HOST-MARKING BEHAVIOUR AND PHEROMONES IN MAJOR FRUIT FLY SPECIES (DIPTERA: TEPHRITIDAE) INFESTING MANGO (*MANGIFERA INDICA*) IN KENYA

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Host-Marking Behaviour and Pheromones in Major Fruit Fly Species (Diptera: Tephritidae) Infesting Mango (*Mangifera indica*) in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

To my parents, David Alfonso Kachigamba (late) and Agness Maudie Putaputa.

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LIST OF ACRONYMS AND ABBREVIATIONS

AFFP	African Fruit Fly Programme
a.m.	ante-meridiem
ANOVA	Analysis of Variance
BCED	Behavioural and Chemical Ecology Department
CRD	Completely Randomized Design
DAAD	Germany Academic Exchange Programme
DARS	Department of Agricultural Research Services (Malawi)
DNA	Deoxyribonucleic Acid
g	gram
GDP	Gross Domestic Product
HPLC	High Pressure Liquid Chromatography
LC-MS	Liquid Chromatography- Mass Spectrometry
ICIPE	International Centre of Insect Physiology and Ecology
IDM	Internal Diameter
JKUAT	Jomo Kenyatta University of Agriculture and Technology
ml	Millimetre
PCR-RFLP	Polymerase Chain Reaction – Restriction Fragment Length Polymorphism
p.m.	post meridiem
s	second

SNK	Student Newman Keuls
USDA	United States Department of Agriculture

ABSTRACT

A laboratory study was conducted on six major fruit fly species infesting mango in Kenya (*Ceratitis capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *Bactrocera invadens*) regarding host-marking behaviour and pheromones in order to get insight into the potential of the host-marking technique for their management. Slices of ripe mango fitted in 50 mm diameter petri dish covers were used as oviposition substrates in bioassays to determine incidence of host-marking behaviour among the fruit fly species and efficacy of their host-marking behaviour and faecal matter in deterring conspecific and heterospecific oviposition. High pressure liquid chromatography was used to determine presence of potential host-marking pheromones of the fruit flies in their faecal matter. Host-marking behaviour was found to be prevalent in *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* only, but duration of host-marking bout was significantly shorter in *C. rosa* than in the other three species. In addition, *C. cosyra* was found to have a unique behaviour of pausing for some time after oviposition before engaging in host-marking. For each host-marking fruit fly species, three chemical components, which could be its host-marking pheromones were found in its faecal matter. However, the chemical components in faecal matter of *C. capitata, C. fasciventris* and *C. rosa* seemed to be similar while for *C. cosyra,* two of the chemical components were those found in faecal matter of the other three species but the third one was specific to it. When tested for behavioural activity, the unique chemical component of *C. cosyra* elicited conspecific oviposition deterrence, suggesting that it was indeed the host-marking pheromone of *C. cosyra*. The findings of the study indicated apparent potential of the

host-marking technique in the management of some of the major fruit fly species infesting mango in Kenya.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Mango in Kenya: Importance of the crop and potential of the host-marking technique for the management of its fruit fly pests

Mango (*Mangifera indica* L.) is one of the most important fruits in Kenya. It is a source of income and nutritional security for many people (ICIPE, 2007). Although mango is an important fruit in Kenya, its production and utilization faces a number of challenges, one of them being infestation by tephritid fruit flies (ICIPE, 2007). It is estimated that Kenya produces 183,486 tons of mango annually but more than 50% of this is lost to fruit flies (Griesbach, 2003). Fruit flies are thus costing Kenya a lot in nutrition and income. There are six main fruit fly species that are infesting mango in Kenya and these are: *Ceratitis capitata* (Wiedemann), *C. fasciventris* (Bezzi), *C. rosa* (Karsch), *C. cosyra* (Walker), *C. anonae* (Graham) and *Bactrocera invadens* (Drew, Tsuruta and White) (Ekesi *et al*., 2009). Currently, farmers in Kenya use a variety of techniques to mitigate the mango fruit fly problem, however, more management techniques need to be developed for enhanced management of the pests (Billah *et al*., 2007; ICIPE, 2007).

Manipulation of insect behaviour using semiochemicals is fast becoming an effective tactic in pest management (Roitberg, 2007). In fruit flies, one such technique is host-marking (Aluja *et al*., 2009). This technique is based on the fact that females of some fruit fly species have a tendency of depositing some specific pheromones on

their host-fruits after oviposition, which deter other prospective ovipositing fruit flies from re-using the resources. This behaviour is called host-marking. By understanding this behaviour, identifying the pheromones involved, producing the pheromones artificially *en mass* and spraying them in orchards, researchers have been able to effectively mitigate fruit fly infestation in orchards (Arredondo and Diaz-Fleischer, 2006; Aluja *et al.,* 2009). As Kenya needed more techniques for managing her mango fruit fly problem, it was envisaged that the host-marking technique was one appropriate tool.

1.2 Problem statement

Although the host-marking technique was envisaged to be one tool that could help to mitigate the mango fruit fly problem in Kenya, there was lack of foundational information regarding host-marking behaviour and pheromones in the major fruit fly species infesting mango in the country, which could actually provide insight into the potential of the host-marking technique for their management. It was, therefore, considered necessary to investigate host-marking behaviour and pheromones in the major fruit fly species infesting mango in Kenya.

1.3 Justification

As stated in section 1.1, fruit flies are a serious problem in mango production in Kenya and more management tools need to be developed to mitigate this problem. On the other hand, the host-marking technique is very effective in fruit fly management, with an efficacy range of 84 – 98% (Katsayannos and Boller, 1976, 1980; Aluja and Boller, 1992; Nufio and Papaj, 2004; Arredondo and Diaz-Fleischer,

2006, Aluja *et al*, 2009). The host-marking technique is also target-specific, environmentally benign (since the host-marking pheromones are biodegradable and non-toxic) and it can use the same equipment for spraying conventional pesticides (Averill and Prokopy, 1989). Hancock (1989) also observed that the fruit fly problem among smallholder fruit farmers in Africa can be better managed using behaviour-based techniques rather than insecticides. In addition, since host-marking pheromones repel prospective ovipositing females, the host-marking technique may also be used compatibly and synergistically in a push-pull system with traps containing synthetic mango volatiles which attract the fruit flies. An investigation on host-marking behaviour and pheromones in the major fruit fly species infesting mango in Kenya was therefore worthwhile because it was going to provide insight into the potential of the host-marking technique for the management of the fruit flies.

1.4 Hypotheses

The following were the hypotheses of this study:

1. Host-marking behaviour is not prevalent in *C. capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens*.
2. Host-marking behaviour and faecal matter of *C. capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens* do not deter conspecific and heterospecific oviposition.
3. Faecal matter of *C. capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens* does not contain potential host-marking pheromones of the fruit fly species.

1.5 Objectives

1.5.1 General objective

The general objective of this study was to investigate host-marking behaviour and pheromones in the major fruit fly species infesting mango in Kenya with the view of getting insight into the potential of the host-marking technique for their management.

1.5.2 Specific objectives

The following were the specific objectives of this study:

1. To determine incidence of host-marking behaviour in *C. capitata*, *C. fasciventris*, *C. rosa*, *C. cosyra*, *C. anonae* and *B. invadens*.
2. To determine efficacy of host-marking behaviour and faecal matter of *C. capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens* in deterring conspecific and heterospecific oviposition.
3. To determine presence of potential host-marking pheromones of *C. capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens* in faecal matter of the fruit flies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mango: A general perspective

2.1.1 Taxonomy and origin of mango

Mango belongs to the genus *Mangifera* and species *indica*. The genus *Mangifera* in turn belongs to the order Sapindales in the family Anacardiaceae (Bompard, 2009). Mango is believed to have originated from the Indian/Burmese monsoon region (Augstburger, *et al*., 2001) and was brought to East Africa by Persians around the 10^{th} century AD (Morton, 1987).

2.1.2 Uses of mango

Mangoes are mostly grown for their fruit, which is predominantly eaten ripe in dessert form and is highly nutritious, containing carbohydrates, proteins, fats, minerals and vitamins A, B1, B2, and C (Bally, 2006). Fresh mangoes are also processed into a wide range of products such as pulps, juices, frozen slices, dried slices, pulp (fruit leather), chutneys, jams, pickles, canned in syrup, and sliced in brine (Parfonry, 2001).

2.1.3 Major pests of mango and their control

Major pests of mango are fruit flies, scale insects and mites (Bissdorf, 2005). Fruit flies damage mangoes by laying their eggs in them and their emerging larvae feed on and burrow the flesh of the fruits, causing them to rot (Van Mele, 2007). Early harvesting, use of baited traps, field sanitation, use of biological agents (parasitoids,

predators and pathogens) and spraying of insecticides are some of the ways of mitigating fruit fly infestation in mangoes (Ekesi *et al*., 2007a). Scale insects suck sup from tender parts of mango trees and they are controlled by spraying the trees with organophosphorous compounds blended with mineral oil (Parfonry, 2001). Mites, which are small and whitish–yellow, attack young leaves of mango trees and cause them to crinkle. They are controlled using acaricides (Parfonry, 2001).

2.2 Fruit flies: A general perspective

2.2.1 Taxonomy of fruit flies

Fruit flies are small insects (about 4 - 7 mm long) belonging to the Order Diptera and Family Tephritidae (White and Elson-Harris, 1992). They usually live in association with plants, using their fruits as oviposition substrates (Jackson and Lee 1985; Dowell and Wange, 1986). Other species of fruit flies, however, attack other parts of plants such as leaves and flowers (Dowell and Wange, 1986). The term “fruit fly” is used for two distantly related families of flies namely Tephritidae and Drosophilidae. The latter are “fruit flies” of geneticists, which are in reality micro-fungi feeders and but erroneously called fruit flies because of their habit of feeding on decaying fruit (Dowell and Wange, 1986). To distinguish them from these fungi-feeding “fruit flies”, tephritid fruit flies are sometimes called the “true fruit flies” since most of their species attack living plants, mainly fruits (Jackson and Lee 1985). There are over 5, 000 described species of tephritid fruit flies and nearly 40% of these species attack intact and growing fruit (Dowell and Wange, 1986). The described species of tephritid fruit flies fall into 500 genera which in turn fall into several subfamilies, the

three most important ones being Trypetinae, Dacinae and Tephritinae (White and Elson-Harris 1992). Most of the fruit-infesting tephritids belong to the Subfamilies Trypetinae and Dacinae while the Tephritinae mostly contains weed-infesting species (White and Elson-Harris 1992). In the Subfamily Trypetinae, *Ceratitis*, *Anastrepha* and *Rhagholetis* are the most economically important genera as fruit pests while in the Subfamily Dacinae, *Bactrocera* and *Dacus* are the most economically important genera as fruit pests (White and Elson-Harris 1992).

2.2.2 World geographic distribution of fruit flies

Ceratitis species are mostly restricted to Africa except for the Mediterranean fruit fly (*C. capitata*), which has spread to many tropical and subtropical parts of the world (Ekesi and Muchugu, 2007). *Ceratitis capitata* is by far the most economically important pest species in the genus, and it is one of the most polyphagous and widespread species of Tephritidae (Liquido *et al*., 1991).

The genus *Anastrepha* is mainly found in the Neotropics and its most economically important pest species are the Mexican fruit fly (*A. ludens*), West Indian fruit fly (*A. obliqua*), and South American fruit fly (*A. fraterculus* complex) (White and Elson-Harris 1992). The genus *Rhagoletis* is mainly found in the Holarctic and Neotropical regions and its most economically important fruit pest species are the apple fruit fly (*R. pomonella*), European and eastern cherry fruit flies (*R. cerasi* and *R. cingulata* respectively), blueberry fruit fly (*R. mendax*), walnut husk fly (*R. completa*), *R. striatella*, a pest of husk tomato, and *R. tomatis*, a pest of tomato (White and Elson-Harris 1992).

Bactrocera, which is the most economically important genus, is native to the Old World tropics. Important species include *B. invadens*, *B. dorsalis*, *B. cucurbitae*, *B. oleae*, *B. tryoni* and *B. zonata* (White and Elson-Harris 1992). *Bactrocera invadens* invaded Africa around 2003 and is currently the most destructive fruit fly pest of mango on the continent (Rwomushana *et al*., 2008). The genus *Dacus* mainly occurs in the Afrotropical region and the most economically important pest species of this genus are *D. bivittatus* and *D. ciliatus* (White and Elson-Harris 1992).

2.2.3 Fruit fly life cycle and the nature of damage they cause in fruits

Fruit flies are holometabolous, i. e. they undergo complete metamorphosis (Plate 2.1). The typical life cycle begins with eggs laid under the skin of a fruit by the female. The female has an ovipositor, similar to the sting of a wasp, with which it punctures the skin of a healthy fruit in which it lays its eggs (Plate 2.1). The eggs hatch into larvae (maggots) in 2 - 3 days. Larval development starts and completes within the fruit, with the larvae feeding on the flesh of the fruit. In the process of feeding, the larvae form galleries in the fruit and these galleries provide entry points for pathogens which increase fruit decay making it unsuitable for human consumption. The larvae feed on the fruit for about two weeks and undergo three instar developmental stages during this time. Third instar larvae then emerge from the rotten fruit and enter the soil where they pupate as fourth instar larvae (Plate 2.1). The pupating larvae stay in the soil for about two weeks after which transformation into an adult fly is complete and young flies hatch. Young females need 1 - 2 weeks to become sexually mature and acquire the protein reserves needed to lay eggs while young males develop to sexual maturity in one week or less (Bronson, 2006).

Adult fruit flies can live for 2 - 11 months depending on species and environmental conditions (White and Elson-Harris, 1992). In temperate regions, fruit flies commonly overwinter as adults, becoming active when weather warms up and gradually the population builds to a peak in late summer (Brunner, 1992; Bronson, 2006). The life cycle of a fruit fly and the nature of damage it causes in fruits (a case of mango) is shown in Plate 2.1.

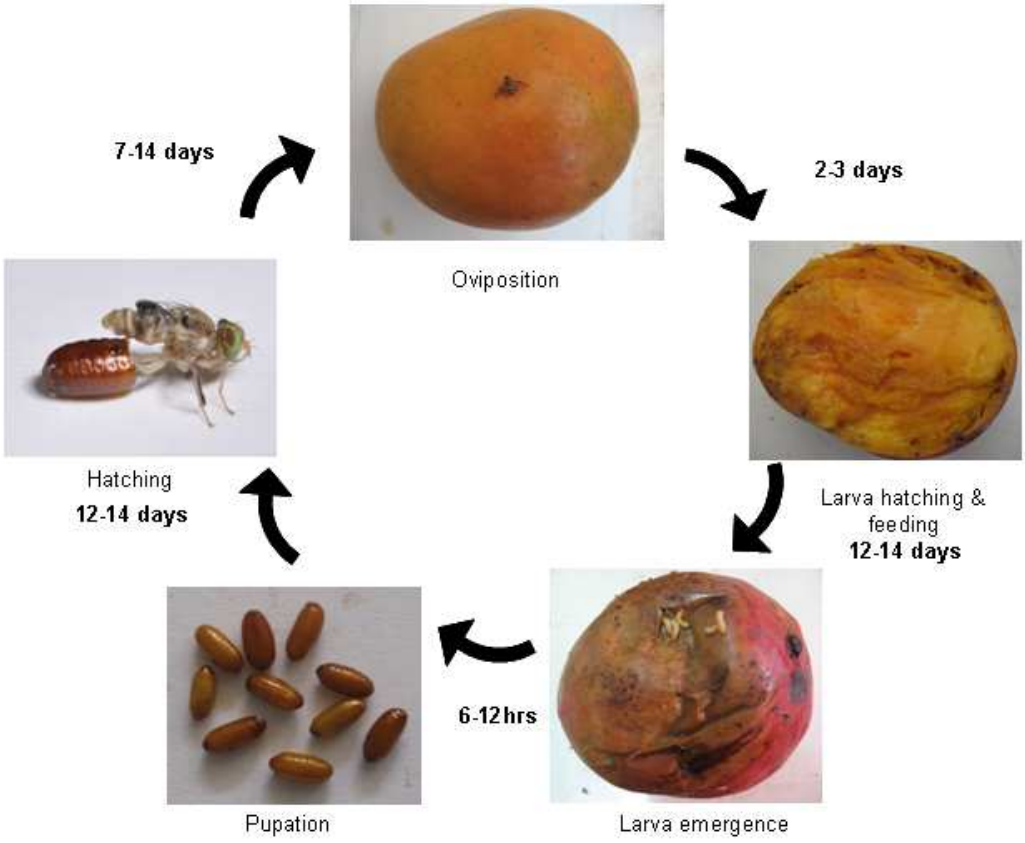

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Plate 2.1: Fruit fly life cycle and damage caused in fruits (a case of mango)

2.2.4 Economic importance of fruit flies

Due to their oviposition and larval feeding habits described in section 2.2.3, fruit flies inflict heavy losses on fruit and vegetable crops worldwide (AliNiazee, 1988). Economic effects of pest species include not only direct loss of yield and increased control costs, but also loss of export markets and / or extra cost of constructing and maintaining fruit treatment and pest eradication facilities. In many countries, exportation of most commercial fruits is severely restricted by quarantine laws to prevent the spread of fruit flies. In Africa, losses in mango yield due to fruit flies are estimated at 50% (ICIPE, 2007). In Bulgaria and Romania, *R. cerasi* was reported to cause losses of up to 100% in cherries (Fischer-Colibrie and Busch-Peterson, 1989). The following damages by fruit flies were reported by Weems (1981): *Ceratitis capitata*, 50% in citrus in Greece; *B. dorsalis*, 50 - 80% in pears, peaches and figs in western Pakistan; *C. rosa*, 50 -100% in plums in South Africa. *Ceratitis cosyra* caused 30 – 90% damage in mango in Kenya (Mukiama and Muraya, 1994). The cost of living with an established infestation of *C. capitata* or several other major fruit fly pests in California was estimated at US$ 910 000 000.00 annually (Jackson and Lee, 1985) while the cost of eradication was estimated at US$ 290 000 000.00 (Dowell and Wange 1986).

2.2.5 Behaviour in fruit flies

The term behaviour refers to neuro-muscularly controlled activities which living things usually do (Forest and Perry, 2006). Tephritid fruit flies exhibit various forms of behaviour in many aspects of their life, both as adults and larvae. The following are some of the forms of behaviour which adult tephritid fruit flies display:

2.2.5.1 Feeding

Fruit flies feed in order to acquire nutrients which their bodies require. They feed on substances such as secretions of plants, nectar, and plant sap exuding from trunk, stem, leaf, or fruit injuries, such as those caused by feeding insects, diseases, or mechanical damage. Rotting fruits, bird dung, decaying insects and honeydew secreted by homopterous insects are other sources of food for fruit flies (Averill and Prokopy, 1987).

2.2.5.2 Mating

Mating is important in tephritid fruit flies to ensure species perpetuation. Males of many fruit fly species produce sex pheromones to attract females for mating. In some species, the males gather in groups (leks) and produce the pheromones together in order to effectively attract females (Kuba and Koyama, 1985; Sivinski and Burk, 1989). Specific fruit fly species usually mate at specific times of the day. For example, in *D. dorsalis*, mating occurs at dusk and is stimulated by decreasing light intensity (Sivinski and Burk, 1989).

2.2.5.3 Host location

Female fruit flies use olfactory and visual cues to locate their potential host fruits (Prokopy and Roitberg, 1984; Aluja and Prokopy, 1993; Quilici *et al*., 1994; Brevault and Quilici, 1999). Olfactory cues are usually used at long range while visual cues, such as colour, shape and size, are used at close range (Aluja and Prokopy, 1993).

2.2.5.4 Oviposition

Oviposition behaviour i. e. egg laying in fruit flies is highly diverse from species to species in aspects such as time of oviposition, ovipuncture clutch size (number of eggs laid in an ovipuncture), positioning of ovipositor, duration of an oviposition bout and parts of plants preferred for oviposition (Shelly, 1999; Averill and Prokopy (1987).

2.2.5.5 Host-marking

Host-marking in fruit flies refers to the tendency of females of some species to deposit some specific pheromones on their host fruits after oviposition so as to deter other prospective ovipositing fruit flies from re-using the fruits. Host-marking behaviour in tephritid fruit flies was first observed by Porter in 1928. Porter (1928) studied *R. pomonella* on apples and observed that immediately after ovipositing, the female walked rapidly around the host fruit with an extended ovipositor and dragged it on the surface of the same. However, it took Hafliger, who studied *R. cerasi*, in 1953 to speculate accurately that the biological significance of the behaviour was to mediate even distribution of offspring in available host fruits (Averill and Prokopy, 1989).

Following the observations of Porter and Hafliger regarding fruit fly host-marking behaviour, the first evidence of host-marking pheromone deposition was produced by Prokopy in 1972. Nufio and Papaj (2004) reported that in *R. juglandis*, apart from deterring conspecific re-use of oviposition substrates, host-marking also serves as a quantitative signal of the anticipated offspring competition such that the more eggs

the fly lays, the longer the duration of marking i.e. the more pheromone it deposits, and any coming fruit fly determines whether to re-use the host or not or just lay a few eggs by gauging the concentration of the marking pheromone on the host. Other forms of behaviour elicited by host-marking pheromones in fruit flies are reduction of residence time of conspecific females on the host, reduction of the desire to oviposit and arresting of males (Papaj *et al*., 1989, 1990).

Host-marking behaviour is, however, not a universal phenomenon in tephritids, it is prevalent in some species, occasional in some and absent in others. Examples of species in which host-marking behaviour is prevalent are *C. capitata, A. ludens, A. obliqua, A. serpentina, R. cerasi, R. basiola, R. pomonella,* and *R. juglandis,* to mention a few (Roitberg and Lalonde, 1991; Papaj *et al*, 1992; Aluja *et al*, 2003; Arredondo and Diaz-Fleischer, 2006; Aluja *et al*, 2009). The behaviour is occasional in some Dacini species (Fitt, 1984), *R. suavis* (Nufio and Papaj, 2004) and *C. catoirii* and *B. zonata* (Duyck *et al*., 2006). Host- marking behaviour is absent in species such as *Dacus cucurbitae, D. opilae and D. cacuminatus* (Prokopy and Koyama, 1982) and *B. invadens* (Fletcher and Prokopy, 1991). Further, although host-marking is generally regarded to cause oviposition deterrence, in some species it has no effect since it is ceremonial, the fruit flies do not actually produce or deposit host-marking pheromones (Papaj, 1994; Nufio *et al*., 2000).

Fruit fly retention of host-marking behaviour and ability to discriminate against host-marking pheromones varies with various factors such as strain and nature of the fruit flies in terms of whether they are wild or laboratoty-reared (Averill and Prokopy,

1989). Boller and Calkins (1984) observed that the Kenyan *C. capitata* has good retention of host-marking behaviour, long duration of ovipositor dragging and high discrimination against host-marking pheromone, unlike strains of the same species from Sardinia and Seibersdorf. Wild fruit flies are known to produce stronger host-marking pheromones than laboratory-reared ones and they are also more sensitive to the pheromones than the laboratory-reared ones (Prokopy *et al*., 1978; Prokopy *et al.,* 1989).

2.2.6 Fruit fly host-marking pheromones

2.2.6.1 Production of host-marking pheromones in fruit flies

Fruit flies produce and store their host-marking pheromones in the posterior half of the midgut and as such, faecal matter of host-marking fruit flies contains large quantities of their pheromones (Averill and Prokopy, 1989).

2.2.6.2 Chemical properties of fruit fly host marking pheromones

Fruit fly host-marking pheromones are low in volatility, highly polar in solution and have molecular weight of less than 10,000 (Mumtaz and AliNiazee, 1983). They are also soluble in water and methanol (Boller, 1981; Averill and Prokopy, 1982; Boller and Hurter, 1985; Hurter *et al.*, 1987; Averill and Prokopy, 1987; Aluja *et al.*, 2003). Fruit fly host-marking pheromones are also persistent on surfaces where they have been deposited regardless of whether they have been deposited directly by the fruit flies or as extracts (Averill and Prokopy, 1987). For example, the half-life of the

host-marking pheromone of *R. pomonella* is 10.7 days with activity persisting for three weeks (Averill and Prokopy, 1987). Persistence of host-marking pheromones of other fruit fly species has been reported as follows: 4 days for *R. indifferens*, (Mumtaz and AliNiazee, 1983), 6 days for *A. suspensa* (Prokopy *et al*., 1977), 6 days for *C. capitata* ((Prokopy *et al*., 1978), 9 days for *R. fausta* (Prokopy, 1975) and 12 days for *R. cerasi* (Katsoyannos, 1975). Aluja *et al*., (2009) observed that the deterrent efficacy of faecal matter extract of *A. ludens* on *A. obliqua* in an orchard of tropical plum dropped by just 10% after 27 days despite heavy rainfall.

2.2.6.3 Perception of host-marking pheromones in fruit flies

Fruit flies perceive host-marking pheromones using the D-sensilla found on the ventral side of the 2nd, 3rd and 4th tarsomeres of pro-thoracic tarsi (Crnjar *et al.*, 1978; Crnjar and Prokopy, 1982; Stadler *et al*., 1992, 1994) and to a lesser extent using the short hairs on labellum and D-sensilla on meso- and meta- thoracic legs (Crnjar *et al.*, 1978; Crnjar and Prokopy, 1982). The sensilla contain contact-chemoreceptor cells which are sensitive to host-marking pheromones and they are present and functional in both male and female flies (Stadler *et al.*, 1994).

Response of fruit flies to host-marking pheromones is influenced by several factors such as activity of pheromone, physiological state of the fly and nature of the fly. Pheromones with higher activity elicit greater response than those with lower activity. Pheromone activity is in turn affected by among other factors age of the producing fly. Older flies (more than twenty-eight days old) produce weaker

pheromones while younger ones (fourteen to twenty-eight days old) produce strong pheromones (Averill and Prokopy, 1987). Physiological states of the fly such as number of mature eggs contained and time elapsed since the last oviposition influence response of the fly in a way that the more mature eggs it contains or the longer the time elapsed since the last oviposition, the more defiant the fly is to host-marking pheromones (Roitberg and Prokopy, 1983). Nature of the fly in terms of whether wild or laboratory-reared influences response of the flies to host-marking pheromones; wild strains are more responsive than laboratory strains (reared for over 200 generations) (Prokopy *et al*., 1978). The ability of fruit flies to discriminate marked hosts is also influenced by type of host (Angermann, 1986).

