DEVELOPMENT AND IMPLEMENTATION OF PRE- AND POST-HARVEST MANAGEMENT MEASURES FOR *BACTROCERA DORSALIS* (HENDEL) AND *CERATITIS COSYRA* (WALKER) (DIPTERA: TEPHRITIDAE) ON MANGO IN KENYA

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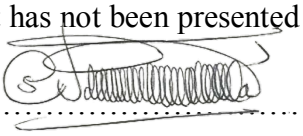
June 2017

DECLARATION

Candidate

I, Shepard Ndlela, hereby declare that this thesis is my original work and to the best of my knowledge, it has not been presented to any other examination body for award of a degree.

Signature:……  …… Date:13/06/2017……

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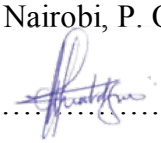
Signature:……  …… Date:13/06/2017……

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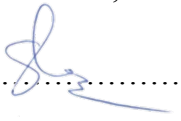
Signature:……  …… Date:13/06/2017……

DEDICATION

To my wife Lee, My son Thabiso Michael and daughter Sisasenkosi Amani Ndlela; for the endless love, support and encouragement in their own different special ways**,** when we were away from home.

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ABBREVIATIONS AND ACRONYMS

a.i	active ingredient
ACET	African Center for Economic Transformation
ANOVA	Analysis of variance
AW-IPM	Area Wide Intergrated Pest Management
CA	Controlled atmosphere
CABI	Centre for Agriculture and Bioscience International
CBD	Convention on Biological Diversity
DDT	Dichloro-diphenyl-trichloroethane
DNA	Deoxyribonucleic acid
EC	Emulsifiable Concentrate
EPPO	European and Mediterranean Plant Protection Organization
EU	European Union
FAO	Food and Agricultural Organisation of the United Nations
FF-ALPPs	Fruit flies-areas of low pest prevalence
FFEZ	Fruit Fly Exclusion Zone
FF-PFAs	Fruit fly-pest free areas

FTD	Flies per Trap per Day
HCDA	Horticultural Crops Development Authority
HORDI	Horticultural Crop Research and Development Institute
HWT	hot water treatment
IAEA	International Atomic Energy Agency
icipe	International Centre of Insect Physiology and Ecology
IITA	International Institute of Tropical Agriculture
IPM	Integrated Pest Management
IPPC	International Plant Protection Commission
ISPM	International Standards for Phytosanitary Measures
KEPHIS	Kenya Plant Health Inspectorate Service
KSTCIE	Kenya Standing Technical Committee on Imports and Exports
MA	Modified Atmosphere
MAT	Male Annihilation Technique
ME	Methyl eugenol
MRL	Maximum Residue Level
NPPOs	National Plant Protection Organisations

PAN–AP	Pesticide Action Network Asia and the Pacific
rANOVA	repeated measures analysis of variance
SIT	Sterile Insect Technique
SSA	Sub-Saharan Africa
ULO	Ultralow Oxygen
UNCTAD	United Nations Conference on Trade and Development
UNEP	United Nations Environment Programme
USA	United States of America
USDA-APHIS	United States Department of Agriculture-Animal and Plant Health Inspection Service
USDA-ARS	United States Department of Agriculture-Agricultural Research Service
US-EPA	United States Environmental Protection Agency
WG	Wettable granules
WTO	World Trade Organisation

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Mango, *Mangifera indica* L (Anacardiaceae) is an important tropical fruit providing opportunities for income generation and improvement of livelihoods of millions of farmers across Africa. However, fruit production is limited by several constraints; ranking high among these is the infestation by Tephritid fruit flies such as *Bactrocera invadens* and *Ceratitis cosyra*. Direct losses caused by these pests, range between 40 and 80% depending on mango variety, locality and season. In addition, quarantine restrictions on fruits from countries where these insects are reported, limit access to lucrative markets abroad, thus impacting negatively on export earnings. Thus pre-harvest management measures such as male annihilation technique (MAT), biological control using parasitoids and post-harvest measures using hot water treatment (HWT) were explored in this study. Male annihilation technique was carried out in mango orchards using methyl eugenol (ME) mixed with deltamethrin for the suppression of *Bactrocera dorsalis* in coastal Kenya. This resulted in 99.5% reduction in the *B. dorsalis* population and significant decline of between 18-25 times lesser infestations in the treatment plots compared to the untreated control. Biological control through the use of parasitoids to complement other existing management measures was also explored. The exotic egg parasitoid *Fopius arisanus* and the larval pupal parasitoid *Diachasmimorpha longicaudata* were released at a rate of 1500 wasps ha^{-1} at a ratio of 2: 1, ♀:♂ for the classical biological control of *B. dorsalis* in mango orchards in Eastern and coastal Kenya. Following sampling of fruits by collecting infested fruit to recover parasitoids, the highest percent parasitism recorded in the Coast and Embu were 33 and 8% for *F. arisanus*; 14 and 16% for *D. longicaudata* respectively. Both parasitoids were recovered 8 km from the central release point in Embu. Following reports that *D. longicaudata* had formed new associations with *C. cosyra*, experiments were conducted in the laboratory to investigate the

interaction between *D. longicaudata* and the native parasitoid *Psyttalia cosyrae* which parasitises *C. cosyra.* Tests were done under three scenarios of *B. dorsalis* only, *C. cosyra* only and mixed infestation of *B. dorsalis* and *C. cosyra.* Hosts were offered to sole, sequential and simultaneous combinations of parasitoids. *Diachasmimorpha longicaudata* was the most efficient parasitoid as shown by searching, probing and ovipositing events on all host combinations evaluated. Findings also indicated that there was strong possibility for the co-existence of the two parasitoids taking cognisance that *P. cosyrae* co-evolved with *C. cosyra* as its sole host. Finally, to facilitate access to export markets, a post-harvest protocol for HWT, to ensure quarantine security against *B. dorsalis*, was developed for "*Apple*"mango variety. Third instar larvae were found to be the most heat-tolerant infesting stage, followed by second and first instar larvae and the egg stage respectively. The duration at 46.1°C for Probit 9 level of mortality was estimated at 81.47 minutes (95% confidence level). There were no survivors in a validation treatment of this regime of 51,101 third instar larvae. In addition, exposure to 46.1°C for 68 minutes resulted in no survivors out of 44,651 third instar larvae. Overall findings of this study demonstrate the suppression of *B. dorsalis* using the MAT, and subsequent reduction in fruit damage by the pest. In addition, results indicate the successful release, establishment and subsequent dispersal of *F. arisanus* and *D. longicaudata* in Kenya. The exotic *D. longicaudata* was shown to be able to co-exist with the indeginous *P. cosyrae* without any detrimental effects. Results of the HWT also provide sound evidence for effective post-harvest disinfestations treatment against *B. dorsalis,* and may facilitate access to export markets for mango fruits from Africa. It is therefore recommended that MAT, Biological control using parasitoids and HWT be adopted within a holistic Integrated Pest Management (IPM) approach in the mango agro-system, preferably practiced over large areas to increase efficacy, especially regarding the pre-harvest techniques.

CHAPTER ONE

INTRODUCTION

1.1 Background

Fruits and vegetables provide an important source of income, nutrition and employment creation in most African countries (Lux *et al*., 2003a; Kenya Ministry of Agriculture, 2012; Selwyn, 2013). The employment created and the direct income to producers is key to poverty alleviation and enhances the health and well being of both consumers and producers (Ganry, 2009). Over the years, global trade of fruits and vegetables has been boosted by several factors such as consumer interest, rising individual incomes, technology advancement, continued decline in market value of traditional crops and year-round availability of most fruits and vegetables. This has made the enterprise one of the most dynamic sectors of international trade (Diop and Jaffee, 2005). World trade of fruits and vegetables appreciated from about US$75 billion in 2000 to over US$150 billion in 2010 (FAO, 2013). The bulk of the world's fruits and vegetables are produced in China (34%), Latin America (11%), the Caribbean (11%), India (10%), with Africa and the European Union contributing 9% each (Diop and Jaffee, 2005).

Mangoes, *Mangifera indica* L (Anacardiaceae), are among the most traded fruits in the world. Currently, Asia is the largest producer of mangoes, with a global production of 72%, followed by Africa (17%), Latin America (10%) and the rest of the world (1%) (UNCTAD INFOCOMM, 2016). Mango production has been growing over the years with India being the world's largest producer of mangoes followed by China, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria and the Philippines (FAO, 2014).

In Sub-Saharan Africa, a greater proportion of mango production is by smallholder farmers (ACET, 2012; Van Melle and Buschmann, 2013). In Kenya for example, small holder farmers produce 80% of the country's total mango production (HCDA, 2009). Their produce is usually consumed in local markets or is sold to middle men who in turn export regionally and globally.

Mango is widely produced in Kenya and ranks third after bananas and pineapples in terms of production (Kenya Ministry of Agriculture, 2012). Most of Kenyan mangoes are produced in the Coast, Eastern, Central and Rift valley Provinces, whereas Nyanza and Western provinces contribute a small fraction to the total production (Griesbach, 2003). The supply is almost year round and this positions Kenya as a potential exporter to Europe, Asia and other African countries where seasons are distinct and non-overlapping. In 2010, Kenya produced 550,000 metric tonnes of mangoes with 61% of this produce originating from smallholder farmers (ACET, 2012; Kenya Ministry of Agriculture, 2012). As a result, regional and international exports accounted for US$ 10.1 million in foreign currency earnings (Kenya Ministry of Agriculture, 2012).

Several factors affect mango production, and key among them are the fruit infesting Tephritids (Mohamed *et al*., 2007; Mwatawala *et al*., 2009b; Suliman *et al*., 2014). Over 40% of mangoes produced every year in Africa are lost due to infestation by fruit flies (Lux *et al*., 2003a). Enormous loses are reported in Kenya where 20-40% of mangoes are lost to fruit flies infestations every year (Lux *et al*., 1998; Ekesi *et al*., 2003). The invasion of Kenya by an alien invasive fruit fly, further compounded the problem (Lux *et al*., 2003b) escalating the loses to over 80% depending on locality, season and mango variety (Ekesi *et al*., 2006; Rwomushana *et al*., 2008). Two years later, this invasive species was described as *Bactrocera invadens* Drew, Tsuruta and White (Drew *et al*., 2005).

Recently, *B. invadens* was synonymised with the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) following several years of intense integrative morphological, molecular, cytogenetic, behavioural and chemoecological research (Bo *et al*., 2014; Schutze *et al*., 2014a; Schutze *et al*., 2014b).

Since its first detection in Africa, *B. dorsalis* continues to cause widespread damage to fruits thereby compelling countries importing fruits originating from regions where the pest has been reported to enforce quarantine restrictions on fruits (Ekesi *et al*., 2006; USDA-APHIS, 2008; Guichard, 2009; Otieno, 2011) resulting in further loses. For example, the annual monetary loss due to fruit flies infestation in Eastern and Southern Africa is estimated to be about US$6 million (Otieno *et al*., 2010). Following initial reports of *B. dorsalis* invasions, the United States of America (USA) through the U.S. Federal Order banned importation of most cultivated vegetables and fruits from countries where the pest had been reported (USDA-APHIS, 2008). On the other hand, the European Union also responded by enacting import tolerance legislation regulating pesticide residue in fruits and vegetables through the harmonised maximum pesticide residue levels (MRL) to ensure a high level of consumer protection (EU, 2005). As a consequence, Kenya continues to lose current and potential export markets in the USA and Europe. The list of African, American and European countries imposing export restrictions on Kenyan fruits is rising gradually and loses are enormous. For example, the country continues to lose at least US$2 million worthy of export earnings every year due to export restrictions imposed by South Africa on avocado (Otieno *et al*., 2010).

Ideally, effective fruit fly management can only be achieved through the application of several harmonised management options that are environmentally friendly and sustainable in reducing fruit fly infestations.

This in turn leads to quality production and improved access to domestic and international markets. Importing countries require tangible evidence that fruits and vegetables being imported are free from pests especially those that are exotic and are of high quarantine concerns. Many countries thus continue to prioritise fruit fly management at pre- and post-harvest levels (Drew, 1992). No single management option is adequate in dealing with the fruit fly menace. Hence several methods of fruit fly suppression and control are being promoted in Kenya. These include baiting techniques, male annihilation, biological control, orchard sanitation, mechanical fruit protection and early harvesting (Ekesi and Billah, 2007; Ekesi *et al*., 2011; Mohamed *et al*., 2012). This kind of systems approach is more effective than single bullet approaches (IPPC, 2012). Effective systems approach depends largely on the targeted fruit fly species, its host fruit and the size of the targeted production area. A combination of at least two or even more management options can be implemented at any given time as may be deemed necessary (IPPC, 2002). Traditionally, *Cerattitis cosyra* (Walker) has been the major pest of mango, guava as well as other wild and cultivated fruits in Kenya (Malio, 1979). However, the invasive *B. dorsalis* has since taken over as the major threat to fruit production and trade (Lux *et al*., 1998; Ekesi *et al*., 2009). Export restrictions are increasingly tightening on Kenyan mangoes as well as other fruits and vegetables. Between the 23rd and 31st of July 2013, a total of 46 interceptions of fruits and vegetables containing quarantine organisms were made at Heathrow and Gatwick Airports in the United Kingdom and 34 of these were contaminated with fruit flies (Food and Environment Reasearch Agency, 2013). The sources of the produce were mainly Pakistan, India, Jamaica, Malaysia, Colombia, Ghana and Kenya.

1.2 Problem statement

In the quest to reduce fruit fly damage and produce fruit fly free fruits, some farmers are still using broad spectrum pesticides such as Dimethoate and Monocrotophos. However, the enactment of legislation advocating against chemical residues in fruits and vegetables further compounded the problem of fruit exports. Thus non chemical methods have to be developed to curb the fruit fly menace.

Since *B. dorsalis* is an exotic pest to Africa and apparently lacking natural enemies in its invaded range, two parasitoid species, an egg parasitoid, *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) and a larval parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) were introduced into Kenya in 2009 for testing and final releases for *B. dorsalis* suppression (Mohamed *et al*., 2010). The egg parasitoid was evaluated and yielded positive results of *B. dorsalis* parasitisation (Mohamed *et al*., 2010). However, for those eggs that may escape parasitisation by the egg parasitoid, there is a need to release a complementary larval parasitoid, *D. longicaudata*, to achieve significant suppression of fruit flies in the field. Although several findings on the complementary nature of these two species have been documented in Hawaii (Harris *et al*., 2007), no similar studies have been carried out in Africa especially on *B. dorsalis* and *C. cosyra* on mango. Therefore, there is need to gather post-release information, especially on dispersal capacity and establishment in target environments.

