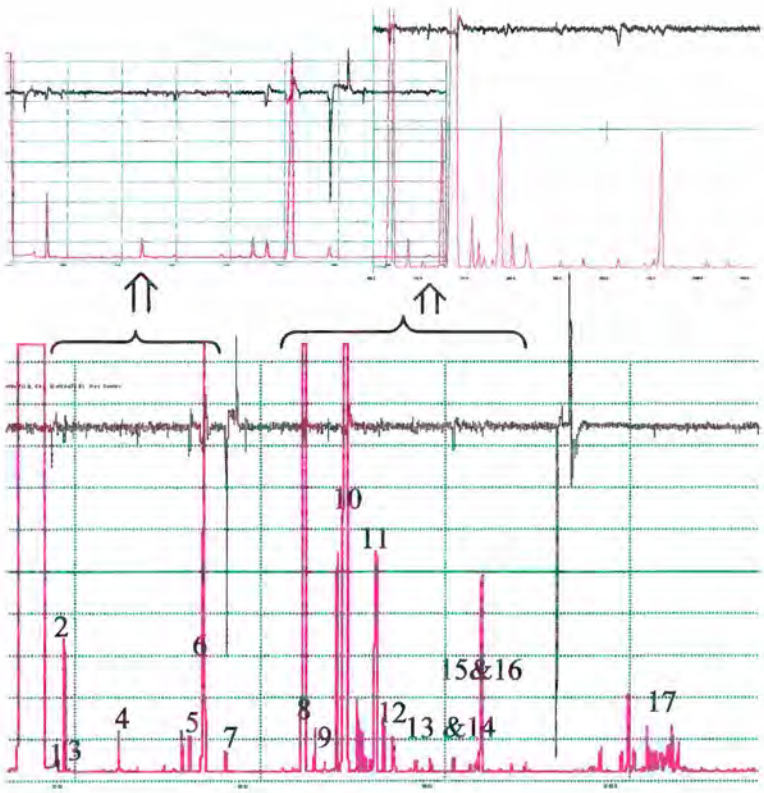


Figure 3.14 GC-EAD Profile of Ripe Mango (*M. indica*) Fruits Volatiles Applied to the Antennae of Male *B. invadens*.

The Numbers Represent Peaks with EAD Responses: 1-3 = (unidentified), 4 = 3-hydroxy-2-butanone, 5 = ethyl butyrate, 6 = *(Z)*-ethyl crotonate, 7 = tricyclene, 8 = α-pinene, 9 = camphene, 10 = β-pinene, 11 = sabinene, 12 = *trans*-β-ocimene, 13 = γ-terpinene, 14 = terpinolene, 15 = *(Z)*-ethyl 4-octenoate, 16 = ethyl octanoate and 17 = α-humulene.

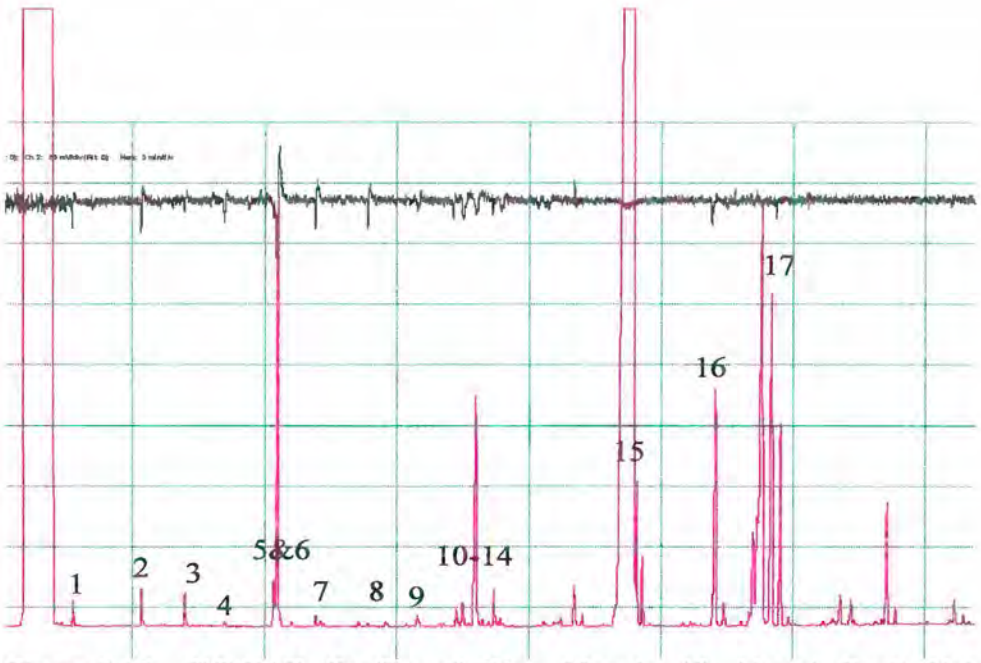


Figure 3.15 GC-EAD Profile of Ripe Marula (*S. birrea)* Fruit Volatiles Applied to the Antennae of Female *B. invadens*.

The Numbers Represent Peaks with EAD Responses: 1 = isopropyl acetate, 2 = ethyl propionate, 3 = ethyl isobutyrate, 4 = ethyl butyrate, 5 = ethyl-2-methylbutyrate, 6 = ethyl isovalerate, 7 = isopropyl valerate, 8 = propyl isovalerate, 9 = *p*-cymene, 10 = *trans*-β-ocimene, 11 = *(Z)*-3-octen-1-ol, 12 = octanol, 13 = 4-undecene, 14 = linalool, 15 = *(Z)*-3-decene-1-ol, 16 = 5-dodecen-1-ol, 17 = (*E,E*)-α-farnesene, 18 = 13-tetradecenal

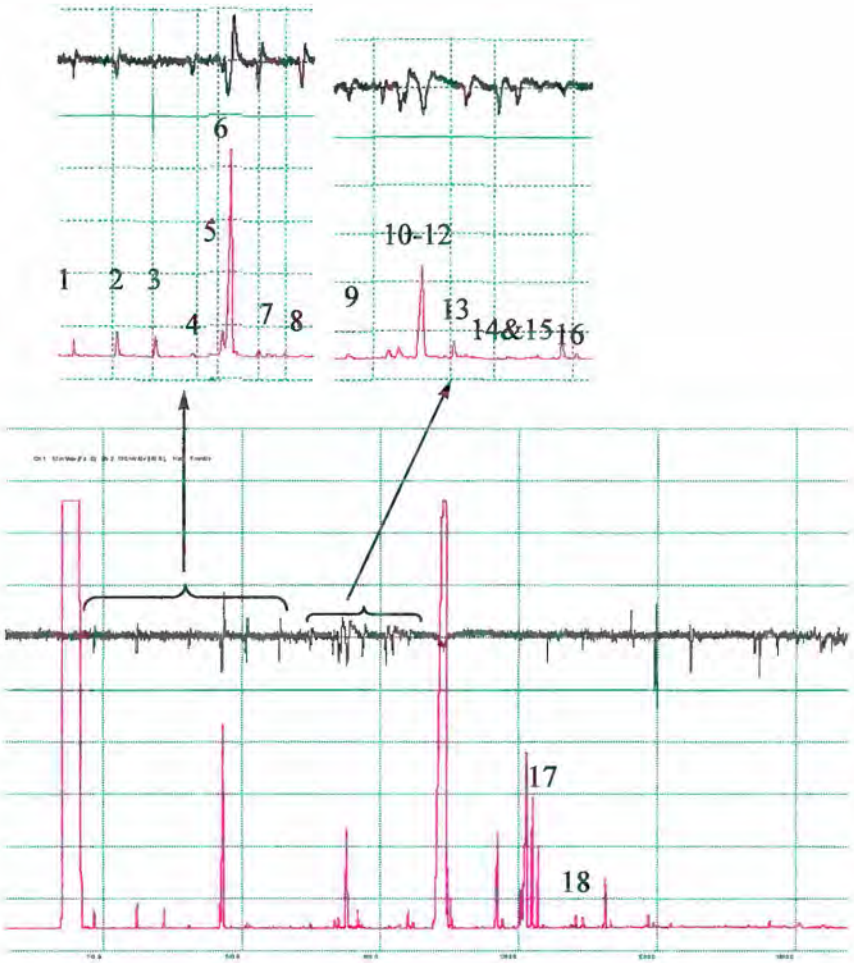


Figure 3.16 GC-EAD Profile of the Ripe Marula (*S. birrea)* Fruits Volatiles Applied to the Antennae of Male *B. invadens*.

The Numbers Represent Peaks with EAD Responses: 1 = isopropyl acetate, 2 = ethyl propionate, 3 = ethyl isobutyrate, 4 = ethyl butyrate, 5 = ethyl 2-methylbutyrate, 6 = ethyl isovalerate, 7 = isopropyl valerate, 8 = propyl isovalerate, 9 = *p*-cymene, 10 = *trans*-β-ocimene, 11 = *(Z)*-3-octen-1-ol, 12 = octanol, 13 = 4-undecene, 14 = linalool, 15 = *(Z)*-3-decene-1-ol, 16 = 5-dodecen-1-ol, 17 = (*E,E*)-α-farnesene and 18 = 13-tetradecenal

CHAPTER FOUR

VOLATILE CONSTITUENTS FROM THE INDIAN ALMOND (*TERMINALIA CATAPPA*) FRUITS AS POTENTIAL ATTRACTANTS FOR THE FRUIT FLY *BACTROCERA INVADENS* (TEPHRITIDAE)

Abstract

GC-MS and GC-EAD analyses were conducted to compare volatile profiles of unripe and ripe fruits of *Terminalia catappa* fruits and to identify compounds in ripe fruits that were electrophysiologically active with respect to antennae of both male and female *Bactrocera invadens*. The volatiles from ripe and unripe fruits showed notable differences in composition. Those from ripe fruits were dominated by esters (89.15 %), with terpenes and alcohols representing only 2.23 and 1.68%, respectively. Volatiles of unripe fruits contained more terpenes (47.24%), followed by esters (37.58%), with alcohols being present in relatively small amount (0.67%). The most prominent peak in the volatiles of ripe fruits was due to 4-penten-1-yl acetate (29.49%) and in that of unripe fruits it was δ-3-carene (29.53%). GC-EAD analyses indicated 19 electrophysiologically active constituents, including esters, terpenes and aldehydes, detected by the *B. invadens* antennae of both males and females. These included isobutyl acetate, 4-pentenol, butyl acetate, 2-butenyl acetate, ethyl isovalerate, isoamyl acetate, 4-penten-1-yl acetate, prenyl acetate, β-myrcene, δ-3-carene, limonene, *trans*-β-ocimene, terpinolene, benzyl acetate, citronellyl acetate, geranyl acetate, methyl eugenol, *(E,E)*-α-farnesene and elemicin. It was concluded that there was a difference in the chemistry of volatiles emanating from ripe and unripe fruits and that both males and females were able to detect similar compounds from volatiles of ripe fruits. The ecological implication of these results is discussed in the light of the current understanding of the dacine lure biology.

4.1 Introduction

Terminalia catappa L. (Indian almond) belongs to the family Combretaceae and in East Africa it originated from Malaysia. The tree is generally confined to mesic and wet coastal habitats and is distributed throughout the Old World tropics and tropical America.[27] It produces fruits (5-10 cm long) with a thin flesh surrounding a large fibrous nut. Natural products reported from *T. catappa* include terpenoids,[28] flavonoids,[29] tannins[30] and esters.[31] The plant is one of the hosts for *B. invadens* and was recently reported to be highly infested, which results from the female's oviposition preference for the plant.

In the literature, it is reported that host odors are critical for the location of potential oviposition sites by mono- and oligophagous insects.[119-121] Adult flies can detect host fruit volatiles from at least several meters away, and can use these olfactory signals to orient upwind toward fruiting host trees.[81,122] For example, leaf extract from *T. catappa* preferentially attracts female oriental fruit flies.[36] Such behaviour can be a useful basis for identifying the attractive constituents emanating from host plants and for developing these as a tool for the control of fruit flies.

Current field control measures for fruit flies in general are based on insecticide cover sprays, bait sprays, physical control (fruit wrapping or bagging), cultural control (crop hygiene, early harvesting, use of resistant varieties, etc.), sterile insect technique (SIT), behavioural control (traps with different colours, shapes, odours, male annihilation, protein bait sprays), and biological control.[70] None of these

focuses directly on reducing oviposition by using deterrents or attractants to alter behavioural responses of females to surfaces of host fruits.[97]

The present investigations were conceived based on the above stated background. The specific aim was to characterize candidate kairomones from *T. catappa*, a wild host of *B. invadens*, which could then be developed further for the fly's management with the envisioned ultimate goal of availing effective and environmentally friendly lures that could provide farmers with a non-toxic method of monitoring and controlling *B. invadens* fruit fly populations in their orchards.

4.2 Materials and Methods

4.2.1 Volatiles Collection

This was carried out as described in Chapter Three.

4.2.2 GC-MS Analysis

This was carried out as described in Chapter Three.

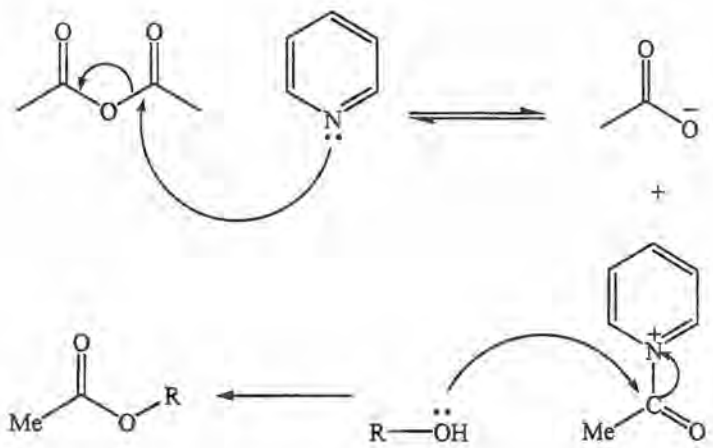
4.2.3 GC-EAD Analysis

This was carried out as described in Chapter Three.

4.2.4 Preparation of Esters

Three esters (geranyl acetate, citronellyl acetate and prenyl acetate) were prepared from their corresponding alcohols: geraniol, citronellol and prenyl alcohol (all purchased from Aldrich, UK) by treating each with acetic anhydride. Acetic anhydride (200 µl, May and Baker Ltd) was placed in a small vial (4 ml), 5 drops of

pyridine was then added as a catalyst followed by 200 µl of the alcohol. The mixture was kept at room temperature for 12 h. The vial was then opened to allow excess pyridine and acetic anhydride to evaporate prior to GC-MS analysis to confirm their identities by comparison with mass spectral data from the library (Wiley275.L and NIST05a.l).



Scheme 4.1 Chemical Reactions for the Preparation of Esters

4.3 Results and Discussion

4.3.1 Volatile Constituents Identified from *Terminalia catappa* Ripe and Unripe Fruits

GC-MS analyses of the volatiles from ripe fruits of *Terminalia catappa* indicated the presence of high percentages of esters (89.15%), with terpenes and alcohols being least abundant constituents, representing only 2.23 and 1.68% respectively. Mature unripe fruits were shown to contain higher percentages of terpenes (47.24%), followed by esters (37.58%), with alcohols being present in relatively small amount

(0.67%, Figure 4.3). The ripe *T. catappa* fruit volatiles contained a high percentage of 4-penten-1-yl acetate (**4.4**, 29.49 %), followed by prenyl acetate (**4.3**, 21.80 %) and geranyl acetate (**4.1**, 19.72 %), while in the unripe fruit volatiles δ-3-carene was found to be present in larger amount (29.53 %), followed by geranyl acetate (9.75 %) and 2-buten-1-ol acetate (8.78 %, Tables 4.1 and 4.2). Thus, some of these compounds were present in both maturity stages of the fruits but in different relative amounts. However, other compounds, like the terpenes β-pinene, γ-terpinene, α-gurjurene, α-humulene, allomadendrene and δ-3-carene were absent in the volatiles of the ripe fruits.

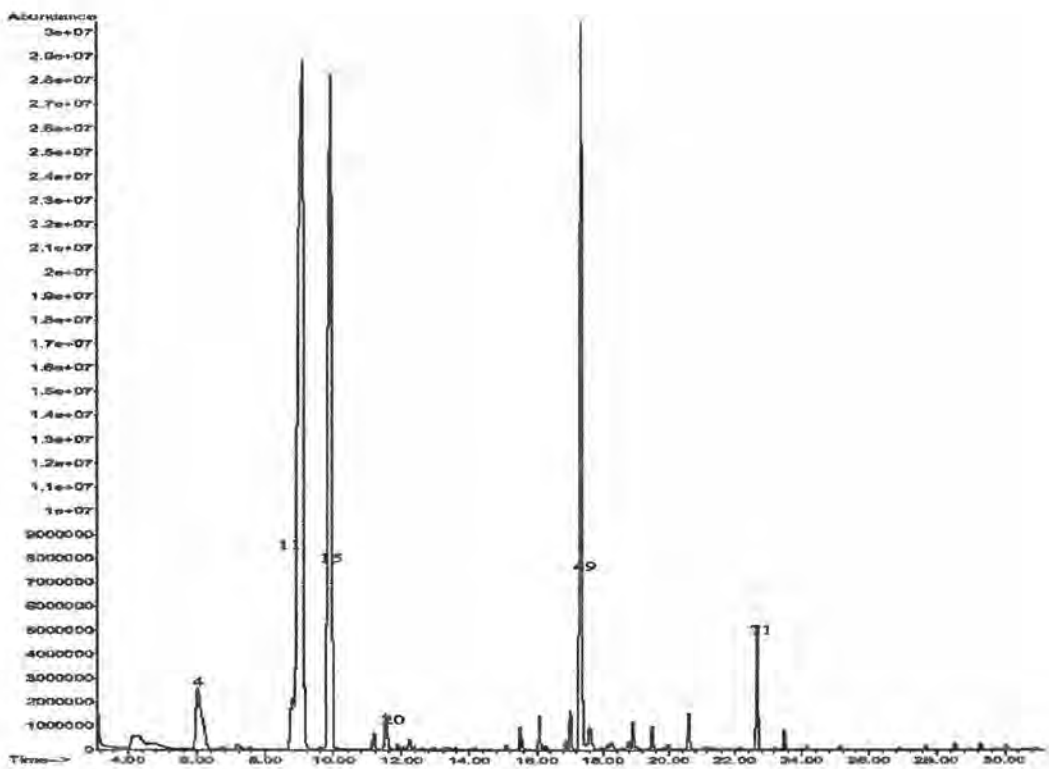


Figure 4.1 Gas Chromatogram of Volatiles Emanating From *T. Catappa* Mature Ripe Fruits (Numbers of the Peaks in the Chromatogram Correspond to Constituents Indicated as S/N in Table 4.1)

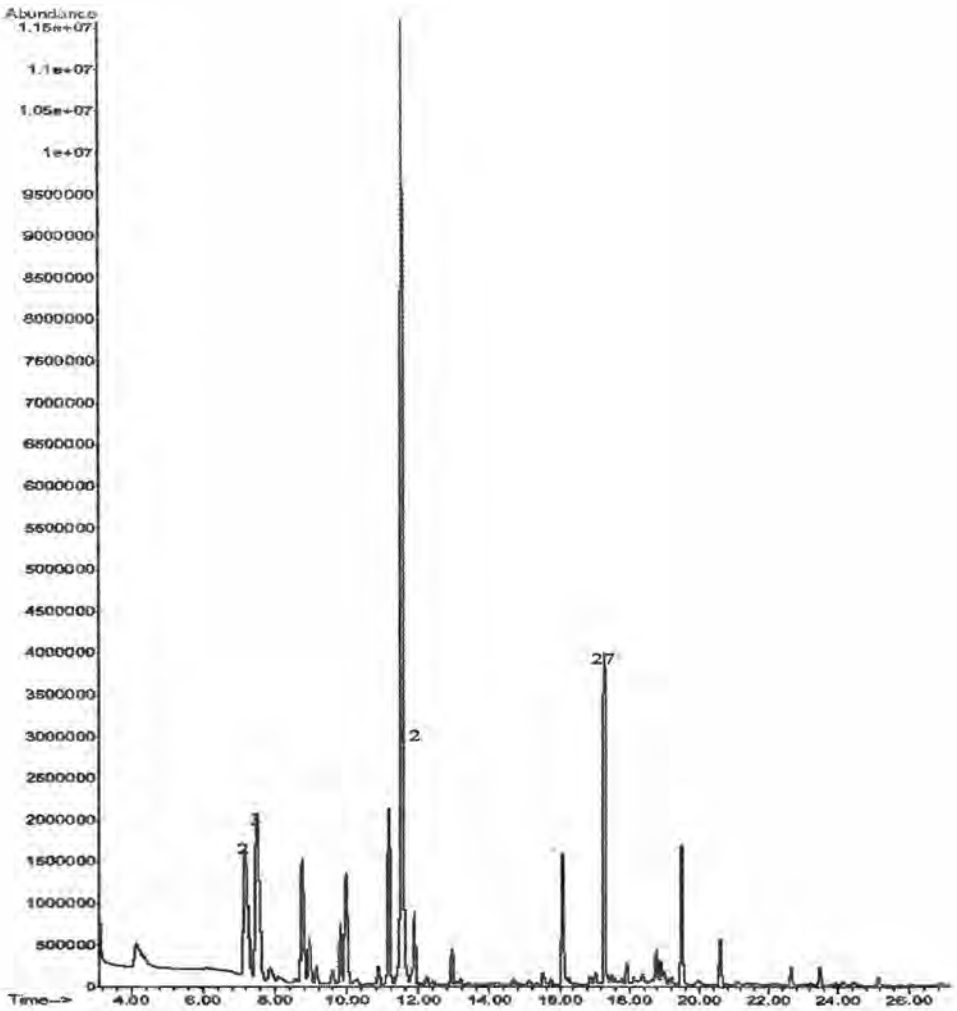


Figure 4.2 Gas Chromatogram of Volatiles Emanating From *T. Catappa* Mature Unripe (Green) Fruit. (Numbers of the Peaks in the Chromatogram Correspond to Constituents Indicated as S/N in Table 4.1)

Table 4.1 Percentage Compositions of the Constituents Identified from Volatiles of Indian Almond (*Terminalia Catappa*) Ripe Fruits

S/N	Compound	Retention time (min)	% Abundance
1	3-hydroxy-2-butanone	4.14	1.83
2	propyl acetate	4.27	0.22
3	isopentanol	4.79	0.09
4	isobutyl acetate	6.04	9.83
5	1,3-butanediol	6.49	0.05
6	methyl 1-methyl-2-propenyl ether	6.72	0.03
7	butyl acetate	7.16	0.43
8	2-buten-1-ol acetate	7.50	0.11
9	hexanol	8.55	0.18
10	isoamyl acetate	8.75	2.55
11	4-penten-1-yl acetate	8.90	29.49
12	*meso*-2,3-butandiol diacetate	9.13	0.26
13	amyl acetate	9.58	0.05
14	octyl acetate	9.60	0.02
15	prenyl acetate	9.83	21.80
16	ethyl butyrate	10.05	0.02
17	6-methyl-5-hepten-2-one	11.08	0.03
18	myrcene	11.17	0.25
19	ethyl hexanoate	11.33	0.07
20	δ-3 carene	11.53	0.42
21	*n*-hexyl acetate	11.58	1.34
22	limonene	11.87	0.08
23	*cis*-ocimene	12.02	0.05
24	*trans*-β-ocimene	12.22	0.15
25	*(5R 9R)*-2,9-trimethyl-1,6-dioxaspiro[4.4]nonane	12.36	0.09
26	*(5R 9S)*-2,9-trimethyl-1,6-dioxaspiro[4.4]nonane	12.58	0.03
27	*cis*-linalool oxide	12.65	0.01
28	α -terpinolene	12.94	0.05
29	nonanal	13.17	0.03
30	*trans*-rose oxide	13.30	0.04
31	*(E)*-4,8-dimethyl-1,3,7-nonatriene	13.37	0.06
32	neoalloocimene	13.59	0.03
33	isobutyl hexanoate	13.90	0.01
34	3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	14.06	0.02
35	benzyl acetate	14.15	0.09
36	dodecane	14.67	0.01
37	octyl ethanoate	14.85	0.02
38	citronellol	15.09	0.04
39	*trans*-geraniol	15.11	0.07

Table 4.1 Continued

S/N	Compound	Retention time (min)	% Abundance
40	*Z*-citral	15.32	0.01
41	geraniol	15.49	0.79
42	geranial	15.74	0.07
43	ethyl nonanoate (IS)	16.08	0.62
44	geraniol formate	16.17	0.01
45	decyl acetate	16.26	0.07
46	citronellyl acetate	16.86	0.32
47	eugenol	17.00	0.28
48	neryl acetate	17.02	0.20
49	geranyl acetate	17.31	19.72
50	*n*-tetradecane	17.49	0.10
51	methyl eugenol	17.58	0.29
52	*trans*-β-caryophyllene	17.91	0.06
53	α-bergamotene	18.05	0.01
54	α–guaiene	18.12	0.07
55	*trans*-isoeugenol	18.20	0.09
56	*trans*-β–farnesene	18.23	0.08
57	*(E)*-7-methyl-1,6-dioxaspiro[4.5]decane	18.29	0.04
58	*cis*-methyl isoeugenol	18.76	0.14
59	*(E,E)*-α-farnesene	18.88	0.98
60	2,3-dimethyl-1,4-pentadiene	18.99	0.05
61	elemicin	19.46	0.36
62	hexadecene	19.86	0.03
63	hexadecane	19.93	0.12
64	*trans*-isoelemicin	20.60	2.00
65	heptadecane	21.07	0.07
66	pentacosane	21.23	0.01
67	farnesol	21.39	0.05
68	octadecane	22.15	0.01
69	nerolidol	22.64	0.46
70	nonadecane	23.20	0.02
71	farnesyl acetate	23.57	2.27
72	methyl hexadecanoate	24.12	0.20
73	methyl hexadecanoate	24.14	0.03
74	1-methylethyl hexadecanoate	24.41	0.01
75	heneicosane	25.10	0.08

Table 4.2 Percentage Compositions of the Constituents Identified from Volatiles of Indian Almond (*Terminalia Catappa*) Unripe Fruits

S/N	Compound	R.T (min)	% Abundance
1	3-hydroxy-2-butanone	4.14	2.48
2	acetic acid, butyl ester	7.16	7.64
3	2-buten-1-ol acetate	7.50	8.78
4	isoamyl acetate	8.75	5.03
5	4-penten-1-yl acetate	8.93	1.63
6	*meso*-2,3-butandiol diacetate	9.13	0.58
7	amyl acetate	9.58	0.50
8	prenyl acetate	9.81	1.84
9	α-pinene,	9.96	4.30
10	β-pinene	10.86	0.57
11	myrcene	11.17	5.20
12	δ-3-carene	11.53	29.53
13	1,3-pentadiene, 3-methyl-	11.64	0.46
14	limonene	11.87	2.43
15	*trans*-β-ocimene	12.23	0.21
16	γ-terpinene	12.40	0.17
17	terpinolene	12.94	1.09
18	nonanal	13.17	0.19
19	methyl salicylate	14.64	0.20
20	citronellol	15.09	0.21
21	geraniol	15.50	0.46
22	geranial	15.74	0.21
23	ethyl nonanoate (IS)	16.08	3.94
24	decyl acetate	16.26	0.26
25	citronellyl acetate	16.86	0.30
26	neryl acetate	17.02	0.42
27	geranyl acetate	17.26	9.75
28	*n*-tetradecane	17.47	0.32
29	methyl eugenol	17.58	0.23
30	α-gurjunene	17.78	0.21
31	*trans*-β-caryophyllene	17.91	0.68
32	α-guaiene	18.12	0.24
33	α-humulene	18.36	0.34
34	alloaromadendrene	18.45	0.12
35	γ-muurolene	18.61	0.22
36	methyl *cis*-isoeugenol	18.77	1.27
37	*(E,Z)*-α-farnesene	18.90	0.76
38	δ-guaiene	18.99	0.37
39	δ-cadinene	19.17	0.29

Table 4.2 Continued

S/N	Compound	R.T (min)	% Abundance
40	elemicin	19.46	2.84
41	hexadecane	19.95	0.17
42	*trans*-isoelemicin	20.60	1.34
43	heptadecane	21.09	0.09
44	β-bisabolene	22.64	0.52
45	methyl hexadecanoate	23.47	0.51
46	ethyl hexadecanoate	24.14	0.13
47	hexadecanoic acid	24.43	0.10
48	heneicosane	25.13	0.25

Key: IS = Internal standard

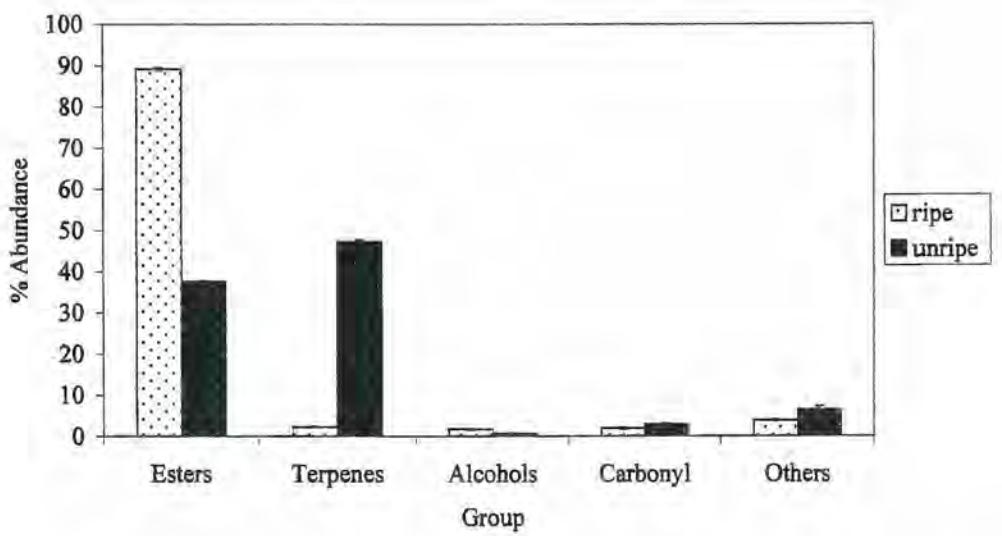
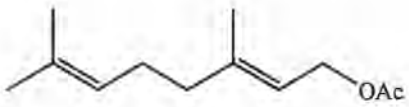


Figure 4.3 Proportions of Compounds in the Volatiles Collected from Ripe and Unripe Fruits of *Terminalia Catappa*

4.3.2 Mass Spectrometry Fragmentation Patterns of Some Compounds from Ripe Fruits of *Terminalia catappa*

4.3.2.1 (E)-3,7-Dimethylocta-2,6-dienyl acetate (geranyl acetate, 4.1)



4.1

The MS of compound **4.1** indicated the molecular ion peak at *m/z* 196.1, which was consistent with the molecular formula $C_{12}H_{20}O_2$. McLafferty rearrangement would have led to the fragmentation leading to the formation of a peak observed at *m/z* 136. Subsequent fragmentation through a retro Diels-Alder process would account for the base peak at *m/z* 69 ascribed to the fragment ion $C_5H_9^+$ while the peak due to loss of CH_3 was observed at *m/z* 121. Another peak appeared at *m/z* 178 corresponding to formula $C_{12}H_{18}O$ was considered to have been formed due to cleavage of a water molecule from compound **4.1**. The fragmentation due to cleavage α to the carbonyl group would produce a peak at *m/z* 43 (Figure 4.4 and Scheme 4.2). The fragmentation pattern as depicted in Scheme 4.2 is consistent with the structure for geranyl acetate (**4.1**).