Although host-marking pheromones of fruit flies are generally perceived to be effective conspecifically only (Fitt, 1984; Nufio and Papaj, 2001), some studies have shown that interspecific recognition of host-marking pheromones also occurs in fruit flies. Prokopy *et al*. (1976) observed cross-recognition of host-marking pheromones among species of the genus *Rhagoletis*. Aluja and Diaz - Fleischer (2006) observed cross-recognition of host-marking pheromones among *A. ludens*, *A. obliqua* and *A. serpentina*. It has also been observed that chemical interference (host - marking) is one important mechanism by which fruit fly species displace one another from ecologies (Giga and Smith, 1985; Vet, 1996; McClure *et al*., 1998; Dicke *et al*., 2004; Aluja and Diaz-Fleischer, 2006). Further, several interspecific ecological displacements based on heterospecific chemical inteference (interspecific host-marking pheromones) have been reported worldwide (Duyck *et al*., 2004). In situations where polyphagous tephritid species have been introduced in areas already

occupied by a polyphagous tephritid, interspecific chemical interference has resulted in a decrease in number and niche shift of the pre-established species with no reciprocal invasions. For example, *C. capitata* displaced *C. catoirii* in the Reunion Island and Mauritius between 1939 and 1942. Several other interspecific and even intergeneric displacements through chemical interference involving *Ceratitis, Bactrocera* and *Anastrepha* species have been reported in various countries from 1950's to 2003 (Duyck *et al.*, 2004).

2.3 The host-marking technique of fruit fly management

2.3.1 History, efficacy and advantages of the host-marking technique

Since the discovery of fruit fly host-marking behaviour and its deterrent effect on prospective oviposition, considerable research has been done to exploit the phenomenon for the management of fruit flies by artificially synthesizing *en mass* the pheromones involved and spraying them in orchards (Sivinski and Calkins, 1986). First field experiments on the subject were conducted in Switzerland by Katsayannos and Boller (1976, 1980) who achieved over 90% control of *R. cerasi* in cherries while Aluja and Boller (1992) achieved 98% control of the same species in the same crop. Later, in the USA, Nufio and Papaj (2004) reduced *R. juglandis* infestation in walnuts to just above 10% using the technique. In Mexico, Arredondo and Diaz-Fleischer (2006) achieved 84% reduction in *C. capitata* infestation in coffee using raw pheromone extract from the flies' faecal matter while in Mexico, Aluja *et al.* (2009) achieved up to 94.1% reduction in *A. obliqua* infestation in mango

and plum orchards by spraying raw pheromone extract from faecal matter of the fruit flies and synthetic mimics of the pheromone.

The host-marking technique is good for fruit fly management because it is highly effective (as indicated in the preceding paragraph). It is also target-specic, environmentally benign since the pheromones are biodegradable and non-toxic, and can use the same equipment used for spraying conventional pesticides (Prokopy, 1980). Behaviour - based insect pest management techniques in general are also better than synthetic pesticides because the pests cannot easily develop resistance against them (Foster and Harris, 1997; Evenden and Haynes, 2001).

2.3.2 The process of developing a host-marking tool for fruit fly management

To develop a host-marking managementnt tool for a given fruit fly species, firstly, it has to be observed and confirmed that host-marking behaviour is prevalent in the species and the behaviour deters conspecific oviposition. If host-marking behaviour is prevalent in the species and the same deters conspecific oviposition, then potential host-marking pheromones of fruit fly are extracted through methanolic washing of used host fruits or faecal matter of adult females. The potential host-marking pheromone can then be isolated and identified from the extract by fractionating the extract through preparative high pressure liquid chromatography (HPLC), then subjecting the fractions to Liquid Chromatography-Mass Spectrometry (LC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy. After the potential host-marking pheromone has been identified, its standard is obtained or synthesized and tested for

behavioural activity in laboratory oviposition choice assays and field pest management trials. If the synthetic compound deters oviposition and consequently curbs infestation in the field, then it is produced commercially for use by farmers (Aluja *et al*., 2003; Aluja *et al*., 2009). However, development of a pheromone-based pest management tool requires a lot of research work and time (Kratt, 2001).

2.3.3 Breakthroughs in isolation and identification of fruit fly host-marking pheromones

To date, host-marking pheromones have been isolated and identified for some fruit flies such as *R. cerasi* and *Anastrepha* species. The taurine (N [15(β-gluco pyranosil) oxy-8-hydroxypalmitoyl] has been identified as the host-marking pheromone of *R. cerasi* (Boller and Hurter, 1985) while 2-(2 ', 14'-Dimethyl-pentadecanoylamino)-pentanedioic acid has been identified as the generic host-marking pheromone of the *Anastrepha* species (Aluja *et al*., 2003).

2.4 Mango production and the fruit fly problem in Kenya

2.4.1 Annual production, economic importance and major producing areas

Kenya produces 183,486 tons of mango annually and the fruit earns the country around US$3.6 million (Ksh 0.3 billion) per annum in foreign exchange (ICIPE, 2007). Mango farming in Kenya is mostly done by smallholder farmers who account for more than 90% of the production (ICIPE, 2007). Main provinces that produce mango in Kenya are Central, Eastern, Western, North-Eastern, Coast, Rift Valley and

Nyanza. Machakos, Meru Central, Meru South and Makueni are the districts known to export substantial quantities of mango in Kenya (Griesbach, 2003).

2.4.2 Types and varieties of mango grown in Kenya

There are two types of mangoes that are grown in Kenya and these are local and exotic or improved (Griesbach, 2003). The local mango varities include Ngowe, Dodo, Boribo and Batawi while the exotic ones include Apple, Kent, Keit, Tommy Atkins, Van Dyke, Haden, Sensation, Sabre, Sabine, Pafin, Maya, Kenston and Gesine (Griesbach, 2003). The Apple variety, which is mainly grown in Lamu, Malindi and Kilifi districts (Griesbach, 2003) is the most economically important (Kehlenbeck *et al*., 2011).

2.4.3 Major fruit fly species infesting mango in Kenya, their economic importance and identification

There are six main fruit fly species that are infesting mango in Kenya and these are: *Ceratitis capitata*, *C. fasciventris*, *C. rosa*, *C. cosyra*, *C. anonae* and *Bactrocera invadens* (Ekesi *et al*., 2009). Loss of mango yield in Kenya due to these fruit flies is estimated to be more than 50% (Griesbach, 2003). The mango fruit fly problem is therefore really serious in Kenya.

2.4.3.1 Ceratitis capitata

This is an indigenous species of Africa, but it has even spread to the Mediterranean area and parts of Central and South America (Ekesi *et al.*, 2007a). In Kenya, *C. capitata* is found at the coastal region and in central and western highlands

(Copeland *et. al.*, 2002). Other countries in Africa where *C. capitata* is found include Algeria, Angola, Benin, Burkina Faso, Burundi, Cameroon, Cape Verde Islands, Congo, Egypt, Ethiopia, Gabon, Ghana, Guinea, Liberia, Libya, Madagascar, Malawi, Mali, Mauritius, Morocco, Mozambique, Niger, Nigeria, Réunion, Senegal, Seychelles, South Africa, St Helena, Sudan, Tanzania, Togo, Tunisia, Uganda, Zaire and Zimbabwe (Ole-Moi Yoi and Lux, 2002; Ekesi *et al*, 2006; Ekesi *et al.*, 2007b; Ekesi *et al.*, 2009). *Ceratitis capitata* is a highly polyphagous species and apart from mango, its other host fruits include apples, avocados, citrus, figs and pears (White and Elson-Harris, 1992; Ole-Moi Yoi and Lux, 2002; Copeland *et al.*, 2006). *Ceratitis capitata* has also been recorded from wild hosts belonging to a large number of families (Ekesi *et al*., 2009).

In general, *Ceratitis* fruit flies differ from fruit flies of other genera by having banded wings, a swollen scutellum and a pattern of grey flecks in basal wing cells (Copeland *et al*., 2002). However, the species c*apitata* can be identified by the following features: the body is yellow; the eyes are reddish purple, the scutellum is entirely black in apical half, with a sinuate yellow line across it sub-basally; the costal band starts beyond the end of vein R1 and is separated from discal cross-band by a hyaline area at the end of R1 while the wing is 4 - 6 mm long (De Meyer and Freidberg, 2006). Males and females of *C. capitata* differ in a way that males have small black diamond-shaped nodules at the apex of their orbital setae, which are not found in females, while females have a characteristic yellow marking on their wings, and the mean apical half of their scutellum is completely black (De Meyer and Freidburg, 2006). Some taxonomic features of *C. capitata* are shown in Plate 2.2.

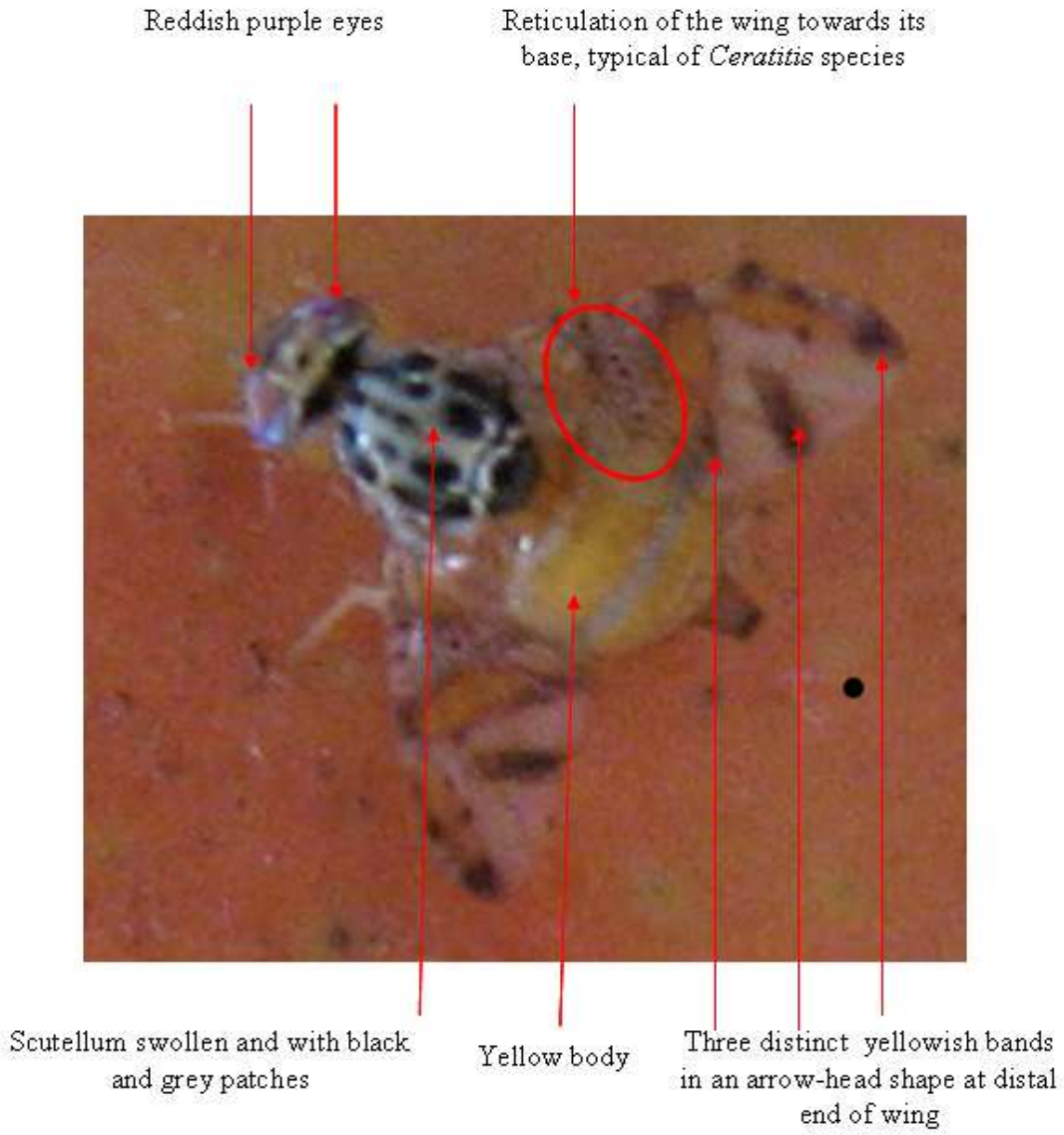

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Plate 2.2: Some taxonomic features in *C. capitata*

2.4.3.2 Ceratitis fasciventris

Ceratitis fasciventris is also an indigenous species of Africa. In Kenya, it is mostly found in the western region (Lux *et al.,* 2006). Other countries where it is found in Africa include Angola, Benin, Congo-Kinshasa, Congo-DRC, Cote d' Ivoire, Ethiopia, Ghana, Guinea, Mali, Namibia, Nigeria, Sao Tome, Seirra Leone, Uganda and Tanzania (White and Elson-Harris, 1992; Ekesi *et al*., 2009).

According to De Meyer and Freidberg (2006), an adult *C. fasciventris* can be distinguished by the following characteristics: the body is 3.95 - 5.15 mm long, the wing is 4.45 - 5.75 mm long while the antennae are yellowish orange. The first flagellomere is 2 - 3 times longer than the pedicel. The arista has short to moderately long rays; ventral rays being shorter and sparser than the dorsal rays, especially basally. The frons is yellow; with short scattered setulae distinctly darker than the frons. Frontal setae are well developed. The face is yellowish white. Genal seta and setulae are dark and well developed. The postpronotal lobe is yellowish white, with no spot. The mesonotum is dark gray, sometimes with an orange tinge; with streaks and darker markings but without distinct spots, except white and separate prescutellar markings, usually with paler gray area in between, occasionally merged. Scapular setae are dark. The scutellum is yellowish white while the anepisternum on ventral half is yellowish brown and setulae pale. Legs are yellow except where otherwise noted. The foreleg is slightly yellow and its femur is without bushy feathering posteriorly, only a row of dispersed, long and usually black setulae. The midleg has its femur with dispersed pale setulae at the base. The femur has long setulae. Wing bands are brown or yellowish brown. Interruption between marginal

and discal bands near vein R1 is clear and complete. The cubital band is free, the medial band is absent; crossvein R-M is opposite the middle of the discal cell, sometimes just proximal to the middle. In female *C. fasciventris* however, the anepisternum on the ventral half is brown or yellowish brown, the crossvein DM-Cu is variable, the legs are not feathery and the oviscape is shorter than the preabdomen. Some taxonomic features of *C. fasciventris* are shown in Plate 2.3.

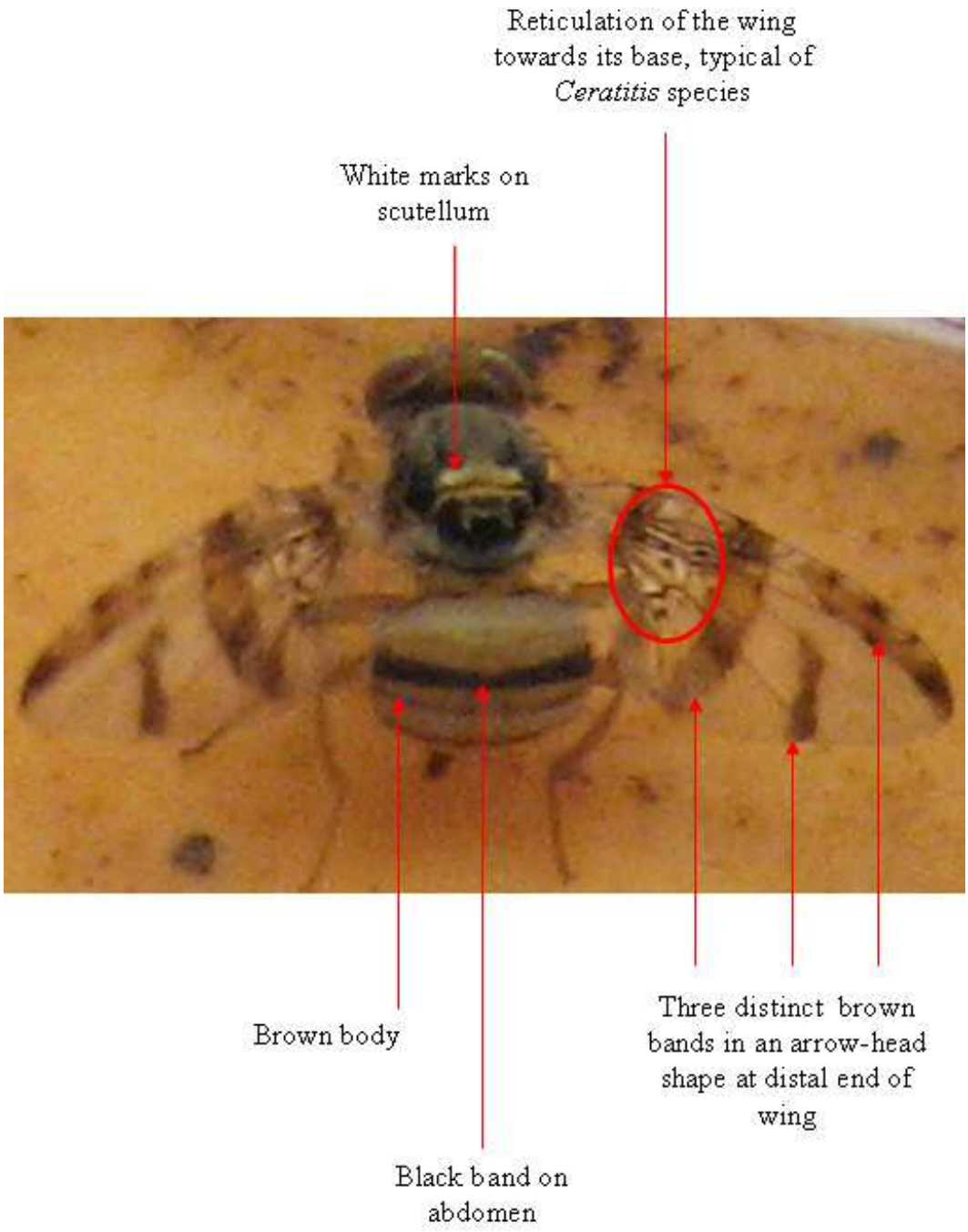

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Plate 2.3: Some taxonomic features in *C. fasciventris*

2.4.3.3 Ceratitis rosa

Ceratitis rosa is also an indigenous species of Africa and in Kenya, it is mostly found at the coastal region (Lux *et al.,* 2006). Other countries in Africa where *C. rosa* has been reported are Angola, Uganda, Ethiopia, Tanzania, Malawi, Mozambique, Nigeria, Réunion, Rwanda, Zambia South Africa, Swaziland, Zaire, Mali, Mauritius and Zimbabwe. *Ceratitis rosa* is a polyphagous species and apart from mango, it also attacks apples, apricots, avocados, citrus, guavas, figs, pawpaws, peaches, pears, plums, quinces, tomatoes and grapes (White and Elson-Harris, 1992; Ekesi *et al*., 2009).

Ceratitis rosa can be distinguished by the following characteristics: wing bands and general body are brown; the wing is 4 - 6 mm long, the body is 4 - 5 mm long, the costal band starts beyond the end of vein R1, and it is separated from discal crossband by a hyaline area at the end of R1. The scutellum is marked black and yellow, with yellow lines or areas meeting the margin, such that each apical scutellar seta is based in or adjacent to a yellow stripe. In males, the mid-tibia has rows of stout setae along the distal half of both the anterior and posterior edges such that it looks feathery (Plant Health Australia, 2011). Some taxonomic features of *C. rosa* are shown in Plate 2.4.

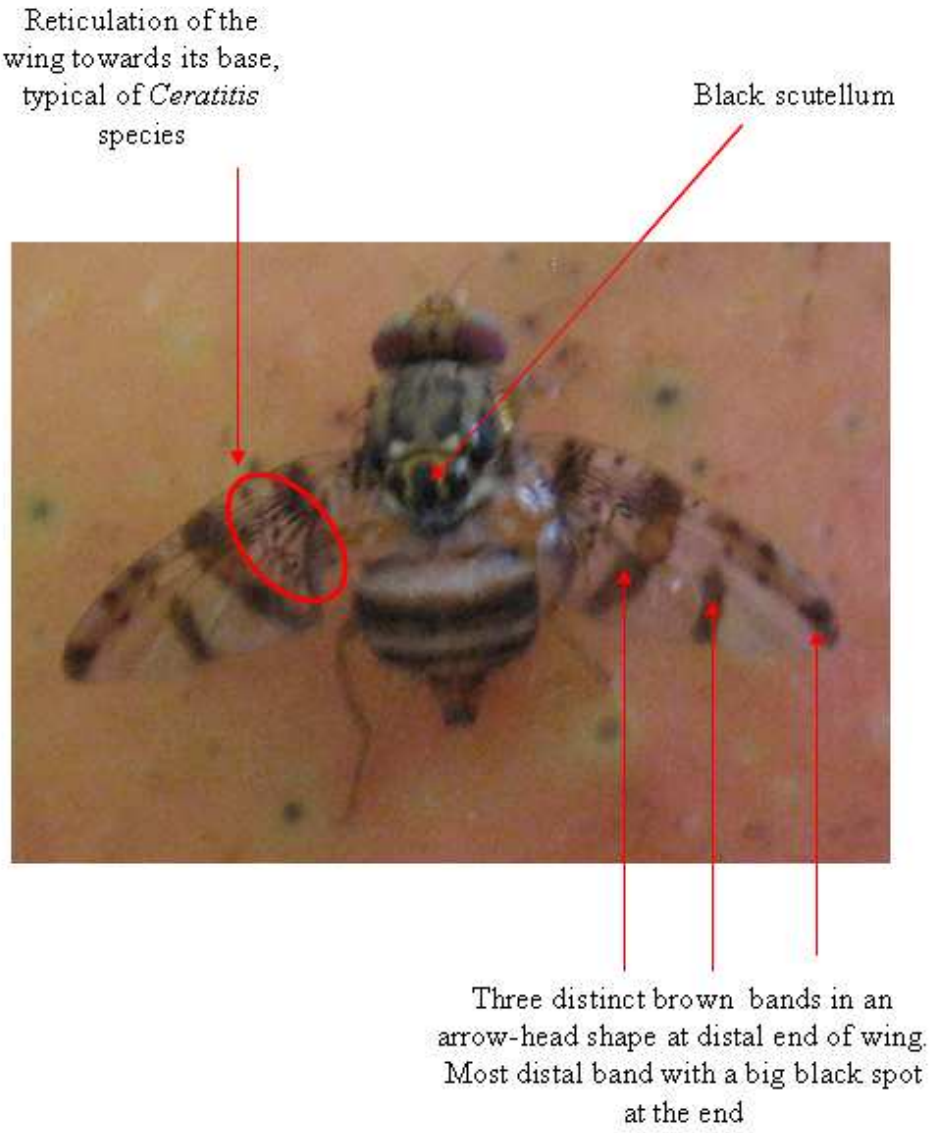

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Plate 2.4: Some taxonomic features in *C. rosa*

2.4.3.4 Ceratitis cosyra

Ceratitis cosyra is another indigenous fruit fly spescies of Africa, found in Kenya, Malawi, Mozambique, South Africa, Sudan, Tanzania, Zaire, Zambia and Zimbabwe (Elson-Harris, 1992; Copeland *et al*., 2006). *Ceratitis cosyra* has a very narrow host range. Apart from mango and marula, which are its main hosts, the species attacks a few other fruits such as avocado, citrus and peach (Ole-Moi Yoi and Lux, 2002). *Ceratitis cosyra* can be identified by the following characteristics: its wing bands and the general body are yellow; the scutum is predominantly yellow or pale-brown, with a pattern of brown to black spots; the scutellum is black and yellow, with yellow lines or areas meeting the margin, such that each apical scutellar seta is based in or adjacent to a yellow stripe and the fore-femur is yellow on both sides (De Meyer and Freidberg, 2006). Some taxonomic features of *C. cosyra* are shown in Plate 2.5.

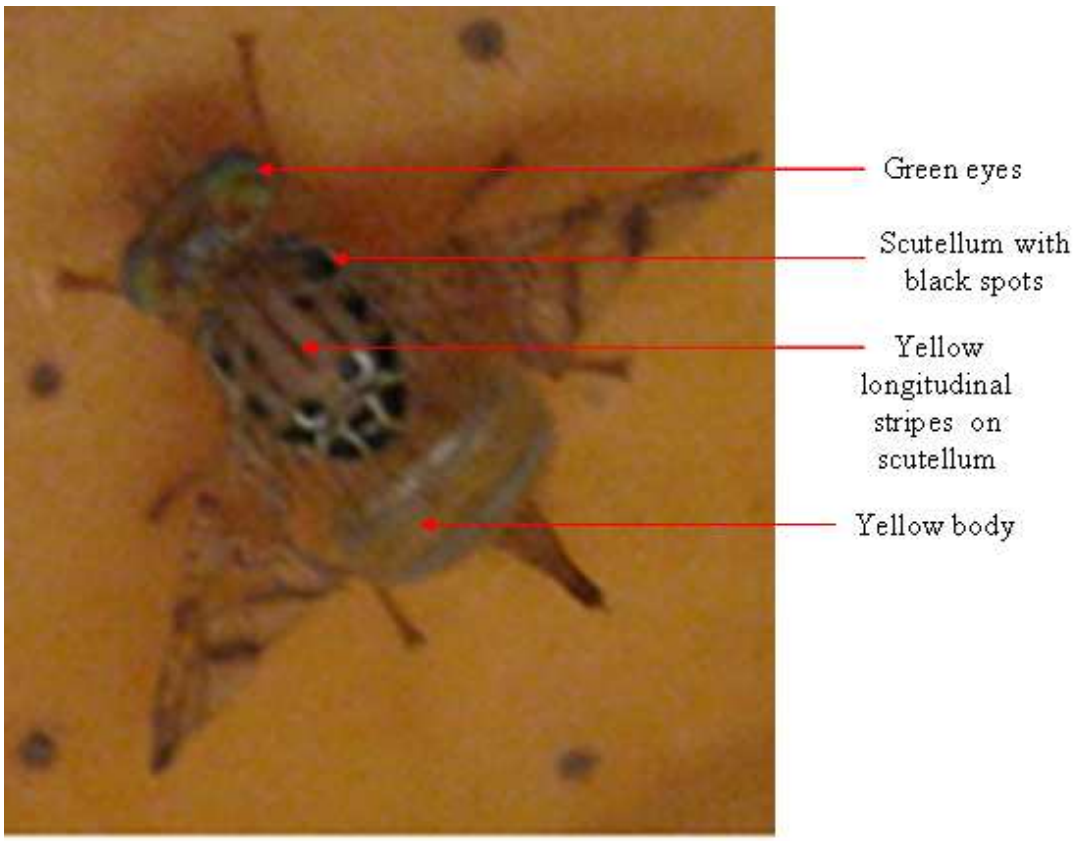

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Plate 2.5: Some taxonomic features in *C. cosyra*

2.4.3.5 Ceratitis anonae

Ceratitis anonae is also indigenous of Africa. In Kenya, it is mostly found in the western part (Lux *et al.,* 2006). Other countries where it is found in Africa include Benin, Cameroon, Central African Republic, Congo-Kinshasa, Congo-DRC, Cote d' Ivoire, Equatorial Guinea, Gabon, Ghana, Guinea, Mali, Sao Tome, Senegal, Tanzania, Togo and Uganda (Elson-Harris, 1992; Copeland *et al*., 2006). Apart from mango, *C. anonae* also attacks robusta coffee, tropical almond, common guava and strawberry guava (Copeland *et al*., 2006; Ekesi *et al*., 2009).