Pre-harvest measures for fruit fly management hardly provide 100% control, especially in high infestation areas. To gain access to export markets, post harvest disinfestation measures need to be implemented to ensure that the exported fruits are free from fruit flies.

Cold disinfestation treatments against *B. dorsalis* in citrus and avocado have been developed to complement other management options (Grout *et al*., 2011, Ware *et al*., 2012). Hot water treatment has also been shown to be an effective disinfestation measure against some fruit fly species (Luaces *et al*., 2005). Harvesting and post harvesting techniques in Kenya still lag behind demands of the ever increasing stringent requirements of the intensely competitive regional as well as European markets. In this regard this work sought to address these research gaps and add knowledge to the management of *B. dorsalis* and *C. cosyra* through pre-harvest (biological control using parasitoids and male annihilation technique) and post-harvest (hot water treatment) options that are compatible with strategies already being implemented.

1.3 Hypotheses

The following were the hypotheses of the study:

1. The male annihilation technique does not suppress *B. dorsalis* population in the field.
2. *Diachasmimorpha longicaudata* and *F. arisanus* do not disperse nor establish following their release in the field.
3. There is no interaction between the introduced parasitoid *D. longicaudata* and the native parasitoid *Psyttalia cosyrae* in the laboratory.
4. No hot water treatment parameters can be developed for post harvest disinfestation of mango infested with *B. dorsalis.*

1.4 Objectives

The overall objective was to develop compatible IPM strategies for *B. dorsalis* and *C.cosyra* for sustainable mango production.

1.4.1 Specific objectives

This study had four specific objectives:

1. To determine *B. dorsalis* suppression in the field using the male annihilation technique.
2. To determine the establishment and dispersal of *D. longicaudata* and *F. arisanus* following field releases.
3. To determine the interaction between the introduced parasitoid *D. longicaudata* and the native parasitoid *Psyttalia cosyrae* in the laboratory.
4. To develop hot water treatment parameters for post harvest disinfestation of mango infested with *B. dorsalis.*

1.5 Justification

The alien invasive pest *B. dorsalis,* has greatly devastated the horticultural industry in Africa, and lacks indegenous natural enemies capable of controlling and managing its populations in the field. Therefore, it represents an ideal target for classical biological control. Bio-control effort through the use of parasitoids targeting both the egg and larval stages is paramount if substantial control is to be achieved. *Fopius arisanus* is an effective parasitoid that parasitises eggs of many fruit fly species including *B. dorsalis*. However, some eggs are able to escape parasitism thus warranting the need to target escapees at the larval stage. The release of *D. Longicaudata*, a larval parasitoid will ultimately complement the action of the egg parasitoid in the management of *B. dorsalis*. Single bullet management options are seldom effective, thus male annihilation and baiting technique are some of the strategies evaluated in this study. These activities also go beyond pre-harvest management measures by developing hot water treatment parameters for *B. dorsalis* to allow export of mangoes to quarantine sensitive markets abroad.

This study explores biological control, male annihilation, as well as hot water treatment as possible systems approach strategies in fruit fly management.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mango production and trade

Mango is considered one of the most important fruits of the world in terms of area under production and its value (Griesbach, 2003; Asif *et al.*, 2011). The native home of mango is tropical Asia, which generates about 70-77% of the world's total production (UNCTAD, 2012). Globally, the crop is widely grown in the tropical and subtropical lowlands and is fast becoming a significant source of foreign exchange for some countries (Jedele *et al*., 2003; Asif *et al*., 2011). India and China are the world's top mango producing countries with Nigeria being the only African country in the coveted top ten (Figure 2.1; FAO, 2013). In 2009, world production of tropical fruits stood at 82.2 million tonnes of which 31.7 million tonnes (39%) was mango (FAO, 2009). Mexico is the leading exporter of mango with a global share of 10.3%, followed by Philippines (7.8%), Pakistan (7.6%), Brazil (6.0%) and India (5.2%) (FAO, 2012).

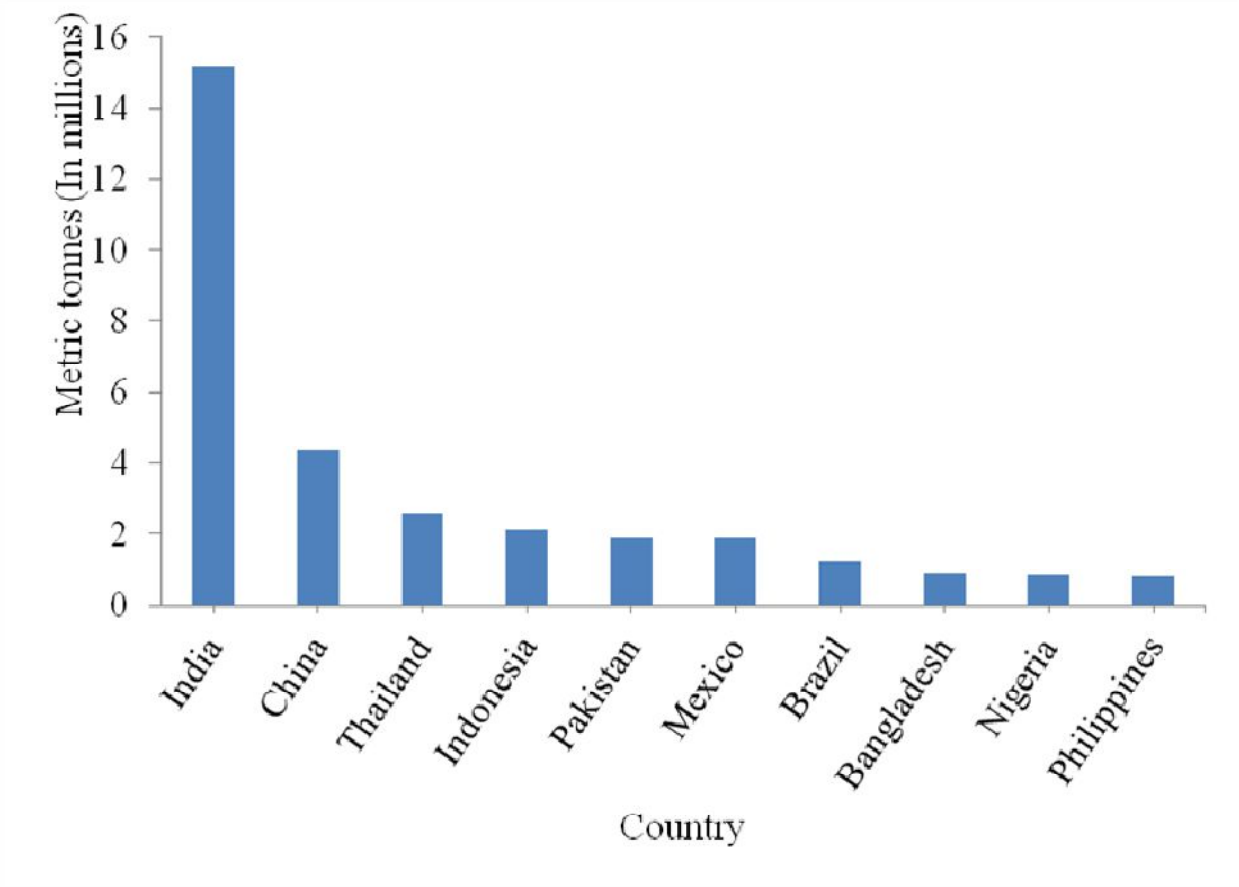

Figure 2.1: The world's top 10 mango producing countries in 2011 (FAO, 2013).

Mango was first introduced to East Africa in the early 14th century (Griesbach, 2003; HCDA, 2011). Due to the Indian Ocean trade that connected Africa to Asia during this era, ivory traders probably introduced it to the coastal fringes of Kenya. Thereafter, its cultivation spread inland to areas such as Kerio valley, Makueni, Meru, Mbeere, Kitui, Machakos, Lower Embu, Taveta, Isiolo, Kajiado, Thika, Murang'a and Lake Victoria basin (HCDA, 2011). These areas are characterised by favourable climatic conditions conducive for cultivation of the mango crop. Mango production in Kenya usually reaches its peak between October and February with an off-peak season from May-October (FAO, 2009; Gitika and Hawkins, 2011). The Coastal region normally has two peak seasons between November and February, while the Eastern and Central

provinces usually supply the market in February and March (Gitika and Hawkins, 2011).

The horticultural sub-sector in Kenya comprises mainly fruits, vegetables and cut flowers, of which mango is one of the key fruits. In 2009, the sub-sector exported 360,474 metric tonnes, generating in excess of Kshs 72 billion (approximately US$900 million) (Ministry of Agriculture, 2010) of which fruits accounted for 12. 5%. Kenyan mangoes are largely consumed locally and only 2% is exported to regional and international markets (Kenya Ministry of Agriculture, 2012). In 2010, the domestic market raked in approximately US$70 million compared to US$10.1 million earned from export. The area under mango production continues to increase and is mainly planted with local varieties (e.g. "*Ngowe*", "*Boribo*", "*Batawi*", "*Dodo*") as well as improved varieties (e.g. "*Apple*", "*Tommy Atkins*", "*Kent*", "*Van Dyke*") (Ouma, 2007). The European Union markets prefer "*Kent*", "*Van Dyke*", and "*Tommy Atkins*", while "*Ngowe"* and "*Apple"*, are more popular with Middle East importers (HCDA, 2011). However, the increased area under cultivation and increased yields from adoption of higher yielding varieties and exports to regional and lesser stringent Middle East markets particularly Saudi Arabia have not necessarily resulted in increased export earnings. Indeed, the loss of the U.S.A and European Union markets have resulted in further decline in export earnings (FAO, 2006). Several factors are responsible for Kenyan mangoes being unable to compete effectively in the world market key among them being high levels of pest infestation (Otieno *et al*., 2010). Ranking high among the pests are fruit flies particularly *Bactrocera dorsalis* (Lux *et al*., 2003a; Mohamed *et al*., 2010).

2.2 Tephritid fruit fly species of ecomomic importance in Africa

Tephritid fruit flies occur globally and pose a major threat to production in fruit and vegetable cropping systems (White and Elson-Harris, 1992).

They comprise 4800 documented species, and are present in all regions of the world except the Antarctica (White and Elson-Harris, 1992; Norrbom, 2004). Approximately 50 of the 1000 species native to Africa are known to cause significant economic damage (De Meyer *et al*., 2007). They attack wild as well as cultivated fruit and have invaded regions far beyond their native areas (White and Elson-Harris, 1992; Mohamed *et al.,* 2006). Five major Tephritid genera are known to be key pests of fruits (White and Elson-Harris 1992). *Ceratitis* spp and *Dacus* spp: are native to tropical Africa while *Bactrocera* spp originated from tropical Asia, Australia and South Pacific regions (White and Elson-Harris 1992). *Anastrepha* spp widely occurs in South and Central America as well as the West Indies while *Rhagoletis* spp are indigenous to South and Central America, North America and Europe. However, these traditional boundaries have since been usurped due to global trade. Many Tephritids are now established in new areas where they have since assumed a new pest status due to the absence of natural enemies and thus impacting negatively on production, markets, and existing ecological balances of indigenous species (Duyck *et al.*, 2006; Jang, 2007). Currently, four *Bactrocera* species are known to have invaded Africa from Asia causing significant direct and indirect damage (De Meyer *et al*., 2007). These include *Zeugodacus cucurbitae (*Coquillett) (formerly *B. cucurbitae*)*, B. dorsalis, Bactrocera latifrons* (Hendel*)* and *Bactrocera zonata* (Saunders). *Bactrocera dorsalis* together with the native *C. cosyra* are the major pests of mango in Kenya (Ekesi *et al*., 2006).

Ceratitis cosyra, commonly known as the mango or marula fruit fly, occurs widely in Tropical Africa (White and Elson-Harris, 1992). It has a limited host range among which includes mango, guava (*Psidium guajava* L), sour orange (*Citrus aurantium* L), marula (*Sclerocarya birrea* Hochst.), wild apricot (*Landolphia* sp) and wild custard apple (*Annona senegalensis* Pers.).

However, mango is the most preferred host (Ekesi *et al*., 2006; Rwomushana, 2008; Mwatawala *et al*., 2009b). Its status as a major pest of mango has gained momentum partly due to the increasing and expanding mango production by smallholder and commercial farmers in sub-Saharan Africa especially in Kenya, Tanzania, Uganda, South Africa, Zambia and Zimbabwe (Malio, 1979; EPPO⁄ CABI, 1996; Steck, 2000). Wherever this pest occurs together with *B. dorsalis*, combined infestation can be as high as 80% on mango (Ekesi *et al*., 2006).

Bactrocera dorsalis belongs to the highly destructive genus, *Bactrocera,* which contains over 450 species most of which are notable pests in horticulture (Abd-El-Samie and El –Fiky, 2011). It is a polyphagous invasive fruit fly pest belonging to a group of high-risk quarantine frugivorous fruit flies in the sub family Dacinae called the "*B. dorsalis* complex"(Clarke *et al*., 2005; Drew *et al*., 2005; Goergen *et al*., 2011). It originated from Southern Asia where it is a pest of economic importance particularly in Sri Lanka (Drew *et al*., 2005; Khamis *et al*., 2009). The pest was first reported in coastal Kenya in 2003 and by 2008 it had spread to at least 20 African countries (Lux *et al*., 2003b; Rwomushana *et al*., 2008). In 2011, twelve new *B. dorsalis* records were reported in West and Central Africa alone bringing the total in that region to 22 affected countries. It was estimated that the pest had spread over a distance of 5,000 km representing a "contiguous area of 8.3 million square km" from the North to the South of the region emphasizing the mobility of the pest (Goergen *et al*., 2011).The pest has a high intrinsic rate of increase coupled with an equally high net reproductive rate which ensures that it overwhelms the invaded area and soon becomes established in a short space of time (Salum *et al*., 2013). It is upon this premise that it continues to spread rapidly to a wide range of ecological and climatic zones and even potentially to subtropical regions (Geurts *et al.,* 2014).