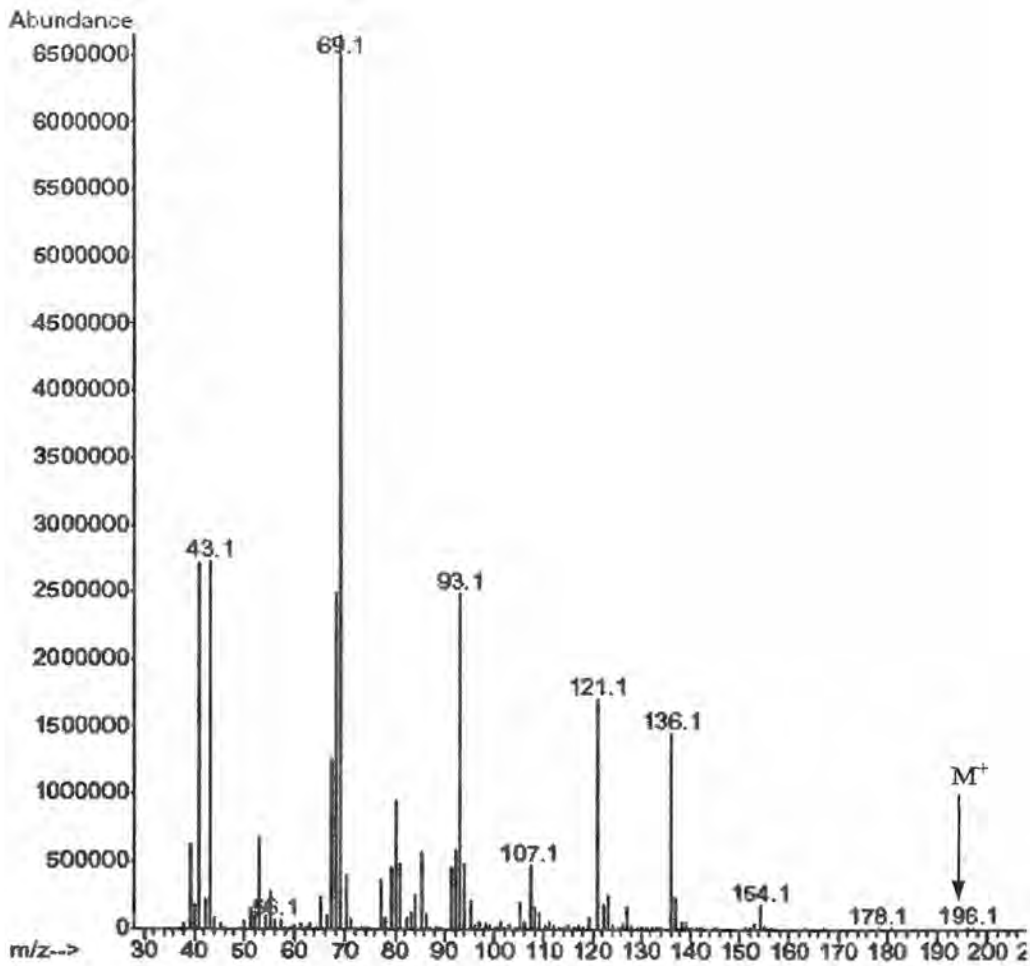
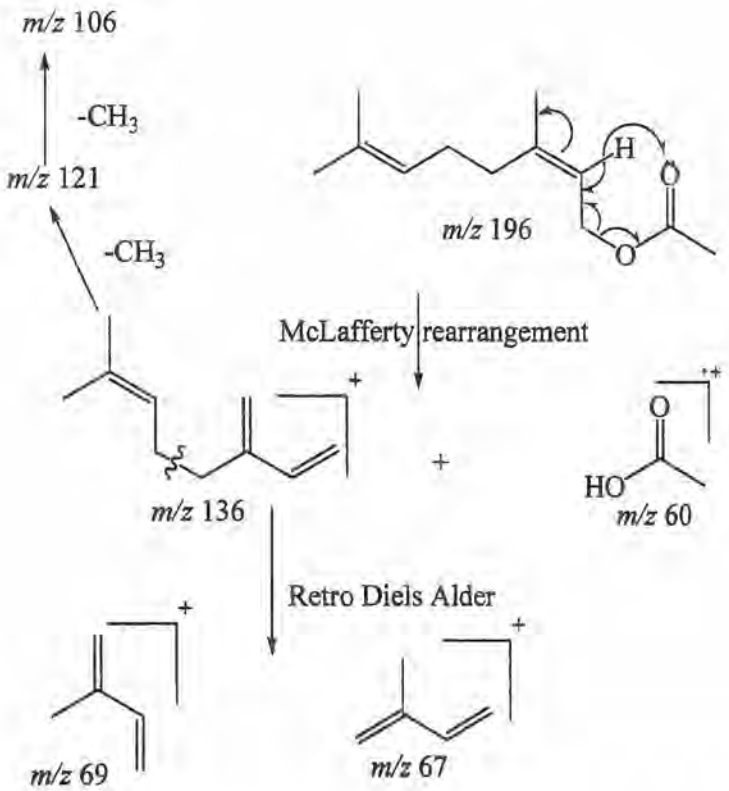
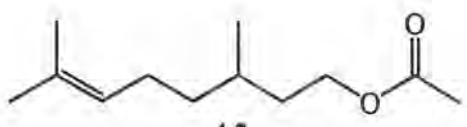


Figure 4.4 Mass Spectrum of *(E)*-3,7-dimethylocta-2,6-dienyl acetate (4.1)



Scheme 4.2 MS Fragmentation Patterns for *(E)*-3,7-dimethylocta-2,6-dienyl acetate (4.1)

4.3.2.2 3,7-Dimethyloct-6-enyl acetate (citronellyl acetate) (4.2)



4.2

The MS of compound **4.2** indicated a very weak molecular ion peak at *m/z* 197.9, being consistent with the molecular formula $C_{12}H_{20}O_2$. Unlike geranyl acetate (**4.1**) compound **4.2** consist of aproton at C-3 on the aliphatic alcoholic side of the chain,

and thus determining the MS fragmentation process for this compound being due to McLafferty +1 rearrangement,[123] which was observed as the key fragmentation process, that produced a fragment ion whose peak appeared at *m/z* 123, corresponding to the formula C_9H_{15}. This fragmentation process is characteristic of esters and amides having aliphatic chains at least three C atoms long, which would involve migration of two protons and would proceed through the same distonic ion intermediate like the one formed during the McLafferty rearrangement. The fragmentation process is known both as the double-hydrogen rearrangement and the McLafferty +1 rearrangement. Migration of the second proton is somewhat unusual in that it involves formation of a five-atom cyclic transition state.[105]

The base peak in the mass spectrum was observed at *m/z* 69, which would result from a homolytic cleavage of the intermediate distonic ion whose peak appeared at *m/z* 138. Two ion peaks were also observed at *m/z* 155 and 43 and these were ascribed to cleavage α to the carbonyl carbon (Figure 4.5 and Scheme 4.3), this being consistent with structure **4.2**.

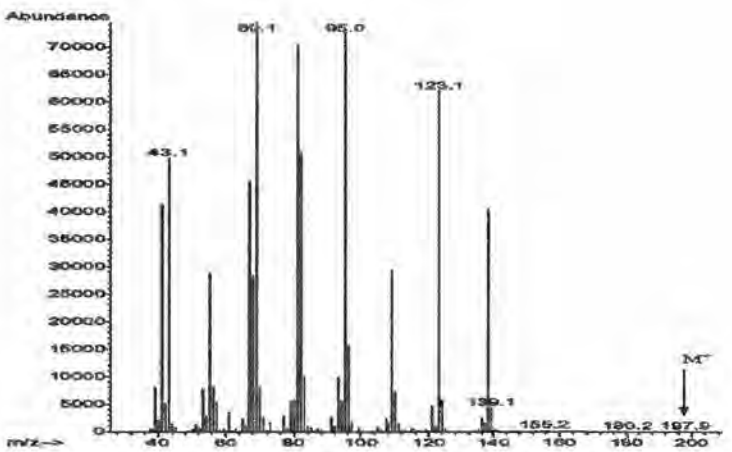
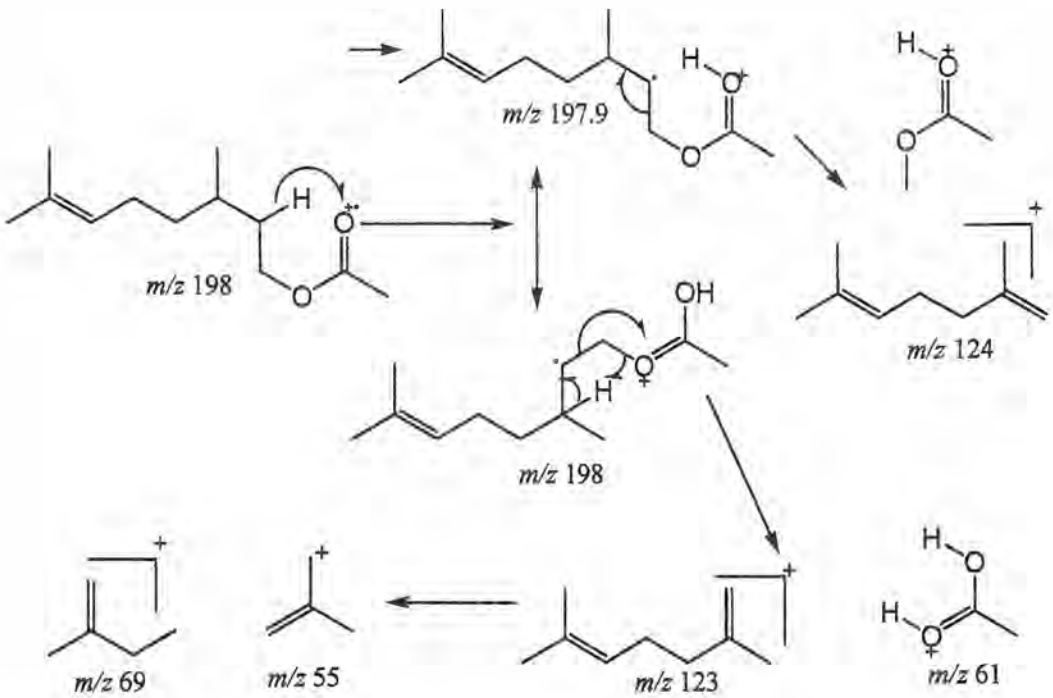
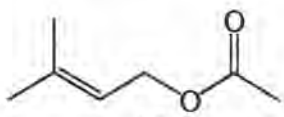


Figure 4.5 Mass Spectrum of *(E)*-3,7-dimethylocta-2,6-dienyl acetate (4.2)



Scheme 4.3 MS Fragmentation Pattern for *(E)*-3,7-dimethylocta-2,6-dienyl acetate (citronellyl acetate, 4.2)

4.3.2.3 3-Methylbut-2-enyl acetate (prenyl acetate, 4.3)



4.3

The MS of compound **4.3** indicated the molecular ion peak at *m/z* 128, which was consistent with the molecular formula $C_7H_{12}O_2$. The MS exhibited the base peak at *m/z* 68, which was ascribed to the fragment ion C_5H_8, formed due to McLafferty rearrangement. Other peaks characteristic for esters were also observed at *m/z* 85 and 43 due to cleavage α to the carbonyl carbon, corresponding to fragment ions $C_5H_{10}O_2^{\cdot}$ and $C_2H_3O^+$ respectively. The fragmentation process as depicted in the MS (Figure 4.6)and Scheme 4.4 is consistent with structure **4.3**.

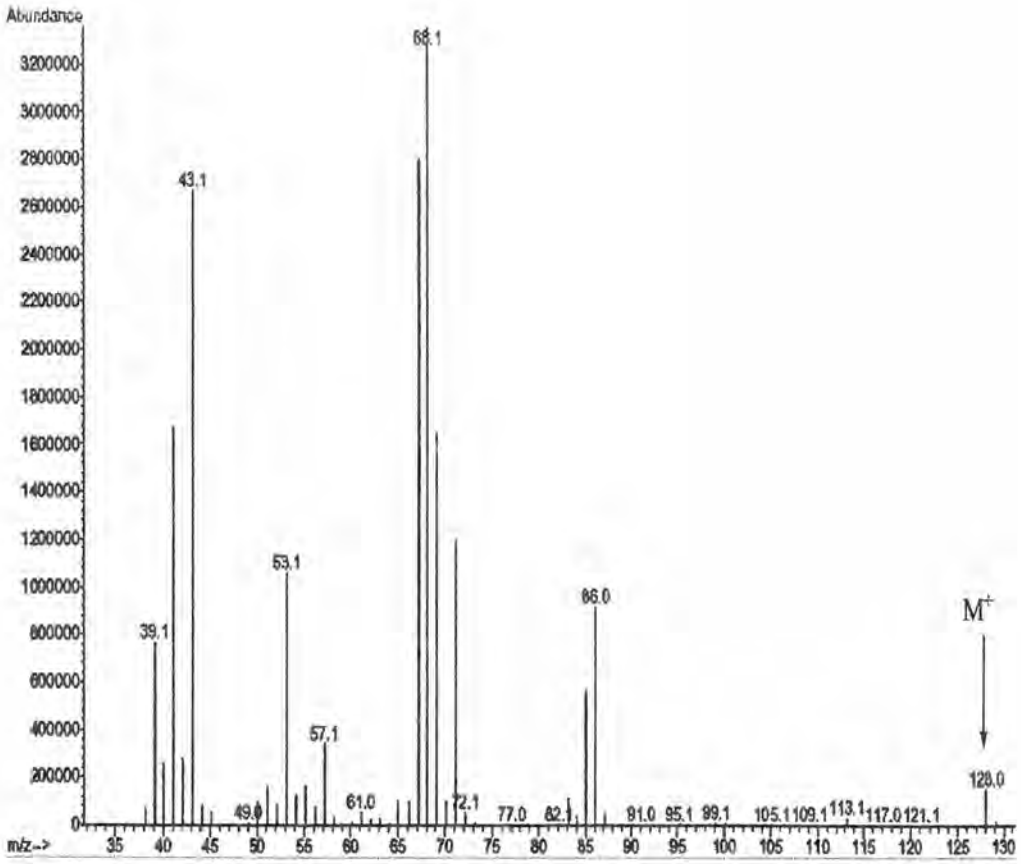
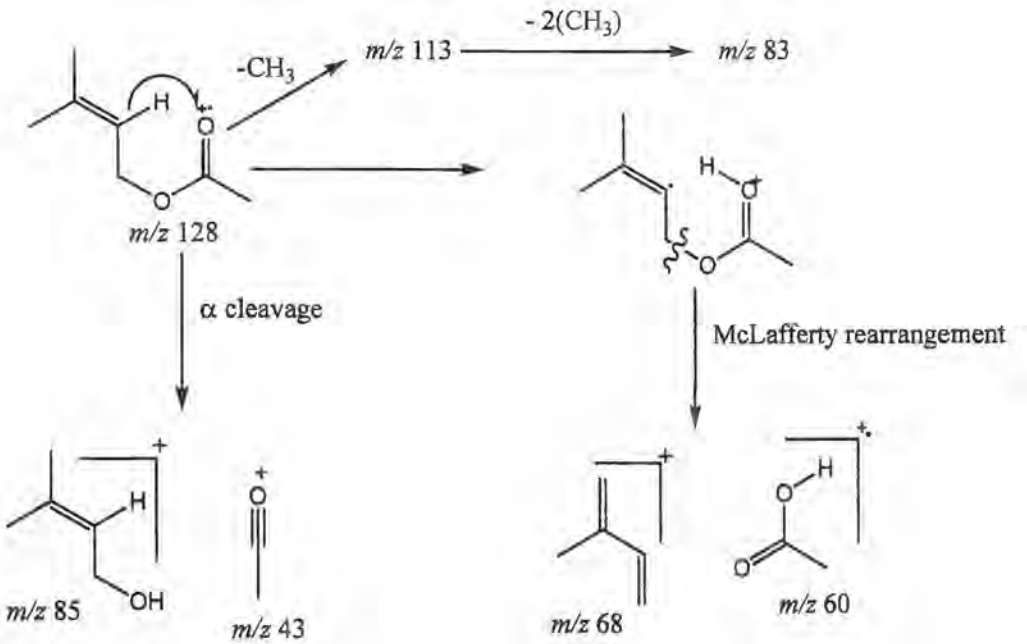
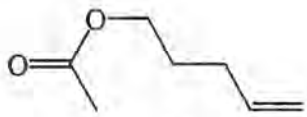


Figure 4.6 Mass Spectrum of 3-methylbut-2-enyl acetate (prenyl acetate, 4.3)



Scheme 4.4 MS Fragmentation Pattern for 3-methylbut-2-enyl acetate (prenyl acetate, 4.3)

4.3.2.4 4-Pentene-1-yl acetate (4.4)



4.4

The MS of compound **4.4** (4-pentene-1-yl acetate) did not exhibit a molecular ion peak but it showed a fragment ion peak due to an M-1 fragment at *m/z* 127. Furthermore, the MS of the compound revealed fragment ion peak at *m/z* 68 and 60 that would be formed through a McLafferty rearrangement. Cleavage α to the carbonyl carbon would have produced the fragment ions that appeared at *m/z* 43 and 85, corresponding to ions C_2H_3O and $C_5H_{10}O_2$ respectively. The fragmentation process is shown in Figure 4.7 and Scheme 4.5, and is consistent with structure **4.4**.

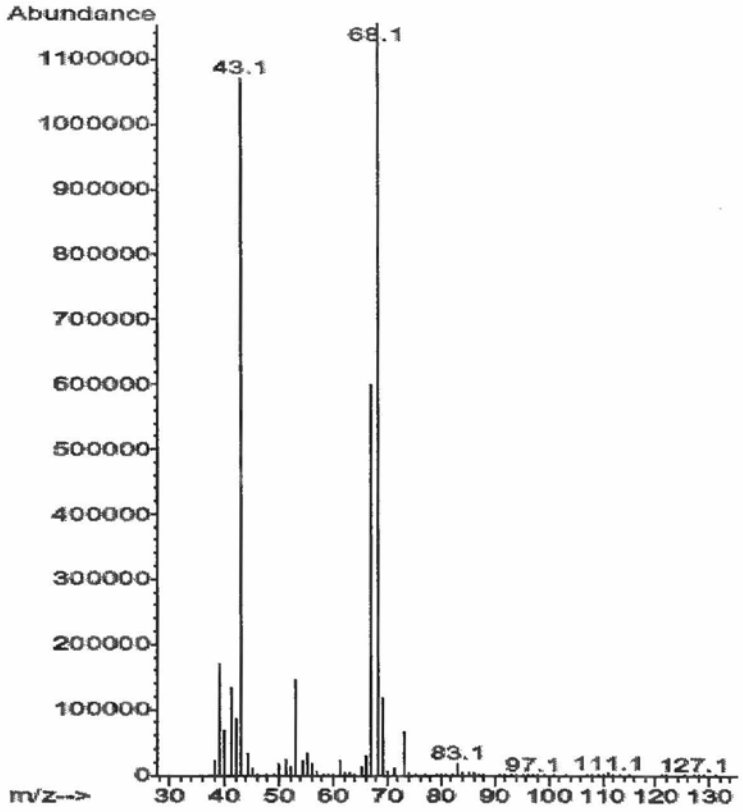
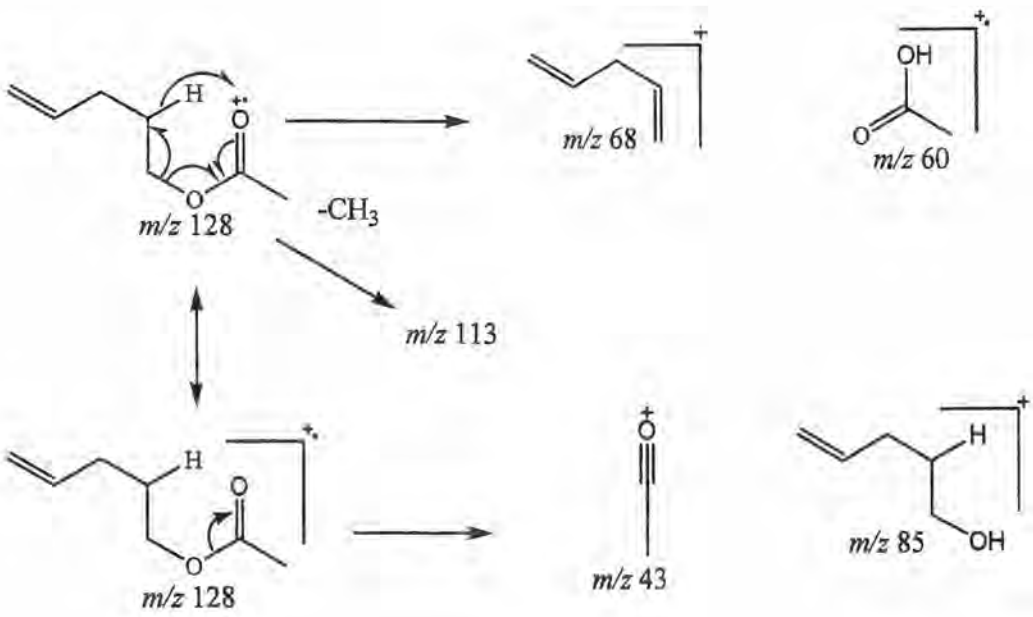
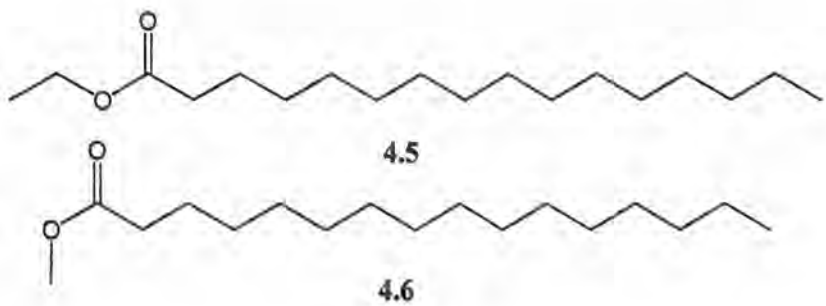


Figure 4.7 Mass Spectrum of 4-pentene-1-yl acetate (4.4)



Scheme 4.5 MS Fragmentation Pattern for 4-pentene-1-yl acetate (4.4)

4.3.2.5 Ethyl hexadecanoate (4.5) and Methyl hexadecanoate (4.6)



4.5

4.6

The MS of compound **4.5** indicated the molecular ion peak at *m/z* 284.3, which was consistent with the molecular formula $C_{18}H_{36}O_2$. The MS also exhibited the base peak at *m/z* 88, which was ascribed to the fragment ion having the formula $C_4H_8O_2$, being formed through a McLafferty rearrangement which is also the characteristic

peak for ethyl esters. Other characteristic peaks for ethyl esters were also observed at *m/z* 101 and 73 due to α and γ cleavage relative to the carbonyl carbon, corresponding to fragment ions with formulae $C_5H_9O_2$ and $C_3H_5O_2$ respectively. Clusters of peaks which were 14 mass units apart were also observed, corresponding to the loss of successive CH_2 units in the aliphatic part of the compound. These included peaks at *m/z* 255, 241, 213, 199, 85, 171, 157, 143, 129 115, 101, 87 and 73, indicating cleavage of 13 CH_2 groups (Figure 4.8 and Scheme 4.6), which was consistent with structure **4.5**.

On the other hand, the MS of compound **4.6** indicated the molecular ion peak at *m/z* 270.3, which was consistent with the molecular formula $C_{17}H_{33}O_2$. The MS further exhibited the base peak at *m/z* 74, which was ascribed to the formula $C_3H_6O_2$, being formed due to a McLafferty rearrangement, and in fact this is normally the base peak for straight-chained methyl esters constituting C_6-C_{26} chains. Other characteristic peaks for methyl esters were also observed in the MS of compound **4.6** at *m/z* 87 and 59, these being due to α and γ cleavage relative to the carbonyl carbon, leading to the formation of the fragment ions with formula $C_4H_7O_2$ and $C_2H_3O_2$, respectively. Clusters of peaks which were 14 mass units apart were also observed and these represented cleavage of CH_2 units, as generally expected for the aliphatic part of the compound. These included peaks that appeared at *m/z* 241, 213, 199, 85, 171, 157, 143, 129, 115, 101, 87 and 73, indicating the presence of 12 CH_2 units in the compound **4.6** (Figure 4.9 and Scheme 4.7), and hence being consistent with structure **4.6**.