An adult *C. anonae* has the following descriptive morphological features (De Meyer and Freidberg, 2006: Body length is 4.35 - 5.90 mm, wing length is 4.45 - 5.75 mm, antennae are yellow and the first flagellomere is three times as long as the pedicel. The arista has short to moderately long rays, the ventral rays being shorter and sparser than dorsal rays, especially basally. The frons is pale, sometimes completely yellow. The frontal setae are well developed, the face is white, sometimes yellowish white while the genal seta and setulae are dark and well developed. The postpronotal lobe is white, sometimes yellowish white and has no spot. The scutal pattern is that the ground color is ash-gray; with streaks and darker markings but without distinct spots or clearly defined stripes except prescutellar white markings. The scapular setae are dark, the scutellum is ash gray, sometimes yellowish white, the legs are yellow except where otherwise noted. Wing markings are yellowish brown, interruption between marginal and discal bands near vein R1 is clear and complete; the discal band is often partly or completely interrupted in the discal cell. The medial band is absent; crossvein R-M is opposite the middle of the discal cell, the

apex of vein R1 is distal to level of crossvein R–M while crossvein DM-Cu is oblique anterobasally. The abdomen is mostly yellow, with the border between tergites 1 and 2 being narrowly black; tergites 2 and 4 have pale gray bands occupying almost entire tergite while tergite 3 has a distinct brownish black band along the posterior half. A female of *C. anonae* is like the male except for the following characteristics: the legs are without feathering, the wing has a complete discal band and the oviscape is shorter than the preabdomen. Some taxonomic features of *C. anonae* are shown in Plate 2.6.

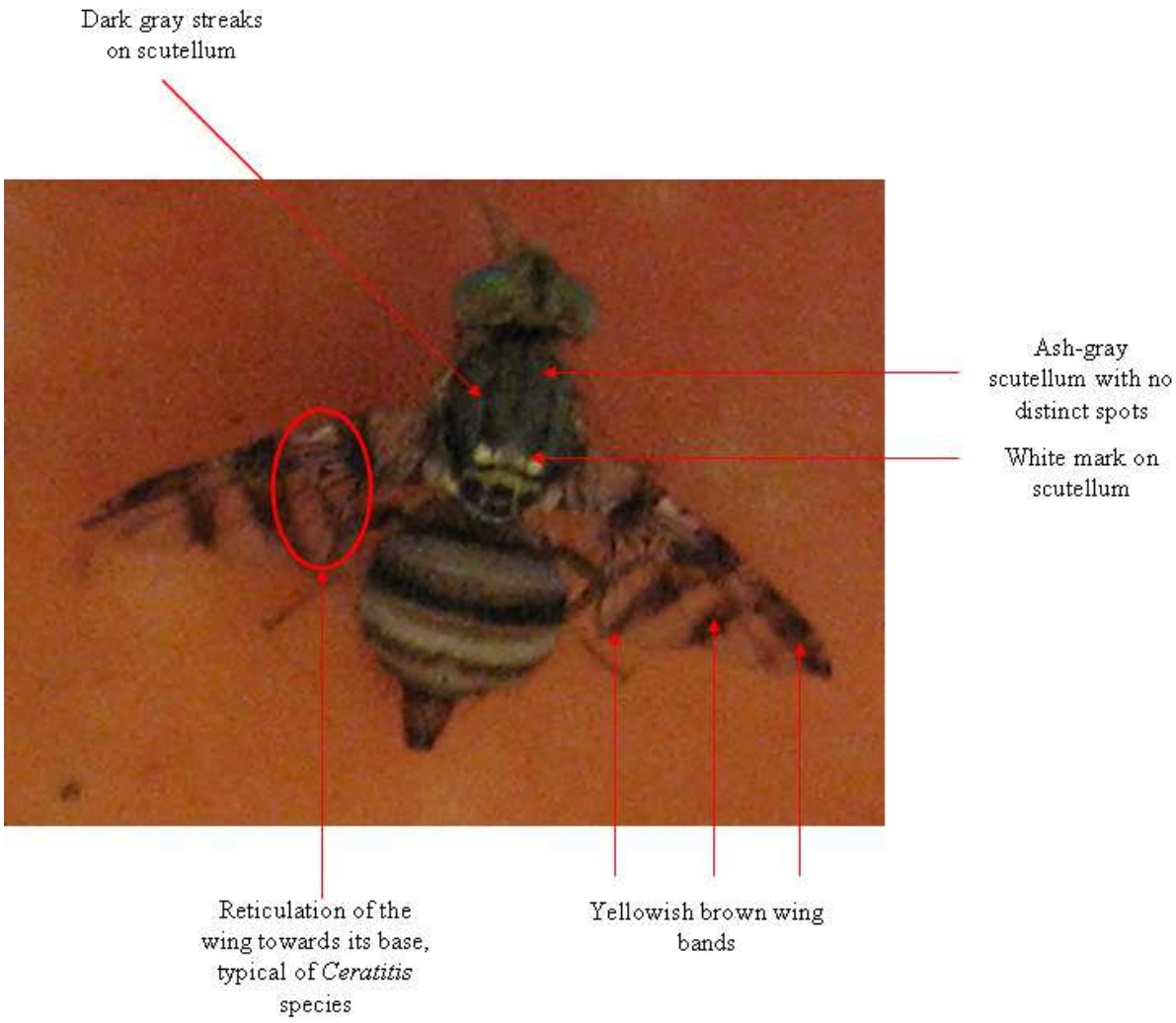

Plate 2.6: Some taxonomic features in *C. anonae*

2.4.3.6 Bactrocera invadens

Bactrocera invadens is an invasive species of Asian origin, first detected in Kenya in 2003, but is now reported in many African countries such as Benin, Cameroon, Comoros, Democratic Republic of Congo, Cote d'Ivoire, Ethiopia, Ghana, Liberia, Mayotte, Mozambique, Nigeria, Senegal, Sudan, Tanzania, Togo, Uganda and Zambia (Rwomushana *et al*., 2008; Ekesi *et al*., 2009). Apart from mango, *B. invadens* attacks banana, guava, pepper and citrus (Rwomushana *et al*., 2008; Ekesi *et al*., 2009).

Bactrocera invadens can be identified by the following features (Drew and Romig, 2007): It is medium in size, the face is fulvous with a pair of medium to large oval black spots while the scutum is red-brown with variable dark fuscous to black patterns (in occasional specimens the scutum base colour is black). The post pronotal lobes and notopleura are yellow, the scutellum is yellow except for a narrow dark basal band and the femora of the legs are entirely fulvous. Its wings have cells bc and c colourless and microtrichia are in the outer corner of cell c only. There is a narrow fuscous costal band confluent with R_{2+3}, remaining narrow around costal margin to end just beyond extremity of R_{4+5}. The abdominal terga III - V are orange brown with a 'T' pattern consisting of a narrow transverse black band across the anterior margin of tergum III. The fly has dark orange-brown shining spots on tergum V. Some taxonomic features of *B. invadens* are shown in Plate 2.7.

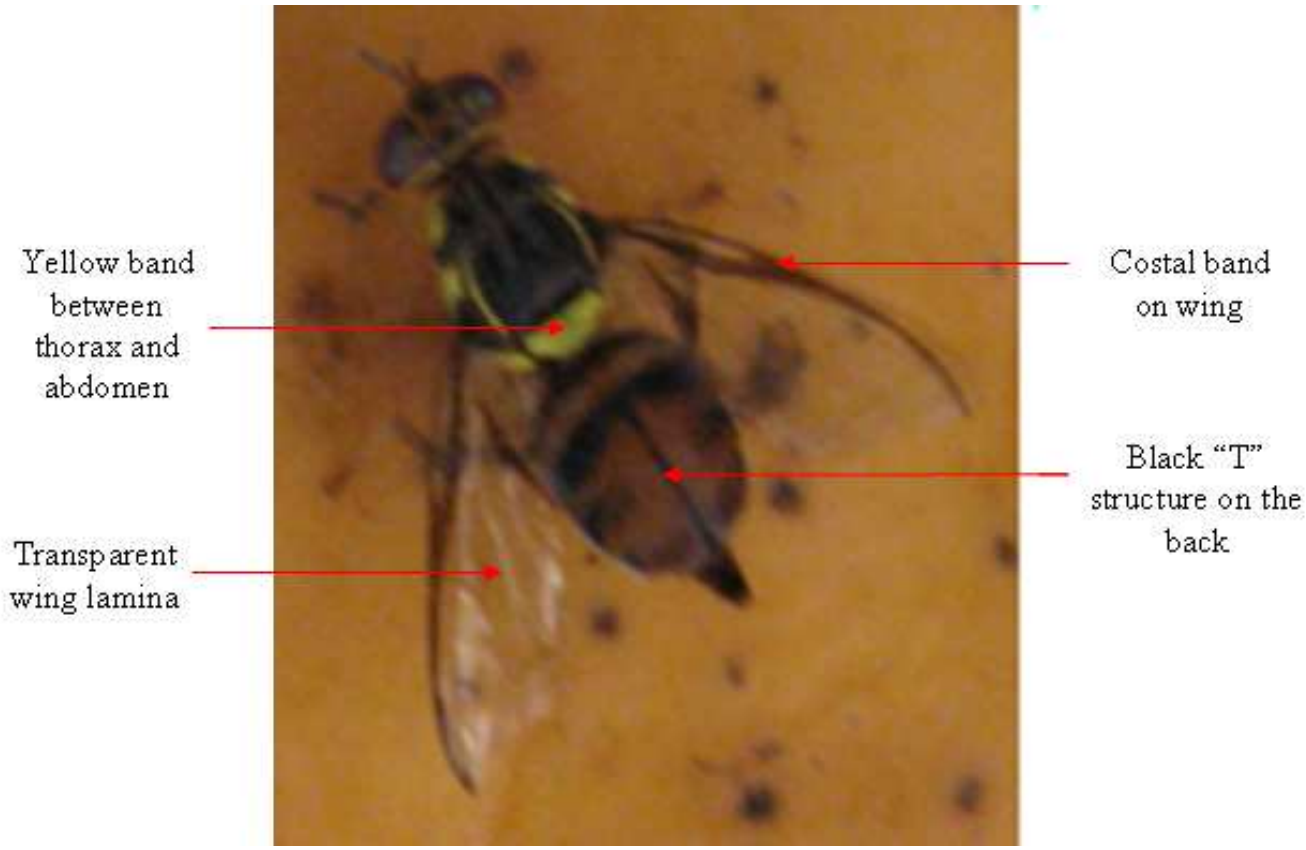

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Plate 2.7: Some taxonomic features in *B. invadens*

2.4.4 Current techniques for managing mango fruit flies in Kenya

Currently, mango farmers in Kenya use a variety of techniques to mitigate the mango fruit fly problem and these include; traps, fruit bagging, early harvesting, orchard sanitation (collection and destruction of infested fruits), use of biological agents (parasitoids, predators and pathogens) and chemical sprays (Billah *et al*., 2007). However, more management techniques need to be developed to enhance management of these pests (Allwood and Drew, 1996; Vargas *et al*., 2001; Billah *et al*., 2007).

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS

3.1 Study site and conditions

All experiments were laboratory-based and carried out at the Duduville Campus of the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. The experiments were run during day, at peak of fruit fly oviposition activity, between 9:00 a.m. and 4:00 p.m. (Smith, 1989) at 23 - 25^{o}C and 40 - 60% relative humidity with LD 12:12 hr cycle.

3.2 Study fruit flies

The fruit flies used in this study came from a laboratory culture that has been maintained for about 100 generations at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya, using the methodology described by Ekesi *et al*. (2003). For experiments, adult flies obtained from the stock culture were transferred into 30 x 30 x 30 cm clear Perspex cages and fed on enzymatic yeast hydrolysate (USB Corporation, Cleveland, OH USA) mixed with sucrose in a ratio of 1:3. Water was provided on pumice granules. The adult fruit flies were then provided with 3 - 5 spiked (1 mm holes) ripe mangoes of Apple variety for 2 days to allow for egg laying. Thereafter, the egg-infested mangoes were removed and kept in incubation cages. The mangoes were held in 1.5 litre rectangular transparent plastic containers (20 x 12.5 x 8 cm) (Kenpoly®, Kenya). The containers were covered with a fine netting material held in place by the perforated covers of the containers. The mangoes were placed on 40 - 60 mm (depth) of moistened sterilized sand at the bottom of the rearing containers. The sand served as pupation medium for the larvae

that exited the fruits in addition to soaking up sap that oozed from the rotting fruits. The rearing cages were checked daily and puparia were picked from the sand with a pair of soft forceps, counted and placed in petri dishes with moistened filter paper. When pupation occurred inside the fruit, the rotting fruits were dissected to completely recover all remaining puparia. The petri dishes with puparia were then held in small-ventilated transparent cylindrical plastic cages (5.5 x 12.5 cm) (No. J-12, GP plastics, Kenya) until eclosion. The emerging fruit flies were provided with an artificial diet that consisted of a volumetric mixture of 1:3 enzymatic yeast hydrolysate and sugar, and water was provided in pumice granules. The colonies were maintained at 23-25^{o}C and 40 - 60% relative humidity with LD 12:12 hr cycle. The flies were reared on mango for at least 3 generation before commencement of experiments. All fruit fly species were used at 15-21 days old, when female fruit are usually at their peak of behavioural and biological activity (Averill and Prokopy, 1989; Nemeye, 2003). All the experiments were conducted between 9:00 a.m. and 4:00 p.m. since fruit flies are diurnal insects (Averill and Prokopy, 1989; Nemeye, 2003). The Apple variety of mango was preferred for rearing the fruit flies and even in all the experiments because of its economic importance to Kenya (Kehlenbeck *et al*., 2011) and susceptibility and appeal to the fruit flies under study (Griesbach, 2003). Appeal of the Apple variety to the fruit flies was of particular importance in this study because is in behavioural studies, it is always important to ensure that the organism being studied is provided with a conducive environment so that it expresses its behaviour fully (Wyatt, 1997).

Since some information was already available, classifying *C. capitata* as a host-marking species (Prokopy *et al*., 1978; Duyck *et al*. 2006) and *B. invadens* as a non host-marking species (Fletcher and Prokopy, 1991), these two species were used as checks in some experiments.

3.3 Oviposition substrates

Slices of ripe mangoes fitted in covers of 50 mm - diameter petri dishes with their peels intact and on the top surface were improvised and used as oviposition substrates for the fruit flies. The mango slices were preferred, unlike the conventional oviposition substrate of whole mangoes, firstly because being horizontal planes, they could restrict the flies in continuous focus of a top-view video-tracking camera, a situation which proved difficult with whole mangoes since the oval or round shape of the fruits allowed the insects to move indiscriminately to their different facets where they could not be effectively observed or video-tracked. Secondly, the mango slices were advantageous over whole mangoes in experiments to determine efficacy of host-marking behaviour of the fruit flies in deterring conspecific and heterospecific oviposition. Since fruit flies are known to deposit their host-marking pheromones in small amounts (Averill and Prokopy, 1989), the smaller surface area of the mango slice, as opposed to that of a whole mango, ensured use of a smaller number of fruit flies for host-marking.

3.4 Data collection

The data collection process involved behavioural observations, dual-choice oviposition assays and high pressure liquid chromatographic (HPLC) shooting of methanolic extracts from faecal matter of the fruit flies. For behavioural observations and dual-choice oviposition assays, sample sizes of the fruit flies to be used were determined through power analysis (sample size calculation) in Statistica 6.0 (Statsoft Inc., 2003) at a power goal of 0.95 and an alpha level of 0.05, with regard to type of experiment i.e. type of statistical test to be used.

Fruit flies to be used in all the investigations were picked randomly. The randomozation was achieved by assigning the six sides of the cage bearing a given fruit fly species with numbers 1 to 6 respectively, then generating a set of random numbers (from 1 to 6) in a computer (Microsoft Excel 2007) to the amount of fruit flies required in a replicate. The order of this set of random numbers determined the order in which the fruit flies were to be collected from the six sides of the cage. In behavioural and oviposition choice assays, where one replicate of each species was to be observed per day, the order in which the species were to be observed was also determined similarly using computer-generated random numbers.

The types of data collected in behavioural observation and dual-choice oviposition experiments included percentages of fruit flies, counts and duration (time). For behavioural observation assays, a video clip was also recorded for each fruit fly (using a Canon Powershot A530 camera) in order to enhance effectiveness in

observation and data collection through playing and replaying the video clips. HPLC data were collected as chemical component profiles.

For experiments involving oviposition, each fruit fly was observed for a maximum of 30 minutes to oviposit on a mango slice. The duration of 30 minutes for observation was chosen just to ensure that all the fruit flies were accommodated because in pilot experiments, one fruit fly took up to 30 minutes to oviposit. However, most of the fruit flies in both pilot and real experiments oviposited in less than 10 minutes.

3.5 Data analysis

Analysis of the data collected in behavioural observation and dual-choice oviposition assays involved analysis of variance (ANOVA) with SNK mean separation, Chi-square test or regression analysis in R 2.8.1 software, with due transformation of the data where necessary. Analysis of HPLC profiles involved comparison of retention times of peaks of chemical components.

CHAPTER FOUR

4.0 INCIDENCE OF HOST-MARKING BEHAVIOUR IN *CERATITIS CAPITATA, CERATITIS FASCIVENTRIS, CERATITIS ROSA, CERATITIS COSYRA, CERATITIS ANONAE* AND *BACTROCERA INVADENS*

4.1 Introduction

Host-marking behaviour is not a universal phenomenon in fruit flies, it is prevalent in some species (Arredondo and Diaz-Fleischer, 2006), occasional in some (Nufio and Papaj, 2004; Duyck *et al.*, 2006) and absent in others (Fitt, 1984; Fletcher and Prokopy, 1991). It therefore follows that the primary condition for applicability of the host-marking technique in the management of a given fruit fly species is that the species must have the host-marking behaviour because if it does not, it may mean that it does not produce a host-marking pheromones which can be exploited for its management.

Although the host-marking technique was thought to be one of the approaches that could help to mitigate the mango fruit fly problem in Kenya, nothing was known yet regarding incidence of host-marking behaviour in most of the major fruit fly species infesting mango in the country since studies had never been conducted to that effect. It was therefore considered necessary to establish incidence of host-marking behaviour in these fruit fly species as the first step of getting insight into the potential of the host-marking technique in the management of the fruit flies. In addition to establishing incidence of host-marking behavioiur in the fruit fly species, it was deemed important to determine their other behavioural traits closely associated with

host-marking namely: duration of host-marking bout, ovipuncture clutch size (number of eggs by a fly per ovipuncture) and duration of ovipuncture oviposition, since these behavioural traits had also not been determined before. Further, in the course of rearing the fruit flies, it had been inadvertently observed that unlike all the other fruit fly species under study, *C. cosyra* apparently had a unique behavioural trait of pausing for some time after oviposition before engaging in host-marking. It was, therefore, also considered important to determine incidence of this pre-mark pausing behaviour among the fruit fly species and the duration of pause. Knowledge of various behavioural traits of insect pests can help in their identification for effective management. For example, behavioural traits have been used to identify different species of stored grain borers and weevils in Canada (Canadian Grain Commission, 2006).

4.2 Materials and methods

4.2.1 Incidence of host-marking behaviour in *C. capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens*

4.2.1.1 Bioassay

Thirty (30) female fruit flies of each species under study (*C. capitata*, *C. fasciventris*, *C. rosa, C. cosyra, C. anonae* and *B. invadens*) were obtained and grouped into three batches of ten individuals each. The batches were treated as replicates. Randomly and one by one, the flies were introduced into a 30 x 30 x 30cm clear Perspex cage containing fruit fly diet, water and a slice of ripe mango fitted in a 50mm petri cover

as an oviposition substrate (Plate 4.1). Each fly was observed for a maximum of 30 minutes to oviposit on the mango slice. Each fruit fly used its own mango slice and if a fruit fly did not oviposit within the designated time (rare cases), it was replaced. The experiment was repeated on ripe avocado pear substrates (Fuerte variety) as another host common to the fruit flies (Copeland *et al.*, 2006) in order to compare the behaviour of the fruit flies on two different hosts (Plate 4.1). The fruit flies to be used in experiments were collected at random from the cages and one replicate of each species was done per day with the replicates observed in a random manner per given day.

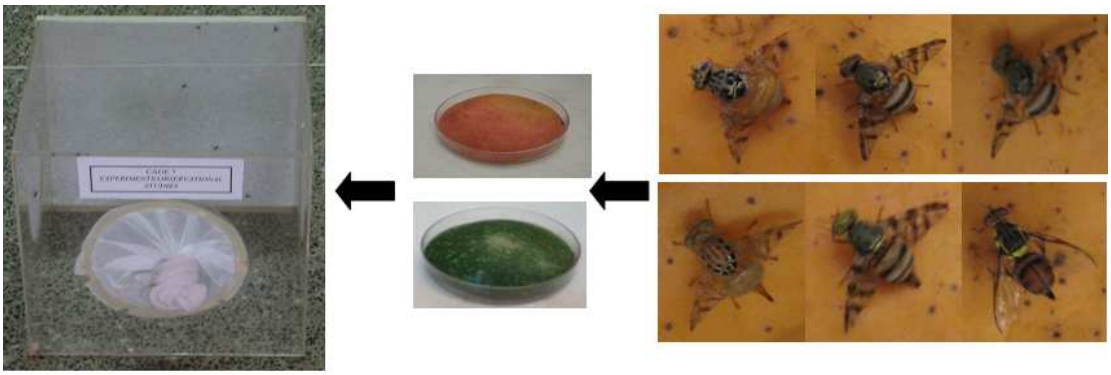

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Plate 4.1: Illustration of the bioassay used to investigate incidence of host-marking behaviour in *C. capitata*, *C. fasciventris*, *C. rosa*, *C. cosyra*, *C. anonae* and *B. invadens*

4.2.1.2 Data collection

Data collected for each replicate were percentage of host-marking (ovipositor dragging) fruit flies, mean duration of host-marking bout, percentage of pre-mark pausing fruit flies and mean duration of pre-mark pause.

4.2.1.3 Data analysis

Data on percentage of host-marking and pre-mark pausing fruit flies were arcsine-transformed and then subjected to two-way ANOVA in order to compare incidence of the behaviour among the species. Data on duration of host-marking bout and pre-mark pause were log (x +1) transformed and then subjected to two-way ANOVA.

4.2.2 Oviposition behaviour in *C. capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens*

4.2.2.1 Bioassay

Forty (40) female fruit flies of each species under study (*C. capitata*, *C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens*) were obtained and grouped into four batches of ten flies each, the batches being treated as replicates. The fruit flies were introduced one by one into a 30 x 30 x 30 cm clear Perspex observation cage containing a mango slice fitted in a 50 mm diameter-Petri dish as an oviposition substrate. Like in the previous experiment, each fruit fly was observed for a

maximum of 30 minutes to oviposit on the mango slice. An oviposition bout was defined, structured and characterized according to Aluja (1994) as having the following four phases of fruit fly action: (i) arrival on the oviposition substrate, (ii) testing the site – characterized by the fruit fly walking on the oviposition substrate and "butting" its head against the same; (iii) puncturing the oviposition substrate – characterized by the fruit fly vertically poking the oviposition substrate with its ovipositor; and (iv) oviposition proper – a still period between the last puncturing stroke and withdrawal of the ovipositor from the oviposition substrate.

4.2.2.2 Data collection

Data were collected in terms of ovipuncture clutch size (number of eggs oviposited per ovipuncture) and oviposition duration (time between last puncturing stroke and withdrawal of the ovipositor from the mango slice). Ovipuncture clutch sizes of the flies were determined by marking the ovipuncture area with a pen immediately the fly withdrew its ovipositor, cutting the oviposition substrates on one side with a scapel and then tearing it with hands continuously to the marked spot. The exposed egg clutch was then picked using an insect pin and spread in a small drop of water on a black plastic sheet and the individual eggs were counted. Oviposition duration data were obtained from recorded video clips. The materials used in this investigation are shown in plate 4.2.

4.2.2.3 Data analysis

Ovipuncture clutch size and oviposition duration data were square root-transformed and then subjected to one-way ANOVA in order to determine if the fruit fly species differed in those behavioural traits.

30 x 30 x 30cm clear Perspex observation cage

Mango slice oviposition substrate

Gravid fruit flies

Pin for picking egg clutches

Scapel for excising oviposition substrates

Water for spreading eggs during counting

Egg counting pad

Plate 4.2: Materials used to investigate oviposition behaviour of *C. capitata*, *C. fasciventris*, *C. rosa*, *C. cosyra*, *C. anonae* and *B. invadens*

4.3 Results

4.3.1 Incidence of host-marking behaviour in *C. capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens*

Host-marking behaviour was significantly prevalent in *C. capitata, C. fasciventris, C. rosa* and *C. cosyra*, (96.7 ± 2.1 %) but not in *C. anonae* (15.0 ± 2.2 %) and *B. invadens* (1.6 ± 1.7 %) ($F_{1,5}$ = 113.551, P < 0.001) (Figure 4.1). Fruit fly host-marking behaviour is illustrated in Plate 4.3. Similarly, duration of host-marking bout was significantly longer in *C. capitata* (62.5 ± 7.6 s), *C. fasciventris* (65.7 ± 8.1 s), *C. rosa* (42.6 ± 2.1 s) and *C. cosyra* (62.8 ± 8.1 s) than in *C. anonae* (4.7 ± 1.1 s) and *B. invadens* (0.4 ± 0.1 s) ($F_{1,5}$ = 137.745, P < 0.001) (Figure 4.2). Interaction between host fruit and fruit fly species had no effect on both incidence of the behaviour and duration of host-marking bout.

Pre-mark pausing behaviour was significantly prevalent in *C. cosyra* only (96.7 ± 2.1 %, ($F_{1,3}$ = 132.578, P < 0.001) (Figure 4.3) and the duration of pause was also significantly longer (26.3 ± 0.1 s) in *C. cosyra* only ($F_{1,3}$ = 69.929, P < 0.001) (Figure 4.4). Interaction between host fruit and fruit fly species had no effect on both incidence of the behaviour and duration of pre-mark pause.