It attacks a wide range of host plants and has impacted heavily on fruit and vegetable production in most African countries (Mwatawala *et al*., 2009b).

2.3 Impact of fruit flies infestation

From 2003, the fruit fly problem in Kenya for which *C. cosyra* was responsible, became further compounded by the arrival of *B. dorsalis*. In addition to direct losses, indirect losses through trade restrictions imposed by importing countries impacted negatively on export earnings. For example, export restrictions imposed in 2008 on mangoes originating from Coastal Kenya resulted in revenue losses of approximately US$ 1 million affecting thousands of smallholder farmers (Ekesi and Mohamed, 2010). Following initial reports of the invasion, the United States of America through the U.S. Federal Order banned importation of most cultivated vegetables and fruits from African countries where *B. dorsalis* had been reported (USDA-APHIS, 2008). On the other hand the European Union also responded by enacting import tolerance legislation regulating pesticide residue in fruits and vegetables to ensure a high level of consumer protection (EU, 2005). Thus Kenya continues to lose current and potential export markets in the USA and Europe. The EU interceptions of African mangoes due to phytosanitary regulations on non-European Tephritidae is tightening. Interception of mangoes due to fruit flies was at 21 rejections in 2008 increasing to 38 by August 2009 (Guichard, 2009). The ripple effects of the fruit fly menace continue to have wide reaching social and economic implications on millions of rural and urban populations involved in mango production and trade.

2.4 Management of fruit flies

Fruit fly management basically falls into two broad catergories namely pre-harvest and post-harvest techniques.

Pre-harvest management techniques are aimed at minimising damage to the product and subsequently suppressing populations in the field while post-harvest techniques supplement the former by disinfesting the fruit or vegetables thereby satisfying quarantine requirements usually of importing countries. Pre-harvest techniques are the most widely employed and there are four strategies mainly used namely suppression, containment, eradication and exclusion (IPPC, 2006; 2008; 2012). Their ultimate aim is to establish fruit fly-pest free areas (FF-PFAs), areas of low pest prevalence for fruit flies (FF-ALPPs) and to develop fruit fly systems approaches through integration of various techniques or strategies (IPPC, 2006; 2008; 2012). The objective of each strategy is to reduce the fruit fly population in an infested area to a level below an economic threshold (suppression), to prevent the spread of the fruit fly from an infested area to an adjacent FF-PFA (containment), to eliminate a fruit fly population from an area (eradication) and to prevent the introduction of a fruit fly into a FF-PFA (exclusion) (IPPC, 2013).

In many parts of the world, blanket cover sprays of synthetic insecticides have traditionally been used for the control of fruit flies. However reliance on these pesticides has not resulted in the total eradication of the pests. Rather the impact has instead been a myriad of problems such as unwanted residues on produce, contamination of the environment, insecticide resistance, human health issues and decline in non target organisms (Guamán, 2009). Concerns about the impacts of pesticides in food and the environment have seen much political and economic pressure being applied in the quest to minimise the use of pesticides and thus develop products and practices that are friendly to the user, consumer and the environment (Rebek *et al*., 2012). Several studies on native and exotic fruit fly species in Africa and other agro-ecological zones in Latin America and the South Pacific have indicated that management of fruit fly species based on a single management technique is unlikely to be successful (Aluja *et al*., 1996; Lux *et al*., 2003a).

Most success stories of fruit fly suppression in the world were due to the use of multiple management techniques that were compatible and effective (Vargas *et al*., 2010a). This approach is widely referred to as Systems Approach or Integrated Pest Management (IPM).

Integrated pest management offers the best methods to improve the economies of production by reducing yield losses and enabling growers to comply with stringent quality standards of the export market (Aluja *et al*., 1996; Lux *et al*., 2003a). Several suppression techniques are currently being implemented against fruit flies and many more are being developed and modified to suit the requirements and objectives of farmers, consumers as well as quarantine authorities. Some of the options available include: application of baiting technique, male annihilation, sterile insect technique, orchard sanitation, and biological control as well as various methods that prevent or reduce damage to fruits such as fruit bagging, early harvesting and post harvest treatment (Ekesi and Billah, 2007). Success stories where these options have employed has been reported in Mauritius, where following the accidental introduction of *B. dorsalis* in 1996, successful containment was achieved through the combined use of bait sprays and male annihilation technique (Seewooruthun *et al*., 1997). Furthermore, a combination of sanitation, baiting, male annihilation, biological control and sterile insect technique achieved between 60-90% suppression of *Ceratitis capitata* (Wiedemann) and *B. dorsalis* in Hawaii (Vargas *et al*., 2009).

2.4.1 Pre- harvest management measures

2.4.1.1 Chemical control

A number of pesticides such as Dimethoate or Fenthion have been used by growers as cover sprays for fruit fly management in both small and large scale mango production with some level of control (Heather *et al*., 1987; Rwomushana, 2008).

The indiscriminate use of pesticides to control fruit flies, however, causes a serious ecological imbalance and triggers the population surge of other pests when natural enemies are eliminated, and also poses human health risks and serious environmental degradation (Mar and Lumyong, 2012).

For example the negative effect of pesticides on pollinators such as bees, wasps, beetles, ants, moths, bats, birds and butteflies is well documented (Bernal *et al*., 2010; Maini *et al*., 2010; Hopwood *et al*., 2012). The overuse and misuse of synthetic pesticides such as organophosphates and carbamates may also lead to insecticide resistance (Elliott *et al*., 1978; Mar and Lumyong, 2012). Moreover, increased public awareness of impact of pesticides on human health has resulted in closer scrutiny of pesticide residues in fruits and vegetables. Sensitive markets such as the European Union have enacted legislation on acceptable maximum residue levels of pesticides in plant products (EU, 2005). The United States of America through the US Environmental Protection Agency (US-EPA) also enforces the judicious use of pesticides because of residue effect on human health (US-EPA, 1997). However, such legislation is missing or poorly enforced in many African countries where ironically much of the production is consumed by the domestic market and often not meeting export requirements. Therefore, other non pesticide options for fruit fly control such as Sterile Insect Technique (SIT) are poised to gain more recognition and investment in the coming decades (Suckling *et al*., 2014). Moreover, increased cost-effectiveness and improvements to technologies are creating new opportunities for tactical combinations in IPM (Suckling *et al*., 2014).

2.4.1.2 Biological control

Biological control largely depends on deploying appropriate biological control agents that can reduce populations of a pest without imparting negative effects to the environment, humans and other non-target organisms (Mooney and Cleland, 2001). Many plant and animal species become invasive when they are introduced in new environments, often through trans-boundary trade and human movement of plant products (Mooney and Cleland, 2001).

The situation becomes more dire because specialized natural enemies that co-evolved with the pest in their regions of origin are absent in the new environment (Torchin *et al.,* 2003; Strayer *et al.,* 2006). A strategy of associating the pest again with its natural enemy therefore becomes an integral component of integrated pest management. Biological control agents include natural enemies, entomopathogenic fungi and predators.

2.4.1.2.1 Parasitoids

Hymenopteran parasitoids have been used widely for the management of economically important fruit fly species of the genera *Ceratitis*, *Anastrepha* and *Bactrocera* (Ovruski *et al.,* 2000; Montoya *et al.,* 2000; Mohamed *et al.,* 2008). They are the most preferred natural enemies for fruit fly suppression because of their host specificity and high parasitism rates (Sivinski, 1996; Sivinski *et al*., 1996; Vargas *et al.,* 2012). The most effective parasitoids belong to the family Braconidae, one of the largest among the Hymenoptera, containing more than 15,000 described species with more still being discovered (Quicke and van Achterberg, 1990; Wharton, 1993). The Opiinae is one of the most economically important subfamilies within the Braconidae (Carmichael *et al.,* 2005). It is a diverse group of koinobiont endoparasitoids of various cyclorrhaphous Diptera (Wharton *et al.,* 2012).

They constitute over 1500 described species wordwide and more than 100 have been reared from fruit-infesting Tephritidae (Wharton and Yoder, 2014).

Some of the Opiinae that have been effective in the control of fruit flies include *Diachasmimorpha longicaudata*, *Diachasmimorpha kraussii* (Fullaway), *Diachasmimorpha fullawayi* (Silvestri), *Biosteres tryoni* (Cameron), *Psyttalia fletcheri* (Silvestri), *Psyttalia humilis* (Silvestri), *Psyttalia concolor* (Szépligeti), *Psyttalia incise* (Silvestri), *F. arisanus*, *Fopius vandenboschi* (Fullaway), (Ramadan *et al.,*1989; Eben *et al.,* 2000; Sivinski *et al.,* 2000a; Wharton *et al.,* 2000; Vargas *et al.,* 2002; Bautista *et al.,* 2004; Mohamed *et al.,* 2008; Ovruski *et al.,* 2011; Yokoyama *et al.,* 2012; Bokonon-Ganta, 2013; Shariff *et al.,* 2014). Among these, *F. arisanus* and *D. longicaudata* are the most widely used parasitoid species.

Fopius arisanus is a solitary egg-pupal endoparasitoid, indigenous to the Indo-Australian region but is now found in many parts of the world (Perez *et al.,* 2013). The parasitoid is known to parasitise at least 21 fruit fly species and is able to complete development in 18 species (Rousse *et al.,* 2005, 2006). It is one of the most effective parasitoids on *B. dorsalis*, *B. latifrons* and *C. capitata* (Zenil *et al.,* 2004; Vargas *et al.,* 2012; Bokonon-Ganta *et al.,* 2013). It is also competitively superior to most larval parasitoids such as *D. kraussii*, *P. concolor, F. vandenboschi, D. longicaudata* and *D. tryoni* (Wang and Messing, 2002). The success story of *F. arisanus* dates back to 1947 when it was released in Hawaii where it successfully established and caused significant suppression of *B. dorsalis* and *C. capitata* (Haramoto and Bess, 1970; Rousse *et al.,* 2006). Apart from Hawaii, *F. arisanus* has been released in many parts of the world with remarkable success (Ovruski *et al.,* 2000; Vargas *et al.,* 2007; Vargas *et al.,* 2012).

Diachasmimorpha longicaudata is the most widely used parasitoid in biological control programs of Tephritidae in North and South America as well as their associated islands (Meirelles, 2013). It has gained widespread usage in integrated pest management programs against fruit flies of the *Bactrocera*, *Anastrepha* and *Ceratitis* genera (Paladino *et al.,* 2010). The parasitoid's original home is the Indo-Pacific region but currently occurs in many parts of the globe as an introduced exotic parasitoid (Duan and Messing, 1997). It is a solitary, koinobiont parasitoid whose females continue to produce mature eggs throughout their entire life (Montoya *et al.,* 2000; Ovruski *et al.,* 2000; Meirelles, 2013). This is an evolutionary adaptation that ensures a successful parasitic life.

The parasitoid was successfully introduced into the Americas and soon became one of the most important parasitoids in the control of *Anastrepha* species and *C. capitata* (Montoya *et al.,* 2012; Suarez *et al.,* 2014). Once released and established *D. longicaudata* populations become self sustaining and persist in the system for many generations. According to Oroño and Ovruski (2007), the parasitoid was recovered in the North western region of Argentina 40 years later after its initial release. This demonstrates successful establishment and possibly widespread dispersal capacity, which is important for an effective natural enemy.

2.4.1.2.2 Fruit fly management using parasitoids in Kenya

Following the detection of *B. dorsalis* in Kenya in 2003, the first efforts were channelled towards finding an effective indigenous natural enemy against the pest. Acceptability studies using three native parasitoid species, *P. cosyrae, Psyttalia phaeostigma* (Wilkinson), (Hymenoptera: Braconidae) and *Tetrastichus giffardii* (Silvestri) (Hymenoptera: Eulophidae), parasitoids of *Ceratitis cosyra* (Walker) (Diptera: Tephritidae), *Dacus ciliatus* (Loew) and *Dacus bivittatus* (Bigot) respectively, were conducted to evaluate possible associations with the pest (Clausen *et*

al., 1965; Mohamed *et al.,* 2003; Mohamed *et al.,* 2006). Although *B. dorsalis* larvae were readily accepted by the above parasitoids, they were unable to complete development due to encapsulation by the host (Mohamed *et al.,* 2006; Mohamed *et al.,* 2010). The study showed that parasitoid immature stages could not evade the superior immune system of *B. dorsalis*. In essence, *B. dorsalis* was acting as an ecological reproductive sink for the indigenous generalist parasitoids thereby likely to reduce their population (Mohamed *et al.,* 2010). Field collected host fruits did not reveal any parasitoid to be effective against this pest (Rwomushana *et al.,* 2008). Thus in the absence of any promising natural enemy locally, *icipe* initiated an exploration mission to Sri Lanka the putative aboriginal home for *B. dorsalis* and its co-evolved biological agents. Among the promising parasitoid species identified were four Braconids: *D. longicaudata, Psyttalia incisi* (Silvestri)*, F. arisanus, Fopius* sp; one Eulophid, *Tetrastichus* sp; one pteromalid, *Spalangia* sp and one diapriid, *Trichopria* sp (Billah *et al.,* 2008). Subsequently two of the above species, *F. arisanus* and *D. longicaudata* were introduced into Kenya. Country-wide releases of the two parasitoids has been done in Central, Eastern, Rift valley and Coastal provinces of Kenya as part of an overall IPM programme for fruit flies in Kenya.

2.4.1.2.3 Entomopathogenic fungi

Several species of entomopathogenic fungi have been commercialised for insect pest management, mainly strains of *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii* and *Isaria fumosorosea* (Mahmoud, 2009; Khashaveh *et al.,* 2011a and b; El-Hawary and Abd El-Salam, 2009). The mode of action by entomopathogenic fungi involves infecting, invading and ultimately killing the host insect purely as a survival mechanism (de Bekker *et al.,* 2013). The relative effectiveness of entomopathogenic fungi thus makes them ideal candidates for IPM programs. The effectiveness of fungal isolates in the control of fruit flies such as *B.*

dorsalis, *C. capitata, C. fasciventris, C. cosyra*, *Anastrepha ludens* (Loew) is well documented (Ekesi *et al.,* 2002; Ekesi *et al.,* 2007a; Toledo *et al.,* 2007). Apart from mycosis of the larvae, pupa and adult flies, entomopathogenic fungi have been shown to be effective in long term suppression of flies through reduced fecundity and fertility (Dimbi *et al.,* 2013; Sookar *et al.,* 2014). Suppression of *B. dorsalis* and *C. cosyra*, the two important fruit fly pests of mango in Kenya was demonstrated using soil inoculation and autodissemination of *M. anisopliae* in mango orchards (Ekesi *et al.,* 2007a).