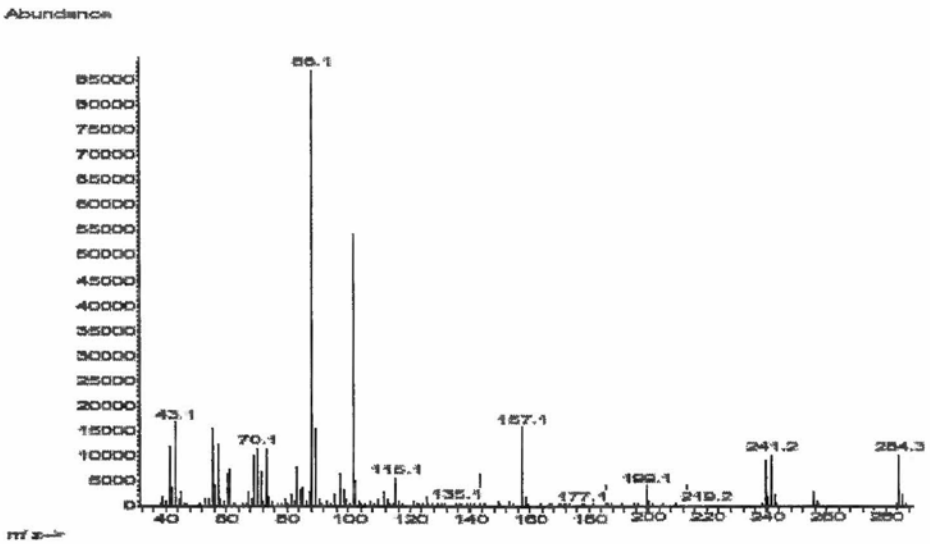
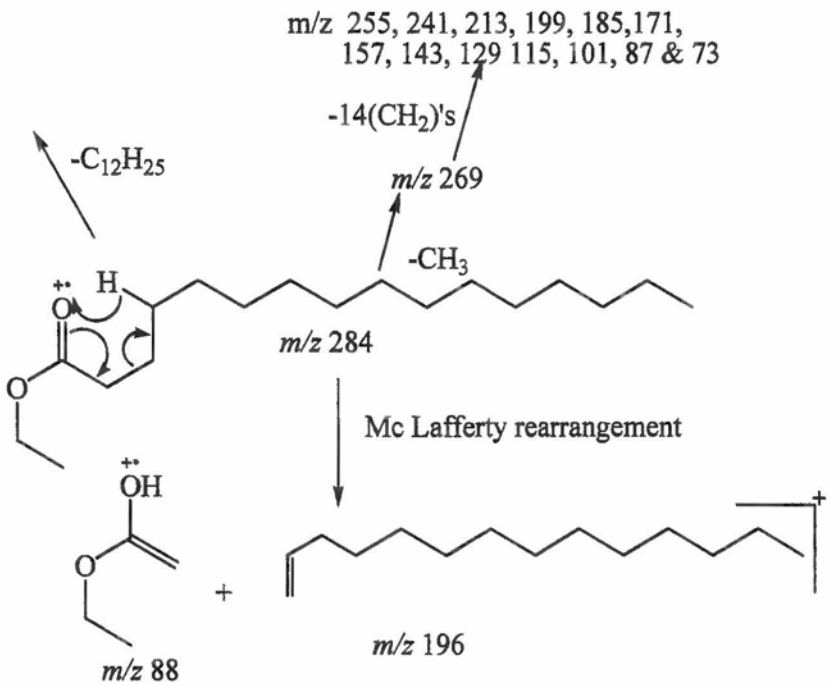


Figure 4.8 Mass Spectrum of ethyl hexadecanoate (4.5)



Scheme 4.6 MS Fragmentation Pattern for ethyl hexadecanoate (4.5)

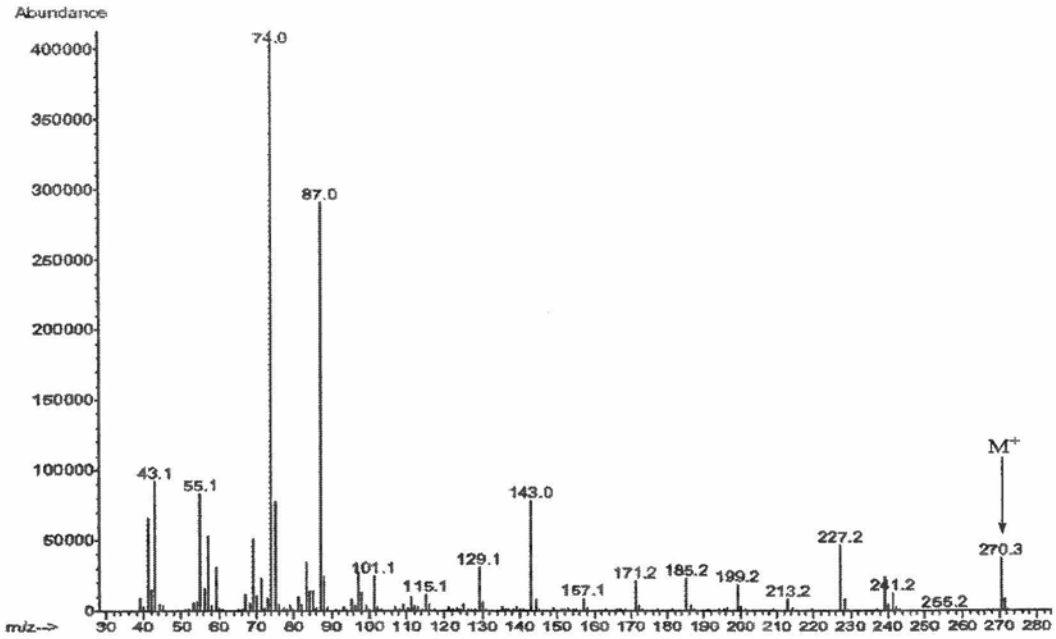
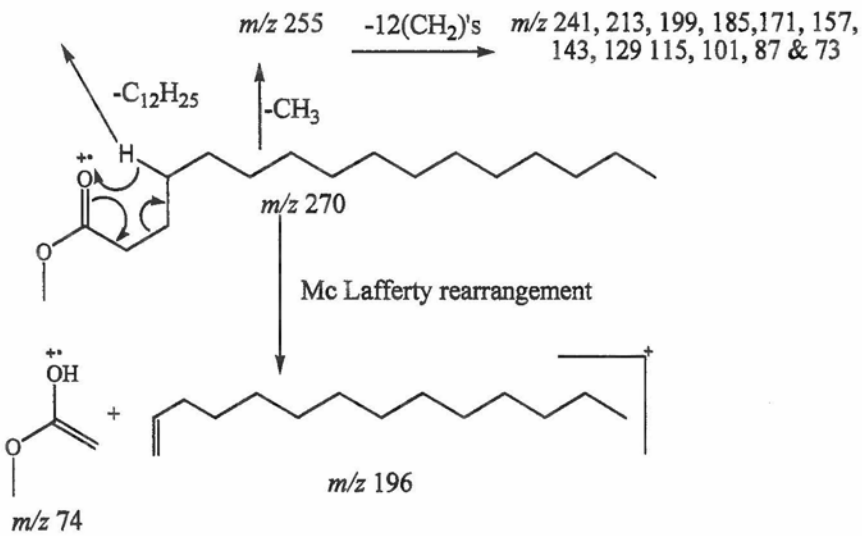


Figure 4.9 Mass Spectrum of methyl hexadecanoate (4.6)



Scheme 4.7 MS Fragmentation Pattern for methyl hexadecanoate (4.6)

4.3.3 Coupled Gas Chromatography-Electroantennographic Detection (GC-EAD) Analysis of Volatiles from Indian almond (*T. catappa*) Ripe Fruits

The volatiles from Indian almond (*Terminalia catappa*) ripe fruits (ca. 200 g) were trapped overnight and then eluted with 150 µl of HPLC grade dichloromethane (Aldrich, UK). GC-EAD analysis on the antennae of both male and female flies led to the detection a total of 19 active peaks from the volatile blends of ripe fruits (Figures 4.10 and 4.11). Identification of the active compounds was facilitated by GC-MS and the identified compounds included esters, terpenes and a ketone and these comprised of 3-hydroxy-2-butanone, propyl acetate, isobutyl acetate, isoamyl acetate, 4-penten-1-yl acetate, prenyl acetate **(4.3)**, β-myrcene, δ-3-carene, *trans*-β-ocimene, terpinolene, benzyl acetate, citronellyl acetate **(4.4)**, geranyl acetate **(4.5)**, and elemicin **(4.6)**. Of these, the EAG activity of eight compounds was confirmed by GC-EAD through comparison with their synthetic analogues, and upon comparison with their mass spectra by GC-MS. The compounds were identified as isobutyl acetate, isoamyl acetate, prenyl acetate, β-myrecene, *trans*-β-ocimene, benzyl acetate, citronellyl acetate and geranyl acetate.

In a recent publication, the *B. dorsalis* female's antennae were reported to have detected 22 compounds from volatiles of *T. catappa* L. fuits using coupled gas chromatography-electroantennographic detector (GC-EAD).[31] In the present investigations, a comparable number of constituents (19) was detected by antennae of both male and female *B. invadens*. Some of these compounds were not detected by *B. dorsalis*[31], including 3-hydroxy-2-butanone, propyl acetate, isobutyl acetate, isoamyl acetate, prenyl acetate, β-myrcene, δ-3-carene, *trans*-β-ocimene,

terpinolene, benzyl acetate, citronellyl acetate, geranyl acetate, and elemicin. *B. dorsalis* is a very closely related to *B. invadens*; in fact, at first *B. invadens* was thought to be *B. dorsalis*. Morever, the two fly species appear to respond to different sets of constituents of the *T. catappa* fruit. Interestingly, esters dominated in the classes of compounds that were perceived electrophysiologically by *B. invadens*; and they also dominated for *B. dorsalis* except that the compounds in the two cases were different.

The presence of methyl eugenol in *T. catappa* fruit volatiles has been reported previously.[31] Since methyl eugenol is one of the very strong lures for a number of fruit flies.[31] its presence and that of other constituents could partly explain why *T. catappa* is a preferred host for *B. invadens* as well as the oriental fruit fly (*B. dorsalis*). However, the compound has been reported to attract only males of these fruit fly species. The observed EAG responses from antennae of female *B. invadens* was surprising and may be supportive of a behavioral role of phenyl propanoids in females of *Bactrocera* species, as suggested by Raghu.[62]

B. invadens detected more than one group of compounds in the volatile blends of the Indian almond fruits. Although there were more esters in the ripe fruits and more terpenes in the unripe fruits, the antennae of the insects were able to detect both the esters and terpenes. This would explain the fact that the insects were not able to discriminate significantly between mature unripe and ripe fruit odours in the olfactrometric assays (see Chapter Two of this Thesis). This may also partly explain the polyphagous nature of this fruit fly. The volatile fruit odors have been used

successfully as attractants for a number of fruit flies and have been investigated as potential attractants for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann),[124-126] the Mexican fruit fly, *Anastrepha ludens* Loew,[127,128] and the Caribbean fruit fly, *Anastrepha suspensa* (Loew).[129]

These results suggest that there is potential for the development of an effective attractant blend from electrophysiologically active compounds identified from the volatiles of this host plant, which could also provide farmers with a non-toxic method for monitoring and controlling *B. invadens* populations in their orchards.

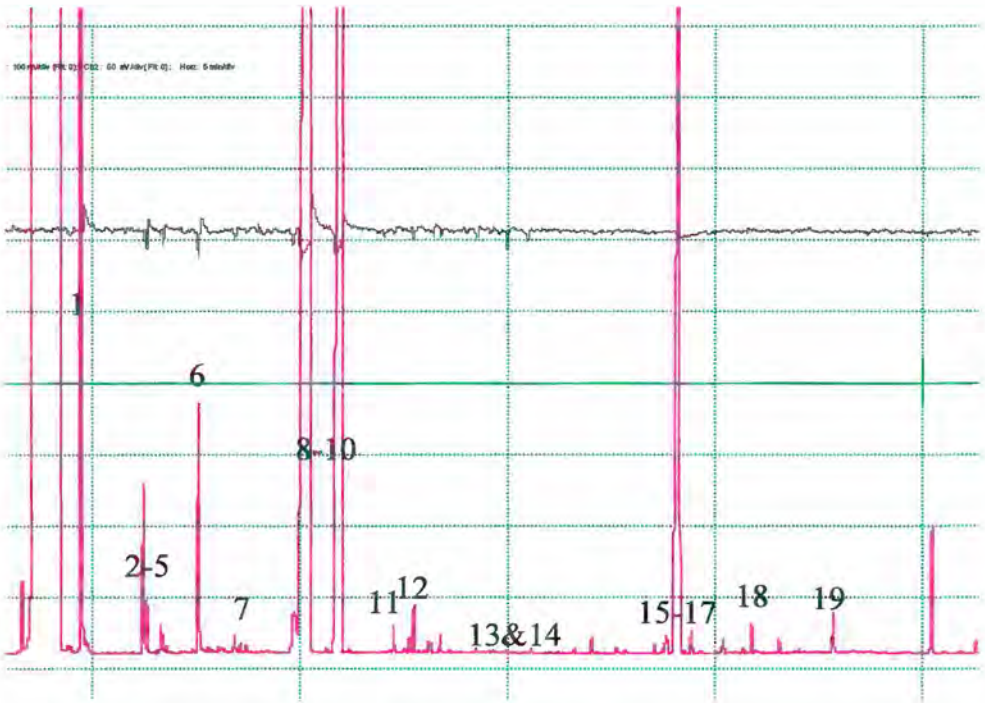


Figure 4.10 GC-EAD of Female *Bactrocera invadens'* Antennae on *Terminalia catappa* Ripe Fruits Volatiles

The Numbers Represent Peaks with EAD Responses: 1 = isobutyl acetate, 2 = 4-pentenol, 3 = butyl acetate, 4 = 2-butenyl acetate, 5 = ethyl isovalerate, 6 = isoamyl acetate, 7 = 4-penten-1-yl acetate, 8 = prenyl acetate, 9 = β-myrcene, 10 = δ-3-carene, 11 = limonene, 12 = *trans*-β-ocimene, 13 = terpinolene, 14 = benzyl acetate, 15 = citronellyl acetate, 16 = geranyl acetate, 17 = methyl eugenol, 18 = *(E,E)*-α-farnesene and 19 = elemicin

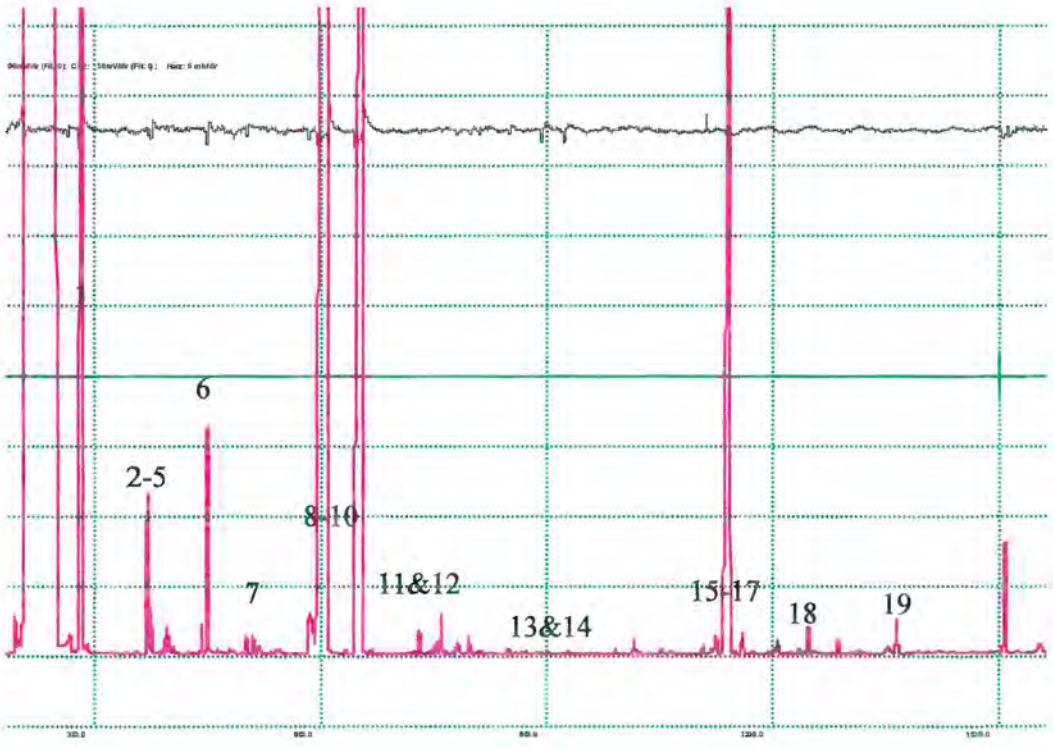


Figure 4.2 GC-EAD of Male *Bactrocera invadens'* Antennae on *Terminalia catappa* Ripe Fruits Volatiles

The Numbers Represent Peaks with EAD Responses: 1 = isobutyl acetate, 2 = 4-pentenol, 3 = butyl acetate, 4 = 2-butenyl acetate, 5 = ethyl isovalerate, 6 = isoamyl acetate, 7 = 4-penten-1-yl acetate, 8 = prenyl acetate, 9 = β-myrcene, 10 = δ-3-carene, 11 = limonene, 12 = *trans*-β-ocimene, 13 = terpinolene, 14 = benzyl acetate, 15 = citronellyl acetate, 16 = geranyl acetate, 17 = methyl eugenol 18 = *(E,E)*-α-farnesene and 19 = elemicin

4.3.4 Summary of Compounds Identified from the Three Hosts

The coupled GC-EAD analysis of identified a total of forty five compounds from the three hosts which included hydrocarbons, esters, alcohols, terpenes, terpenoids and phenylpropanoids others. Specific compounds identified from the three hosts are indicated in Table 4.3.

Table 4.3 Summary of all the Compounds Identified from the Three Hosts

	Hosts		
S/N	***M. indica***	***S. birrea***	***T. catappa***
1	Unidentified	isopropyl acetate	isobutyl acetate
2	Unidentified	ethyl propionate	4-pentenol
3	Unidentified	ethyl isobutyrate	butyl acetate
4	3-hydroxy-2-butanone	ethyl butyrate	2-butenyl acetate
5	ethyl butyrate	ethyl-2-methylbutyrate	ethyl isovalerate
6	*(Z)*-ethyl crotonate	ethyl isovalerate	isoamyl acetate
7	tricyclene	isopropyl valerate	4-penten-1-yl acetate
8	α-pinene	propyl isovalerate	prenyl acetate
9	camphene	*p*-cymene	β-myrcene
10	β-pinene	*trans*-β-ocimene	δ-3-carene
11	sabinene	*(Z)*-3-octen-1-ol	limonene
12	*trans*-β-ocimene	octanol	*trans*-β-ocimene
13	γ-terpinene	4-undecene	terpinolene
14	terpinolene	linalool	benzyl acetate
15	*(Z)*-ethyl 4-octenoate	*(Z)*-3-decene-1-ol	citronellyl acetate
16	ethyl octanoate	5-dodecen-1-ol	geranyl acetate
17	α-humulene	*(E,E)*-α-farnesene	methyl eugenol
18		13-tetradecenal	*(E,E)*-α-farnesene
19			elemicin

From the above results, it is evident that there was overlap in the chemical composition of fruit volatiles from the three host plants. For example, the compounds that were identified in mango and almond included myrcene, 3-hydroxy-2-butanone and *trans*-β-ocimene; the compound found in mango and marula was *trans*-β-ocimene while the compounds occurring in almond and marula included ethyl isovalerate, *trans*-β-ocimene and *(E,E)*-α-farnesene. However, *trans*-β-ocimene was present in volatiles from all the three host fruits. The results demonstrated a low degree of overlapping of the active constituents among the different hosts, implying a chance for *B. invadens* to infest a relatively large number of hosts. In general, esters

represented a larger group of compounds that elicited antennal responses of the flies; the other groups included terpenes and alcohols.

CHAPTER FIVE

CANDIDATE ATTRACTANTS FOR *BACTROCERA INVADENS* (DIPTERA; TEPHRITIDAE) MALE FLIES FROM *GYNANDROPSIS GYNANDRA* (CAPPARIDACEAE)

Abstract

This Chapter reports candidate attractants of *Bactrocera invadens* male flies associated with *Gynandropsis gynandra*. In addition, the presence of the candidate attractive compounds influencing the behaviour of the insect was investigated in extracts of the plant and those from guts of the flies. Field observations on the plant showed that the male fruit flies were attracted to *G. gynandra* plants beginning at around 06:30 am to 12:30 pm. The maximum mean number of flies attracted was 65.26 ± 1.06/plant/day recorded between 08:01-08:15 hrs, showing a strong positive correlation to the time of the day ($r^2 = 0.9423$). In GC-EAD and GC-MS analyses, two compounds 4-methyl-3-penten-2-one, and 4-hydroxy-4-methyl-2-pentanone were identified in both *G. gynandra* and male *B. invadens* gut extracts to be potential candidate attractants and they have not been reported before. The analyses of the extracts from *G. gynandra* and gut of both laboratory reared and field flies revealed that, 4-hydroxy-4-methyl-2-pentanone was present in both sources, however, 4-methyl-3-penten-2-one was only present in the extracts from the plant and from the gut extract of insects collected from the field while feeding on the plant. It was concluded that attraction of *B. invadens* to *G. gynandra* could be mediated by two compounds, *viz*, 4-hydroxy-4-methyl-2-pentanone and 4-methyl-3-penten-2-one, which could also be candidate attractants for the fruit fly.

5.1 Introduction

Gynandropsis gynandra belongs to the plant family Capparidaceae that include erect, branched, somewhat hairy herbs that grow 0.4 - 1 meter high, and usually have purple stems.[130] The plant has been used for many years in African traditional medicinal practices. For example, the leaves which have a high content of Vitamin C are taken as a pot herb in soups, either fresh or dried.[131-133] Extracts from the leaves and stems of *G. gynandra* have antibacterial activity against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Streptococcus faecalis,* and antifungal activity against *Candida albicans, Penicillium sp, Fusarium oxyposirum, Aspergillus flavus and Aspergillus niger.*[134] In a tick-climbing repellency bioassay, the oil from *G. gynandra* was repellent to *Rhipicephalus appendiculatus,* which at the highest treatment levels was higher than that by the commercial arthropod repellent *N,N*-diethyltoluamide.[135] In studies whose results are reported in this Chapter, this plant species was observed to attract male fruit flies, *Bactrocera invadens* in the field.

Males of several economically important Tephritid species are strongly attracted by specific chemical compounds that are referred to as parapheromones or male lures. These lures either occur naturally in plants or have been suggested to be synthetic analogues of plant derived substances.[54] Because of their powerful attractiveness to flies, parapheromones are frequently used in control programmes for detecting and monitoring fruit fly populations in the wild.

Most pest species of *Bactrocera* are attracted to two major natural attractants, namely the raspberry ketone (RK, cue-lure) and methyl eugenol (ME).[136-138] Males of the pest fruit fly species such as *Bactrocera cucurbitae* and *Bactrocera tryoni*, are attracted by raspberry ketone and sequester the chemical into their pheromonal system. Ingestion of methyl eugenol by *Bactrocera dorsalis* has been studied intensively, which showed that the compound is converted in the crop into two major booster sex pheromonal components namely *trans*-coniferyl alcohol and 2-allyl-4,5-dimethoxyphenol.[54] The metabolites are stored in the rectal gland and subsequently released during fanning, which is performed during courtship. These components improve the mating competitiveness of males by at least three-fold when compared with methyl eugenol-deprived males. On the other hand, attractants for male *B. dorsalis* also play a role in the survival of the flies since one of the components, 2-allyl-4,5-dimethoxyphenol, is a very potent allomone, that is a feeding deterent to vertebrate predators.[55]

Field observations showed that *G. gynandra* attracted only males of *B. invadens*. The study described here was conceptualized based on this observation. The objective was to undertake appropriate investigations on the interactions between the plant and the fruit fly species so as to establish the active chemical constituents mediating these interactions.

5.2 Materials and Methods

5.2.1 Insects

Larvae of *B. invadens* were collected from infested mangoes in the field at Nguruman, 180 km South West of Nairobi and from Embu, about 140 km from Nairobi in Eastern Kenya. The larvae were reared in the insectaries at *icipe* Kasarani campus to obtain mature flies that were used for wind tunnel assays. The rearing procedure and maintenance of the fly colony were as previously described in detail in Chapter Two of this thesis.

5.2.2 Response of Flies to *Gynandopsis gynandra* in the Field

Observations on the behavioural responses of fruit flies *B. invadens* towards *G. gynandra* were done in the field at Nguruman, Kenya. Four healthy plants growing in the field were selected and the numbers of the flies on them were counted at intervals of 15 min beginning at 06:30 am to 12:30 pm, for 9 days. On each day, flies on the four plants were counted and their mean constituted one replicate. The average number of flies on the plant for the nine days was calculated. The age of the plants was not taken into account because these were wild plants whose ages were not known. The relative humidity and temperatures were also recorded and their means calculated.

5.2.3 Dual Choice Olfactometric Bioassays

These were carried out as described in detail in Chapter Two of the Thesis. However, in this case plant parts (10 g) including leaves, stems, flowers and pods were used all together in the bioassays as the source of volatiles.

5.2.4 Solvent Extracts from *Gynandropsis gynandra*

Extraction of compounds from *G. gynandra* was carried out in the field at Nguruman in one of the mango orchards. Plant parts (leaves, pods, flowers and stems) were cut and dipped in HPLC grade hexane (Aldrich) for 5 min, then decanted to obtain extracts that were stored in 8 ml glass vials. Vials containing the extracts were then wrapped with aluminum foil and placed in a cool box containing ice, which was then quickly transported to the laboratory for analysis. The residuals were also transferred to the laboratory for weighing. The average weight of the extracted plant materials was approximately 8.9 g.

5.2.5 Solvent Extracts from the Gut of Male Flies, *Bactrocera invadens*

Compounds were extracted from the gut of laboratory-reared fruit flies that had been fed on artificial diet as well as from flies collected from the field while feeding on *G. gynandra*. The gut was removed using forceps by pulling gently from the neck while holding the abdomen. Gut materials from 10 insects were extracted using 1 ml of HPLC grade acetone (Aldrich), and then concentrated to remove the acetone. The extract was dissolved in HPLC grade dichloromethane (Aldrich) and the sample was stored below 20°C prior to analysis on GC-MS.

5.2.6 GC-EAD and GC-MS Analyses

These were carried out as described in Chapter Three of the Thesis.

5.3 Results

5.3.1 Attraction of Males of *Bactrocera invadens* to *G. gynandra* in the Field

Male flies, *B. invadens* were fortuitously observed to visit *Gynandropsis gynandra* in large numbers in the field during the day, which warranted further investigations. Results on observations on males of *B. invadens* visiting *G. gynandra* over a period of nine days showed a strong positive correlation between the number of flies visiting the plants and the time of day (r^2 = 0.9423) for a non-linear model. Observations were made from 06:30 am to 12:30 pm (Figure 5.1). The number of visiting flies increased gradually and reached a maximum of 65.3 ± 1.0 flies/plant/day between 08:01 and 08:15 hrs. Fly numbers then decreased gradually to 2 insects/plant/day between 12:16-12:30 hrs. In addition, the other ambient factors recorded were temperature and relative humidity (Figure 5.2). The number of flies on the plants and the relative humidity were found to decrease with the increase in the ambient temperature. Thus, there was a strong positive correlation between the flies on the plants and the relative humidity. While on the plant, flies were observed to feed on the surface of the plant parts, *viz.* leaves, flowers, stems and pods (Plate 5.1). Results of these observations were an indication that, male *B. invadens* could be ingesting some chemicals (in the dew on these plant parts) that may play an important role in the life processes of this species. This is probably part of the reason

why the numbers of the flies decreased with the decrease in relative humidity associated with the rise in temperature.