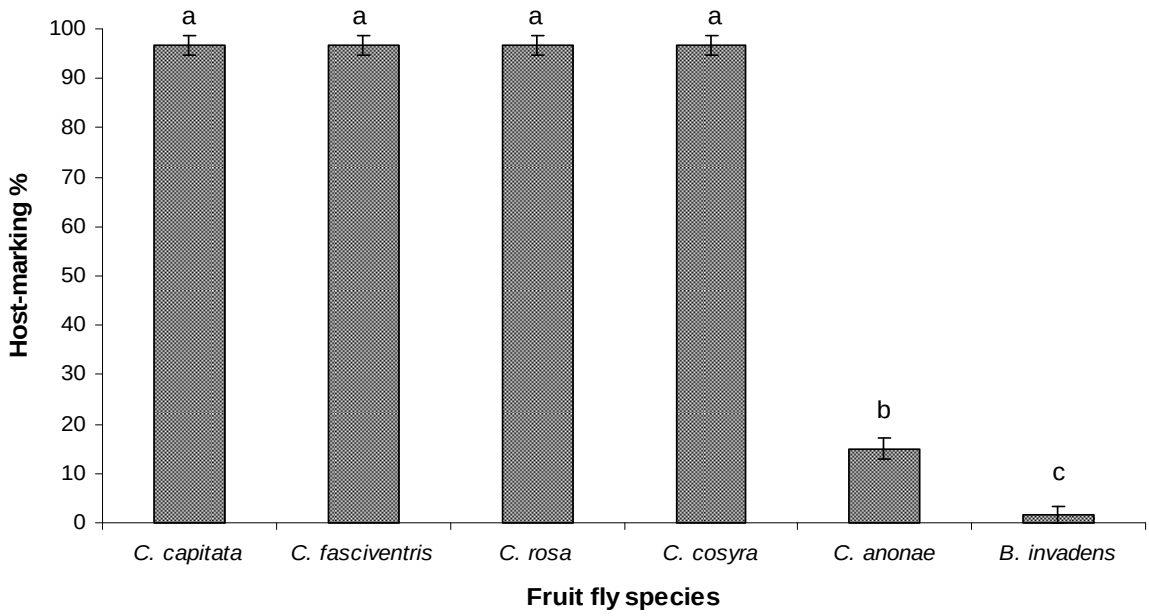


Figure 4.1: Incidence of host-marking behaviour in *C. capitata*, *C. fasciventris*, *C. rosa*, *C. cosyra*, *C. anonae* and *B. invadens*. Means accompanied by similar letters are not significantly different ($F_{1,5} = 113.551$, $P < 0.001$, $n = 60$ for each species).

Plate 4.3: Illustration of fruit fly host-marking behaviour - *C. fasciventris* dragging a protracted ovipositor on the surface of a mango oviposition substrate immediately after oviposition.

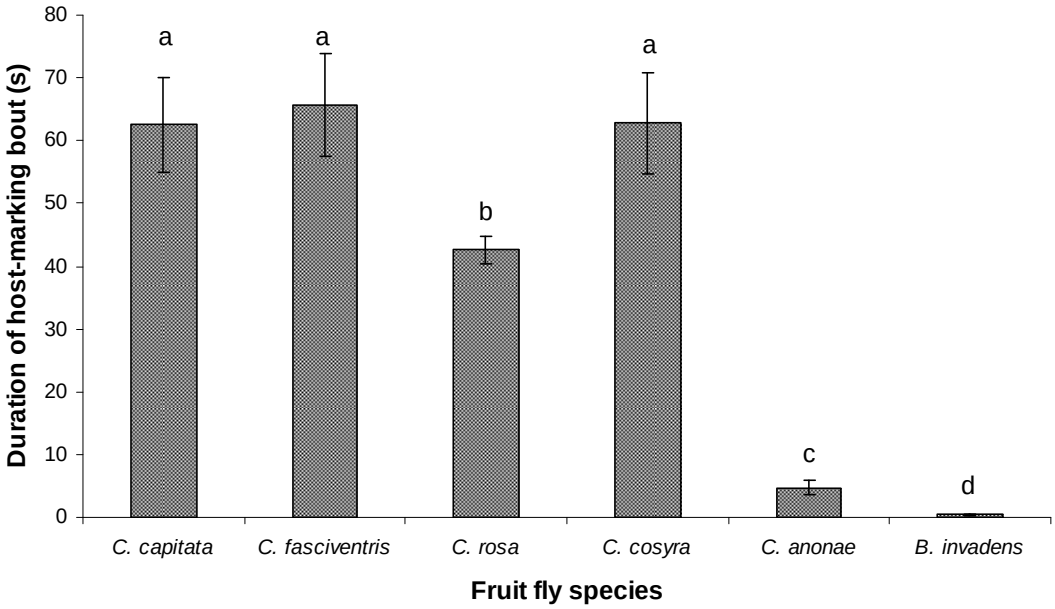


Figure 4.2: Duration of host-marking bout in *C. capitata*, *C. fasciventris*, *C. rosa*, *C. cosyra*, *C. anonae* and *B. invadens*. Means accompanied by similar letters are not significantly different ($F_{1,5}$ = 137.745, P < 0.001, n = 60 for each fruit fly species).

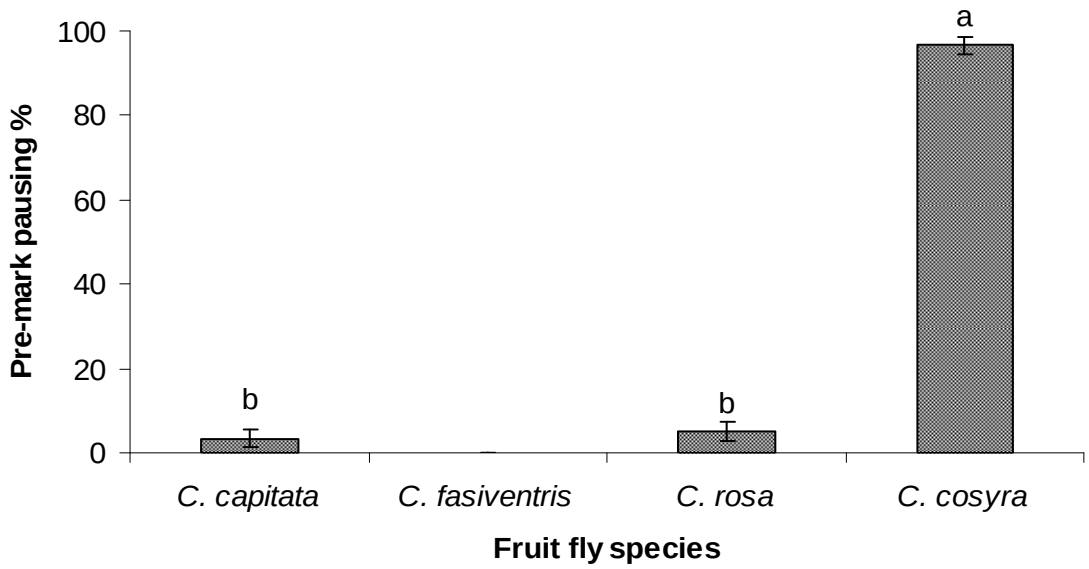


Figure 4.3: Incidence of pre-mark pausing behaviour in *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra*. Means accompanied by similar letters are not significantly different ($F_{1,3}$ = 132.578, P < 0.001, n = 60 for each species). *Ceratitis anonae* and *Bactrocera invadens* were not included because host-marking behaviour had been found to be insignificant in the two species.

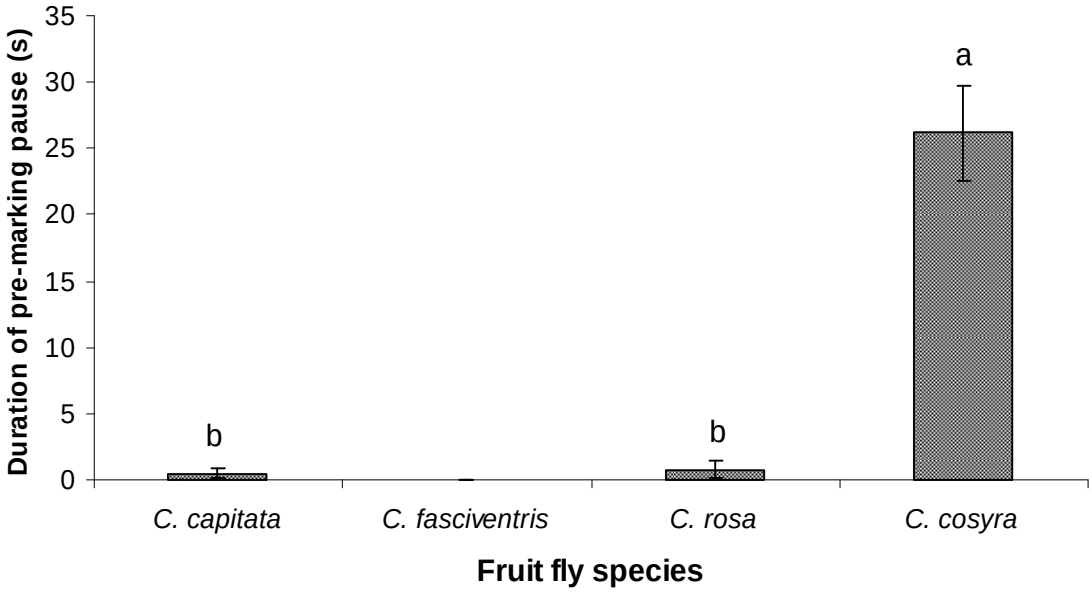


Figure 4.4: Duration of pre-marking pause in *C. capitata*, *C. fasciventris* and *C. rosa* and *C. cosyra*. Means accompanied by similar letters are not significantly different ($F_{1,3}$ = 69.9295, P < 0.001, n = 60 for each species). *Ceratitis anonae* and *Bactrocera invadens* were not included because host-marking behaviour had been found to be insignificant in the two species.

4.3.2 Oviposition behaviour in *C. capitata, C. fasciventris, C. rosa, C. cosyra, C. anonae* and *B. invadens*

Ovipuncture clutches were significantly larger in *C. cosyra* (19.2 ± 0.9 eggs) and *B. invadens* (13.8 ± 1.5 eggs) than in *C. anonae* (9.8 ± 0.7 eggs), *C. capitata* (6.3 ± 0.3 eggs), *C. rosa* (3.8 ± 0.2 eggs) and *C. fasciventris* (3.6 ± 0.9 eggs) ($F_5 = 45.358$, $P < 0.001$) (Figure 4.5). Similarly, oviposition duration was significantly longer in *C. cosyra* (298.8 ± 0.3 s) and *B. invadens* (267.8 ± 0.5 s) than in *C. anonae* (135.7 ± 0.1 s), *C. capitata* (186.8 ± 0.5 s), *C. fasciventris* (108.3 ± 0.3 s) and *C. rosa* (72.9 ± 0.5 s) ($F_5 = 67.583$, $P < 0.001$) (Figure 4.6).

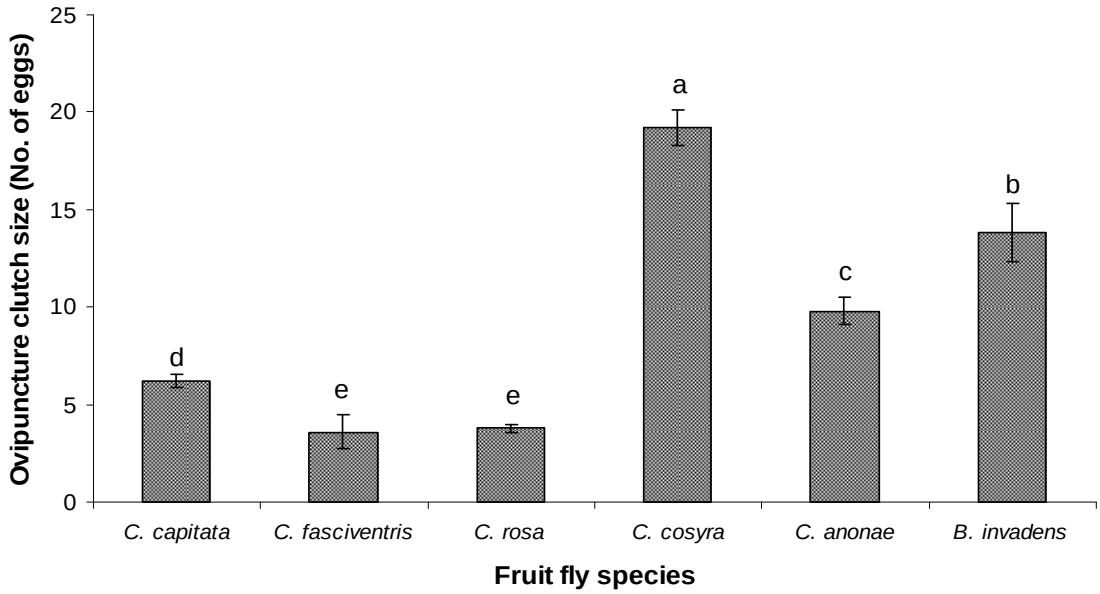


Figure 4.5: Ovipuncture clutch size (number of eggs per ovipuncture) in *C. capitata*, *C. fasciventris*, *C. rosa*, *C. cosyra*, *C. anonae* and *B. invadens*. Means accompanied by similar letters are not significantly different (F_5 = 45.358, P < 0.001, n = 40 for each species).

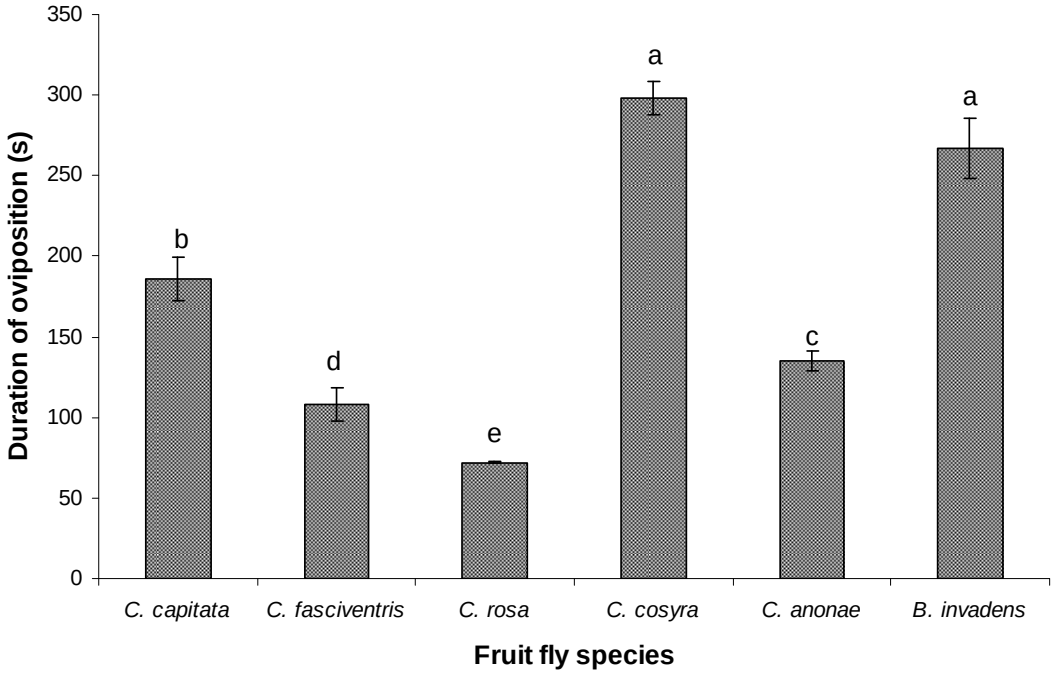


Figure 4.6: Oviposition duration (seconds) in *C. capitata*, *C. fasciventris*, *C. rosa*, *C. cosyra*, *C. anonae* and *B. invadens*. Means accompanied by similar letters are not significantly different ($F_5 = 67.583$, $P < 0.001$, $n = 40$ for each species).

4.4 Discussion

In this investigation, *C. capitata* and *B. invadens* were used as a checks since some information regarding host-marking behaviour in these two species was already available. Duyck *et al*. (2006) observed *C. capitata* (Reunion Island strain) to be a host-marking species with 72.3% incidence of the behaviour. Prokopy *et al*. (1978) observed the mean duration of host-marking bout (duration of ovipositor dragging) in *C. capitata* (Hawaiian strain) to be 77 s. Fletcher and Prokopy (1991) reported that host-marking behaviour was rare in *B. invadens*. These previous observations are comparable to those of the present study. The comparability of these observations, therefore, indicates sensitivity and effectiveness of the bioassays used in the present study.

The high incidence of host-marking behaviour in *C. fasciventris* and *C. rosa and C. cosyra* of 96.7% (Figure 4.1), which compares favourably with that of *C. capitata*, a known host-marking species, indicates that these three species are also host-markers. On the other hand, low occurrence of host-marking behaviour like in *C. anonae* and *B. invadens* (Figure 4.1) has already been observed in other species. Fitt (1984) and Nufio and Papaj (2004) made similar observations in some *Dacini* species and *R. suavis* respectively. Duyck *et al.,* (2006) observed incidence of host-marking behaviour to be 18.9% in *C. catoirii* and 12.9% in *B. zonata*. Low occurrence of host-marking behaviour in fruit flies may be associated with evolution, expansion of host range or development of other means of competing for oviposition resources.

The high occurrence of host-marking behaviour in *C. fasciventris* and *C. rosa* on one hand and low in *C. anonae* on the other (Figure 4.1) may be a reflection of the genetic differences and similarities among the species. These three species are phylogenetically closely related and morphologically alike such that they are not easily distinguishable from each other visually (Barr *et al*., 2006). As such, they are commonly referred to as the "FAR" complex of *Ceratitis*. However, molecular diagnostic work (PCR-RFLP analysis of mitochondrial DNA) by Barr *et al.* (2006) on this species cluster revealed that although the three were close relatives, *C. fasciventris* and *C. rosa* were more closely related to each other than to *C. anonae*. The finding of the present study that host-marking behaviour is equally high in *C. fasciventris* and *C. rosa* but significantly low in *C. anonae* may therefore support the finding of Barr *et al.* (2006) but from a behavioural angle.

The differences among *C. capitata*, *C. cosyra*, *C. fasciventris* and *C. rosa* regarding duration of host-marking bout (Figure 4.2) may be attributed to genetic differences among the species. Intrageneric differences in host-marking durations have also been reported in the Genus *Rhagoletis* as follows: *R. pomonella* marks for 30 s (Averill and Prokopy, 1987), *R. indifferens* for 51 s (Mumtaz and AliNiazee, 1983), *R. cerasi* for 30 s (Katsayannos, 1975) and *R. fausta* for 17 s (Prokopy, 1975).

The high incidence of pre-mark pausing behaviour in *C. cosyra* unlike in the rest of the host marking species (Figure 4.3) may also be attributed to genetic differences among the species. Probably, the purpose of this behaviour is to rid the ovipositor of sap from the oviposition substrate because during the pause, the flies systematically

rub the protracted ovipositor with the hind legs and then the hind legs against the wings. This unique behaviour of *C. cosyra* may help in distinguishing the species from *C. capitata* since these two species somehow look alike.

The ovipuncture clutch size of 6.3 eggs observed for *C. capitata* in this investigation (Figure 4.5) is comparable to 4.2 eggs which Prokopy *et al.* (1978) observed in a Hawaiian strain of the species. The comparability of these observations also indicates that the bioassay used in the present study was sensitive and effective. The significant differences in ovipuncture clutch size among the species may be attributed to different genetic bases of the species. The similarity in ovipuncture clutch size between *C. fasciventris* (3.6 eggs) and *C. rosa* (3.8 eggs) as opposed to *C. anonae* (9.8 eggs) further supports the finding of Barr *et al*., 2006 that despite these three species belonging to the FAR complex of *Ceratitis* species, the former two are more closely related to each other phylogenetically than to the latter.

The significantly large ovipuncture clutch sizes of *C. cosyra* and *B. invadens* as opposed to the other species may mean that the two species have higher reproductive potential. This may also help to explain why the two species are the most abundant (commonly intercepted) and destructive in mangoes in Kenya (Ekesi *et al*., 2009).

4.5 Conclusion

In conclusion, this investigation established that among the major fruit fly species infesting mango in Kenya, host-marking behaviour is prevalent in *C. capitata*, *C. cosyra*, *C. fasciventris*, and *C. rosa* but not in *C. anonae* and *B. invadens*. However, among the host-marking species, duration of host-marking bout is relatively longer in *C. capitata*, *C. fasciventris* and *C. cosyra* than in *C. rosa*. In addition, *C. cosyra* has a unique behaviour of pausing for some time after oviposition before engaging in host-marking. The six species also differ in their oviposition behaviour in that *C. cosyra* and *B. invadens* oviposit more eggs per ovipuncture than *C. capitata*, *C. fasciventris*, *C. rosa* and *C. anonae* and correspondingly take longer to oviposit.

CHAPTER FIVE

5.0 EFFICACY OF HOST-MARKING BEHAVIOUR AND FAECAL MATTER OF *CERATITIS CAPITATA*, *CERATITIS FASCIVENTRIS*, *CERATITIS ROSA* AND *CERATITIS COSYRA* IN DETERRING CONSPECIFIC AND HETEROSPECIFIC OVIPOSITION

5.1 Introduction

Although they engage in host-marking behaviour after oviposition, some fruit fly species do not produce host-marking pheromones, let alone deposit them on their their host substrates. The host-marking behaviour of such fruit fly species does not deter other prospective ovipositing fruit flies (Nufio and Papaj, 2004). It therefore follows that for fruit fly species which engage in host-marking behaviour but do not produce or deposit host-marking pheromones, it can be practically difficult, if not impossible, to manage them using the host-marking technique since there are no host-marking pheromones which can be exploited.

Also, cross-recognition of host-marking pheromones occurs among some fruit fly species such that the host-marking behaviour of one fruit fly species deters fruit flies of other species (Aluja and Diaz – Fleischer, 2006; Aluja *et al*, 2009). In the context of host-marking management of fruit flies, this phenomenon can have an economical advantage because a host-marking pheromone of one fruit fly species can be used to manage two or more fruit fly species.

Further, for fruit fly species that produce host-marking pheromones, these pheromones are also found in their faecal matter (Averill and Prokopy, 1989). These host-marking pheromones are soluble in water and methanol such that aqueous or methanolic solutions of the faecal matter of the fruit flies deter oviposition if applied on oviposition substrates (Arredondo and Diaz-Fleischer, 2006; Aluja *et al*., 2009). It therefore follows that when searching for fruit fly host-marking pheromones, towards their management using the host-marking technique, determining efficacy of the faecal matter of the fruit flies in deterring oviposition can help to provide insight regarding presence of the host-marking pheromones of the fruit flies in their faecal matter.

In the previous chapter (Figure 4.1), it was established that among the major fruit fly species infesting mango in Kenya, host-marking behaviour is prevalent in *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra*. It was, therefore, deemed necessary to determine efficacy of the host-marking behaviour and faecal matter of these fruit fly species in deterring conspecific and heterospecifics oviposition. The ultimate objective of the investigation was to get insight regarding production and deposition of host-marking pheromones by the fruit fly species after oviposition, cross-recognition of host-marking pheromones among the fruit fly species, and presence of their host-marking pheromones in their faecal matter. Basing on results of the experiments on efficacy of the host-marking behaviour and faecal matter of the fruit fly species in deterring conspecific and heterospecifics oviposition, another bioassay

was conducted to determine efficacy of various doses of faecal matter of *C. cosyra* in deterring conspecific and heterospecific oviposition.

5.2 Materials and methods

5.2.1 Efficacy of host-marking behaviour of *C. capitata, C. fasciventris, C. rosa* and *C. cosyra* in deterring conspecific and heterospecific oviposition

5.2.2 Bioassay

A dual - choice oviposition assay adapted from Prokopy *et al*. (1978) was used in this study. In a completely randomized design (CRD), a pair of "marked" and "non - marked" oviposition substrates was given to a gravid fruit fly enclosed in a 30 x 30 x 30cm clear Perspex observation cage. While Prokopy used 25 flies to "mark" one hawthorn fruit (surface area ≈ 7.1cm^2), by proportion, 90 flies were used to do the same in this study (surface area of oviposition substrate ≈ 23.8cm^2). The marked substrate was prepared by subjecting a mango slice fitted in a 50mm diameter petri cover to 90 flies to oviposit on and drag their ovipositors over it, with each fly ovipositing once. Non-marked mango slices were prepared similarly but the fruit flies were not allowed to drag their ovipositors over them. This was done by taking them out of the cage immediately after they oviposited. The mango slices were placed at the centre of the cage, side by side and in contact so as to allow the fruit flies to move easily across in search of oviposition sites. For each species, 100 fruit flies were observed and the fruit flies were grouped into 10 batches of ten each, each batch being a replicate. The fruit flies were, however, observed one at a time and

each replicate used one pair of mango slices, which was prepared from one mango in order to minimize differences in their chemistry, which could probably influence choice of the fruit flies. Each fruit fly was observed for a maximum of 30 minutes to oviposit once on any of the two substrates after which it was taken out. If a fly did not oviposit within the designated time, it was replaced. For each replicate, relative positions of the oviposition plates were randomly changed before introducing the next fruit fly in order to eliminate positional bias. The bioassay used in this investigation is illustrated in plate 5.1.

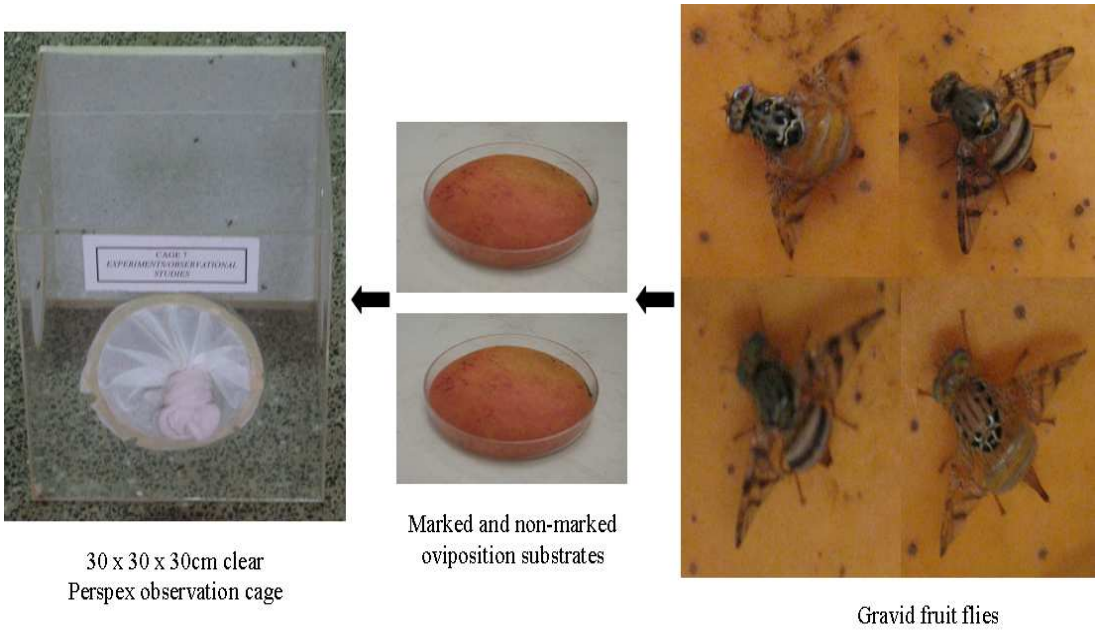


Plate 5.1: Illustration of the bioassay used to investigate efficacy of the host-marking behaviour of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* in deterring conspecific and heterospecific oviposition

5.2.3 Data collection and analysis

Data were collected in terms of percentages of fruit flies that oviposited on each type of oviposition substrate for each replicate. The data were subjected to chi-square test in order to determine efficacy of the host-marking behaviour of the fruit fly species in deterring conspecific and heterospecific oviposition.