Deployment of the fungi in the field has been achieved using autodissemination devices, combining conidia with fruit fly attractants in baiting stations and inoculation of soil at strategic places targeting fruit fly adults as well as pupariating larvae and puparia respectively (Ekesi *et al.,* 2007a; Pell *et al.,* 2010).

2.4.1.2.4 Predatory ants

The use of predators in biological control has not gained widespread research and application in Africa compared to Asia despite their great potential and effectiveness in fending off pests especially on tree crops (Van Mele *et al.,* 2007; Van Mele *et al.,* 2009). Predators such as weaver ants are potential control agents that are naturally available or could be introduced as effective natural enemies in the management of insect pests through predation, physical deterrence or indirectly when pests seek to avoid territories marked by ant chemical trails (Offenberg *et al.,* 2004; Van Mele 2008a; Van Mele *et al.,* 2009a; Seguni *et al.,* 2011). There are only two species of the weaver ant *Oecophylla* (Hymenoptera: Formicidae); the African weaver ant *O. longinoda* and the Asian weaver ant *O. smaragdina* (Van Mele *et al.,* 2009a).

The former species is found in most tropical regions of Africa where it occurs naturally and builds nests using leaves while the latter has been used in Asia for centuries especially in plantations (Van Wijngaarden *et al.,* 2007). The first documented record of biological control using *Oecophylla* sp dates back to 304AD in Asia when the species was used to fend off fruit flies in plantations (Van Mele, 2008b). *Oecophylla* usually colonises a wide range of trees such as mango, citrus, cashew, cocoa and coconut (Van Mele, 2008a). They are generalist predators capable of controlling fruit flies, beetles, sap sucking bugs, caterpillars, and thrips (Van Mele and Vayssières, 2007).

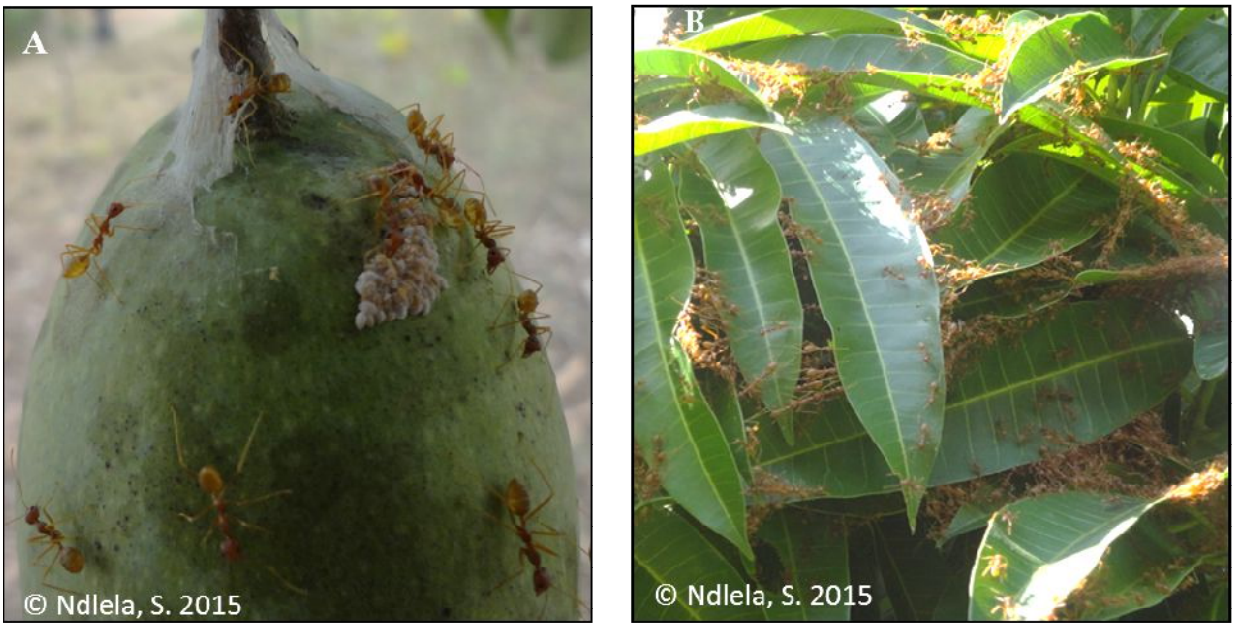

Figure 2.2: The African weaver ant *O. longinoda* on mango fruit (A) and on mango tree leaves (B)

Although potentially effective, weaver ants have not been readily adopted as a component of fruit fly IPM in Africa. Weaver ants are known to be aggressive and are not friendly to regular farm operations and other IPM options such as parasitoids (Sinzogan *et al.,* 2008; Van Mele *et al.,* 2009b; Van Mele, 2008b). However, several studies have shown the benefits of weaver ants on reduction of several pest species in different agro-systems (Vayssières *et al.,* 2013; Van Mele *et al.,* 2007). In Kenya, *O. longinoda* is prevalent in the Coastal province where farmers treat it as nuisance especially during harvesting periods. It is imperative that farmers be engaged in on-farm participatory training and research to change their perceptions and adopt pest control measures that are natural and self sustainable.

2.4.1.3 Physical control: Fruit bagging

Pre-harvest bagging of fruit is one of the best approaches of physically excluding pests attack on the fruit and has been practiced worldwide with consistent positive results (Sharma *et al.,* 2013). The technique involves enclosing individual or a bunch of immature fruits in paper bags (Figure 2.4) to prevent adult flies from laying eggs on the fruits (Ekesi *et al.,* 2007b The technique not only physically excludes insect pests but also optimizes fruit quality through reduced physiological and pathological disorders thereby improving fruit colouration and market value (Sharma *et al.,* 2013; Feng *et al.,* 2014). Fruit bagging provides 100% protection of mango fruits against fruit fly infestation and maintains quality of fruit especially if brown paper bags are used (Sarker *et al.,* 2009). For the technique to be effective, fruits have to be bagged before the onset of fruit fly attack when the mangoes are still hard green and unattractive for egg laying, i.e. at least one month before harvest (Ekesi *et al.,* 2007b). The practice is quite common in South-East Asian countries for large high value fruit such as avocado, grapefruit, *Annona* spp. and *Rollinia* spp (Smith and Brown, 2014). In Kenya, the technique is made difficult or impossible by the height of mango trees, for example mango trees in the Coast and Rift valley provinces are very tall (more than 10 m) to the extent that it is imposible to carry out fruit bagging operations (Rwomushana, 2008). For the operation to be practical, the trees have to be at a manageable height, preferably in a relatively small orchard where the objective is to produce high quality fruits for export and high returns (Rwomushana, 2008).

Figure 2.3: Fruit bagging using brown bags to prevent adult flies from laying eggs on the fruits

2.4.1.4 Sterile insect technique (SIT)

Economically important pest species can be contained, suppressed and managed through ecologically friendly techniques such as the Sterile Insect Technique (SIT). This strategy is amongst the most non-disruptive pest control methods. It is species specific, does not release exotic agents into new environments and neither does it introduce new genetic material into existing populations as the released organisms are not self-replicating (Hendrichs *et al.,* 2002; Vreysen *et al.,* 2006). The technique involves mass production of male fruit flies, sterilization by irradiation and sustained release of the sterilized insects over a large area (Wimmer, 2005).

The goal is to provide a sufficient number of sterile male flies in the environment to increase the probability that unmated wild females will mate with the sterile male and produce infertile eggs (Dowel *et al.,* 1999; Van der Vloedt and Klassen, 1991). Sterile Insect Technique has been employed in the eradication of the Melon fruit fly *B. curcubitae* in Asia, the Mediterranean fruit fly *Ceratitis capitata* in Mexico, Tunisia and South Africa, the Queensland fruit fly *B. tryoni* in Australia and *B. dorsalis* in Mauritius (Hendrichs *et al.,* 1983; Seewooruthun *et al*., 1997; Koyama *et al.,* 2004; Barnes *et al.,* 2007; Ogaugwu, 2014). In the wake of the devastating effects of the Oriental fruit fly, *B. dorsalis* in sub-Saharan Africa, there are prospects of employing SIT in Area Wide Intergrated Pest Management (AW-IPM) of the pest (Ogaugwu, 2014). However, the positive effects of SIT can only be realised when the technique is incorporated in an area wide scale dealing with the pest population as a whole rather than isolated pockets of the pest (Hendrichs *et al.,* 2002). This makes it an expensive enterprise and unusable at farm level. The cost of the SIT is strongly influenced by the need for initial setup which include radiation equipment, bio-safety concerns and mass-rearing, which may initially require higher capital and expertise (Suckling, 2003).

2.4.1.5 Male Annihilation Technique (MAT)

The concept of lure and kill has been practiced for many years in eradicating and suppression of many different pests but mostly fruit flies (El-sayed *et al.,* 2009). Male Annihilation Technique (MAT) is one such technique that involves the use of high density bait stations consisting of a male lure combined with an insecticide for the purpose of suppressing or completely eliminating the male fruit fly population (Vargas *et al.,* 2000; Ekesi *et al.,* 2007b). The technique has a profound effect on the male: female ratio thus reduces the insects' chances of finding mates thereby females produce unviable eggs (Ghanim, 2013).

The male lures are mostly synthetic complex chemicals called parapheromones which elicit responses similar to pheromones and are available either in liquid or polymeric plugs form (Sivinski and Calkins, 1986; Ekesi *et al.,* 2007b). The commonly used male lures against the major fruit fly pests are Methyl eugenol (ME), Cuelure (CUE), Alpha-ionol, Spiroketal, Trimedlure, Terpinyl acetate, Capilure and Vertilure. These are used against *B. dorsalis* and *B. zonata*, *B. cucurbitae*, *B. latifrons*, *B. oleae*, *C. capitata*, *C. cosyra*, *C. rosa*, *C. fasciventris* and *Dacus vertebratus* respectively (Manrakhan, 2007).

Several success stories of MAT using Methyl eugenol have been reported. For example, the eradication of the Oriental fruit fly, *B. dorsalis,* in Guam in the Western Pacific Ocean and the Commonwealth of the Northern Mariana Islands in the 1960s was achieved using the organophosphate Naled as the toxicant (SPC Land Resources Division, 2010). Additionally, *B. dorsalis* was eradicated from California and Amami Islands using the same method. Lengths of string soaked in ME and the organophosphate Malathion were used in the successfull eradication of the Asian papaya fruit fly *B. papaya* from several Torres Strait Islands separating far northern continental Australia's Cape York Peninsula and the island of New Guinea (SPC Land Resources Division, 2010). In the 1990s, Cane-ite blocks were used to successfully eradicate the Asian papaya fruit fly from the Cairns area of northern Queensland. The same blocks impregnated with ME and Fipronil were used in the eradication of *B. dorsalis*, Pacific fruit fly *B. xanthodes*, and melon fly *B. cucurbitae* from the Republic of Nauru in the South Pacific in 1999 (Allwood *et al.,* 2002). More success stories of the MAT include the eradication of the oriental fruit fly *B. dorsalis* in Rota, Saipan and Okinawa, the Asian papaya fruit fly, *B. papayae* in Australia, *B. dorsalis* and *C capitata* in Kamuela Hawaii (Cantrell *et al.,* 2002, Vargas *et al.,* 2010a, Ghanim, 2013).

Effective suppression of *B. dorsalis* (Hendel) and the melon fly, *B. cucurbitae* (Coquillett), was also demonstrated using Methyl eugenol and Cue-Lure Traps in Hawaii (Vargas *et al.,* 2000; Vargas *et al.,* 2007).

In Kenya and Africa in general, the adoption of MAT is being promoted by the International Centre of Insect Physiology and Ecology (*icipe*) together with the International Institute of Tropical Agriculture (IITA) for the control of *Bactrocera*, *Ceratitis* and *Dacus* fruit fly pests (Ekesi *et al.,* 2007b). If integrated with other management techniques such as orchard sanitation, biological control with parasitoids and entomopathogens as well as baiting, significant suppression of fruit fly populations can be achieved especially if applied over considerably large areas (Ekesi *et al.,* 2007b).

2.4.1.6 Protein Bait Technique

The baiting technique in which food baits are mixed with an insecticide has been used to suppress tephritid fruit flies with significant success over the years (Steiner, 1955; Burns *et al.,* 2001; Moreno *et al.,* 2001; Vargas *et al.,* 2001; Prokopy *et al.,* 2003; Vargas *et al.,* 2002). This lure-and-kill technique works by using a protein food component to attract flies to a spot to feed, and the insecticide kills the insect soon after ingestion (Balagawi *et al.,* 2014). The rationale of the technique is based on the fact that immature fruit fly females require a protein meal for the subsequent development and maturation of their eggs (Hagen and Finney, 1950). The protein attracts mostly but not exclusively female fruit flies which in turn ingest the poisoned bait which is usually sprayed on small portions of the tree canopy, usually one square meter, preferably on the underside of leaves to reduce wash-down and enhance persistence of the bait (Prokopy *et al.,* 2003; Ekesi *et al.,* 2007b).

Unlike cover sprays or broadcast application in conventional sprays, this practice reduces the amount of pesticide used as well as the area receiving treatment (Prokopy *et al.,* 2003).

Several commercial baits are available on the market and these include: Nu-Lure®, Mazoferm®, Hymlure®, Buminal®, Solbait® and GF-120®, (all are made of several components that attract fruit flies) but most of them have to be imported into Africa, making them expensive and inaccessible to smallholder farmers (Ekesi *et al.,* 2007b).