Plate 5.1 Males of *Bactrocera invadens* on *Gynandropsis gynandra* in the Field (By Fikira Kimbokota, 2007)

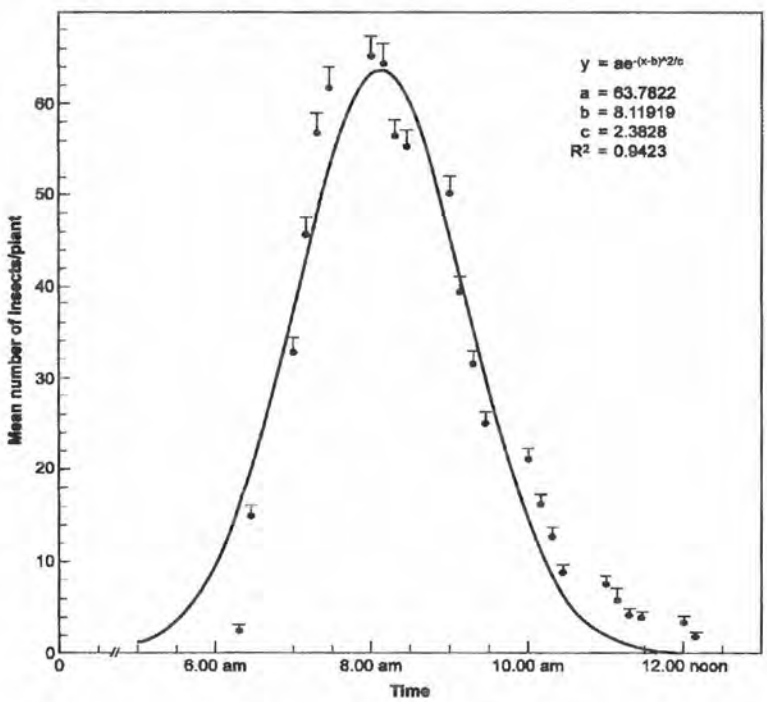


Figure 5.1 Mean Number of Males of *Bactrocera invadens* Per Plant Per Day Observed on *Gynandropsis gynandra*

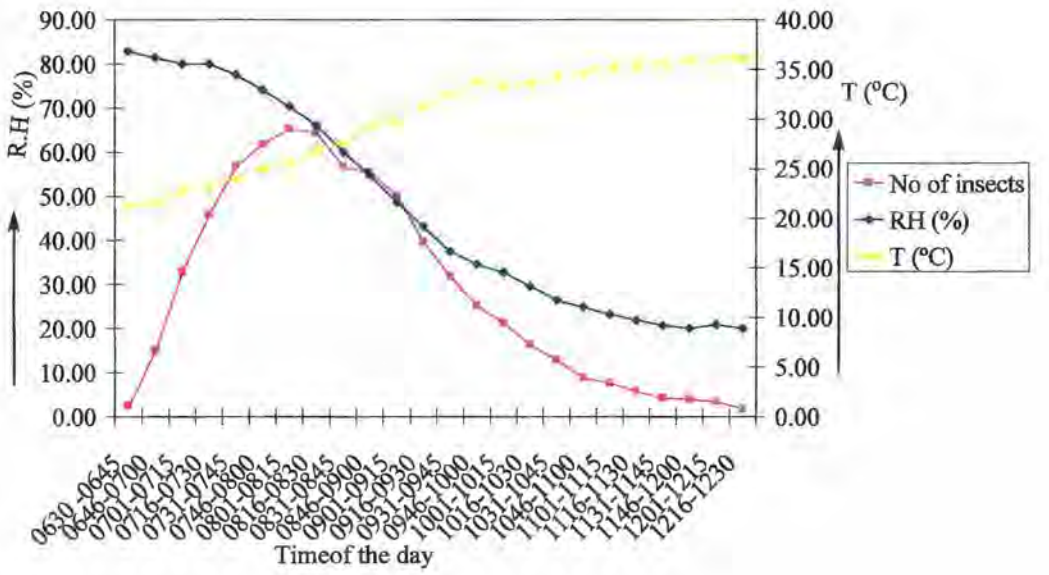


Figure 5.2 Changes in Temperature, Relative Humidity and Number of Flies on the Plant at Different Times of the Day

5.3.2 Dual Choice Olfactometric Bioassays

Assessment in the dual choice olfactometer to evaluate the attractiveness of volatiles from *Gynandropsis gynandra* indicated higher but non-significant response to *G. gynandra* volatiles relative to control ($P > 0.05$) (Figures. 5.3 and 5.4).

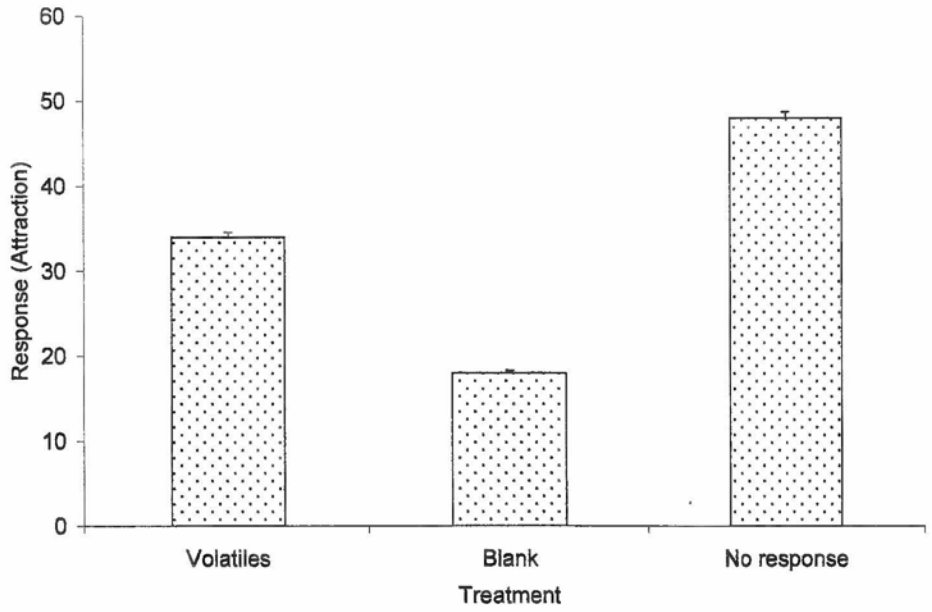


Figure 5.3 **Mean Attraction (± S.E. %) of Male *Bactrocera invadens* to Volatiles from *Gynandropsis gynandra* in a Dual Choice Olfactometer**

5.3.3 GC-MS and GC-EAD Analyses of Plant and Gut Extracts

GC-MS analyses of the hexane extracts of *Gynandropsis gynandra* indicated the presence of a number of compounds including monoterpene and diterpene hydrocarbons, esters, alcohols, ketones, saturated and unsaturated hydrocarbons and others (Figure 5.4). The main constituent was 4-hydroxy-4-methyl-2-pentanone at 69.7% followed by δ-3-carene (6.13 %). With regard to groups of compounds present, ketone was the most abundant at (70.2%) followed by terpenes at (9.66 %). Other groups constituted 9.10 % of compounds in the extracts.

In GC-EAD analyses, antennal olfactory receptors of male flies detected two peaks in extracts from *G. gynandra* that were identified as 4-methyl-3-penten-2-one (**5.1**) and 4-hydroxy-4-methyl-2-pentanone (**5.2**) (Figures 5.5 and 5.6). The identity of these compounds was confirmed by comparison with MS results of gut extracts there had been collected from flies on the same day (Figure 5.7). Analyses of gut extracts from laboratory-reared flies and flies collected from the field indicated the presence of 4-hydroxy-4-methyl-2-pentanone (**5.2**) in both extracts (Figures 5.7 and 5.8). However, 4-methyl-3-penten-2-one (**5.1**) was only obtained from flies collected from the field (Figure 5.8). Further analysis with GC-EAD to confirm the electroantenographic activities of these compounds was performed using their synthetic analogues. However, compound **5.2** exhibited a relatively weak EAG response at the dose tested.

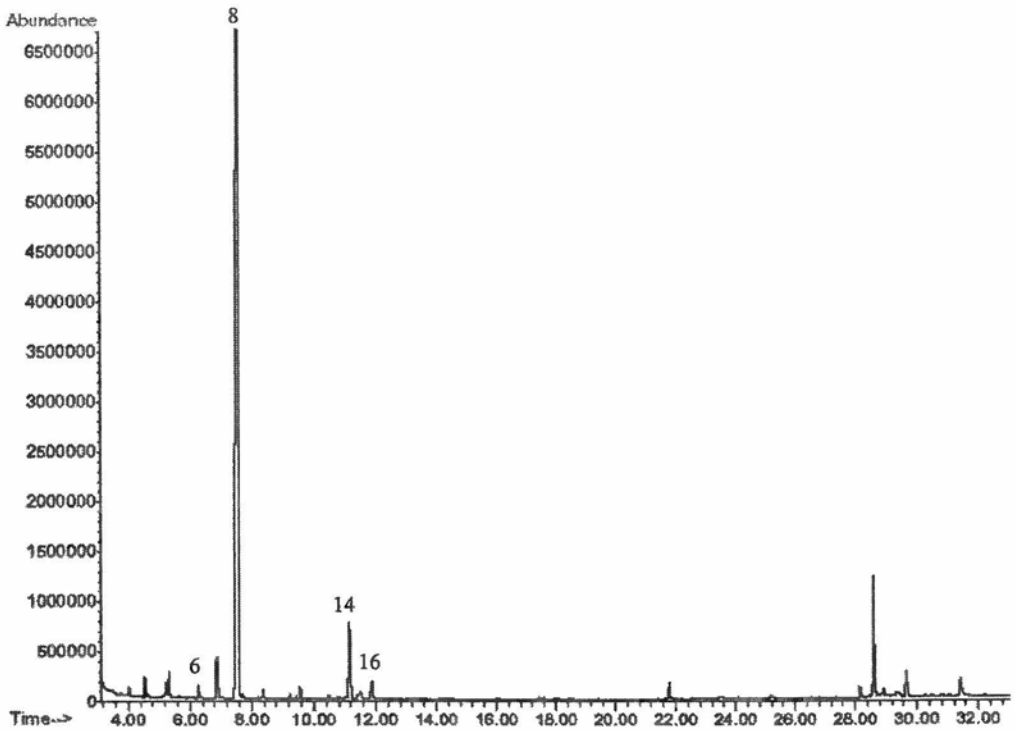
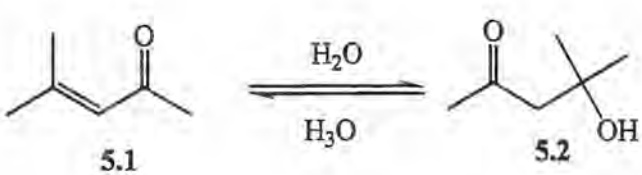


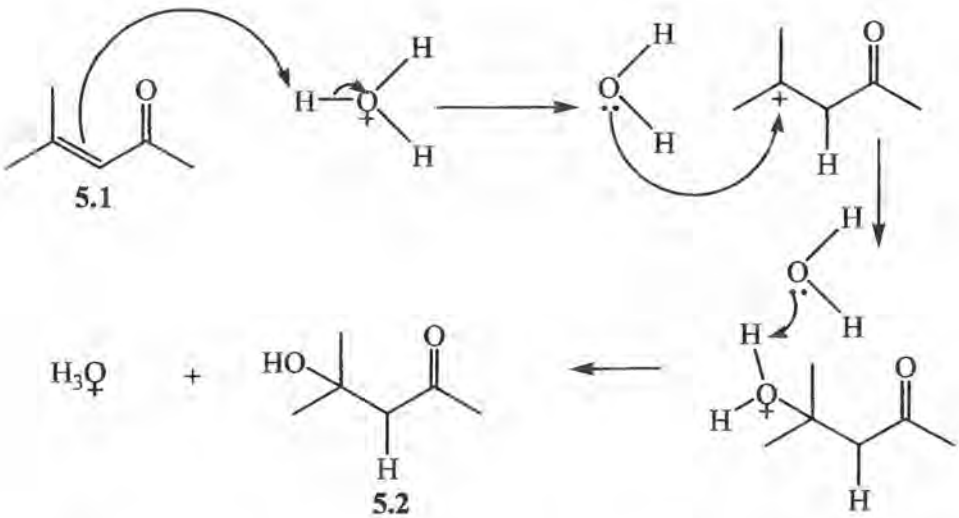
Figure 5.4 Representative Gas chromatogram of hexane Extracts from *Gynandropsis gynandra.*

Table 5.1 Relative Amounts (%) of Compounds Identified in hexane Extracts of *Gynandropsis gynandra*

S/N	Compound	R.T. (min)	% Composition
1	ethyl propanoate	4.03	0.34
2	3-methyl-1-butanol	4.54	0.89
3	4-methyl-2-pentene	4.63	0.20
4	ethyl isobutanoate	5.26	0.68
5	toluene	5.33	1.11
6	4-methyl-3-penten-2-one	6.29	0.48
7	isopropyl hydroperoxide	6.87	2.35
8	4-hydroxy-4-methyl-2-pentanone	7.57	69.71
9	Ethyl-2-methylbutanoate	7.7	0.34
10	3-methylbutyl acetate	8.37	0.32
11	α-phellandrene	9.45	0.14
12	α-pinene	9.56	0.46
13	β-pinene	10.48	0.24
14	δ-3-carene	11.15	6.13
15	limonene	11.51	0.99
16	*trans*-β-ocimene	11.89	1.45
17	α-gurjunene	17.44	0.13
18	β-caryophyllene	17.58	0.12
19	*trans*-methyl isoeugenol	21.77	0.88
20	1,13-tetradecadiene	22.55	0.14
21	hexadecanoic acid	23.47	0.20
22	tricosane	26.56	0.08
23	pentacosane	28.17	0.66
24	heptacosane	29.67	1.66
25	nonadecane	30.48	0.29
26	eicosane	31.4	1.64
27	hexacosane	33.82	0.08



5.1 5.2



Scheme 5.1 Proposed Mechanisms for Hydration of 5.1 to 5.2

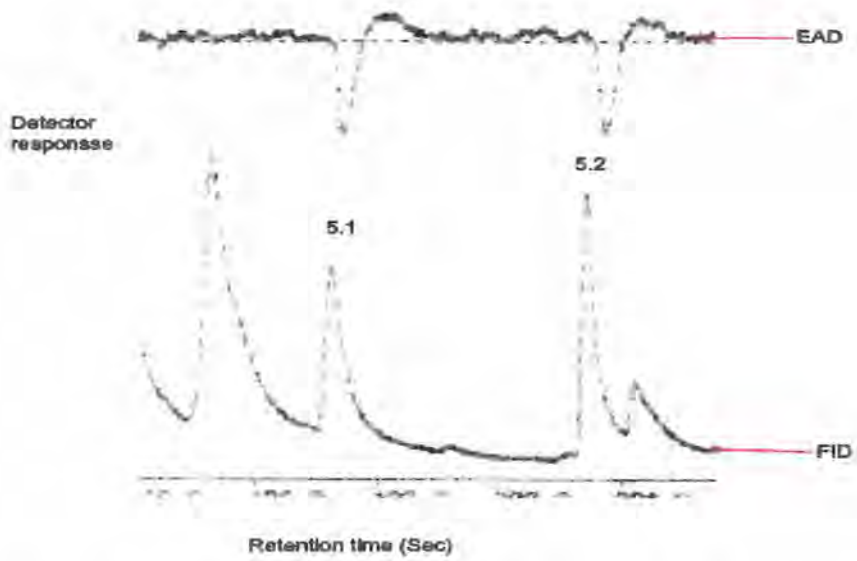


Figure 5.5 Representative GC-EAD Profile of Compounds in dichloromethane Extract of *Gynandropsis gynandra* Tested on the Antenna of Male *B. invadens* Showing the EAG-Active Peaks (5.1 and 5.2)

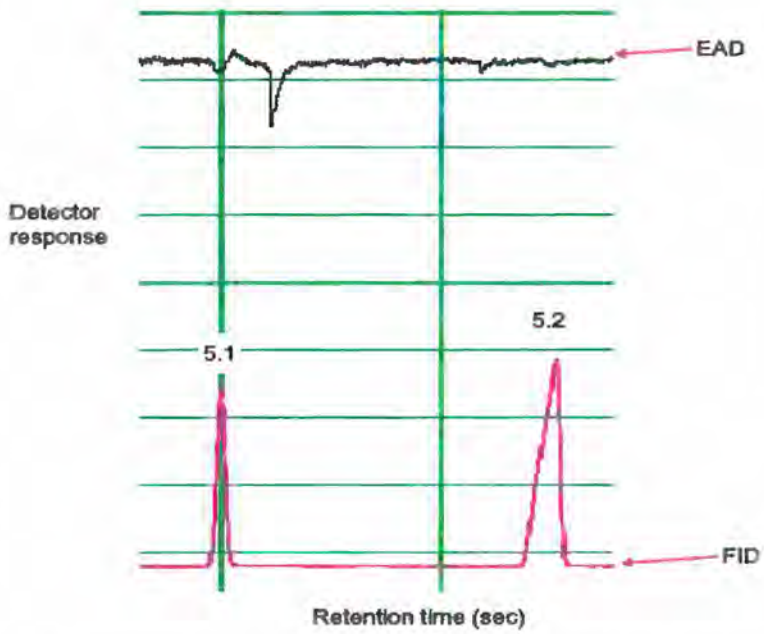


Figure 5.6 **Representative GC-EAD Profile of the Synthetic Analogues of the Two Identified EAG-Active Compounds (5.1 = 4-methyl-3-penten-2-one and 5.2 = 4-hydroxy-4-methyl-2-pentanone)**

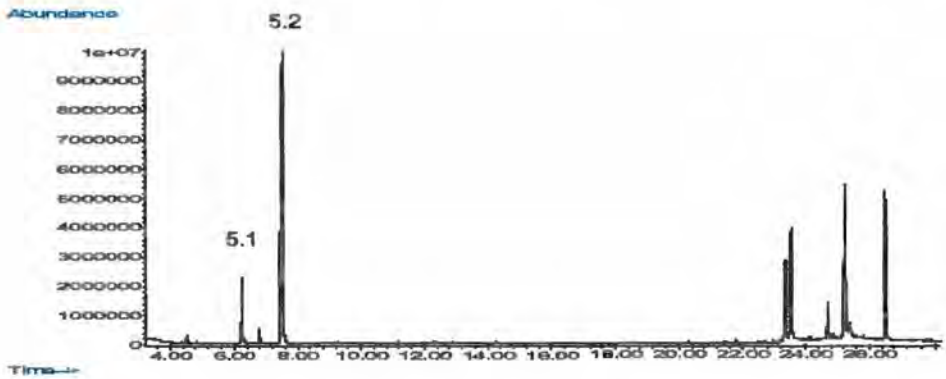


Figure 5.7 **Representative Gas Chromatogram of Gut Extracts from Male Flies/ *B. invadens* after feeding on *Gynandropsis gynandra* in the Field**

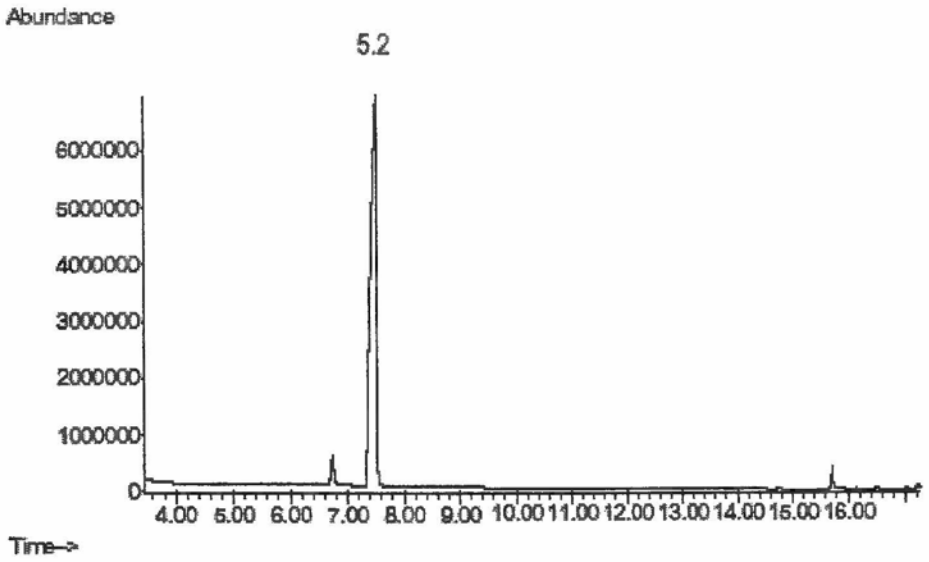


Figure 5.8 Representative Gas Chromatogram of Gut Extracts from Male Flies, *B. invadens* Reared on Artificial Diet in the Laboratory

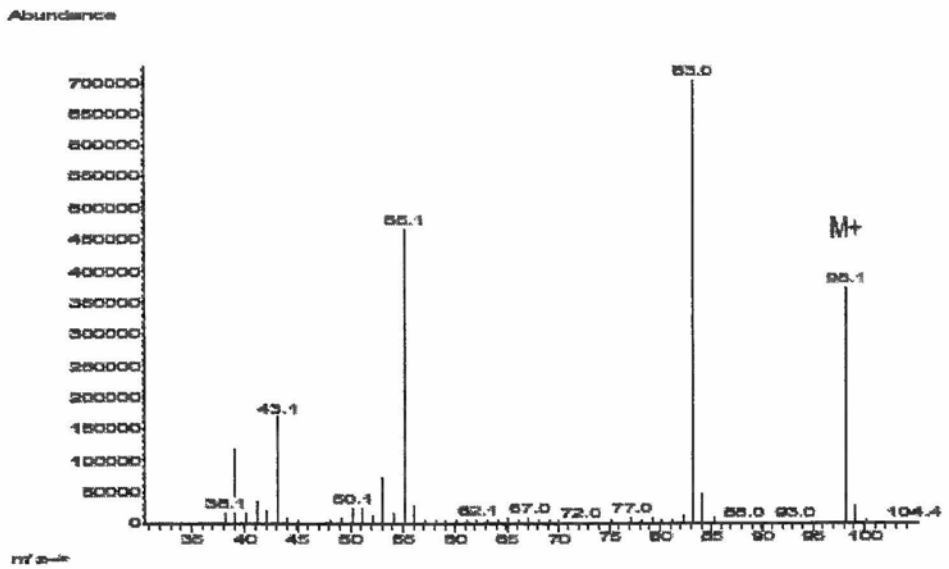
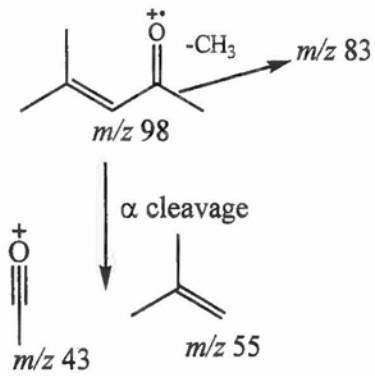


Figure 5.10 Mass Spectrum of Compound 5.1



Scheme 5.2 Mass Spectral Fragmentation Pattern for 4-methyl-3-penten-2-one (5.1)

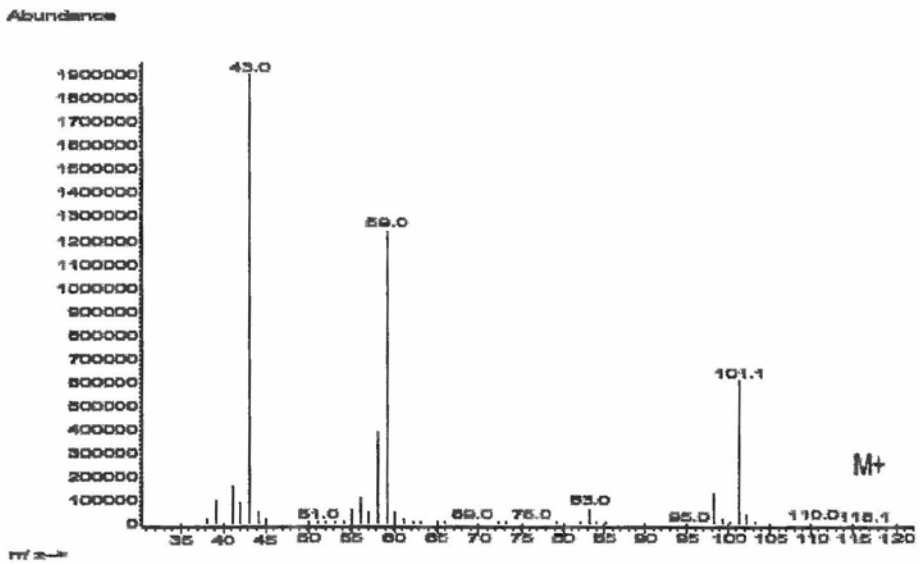
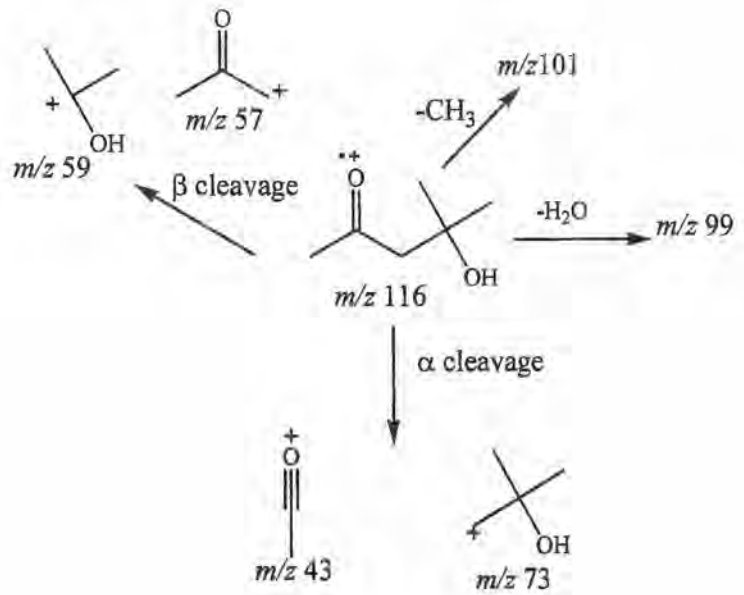


Figure 5.11 Mass Spectrum of Compound 5.2



Scheme 5.3 Mass Spectral Fragmentation Pattern for 4-hydroxy-4-methyl-2-pentanone (5.2)

5.4 Discussion

In recent years, parapheromones have been documented as significant lures for the management of fruit flies, in particular those of the *Bactrocera* complex of fruit flies.[54] Thus, the results reported in this work on the attractiveness of *Gynandropsis gynandra* to *Bactrocera invadens* males in the field are a contribution to the relentless search for plant-derived chemical attractants for monitoring and management of fruit fly pests. Overall, the results suggest that *G. gynandra* contains candidate attractants for male flies, *B. invadens*. From literature search, this is the first report that has documented observations on and evaluation of the attractiveness of *G. gynandra* to males of *B. invadens*. However, attractiveness of the plant volatiles under laboratory conditions was not statistically significant, which could be due to different factors, including inappropriate dose emission of the volatiles from

harvested plant materials, changed composition of volatiles from these materials, inappropriate diurnal physiological state of the laboratory insects, and artificial environmental conditions of the olfactometer used. Interestingly, the results obtained in this study coincided with what was previously reported for other species of fruit flies, where many males were reported to be attracted by compounds emitted by non-host plants.[54] It has been speculated that these compounds act as precursors of pheromone constituents of the respective species or, are used for defense against predators of the flies.[54] It has recently been demonstrated that plant-derived chemicals may also influence the mating success of a number of fruit flies. Thus, in some of such studies male medflies, *Ceratitis capitata*, exposed to oranges were found to attain significantly more copulations than non-exposed males.[139,140] Also, male flies of this species that were exposed to the bark and fruits of guava trees, *Psidium guajava* L. had a significant mating advantage over their male conspecifics that were deprived access to these substrates.[94]

In the present study, the identified compounds that were considered to be responsible for the attraction of male *B. invadens* are two low molecular weight ketones, namely 4-methyl-3-penten-2-one (**5.1**) and 4-hydroxy-4-methyl-2-pentanone (**5.2**). It is the first time that these compounds are being reported to be candidate attractants for this fruit fly and may perhaps play a similar role in other fruit fly species. However, it was interesting to observe that these compounds are the close intermediates in the synthesis of methyl–isobutyl ketone (MIBK). The compound is widely used in the industrial manufacture of a large number of products, such as inks, varnishes, etc.[141,142] Its synthesis involves aldolization of two acetone molecules to form 4-

hydroxy-4-methyl-2-pentanone (**5.2**) followed by dehydration of this compoundto give 4-methyl-3-penten-2-one, which is then selectively hydrogenated to give methyl isobutyl ketone (MIBK).[141,142]

From the above results, it is also possible that, once compound **5.1** is emitted by the plant in a humid environment it readily hydrolyses to compound **5.2**. This process could also be explained from the fact that the insects were mostly attracted in the morning when the environment was much more humid and the temperature was low compared to the day time when temperatures were high and the air was dry. From results on the observations on the behaviour of flies on *G. gynandra* plants in the field and the chemical nature of the identified compounds, the following factors are hypothesized:

a) It is possible that, the insects ingest compound **5.1** from the surface of the plant which acts as a precursor for compound **5.2,** which was also present in the gut of both wild flies that had been feeding from these plants and laboratory reared male flies. However, the evolutionary significance of this compound in the behavioural and chemical ecology of these flies remains to be investigated.

b) Also, it is possible that, these compounds are associative signal(s) to the insects that indicate the presence of other less volatile compound(s) on the plant that are beneficial to the fruit flies.