5.2.4 Efficacy of faecal matter of *C. capitata, C. fasciventris, C. rosa* and *C. cosyra* in deterring conspecific and heterospecific oviposition

5.2.4.1 Collection of faecal matter of the fruit flies

Approximately, 1500 females of each fruit fly species were introduced into four respective clear Perspex cages each measuring 30 x 30 x 30 cm. Each cage contained two 13 × 25 cm glass pieces (placed at the base), one petri dish of water and another one with hydrolyzed yeast meal as food for the fruit flies. The fruit flies were reared in the cages for 30 days after which all living and dead flies and broken body parts were removed. Then, the faecal matter remaining on the glass plates was scrapped using separate spatulas into separate glass petri dishes, covered accordingly and then stored at -20°C for subsequent use.

5.2.4.2 Preparation of faecal matter solutions

An aqueous solution of faecal matter of each fruit fly species was prepared by dissolving the faecal matter in distilled water at a rate of 20 mg of faecal matter in 1ml of distilled water. The 20 mg/ml rate was based on the fact that Arredondo and Diaz-Fleischer (2006) observed 10 mg/ml to be the minimum effective dose for *C.*

capitata, the check species in this investigation. The mixture was vortexed for 5 minutes before use. An aliquot of distilled water was used as a control against the faecal matter solution.

5.2.4.3 Bioassays

Bioassays were conducted as in the previous investigation (section 5.2.2) but in this case, treatment and control mango slices were prepared by swabbing intact mango slices with separate pieces of cotton soaked in faecal matter solution and distilled water respectively. The mango slices were air-dried for five minutes before being used.

5.2.4.4 Data collection and analysis

Data collection and analysis were done as in the previous investigation (section 5.2.3).

5.2.5 Efficacy of various doses of faecal matter of *C. cosyra* in deterring oviposition in *C. capitata, C. fasciventris, C. rosa* and conspecifics

5.2.5.1 Faecal matter doses

Five doses, viz 0, 5, 10, 15 and 20 mg of faecal matter in 1ml of distilled water were prepared. The dose 0 mg/ml was distilled water only. The solutions were vortexed for 5 minutes before use. For each dose, a control of distilled water was used.

5.2.5.2 Bioassays

Bioassays were conducted as in section 5.2.2. but in this case, the faecal matter (of *C. cosyra*) was tested at five levels of concentration as indicated in section 5.2.5.1 above.

5.2.5.3 Data collection and analysis

Data collection and analysis were done as in section 5.2.3. However, in this case, the data were further subjected to regression analysis in order to determine relationship between faecal matter dose and efficacy in deterring oviposition.

5.3 Results

5.3.1 Efficacy of host-marking behaviour of *C. capitata, C. fasciventris, C. rosa* and *C. cosyra* in deterring conspecific and heterospecific oviposition

The host-marking behaviour of *C. capitata* significantly deterred conspecifics (74%, $P < 0.001$), significantly attracted *C. rosa* (72%, $P < 0.001$) but had no effect on *C. cosyra* and *C. fasciventris*. The host-marking behaviour of *C. fasciventris* significantly deterred conspecifics (69%, $P < 0.001$), significantly attracted *C. rosa* (64%, P = 0.009) but had no effect on *C. capitata* and *C. cosyra*. The host-marking behaviour of *C. rosa* had no effect on conspecifics, but significantly deterred *C. capitata* (66%, P = 0.001) and significantly attracted *C. cosyra* (64%, P = 0.009) and *C. fasciventris* 61%, P = 0.046). The host-marking behaviour of *C. cosyra* significantly deterred conspecifics (64%, P = 0.005) and *C. fasciventris* (88%, $P < 0.001$) but had no effect on *C. capitata* and *C. rosa* (Chi-square test) (Table 5.1).

Table 5.1: Efficacy of host-marking behaviour of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* in deterring conspecific and heterospecific oviposition (Chi-square test). Entries in red ink are cases of significant deterrence

Marking species	Respondent species	*n*	Mean % of ovipositing flies		Chi-square value	P- value
			Marked substrate	Non-marked substrate		
C. capitata	*C. capitata*	100	26	74	27.0400	< 0.001
	C. fasciventris	100	51	49	0.4000	0.842
	C. rosa	100	72	28	19.3600	< 0.001
	C. cosyra	100	57	43	1.4400	0.230
C. fasciventris	*C. fasciventris*	100	31	69	14.4400	< 0.001
	C. capitata	100	52	48	0.1600	0.689
	C. rosa	100	63	37	6.7600	0.009
	C. cosyra	100	51	49	0.0400	0.842
C. rosa	*C. rosa*	100	54	46	0.6400	0.424
	C. capitata	100	34	66	10.2400	0.001
	C. fasciventris	100	61	39	4.0000	0.046
	C. cosyra	100	64	36	6.7600	0.009
C. cosyra	*C. cosyra*	100	36	64	7.8400	0.005
	C. capitata	100	45	55	0.6400	0.424
	C. fasciventris	100	12	88	57.7600	< 0.001
	C. rosa	100	42	58	1.9600	0.162

5.3.2 Efficacy of faecal matter of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. rosa* in deterring conspecific and heterospecific oviposition

The faecal matter of *C. capitata* significantly deterred conspecifics (84%, $P < 0.001$) but had no effect on *C. fasciventris*, *C. rosa* and *C. cosyra*. Faecal matter of *C. fasciventris* significantly deterred conspecifics (85%, $P < 0.001$) and *C. capitata* (74%, $P = 0.0000$) but had no effect on *C. rosa* and *C. cosyra*. Faecal matter of *C. rosa* significantly deterred conspecifics (72%, $P < 0.001$), *C. capitata* (76%, $P < 0.001$) and *C. fasciventris* (74%, $P < 0.001$) but had no effect on *C. cosyra*. Faecal matter of *C. cosyra* significantly deterred conspecifics (83%, $P < 0.001$), *C. capitata* (86%, $P < 0.001$) and *C. fasciventris* (71%, $P < 0.001$) but had no effect on *C. rosa* (Chi-square test) (Table 5.2).

Table 5.2: Efficacy of faecal matter of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* in deterring conspecific and heterospecific oviposition (Chi-square test). Entries in red ink are cases of significant deterrence.

Type of faecal matter	Respondent species	*n*	Mean % of ovipositing flies		Chi-square value	P- value
			Faecal matter treatment	Control		
C. capitata	*C. capitata*	100	16	84	46.2400	< 0.001
	C. fasciventris	100	51	49	0.0400	0.842
	C. rosa	100	57	43	1.9600	0.162
	C. cosyra	100	44	56	1.4400	0.230
C. fasciventris	*C. fasciventris*	100	15	85	49.0000	< 0.001
	C. capitata	100	26	74	23.0400	< 0.001
	C. rosa	100	54	46	0.6400	0.424
	C. cosyra	100	47	53	0.3600	0.549
C. rosa	*C. rosa*	100	28	72	19.3600	< 0.001
	C. capitata	100	24	76	27.0400	< 0.001
	C. fasciventris	100	26	74	23.0400	< 0.001
	C. cosyra	100	47	53	0.3600	0.549
C. cosyra	*C. cosyra*	100	17	83	43.5600	< 0.001
	C. capitata	100	14	86	51.8400	< 0.001
	C. fasciventris	100	29	71	17.6400	< 0.001
	C. rosa	100	47	53	0.3600	0.5485

5.3.3 Efficacy of various doses of faecal matter of *C. cosyra* in deterring oviposition in *C. capitata, C. fasciventris, C. rosa* and conspecifics

Significant oviposition deterrence was observed in *C. capitata*, *C. fasciventris* and *C. cosyra*, but not in *C. rosa*. The minimum effective dose for *C. cosyra* was 10 mg/ml while for *C. capitata* and *C. fasciventris* it was 5 mg/ml (Table 5.3). There was strong positive relationship between faecal matter dose and oviposition deterrence in *C. capitata*, and *C. fasciventris* and *C. cosyra* but not in *C. rosa* (R^2 = 0.96, 0.92, 0.93 and 0.21 respectively (Figures 5.1 – 5.4).

Table 5.3: Efficacy of various doses of *C. cosyra* faecal matter in deterring oviposition in *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* (Chi-square test). Entries in red ink are cases of significant deterrence.

Respondent species	*n*	Dose of faecal matter (mg/ml)	Mean % of ovipositing flies		Chi-square value	P-value
			Treatment	Control		
	60	0	55	45	0.6000	0.439
	60	5	35	65	5.4000	0.021
C. capitata	60	10	33	67	6.6667	0.010
	60	15	27	73	16.7046	< 0.001
	60	20	28	72	13.8714	< 0.001
	60	0	47	53	0.2667	0.605
	60	5	23	77	17.0667	< 0.001
C. fasciventris	60	10	25	75	15.0000	< 0.001
	60	15	18	82	24.0667	< 0.001
	60	20	13	87	32.2667	< 0.001
	60	0	47	53	0.2667	0.606
	60	5	40	60	2.4000	0.121
C. rosa	60	10	43	57	1.0667	0.302
	60	15	45	55	0.6000	0.439
	60	20	42	58	1.6667	0.197
	60	0	48	52	0.0667	0.796
	60	5	42	58	1.6667	0.197
C. cosyra	60	10	37	63	4.2667	0.039
	60	15	37	63	4.2667	0.039
	60	20	27	73	13.0667	< 0.001

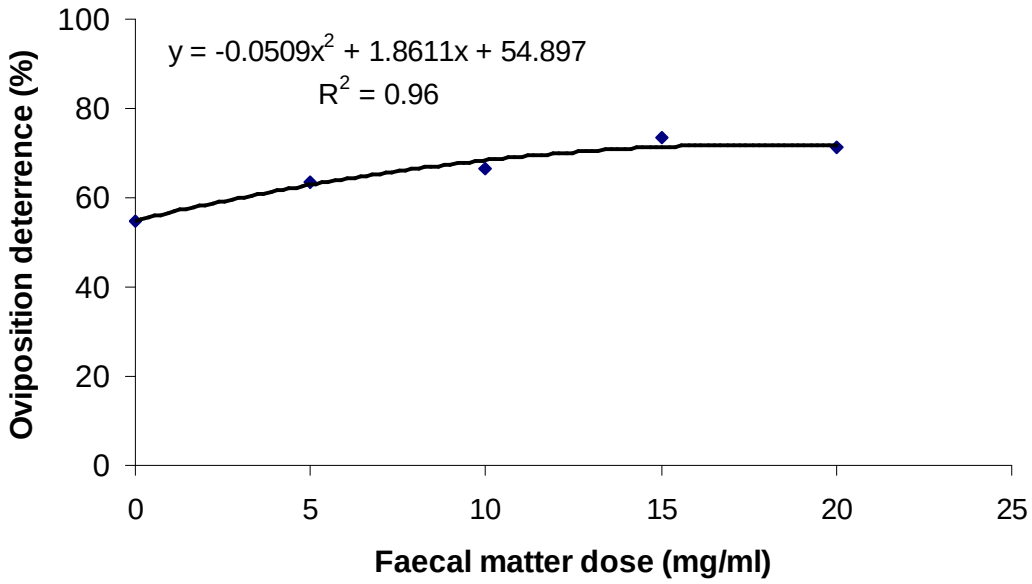


Figure 5.1: Relationship between dose of *C. cosyra* faecal matter and oviposition deterrence in *C. capitata*

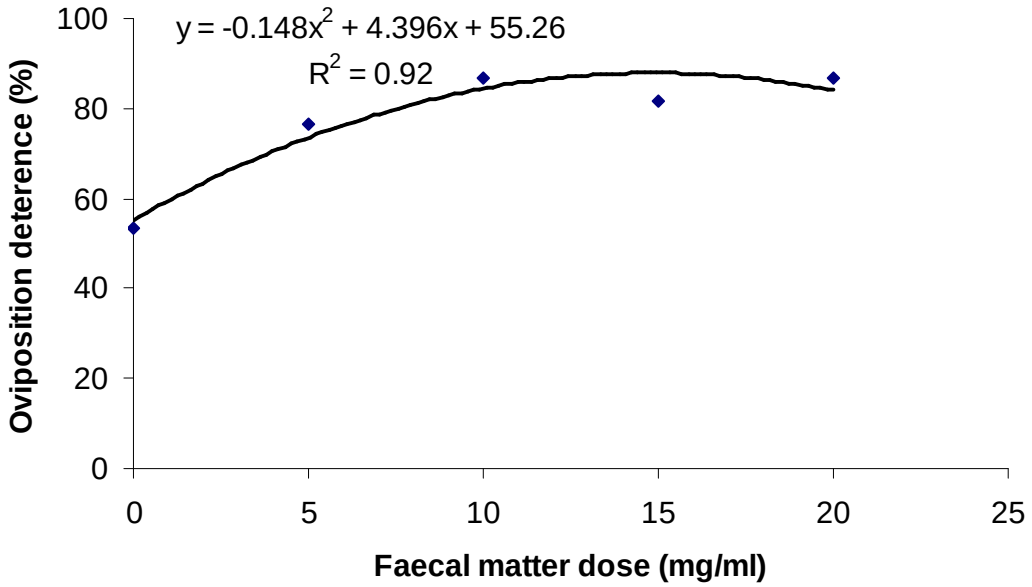


Figure 5.2: Relationship between dose of *C. cosyra* faecal matter and oviposition deterrence in *C. fasciventris*

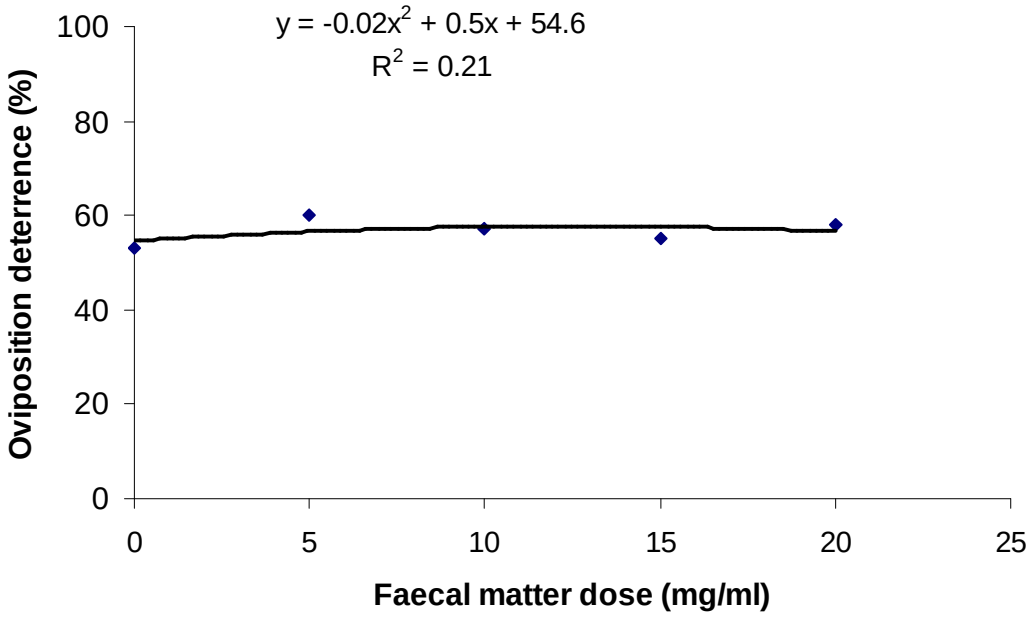


Figure 5.3: Relationship between dose of *C. cosyra* faecal matter and oviposition deterrence in *C. rosa*.

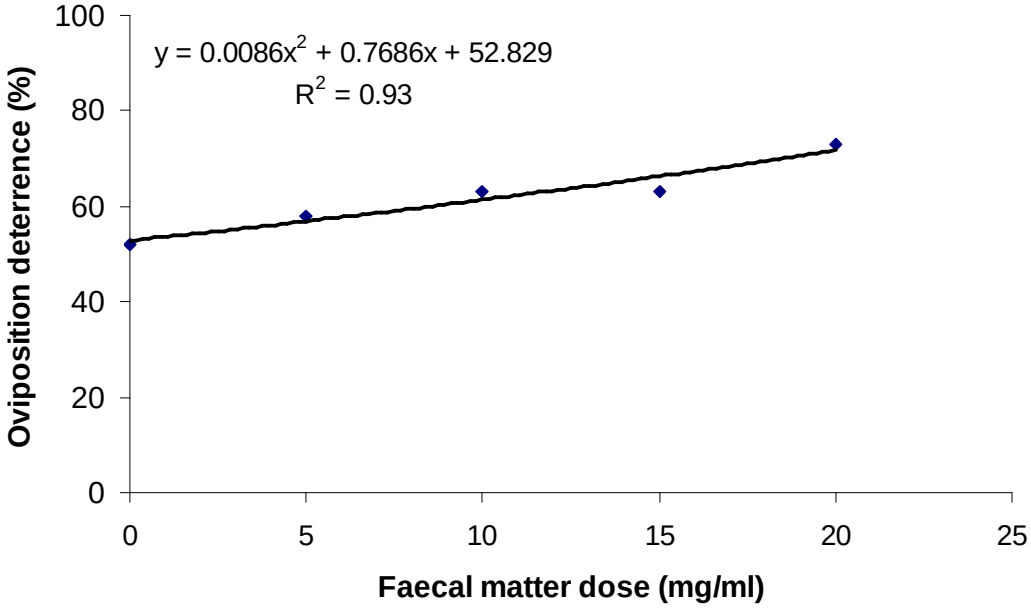


Figure 5.4: Relationship between dose of *C. cosyra* faecal matter and oviposition deterrence in *C. cosyra*

5.4 Discussion

In this investigation, *C. capitata* was used as a check since it was already known that the species produces host-marking pheromones, deposits them on its host fruits after oviposition and the pheromones deter other prospective ovipositing conspecifics (Prokopy *et al.,* 1978; Aluja and Diaz-Fleischer, 2006 and Arredondo and Diaz-Fleischer, 2006). It was also already known that faecal matter of *C. capitata* contains host-marking pheromones of the species such that an aqueous or methanolic solution of faecal matter of the species deters prospective ovipositing conspecifics if applied on host fruits (Prokopy *et al.* 1978; Arredondo and Diaz-Fleischer, 2006). In the present study, both the host-marking behaviour and faecal matter (aqueous solution) of *C. capitata* deterred conspecific oviposition (Tables 5.1 and 5.2). These results compare favourably with those of previous studies. This comparability indicates sensitivity and effectiveness of the bioassay used in the present study.

Since oviposition deterrence by host-marking behaviour and faecal matter of *C. capitata* stems from the fact that the species produces host-marking phromones, deposits them on its hosts during post-oviposition ovipositor dragging and the pheromones are also found in its faecal matter (Prokopy *et al.,* 1978; Aluja and Diaz-Fleischer, 2006; Arredondo and Diaz-Fleischer, 2006), conspecific oviposition deterrence by the host-marking behaviour and faecal matter of *C. cosyra* and *C. fasciventris* (Tables 5.1 and 5.2) suggests that these two fruit fly species also produce host-marking phromones, deposit them on their hosts after oviposition and the pheromones are also found in their faecal matter. On the other hand, failure of the host-marking behaviour of *C. rosa* to elicit conspecific oviposition deterrence (Table

5.1) could mean that the deposited pheromone was not sufficient since in fruit flies, host-marking pheromones are deterrent when in high concentration but attractive when concentration is low (Papaj and Aluja, 1993). However, this could not be verified because the host-marking technique used in this investigation was based on number of flies and not pheromone amount owing to the fact that the actual presence of host-marking pheromones had not yet been established.

Heterospecific oviposition deterrence by the host-marking behaviour and faecal matter of the fruit fly species (Tables 5.1 and 5.2) could mean interspecific recognition of the host-marking pheromone or pheromone components among the fruit fly species. Interspecific recognition of host-marking pheromones in fruit flies has already been reported in species of the genera *Rhagoletis* (Prokopy *et al*., 1976) and *Anastrepha* (Aluja and Diaz-Fleischer, 2006). Similarly, heterospecific oviposition attractions of *C. cosyra* to *C. capitata*; *C. fasciventris* to *C. rosa*; *C. rosa* to *C. capitata* and *C. fasciventris* could mean interspecific recognition of host-marking pheromones or pheromone components among the species but maybe the pheromones were in low concentration. It is known that, when in low concentration, fruit fly host-marking pheromones are attractive to prospective ovipositing females (Papaj and Aluja, 1993).

Although 90 fruit flies were used in the host-marking experiment to mark a mango slice for oviposition, less fruit flies may be required in a natural situation because wild flies produce more potent pheromones than laboratory-reared ones (Prokopy *et*

al., 1978) and they are also more sensitive to the pheromones than their laboratory-reared counterparts (Prokopy *et al*., 1978; Prokopy *et al*., 1989).

The most intriguing observation in this investigation was that in both host-marking and faecal matter experiments, *C. cosyra* was not deterred by any other species except itself but it deterred other species namely: *C. capitata* and *C. fasciventris* (Tables 5.1 and 5.2). *Ceratitis cosyra* is the most destructive of the four host-marking species on mango (Rwomushana *et al*., 2009). The fact that no other fruit fly species could deter it yet it could deter others could mean that the host-marking pheromone of *C. cosyra* is a chemical compound or compound blend which is not produced by any of the other species but the pheromone is deterrent to them. In the context of developing a host-marking tool for the management of these fruit fly species, this may be an advantageous phenomenon because it may mean ability to control the most destructive (economically important) fruit fly species as well as other species just using the host-marking pheromone of the former. This observation, therefore, suggests that as far as application of the host-marking technique in the management of these fruit fly pests is concerned, the host-marking pheromone of *C. cosyra* may be the best to pursue and exploit.

In ecological terms, the ability of *C. cosyra* (its host-marking behaviour and faecal matter) to deter other species without reciprocation may corroborate with fact that *C. cosyra* has a very narrow host range (mango and marula as main hosts) compared to the other species (White and Elson-Harris, 1992; Vargas *et al*, 2001; Rwomushana *et al.*, 2009). *Ceratitis cosyra* may thus have a unique host-marking pheromone which

deters the other species with it (*C. cosyra*) not recognizing their host-marking pheromones as a means of protecting its narrow niche and maximizing its utility.

The strong positive relationship between dose of *C. cosyra* faecal matter and oviposition deterrence in conspecifics, *C. capitata* and *C. fasciventris* (Figures 5.1, 5.2 and 5.4) provides another hint that the faecal matter of *C. cosyra* contains a host-marking pheromone of the species, to whose increasing concentration the flies were responding. The relationship further suggests potential of the host-marking pheromone of *C. cosyra* alone for the management of *C. cosyra, C. capitata* and C. *fasciventris* using the host-marking technique.

The minimum effective dose of 10 mg of faecal matter per ml of water observed for *C. cosyra* this study (Table 5.3) is the same as what Arredondo and Diaz-Fleischer (2006) observed for *C. capitata* although they used methanol as a solvent. The minimum effective dose of 5 mg/ml for *C. capitata* and *C. fasciventris* as compared to 10 mg/ml for *C. cosyra* (Table 5.3) may mean that *C. capitata* and *C. fasciventris* are more sensitive to the marking pheromone of *C. cosyra*. This difference in sensitivity among these three fruit fly species may also work to the ecological advantage of *C. cosyra*, as a narrow host range species, to effectively keep the other fruit fly species away from its narrow niche.

Although the minimum effective dose for the species has been found to range between 5 and 10 mg/ml, a lower dose may be required in a natural situation because wild flies produce more potent pheromones than laboratory-reared ones (Prokopy *et*

al., 1978) and they are also more sensitive to host-marking pheromones (Prokopy *et al*., 1978; Prokopy *et al.*, 1989).

5.5 Conclusions

In conclusion, this investigation established that the host-marking behaviour and faecal matter of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* deter at least conspecific or heterospecific oviposition, thus suggesting that the fruit fly species produce host-marking pheromones, deposit the host-marking pheromones on their host substrates after oviposition and the pheromones are also present in their faecal matter. The investigation also established that the host-marking behaviour and faecal matter of *C. cosyra* deter *C. cosyra* and other species (*C. capitata* and *C. fasciventris*) without reciprocation, suggesting that the host-marking pheromone of *C. cosyra* is chemically unique but also deterrent to *C. capitata* and *C. fasciventris*.

CHAPTER SIX

6.0 PRESENCE OF POTENTIAL HOST-MARKING PHEROMONES OF *C. CAPITATA, C. COSYRA, C. FASCIVENTRIS* AND *C. ROSA* IN FAECAL MATTER OF THE FRUIT FLY SPECIES

6.1 Introduction

Host-marking pheromones of fruit flies are found in large quantities in faecal matter their mature females. This is so, because fruit flies produce their host-marking pheromones in the posterior half of the midgut such that when being voided, their faecal matter carries with it, a lot of these compounds (Averill and Prokopy, 1989). Fruit fly host-marking pheromones are also water- and methanol-soluble (Aluja *et al*., 2003). Owing to the fact that host-marking pheromones of fruit flies are present in their faecal matter and the compounds are soluble in water and methanol, aqueous and methanolic solutions of the faecal matter deter oviposion if applied on oviposition substrates while the faecal matter is a good source for isolating and identifying fruit fly host-marking pheromones (Aluja *et al*., 2009).

In the previous chapter (chapter 5), the host-marking behaviour and faecal matter of *C. capitata, C. fasciventris, C. rosa* and *C. cosyra* deterred at least conspecific or heterospecific oviposition, thus suggesting that the fruit flies produce host-marking pheromones, deposit them on their host fruits after oviposition and the pheromones are also present in their faecal matter. It was therefore considered necessary to determine presence of potential host-marking pheromones of the four fruit fly species

in their faecal matter. Since male fruit flies are reported not to produce host-marking pheromones (Stadler, *et al*., 1992), faecal matter of male *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* was analysed in comparison with that of their females in order to help in closing down more easily on potential host-marking pheromones of the fruit flies.

Basing on results of this investigation and in consideration of the results of the experiments on efficacy of the host-marking behaviour and faecal matter of the fruit flies in deterring conspecific and heterospecific oviposition, one of the chemical components that were found in faecal matter of *C. cosyra* and suggested to be the host-marking pheromone of species was isolated and tested for behavioural activity against conspecifics.