Baiting has been used extensively in the control and management of economically important tephritid fruit flies such as *Anastrepha suspensa* (Loew), *B. cucurbitae* Coquillet; *C. capitata* (Wiedemann), *B. dorsalis* and *C. cosyra* (Walker) (Epsky *et al.,* 1993; Prokopy *et al.,* 2004; McQuate *et al.,* 2005; Barry *et al.,* 2006; Vayssieres *et al.,* 2009; Bockmann *et al.,* 2014). Although quite effective, protein bait sprays may be inadequate especially in high infestation areas and, high rainfall seasons as well as in cases where the area applied is relatively small in relation to surrounding untreated areas (Allwood *et al.,* 2001). However, overally the benefits far outweigh the shortcomings. Due to the minimal and judicious use of insecticide, bait sprays are less harmful to beneficial insects such as parasitoids, costs are relatively lower and residues on fruit are minimised as sprays can be directed away from the fruits onto the leaves alone thus conforming to requirements of environmental friendliness and consumer requirements (Allwood *et al.,* 2001). As an alternative to aerial and ground bait sprays, bait station devices were developed to lure and kill fruit flies without retaining them in the device (Navarro-Llopis *et al*., 2008). Bait stations are discrete containers of attractants and toxins that are retrievable and biodegradable or, devices based on direct application to a substrate for the aim of targeting specific pests (Ekesi *et al.,* 2007b; El-Sayed *et al.,* 2009; Epsky *et al.,* 2011).

Male and female food baits such as Mazoferm can be used together with a contact pesticide or an entomopathogen such as *M. anisopliae* (Ekesi *et al.,* 2007a) in the bait station. Compared with current bait spray applications, bait stations are costly but minimise risks to the environment because they are placed in the insect host habitat at spatially localised sites and can be left in the field to provide maintenance-free protection over a long period (IAEA, 2009; Epsky *et al.,* 2011; Navarro-Llopis *et al*., 2014). However, the use of baiting stations is still in its developmental stages.

2.4.1.7 Orchard sanitation/ crop hygiene

Orchard sanitation involves the regular collection and destruction of infested fruit either on the tree or fallen on the ground (Ekesi *et al.,* 2007b). This requires the full participation of the farmer if the operation is to be sustainable and successful. When used in combination with other management options, it is effective in controlling fruit flies (Pinero *et al.,* 2009). For example, the most effective method in the management of *B. cucurbitae* involves field sanitation as the primary component (Dhillon *et al.,* 2005). All unharvested fruit is buried at least half a metre underground to prevent adult fly eclosion and thereby reduce population increase (Dhillon *et al.,* 2005). However, in as much as the infested fruit contains eggs and larvae of fruit flies, there is a high possibility that egg and larval parasitoids might be in the fruit lot earmarked for destruction. Therefore, it is imperative that an Augmentorium (Fig 2.4) be used in disposing of the infested fruit.

An Augmentorium is a tent like structure in which infested fruit are dumped inside for the purpose of sanitation and conservation of natural enemies (Klungness *et al.,* 2005; Ekesi *et al.,* 2007b; Deguine *et al.,* 2011).

This structure sequesters adult flies emerging from infested fruit while allowing the parasitoids to escape, via a net placed at the top of the structure, back into the cropping system (Jang *et al.,* 2007; Deguine *et al.,* 2011). Working in Reunion Island, Deguine *et al.,* (2011) demonstrated that 100% sequestration of adult flies (*C. capitata, B. cucurbitae, B. zonata*) was possible if the right mesh size was used in the construction of the Augmentorium. In addition, 100% of the parasitoids (*F. arisanus* and *Psyttalia fletcher*i) were also able to escape from the cage through the mesh. Field sanitation can only be effective if practised by all farmers within a specified farming area. It is even more effective if adopted alongside other fruit fly management measures such as biological control, baiting technique, MAT, SIT and others.

Figure 2.4: A farmer placing infested mango fruit picked from the ground into an augmentorium.

2.4.2 Post-harvest disinfestation treatments

In fruits such as mango, tephritid fruit flies are one of the major factors contributing to direct loss of fruit through the feeding and burrowing of their larvae as well as loss of export markets through quarantine restrictions (Mwatawala *et al.,* 2004; Verghese *et al.,* 2006).

In recent years, increased international trade in agricultural commodities has multiplied the risk of introducing exotic insects into new areas (Paul, 1994; Krugman *et al.,* 1995; Feenstra, 1998; Follet and Neven, 2006). Therefore, post-harvest treatments become necessary especially if fruit is to be exported. These treatments provide a means for the killing, inactivation or removal of pests, rendering pests infertile or for devitalization, at a stated efficacy, and are relevant primarily to international trade (Follet and Neven, 2006; IPPC, 2009a).

Several techniques are currently available for post-harvest disinfestations of insect pests depending on the type of the fruit and the maturity stages. These include cold (refrigeration) treatment, controlled or modified atmosphere, irradiation, and heat treatments, among others (Sharp and Spalding, 1984; Sharp *et al*., 1989a, b, c; Sharp, 1993; Wang and Tang, 2001; Bustos *et al*., 2004; Torres-Rivera, 2007; De Lima *et al.,* 2007; Pryke and Pringle, 2008; Massa *et al.,* 2011; Grout *et al.,* 2011; Johnson and Neven, 2011; Ware *et al.,* 2012; Follet *et al.,* 2013). In most cases, only a single post-harvest treatment is usually applied to a commodity but a number of options, such as multiple or combination treatments and systems approaches have been found to be highly effective (Follet and Neven, 2006). The combinations of treatments used largely depends on the commodity and pest involved.

Some of the widely used combinations include cold and low oxygen, heat and high carbon dioxide, sequential fumigants, heat and irradiation, heat followed by cold storage, low temperature and fumigation, or low temperature storage with slow release of sulphur dioxide pads (UNEP, 2000; Fields and White, 2002). As technology advances and various options become widely available, chemically based post-harvest treatments are bound to become less available as they are replaced by physical treatments and systems approach (Follett and Neven, 2006).

2.4.2.1 Heat treatment

In the last 30 years, there has been renewed interest in heat treatment through immersing commodities in hot water, subjecting them to direct hot air or saturated hot vapour (Sharp, 1993; Sharp and Hallman, 1992; Hansen *et al.,* 2011). Heat treatment in its different forms is increasingly becoming a common method for post-harvest disinfestation of commodities destined for export (USDA-APHIS, 2010). However, certain factors should be considered before choosing a particular disinfestation treatment. Heat disinfestation protocols should be employed after considering the impact on the commodity, effectiveness on the target pests, post-harvest shelf-life, feasibility (including cost) and requirements of the importing country (FAO, 2004).

It is for this reason that protocols have to be precise, effective and able to retain physical and biochemical quality of the commodity (Hansen *et al.,* 2011).

2.4.2.1.1 Hot water treatment

Hot water immersion treatment, also known as hydrothermal treatment, involves wholly dipping a commodity such as fruits in hot water, set at a specific temperature for pre-determined duration (USDA, 2005). It is an efficient disinfestation treatment against fruit flies on mango (Miller *et al.,* 1988; Mitcham and Yahia, 2008; USDA-APHIS, 2010). There are several steps and considerations involved in developing an effective a hot water disinfestation treatment. Foremost, a protocol can only be developed for a pest whose taxonomy and biological data are fully understood. The most heat-tolerant stage of the insect is then established through experimentation, followed by large scale confirmatory tests to demonstrate mortality (usually probit 8.7 or 9, which is 99.99% or 99.9968%) or efficacy of the treatment to the level required by the importing country (FAO, 2004).

Hot water treatment protocols have been developed for fruit flies such as *C. capitata* and *A. serpentine* (Sharp *et al.,* 1989b) as well as *B. dorsalis* (Verghese *et al.,* 2006; Armstrong and Follett, 2007; Hernández *et al.,* 2012) in mangoes and litchi, respectively. Temperatures of 46.1°C over specified periods for specific pests and fruits are often adequate in hot water treatment procedures (USDA-APHIS, 2009). The length of immersion varies depending on the fruit shape and weight. Submerging most perishable fruits in a hot water bath at temperatures of between 43-46.7°C for 35-90 minutes is equally effective (De La Cruz Medina and Garcia, 2002). For example, hot water at 46.1°C for 35 minutes was shown to effect probit 9 mortality in guavas (*Psidium guajava* L.), infested with Caribbean fruit fly (*A. suspense* (Loew) (Gould, 1994). In Mexico, dipping fruits in hot water at 46.1°C for 65-90 minutes is recommeded (Yahia *et al.,* 1999).

Besides disinfesting fruits of fruit flies, hot water treatment has been reported to increase tolerance of fruit to chilling injury as well as enhance post harvest shelf life of mangoes (Grové *et al.,* 1999; Irtwange, 2006; Yimyong *et al.,* 2011; Çandir *et al.,* 2012). Moreover, hot water immersion also has the additional benefit of controlling post-harvest microbial diseases such as anthracnose and stem end rot (McGuire, 1991; Kumah *et al.,* 2011). Hot water treatment therefore is an effective alternative to disinfesting fruits but there are some concerns associated with the treatment method. Current hot water treatment protocols are believed to be contributing factors in the loss of fruit quality in the market (Mitcham and Yahia, 2008). During hot water treatment, the fruit cuticle expands causing fissures and enlarged pores, although these can return to normal if treated fruits are cooled in water at room temperature (preferably 21-27°C), 30 minutes post-treatment (Shellie and Mangan, 2002, Mitcham and Yahia, 2008). If post-harvest treatments are effected well, it offers the opportunity for exporting countries to retain lucrative markets in Europe and USA for their fresh fruit produce.

2.4.2.1.2 Hot air and Vapour Heat Disinfestation

Methods such as hot air and vapour treatments have been found to be less damaging to fruits and other commodities but have the disadvantage of being more expensive beyond the reach of most farmers requiring commodity treatment for the export market (De La Cruz Medina and Garcia, 2002). Hot air treatment involves disinfesting fruits by application of re-circulated air that has been heated, humidified and then forced over fruit surfaces thereby raising the fruit pulp temperature beyond the thermal limits of the pest (Sharp, 1994).Vapor-heat (VHT) differs from high-temperature, forced-air in that moisture accumulates on the surface of the fruit. The water droplets transfer heat more efficiently than air, allowing the fruit to heat quickly but there may also be increased physical injury to the fruit (Lurie *et al.,* 2003).

2.4.2.2 Cold treatment

Cold treatment decreases the temperature of the commodity below the thermal limits tolerated by the pest (Follet and Neven, 2006). Low temperatures are known to retard the development of insect pests thus causing mortality (Maier, 1994). Cold treatment protocols have been developed for use against tephritid fruit flies such as *B. dorsalis*, *C. capitata*, *B. zonata*, *B. tryoni* mostly (De Lima *et al.,* 2007; Grout *et al.,* 2011; Fallik *et al.,* 2012; Ware *et al.,* 2012; Hallman *et al.,* 2013). Cold treatment has also been shown to be effective against *Anastrepha ludens* and *A. suspensa* in avocado and other fruits such as *Psidium guajava* (EPPO/CABI, 1996); Aluja *et al.,* 2010). Due to the lengthy treatment times required to effectively disinfest fruits, the practice is best incorporated into existing storage or shipping regimes (Wang and Tang, 2001). For example, to achieve probit 9 disinfestation level of *B. dorsalis* in Hass avocado (*Persea Americana* Miller), a continuous cold treatment of 1.5°C or lower for 18 days is required (Ware *et al.,* 2012). Cold disinfestation has also been combined with hot water or hot air treatment as a post-treatment strategy to ameliorate possible damage due to hot treatment (Kremer-Kohne, 1999).

2.4.2.3 Fumigation with chemicals

Chemical fumigation has always been the post-harvest control measure of choice in many commodities because of its ease of use, low cost and effectiveness. However, health hazard concerns have forced the abandonment of this method in favour of more environmentally friendly alternatives such as controlled atmospheres, cold and hot treatments (Wang and Tang, 2001).

In the past, methyl bromide fumigation was the most common phytosanitary treatment especially in stored commodities and some fresh produce such as watermelons.

The greatest undoing of methyl bromide fumigation is its toxicity to the environment. Normally, products to be fumigated were enclosed in a special chamber or covered with tarpaulin in which the gas was then injected. However, 80-90% of the injected gas would escape into the atmosphere causing damage to the ozone layer (Wang and Tang, 2001).

2.4.2.4 Irradiation

Irradiation is the process by which commodities are exposed to ionizing radiation for the sole purpose of sterilising, killing or preventing the emergence of insect pests by damaging their genetic material (IPPC, 2003). Generally, irradiation phytosanitary treatment is aimed at preventing egg and larval stages of holometabolous insects from developing into adults or, where adults and pupae are present, to prevent reproduction (Hossain *et al.,* 2011).

A generic radiation dose of 150 Gy for all tephritid fruit flies is recommended for USA (USDA-APHIS, 2006; Follet *et al.,* 2008). Similarly, in Australia the recommended irradiation dose is 150 Gy for tephritid fruit flies when treating mango and papaya from northern Queensland exported to New Zealand. The International Plant Protection Commission (IPPC) has recommended an irradiation treatment dose of 150 Gy for tephritid fruit flies and specific doses for a number of other pest species (Follet *et al.,* 2008; IPPC, 2009a). In mango, irradiation using Co-60 gamma rays at a dose of 150 Gy was found to be sufficient in providing quarantine security (99.9968% mortality) against four fruit flies species namely *A. ludens, A. obliqua, A. serpentina* and *C. capitata* and had no adverse effect on the quality of the treated fruits (Bustos *et al.,* 2004).

The greatest challenge of widespread use of irradiation, however pertains to large sums of capital investment for initial set up of facilities which might be detrimental especially to individuals or poor third world countries. It is also common to find live insects inside treated product consignments, even though the insects may be harmless. This is where irradiation differs with other disinfestation treatments: it does not necessarily kill the pest instantly but prevents successful development or reproduction of the pest, which is as good as imparting mortality (Hossain *et al.,* 2011).

2.4.2.5 Controlled Atmosphere (CA) or Modified Atmosphere (MA) Treatments

Controlled atmosphere (CA) has been used for many years to extend commodity shelf life especially in stored grains but recent research has shown great promise in disinfesting fresh produce (Ke and Kader, 1992a; Whiting *et al.,* 1992; Wang *et al.,* 2001). Normal composition of atmospheric air is 21% oxygen, 0.03% carbon dioxide and 78% nitrogen (Banks and Fields, 1995; Das *et al.,* 2013). This composition can be altered appropriately for the purposes of disinfesting products such as fruits from insect pests. For example, subjecting fruits and vegetables to low oxygen and/or very high carbon dioxide atmospheres imparts beneficial effects such as the reduction of respiration rate and inhibition of ethylene production, colour change, softening (thus ripening retarded) as well as inhibition of decay and the maintenance of nutritional value (Ke and Kader, 1992a; 1992b). The atmosphere around the fruits of interest can be modified inside a package either passively through product respiration (consumption of oxygen and production of carbon dioxide), or actively by replacing the atmosphere in the package with a desired mixture of gases respectively (Follett *et al.,* 2013).