Furthermore, the presence of compound **5.1** in the gut of the insects obtained in the field when feeding on the plant and its absence in the gut of insects reared in the

laboratory might indicate that, this compound has a biological significance to the insect. The presence of compound **5.2** in the insect gut was probably because of the possibility of reversible hydration and dehydration reactions between the two compounds **5.1** and **5.2**.

In tephritid fruit flies, sexual selection has been hypothesized to occur through a mechanism of female choice.[143,144] Thus, female flies are believed to preferentially mate with flies that have fed on certain chemicals that are collectively denoted by fruit fly biologists as parapheromones or male-lures. However, there are no observable benefits to females mating with lure-fed males with regard to fecundity or subsequent hatchability of eggs.[143] Most of these chemicals such as methyl eugenol occur naturally in plants or are close analogues of plant-derived chemicals (*e.g.* cuelure).[82,145,146] The ingested substances are hypothesized to be precursors of the male-produced sex pheromone which subsequently make the emitting males more attractive to females.[143,147-149] Although a lot of effort has been invested on this area of chemical ecology of fruit flies, there is still no concrete explanation on the exact role that is played by parapheromones in the biology of these flies. Hence, more research is needed in this area.

In conclusion, the results reported in this chapter show that the emission of 4-methyl-3-penten-2-one (**5.1**) may play a major role in the attraction of male *B. invadens* to *G. gynandra* plants in the field. In addition, the presence of 4-hydroxy-4-methyl-2-pentanone (**5.2**) in the guts of male flies that were found feeding on the plant as well as in the plant extract indicates the possibility of facile hydration of compound **5.1** to

form **5.2**. The latter may play a significant role in the biology of the flies that remains to be elucidated.

CHAPTER SIX

RESPONSES OF *BACTROCERA INVADENS* TO CONSPECIFIC VOLATILES AND IDENTIFICATION OF THEIR CONSTITUENTS

Abstract

Responses of both male and female *Bactrocera invadens* to their volatiles and to those of each other were evaluated in a dual choice olfactometer. Volatiles from male flies were significantly ($P<0.05$) more attractive to both male and female flies. Further, in GC-MS analyses these volatiles were found to constitute of various groups of compounds in which esters and spiroacetals were the main constituents. The identified spiroacetals included some compounds that had previously been reported to be constituents of the sex pheromones of other fruit flies. These include 2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane, (E,E)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, 2,8-diethyl-1,7-dioxaspiro[5.5]undecane and 2-(*n*-propyl)-8-methyl-1,7-dioxaspiro[5.5]undecane. The esters were the most abundant constituting 59.89% of the volatiles from female flies and 35.73% in those from males. The major peak in the volatile emission was identified to be ethyl dodecanoate which constituted 35.9% and 18.0% in volatiles from female and male flies respectively. The second major peak was for 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (10.67%) from females and *N*-(3-methylbutyl)acetamide (14.75%) from males. Most of the compounds identified were present in the volatile emissions from both male and female flies. The attraction of both male and female flies to the volatiles produced by males and the production of similar constituents by flies of the two sexes suggested that, the volatiles produced by male flies may play a dual role in aggregation for the formation of leks at dusk and for mate recognition.

6.1 Introduction

Individual organisms are surrounded by large and complex ecological communities, and chemical communication may play an important role in inter-specific interaction communication between individuals.[150] This may enable the emitting organism to influence interactions with others at a relatively low energy expenditure.[151] Inter- and intra- specific chemical communication:, mediated by signals, referred to as infochemicals, refers to the act of giving information on the locality and 'species identity' of the emitter, thereby enabling espionage and exploitation by conspecifics, natural enemies, and competing species.[152] In insects, pheromones that regulate intra-specific communication are highly specific and may have a large action radius.However, the overall mechanism involving release of pheromones for infochemical communication depends on the insect species. Several types of pheromones in fruit fly species have been recognized, which include sex and aggregation pheromones, among others.

There is considerable evidence that male fruit fly of the *Dacus* spp. synthesizes and store pheromones in a sac and reservoir associated with the posterior region of the rectum, generally referred to as the rectal glands.[153] Furthermore, sex pheromones are mostly produced by male fruit files, with a few exceptions of species in the genus *Bactrocera*. However, female flies of other species, including those of *Bactrocera invadens* produce some components of sex pheromones.[66] Male fruit flies of *Bactrocera carambolae* produce a major component (6-oxo-1-nonanol) and a minor compound (*N*-3-methylbutyl acetamide) in their rectal glands. These male emissions

were shown to elicit anemotactic flights and attracted more conspecific virgin female flies than males in wind tunnel assays.[154]

The investigations results of which are reported in this Chapter were conceptualized based on the background outlined above, with the aim of characterizing the components in volatiles produced by the fruit fly *Bactrocera invadens*. It was hypothesized that some of these compounds may play a role as a sex pheromone in the biology of this insect.

6.2 Materials and Methods

6.2.1 Dual Choice Olfactometric Analysis

The bioassays were carried out in a dual-choice olfactometer as described in detail in Chapter Two of the thesis. The sources of volatiles were either 50 sexually mature male or female flies that were 10-15 days old and were held in a 2 l flask (ARS Gainesville, FL, USA). Test flies were virgin conspecifics that were also of the same age and 10 flies were tested in each replicate and each test was replicated five times.

6.2.2 Collection of Volatiles from Flies

The volatiles emitted by virgin females and calling male flies were collected using an air entrainment apparatus. A humidified and activated charcoal-filtered (Chrompack, Gas-clean Charcoal, The Netherlands) air stream was passed over live flies in a quickfit glass container (ARS Gainesville, FL, USA, 23 long x 4 cm o.d.). The

volatile compounds emitted by the insects were trapped onto Super-Q adsorbent polymer (*ca.* 30 mg, 80-100 mesh; Alltech, Nicholasville, KY). The preparation of the adsorbent involved washing of the Super-Q with HPLC grade dichloromethane (Aldrich), by pushing it with high purity nitrogen gas (BOC, Kenya). The volume or mass flow was ca. 260 ml/min, flowing constantly and measured with an airflow meter (AALBORG, 1-800-866-3837, Orangeburg, NY). All glassware used were previously washed and rinsed with acetone and dried in an oven. The aeration process was carried out from 03:00 to 06:00 hrs using 50 virgin flies aged between 10 and 15 days. After aeration, the trapped compounds were eluted from the adsorbent using 150 µl of HPLC-grade dichloromethane (99.9%, Aldrich), by passing nitrogen gas over it. The eluates was then stored in a freezer until analyses.

6.2.3 GC-MS Analyses

Odours collected from 10-15 day-old sexually mature male and female flies respectively, were analysed as described in Chapter Three.

6.3 Results and Discussion

6.3.1 Dual-Choice Olfactometer

In the olfactometric tests with volatiles from male *B. invadens*, flies were more attracted to the volatile from conspecifics than to the control (clean air). Up to 74% of the female flies were attracted to the volatiles, while only 6% flew to the control (χ^2 =17.3000, P = 0.0040) (Fig. 6.1). For the male flies, 66% flew to the volatiles and 8% to the control (χ^2 =15.0270, P = 0.0046). Up to 50% of flies when females were

used as sources of odour, flew toward the volatiles and 18% to the control. For male flies, 54% flew to the volatiles emitted by females and 14% flew to the control side of the olfactometer (Fig. 6.1). However, these differences were not statistically significant ($\chi^2 = 9.6$, P = 0.1425 and $\chi^2 = 8.7059$, P = 0.1214 respectively).

These results suggest that, it is most likely that male flies were the ones producing a pheromone that was being detected by both female and male conspecifics. It has been reported that, with the exception of *Dacus oleae*, attractant pheromones in tropical frugivorous tephritids are produced by male flies.[66,155] On the other hand, attraction of both males and females of *B. invadens* by the emissions from conspecific males suggest the possibility that, these volatiles could play two major roles, as sex and aggregation pheromones. In other species of fruit flies, in particular those whose males form leks, male flies release pheromones that attract both males and virgin females.[146] Furthermore, the production of pheromonal constituents by females as it is hereby reported, is also common to some tephritid species.[66] Volatile emissions are also believed to be used as aggregation pheromones for gathering females at the courtship sites. Several males have been observed to gather in a small space under tree leaves and then attract other males to form lek.[156,157]

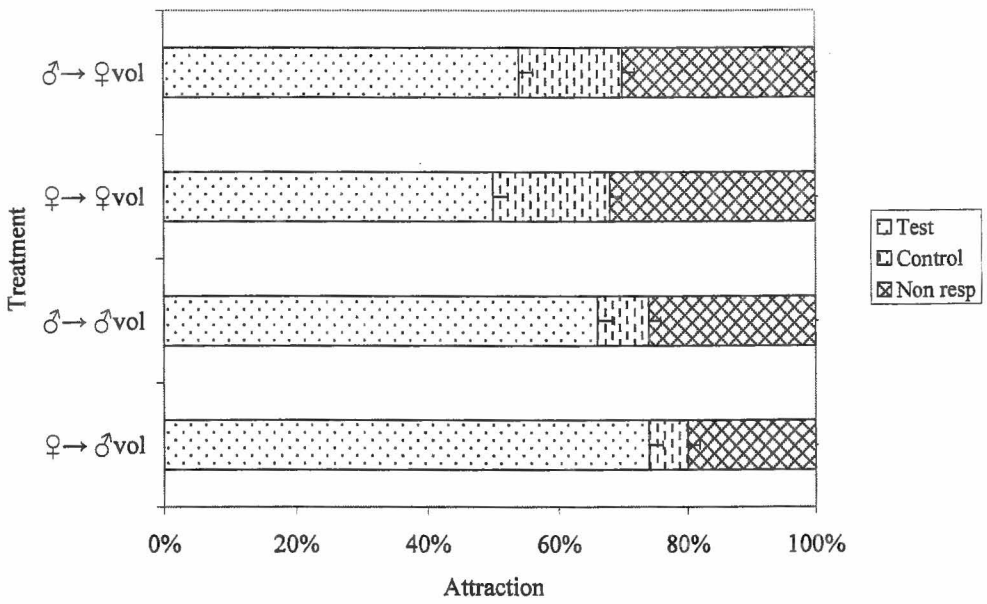


Figure 6.1 Response of Male and Female Flies, *B. invadens* to Volatiles Emitted by their Live Conspecifics in a Dual-Choice Olfactomer.

Key; ♀ = female flies, ♂ = male flies, ♀vol = volatiles produced by females and ♂vol = volatiles produced by males.

6.3.2 GC-MS Analysis

Mass spectrometric GC-MS analysis of volatiles produced by male and female flies, *B. invadens* showed the presence of similar compounds that only differed in quantities (Table 6.1). A detailed analysis of the mass spectral data and upon comparison with mass spectra of previously reported compounds from a closely related fruit fly indicated the presence of compounds that have been reported to be constituents of sex pheromones from other closely related fruit fly species.[154,158]

The compounds identified in the volatile mixtures included unsaturated and mono-saturated fatty acid esters, spiroacetals, amides, keto alcohols, aldehydes, alcohols and other unidentified compounds. In the GC- MS profile (Figure 6.2) the major peak was established to be methyl dodecanoate (35.91% and 17.96%) for females and males respectively. The second major peaks were due to 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (10.67%) for females and *N*-(3-methylbutyl)acetamide (14.75%) for males. Esters represented high percentages (59.89% and 35.73%) for females and males respectively (Figure 6.2).

The identified spiroacetals in the present study included 2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane **(6.1)**, (*E,E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane **(6.2)**, 2,8-diethyl-1,7-dioxaspiro[5.5]undecane **(6.3)** and 2-*n*-propyl-8-methyl-1,7-dioxaspiro[5.5]undecane **(6.4)**. Other compounds identified were C_{14}, C_{16} and C_{18} ethyl and methyl esters, as well as acetamides. In other previous studies, it was observed that except for a few cases, male fruit flies were the main producers of sex pheromones. Furthermore, it was reported that GC-MS analysis of SPME-trapped volatiles from *Bactrocera tryoni* females showed that the major components of these emissions were *N*-(3-methylbutyl)propanamide and the spiroacetal (*E,E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane.[158]

In another study, GC-MS analysis of the acetone extract of *Bactrocera dorsalis* male rectal glands showed the presence of fatty acids, esters, amides, keto alcohols, diols and spiroacetals, and the major components were long chain fatty acids.[159] These results are in agreement with the results of the present study. However, some

compounds that were identified in the previous study such as 6-oxo-1-nonanol were not obtained in this study, while compounds such as 3-methylbutanal, 2-methyl butanal and 3-hydroxy-2-butanone were not detected in previous studies mentioned above.[158,159]

The results from these investigations suggest that some of the volatile components could be involved in intraspecific communication process and may play a dual role as sex and/or aggregation pheromone during courtship of *B. invadens.* The results are in agreement with previous reports on *B. dorsalis* complex of fruit flies. This in turn reflects on the close relatedness of the two fruit fly species, namely *B. dorsalis* and *B. invadens*.

In the following section of this Chapter, results of the identification of compounds in volatiles shown in GC-MS profiles from male and female flies are discussed in details. Fragmentation patterns of some of the compounds are also presented.

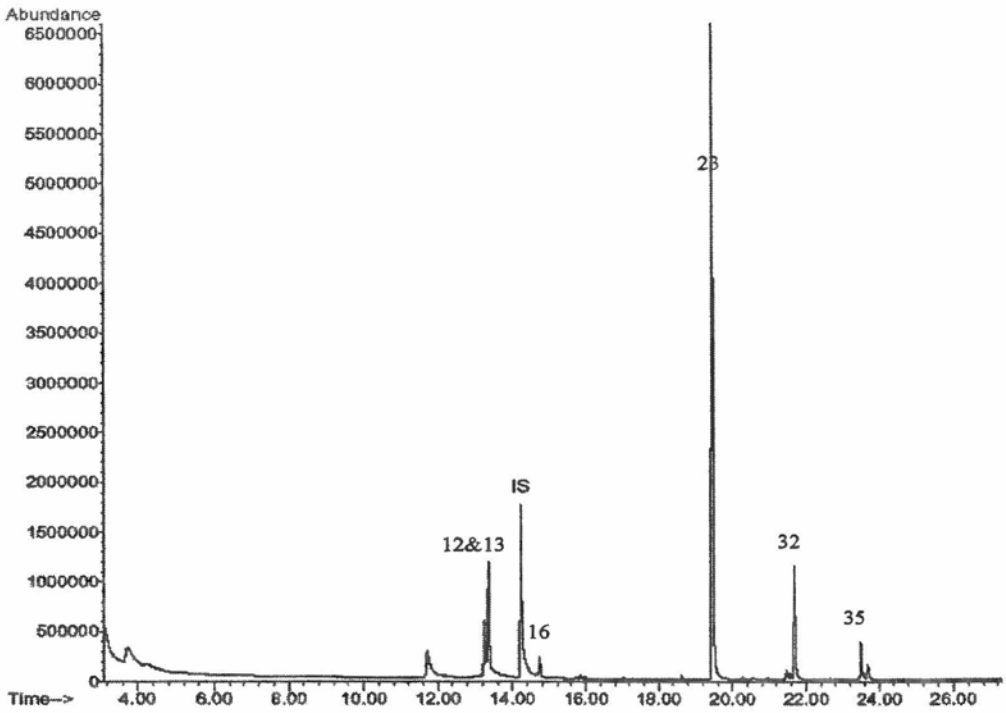


Figure 6.2 **Representative Gas Chromatogram of Volatiles Emitted by Female *Bactrocera invadens*. Numbers Above Peaks Indicate the Identified Compounds Corresponding to those in Table 6.1.**

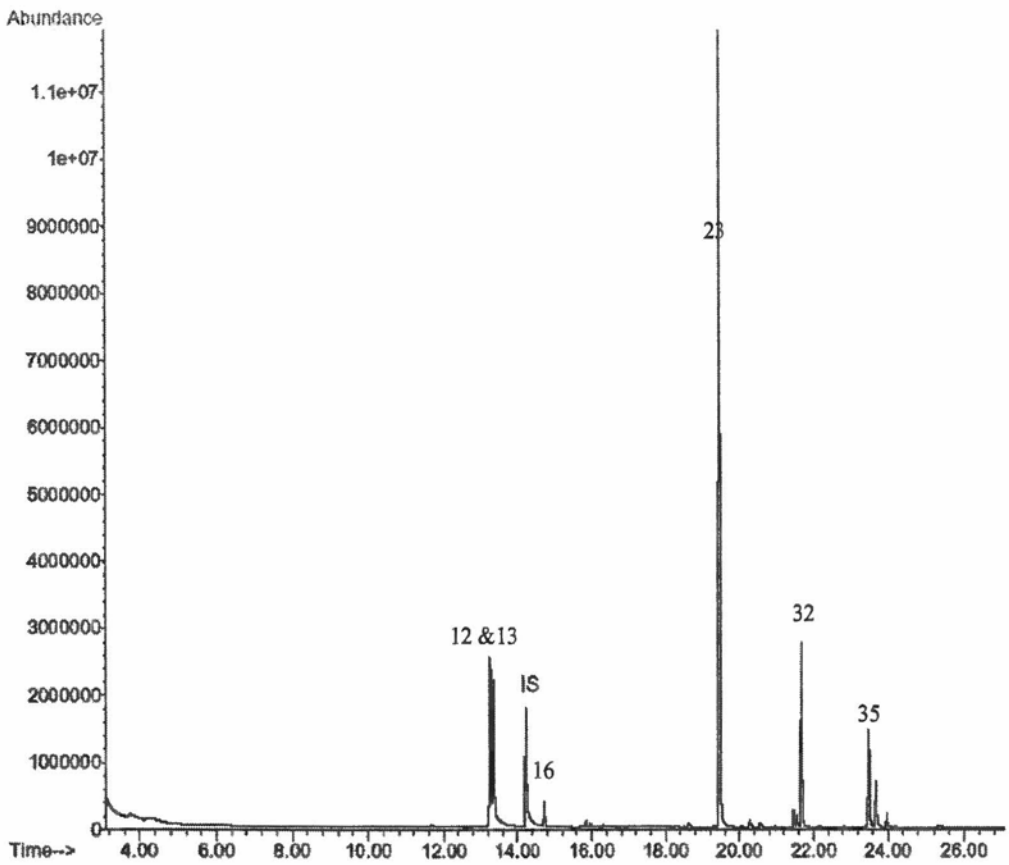


Figure 6.3 **Representative Gas Chromatogram of Volatiles Emitted by Male *Bactrocera invadens*. Numbers Above Peaks Indicate the Identified Compounds Corresponding to those in Table 6.1.**

Table 6.1 Percent Compositions of Compounds Identified in Volatile Emissions from *Bactrocera invadens*

S/N	Compound	R.T. (min)		% Composition	
		Females	Males	Females	Males
1	3-methyl butanal	3.43	3.45	3.43	1.61
2	2-methyl butanal	3.56	3.59	3.56	1.28
3	3-hydroxy-2-butanone	4.52	3.77	4.52	13.56
4	isoamyl alcohol	-	4.26	-	0.06
5	3-methyl-1-butanol	5.11	-	1.76	-
6	hexanal	6.95	-	1.39	-
7	cyclohexanone	9.26	-	0.53	-
8	benzeneacetaldehyde	12.32	-	1.88	1.01
9	nonanal	13.33	13.19	0.97	0.15
10	benzeneethanol	13.55	13.34	1.23	1.01
11	isopropylbutylamine	-	13.57	-	0.69
12	*N*-(3-methylbutyl)acetamide	13.86	13.26	2.03	14.75
13	2,8-dimethyl-1,7-dioxaspiro[5.5]undecane	13.96	13.79	10.67	5.61
14	methyl salycilate (IS)	14.82	14.23	0.49	23.92
15	decyl aldehyde	14.91	-	0.57	-
16	2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane	15.33	14.79	1.14	0.43
17	2,8-diethyl-1,7-dioxaspiro[5.5]undecane	15.87	15.33	0.34	2.35
18	tridecane	-	15.72	-	0.03
19	2-*n*-propyl-8-methyl-1,7-dioxaspiro[5.5]undecane	15.97	15.97	0.2	0.1
20	3-octene	16.32	16.32	-	0.8
21	ethyl decanoate	17.53	17.04	0.57	0.23
22	methyl dodecanoate	19.14	18.63	1.68	0.31

Table 5.1 Continued

S/N	Compound	R.T. (min)		% Composition	
		Females	Males	Females	Males
23	ethyl dodecanoate	20.02	19.45	35.91	17.96
24	ethyl tridecanoate	20.77	20.27	0.82	0.37
25	propyl dodecanoate	21.05	20.55	0.86	0.27
26	methyl 12-methyltridecanoate	-	20.94	-	0.08
27	*(Z)*-methyl 9-octadecenoate	21.22	21.45	0.24	0.78
28	ethyl 9-hexadecenoate	-	21.55	-	0.48
29	methyl tetradecanoate	21.44	-	0.54	-
30	methyl 3-oxo-6-heptenoate	21.95	-	2.66	-
31	hexyl cyclopentenone	22.04	-	1.16	-
32	ethyl tetradecanoate	22.17	-	8.42	3.71
33	ethyl dodecanoate	-	22.21	-	0.11
34	*(Z)*-methyl-9-hexadecenoate	23.32	22.83	0.3	0.06
35	ethyl 9-hexadecanoate	23.98	23.49	5.99	4.39
37	ethyl hexadecanoate	24.17	23.68	1.41	1.89
38	ethyl *cis*-9-octadecenoate	-	25.36	-	0.09

Key: R.T. = Retention Time

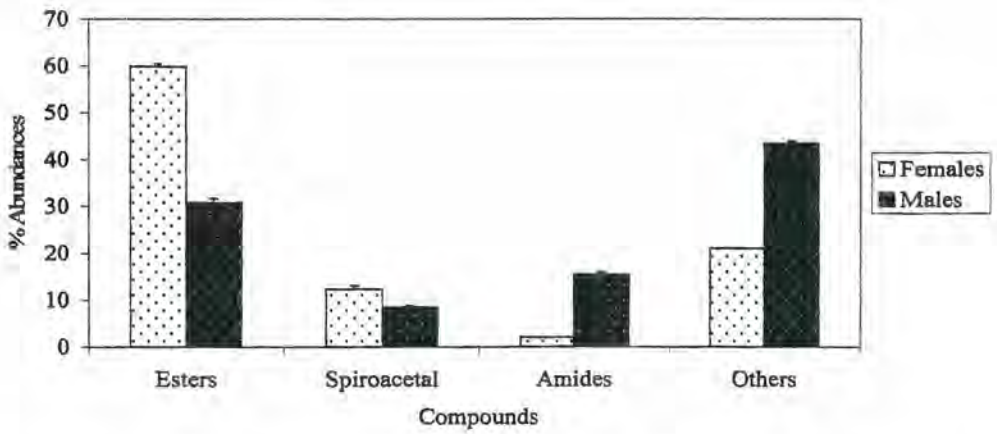
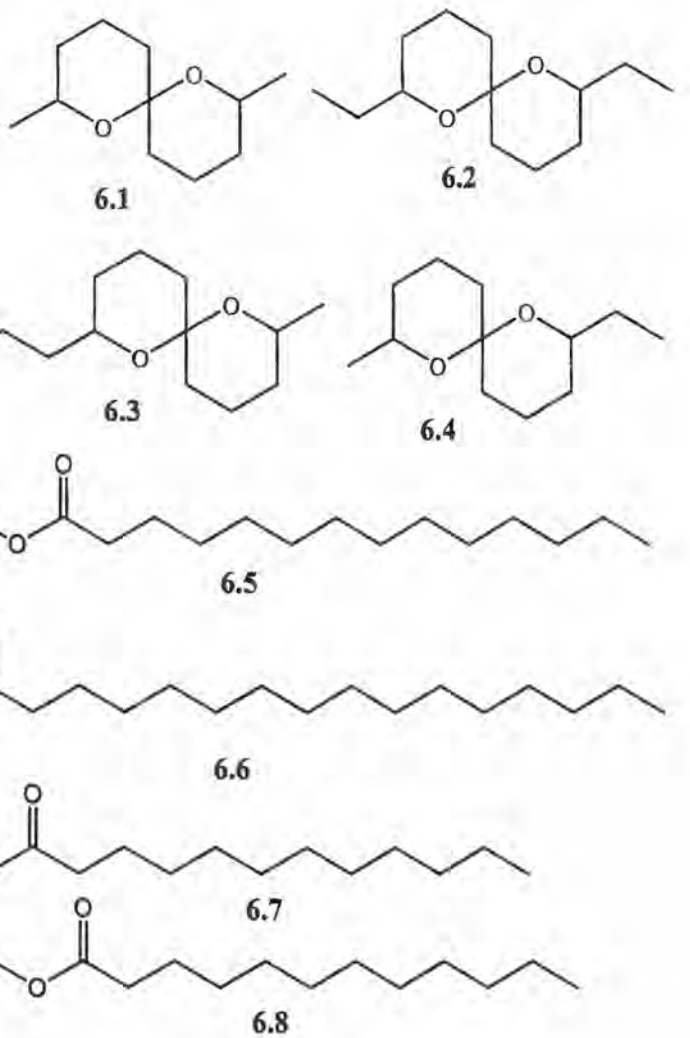


Figure 6.4 Average Relative Proportion (± S.D.) of Different Groups of Compounds Identified in Volatile Emissions from *Bactrocera invadens*

6.3.3 Spiroacetals and Some Esters Identified in Volatile Emissions from *Bactrocera invadens*



6.3.3.1 2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane (6.1)

The MS of compound **6.1** indicated the molecular ion peak at *m/z* 184.1 corresponding to the formula $C_{11}H_{20}O_2$. The MS also exhibited a fragment ion peak at *m/z* 169 that would have resulted from the loss of a methyl unit from the molecular

ion. Furthermore, subsequent extrusion of ethanal would give a fragment ion whose peak appeared at *m/z* 125, corresponding to the formula $C_8H_{13}O$. A peak at *m/z* 140 was also observed in the MS and this was envisioned to have been produced due to extrusion of ethanal from the molecular ion of compound **6.1**. The base peak was observed at *m/z* 112 in the MS resulting from fragmentation of one of the pyran rings at C-6 and C-10 to produce an ion with formula $C_7H_{12}O$. On the other hand fragmentation of a pyran ring at C-7 and C-11 would produce a fragment ion whose peak appeared at *m/z* 114, corresponding to the formula $C_6H_{10}O_2$. The MS fragmentation pattern is summarized in Scheme 6.1, and is consistent with structure **6.1**.