6.2 Materials and methods

6.2.1 Presence of potential host-marking pheromones in faecal matter of the fruit fly species

6.2.1.1 Technique

The technique used was HPLC analysis of methanolic extracts from faecal matter of female fruit flies, adapted from Aluja *et al.,* (2003) and Arredondo and Diaz-Fleischer (2006). This technique is based on the fact that fruit fly host-marking pheromones are soluble in methanol (Averill and Prokopy, 1982; Boller and Hurter, 1985; Hurter *et al*., 1987; Averill and Prokopy, 1987; Aluja *et al*., 2003: Aluja *et al*.,

2009). The technique involved the following four steps: (1) faecal matter collection, (2) preparation of host-marking pheromone extract, (3) HPLC data acquisition and (4) analysis of the data.

6.2.1.2 Faecal matter collection

Faecal matter of each species was collected by putting 150 flies of a given species and sex in a clean glass bottle (≈ 200 cm^3) with a net-fitted lid for five consecutive periods of 4:00 p.m. – 8:00 a.m. so as to deposit their faecal matter in the vessel. Everyday at 8:00 a.m., the flies were released into respective 30 x 30 x 30 cm clear perspex cages containing food, water and ripe mangoes until 4:00 pm when they were taken back into their respective bottles. During day, in the cages, the fruit flies were provided with ripe mangoes so that they oviposited therein. It had been observed that if the fruit flies were not provided with mangoes during day, they were ovipositing in the faecal matter collection bottles at night, thus contaminating the faecal matter. After five days, the faecal matter in the bottles was collected by pouring 5 ml of HPLC grade methanol into the vessels and scraping it from the sides of the bottle using respective clean spatulas into the methanol. The contents were then poured into respective pre-weighed glass vials and finally evaporated to dryness in a vacuum hood. The mass of the collected faecal matter was determined by subtracting the mass of the empty vial from that of the vial together with faecal matter. Collection of faecal matter was replicated four times for each species and sex.

6.2.1.3 Preparation of fruit fly faecal matter extract

The collected faecal matter was dissolved in HPLC grade methanol at a rate of 100 mg of faecal matter in 1ml of methanol. The dissolution rate was determined through pilot HPLC analyses with serial dilutions of 100, 50, 25 and 12.5 mg per ml with respect to expression of peaks of chemical components. The faecal matter – methanol mixture was then vortexed for 5 minutes, sonicated for 5 minutes and then 80 µl pipetted into a centrifugation vial and centrifuged at 12000 rpm for 5 minutes. The supernatant was taken as the extract to be analyzed.

Since fruit fly host-marking pheromones are also soluble in water (Averill and Prokopy, 1987; Aluja *et al.*, 2003), four replicates of water-based extract were similarly prepared for *C. fasciventris* and *C. cosyra* faecal matter in order to determine solubility, in water, of the potential chemical components that had been observed in methanolic extracts from faecal matter of the fruit flies. In the case of aqueous extracts, distilled water, methanol and aqueous extract from fruit fly diet were used as controls. The choice of *C. cosyra* and *C. fasciventris* only for this investigation was based on the fact that results of methanolic extract analyses were consistently similar for *C. fasciventris*, *C. capitata* and *C. rosa*, only *C. cosyra* had a different one. So, *C. fasciventris* was randomly chosen as a representative of itself, *C. capitata* and *C. rosa*.

6.2.1.4 HPLC data acquisition

HPLC data was acquired using a Shimadzu Prominence HPLC system with the following operation parameters: diode array detector (at 350 nm), Ace C18 reverse phase column (25 x 4.6 mm IDM, 5 µm bead size), 20 ^{o}C column temperature, water-methanol as the mobile phase at a gradient of 5 – 100 % in 43 minutes, 40 µl injection volume and a flow rate of 1 ml / minute. Control HPLC data was acquired by running similar aliquots of distilled water, methanol and methanolic extract of fruit fly diet (yeast hydrolysate) in four replicates also. For the aqueous extracts, controls were distilled water, methanol, and aqueous extract of fruit fly diet. The distilled water used contained 5% formic acid while the actual methanol concentration gradient was 5, 15, 25, 30, 55 and 100 % at 0, 3, 13, 25, 35 and 36 minutes respectively.

6.2.1.5 Determination of potential host-marking pheromones of the fruit fly species

Potential host-marking pheromones of the fruit flies were considered as those chemical components which were consistently present in chemical profiles of fruit fly faecal matter extract but consistently lacked in control profiles of solvent and fruit fly diet extract. The observed potential host-marking pheromones were considered similar or differrent depending on similarity or difference in retention time. The technique used to determine presence of potential host-marking pheromones of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* in faecal matter of the fruit fly species is illustrated in plate 6.1.

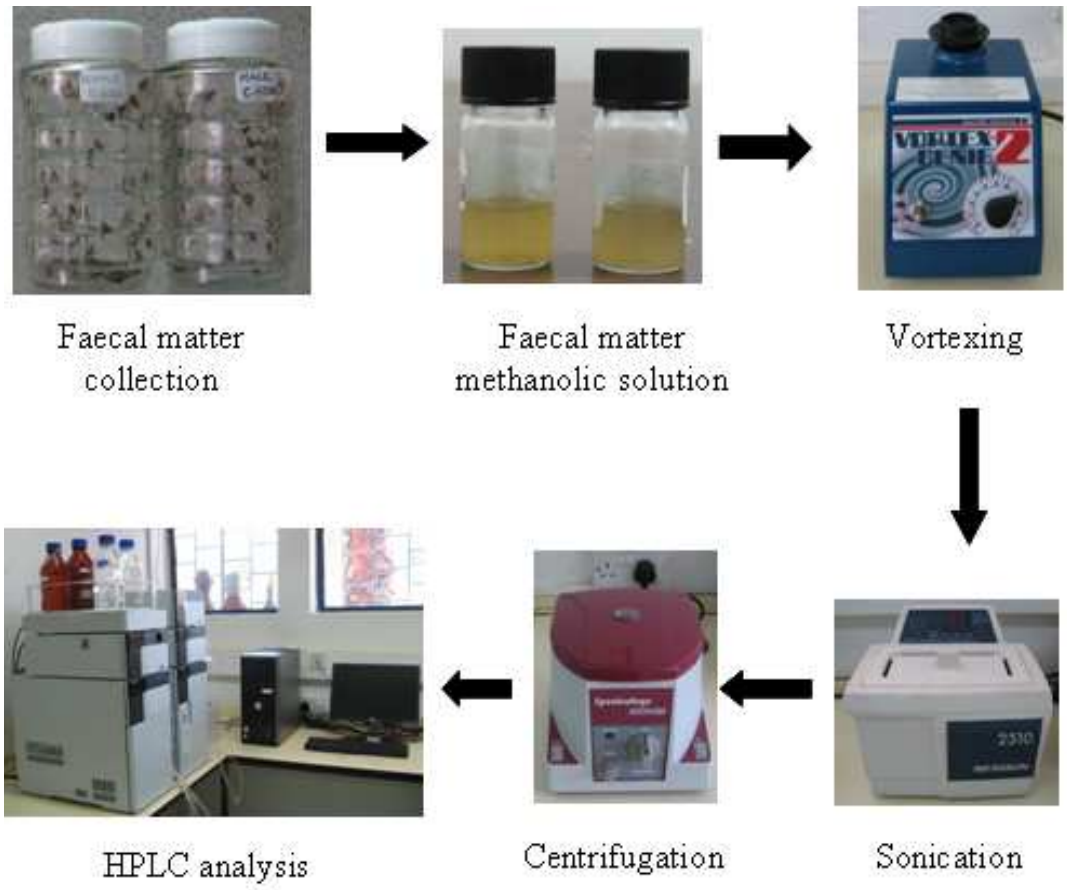


Plate 6.1: Illustration of the technique used to determine presence of potential host-marking pheromones of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* in faecal matter of the fruit fly species.

6.2.2 Behavioural activity of a potential host-marking pheromone of *C. cosyra* against conspecifics

6.2.2.1 Collection of *C. cosyra* faecal matter and isolation and preparation of the potential host-marking pheromone

Faecal matter of *C. cosyra* (1g) was collected and a pheromone extract prepared as in the previous investigation. The three fly-produced chemical components of the extract were fractionated as in the previous investigation but the program was shortened to 27 minutes such that the chemical components eluted respectively a little earlier (Figure 6.9). The fractionation program was reduced in order to save time and solvents. The collected fractions were then evaporated to dryness and their masses determined. The one unique chemical component of *C. cosyra* (fraction with retention time 21.8 min) was then dissolved in distilled water at a rate of 0.1mg/ml and used in dual-choice oviposition assays. The dissolution rate was based on the one used in the faecal matter experiment of 20mg of faecal matter in 1ml of distilled water, where it was found out that this chemical component of interest occurs at a rate of 0.005mg per mg of faecal matter. This therefore translated the rate of 20mg of faecal matter per ml to 0.1mg of the chemical component per ml of distilled water.

6.2.2.2 Bioassay

Bioassays were conducted as in section 5.2.2, but in this case, treatment and control mango slices were prepared by swabbing intact mango slices with separate pieces of cotton soaked in “host-marking pheromone solution” and distilled water respectively. The mango slices were air-dried for five minutes before being used.

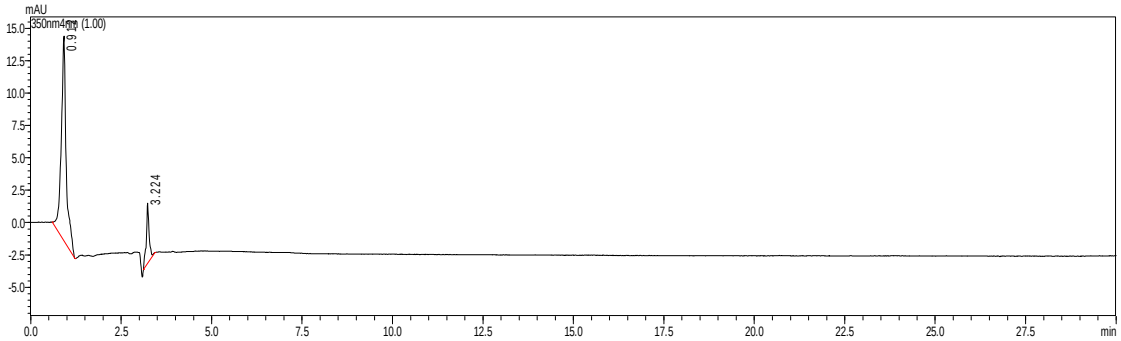
6.2.2.3 Data collection and analysis

Data collected were percentages of deterred flies for each replicate. Deterred flies were regarded as those that opted to oviposit on the control substrate. The data were subjected to Chi-square test.

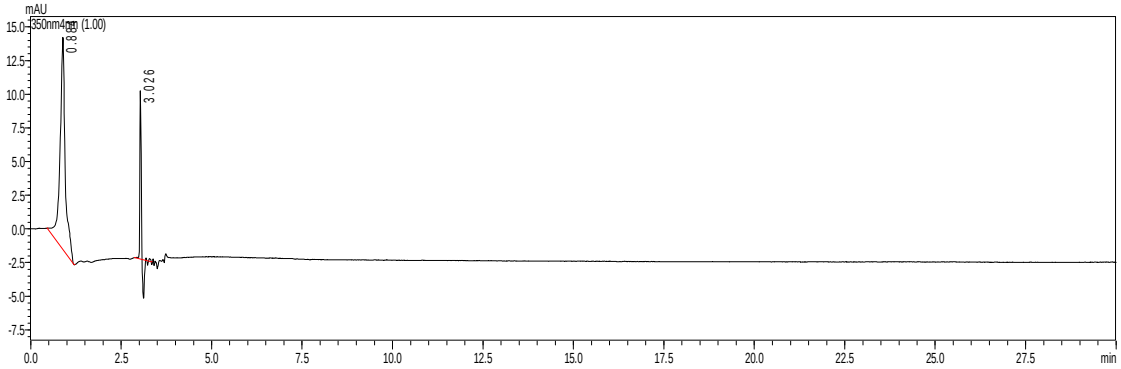
6.3 Results

6.3.1 Presence of potential host-marking pheromones in faecal matter of the fruit fly species

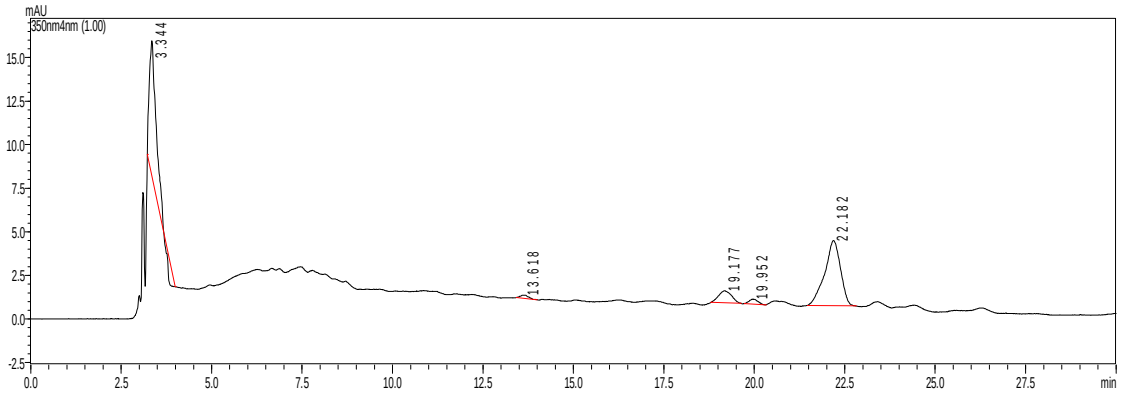
Across all the four replicates, similar profiles of fly-produced methanol-soluble chemical components were consistently observed for female *C. capitata*, *C. fasciventris* and *C. rosa*, with each species having three fly-produced chemical components which eluted at 10, 11 and 15 minutes after injection, respectively (Figures 6.1 – 6.5). On the other hand, female *C. cosyra* differed from the other three species by consistently having only two of these chemical components (10 and 15) and an additional unique and less polar component which eluted at 24 minutes (Figure 6.5). For all the species, profiles of chemical components were similar between male and females (Figures 6.2 - 6.5). Similar results as with methanolic extracts were obtained for aqueous extracts from faecal matter of *C. fasciventris* and *C. cosyra*. However, water was more efficient in extracting diet-based chemical components (Figures 6.6 – 6.8).



Distilled water (Control 1)

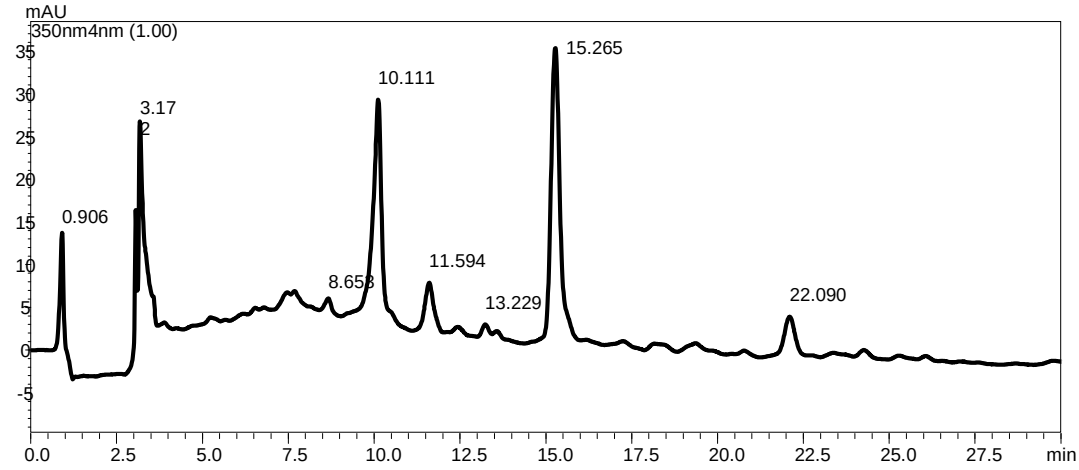


Methanol (Control 2)

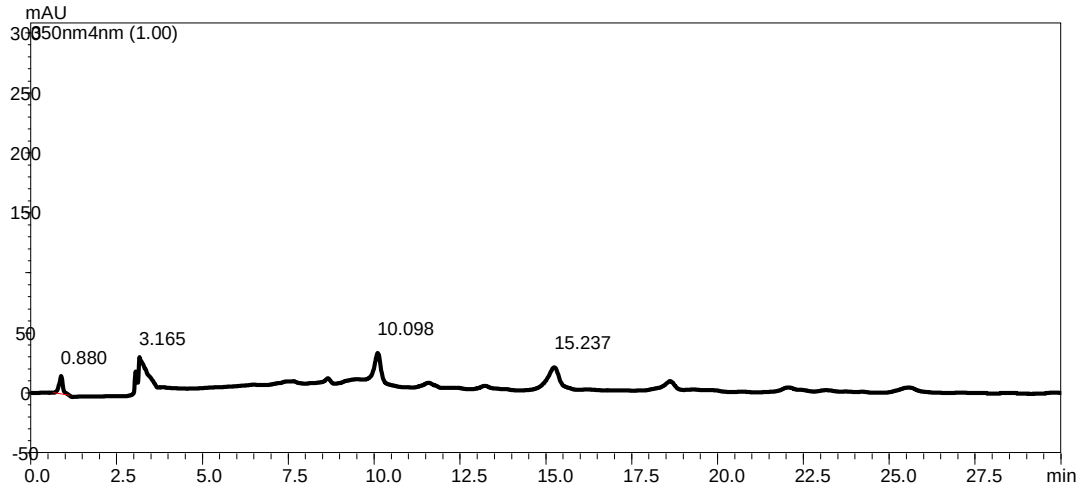


Fruit fly diet extract (Control 3)

Figure 6.1: Representative HPLC profiles of distilled water, methanol and methanolic extracts from fruit fly diet as controls for methanol-soluble fly-produced chemical components found in faecal matter of female and male *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra.*

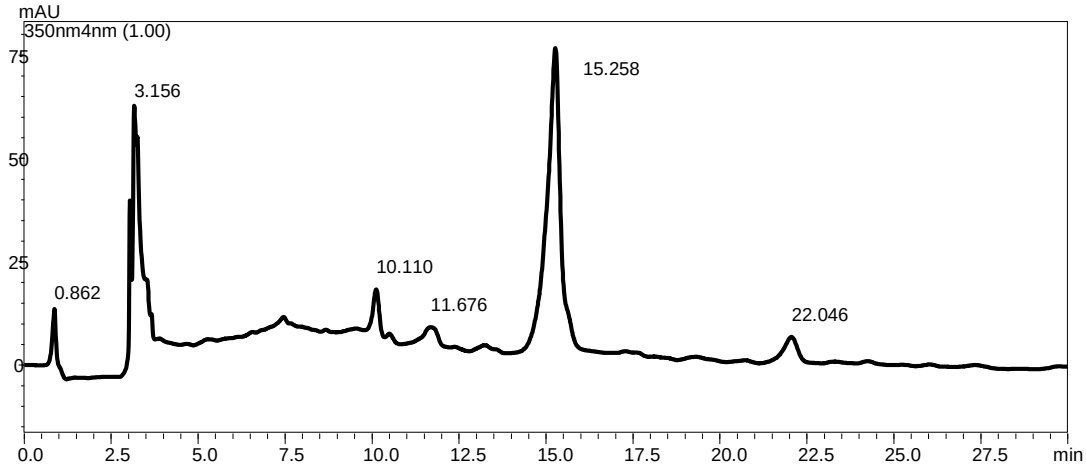


Female

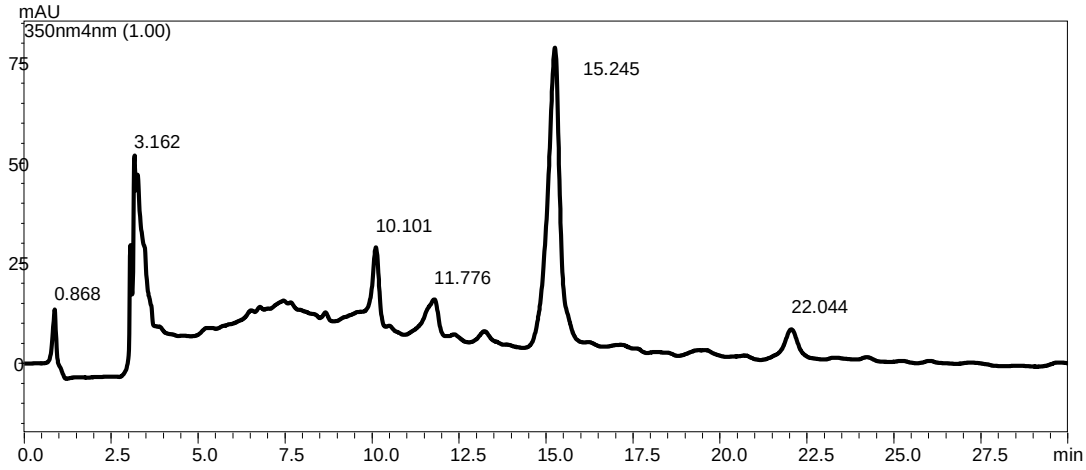


Male

Figure 6.2: Representative HPLC profiles of methanolic extracts from faecal matter of female and male *C. capitata*.

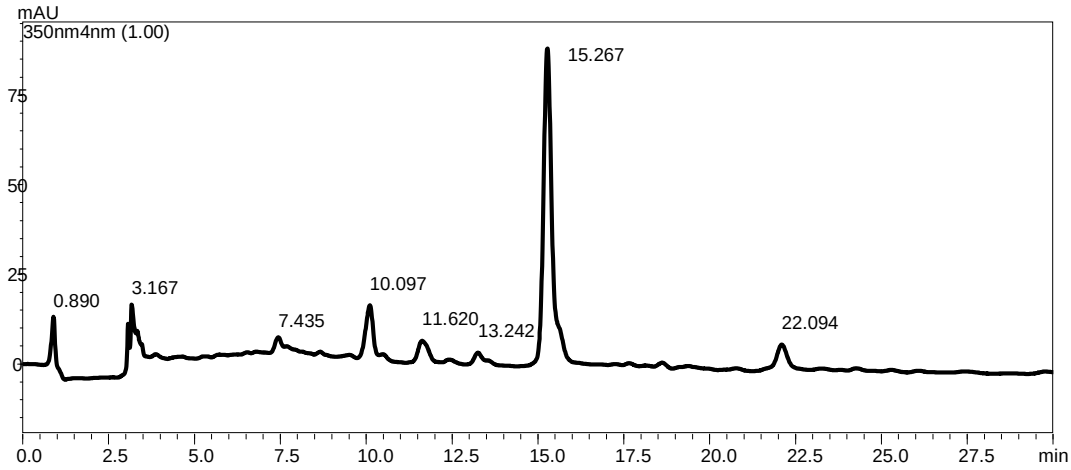


Female

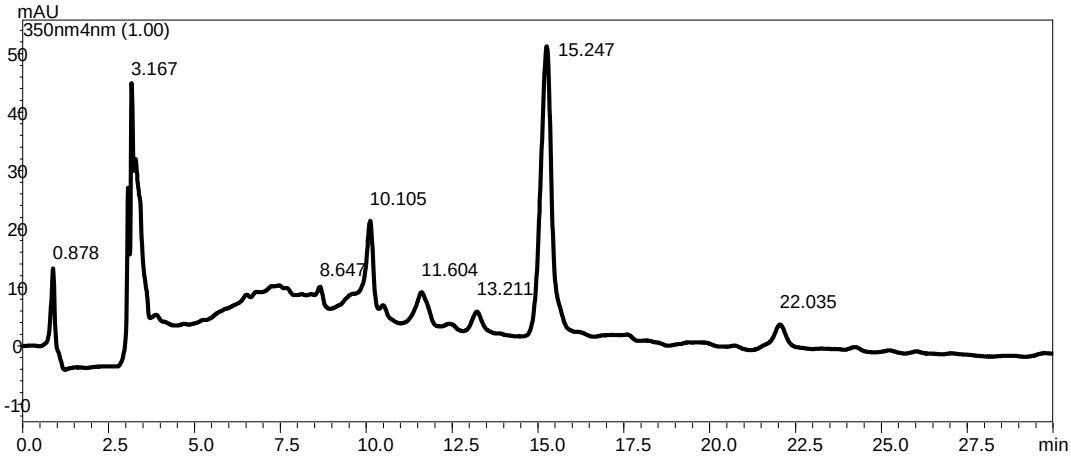


Male

Figure 6.3: Representative HPLC profiles of methanolic extracts from faecal matter of female and male *C. fasciventris*.

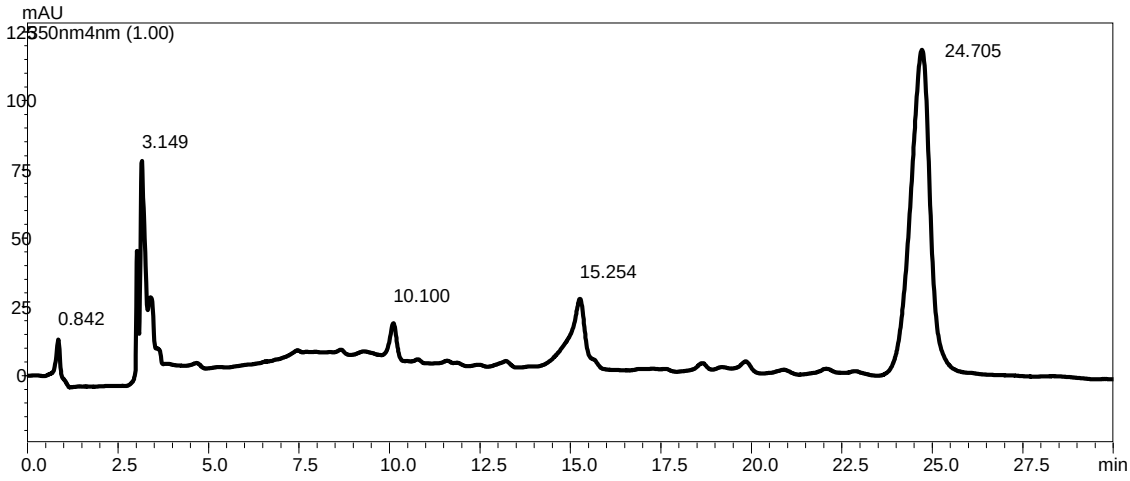


Female

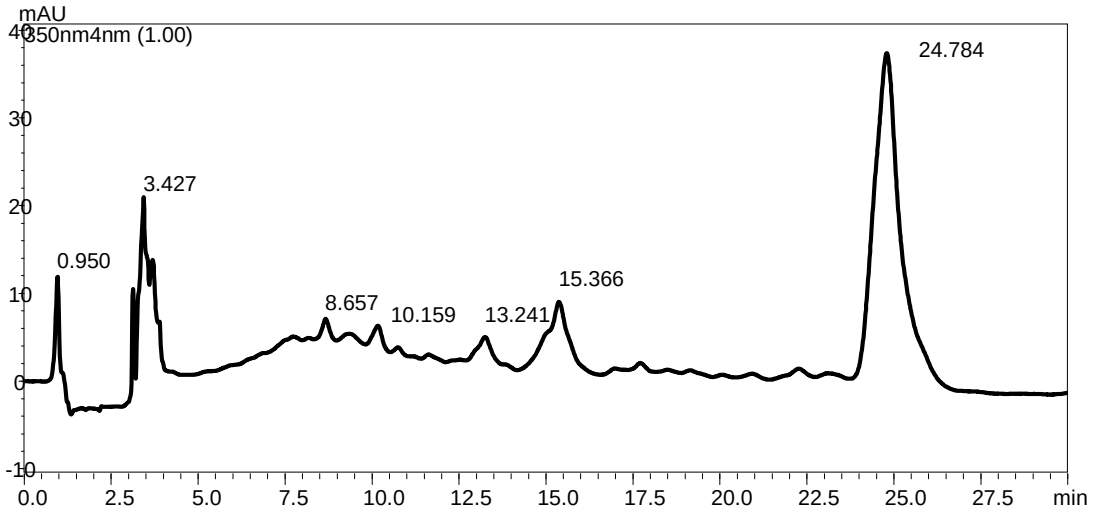


Male

Figure 6.4: Representative HPLC profiles of methanolic extracts from faecal matter of female and male *C. rosa*.