At very low oxygen concentrations (Ultralow Oxygen, ULO), infested fruits are exposed to oxygen levels below 1% for particular duration of time to kill insect pests (Liu, 2010). In some instances, low levels of oxygen are combined with elevated carbon dioxide concentrations to create conditions that are unfavourable to survival of insect pests (Liu, 2010). In order for CA to be effective, oxygen concentrations must be below 1% and carbon dioxide concentration above 20% for at least 24 hr. However, prolonged exposure to these conditions might cause undesirable effects to the fruits. In some instances fruits exposed to CA treatment might suffer physical injury, fail to ripen, show increased susceptibility to decay and development of undesirable flavour (Ke and Kader, 1992b). Controlled atmosphere of 0-0.5 kPa oxygen and 50 kPa carbon dioxide at high temperature of 44-55°C is effective in disinfesting mangoes infested with *A. ludens* and *A. oblique* (Yahia *et al.,* 1999). Controlled atmosphere is a viable alternative to conventional disinfestation methods due to their usability and practicability but are not suitable in heavily infested products (Das *et al.,* 2013).

2.5 Combined treatments

Several disinfestation treatments described above are quite effective but are somehow known to cause some undesirable effects to treated commodities. To overcome this shortcoming, there has been great interest in combining compatible treatments either simultaneously or applying them one after the other (UNEP, 2000). Von Windeguth and Gould (1990) demonstrated the effectiveness of irradiation followed by cold treatment in disinfesting the Caribbean fruit fly in grape fruit. This kind of combination treatments have the enhanced advantage of speeding up the disinfestation process as well as saving energy consumed, for example a 50 Gy treatment is required if cold treatment is incorporated over the 150 Gy treatment required in routine treatments. This represents a 33% saving in energy costs.

Similarly, low doses of X-ray irradiation followed by cold exposure for short periods effectively disinfested 'Clemenules' mandarins from the Mediterranean fruit fly and had no detrimental effects on fruit quality (Palou *et al.,* 2007). Treating fruits by hot water immersion followed by cold treatment has also been shown to improve the tolerance of mangoes to chilling injury (McCollum *et al.,* 1993). Thus various combinations are currently being tried inorder to save energy, increase efficiency and lower undesirable effects on treated fruit.

CHAPTER THREE

Male annihilation technique using methyl eugenol for field suppression of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) on mango in Kenya[1]

3.1 Abstract

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), is one of the most devastating tephritid fruit flies of horticulture worldwide. Field trials were carried out for two seasons on mango (*Mangifera indica* L.) to evaluate the use of male annihilation technique (MAT) using methyl eugenol laced with deltamethrin instead of the commonly used malathion for the suppression of the pest on mango in coastal Kenya. Prior to application of the MAT, mean total numbers of *B. dorsalis* flies per trap per day (FTD) in pre-suppression monitoring data were comparable in orchards assigned to MAT treatment (FTD = 3.5) and those assigned to the control (FTD = 3.5) in season 1 and 12.4 and 10.5 FTD, respectively, in season 2. Following the application of MAT systems, total FTD were significantly lower in MAT-treated orchards (0.1 and 2.7 FTD, for seasons 1 and 2, respectively) compared to that in the control (18.6 and 21.5 FTD, for seasons 1 and 2, respectively) at 49 days after deployment of the control measures. This represented a reduction in the *B. dorsalis* population of 99.5% in both seasons, resulting in a significant reduction of fruit infestation in the MAT-treated orchards compared to the control. The percentage of infested fruit was 25 and 18 times lower in MAT-treated orchards compared to the control for the first and second season, respectively. The number of puparia/kg of mango fruit was 17 and 24-fold lower in MAT-treated orchards compared to the control for the two consecutive seasons.

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These findings demonstrate the suppression of *B. dorsalis* using the MAT, and subsequent reduction in fruit damage by the pest. It is therefore recommended that MAT be adopted within a holistic Integrated Pest Management (IPM) approach in the mango agro-system, preferably covering large areas.

3.2 Introduction

Globally, Tephritidae fruit flies such as *Ceratitis capitata* (Wiedemann), *Bactrocera cucurbitae* (Coquillett) and *Dacus* sp. represent a serious threat to the horticulture industry (White and Elson-Harris, 1992; Vargas *et al*., 2013). In the tropics, the fruit fly problem is further compounded by predominantly conducive weather conditions and the availability of host fruits throughout the year (Purcell, 1998). Some species of this family have of late attracted much attention due to their ability to invade new areas, where they are relatively unknown, and causing significant damage before control measures can be initiated. The best example of these is a member of the genus *Bactrocera*, that was first detected in Africa in 2003 (Lux *et al*., 2003b). Two years later, the pest was described as *B. invadens* Drew, Tsuruta and White (Drew *et al*., 2005). However, recently *B. invadens* was synonymised with the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) following several years of intense integrative morphological, molecular, cytogenetic, behavioural and chemoecological research (Bo *et al*., 2014; Schutze *et al*., 2014a; Schutze *et al*., 2014b).

Since its first detection in Africa, *B. dorsalis* continues to cause widespread damage to various commercially-grown fruit varieties, thereby compelling importing countries to enforce quarantine restrictions on fruit originating from regions where the pest has been reported (Ekesi *et al*., 2006; USDA-APHIS, 2008; Guichard, 2009; Otieno, 2011).

For example in Kenya, direct mango fruit losses due to fruit flies infestation have doubled (from approximately 40% to 80%) following the invasion by *B. dorsalis* (Ekesi *et al*., 2006; Rwomushana *et al*., 2008), in addition to the indirect losses incurred at the regional and international markets.

Various management strategies such as the use of food baits, parasitoids, pathogens, field sanitation, fruit bagging and male annihilation technique (MAT) are currently available for the management of different fruit flies in many countries (Ekesi and Billah, 2007). The use of MAT has been recommended specifically for fruit flies that respond to male attractants (Wee *et al*., 2007; Tan *et al*., 2014).

Male annihilation technique (MAT) involves the deployment of high density trapping stations consisting of the male attractant (*e.g.* methyl eugenol (ME), cue lure, trimedlure, among others) combined with an insecticide, to considerably reduce male populations to a level that mating does not occur at all or is extremely diminished. MAT was first used to successfully eradicate *B. dorsalis* from the island of Rota (Steiner *et al*., 1965). Also *B. dorsalis* and its close relatives were successfully eradicated using MAT and protein bait sprays, from the Mariana Islands (Steiner *et al*., 1970), the Ryukus in Japan (Koyama *et al*., 1984), Nauru (Allwood *et al*., 2002), and Australia (Cantrell *et al*., 2002). Despite the successful implementation of MAT for management of various species of fruit flies, its use has not gone without scrutiny, due to the environmental and health hazards associated with the toxicant component of MAT that is required for killing the flies. Traditionally, organophosphate insecticides such as malathion, dichlorvos (DDVP) and naled have been used as toxicants in male annihilation technique using ME (Vargas *et al*., 2003; Vargas *et al*., 2009).

In the present study, we used deltamethrin (Decis® 025 EC), a synthetic pyrethroid that acts by both contact and ingestion giving a fast knockdown effect (Bayer Crop Science, 2015). We also used this insecticide primarily because of its availability and affordability to smallholder farmers. In some sterile insect technique (SIT) programmes, MAT is also deployed to substantially reduce the fruit flies populations prior to the release of sterile males to ensure a very high ratio of sterile males to their wild counterparts (100:1) which is a pre-requisite, among other factors, for successful implementation of SIT. For example, the melon fly, *Bactrocera cucurbitae* (Coquillett), was eradicated from Okinawa Island, Japan, following the deployment of MAT using cuelure and toxicants (Koyama *et al*., 1984). MAT is also recommended as part of pest suppression techniques to maintain a buffer zone around fruit fly pest-free areas (FF-PFA), in areas with no geographic isolation, or in locations where natural barriers are not adequate to safeguard against re-infestation of a PFA (ISPM 26, 2006).

Within the African context, the eradication of *B. dorsalis* from the Indian Ocean island of Mauritius in 2000 was primarily credited to the use of MAT and bait spray application (Seewooruthun *et al.,* 2000). The same approaches were used to eradicate this pest from the same island following its reinvasion in 2013 (Sookar *et al*., 2014). In mainland Africa, MAT was also used as an important component of a package targeting *B. dorsalis* when it was first detected in Limpopo province of the South Africa, resulting in successful eradication of the pest in 2010 (Manrakhan *et al*., 2011), and in a few subsequent incursions (Manrakhan, pers. comm.). However, the pest is currently established in the northern part of the country where MAT is still being used in a programme aiming at the fly's exclusion and curbing its movements southwards (Manrakhan, pers. comm.).

In sub-Saharan Africa (SSA), outside South Africa, where the pest is well established, MAT is used on a limited scale generally in combination with other management tactics (*e.g*. Hanna *et al*., 2008; Ekesi, unpubl.). In Kenya, an IPM package, targeting various species and stages of fruit flies, consisting of (1) spot application of bait spray, (2) MAT, (3) parasitoid release, (4) biopesticide application and (5) orchard sanitation, is being promoted and implemented in different major mango-growing zones (Ekesi and Billah, 2007; Ekesi *et al*., 2011; Mohamed *et al*., 2012). This initiative has resulted in a drastic reduction of target fruit fly pest populations, especially *B. dorsalis*. This has led to production of high-quality fruit that has also opened access to export markets. However, among the various management packages, the majority of growers tend to rely more on the use of MAT through deployment of ME baited traps. This is perhaps not surprising considering that growers are more convinced when they see dead flies inside traps. Although ME-based MAT has been used either alone or in combination with other methods for management of fruit flies in the *B. dorsalis* complex, its sole use for suppression of fruit flies in Africa has not been evaluated, which is an important aspect required to guide farmer practices for fruit fly management. It is also possible that the African population of *B. dorsalis* might respond differently to ME compared with *B. dorsalis sensu stricto*, even though they were recently synonymised. For example, *B. papaya*, which was also recently synonymised with *B. dorsalis s.s.,* is reported to be least sensitive to ME compared with other species within the complex (Wee *et al*., 2002). Therefore the objective of this study was to evaluate the use of ME-based MAT for suppression of *B. dorsalis* populations in mango-production areas, and to assess whether fly population reduction subsequently translates into a reduction in fruit damage by the pest.

3.3 Material and methods

3.3.1 Study site

The study was conducted in mango orchards in Muhaka (04°32'S 039°52'E, 38 m a.s.l.) in Kwale County of Kenya over two consecutive mango fruiting seasons. Muhaka is located south of Mombasa and receives an average annual rainfall of 1212 mm with temperature ranging between 22.0–30.4°C throughout the year (Otieno *et al*., 2006). Traditionally, Kwale County experiences two mango seasons from November to March and May to August.

The main mango varieties that are cultivated in this area include Apple, Ngowe and Boribo, which are usually grown as mixtures in the same orchard by most smallholder farmers. Four localities (~3–4 km apart) along the Ukunda–Lunga-lunga road were identified in Muhaka and environs (Figure 3.1).

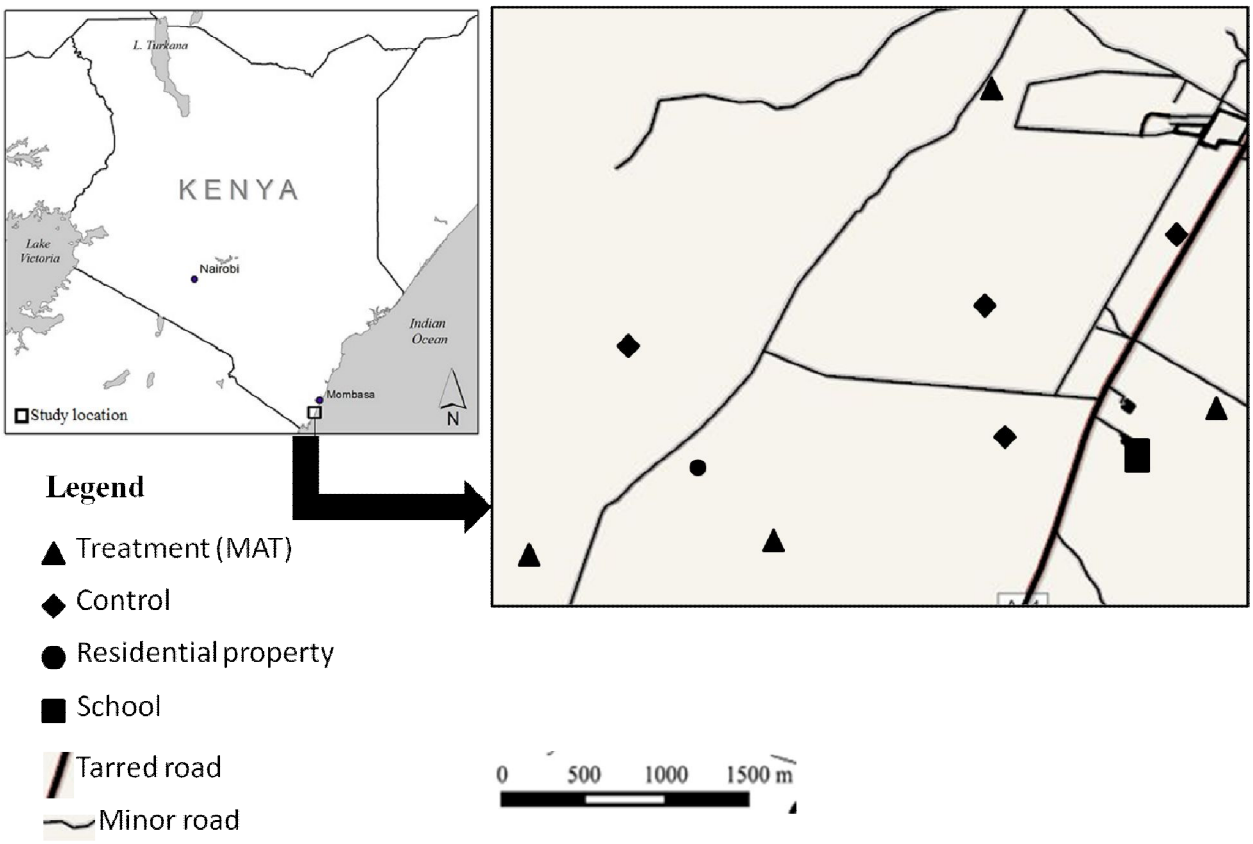

Figure 3.1: Map of Kenya indicating the enlarged location of orchards in Muhaka used in male annihilation, either as treatment plots (with MAT systems) or control plots (without MAT systems).