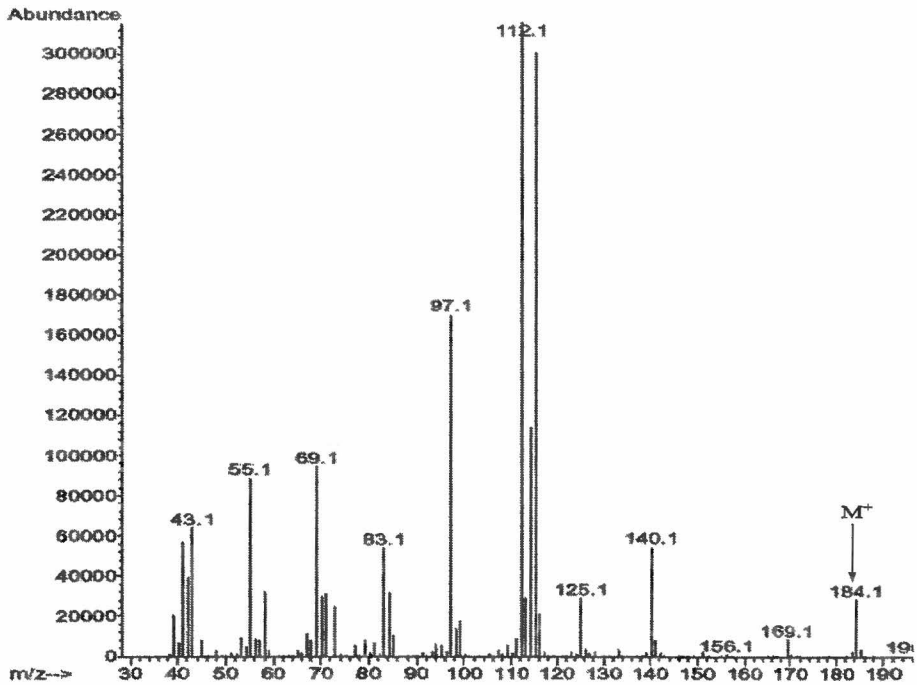
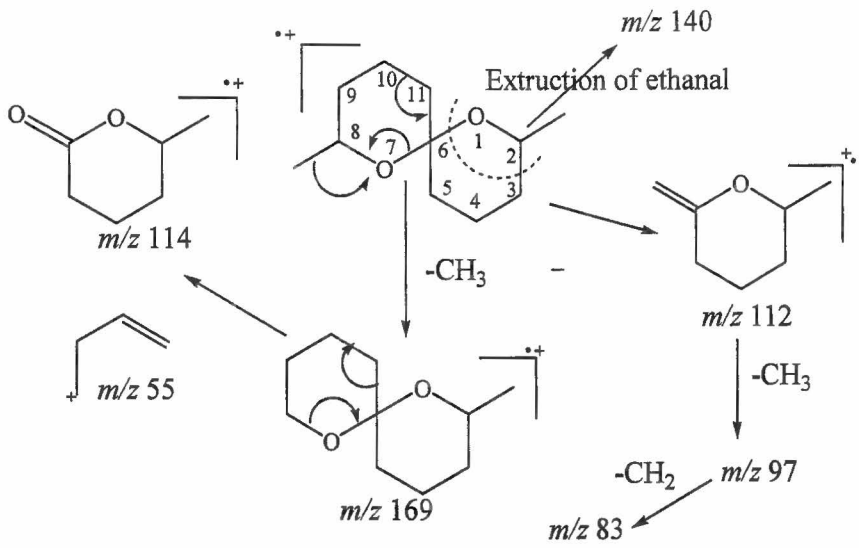


Figure 6.5 **Mass Spectrum of 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (6.1)**



Scheme 6.1 MS Fragmentation Pattern for 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (6.1)

6.3.3.2 2,8-Diethyl-1,7-dioxaspiro[5.5]undecane (6.2)

The MS of compound **6.2** consisted of the molecular ion peak at *m/z* 212.2, corresponding to the formula $C_{13}H_{24}O_2$. A peak due to loss of a methyl and an ethyl group was observed at *m/z* 183 and 197 respectively, while a very weak peak due to extrusion of propanal appeared at *m/z* 154. The fact that only one peak due to extrusion of an alkyl group was observed suggested that the compound was symmetrical as shown by structure **6.2**. The base peak was observed at *m/z* 125 and this was ascribed to the fragmentation of one of the pyran rings from the fragment ion whose peak appeared at *m/z* 183 (Scheme 6.2). The fragmentation process as shown in Scheme 6.2 was consistent with structure **6.2**.

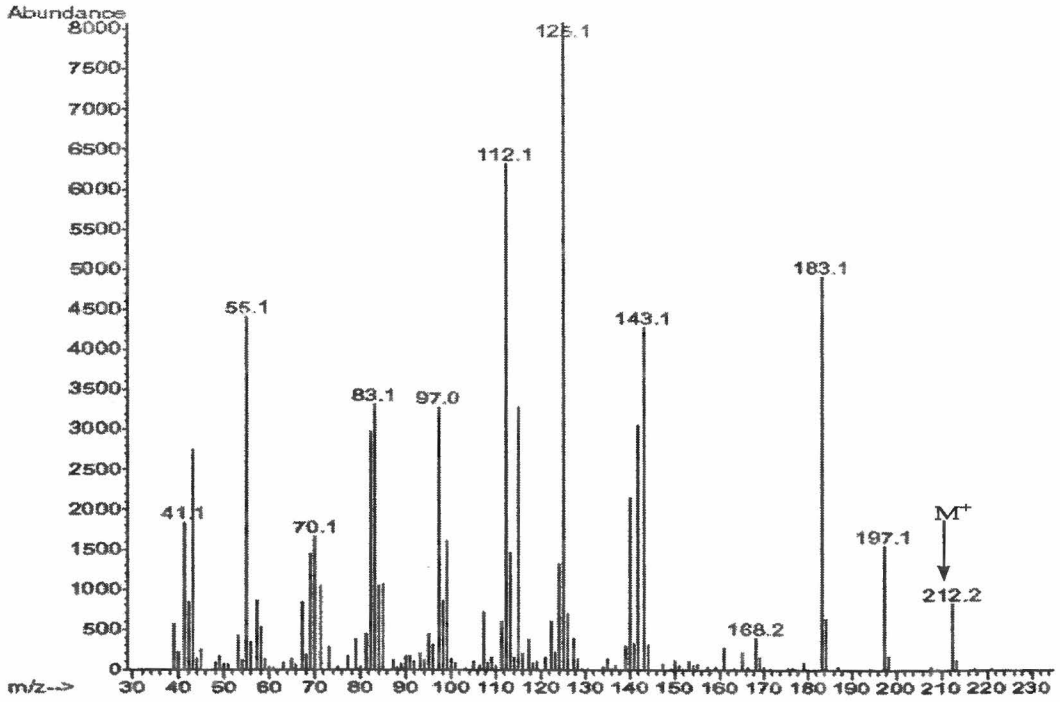
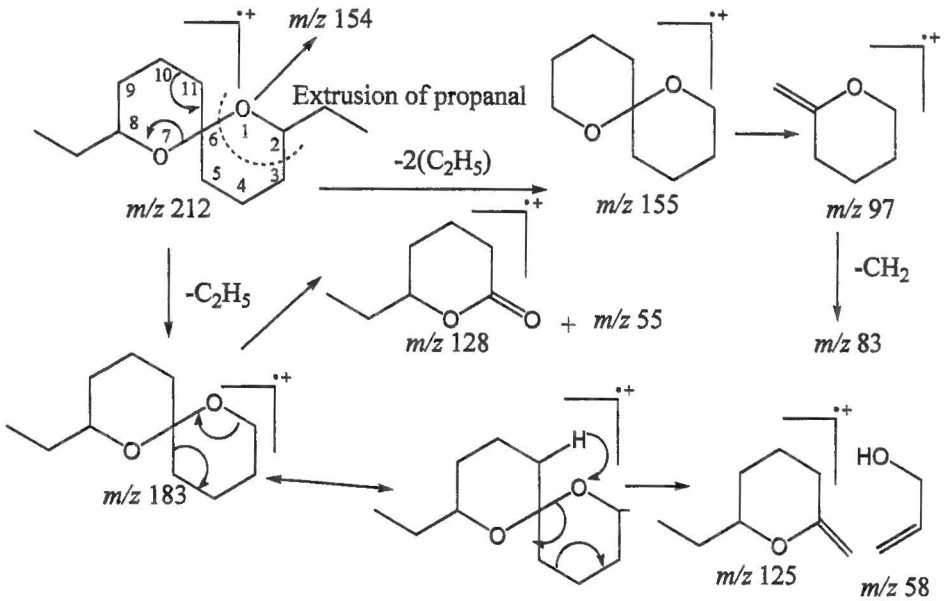


Figure 6.6 Mass Spectrum of 2,8-diethyl-1,7-dioxaspiro[5.5]undecane (6.2)



Scheme 6.2 MS Fragmentation Pattern for 2,8-diethyl-1,7-dioxaspiro[5.5]undecane (6.2)

6.3.3.3 2-(*n*-Propyl)-8-methyl-1,7-dioxaspiro[5.5]undecane (6.3)

The MS of compound **6.3** indicated the molecular ion peak to be at *m/z* 212.1, being consistent with the molecular formula $C_{13}H_{24}O_2$. Subsequent fragmentation of the pyran ring at C-1 and C-5 would account for the base peak appearing at *m/z* 115, corresponding to the fragment ion $C_6H_{11}O_2^+$. On the other hand cleavage of a methyl group from the molecular ion would yield a fragment ion whose peak appeared at *m/z* 197, corresponding to the fragment ion $C_{12}H_{21}O_2^+$. The MS fragmentation pattern as summarized in Scheme 6.4 was found to be consistent with structure **6.3**.

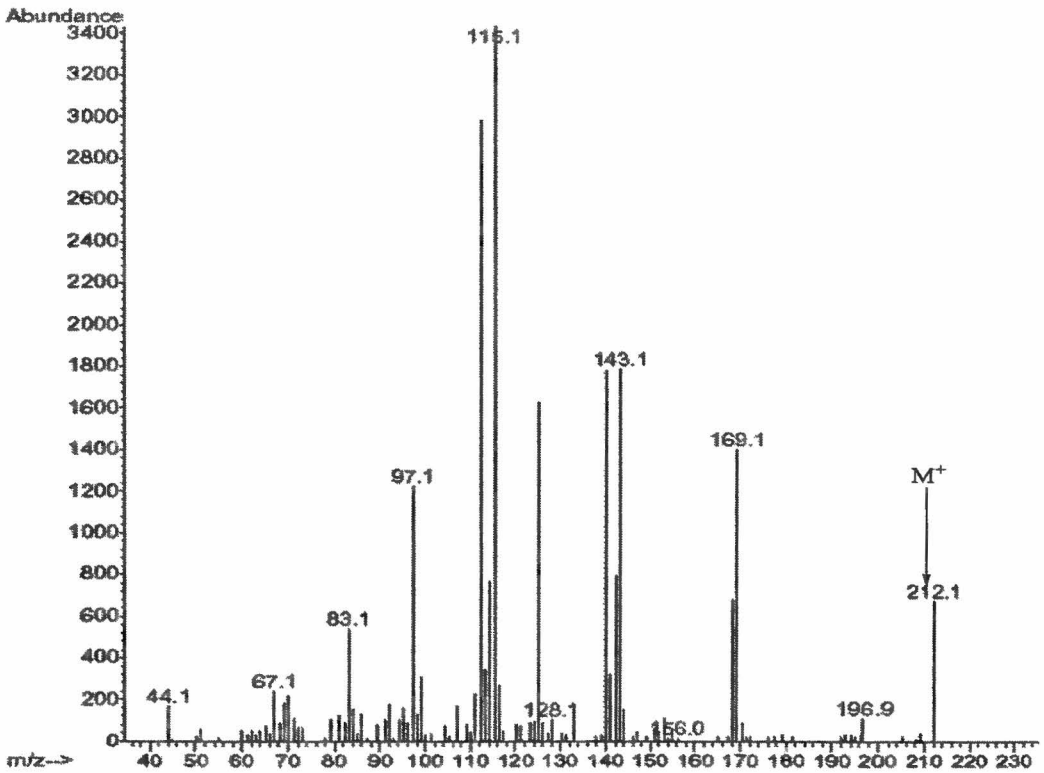
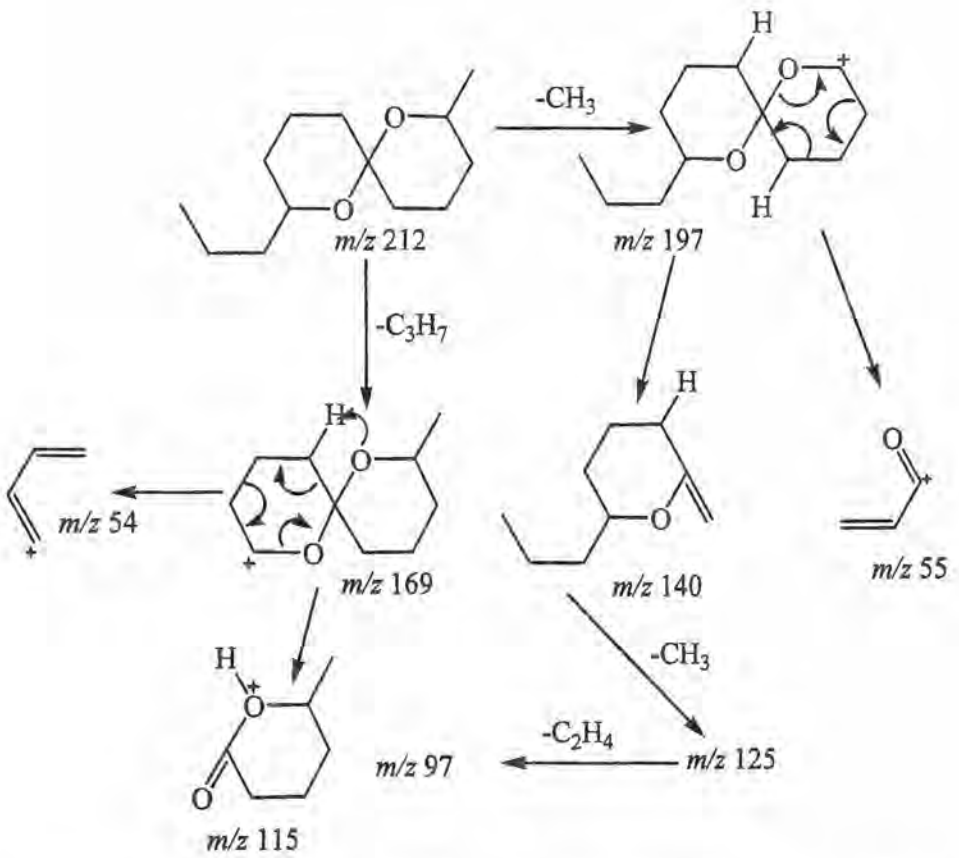


Figure 6.7 Mass Spectrum of 2-(*n*-propyl)-8-methyl-1,7-dioxaspiro[5.5]undecane (6.3)



Scheme 6.3 MS Fragmentation Pattern for 2-(*n*-propyl)-8-methyl-1,7-dioxaspiro[5.5]undecane (6.3)

6.3.3.4 2-Ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane (6.4)

The MS of compound **6.4** as generated from GC-MS analysis indicated the molecular ion peak at *m/z* 198.1, corresponding to the molecular formula $C_{12}H_{22}O_2$. Loss of an ethyl group from the molecular ion would produce a fragment ion whose peak appeared at *m/z* 169, corresponding to the formula $C_{11}H_{19}O_2^+$. Extrusion of ethanal and propanal from the molecular ion would produce fragment ions whose peaks appeared at *m/z* 154 and 140 respectively. On the other hand rearrangement within

one of the pyranoid ring through the spiran system would lead to the formation of a diene fragment ion whose peak appeared at *m/z* 55, corresponding to the formula $C_4H_7^+$ and a protonated lactone whose peak was observed at *m/z* 115, corresponding to the formula $C_6H_{11}O_2$, which was also the base peak. Subsequent loss of water from the protonated lactone would have produced a fragment ion whose peak appeared at *m/z* 97. The MS fragmentation pattern for compound **6.4** is depicted in Scheme 6.4 and it is in agreement with the structure.

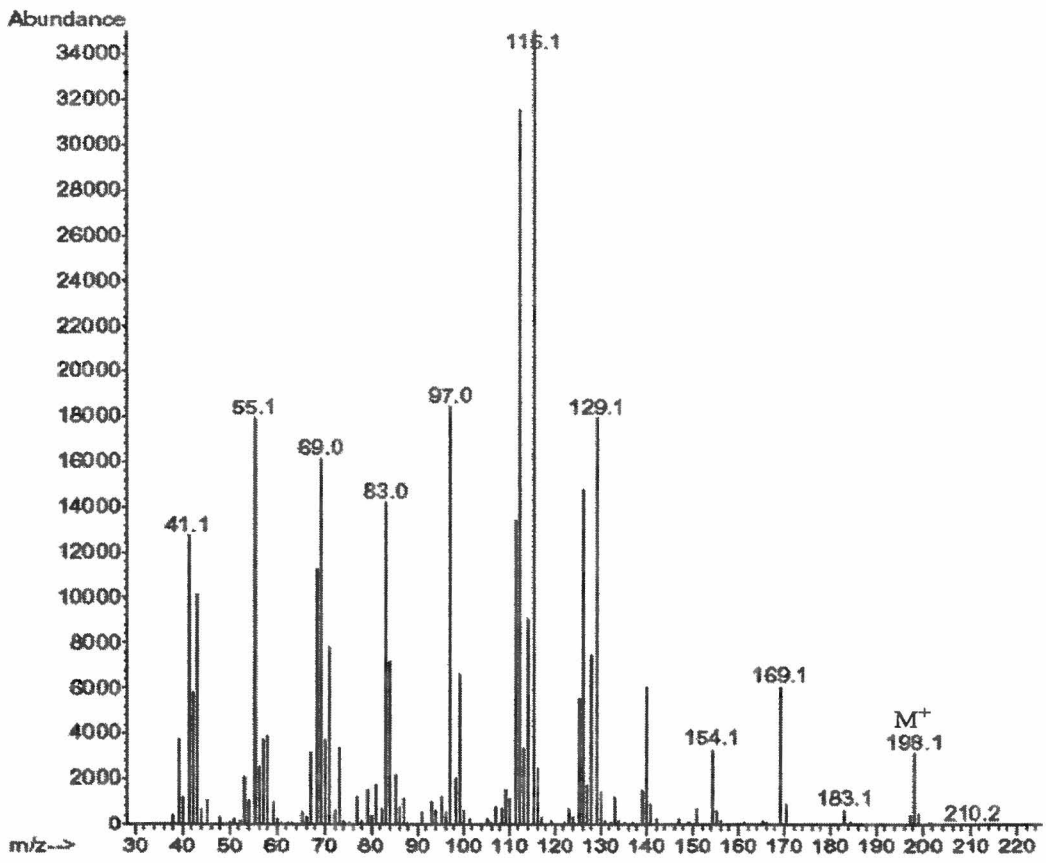
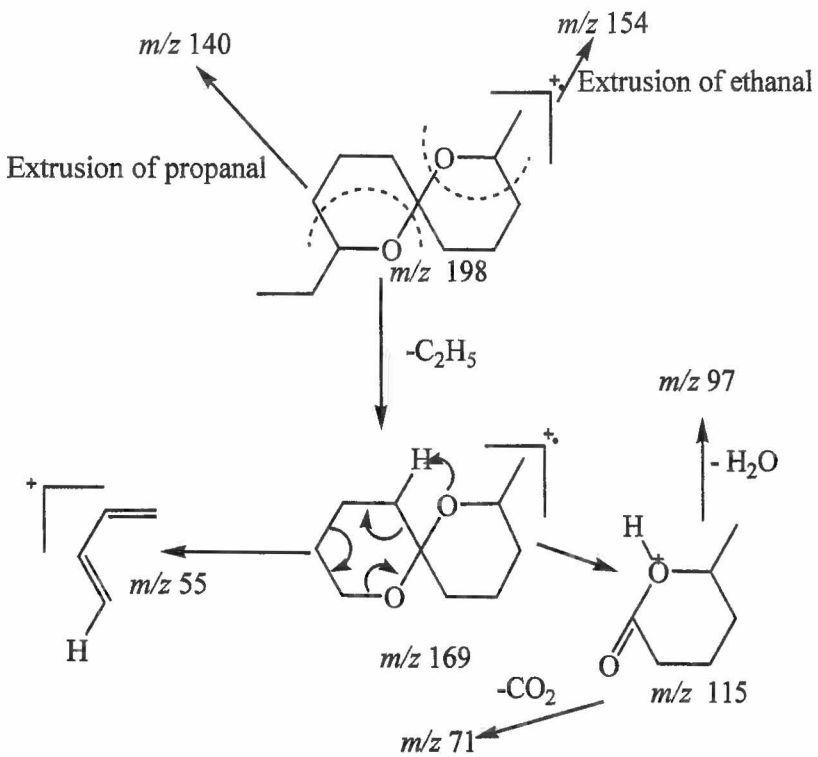


Figure 6.8 Mass Spectrum of 2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane (6.4)



Scheme 6.4 MS Fragmentation Pattern for 2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane (6.4)

6.3.3.3 Ethyl tetradecanoate (6.5)

The MS of compound **6.5** showed the molecular ion peak at *m/z* 256.1 and this was consistent with the molecular formula $C_{16}H_{32}O_2$. The MS exhibited the base peak at *m/z* 88 that was ascribed to the fragment ion $C_4H_8O_2^+$, being formed through a McLafferty rearrangement characteristic of ethyl esters.[102,105] Other characteristic peaks for ethyl esters were also observed at *m/z* 101 and 73 due to α and γ cleavage relative to the carbonyl carbon, corresponding to fragment ions $C_5H_{10}O_2^+$ and

$C_3H_5O_2^+$ respectively. Furthermore, clusters of peaks that were 14 atomic mass units apart were observed, these corresponding to loss of CH_2 units in the aliphatic part of the compound. These included fragment ion peaks at *m/z* 241, 213, 199, 185, 171, 157, 143, 129, 115, 101 indicating the presence of 10 CH_2 groups after the γ carbon in compound **6.5** (Scheme 6.5), which was consistent with structure **6.5.**

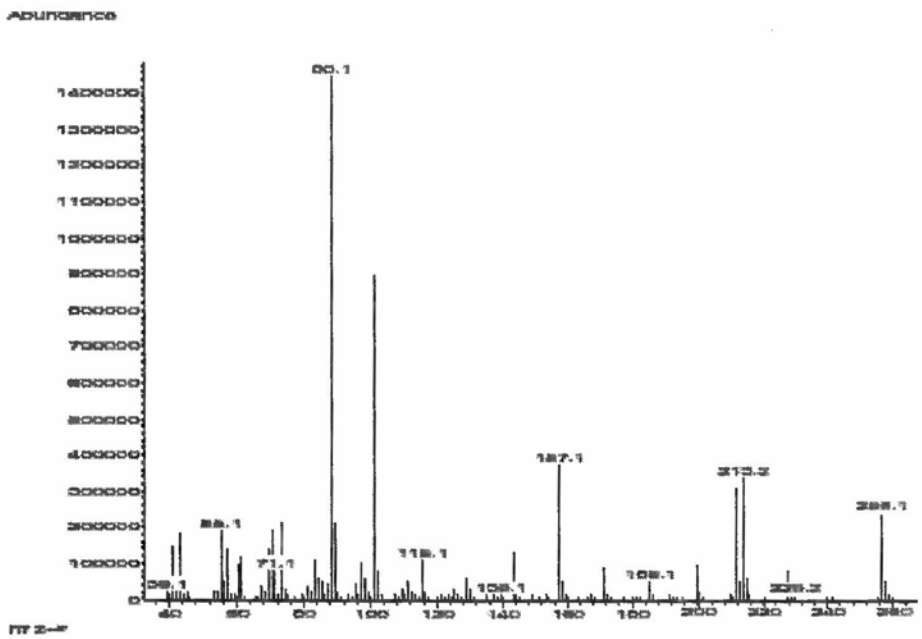
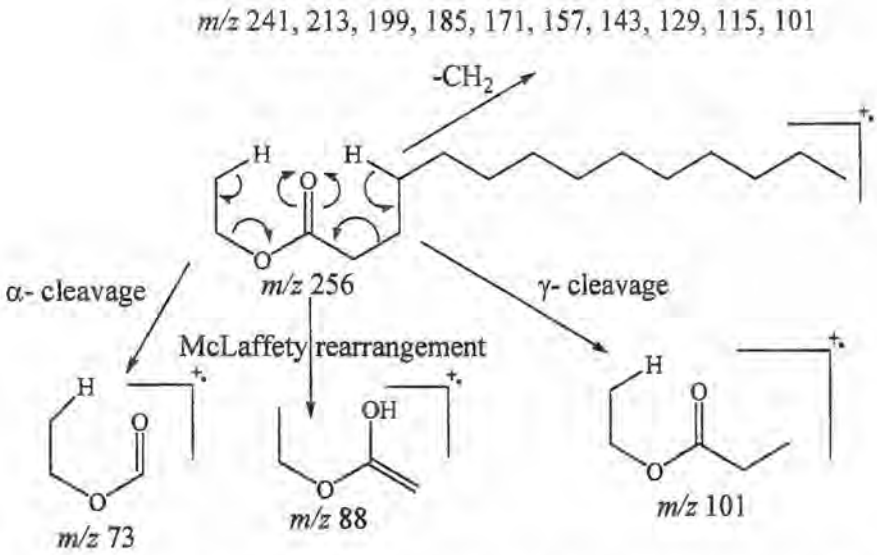


Figure 6.9 Mass Spectrum of ethyl tetradecanoate (6.5)



Scheme 6.5 MS Fragmentation Pattern for ethyl tetradecanoate (6.5)

6.3.3.4 Methyl dodecanoate (6.6) and Ethyl dodecanoate (6.7)

The MS of methyl dodecanoate **(6.6)** as generated from the GC-MS spectrum indicated the molecular ion peak at *m/z* 214.2, corresponding to the molecular formula $C_{13}H_{26}O_2$. Loss of an ethyl group from the molecular ion would produce a fragment ion whose peak appeared at *m/z* 185, having the formula $C_{11}H_{19}O_2^+$. Subsequent loss of a cluster of peaks that were 14 atomic mass units apart which corresponded to loss of CH_2 units in the aliphatic part of the compound was observed. The peaks appeared at *m/z* 185, 171, 157, 143, 129 and 115. McLafferty rearrangement was evident due to the appearance of a fragment ion that indicated a peak at *m/z* 74 and other peaks characteristic of methyl esters were also observed at

m/z 87 and 59. The MS fragmentation pattern for compound **6.6** is depicted in Scheme 6.6 and it is in agreement with the structure.

On the other hand the MS of ethyl dodecanoate (**6.7**) indicated a molecular ion peak at *m/z* 228.2 corresponding to the formula $C_{14}H_{28}O_2$, having one CH_2 unit more than in compound **6.6**. Thus, a McLafferty rearrangement type fragmentation was indicated by the appearance of a fragment ion peak at *m/z* 88 which was also the base peak, and other characteristic fragment ion peaks that appeared at *m/z* 101 and 73 corresponding to formula $C_5H_9O_2$ and $C_3H_5O_2$ respectively. Cluster of peaks which were 14 mass units apart were also observed, corresponding to loss of CH_2 units in the aliphatic part of the compound. These included peaks at *m/z* 214, 195, 185, 171, 157, 143, 129 and 115. The MS fragmentation pattern as depicted in Scheme 6.8 was in agreement with the structure **6.7**.