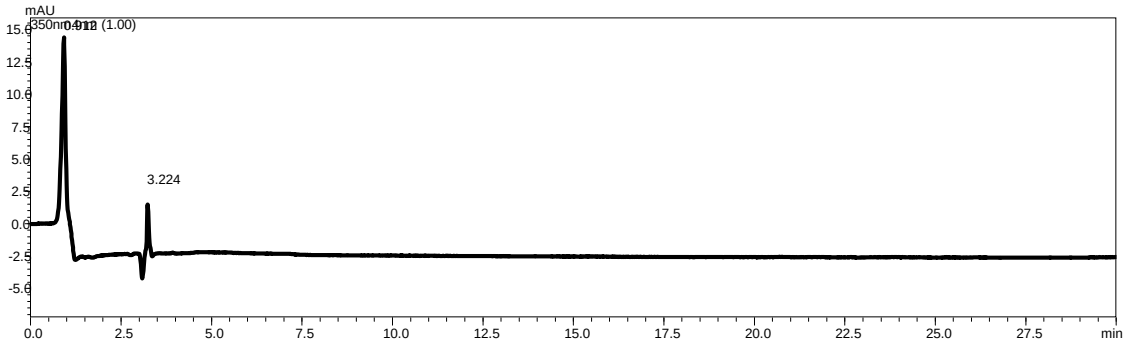


Female

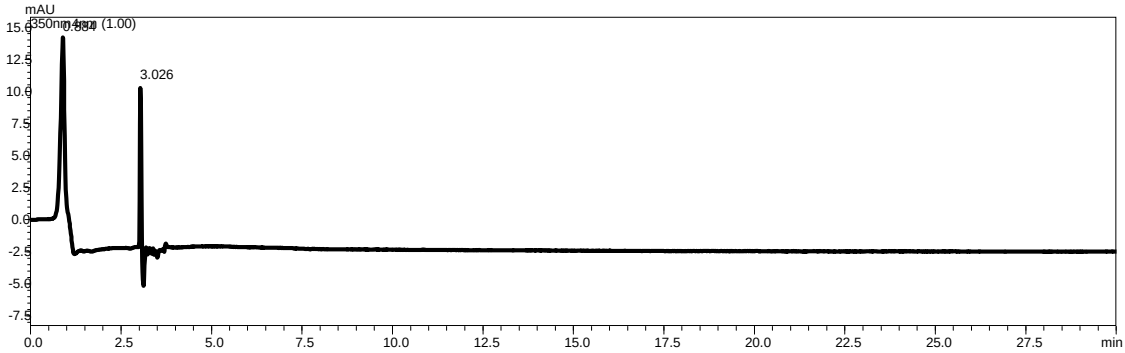


Male

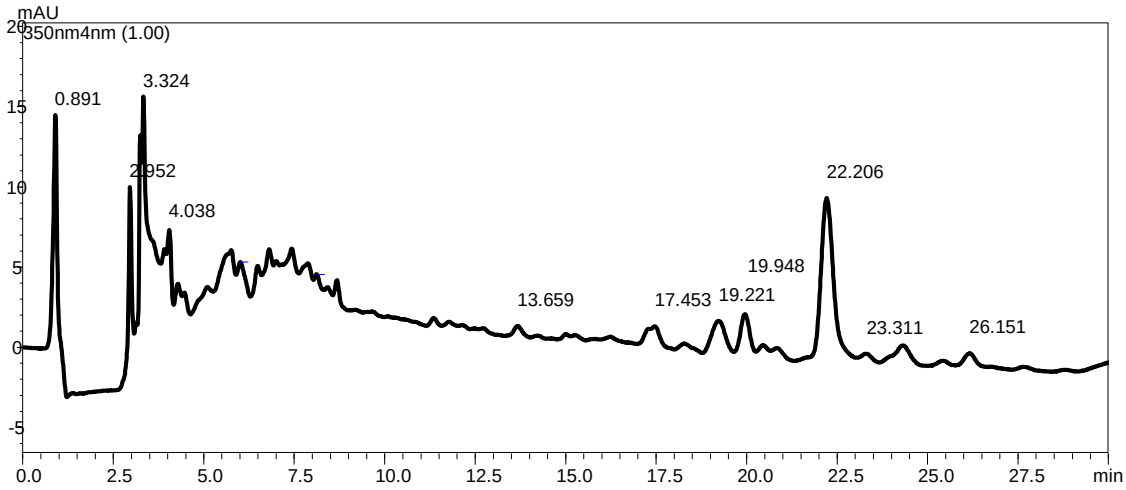
Figure 6.5: Representative HPLC profiles of methanolic extracts from faecal matter of female and male *C. cosyra*.



Distilled water (Control 1)

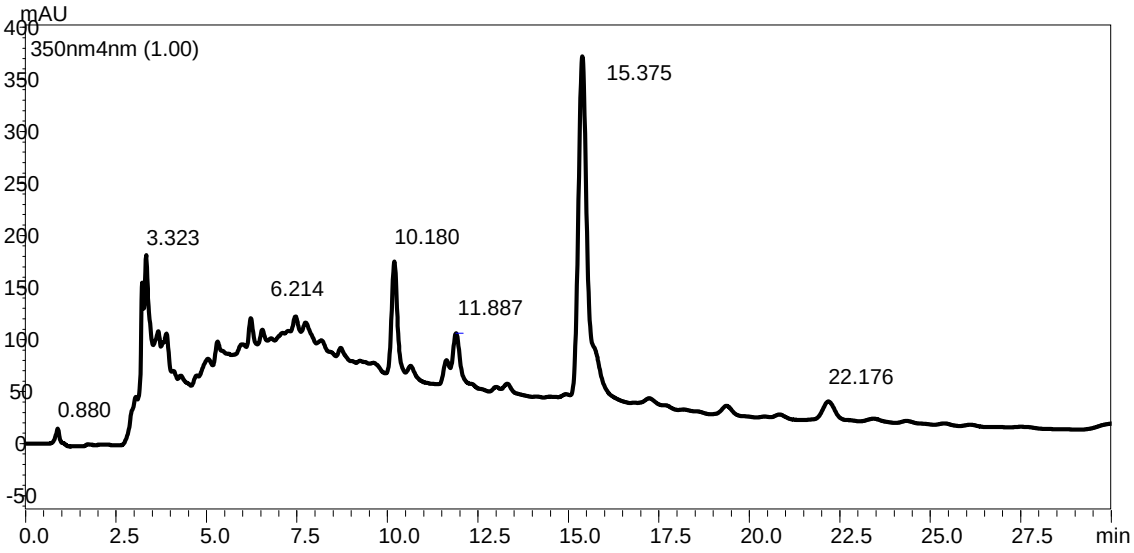


Methanol (Control 2)

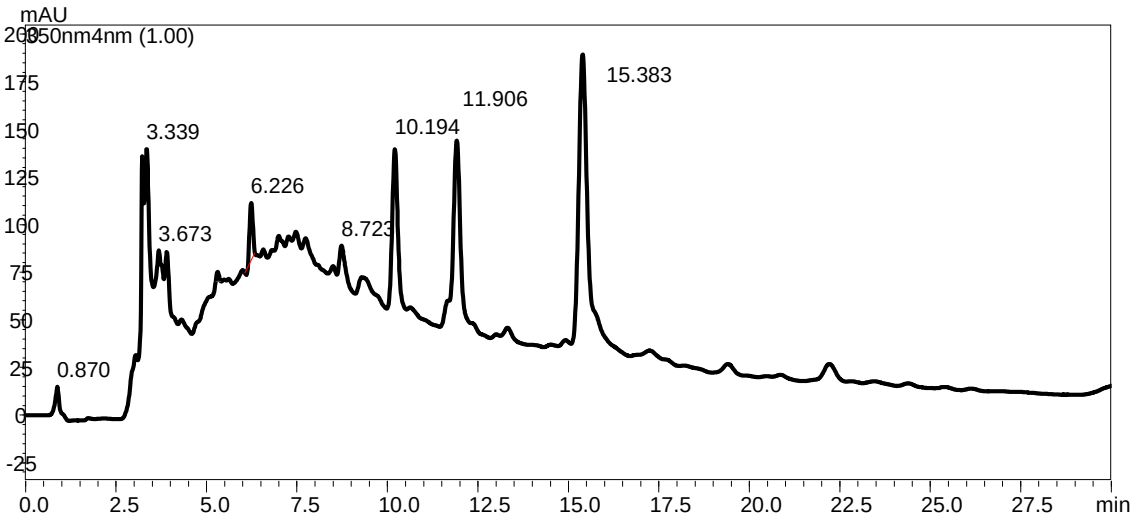


Fruit fly diet extract (aqueous)

Figure 6.6: Representative HPLC profiles of distilled water, methanol and aqueous extracts from fruit fly diet as controls for water-soluble fly-produced chemical components found in faecal matter of female and male *C. fasciventris* and *C. cosyra*.

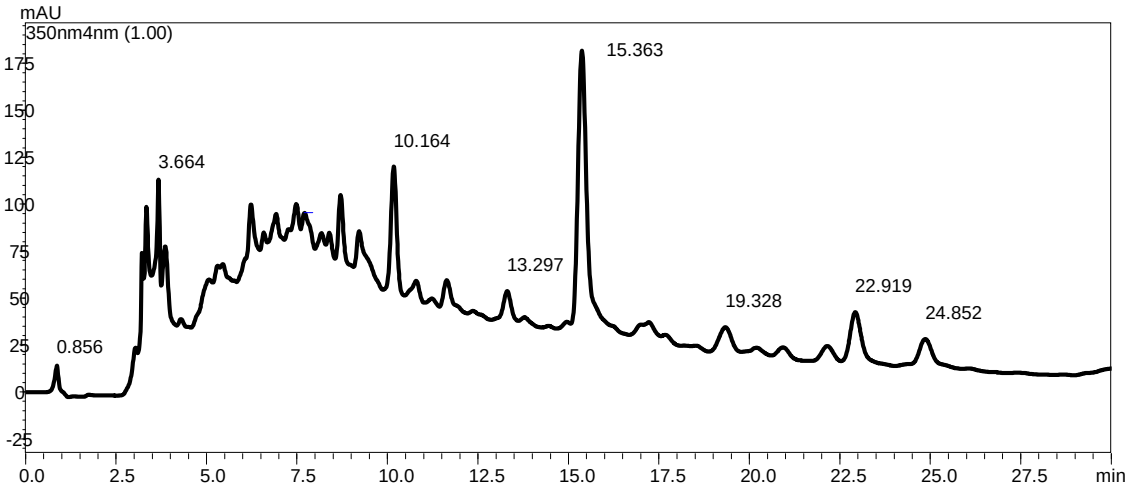


Female

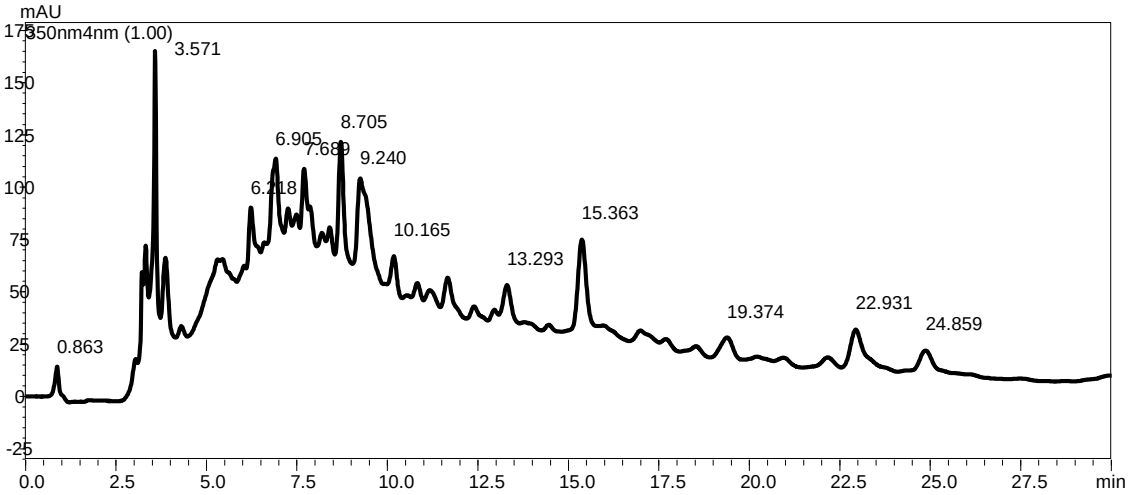


Male

Figure 6.7: Representative HPLC profiles of aqueous extracts from faecal matter of female and male *C. fasciventris*



Female



Male

Figure 6.8: Representative HPLC profiles of aqueous extracts from faecal matter of female and male *C. cosyra*.

6.3.2 Behavioural activity of a potential host-marking pheromone of *C. cosyra* against conspecifics

The potential host-marking pheromone was successfully isolated (Figure 6.9). The potential host-marking pheromone elicited significant conspecific oviposition deterrence with mean deterrent efficacy of 70% (χ^2 test, df = 1, $P < 0.001$).

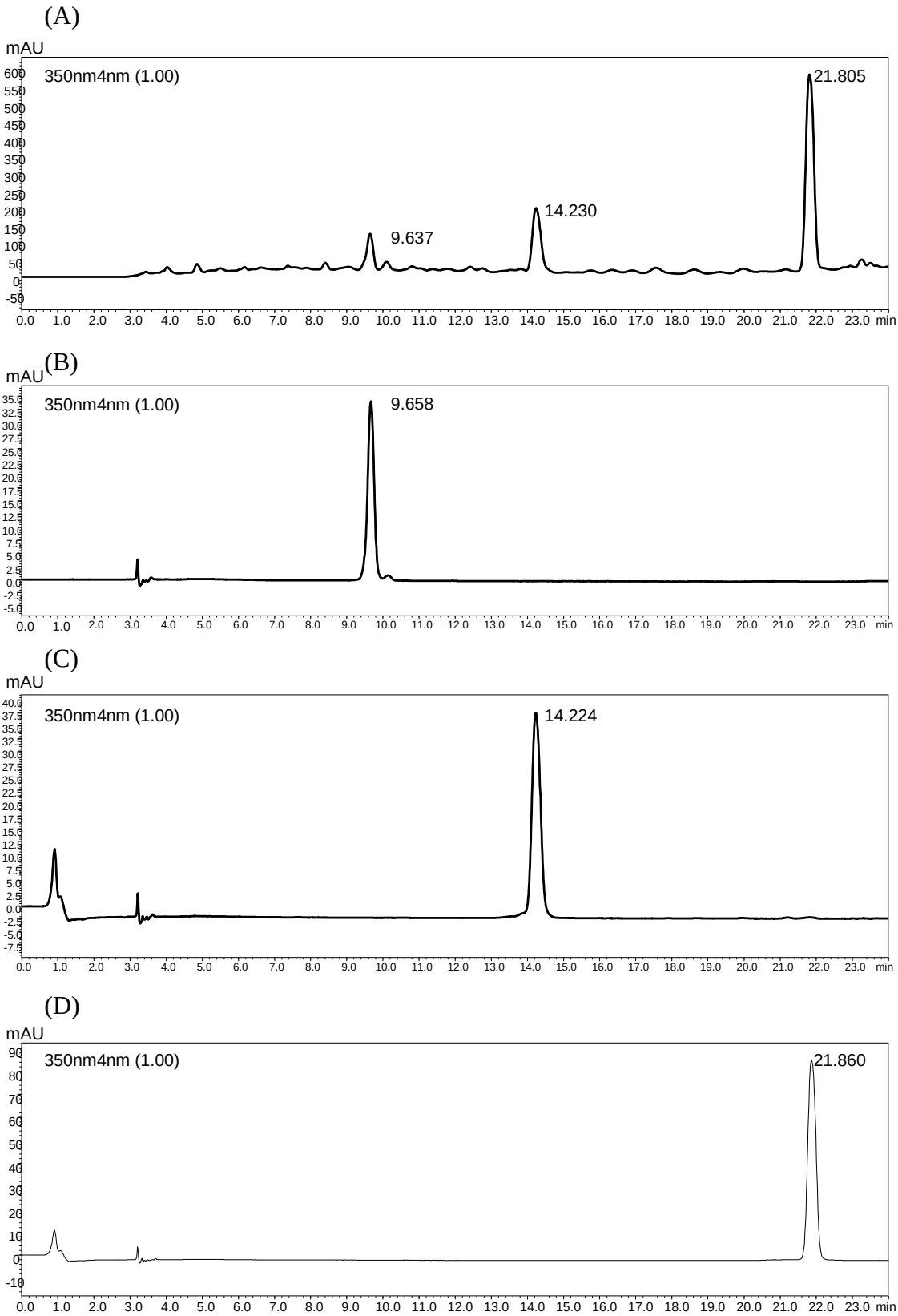


Figure 6.9: Isolation of potential host-marking pheromone of *C. cosyra* (A) parent profile, (B) peak 10, (C) peak 15, (D) potential host-marking pheromone (peak 24)

6.4 Discussion

It is likely that some, if not all, of the fly-produced chemical components observed in methanolic extracts from faecal matter of the flies are their host-marking pheromones for the following reasons:

1. The fruit flies (species) actively engage in host-marking behaviour after oviposition (Figure 4.1) and their host-marking behaviour deters at least conspecific or heterospecific oviposition (Table 5.1). This, therefore, associates the flies with production and deposition of host-marking pheromones on their hosts after oviposition as it has been observed to be the case in other fruit fly species (Nufio and Papaj, 2004; Arredondo and Diaz-Fleischer, 2006).

2. For fruit fly species which produce host-marking pheromones, these pheromones are also found in their faecal matter such that an aqueous or methanolic solution of their faecal matter elicits oviposition deterrence if applied on oviposition substrates (Arredondo and Diaz-Fleischer, 2006; Aluja *et al*., 2009). Faecal matter of each of the fruit fly species (*C. capitata*, C. *fasciventris*, *C. rosa* and *C. cosyra*) deterred at least conspecific or heterospecific oviposition (Table 5.2). This, therefore, suggested presence of host-marking pheromones of the fruit flies in their faecal matter.

3. Fruit fly host-marking pheromones are water- and methanol-soluble (Hurter *et al*., 1987; Aluja *et al*., 2009). In this investigation, all the observed

chemical components have been extracted with methanol, meaning that they are soluble in methanol (Figures 6.1 - 6.5). The chemical components have also been found to be soluble in water (Figures 6.6 - 6.8). The solubility of these chemical components in both methanol and water, therefore, further suggests them to be the host-marking pheromones of the fruit flies.

4. The chemistry of the observed chemical components is to some extent dependent on fruit fly species because despite being fed on the same diet and reared in the same conditions, the fruit fly species consistently produced different chemical components, particularly *C. capitata*, *C. fasciventris* and *C. rosa* on one hand and C. *cosyra* on the other (Figures 6.1 – 6.8). This difference in chemistry may therefore mean that chemical components are of specific use, probably host-marking.

5. For *C. capitata*, which was used as a check in this investigation, it is already known that its host-marking pheromone is water- and methanol-soluble and also present in faecal matter of the fruit fly (Arredondo and Diaz-Fleischer, 2006). In this investigation, the observed potential host-marking pheromones of *C. capitata* are also soluble in methanol (Figure 6.2). It is therefore likely that at least one of the chemical components observed in methanolic extracts from faecal matter of *C. capitata* is really the host-marking pheromone of the fruit fly species.

6. In the particular case of *C. cosyra*, the fact that the potential host-marking pheromone deterred conspecific oviposition (section 6.3.2) suggests the chemical component to be really the host-marking pheromone or a component of the host-marking pheromone of the fruit fly species.

The similarity in faecal extract profiles of *C. capitata*, *C. fasciventris* and *C. rosa* may mean that the species have a common host-marking pheromone or their pheromones are chemically similar. A generic host-marking pheromone, 2-(2 ', 14'-Dimethyl-pentadecanoylamino)-pentanedioic acid, has already been reported for some species of the Genus *Anastrepha* (Aluja *et al.,* 2003).

The presence of the unique chemical component, which eluted at 24 minutes, in the extract from faecal matter of *C. cosyra* may mean that the chemistry of the host-marking pheromone of this species is different from that of the marking pheromones of *C. capitata*, *C. fasciventris* and *C. rosa*. However, the presence of the two chemical components, which eluted at 10 and 15 minutes, in extracts from faecal matter of all the four fruit fly species may also indicate some similarity in the chemistry of the host-marking pheromones of all the species.

Since in both host-marking and faecal matter experiments, C. cosyra was not deterred by any other species except itself (Tables 5.1 and 5.2) and the only difference observed between faecal matter extracts of *C. cosyra* and the other species is that faecal matter of *C. cosyra* has a unique chemical component which eluted at 24 minutes, it may mean that this unique chemical component is the host-marking

pheromone of *C. cosyra*. This is further supported by the fact that in a behavioural assay, the chemical component elicited conspecific oviposition deterrence (section 6.3.2). In the same line of thinking, possibly, faecal matter of *C. cosyra* was able to deter the other species (Tables 5.1 and 5.2) because it has the two chemical components which eluted at 10 and 15 minutes respectively, which are also found in faecal matter of those species. Still, it could be that the other species are also sensitive to the unique chemical component of found in the faecal matter of *C. cosyra*.

The presence of the potential host-marking pheromones in extracts from faecal matter of male flies may mean that the males also produce these chemicals but they "waste" them in faeces since they are not involved in oviposition. This could be so because even in female fruit flies, host-marking pheromones are not produced by a special organ but the posterior half of the midgut (Averill and Prokopy, 1989), an organ which is also found in male flies. In addition, Prokopy (1982) observed that faecal matter of male *R. pomonella* also elicits conspecific oviposition deterrence. Furthermore, Brevault and Quilici (1999) observed that usually host-marking pheromones in insects are not special chemical compounds but simply by-products of the process of digestion.

6.5 Conclusion

In conclusion, this investigation established that faecal matter of female *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* each contains three unknown fly-produced methanol-soluble chemical components which may be host-marking pheromones of the fruit flies. However, all the chemical components in faecal matter of *C. capitata*, *C. fasciventris* and *C. rosa* seem to be chemically similar while for *C. cosyra* two of the components seem to be those found in faecal matter of *C. capitata*, *C. fasciventris* and *C. rosa* but the third one is specific to it.

CHAPTER SEVEN

7.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

The finding that among the major fruit fly species infesting mango in Kenya, host-marking behaviour is prevalent in *C. capitata*, *C. cosyra*, *C. fasciventris* and *C. rosa* is significant as a preliminary indicator for potential of the host-marking technique in the management of these fruit fly species. This is so because host-marking behaviour is not a universal phenomenon among fruit fly species, it occurs in some species and lacks in others (Fletcher and Prokopy 1991; Nufio and Papaj, 2004; Duyck *et al*., 2006), thus for fruit fruit fly species which do not have this behaviour it may be difficult, if not impossible, to manage them using the host-marking technique.

By establishing that the host-marking behaviour of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* deters at least conspecific or heterospecific oviposition, this study hints further to potential of the host-marking technique in the management of these pests. This is so because in some fruit fly species, host-marking behaviour is ceremonial, no pheromone is deposited and consequently the behaviour does not deter oviposition (Nufio and Papaj, 2004). For fruit fly species whose host-marking behaviour is ceremonial, it may be difficult, if not impossible, to manage them using the host-marking technique since there is no marking pheromone which can be exploited. The deterrent effect of the host-marking behaviour each species on at least

conspecific or heterospecific oviposition therefore suggests that indeed these species produce and deposit on their hosts, host-marking pheromones which may be exploited against them.

The finding that faecal matter of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* deters at least conspecific oviposition also hints towards potential of the host-marking technique in the management of these pests. This is so because for most fruit fly species whose host-marking behaviour deters oviposition and has consequently been exploited for the management of the pests through the host-marking technique, their marking pheromones have been found in their faecal matter, which also deters oviposition if applied on oviposition substrates due to the marking pheromones it contains (Averill and Prokopy, 1989; Aluja *et al*., 2003; Arredondo and Diaz-Fleischer, 2006; Aluja *et al*., 2009). The ability of the faecal matter of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* to deter at least conspecific oviposition therefore suggests presence of host-marking pheromones of the fruit flies in their faecal matter.

The finding of some unknown water- and methanol-soluble chemical components in faecal matter of *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra*, which may be the host-marking pheromones of the flies is another hint towards potential of the host-marking technique in the management of these fruit fly species. This is so because host-marking pheromones of fruit flies are soluble in water and methanol and found in faecal matter of the flies (Boller and Hurter, 1985; Hurter *et al*., 1987; Averill and Prokopy, 1987; Aluja *et al*., 2003: Aluja *et al*., 2009). This finding, therefore,

suggests the possibility of isolating and identifying host-marking pheromones of the fruit flies from their faecal matter as it has been done for other host-marking fruit fly species (Aluja *et al*., 2003; Arredondo and Diaz-Fleischer, 2006; Aluja *et al*., 2009).