Within each locality, two different mango orchards (1 ha in size, with ~100 mango trees/ha) and approximately 1.5–2 km apart were selected each mango season (2013/2014 and 2014/2015, corresponding to seasons 1 and 2, respectively) and allocated either to MAT treatment or to the control. Mango orchards selected for the study were composed of approximately 99% Ngowe variety. The distance between localities and the treatments within them was maximized to reduce the chance of movement of flies between treatments.

Testing both the treatment and the control in the same orchard was avoided due to the mobility of fruit flies (Peck and McQuate, 2000; Piñero *et al*., 2009; Vayssières *et al*., 2009; Ekesi *et al*., 2011; Ekesi *et al*., 2014). No pesticides had been applied in any of the experimental fields prior to this study and trials were conducted with permission from the growers. The experimental orchards were more than five kilometers from large-scale and smallholder mango orchards that were applying pesticides. At full bloom, mango trees were treated with a broad-spectrum non-systemic fungicide (mancozeb 750 WG, Cheminova Australia Pvt Ltd, North Ryde NSW, Australia) at the label rate of 0.8 kg a.i./ha. Mancozeb is a protectant fungicide for the control of fungal diseases such as anthracnose which causes flower abortion.

3.3.2 Population monitoring with baited traps

Fruit fly populations were monitored in both mango growing seasons using the liquid food bait Dudulure® (a fruit fly food bait based on waste brewers' yeast; *icipe*, Nairobi, Kenya) from 25 December 2013 to 26 March 2014 and from 9 December 2014 to 03 February 2015 in seasons 1 and 2, respectively, before MAT application and continued until mango fruit harvest. The food bait was diluted to 7% and borax added to the bait/ water solution at the rate of 30 g/l to preserve the captured flies. In each mango orchard, 10 Lynfield traps (Fig 3. 2) containing 250 ml of bait were positioned on randomly selected trees at 2 m above ground level and at least 25 ± 5 m away from each other.

Figure 3.2: Lynfield trap with 250 ml of Dudulure fruit fly bait for monitoring fruit fly population in the field.

Bait was removed and replaced weekly and flies collected from the traps, preserved in 70% ethanol and transported to the laboratory where their identity was determined and a daily capture rate estimated using the equation:

$$FTD = \frac{F}{(T \times D)}$$

Where F = total number of flies; T = number of serviced traps and D = average number of days traps were exposed in the field (IAEA, 2003).

3.3.3 Suppression with MAT

MAT systems were made from cotton wicks (4 cm long and 1 cm thick) soaked in ME (lure) and mixed with toxicant (Decis® 025 EC; deltamethrin at the label rate of 0.5 l a.i./ha, Bayer Crop Science AG, Monheim am Rhein, Germany) at a ratio of 4:1 until saturation but without dripping. The cotton wick dispensers were suspended inside Lynfield traps using a piece of galvanised wire. The traps were then randomly distributed in the orchard assigned to the MAT treatment at a density of five traps per hectare. Suppression by deployment of MAT commenced on 26 March 2014 and 3 February 2015, when fly density was at 11.61 and 22.1 FTD, in the first and second season, respectively (Fig. 2A, B). This was at a stage when the fruits were reaching maturity and monitoring data indicated that flies had begun the characteristic seasonal population build-up. The Methyl eugenol impregnated cotton wicks were changed after six weeks (the average time when they start losing effectiveness) and the duration of the suppression phase was seven weeks (representing 2-3 generations of *B. dorsalis* (Fig 3.3)) in both seasons.

Figure 3.3: *Bactrocera dorsalis* adult flies. (A) Female (B) Male

3.3.4 Fruit infestation assessment

At harvest, 100 mangoes (Ngowe variety) were randomly picked from each orchard using longarm pruners and packed in brown paper bags (20 × 14 × 40 cm; Paperbags Ltd, Nairobi, Kenya). Mangoes were harvested on 14 May 2014 and 24 March 2015 in the first and second seasons, respectively. They were then transported to the laboratory where they were weighed and placed individually in 2 L white rectangular plastic containers whose base measured (14.7 × 14.2 × 7.7 cm; Kenpoly Manufacturers Ltd) and open top (20.7 × 14.3 × 14.7 cm). The base of the container was fitted into another container of the same size but from the top, leaving a 7 cm clearance between the base of the top and bottom container. Inside the base of the larger container was a thin layer (10–15 mm) of moist sterilised sand which served as pupation medium for third instar larvae popping out of fruits. The fruits were then incubated in a room at ambient conditions 26-28°C, 60-70 RH% and photoperiod of L12: D12 for 2–3 weeks. Pupae were periodically sieved from the sand and placed in petri dishes (8.6 cm in diameter). After three weeks, when all larvae were deemed to have exited the fruit, fruit remains were dissected, washed and remaining larvae as well as any puparia inside were removed, also placed in Petri dishes and the number of puparia/kg fruits was computed. Petri dishes containing puparia were then placed into Perspex cages (30 × 30 × 30 cm) to allow emergence of fruit fly imago. Upon emergence, adult flies were provided with water on moist cotton wool and fed on a mixture of artificial diet consisting of ground sugar and enzymatic yeast hydrolysate ultrapure (USB Corporation, Cleveland, Ohio, U.S.A.) at the ratio 3:1 by volume, for four days to attain full body colouration necessary for proper identification. Thereafter, the flies were killed by freezing and preserved in 70% ethanol for storage and further identification.

The number of infested fruit as well as the number and sex of the emerging fruit fly species were recorded in order to estimate infestation levels and sex ratio.

3.3.5 Data analysis

The mean number of *B. dorsalis* expressed as FTD were transformed using log10 (x + 1) to normalize the data. Data on number of *B. dorsalis* puparia recovered per mango fruit as well as number of puparia/kg of mango fruits from treated and control orchards were also transformed in the same way before subjecting them to ANOVA to test for treatment effect on infestation. Pre-suppression monitoring data (corresponding to week 11 (19 March 2014) for season 1 and week 8 (27 January 2015) for season 2) were compared using a two sample *t*-test. Trap catches during the suppression period, were subjected to repeated measures analysis of variance (rANOVA) to evaluate the effect of male annihilation on *B. dorsalis* populations over time as compared to the control. Percentage reduction in fruit fly populations relative to the control for the first and second season trials was calculated using the equation of Henderson and Tilton (1955):

$$\{1 - [\frac{\text{Number in control unit before treatment} \times \text{number in treatment unit after treatment}}{\text{Number in control unit after treatment} \times \text{number in treatment unit before treatment}}] \times 100\}$$

Values corresponding to weeks 12 (26 March 2014) and 19 (14 May 2014) for season 1 and weeks 9 (3 February 2015) and 16 (24 March 2015) for the second season trials were used as points on which treatment was initiated and terminated as required in the formula. The data on number of infested mangoes in treated and control orchards were analysed using a generalised linear model with logit link and quasi-binomial distribution error to test for treatment effects on infestation. All analyses were performed using R software version 3.1.1 (R Development-Core-Team, 2014).

3.4 Results

Prior to application of the MAT system, data from baseline monitoring of *B. dorsalis* expressed as FTD showed that fly catches were comparable among the two test sites for the two mango fruiting seasons (Table 3.1). Over 99.8% of the fruit flies caught in monitoring traps were *B. dorsalis,* with insignificant numbers of the native mango fruit fly, *Ceratitis cosyra* (Walker). Generally, mean FTD increased gradually as the mango fruiting season progressed (Figure 3.4A, 3.4B). In Season 1, the mean number of male, female and total (male and female) FTD were significantly lower in MAT-treated orchards compared to those in the control (Table 3.2). Similarly, time after deployment of MAT (in weeks) had a significant effect on mean number of male FTD ($F_{6,36} = 9.10$, $p < 0.0001$), female FTD ($F_{6,36} = 30.35$, $p < 0.0001$), and total ($F_{6,36} = 27.59$, $p < 0.0001$), with population being lower in the MAT-treated orchards for the three categories. Also, the interaction between treatment and days after MAT deployment had a significant effect on fly catches for the three categories (male FTD: $F_{6,36} = 9.63$, $p < 0.0001$; female FTD: $F_{6,36} = 39.87$, $p < 0.0001$; total FTD: $F_{6,36} = 33.07$, $p < 0.0001$, again being lower in MAT-treated orchards. The overall percentage reduction in *B. dorsalis* catches in the treatment relative to the control was 99.04, 99.60 and 99.45% for the male, female and total flies, respectively.

Table 3.1. Mean number of *Bactrocera dorsalis* flies captured per trap per day (FTD) in mango orchards during the pre-suppression phase prior to the application of MAT during 2013/2014 and 2014/2015 mango seasons.

Mango season		Mean *B. Dorsalis* FTD ± SE		Statistics		
		Control	MAT-treated orchards	*t*-value	df	Probability *p*
2013/2014	Male	1.33 ± 0.40a	1.36 ± 0.40a	0.42	6	0.69
	Female	2.14 ± 0.61a	2.13 ± 0.59a	0.19	6	0.85
	Total	3.47 ± 1.00a	3.49 ± 0.98a	0.41	6	0.70
2014/2015	Male	5.43 ± 0.43a	4.80 ± 0.59a	0.098	6	0.93
	Female	7.00 ± 0.93a	5.77 ± 1.06a	0.22	6	0.84
	Total	12.43 ± 1.34a	10.57 ± 1.63a	0.18	6	0.86

Means within a row followed by the same letter are not significantly different from each other by t-test (P = 0.05).

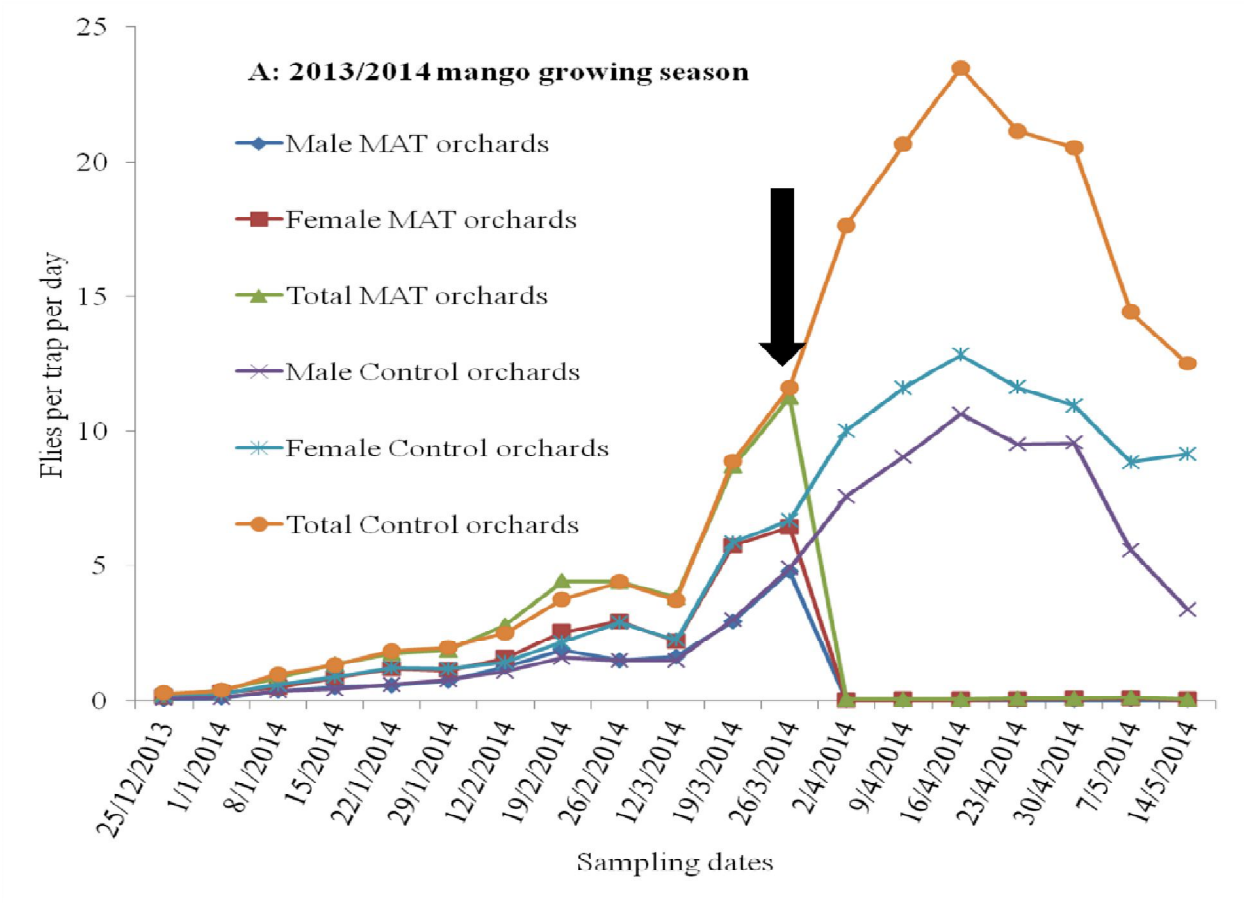

Figure 3.4A: Mean number of *Bactrocera dorsalis* flies captured per trap per day in treatment plots (with MAT systems) and control plots (without MAT systems) in Muhaka during the mango fruiting seasons of 2013/2014.