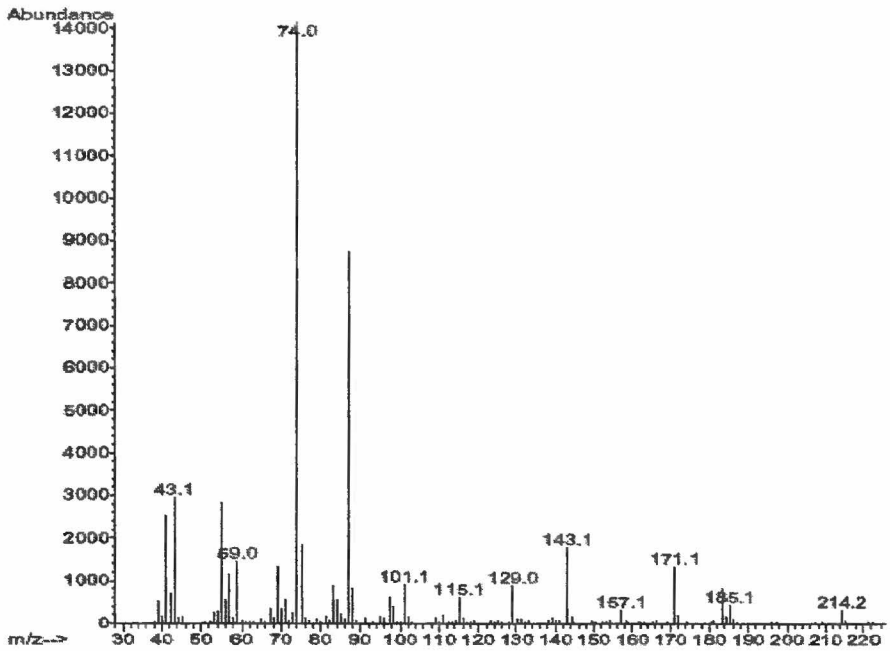
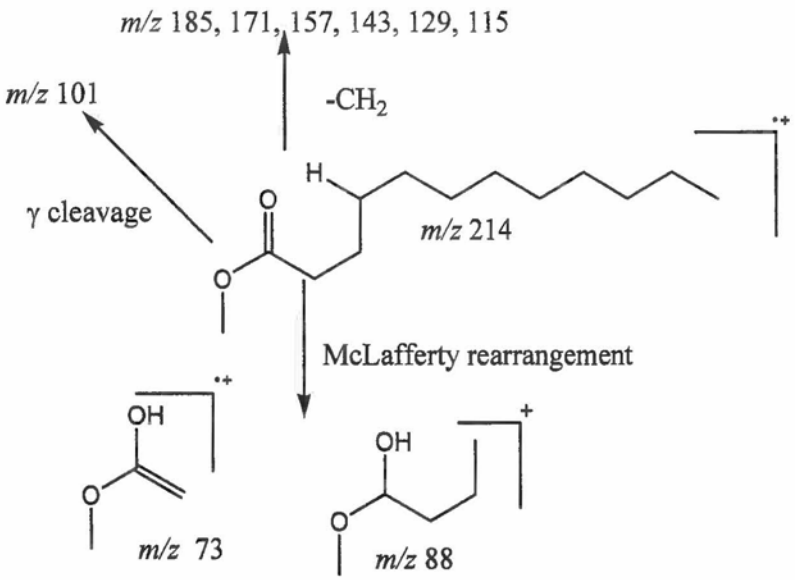


Figure 6.10 Mass Spectrum of methyl dodecanoate (6.6)



Scheme 6.6 MS Fragmentation Pattern for methyl dodecanoate (6.6)

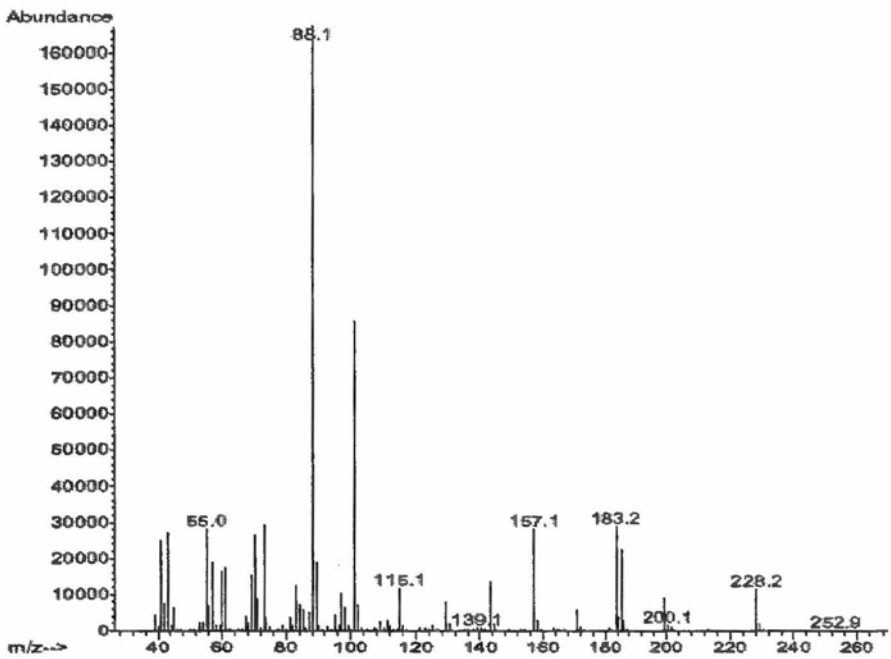
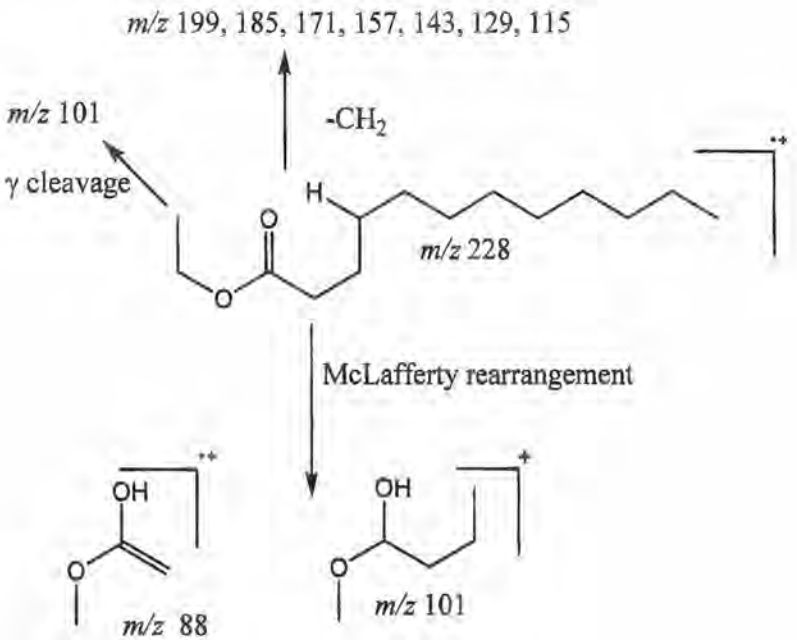


Figure 6.11 Mass Spectrum of ethyl dodecanoate (6.7)



Scheme 6.7 MS Fragmentation Pattern for ethyl dodecanoate (6.7)

CHAPTER SEVEN

CONCLUSION AND RECOMMENDATIONS

Conclusion

The investigations whose results are reported in this thesis were aimed at studying the responses of *Bactrocera invadens* to volatile blends of some hosts and one non-host, to see if there are any inter-specific interaction and to undertake GC-EAD and GC-MS analyses so as to identify candidate behaviourally-active constituents of the volatiles emitted by the different hosts. The study also evaluated responses of both male and female flies to the blends their own volatiles emission in order to see if there was any intra-specific interaction and GC-MS was carried out to identify the constituents that composed of their own volatiles. *Bactrocera invadens,* a fruit fly which was recently reported in East Africa as a serious pest of fruits and vegetables. Attention was given to highly infested hosts (cultivated and wild fruit plants), which included mango (*Mangifera indica*), marula (*Scelocarya birrea*) and Indian almond (*Terminalia catappa*), as well as *Gynandropsis gynandra,* a non-host plant that attracts only males in the field. Behavioural responses of male and female fruit flies to volatile emissions of three host plants and a non host plant, *G. gynandra*, and from conspecifics were studied in a two-choice wind tunnel. In addition, analyses were conducted to identify behaviourally active compounds by GC-EAD (using antennae of both male and female flies) and GC-MS experiments.

Bioassays with natural volatiles emitted by the fruits in the wind tunnel experiments indicated the attraction of both male and female flies. There was no apparent difference in the responses of the flies to the three hosts, probably reflecting the polyphagous habits of the insect. However, for mangoes, of the three varieties that were tested the flies were able to discriminate between sensation and kent varieties. This could be due to the differences in the quality and quantity of semiochemicals in the volatiles emitted by the two mango varieties. Attraction of both male and female flies to crude volatiles may indicate the production of a similar range of attractant constituents by different hosts that are perceived by both sexes. The male flies were assumed to be using some components of kairomones for the purposes of sex or aggregation pheromone synthesis. The preference of flies for mature unripe and mature ripe fruits indicated the presence of a certain group of compounds that are produced at a certain level of maturity of the fruits that are perceived by the insects by olfaction and guide them to their hosts. This may also signal the presence of enough nutrient resource for the insect's survival. This and previously reported evidence indicates that fruit volatiles are important in enabling flies not only to locate the host plants, but also to discriminate between hosts and non-hosts and among fruits at different stages of ripeness. This was further confirmed by GC-EAD analysis, since both male and female flies responded to similar chemical constituents.

The coupled GC-EAD analysis of volatiles was conducted in order to identify compounds in volatiles from ripe fruits that were detected by the antennal olfactory receptors of the fruit flies. Results showed that, olfactory receptors in the antennae of male and female flies detected a wide range of compounds in the crude volatiles

trapped from ripe fruits of the three hosts investigated. The identified compounds included acetates, ethyl esters, terpenoids, and phenylpropanoids, all of which being widely found in odors of a variety of fruits and flowers. Specific compounds identified from the three hosts were (i) isopropyl acetate, ethyl propionate, isopropyl valerate, propyl isovalerate, *p*-cymene, *trans*-β-ocimene, 3-*(Z)*-octen-1-ol, octanol, linalool, *(Z)*-3-decene-1-ol, 5-dodecen-1-ol, *(E,E)*-α-farnesene, and 13-tetradecenal from marula *(Sclerocarya birrea)* fruits; (ii) isobutyl acetate, 4-pentenol, butyl acetate, 2-butenyl acetate, ethyl isovalerate, isoamyl acetate, 4-penten-1-yl acetate, prenyl acetate, β-myrcene, δ-3-carene, limonene, *trans*-β-ocimene, terpinolene, benzyl acetate, citronellyl acetate, geranyl acetate, methyl eugenol, *(E,E)*-α-farnesene and elemicin from the Indian almond (*Terminalia catappa*); and (iii) 3-hydroxy-2-butanone, crotenoic acid, ethyl *cis*-crotonate, tricyclene, α-pinene, camphene, myrcene, sabenene, *trans*-β-ocimene, γ-terpinene, terpinolene, ethyl *cis*-4-octenoate, ethyl octanoate and α-humulene from mangoes *(Mangifera indica)*. From the above results, it is evident that there was overlap in the chemical composition of fruit volatiles from the three host plants. For example, the compounds that were identified in mango and almond included ethyl butyrate and *trans*-β-ocimene; the compound found in mango and marula was *trans*-β-ocimene while the compounds occurring in almond and marula included ethyl isovalerate, *trans*-β-ocimene and *(E,E)*-α-farnesene. *Trans*-β-ocimene was present in volatiles from all the three host fruits. The results demonstrated a low degree of overlapping of the active constituents among the different hosts, implying a chance for *B. invadens* to infest a relatively large number of hosts. In general, esters represented a larger group

of compounds that elicited antennal responses of the flies; the other groups included terpenes and alcohols.

The response of flies to different hosts as well as to a wide range of compounds from different hosts on GC-EAD reflects polyphagous trait of *Bactrocera invadens*, capable of infesting a wide range of hosts. Indeed, the host range appears to keep on growing. Polyphagous species may respond to a wide range of fruit volatile combinations, unlike monophagous species that respond to more specific group of host constituents.

The observations on the attraction of male fruit flies, *B. invadens* to *Gynandropsis gynandra* showed that, male flies visited the plants in the field from 06:00 am to 12:30 pm. Analyses with GC-MS and GC-EAD of the volatiles led to the identification of two EAG-active compounds, namely 4-hydroxy-4-methyl-2-pentanone and 4-methyl-3-penten-2-one. This is the first time that these compounds are being reported to be olfactory stimuli from a non-host plant for the antennal receptors of a fruit fly species. In most cases, phenyl propanoids have been found to be the attractants and some of them are already available in the market for the control of fruit flies. A number of male flies from the genus Tephritidae and especially from the genus *Bactrocera* are known to be attracted to plants that are non-hosts and in the process they ingest certain compounds the biological significance of which has so far not been clearly defined. In any case, despite the wide use of male lures in the control of fruit flies, relatively little attention has been given to explaining the underlying biological basis of this sex-specific, chemical attraction. However, it has

been speculated that the insects might be using compounds that they ingest from non-host plants as precursors for their pheromones, or for self-defense against potential predators.[54,55]

The analyses of volatile emissions from flies were conducted in order to identify the components that the insects emit at dusk, the underlying rationale being that some of the compounds might be constituents of sex and/or aggregation pheromones. The olfactometric tests with volatile emissions indicated that the emissions attracted both sexes although the male volatiles attracted more flies of both sexes than those emitted by female flies. The attraction of both female and male flies to their own volatiles suggested that the emitted volatiles may play a dual role as sex pheromone and as an aggregant. The analysis of volatile emissions on GC-MS indicated the presence of a number of compounds most of which have been reported as pheromone components of other closely related fruit flies, such as *Bactrocera dorsalis*. The analysis was conducted on both male and female volatiles and the results there from indicated similar composition of the constituents that differed in quantities. The identified compounds mainly included esters and spiroacetals. The later compounds included those previously reported as fruit fly pheromone constituents, namely 2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane, *(E,E)*-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, 2,8-diethyl-1,7-dioxaspiro[5.5]undecane and 2-*n*-propyl-8-methyl-1,7-dioxaspiro[5.5]undecane. The esters represented relatively high percentages for both female (59.89%) and male (35.73%) flies. GC-MS analyses of the emitted volatiles indicated the major peak to be due to ethyl dodecanoate (35.91% and 17.96 % for female and male flies respectively). The second major

peaks were shown to be due to 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (10.67%) for female and *N*-(3-methylbutyl)acetamide (14.75%) for male flies.

There were some compounds that were present in volatiles of host fruits and those produced by the flies. These included 3-hydroxy-2-butanone, methyl and ethyl tetradecanoate, ethyl hexadecanoate, ethyl decanoate and ethyl dodecanoate. Male and female flies were observed to use the plant as a rendezvous site for courtship and mating, a mode of mating behavior typical of many members of the family Terphtitidae. This further corroborated the possibility that the search for mates by both sexes is closely linked to the search for the 'host' plant. Thus, it is generally believed that chemical cues used to locate places for mating could also be involved in the search for hosts. As reported in other studies, the males of many phytophagous insects tend to aggregate at the primary feeding and oviposition sites and what guides them are the chemical stimuli emitted not only by a host plant species, but also by any other plant that is preferred by females. This would ensure high chances for mating and hence the flies' survival and proliferation.

In conclusion, the foregoing results constitute a significant contribution toward understanding the chemical ecology of interspecific interactions between the fruit fly *Bactrocera invadens* and some of its host plants and intraspecific communication between the flies themselves. The results lay down the foundation for the development of host plant-derived attractants for the management of both male and female *Bactrocera invadens*, as an important tool in Integrated Pest Management (IPM)

Recommendations

The results from investigations reported in this Thesis on the candidate semiochemicals that mediate the oviposition and mating behavior of the fruit fly *Bactrocera invadens* indicated the attraction of the fruit fly to volatiles emitted by the three host plants, namely *Mangifera indica, Terminalia catappa* and *Sclerocarya birrea,* as well as volatile emissions from the insects (pheromones). GC-EAD analysis of the three host fruit volatiles led to the identification of a number of compounds, the identity of some of which were confirmed by GC-EAD as well as GC-MS. However, further research is needed to establish which of the identified compounds contribute to behavioural attraction of the flies and to establish the blend that would be effective in controlling this fruit fly. With regard to non-host *Gynandropsis gynandra,* more studies are still required to evaluate the possibility of reversible reaction that could be taking place between the two compounds identified during these investigations, namely 4-hydroxy-4-methyl-2-pentanone and 4-methyl-3-penten-2-one and their ecological significance.

In addition, the candidate pheromone constituents need to be evaluated by GC-EAD and bioassays and to establish if they could also be effectively incorporated in the formulation of potent attractive blend(s). The overall conclusion and recommendation from these investigations is that the volatiles from host plants, candidate pheromonal constituents and non-host plants are potential attractants for both male and female *Bactrocera invadens*.

REFERENCES

1. Norin, T. Pheromones and Kairomones for Control of Pest Insects. Some Current Results from a Swedish Research Program. *Pure. Appl. Chem.*, **2001**, *73*, 607-612.
2. Mandava, N.B. *Handbook of Natural Pesticides: Methods. Volume I. Theory, Practice and Detection.* CRC Press, Inc. Florida, **1985**, pp 482.
3. Hardle, J. and Minks, A.L. *Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants*, CABI Publishing, Wallingford, UK, **1999**.
4. (IPC) Investment Promotion Centre, Kenya Kenya *–A Guide for Investors. Sector Profile on Agriculture*. URL http://www.ipckenya/ipcpm.htm, 10th September, **2006**.
5. Lux, S.A., Ekesi, S., Dimbi, S., Mohamed, S. and Billah, M.K. Mango Infesting Fruit Flies in Africa-Perspectives and Limitations of Biological Approaches to Their Management Management.. *In* Neuenschwander, P., Borgemeister, C. and Langewald, J. [eds.], *Biological Control in Integrated Pest Management Systems in Africa.* CABI, Wallingford, United Kingdom, **2003**.
6. White, I.M and Elson-Harris, M.M. *Fruit Flies of Economic Significance: Their Identification and Bionomics*. CAB International, Wallingford, U.K, **1992**.
7. De Meyer, M. Distribution Patterns and Host-Relationships within the Genus *Ceratitis* (Diptera: Tephritidae) in Africa. *Cimbebasia*, **2001**, 17, 219-228.

8. Averill, A.L. and Prokopy, R.J. Host Marking Pheromones. *World Crop Pests: Fruit Flies. Their Biology, Natural Enemies and Control* (ed. by A. S. Robinson and G. Hooper), Elsevier, Amsterdam, **1989.**

9. Zalom, F.G. IPM Practices for Reducing Insecticide Use in US Fruit Crops. In. Pimentel, D. (Ed.). *Techniques for Reducing Pesticide Use: Economic and Environmental Benefits.* John Wiley and Sons Ltd., UK, **1997.**

10. Mwatawala, M.W., White, I.M., Maerere, A.P., Senkondo, F.J. & De Meyer, M. A New Invasive *Bactrocera* Species (Diptera: Tephritidae) in Tanzania. *African Entomol.*, **2004,** *12,* 154-156.

11. Drew, R.A.I. and Hancock, D.L. The *Bactrocera dorsalis* complex of Fruit Flies (Diptera: Tephritidae: Dacinae) in Asia. *Bull. Entomol. Res.*, **1994,** *Supplement 2,* 1-68.

12. Rwomushana, I., Ekesi, S., Gordon, I. and Ogol, C.K.P.O. Host Plants and Host Plant Preference Studies for *Bactrocera invadens* (Diptera: Tephritidae) in Kenya, a New Invasive Fruit Fly Species in Africa. *Ann. Entomol. Soc. Am.*, **2008,** *101,* 331-340.

13. Jang, E.B. and Light, D.M. Olfactory Semiochemicals of Tephritids, pp. 73-90. *In.* McPheron, B.A. and Steck, G.J. (eds), Economic Fruit Flies: A World Assessment of their Biology and Management. *Proceedings of the 4th International Symposium on Fruit Flies of Economic Importance.* St. Lucie Press, Delray Beach, FL. **1996.**

14. Epsky, N.D., Heath, R.R., Guzman, A. and Meyer, W.L. Visual Cue and Chemical Cue Interactions in Dry Trap With Food-Based Synthetic

Attractant for *Ceratitis capitata* and *Anastrepha ludens* (Diptera: Tephritidae). *Environ. Entomol.*, **1995,** *24*, 1387-1395.

15. Heath, R.R., Epsky, N.D., Guzman, A., Deuben, B.D., Manukian, A. and Mayer, W.L. Development of a Dry Plastic Insect Trap With Food-Based Synthetic Attractant for the Mediterranean and Mexican Fruit Flies. (Diptera: Tephritidae). *J. Econ. Entomol.*, **1995,** *88*, 1307-1315.

16. Robacker, D.C. Attractiveness of a Mixture of Ammonia, Methylamine and Putrescine to Mexican Fruit Flies (Diptera: Tephritidae) in a Citrus Orchard. *Florida Entomol.*, **1995,** *78*, 571-578.

17. Cornelius, M.L., Nergel, L., Duan J.J. and Messing R.H. Responses of Female Oriental Fruit Flies to Protein and Host Fruit Odors in Field Cage and Open Field Tests. *Environ. Entomol.*, **2000,** *29,* 14-19.

18. Clarke, A.R., Armstrong, K.F., Carmichael, A.E., Milne, J.R., Raghu, S., Roderick, G.K. and Yeates, D.K. Invasive Phytophagous Pests Arising Through A Recent Tropical Evolutionary Radiation: The *Bactrocera Dorsalis* Complex of Fruit Flies. *Annu. Rev. Entomol.*, **2005,** *50*, 293–319.

19. Lux, S.A., Copeland, R.S., White, I.M., Manrakhan, A. and Billah, M.K. A New Invasive Fruit Fly from the *Bactrocera dorsalis* (Hendel) Group Detected in East Africa. *Insect Sci. Appl.*, **2003,** *23*, 355-360.

20. Drew, R.A.I., Tsuruta, K. and White, I.M. A New Species of Pest Fruit Fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. *African Entomol.*, **2005,** *13*, 149-154.

21. Cornelius, M.L., Duan, J.J., Messing, R.H. and Cornelius, M.L. Visual Stimuli and the Response of Female Oriental Fruit Flies (Diptera: Tephritidae) to Fruit-Mimicking Traps. *J. Econ. Entomol.*, **1999,** *92,* 121-129.

22. Pal R.K. Ripening and Rheological Properties of Mango as Influenced by Ethereal and Carbide. *J. Food Sci. Technol.*, **1998,** *35,* 358-360.

23. Pino, J.A. and Mesa, J. Contribution of Volatile Compounds to Mango (*Mangifera indica* L.) Aroma. *Flavour. Fragr. J.*, **2006,** *21,* 207–213.

24. Macleod, A.J. and Troconis, N.G. Volatiles Flavour Components of Mango. *Phytochemistry*, **1982,** *21*, 2523-2526.

25. Gikonyo, N.K., Lux, S.A. and Nemeye, P.S. Variation in Volatiles from Fruits of Mango and Marula Attractive to the Mango Fruit Fly, *Certitis cosyra* (Walker). *East Cent. African J. Pharm. Sci.*, **2003,** *6,* 3-8.

26. Hernandez-Sanchez G., Sanz-Berzosa, I., Casana-Giner, V. and Primo-Yufera, E. Attractiveness for *Ceratitis capitata* (Wiedemann) (Dipt., Tephritidae) of Mango (*Mangifera indica*, cv. Tommy Atkins) Airborne Terpenes. *J. Apl. Enoml.*, **2001,** *125*, 189-192.

27. Morton, J.F. Indian almond (*Terminalia catappa*), Salt-Tolerant, Useful, Tropical Tree with "Nut" Worthy of Improvement. *Econ. Bot.*, **1985,** *39,* 101-112.

28. Jeng-Leun, M., Pei-Tzn, K. and Chamg-Chamg, C. Aroma Characterization and Antioxidant Activity of Supercritical Carbon Dioxide Extracts from *Terminalia catappa* Leaves. *Food Res. Int.*, **2003,** *36*, 97-104.

29. Yun-lian, L., Yuch-Hsiung, K., Ming-Shi, S., Chein-Chin, C. and Jun-Chio, Flavonoid Glycosides from *Terminalia catappa* L. *Chinese Chem. Soc.*, **2000,** *47,* 253-256.

30. Fan, Y.M., Xu, L.Z., Gao, J., Wang, Y., Tang, S.H., Zhao, S.N. and Zhang, Z.X. Phytochemical and Antiinflammatory Studies on *Terminalia catappa. Fitoterapia,* **2004,** *75,* 253-260.

31. Siderhurst, M,S. and Jang, E.B., Female-Biased Attraction of Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel), to a Blend of Host Fruit Volatiles From *Terminalia catappa* L. *J. Chem. Ecol.*, **2006,** 32: 2513–2524.

32. Siderhurst, M,S. and Jang, E.B. Attraction of Female Oriental Fruit Fly, *Bactrocera dorsalis*, to *Terminalia catappa* Fruit Extracts in Wind Tunnel and Olfactometer Tests. *Formosan Entomol.,* **2006,** *26,* 45-55.

33. Chyau, C.C., Ko, P.T., Mau, J.L., and Kao, M.D. Volatile Components of a Tea of *Terminalia catappa, Food Res. Int.*, **2003,** *36,* 97.

34. Van Wyk, P. *Sclerocarya caffra* Trees of the Kruger National Park. Purnell, Cape Town, **1974**.

35. Van Wyk, B-E., Van Oudtshoorn, B. and Gericke, N. *Medicinal Plants of South Africa*, Briza Publications, Pretoria, **1997**.

36. Burger, A.E.C., De Villiers, J.B.M. and Du Plessis, L.M. Composition of the Kernel Oil and Protein of the Marula Seed. *South African J. Sci.,* **1987,** *83,* 733–735.

37. Viljoena, A.M., Kamatoua, G.P.P. and Başerb, K.H.C. Head-space Volatiles of Marula (*Sclerocarya birrea* subsp. caffra). *South African J. Bot.*, **2008,** *74,* 325–326.

38. Ware G.W. and Whitacre D.M. The Pesticide Book, 6th Ed. Meister Media Worldwide, Willoughby, Ohio, **2004.**

39. Glen, A. Pesticides and Human Health. URL:http,www.ncsl.org/programs/environ/envHeath/serpest, 2nd October, **2006.**

40. Hodgson, E. and Kuhr, R.J. Safer Insecticides. Development and Use; *In.* Hodgson, E. and Kuhr, R. J., Ed.; Marcel Dekker, Inc. New York, *Vol. 7,* **1990,** pp 1-18.

41. Brader L. Integrated Pest Control in the Developing World. *Ann. Rev. Entomol.,* **1979,** *24,* 225-254.

42. International Conference on Pesticide Use in Developing Countries: Environmental Fate, Effects and Public Health Implications *A Conference Organized by ANCAP in Collaboration with SETAC AFRICA BRANCH,* 16th - 20th October 2006. URL: http://www.ancap.org/, 27th January, **2007.**

43. Cardé, R.T. and A.K. Minks. Control of Moth Pests by Mating Disruption: Successes and Constraints. *Annu. Rev. Entomol.*, **1995,** *40,* 559-585.

44. Bueno, A.M. and Jones, O. Alternative Methods for Controlling the Olive fly, *Bactrocera oleae*, Involving Semiochemicals. *IOBC, WPRS (West Palaearctic Regional Section) Bulletin,* **2002,** *vol. 25,* 1-10.