Since among the four host-marking species, *C. cosyra* is the most destructive on mango (Rwomushana *et al*., 2009) and it can only be deterred by its host-marking behaviour (Table 5.1) and faecal matter (Table 6.1), which are also effective on some of the other species (Tables 5.1 and 6.1), it may be necessary in the quest to develop a host-marking management technique against these fruit flies to prioritize the search and research on the host-marking pheromone of this species. Considering that *C. cosyra* was not deterred by the host-marking behaviour or faecal matter of any other species except itself (Tables 5.1 and 5.2) and the only difference observed between faecal matter extracts of *C. cosyra* and the other fruit fly species is that faecal matter of *C. cosyra* has a unique chemical component which eluted at 24 minutes, this chemical component may be the host-marking pheromone of *C. cosyra* and it may be the one to be prioritized.

Although host-marking behaviour has been found to be rare in *B. invadens* and *C. anonae*, if the host-marking pheromone of *C. cosyra* or that of any species is discovered, it may be necessary to test it on these two species as well since some species which do not have the host-marking behaviour seem to recognize host-marking pheromones of other species (Duyck *et al*., 2006). This may be particulary necessary on *B. invadens* because it is the most destructive fruit fly species on mango in Kenya and Africa at large (Rwomushana *et al*., 2009).

7.2 Conclusions

This study has established that among the major fruit fly species infesting mango in Kenya, host-marking behaviour is prevalent in *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* and faecal matter of these fruit flies contains some chemical components which may be their host-marking pheromones. These findings indicate apparent potential of the host-marking technique in the management of some of the major fruit fly species infesting mango in Kenya.

7.3 Recommendations for future research

The following recommendations are drawn from this study for future research:

1. Isolation and behavioural activity testing of the observed potential host-marking pheromones across all the species, with priority on peak 24 of *C. cosyra*)
2. Chemical identification of the behaviourally active peaks that may be found in recommendation 1.
3. Procurement and testing of standards of the behaviourally active peaks identified in recommendation 2 (and their analogues) for behavioural activity in laboratory, semi-field and field experiments at various doses across all the six fruit fly species.

REFERENCES

AlliNiazee, M. T. 1988. Introduction. In: AliNiazee M. T. (Ed.). *Ecology and management of economically important fruit flies*: A special report of the Oregon State University Agricultural Experiment Station. pp.118.

Allwood, A. J. 1996. Biology and ecology: Prerequisites for understanding and managing fruit flies (Diptera: Tephritidae) pp 95-101 in Allwood, A. J. and Drew, R. A. I. (Eds.). *Proceedings of a Regional Symposium on Management of Fruit Flies in the Pacific,* Nadi, 28 - 31 October 1996, Australian Centre for International Agricultural Research.

Aluja, M. and Boller, E. F. 1992. Host-marking pheromones of *Rhagoletis cerasi*: field deployment of synthetic pheromones as a novel cherry fruit fly management strategy. *Entomologia Experimentalis et Applicata*, **65**: 141 – 147.

Aluja, M. and Diaz-Fleischer, F. 2006. Foraging behaviour of *A. ludens, A. obliqua* and *A. serpentina* in response to faeces extracts containing host-marking pheromones. *Journal of Chemical Ecology*, **32**: 367 - 389.

Aluja, M. and Prokopy, R. J. 1993. Host odour and visual stimulus interaction during intratree host finding behaviour of *Rhagoletis pomonella* flies. *Journal of Chemical Ecology,* **19**: 2671 – 2686.

Aluja, M., Diaz-Fleischer, F., Boller, E. F., Hurter, J., Edmunds A. J. F., Hagmann, L., Patrian, B. and Reyes, J. 2009. Application of faeces extracts and synthetic analogues of the host-marking pheromone of *Anastrepha ludens*

significantly reduces fruit infestation by *A. obliqua* in tropical plum and mango backyard orchards. *Journal of Economic Entomology*, **102**: 2268 - 2278.

Aluja, M., Diaz-Fleischer, F., Edmunds, A. J. F. and Hagmann, L. 2003. Isolation, structural determination, synthesis, biological activity and application as control agent of the host-marking pheromone (and derivatives thereof) of the fruit flies of the type *Anastrepha* (Diptera: Tephritidae). United States Patent No. US 6 555 120 BI. 30p.

Angermann, H. J. 1986. Ecological differentiation of the tephritid flies *Xyphosia miliaria* and *Orellia ruficauda* (Diptera: Tephritidae) in flowerheads of Canada thistle (*Cirsium arvense*). *Entomologia Generalis*, **11**: 249 – 262.

Arredondo, J. and Diaz-Fleischer, F. 2006 Oviposition deterrents for the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae) from fly faeces extracts. *Bulletin of Entomological Research,* **96:** 35 – 42.

Augstburger, F., Berger, J., Censkowsky, U., Heid, H., Milz, J. and Streit, C. 2001. Organic farming in the tropics and subtropics: Mango (2nd Ed.). Naturland e.V., Germany.

Averill, A. L. and Prokopy, R. J. 1982. Oviposition-deterring fruit-marking pheromone in *Rhagoletis zephyria*. *Journal of the Georgia Entomological Society*, **17**: 315 – 319.

Averill, A. L. and Prokopy, R. J. 1987. Residual activity of oviposition deterring pheromone in *Rhagoletis pomonella* (Diptera, Tephritidae) and female response to infested fruit. *Journal of Chemical Ecology,* **13:** 167 – 177.

Averill, A. L. and Prokopy, R. J. 1989. Host-marking Pheromones. In: Robinson, A. S. and Hooper, G. (Eds.). *World Crop Pests: Fruit Flies - Their Biology, Natural Enemies and Control*, Elsevier, New York. pp. 207 – 219.

Bally, I. S. E. 2006. *Mangifera indica* (mango). In: Elevitch, C. R. (Ed.). Species profiles for Pacific Island Agroforestry. Permanent Agriculture Resources (PAR), Hawaii.

Barr, N. B., Copeland, R. S., De Meyer, M., Masiga, D., Kibogo, H. G., Billah, M. K., Osir E., Wharton R. A. and McPheron, B. A. 2006. Molecular diagnostics of economically important *Ceratitis* fruit fly species (Diptera: Tephritidae) in Africa using PCR and RLFP Analyses. *Bulletin of Entomological Research*, **96:** 505 - 521.

Billah, M. K., Mansell, M. W., De Meyer, M. and Goergen, G. 2007. Fruit fly taxonomy. In: Ekesi, S. and Billah, K. (Eds.). *A field guide to the management of economically important tephritid fruit flies in Africa*, ICIPE Science Press, Nairobi, Kenya. pp. H-1 - I-4.

Bissdorf, J. 2005. Field guide to non-chemical pest management in mango production. Pesticide Action Network. Hamburg, Germany.

Boller, E. F. 1981. Oviposition deterring pheromone of the European cherry fruit fly: status of research and potential applications. In: Mitchell E. R. (Ed.).

Management of Insect Pests with Semiochemicals. Plenum Press, New York, pp. 457 – 462.

Boller, E. F. and Calkins, C. O. 1984. Measuring, monitoring and improving the quality of mass-reared Mediterranean fruit flies, *Ceratitis capitata*: Improvement of quality by selection. *Journal of Applied Entomology*, **98**: 1 - 15.

Boller, E. F. and Hurter, J. 1985. Oviposition deterring pheromone in *Rhagoletis cerasi*: Behavioural laboratory test to measure pheromone activity. *Entomologia Experimentalis et Applicata*, **39**: 163 – 169.

Bompard, J. M 2009. Taxonomy and Systematics. In: Litz, R. E. (Ed.). *The Mango: Botany, Production and Uses*, 2nd Ed. CAB International, Wallingford, UK. pp. 19 – 41

Brevault, T. and Quilici, S. 1999. Factors affecting behavioural responses to visual stimuli in the tomato fruit fly, *Neoceratitis cyanescens*. *Physiological Entomology*, **24**: 333 – 338.

Bronson, C. H. 2006. Fruit fly pests: A brochure. Florida Department of Agriculture and Consumer Services. pp. 1 - 2.

Brunner, J. F. 1992. Biology and distribution of the western and black cherry fruit flies. In: AliNiazee, M.T. (Ed.). *Biology and control of the cherry fruit flies: A world perspective*. Department of Entomology, Oregon State University. pp. 25 – 30.

Canadian Grain Commission. 2006. Managing the quality of stored grain: Preventing insect infestation. www.grainscanada.gc/Entomology/identify01-e.htm Retrieved: 5/05/2012.

Copeland, R. S., Wharton, R. A., Luke, Q, De Meyer, M. 2002. Indigenous hosts of *Ceratitis capitata* (Diptera:Tephritidae) in Kenya. *Annals of the Entomological Society of Ammerica*, **95**: 672 – 694.

Copeland, R. S., Wharton, R. A., Luke, Q., De Meyer, M., Lux, S., Zenz, N., Machera, P., and Okumu, P. 2006. Geographic distribution, host fruits, and parasitoids of African fruit fly pests: *Ceratitis anonae*, *Ceratitis cosyra*, *Ceratitis fasciventris* and *Ceratitis rosa* (Diptera: Tephritidae) in Kenya. *Annals of the Entomological Society of America*, **99**: 261 - 278.

Crnjar R. M., Prokopy, R. J. and Dethier, V. G. 1978. Electrophysiological identification of oviposition-deterring pheromone receptors in *Rhagoletis pomonella*. *Journal of the New York Entomological Society*, **86**: 283 – 284.

Crnjar, R. M. and Prokopy, R. J. 1982. Morphological and electrophysiological mapping of tarsal chemoreceptors in *Rhagoletis pomonella* flies. *Journal of Insect Physiology*, **28**: 393 – 400.

De Meyer M and **Freidberg A. 2006.** Revision of the subgenus *Ceratitis* (*Pterandrus*) (Diptera, Tephritidae). *Israel Journal of Entomology*, **35:** 197 – 315.

Dicke, M., Van Loon, J. J. A. and De Jong, P.W., 2004. Ecogenomics benefits community ecology. *Science*, **305**: 618 - 619.

Dowell, R. V. and Wange, K. L. 1986. Process analysis and failure avoidance in fruit fly programs. In: Mangel, M. Carey, J. and Plant, R. Pest (Eds.). *Control Operations and Systems Analysis in Fruit Fly Management*. Springer-Verlag, Berlin.

Drew, R. A. I. and Romig, M. C. 2007. Records of Dacine fruit flies and new species of Dacus (Diptera: Tephritidae) in Bhutan. *The Raffles Bulletin of Zoology,* **55**: 1 - 21.

Duyck, P. F., David, P. and Quilici, S. 2004. A Review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephridae) *Ecological Entomology,* **29:** 511 – 520.

Duyck, P. F., David, P., Junod, G., Brunel C., Dupont, R. and Quilici, S. 2006. Importance of competition mechanisms in successive invasions by polyphagous tephritids in La Reunion. *Ecology,* **87**: 1770 – 1780.

Ekesi, S and Muchugu, E. 2007. Tephritid fruit flies in Africa – Fact sheets of some economically important species. In: Ekesi, S. and Billah, K. (Eds.). *A field guide to the management economically important tephritid fruit flies in Africa* (2nd edition) ICIPE Science Press, Nairobi, Kenya.

Ekesi, S., Billah, M. K., Nderitu, P. W., Lux, S. A. and Rwomushana, I. 2009. Evidence for competitive displacement of *Ceratitis cosyra* by the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) on mango and mechanisms contributing to the displacement. *Journal of Economic Entomology,* **102**: 981 - 991.

Ekesi, S., Dimbi S. and Maniania, N. K. 2007b. The role of entomopathogenic fungi in the integrated management of fruit flies (Diptera: Tephritidae) with

emphasis on species occurring in Africa. In: Ekesi, S., Maniania, N. K. (Eds.). *Use of Entomopathogenic Fungi in Biological Pest Management*. Research SignPost, Kerala, India, pp. 239 - 274.

Ekesi, S., Mohamed, S. A., Hanna, R., Lux, S. A., Gnanvossou, D. and Bokonon-Ganta, A. 2007a. Fruit fly suppression – Purpose, tools and methodology. In: Ekesi, S. and Billah, K. (Eds.). *A field guide to the management economically important tephritid fruit flies in Africa* (2nd edition) ICIPE Science Press, Nairobi, Kenya.

Ekesi, S., Nderitu, P. W. and Rwomushana, I. 2006. Field infestation, life history and demographic parameters of the fruit fly *Bactrocera invadens* (Diptera: Tephritidae) in Africa. *Bulletin of Entomological Research,* **96**: 379 - 386.

Evenden, M. and Haynes, K. 2001. Potential for the evolution of resistance to pheromone-based mating disruption tested using two pheromone strains of the cabbage looper, *Trichoplusia ni*. *Entomologia Experimentalis et Applicata,* **100**: 131 - 134.

Fischer-Colibrie, P. and Busch-Peterson, E. 1989. Pest status: Temperate Europe and West Asia. In Robinson, A. S. and Hooper, G. (Eds.). *World crop pests, volume 3A: Fruit flies - their biology, natural enemies and control*. Elsevier, Amsterdam.

Fitt, G. P. 1984. Oviposition behaviour of two tephritid flies *Dacus tyroni* and *Dacus jarvisi*, as influenced by the presence of larvae in the host fruit. *Oecologia*, **62**: 37 – 46.

Fletcher, B. S. and Prokopy, R. J. 1991. Host location and oviposition in tephritid fruit flies. In: Bailey, W. J. and Ridsdill-Smith J. (Eds.). *Reproductive Behaviour of lnsects: Individuals and Populations*. Chapman and Hall, New York. pp. 139-171.

Forest, A. R. and Perry, R. N. 2006 Nematode behaviour and sensory perception In: Perry, R. N. and Moens M. (Eds.) *Plant Nematology*. CABI Publishing, UK. pp. 210 - 233.

Foster, S. P. and Harris, M. O. 1997. Behavioural manipulation methods for insect pest management. *Annual Reviews of Entomology,* **42**: 123 – 146.

Giga, D. P. and Smith, R. H. 1985. Oviposition markers in *Callosobruchus maculatus* F and *Callosobruchus rhodesianus* Pic (Coleoptera: Bruchidae): asymmetry of interspecific responses. *Agriculture Ecosystems & Environment,* **12**: 229 – 233.

Griesbach, J. 2003. Mango growing in Kenya. Kul Graphics, Nairobi, Kenya. 144p.

Hancock D. L. 1989. Pest Status of Fruit Flies in Southern Africa. In: Robinson, A. S. and Hooper, G. (Eds.). *World crop pests: Fruit flies - Their biology, natural enemies and control*, Elsevier, New York. pp. 51 – 58.

Hurter, J, Boller, E. F., Stadler, E., Blattman, H., Buser, R., Boshard, N. U., Damm, L., Kozlowski, M. W., Schoni, R., Raschdorf, F., Dahinden, R., Schlumpf, E., Fritz, H., Richter, W. J. and Schreiber, J. 1987. Oviposition-deterring pheromone in *Rhagoletis cerasi* L.: Purification and determination of the chemical constitution. *Experientia,* **43**: 157 - 164.

ICIPE. 2007. Development and implementation of a sustainable IPM program for major mango pests and opportunity for improving market information and processing in sub-Saharan Africa – A fact sheet. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH.

Jackson, D. S. and Lee, B. G. 1985. Medfly in California 1980-1982. *Bulletin of the Entomological Society of America*, **31**: 29 - 37.

Katsayannos, B. I. 1975. Oviposition-deterring, male arresting, fruit-marking pheromone in *Rhagoletis cerasi*. *Environmental Entomology*, **4**: 801 – 807.

Katsayannos, B. I. and Boller E. F. 1980. Second field application of oviposition deterring marking pheromone of the European cherry fruit fly, *Rhagoletis cerasi* L. (Diptera: Tephritidae). *Zeitschrift fur Angewandte Entomologie*, **96**: 83 - 93.

Katsayannos, B. I. and Boller, E. F. 1976. First field application of oviposition deterring marking pheromone of the European cherry fruit fly. *Environmental Entomology*, **5**: 151 – 152.

Kehlenbeck, K., Rohde, E., Njuguna, J. K., Omari F., Wasilwa, L. and R. Jamnadass R. 2011. Mango cultivar diversity and its potential for improving mango productivity in Kenya. World Agroforestry Centre ICRAF, Tree Genetic Resources and Domestication, Nairobi, Kenya.

Kratt, A. 2001. Pheromones - A long way from identification to application. In: Nogueira, M. A. S. and M. Palmerio (Eds.). *Practice oriented results on use and production of plant extracts and pheromones in integrated and biological pest*

control: Proceedings of the 2nd workshop "Neem and Pheromones" held at University of Uberaba, Brazil, 15 – 16th May 2001.

Kuba, H. and Koyama, J. 1985. Mating behaviour of wild melon flies *Dacus cucurbitae* Coquillett (Diptera: Tephritidae) in a field cage: Courtship behaviour. *Applied Entomology and Zoology,* **20:** 365 – 372.

Liquido, N. J. 1991. Fruits on the ground as a reservoir of resident melon fly populations in papaya orchards. *Environmental Entomology*, **20**: 620 - 625.

Lux, S. A., Ekesi, S., Dimbi, S., Mohamed, S. and Billah, M. 2006. Mango-infesting fruit flies in Africa: Perspectives and limitations of biological approaches to their management. ICIPE, Nairobi, Kenya.

McClure, M., Quiring, D. T. and Turgeon, J. J. 1998. Proximate and ultimate factors influencing oviposition site selection by endoparasites on conifer seed cones: two sympatric dipteran species on larch. *Entomologia Experimentalis et Applicata,* **87**: 1 – 13.

Morton, J., 1987. Mango. In: Julia, F. and Morton, J. (Eds.). *Fruits of Warm Climates*, Miami, Florida. pp. 27 – 56.

Mukiama, T. K. and Muraya, J. K. 1994. Ceratitid fruit flies infesting fruit crops in Kenya. *Insect Science and its Application*, **15:** 155 - 159.

Mumtaz, M. M. and AlliNiazee, M. T. 1983. The oviposition-deterring pheromone of the western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera.: Tephritidae).

Chemical characterization and partial purification. *Zeitschriftfur Angewandte Entomologie,* **96**: 93 – 99.

Nemeye, P. 2003. The behavioural ecology of three African fruit fly species, *Ceratitis cosyra*, *C. fasciventris* and *C. capitata*, with emphasis on their sexual behaviour and host plant relationships. Ph. D. thesis. Makerere University.

Nufio, C. R. and Papaj, D. R. 2001. Host-marking behavior in phytophagous insects and parasitoids. *Entomologia Experimentalis et Applicata,* **99**: 273 – 293.

Nufio, C. R. and Papaj, D. R. 2004. Host-marking behaviour as a quantitative signal of competition in the walnut fly *Rhagoletis juglandis. Ecological Entomology*, **29**: 336 – 344.

Nufio, C.R., Papaj, D.R. and Alonso-Pimentel, H. **2000.** Host utilization by the walnut fly, *Rhagoletis juglandis* (Diptera: Tephritidae). *Environmental Entomology*, **29:** 994 – 1001.

Ole-Moi Yoi, O. K. and Lux, S. A. 2002. Fruit flies in sub-Saharan Africa: A long-neglected problem devastating local fruit production and a threat to horticulture beyond Africa In: Barnes, B. N. (Ed.). *Proceedings of the 6th international symposium on fruit flies of economic importance. Stellenbosch, South Africa*, 6th – 10th May 2002. Isteg Scientific Publications, Cape Town , South Africa pp. 5 – 10.

Papaj, D. R. 1994. Use and avoidance of occupied host as a dynamic process in tephritid flies. In: Bernays, E. A. (Ed.). *Insect–plant interactions,* vol. 5. CRC Press, Boca Raton, Florida. pp. 25 – 46.

Papaj, D. R. and Aluja, M. 1993. Temporal dynamics of host-marking in the tropical tephritid fly, *Anastrepha ludens*. *Physiological Entomology*, **18:** 279 - 284.

Papaj, D. R., Averill, A. L., Prokopy, R. J, and Wong, T. T. Y. 1992. Host-Marking pheromones and use of previously established oviposition sites by the Mediterranean fruit fly (Diptera: Tephritidae) *Journal of Insect Behaviour*, **5**: 583 – 598.

Papaj, D. R., Roitberg, B. D. and Opp, S. B. 1989 Serial effects of host infestation on egg allocation by the Mediterranean fruit fly: A rule of a thumb and its functional significance. *Journal of Animal Ecology,* **58**: 955 – 970.

Parfonry, R. 2001. Mango. In: Raemaekers R. H. (Ed.). *Crop Production in Tropical Africa*. Goenkit Graphics nv. Belgium. pp. 596 – 609.

Plant Health Australia 2011. *The Australian handbook for the identification of fruit flies. Version 1.0.* Plant Health Australia. Canberra, ACT.

Porter, B. A. 1928. The apple maggot. *United States Department of Agriculture Technical Bulletin,* **66**: 1 – 47.

Prokopy, R. J. 1972. Evidence for a marking pheromone deterring repeated oviposition in apple maggot flies. *Environmental Entomology*, **1**: 326 - 332.

Prokopy, R. J. 1975. Oviposition deterring fruit marking pheromone in *R. fausta*. *Environmental Entomology*, **4:** 298 – 300.

Prokopy, R. J. and Koyama, J 1982. Oviposition site partitioning in *Dacus cucurbitae*. *Entomologia Experimentalis et Applicata*, **31**: 428 -432.

Prokopy, R. J. and Roitberg, B. D. 1984. Foraging behaviour of true fruit flies (Tephritidae). *American Scientist*, **72**: 41 – 49.

Prokopy, R. J., Aluja, M. and Wong, T. T. Y. 1989. Foraging behaviour of laboratory –cultured Mediterranean fruit flies on field-caged host trees. *Proceedings of the Hawaiian Entomological Society*, **29**: 103 - 110.

Prokopy, R. J., Reissig, W. H. and Moericke, V. 1976. Marking pheromones deterring repeated oviposition in *Rhagoletis flies*. *Entomologia Experimentalis et Applicata*, **20**: 170 – 178.

Prokopy, R. J., Ziegler, J. R. and Wong T. Y. 1978. Deterrence of repeated oviposition by fruit-marking pheromone in *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Chemical Ecology*, **4**: 55 – 63.

Prokopy, R., Greany, P. D. and Chambers, D. L. 1977. Oviposition-deterring pheromone in *Anastrepha suspensa*. *Environmental Entomology*, **6**: 463 – 465.

Quilici, S., Rivry, L. and Rossolin, G. 1994 Visual stimuli influencing the choice of oviposition site in *Ceratitis rosa* Karsch (Diptera: Tephritidae). In: Yong, H.S and Khoo, S.G. (Eds.). *Proceedings of a symposium on tropical fruit flies - Current research on tropical fruit flies and their management* pp. 9–21. Kuala Lumpur, Malaysia. Eagle Trading Sdn., Bhd., Petaling, Jaya, Malaysia.

Roitberg, B. D. 2007. Why pest management needs behavioural ecology and vice versa. *Entomological Research*, **37**: 14-18.

Roitberg, B. D. and Lalonde, R. G. 1991 Host-marking enhances parasitism risk for a fruit-infesting fly *Rhagoletis basiola*. *OIKOS*, **61**: 389 – 393.

Roitberg, B. D. and Prokopy R. J. 1983. Host deprivation influence on response of *Rhagoletis pomonella* to its oviposition-deterring pheromone. *Physiological Entomology*, **8**: 69 – 72.

Rwomushana, I., Ekesi, S., Gordon, I. and Ogol, K. P. O. 2008. Host plants and host plant preference studies for *Bactrocera invadens* (Diptera: Tephritidae) in Kenya, A new invasive fruit fly species in Africa. *Annals of the Entomological Society of America*, **101:** 331 - 3340.

Rwomushana, I., Ekesi, S., Ogol, C. K .P. O. and Gordon I. 2009. Mechanisms contributing to the competitive success of the invasive fruit fly *Bactrocera invadens* over the indigenous fruit fly, *C. cosyra*: the role of temperature and resource pre-emption. *Entomologia Experimentalis et Applicata*, **133**: 27 - 37

Shelly, T. E. 1999. Defence of oviposition sites by female oriental fruit flies (*Bactrocera dorsalis*) (Diptera: Tephritidae) University of Hawaii.

Sivinski, J. and Burk, T. 1989. Reproductive and mating behaviour. In: Robinson, A. S. and Hooper, G. (Eds.). *World crop pests: Fruit flies - Their biology, natural enemies and control Volume 3*, Elsevier, New York. pp. 343 – 351.

Sivinski, J. M., and Calkins, C. 1986. Pheromones and parapheromones in the control of Tephritids. *Florida Entomologist,* **69**: 157 - 168.

Smith, P. 1989. Behavioural partitioning of the day and circadian rhythmicity. In: Robinson, A. S. and Hooper, G. (Eds.). *World crop pests: Fruit flies - Their biology, natural enemies and control,* Elsevier, New York. pp. 325 – 341.

Stadler, E., Ernest, B., Hurter, J., Boller, E. & Kozlowski, M. 1992. Tarsal contact chemoreceptors of the cherry fruit fly, *Rhagoletis cerasi*: specificity, correlation with oviposition behaviour, and response to the synthetic pheromone. In: Menken, S. B. J., Visser, J. H. and Harrewijn, P. (Eds). *Proceedings of the 8th Symposium of Insect–Plant Relationships*. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 143 – 145.

Stadler, E., Ernst, B., Hurter, J. and Boller, E. 1994. Tarsal contact chemoreceptor for the host-marking pheromone of the cherry fruit fly, *Rhagoletis cerasi*: responses to natural and synthetic compounds. *Physiological Entomology*, **19**: 139 - 151.

Van Mele, P., Vayssieres, J. F., Adandonon, A. and Sinzogan, A. 2009. Ant cues affect the oviposition behaviour of fruit flies (Diptera: Tephritidae) in Africa. *Physiological Entomology*, **34:** 256 – 261.

Van Mele, P., Vayssieres, J. F., Van Tellingen, E. and Vrolijks, J. 2007. Effects of an African weaver ant, *Oecophylla longinoda*, in controlling mango fruit flies (Diptera: Tephritidae) in Benin. *Journal of Economic Entomology*, **100**: 695 - 701.

Vargas, R. I., Peck, S. L., McQuate, G. T., Jackson, C. G., Stark J. D. and Armstrong J. W. 2001. Potential for areawide integrated management of the Mediterranean fruit fly (Diptera: Tephritidae) with a braconid parasitoid and a novel bait spray. *Journal of Economic Entomology,* **94**: 817 - 825.

Vet, L. E. M., 1996. Parasitoid foraging: The importance of variation in individual behaviour for population dynamics. In: Floyd, R. B., Sheppard, A.W. and De Barro, P. J. (Eds.). *Frontiers of population ecology*. CSIRO Publishing, Collingwood, pp. 245 - 256.

White, I. M. and Elson-Harris, M. M. 1992. Fruit flies of economic significance: Their identification and bionomics. CAB International, Wallingford, 602p.

Wyatt, D. T. 1997. Methods in studying insect behaviour. In: Dent D. R. and Walton, M. P. (Eds.). *Methods in ecological and agricultural entomology*. CAB International. pp. 27 – 50.