45. Gikonyo, N.K. and Lux, S.A. Evidence of Male Attractants for Fruit Flies in the leaf Extract of *Schinus molle, East Cent. Afr. J. Pharm. Sci., 6*, 3-8.

46. Suckling, D.M. Issues affecting the Use of Pheromones and other Semiochemicals in Orchards. *Crop Protection,* **2000,** *19,* 677-683.

47. Russell J. Insect Pheromone Biosynthesis. *Top. Curr. Chem.,* **2004,** *239,* 97-132.

48. Jang, E.B., Light, D.M., Binder, R.G., Flath, R.A. and Carvalho, L.A., Attraction of Female Mediterranean Fruit Flies to the Five Major Components of Male-Produced Pheromone in A Laboratory Flight Tunnel. *J. Chem. Ecol.,* **1994,** *20,* 1573-1561.

49. Baker R., Herbert, R., Howse, P.E., Jones, O.T., Franke, W. and Reith, W. Identification and Synthesis of the Major Sex Pheromone of the Olive Fly (*Dacus oleae*). *J. Chem. Soc. Chem. Commun.*, **1980,** *1,* 52-53.

50. Grasswitz, T.R. and G.R. Jones "Chemical Ecology", *Encyclopedia of Life Sciences,* John Wiley & Sons, Ltd, **2002.**

51. Fitt, G. P. The Influence of a Shortage of Hosts on the Specificity of Oviposition Behaviour in Species of *Dacus* (Diptera: Tephritidae). *Physiol. Entomol.,* **1986,** *11,* 133-143.

52. Fowler, A.J. Host-Selection in Wild and Laboratory-Culture *Dacus tryoni. Honours Thesis.* Univ. Sydney, Australia. **1977.**

53. Prokopy, R.J. and Vargas, R.I. Attraction of *Ceratitis capitata* (Diptera: Tephritidae) Flies to Odor of Coffee Fruit. *J. Chem. Ecol.,* **1996,** *22,* 807–820.

54. Shelly, T.E. Consumption of Methyl Eugenol by Male *Bactrocera dorsalis* (Diptera: Tephritidae): Low Incidence of Repeat Feeding. *Florida Entomol.*, **1994,** *77,* 201-208.

55. Tan, K.H. Sex Pheromone Components in Defense of Melon Fly, *Bactrocera cucurbitae* against Asian House Gecko, *Hemidactylus frenatus. J. Chem. Ecol.,* **2000,** *26,* 697-704.

56. Cunningham, R.T. Population detection. In Robinson, A.S. and Hooper, G. (Eds) *Fruit flies: Their Biology, Natural Enemies and Control.* Amsterdam, Elsevier, **1989**.

57. Beroza, M., Green, N., Gertler, S.I., Steiner, L.F. and Miyashita, D.H. Insect Attractants: New Attractants for the Mediterranean fruit fly. *J. Agric. Food Chem.*, **1961,** *9,* 361-365.

58. Drew, R.A.I. Fruit Fly Collecting. In: *Economic fruit flies of the South Pacific Region* (Ed. by Drew, R.A.I.; Hooper, G.H.S.; Bateman, M.A.) 2nd edition, Queensland Department of Primary Industries, Brisbane, Australia, **1982**.

59. Alexander, B.H., Eroza, M.B., Oda, T.A., Steiner, L.F., Miyashita, D.H. and Mitchell. W.C. The Development of Male Melon Fly Attractants. *J. Agric. Food Chem.*, **1962,** *10,* 270-276.

60. Howlett, F.M., Chemical Reactions of Fruit Flies. *Bull. Entomol. Res.*, **1915,** *6,* 297-305.

61. Chambers, D.L. Attractants for Fruit Fly Survey and Control. *In* Shorey H.H. and McKelvey J.J. [eds.]. *Chemical Control of Insect Behavior.* Wiley, New York, **1977**.

62. Raghu, S. Functional Significance of Phytochemical Lures to Dacine Fruit Flies (Diptera: Tephritidae: An Ecological and Evolutionary Synthesis. *Bull. Entomol. Res.*, **2004,** *94,* 385-399.

63. Shelly, T.E and Villalobos, E.M. Cue Lure and the Mating Behavior of Male Melon Flies (Diptera: Tephritidae). *Florida Entomol.,* **1995,** *78,* 473-482.

64. Schultz, G.A., and Bousch, G.M. Suspected Sex Pheromone Glands in Three Economically Important Species of *Dacus. J. Econ. Entomol.,* **1971,** *64,* 347-349.

65. Kobayashi, R. M., K. Ohinata, D. L. Chambers, and M. S. Fujimoto. Sex Pheromones of the Oriental Fruit Fly and the Melon Fly: Mating Behavior, Bioassay Method, and Attraction of Females by Live Males and by Suspected Pheromone Glands of Males. *Environ. Entomol.,* **1978,** *7,* 107-112.

66. Baker, R., Herbert, R.H. and Lomer, R.A. Chemical components of the rectal gland secretions of male *Dacus cucurbitae,* the melon fly. *Experientia,* **1982,** *38,* 232-233.

67. Christenson, L.D. and Foote, R.H. Biology of Fruit Flies. *Ann. Rev. Entomol.,* **1960,** *5,* 171-192.

68. Fitt, G.P. Oviposition behaviour of two tephritid fruit flies, *Dacus tryoni* and *Dacus jarvisi,* as Influenced by the Presence of Larvae in the Host Fruit. *Oecologia,* **1984,** *62,* 37–46.

69. Fletcher, B.S. and Prokopy, R.J. Host Location and Oviposition in Tephritid Fruit Flies. Reproductive Behaviour of Insects (eds. by Bailey, W.J and Ridsdill-Smith, J), Chapman & Hall, London, UK, **1991**.

70. Allwood A.J. Control strategies for fruit flies (Family Tephritidae). Management of Fruit Flies in the Pacific. *In* Allwood, A.J. and Drew, R.A.I. (eds) ACIAR Proceedings. Nadi, Fiji 28–31 October 1996. **1997**.

71. Averill, A.L. and Prokopy, R.J. Host Marking Pheromones. *In* World Crop Pests: Fruit Flies. Their Biology, Natural Enemies and Control by Robinson, A.S. and Hooper, G. (eds). Elsevier, Amsterdam, **1989**.

72. Prokopy, R.J. Evidence for a Pheromone Deterring Repeated Oviposition in Apple Maggot Flies. *Environ. Entomol.*, **1972,** *1,* 326–332.

73. Prokopy, R.J., Malavasi, A. and Morgante, J.S. Oviposition Deterring Pheromone in *Anastrepha fraterculus* Flies. *J. Chem. Ecol.*, **1982,** *8,* 763–771.

74. Prokopy, R.J., Ziegler, J.R. and Wong, T.T.Y. Deterrence of Repeated Oviposition by Fruit Marking Pheromone in *Ceratitis capitata* (Diptera: Tephritidae). *J. Chem. Ecol.*, **1978,** 4, 55–63.

75. De Meyer, M., Copeland, R.S., Lux, S.A., Mansell, M., Quilici, S., Wharton, R., White, I.M. and Zenz, J.N. Annotated Checklist of Host Plants for Afro-Tropical Fruit Flies (Diptera: Tephritidae) of the Genus *Ceratitis. Documentation Zoologique,* **2002,** *27,* 1-91.

76. De Meyer, M. and Copeland, R.S. Description of New *Ceratitis* MacLeay (Diptera: Tephritidae) Species from Africa. *J. Nat. Hist.,* **2005,** *39,* 1283–1297.

77. White, I.M. Taxonomy of the Dacine (Diptera: Tephritidae) of Africa and the Middle East. *African Entomol. Memoir.,* **2006,** *2,* 1–156.

78. Vayssie`res, J.F., Goergen, G., Lokossou, O., Dossa, P. and Akponon, C. A new *Bactrocera* species in Benin among Mango Fruit Fly (Diptera: Tephritidae) Species. *Fruits*, **2005,** *60,* 371-377.

79. Ekesi, S., Nderitu, P.W. and Rwomushana, I. Field Infestation, Life History and Demographic Parameters of *Bactrocera invadens* Drew, Tsuruta & White, A New Invasive Fruit Fly Species in Africa. *Bull. Entomol. Res.,* **2006,** *96,* 379-386.

80. Mwatawala, M.W., De Meyer, M., Makundi, R.H. and Maerere, A.P. Seasonality and Host Utilization of the Invasive Fruit fly *Bactrocera invadens* (Dipt., Tephritidae) In Central Tanzania. *J. Appl. Entomol.,* **2006,** *130,* 530-537.

81. Aluja, M. and Prokopy, R.J. Host Search Behaviour by *Rhagoletis pomonella* Flies: Inter-Tree Movement Pattern in Response to Wind-Borne Fruit Volatiles under field conditions. *Physiol. Entomol.*, **1992,** *17,* 1-8.

82. Fletcher, B.S. The Biology of Dacine Fruit Flies. *Ann. Rev. Entomol.*, **1987,** *32*, 115-144.

83. Metcalf, R.L. Chemical Ecology of Dacine Fruit Flies (Diptera:tephritidae). *Ann. Entomol. Soc. Am.*, **1990,** *83,* 1017-1030.

84. SAS Institute Users Guide: Statistics. SAS Institute, Release 9.2 edition, Carry, Inc., USA, **1999 - 2000,**

85. Liquido, N.J., Cunningham, R.T. and Couey, H.M. Infestation Rates of Papayas by Fruit Flies (Diptera: Tephritidae) in Relation to the Degree of Fruit Ripeness. *J. Econ. Entomol.*, **1989,** *82,* 213-219.

86. Seo, S.T., Farias, G.J. and Harris, E.J. Oriental Fruit Fly: Ripening of Fruit and its Effect on Index of Infestation of Hawaiian Papayas. *J. Econ. Entomol.*, **1982,** *75,* 173-175.

87. Thompson, J.N. and Pelmyr, O. Evolution of Oviposition Behavior and Host Preference in Lepidoptera. *Ann. Rev. Entomol.*, **1991,** *36,* 65-89.

88. Narain, N. and Galvã, M.D.E.S. Volatile Aroma Compounds in Mango Fruit cv. 'Tommy Atkins' – A Preliminary Study. *Acta. Hortc.*, **2004,** *645, 671*-676.

89. Prokopy, R.J., Poramarcom, R., Sutantawong, M., Dokmaihom R. and Hendrichs. J. Localization of Mating Behavior of Released *Bactrocera dorsalis* Flies on Host Fruit in an Orchard. *J. Insect Behav.*, **1996,** *9,* 133-142.

90. Landolt, P.J. Host Plant Influences on Sex Pheromone Behaviour of Phytophagous Insects. *Ann. Rev. Entomol.*, **1997,** *42,* 371-391.

91. Bush, G.L. Sympatric Host Race Formation and Speciation in Frugivorous Flies of the Genus *Rhagoletis* (Diptera, Tephritidae). *Evolution*, **1969,** *23,* 237-251.

92. Bush, G. L. The Mechanism of Sympatric Host Race Formation of the True Fruit Flies (Tephritidae). *In* White, M.J.D. (ed). *Genetic Mechanisms of Speciation in Insects.* Australia and New Zealand Book Co, Sydney, **1974.**

93. Katsoyannos, B.I., Kouloussis, N.A. and Papadopoulos, N.T. Responses of *Ceratitits capitata* to Citrus Chemicals under Semi-Natural Conditions. *Entomol. Exper. Appl.*, **1997,** *82,* 181–188.

94. Shelly, T.E. Exposure To α-copaene and α-copaene- Containing Oils Enhances Mating Success of Male Mediterranean Fruit Flies (Diptera Tephritidae). *Ann. Entomol. Soc. Am.*, **2001,** *94,* 497-502.

95. Shelly, T.E. and Villalobos, E. Host plant influence on the mating success of male Mediterranean fruit flies: variable effects within and between individual plants. *Anim. Behav.*, **2004,** *68,* 417–426.

96. Andrade E.H.A., Maia J.H.G.S. and Zoghbi M.B.G. Aroma Volatile Constituents of Brazilian Varieties of Mango Fruit. *J. Food Comp. Anal.*, **2000,** 13, 27-33.

97. Nguyen, V.L., Meats, A., Beattie G.A.C., Spooner-Hart, R., Liu Z.M. and Jiang, L. Behavioural Responses of Female Queensland Fruit Fly, *Bactrocera tryoni,* to Mineral Oil Deposits. *Entomol. Exper. Appl.*, **2007,** *122,* 215–221

98. Macleod, A.J. and Gonzale de Troconis, N. Volatile Flavour Components of Mango Fruit. *Phytochemistry*, **1982,** *21*, 2523-2526.

99. Macleod, A.J., Macleod, G. and Snyder, C.H. Volatile aroma constituents of mango (cv Kensington). *Phytochemistry*, **1988,** *27*, 2189-2193.

100. Winterhalter, P. *Fruit IV.* In: Maarse, H. (Ed.). *Volatile Compounds in Foods and Beverages*. Marcel Dekker, New York, USA, **1991.**

101. MacLeod, A.J. and Snyder, C.H. Volatile Components of Two Cultivars of Mango from Florida. *J. Agric. Food Chem.*, **1985,** *33,* 380-384.

102. MacLeod, A.J. and Pieris, N.M., Comparison of the Volatile Components of Some Mango Cultivars. *Phytochemistry,* **1984,** *23,* 361-366.

103. Sandoval, I.S., Hernández, A.S. Valdez, G.V. and Cruz-López, L. Volatiles of Mango var. Ataulfo Characterized by SPME and Capillary GC/MS Spectroscopy. *J. Mexican Chem. Soc.,* 2007, *51,* 145-147.

104. Silverstein, R.M., Webster, F.X and Kiemle, D.J. *Spectroscopic Identification of Organic Compounds.* Seventh edition. John wiley & Sons. U.S.A. **2005.**

105. Smith, R.M. *Understanding Mass Spectra: A Basic Approach.* Second Edition. A John Wiley & Sons, Inc., Publication, **2005.**

106. Light, D. M., Jang, E. B. and Dickens, J. C. Electroantennogram Responses of The Mediterranean Fruit Fly, *Ceratitis capitata,* to A Spectrum of Plant Volatiles. *J. Chem. Ecol.*, **1988,** *14,* 159 –180.

107. Light, D.M., Jang, E.B. and Flath, R.A. Electroantennogram Responses of the Mediterranean Fruit Fly, *Ceratitis capitata,* to The Volatile Constituents of Nectarines. *Entomol. Exp. Appl.*, **1992,** *63,* 13–26.

108. Jang, E.B., Light, D.M., Flath, R.A., Nagata, J.T. and Mon, T.R. Electroantennogram Responses of the Mediterranean Fruit Fly, *Ceratitis capitata* to Identified Volatile Constituents from Calling Males. *Entomol. Exp. Appl.*, **1989,** *50,* 7–19.

109. Nojima, S., Linn, J. and Roelofs, W. Identification of Host Fruit Volatiles from Flowering Dogwood (*Cornus Florida*) Attractive to Dogwood-Origin *Rhagoletis pomonella* flies. *J. Chem. Ecol.*, **2003,** *29,* 2347–2357.

110. Nojima, S., Morris, B., Roelofs, W., Zhang, A. and Linn, J. Identification of Host Fruit Volatiles from Hawthorn (*Crataegus Spp.*) Attractive to Hawthorn-Origin *Rhagoletis pomonella* Flies. *J. Chem. Ecol.*, **2003,** *29,* 321–336.

111. Hull, C.D. and Cribb, B.W. Olfaction in the Queensland Fruit Fly, *Bactrocera tryoni.* Identification of Olfactory Receptor Neuron Types Responding to Environmental Odors. *J. Chem. Ecol.,* **2001,** *27,* 871-887.

112. Hernández-Sánchez, G., Sanz-Berzosa, I., Casañaginer, V. and Primo-Yúfera, E. Attractiveness for *Ceratitis capitata* (Wiedemann) (Dipt., Tephritidae) of Mango (*Mangifera indica,* cv. Tommy Atkins) Airborne Terpenes. *J. Appl. Entomol.,* **2001,** *125,* 189-192.

113. Cornelius, M.L., Duan, J.J. and Messing R.H. Volatile Host Fruit Odors as Attractants for the Oriental Fruit Fly (Diptera: Tephritidae). *J. Econ. Entomol.,* **2000,** *93,* 93-100.

114. Haniotakis, G. and Voyadjoglou, A. Oviposition Regulation in *Dacus oleae* by Various Olive Fruit Characters. *Entomol. Exp. Appl.,* **1978,** *24,* 387-392.

115. Fowler, A.J. Host-selection in Wild and Laboratory-Culture *Dacus tryoni.* Honours *Thesis.* Univ. Sydney, Australia. **1977.**

116. Gonza´ Lez, R., Toledo, J., Cruz-Lopez, L., Virgen, A., Santiesteban, A. and Malo, E.A. A New Blend of White Sapote Fruit Volatiles as Potential Attractant to *Anastrepha ludens* (Diptera: Tephritidae). *J. Econ. Entomol.,* **2006,** *99,* 1994-2001.

117. Cossé, A.A., Todd, J.L. Millar, J.G., Martínez, L.A. and. Baker, T.C. Electroantennographic and Coupled Gas Chromatographic Electroantennographic Response of the Mediterranean Fruit Fly, *Ceratitis capitata, to* Male-Produced Volatiles and Mango Odour. *J. Chem. Ecol.,* **1995,** *21,* 1823-1836.

118. Du, Y. and Millar, J.G. Electroantennogram and Oviposition Bioassay Responses of *Culex quinquefasciatus* and *Culex tarsalis* (Diptera: culicidae) to Chemicals in Odors of Bermuda Grass Infusions. *J. Med. Entomol.*, **1999,** *36,* 158-166.

119. Feder, J.L. and Bush, G.L. A Field Test of Differential Host-Plant Usage between Two Sibling Species of *Rhagoletis pomonella* Fruit Flies and its Consequences for Sympatric Models of Speciation. *Evolution,* **1989,** *43,* 1813 –1819.

120. Feder, J.L., Opp, S., Wlazlo, B., Reynolds, K., Go, W. and Spisak, S. Host Fidelity is an Effective Pre-Mating Barrier between Sympatric Races of the Apple Maggot Fly. *Proc. Natl. Acad. Sci.,* **1994,** *91,* 7990 –7994.

121. Frey, J.E. and Bush, G. L. Impaired Host Odor Perception in Hybrids between the Sibling Species *Rhagoletis pomonella* and *R. mendax. Entomol. Exp. Appl.,* **1996,** *80,* 163 –165.

122. Green, T.A., Prokopy, R. J. and Hosmer, D.W. Distance of Response to Host Tree Models by Female Apple Maggot Flies, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae): Interaction of Visual and Olfactory Stimuli. *J. Chem. Ecol.,* **1994,** *20,* 2393-2413.

123. McLafferty, F.W. and Turecek, F. (eds). Interpretation of Mass Spectra, 4th edition. Sausalito, CA: University Science Books, **1993**.

124. Prokopy, R.J., and Vargas, R.I. Attraction of *Ceratitis capitata* (Diptera: Tephritidae). Flies to Odor of Coffee Fruit. *J. Chem. Ecol.*, **1996,** *22,* 807-820.

125. Warthen, J.D., Lee, C.-J., Jang E.B., Lance, D.R. and McInnis, D.O. Volatile, Potential Attractants from Ripe Coffee Fruit for Female Mediterranean Fruit Fly. *J. Chem. Ecol.*, **1997,** *23,* 1891-1900.

126. Prokopy, R.J., Hu, X.P., Jang, E.B., Vargas, R.I. and Warthen, J.D. Attraction of Mature *Ceratitis capitata* Females to 2-Heptanone, a Component of Coffee Fruit Odor. *J. Chem. Ecol.*, **1998,** *24,* 1293-1304.

127. Robacker, D.C. and Heath, R.R. Attraction of Mexican Fruit Flies (Diptera: Tephritidae) to Lures Emitting Host-Fruit Volatiles in a Citrus Orchard. *Florida Entomol.*, **1996.** *79,* 600-602.

128. Robacker, D.C., Warfield, W.C. and Flath, R.A. A Four-Component Attractant for the Mexican Fruit Fly, *Anastrepha ludens* (Diptera: Tephritidae), From Host Fruit. *J. Chem. Ecol.*, **1992,** *18,* 1239-1254.

129. Nigg, H.N., Mallory, L.L., Simpson, S.E., Callaham, S.B., Toth, J P., Fraser, S., Klim, M., Nagy, S., Nation, J.L. andAttaway, J.A. Caribbean Fruit Fly, *Anastrepha suspense* (Loew), Attraction to Host Fruit and Host Kairomones. *J. Chem. Ecol.*, **1994,** *20,* 727–743.

130. Hubbard C.E. and Milne-Redhead, E. Flora of Tropical East Africa. London, Whitefriars Press, **1964**.

131. Ainsle, J.R. *List of plants used in Native Medicine in Nigeria.* Oxford University Press, London, **1937**.

132. Watt, J.M. and Breyer-Brandwijk, M.G. *The Medicinal and Poisonous Plants of Southern and Eastern Africa.* Second Edition, Livingstone, London, **1962**.

133. Kokwaro, J.O. *Medicinal plants of East Africa., Nairobi,* East African Literature Bureau. **1976**.

134. Ajaiyeoba, E.O. Phytochemical and Antimicrobial Studies of *Gynandropsis gynandra* and *Buchholzia coriaceae* Extracts. *African J. Biomed. Res.*, **2000,** *3,* 161 – 165.

135. Lwande, W., Ndakala, A.J., Hassanali, A., Moreka, L., Nyandat, E., Ndungu, M., Amiani, H., Gitu, P.M., Malonza, M.M. and Punyua, D.K. *Gynandropsis gynandra* Essential Oil and Its Constituents as Tick (*Rhipicephalus appendiculatus*) Repellents. *Phytochemistry*, **1999,** *50,* 401-405.

136. Tan, K.H. Fruit Flies (*Bactrocera* spp.) as Pests and Pollinators. *Sabah Soc. J.*, **2000,** *17,* 37–56.

137. Tan, K.H. Behaviour and Chemical Ecology of *Bactrocera* flies. *In*: Tan, K.H. (eds.), *Area-Wide Control of Fruit Flies and Other Insect Pests.* USM Press, Penang, **2000**.

138. Tan, K.H., Nishida, R. and Toong, Y.C. Floral Synomone of a Wild Orchid, *Bulbophyllum cheiri*, Lures *Bactrocera* Fruit Flies for Pollination. *J. Chem. Ecol.*, **2002,** *28,* 1161–1172.

139. Papadopoulos, N.T., Shelly, T.E., Niyazi, N. and Jang, E.B. Olfactory and Behavioral Mechanisms Underlying Enhanced Mating Competitiveness Following Exposure to Ginger Root Oil and Orange Oil in Males of the Mediterranean Fruit Fly, *Ceratitis capitata* (Diptera: Tephritidae). *J. Insect Behav.*, **2006,** *19,* 403–418.

140. Shelly, T.E., Dang, C. and Kennelly, S. Exposure to Orange (*Citrus sinensis* L.) Trees, Fruit, and Oil Enhances Mating Success of Male Mediterranean Fruit Flies (*Ceratitis capitata* [Wiedemann]). *J. Insect. Behav.*, **2004,** *17,* 303–315.

141. Chikán, V., Molnár, Á. and Balázsik, K. One-Step Synthesis of Methyl Isobutyl Ketone from Acetone and Hydrogen over Cu-on-MgO Catalysts. *J. Catalysis*, **1999,** *184,* 134–143.

142. Melo, L., Llanos, A., Mediavilla, M. and Moronta, D. Acetone Transformation over Pt/H[Al]ZSM5 and Pt/H[Ga]ZSM5 Catalysts Evidences of a Pt–Ga Interaction. *J. Mol. Catal. Chem.*, **2002,** *177,* 281–287.

143. Shelly, T.E. Flower-Feeding Affects Mating Performance in Male Oriental Fruit Flies *Bactrocera dorsalis*. *Ecol. Entomol.*, **2000,** *25,* 109–114.

144. Shelly, T.E. Lek Size and Female Visitation in Two Species of Tephritid Fruit Flies. *Anim. Behav.*, **2001,** *62,* 33–40.

145. Fletcher B.S., Bateman, M.A., Hart, N.K. and Lamberton, J.A. Identification of Fruit Fly Attractant in an Australian Plant, *Zieria Smithii*, as *O*-Methyl Eugenol. *J. Econ. Entomol.*, **1975,** *68,* 815–816.

146. Sivinski, J.M. and Calkins, C. Pheromones and Parapheromones in the Control of Tephritids. *Florida Entomol.*, **1986,** *69,* 157-168.

147. Nishida, R., Tan, K.H., Serit, M., Lajis, N.H., Sukari, S., Takahashi, S., Fukami, H. Accumulation of Phenylpropanoids in the Rectal Glands of Males of the Oriental Fruit Fly, *Dacus dorsalis. Experientia,* **1988,** *44,* 534–536.

148. Nishida, R., Iwahashi, O. and Tan, K.H. Accumulation of *Dendrobium superbum* (Orchidaceae) Fragrance in the Rectal Glands by Males of the Melon Fly, *Dacus cucurbitae. J. Chem. Ecol.*, **1993,** *19,* 713-722.

149. Nishida, R., Shelly, T.E., Kaneshiro, K.Y., Acquisition of Female-Attracting Fragrance from a Hawaiian Lei Flower, *Fagraea berteriana,* by Males of the Oriental Fruit Fly. *J. Chem. Ecol.,* **1997,** *23,* 2275–2285.

150. Wertheim, B., Allemand, R., Louise E., Vet, M. and Dicke, A. Effects of Aggregation Pheromone on Individual Behaviour and Food Web Interactions: A Field Study on Drosophila. *Ecol. Entomol.,* **2006,** *31,* 216–226.

151. Baker, T.C. *Chemical control of behavior. Comprehensive Insect Physiology, Biochemistry and Pharmacology* (eds. by G. A. Kerkut and L. I. Gilbert). Pergamon, Oxford, **1985.**

152. Dicke, M. and Sabelis, M.W. Costs and Benefits of Chemical Information Conveyance: Proximate and Ultimate Factors. *Insect Chemical Ecology: An Evolutionary Approach* (ed. by B.D. Roitberg and M. B. Isman). Chapman and Hall, New York, **1992.**

153. Fletcher, B.S. The Structure and Function of the Sex Pheromone Glands of the Male Queensland Fruit Fly, *Dacus tryoni*. *J. Insect Physiol.*, **1969,** *15,* 1309-1322.

154. Wee, S-L. And Tan, K-H. Female Sexual Response to Male Rectal Volatile Constituents in the Fruit Fly, *Bactrocera carambolae* (Diptera: Tephritidae). *Appl. Entomol. Zool.*, **2005,** *40,* 365–372.

155. Mazamenos, B.E., and Pomonis, J.E. Male Olive Fruit Fly Pheromone: Isolation, Identification and Laboratory Bioassays. *In* International Symposium on Fruit Flies of Economic Importance. CEC/IOBC, Athens, Greece, **1982.**

156. Iwahashi, O. and Majima, T. Lek Formation and Malemale Competition in the Melon Fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). *Appl. Entomol. Zool.*, **1986,** *21,* 70–75.

157. Shelly, T.E. and Kaneshiro, K.Y. Lek Behavior of the Oriental Fruit Fly in Hawaii. *J. Insect Behav.*, **1991,** *4,* 235–241.

158. Booth, Y.K., Schwartz, B.D., Fletcher, M.T., Lambert, L.K., Kitching, W. and De Voss, J.J. A Diverse Suite of Spiroacetals, Including a Novel Branched Representative, Released by Female *Bactrocera tryoni* (Queensland fruit fly). *Chem. Commun.* **2006,** *38,* 3975–3977.

159. Perkins, M.V., Fletcher, M.T., Drew, R.A.I. and Moore, C.J. Chemical Studies of Rectal Gland Secretions of Some Species of *Bactrocera dorsalis* Complexes of Fruit Flies (Diptera: Tephritidae). *J. Chem. Ecol.*, **1990,** *16,* 2475-2487.