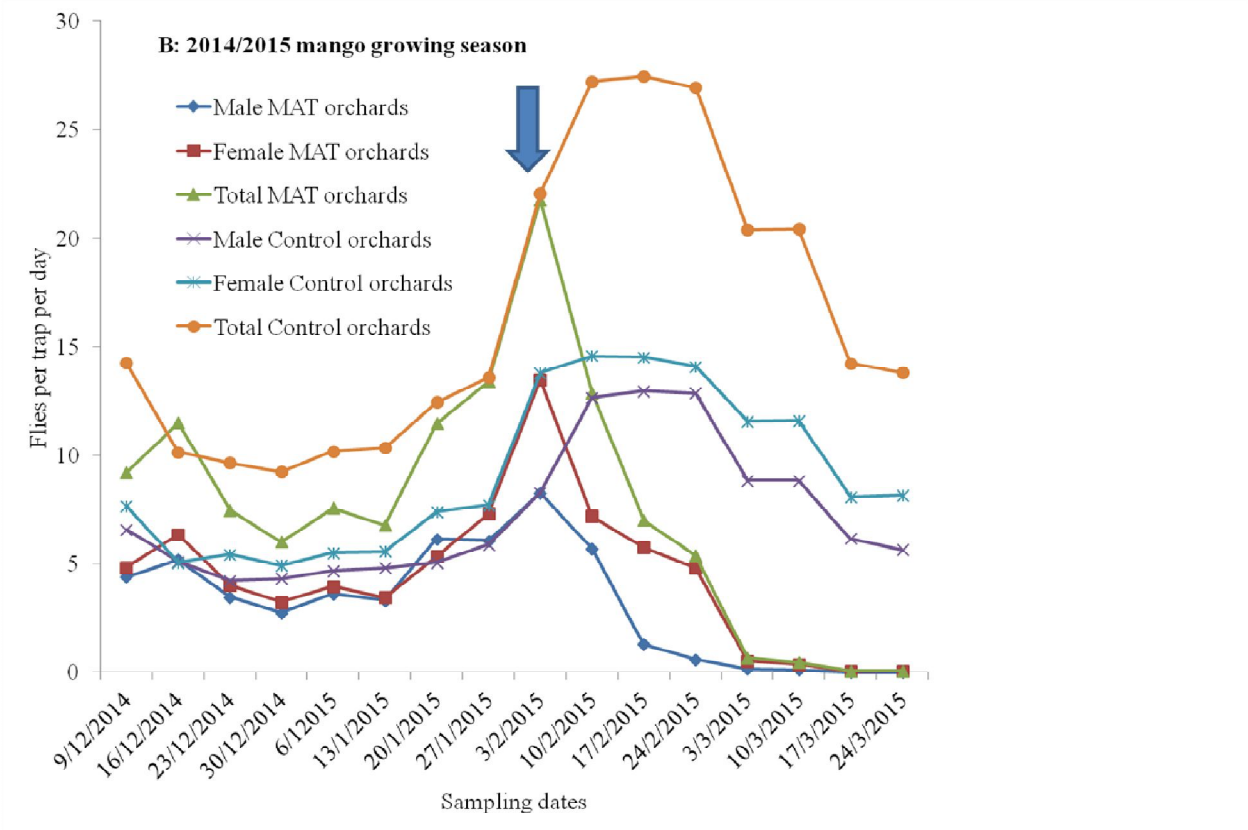

Figure 3.4B: Mean number of *Bactrocera dorsalis* flies captured per trap per day in treatment plots (with MAT systems) and control plots (without MAT systems) in Muhaka during the mango fruiting seasons of 2014/2015.

Arrows in figures, 3.4A and 3.4B denote the point at which male annihilation using methyl eugenol in MAT orchards was initiated.

Percentage infested fruit, mean number of puparia/mango fruit and mean number of puparia/kg of fruits were significantly lower in MAT-treated orchards compared to the control ($F_{1,6} = 59.8$, $p < 0.001$; $F_{1,6} = 95.7$, $p < 0.001$; $F_{1,6} = 6.\ 92.41$, $p < 0.001$, for percentage infested fruits, mean number of puparia/fruit and mean number of puparia/kg of fruits, respectively (Fig. 3.5A).

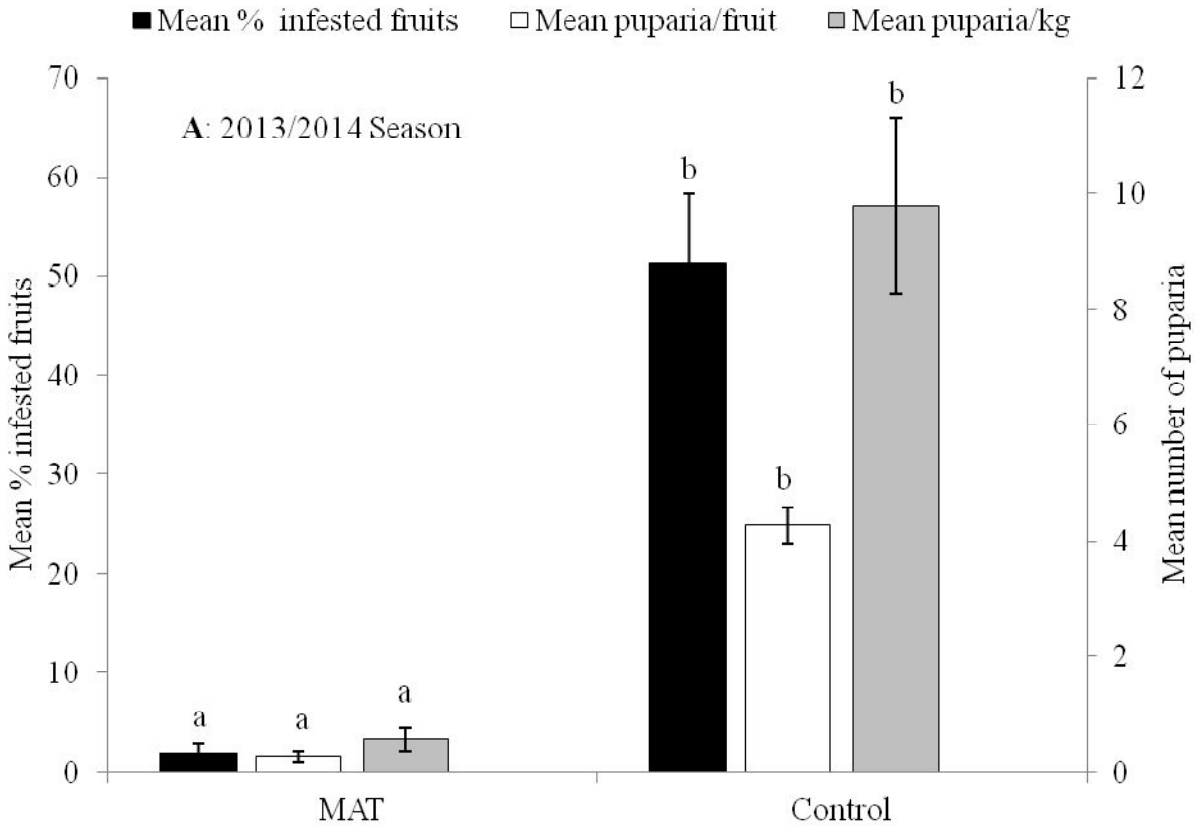

Figure 3.5A: Infestation levels of mango fruits by B. dorsalis in trteatment orchards (with MAT systems) and control orchards (without MAT systems) in Muhaka during the 2013/2014 mango fruiting season.

Error bars denote standard error of the mean (S.E.). For each parameter, means bearing the same letter do not differ significantly at p = 0.05 (Tukey HSD test).

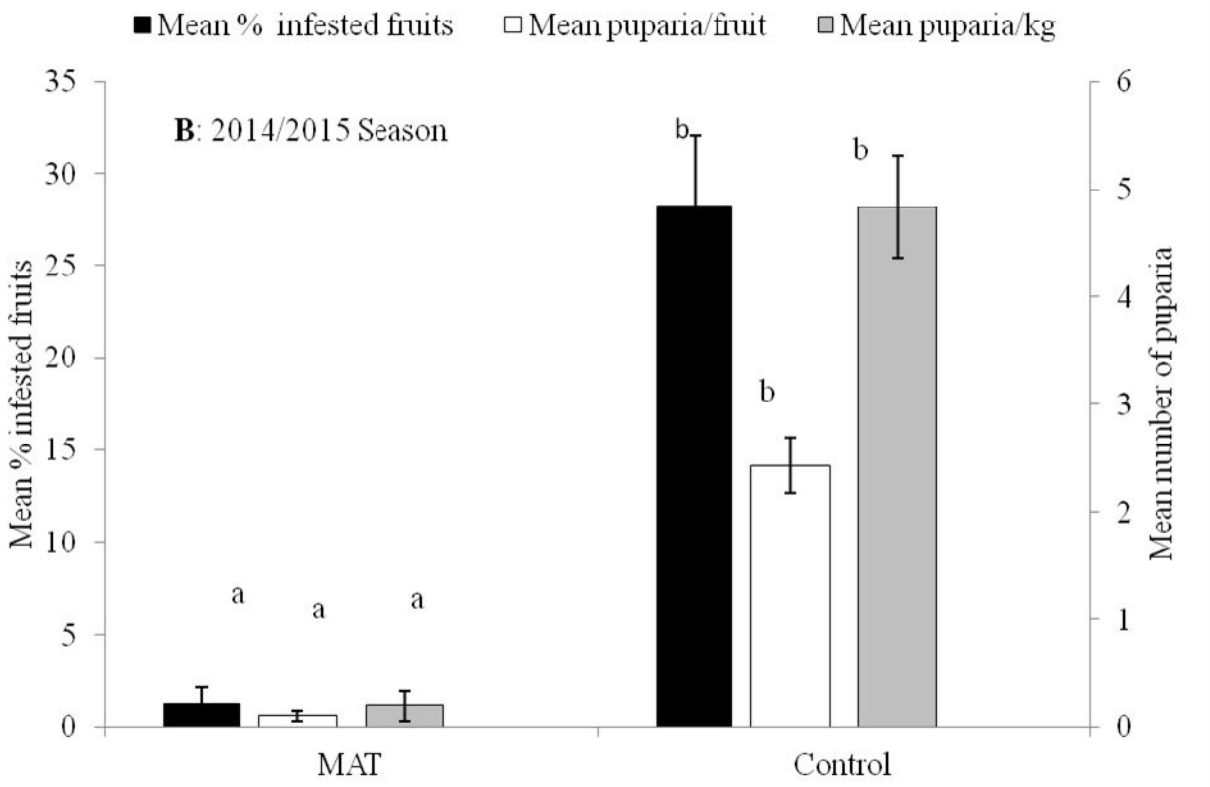

Figure 3.5B: Infestation levels of mango fruits by B. dorsalis in trteatment orchards (with MAT systems) and control orchards (without MAT systems) in Muhaka during the 2014/2015 mango fruiting season.

Error bars denote standard error of the mean (S.E.). For each parameter, means bearing the same letter do not differ significantly at p = 0.05 (Tukey HSD test).

In Season 2, the trend was similar, whereby the MAT-treated orchards had significantly lower mean number of male, female and total FTD compared to those in the control (Table 3.2).

Table 3.2. Mean number of *Bactrocera dorsalis* flies captured per trap per day (FTD) in mango orchards in Muhaka in the 2013/2014 and 2014/2015 mango growing seasons during the suppression phase.

Mango season		Mean *B. dorsalis* FTD ± SE		Statistics		
		Control	MAT-treated orchards	*F*-value	df	Probability *p*
2013/2014	Female	10.71 ± 0.55 a	0.05 ± 0.009 b	62945	1, 6	< 0.0001
	Male	7.90 ± 0.975 a	0.03 ± 0.003 b	1676	1, 6	< 0.0001
	Total	18.61 ± 1.49 a	0.08 ± 0.012 b	48886	1, 6	< 0.0001
2014/2015	Female	11.3 ± 1.134 a	1.91 ± 1.069 b	1273	1, 6	< 0.0001
	Male	9.21 ± 1.289 a	0.36 ± 0.204 b	1527	1, 6	< 0.0001
	Total	20.5 ±2.414 a	2.27 ± 1.263 b	1334	1, 6	< 0.0001

Means within a row followed by different letters are significantly different at $p = 0.05$ (Tukey's test).

Time after deployment of MAT had a significant effect on mean FTD, with male ($F_{6,36} = 159.84$, $p < 0.0001$), female ($F_{6,36}$=215.83, $p < 0.0001$), and total ($F_{6,36}$= 278.4, $p < 0.0001$) captures being lower in the MAT-treated orchard for the three categories. Similarly, the interaction between treatment and days after MAT deployment had a significant effect on fly catches for the three categories ($F_{6,36}$=48.04, $p < 0.0001$; $F_{6,36} = 86.57$, $p < 0.0001$; $F_{6,36}$ =105.7, P < 0.0001, for males, females and total flies, respectively), again being lower in MAT-treated orchards.

This resulted in overall percentage reduction of 99.8%, 99.3% and 99.5%, for males, female and total flies, respectively, and subsequent reduction of fruit infestation in terms of percentage infested fruit ($F_{1,6}$= 48.3, $p < 0.001$), mean number of puparia/fruit ($F_{1,6}$ = 158.03, $p < 0.001$) and mean number of puparia/kg of fruit ($F_{1,6}$ =133, $p < 0.001$) (Fig. 3.5B).

3.5 Discussion

The suppression of *B. dorsalis* in Africa will require an area-wide approach using various management tools of which MAT using methyl eugenol laced with a toxicant have been identified as key to a successful campaign (Ekesi and Billah, 2007). In this study we evaluated the effect of male annihilation for suppression of this pest over two mango fruiting seasons and application of MAT resulted in a significant drop in *B. dorsalis* population within one week of commencement in the first season while in the following season a sudden drop in population density was observed after three weeks of suppression. In both seasons we observed that mean FTD declined to <1 after six weeks of MAT application. At 49 days after deployment of the MAT systems, overall percentage reduction in fruit flies was 99.5% and 99.5%, for seasons 1 and 2, respectively, in the MAT-treated orchards relative to the control. A similar trend in the suppression of *B. dorsalis* was reported by Vargas *et al.,*(2010a), in Kamuela, Hawaii, where flies captured per day declined from a peak of 35.6 FTD to 0.15 FTD, (a percentage reduction of 99.5%). This was, however, a result of several suppression techniques such as the use of parasitoid, baiting, SIT combined with MAT. In earlier studies by Cunningham and Suda (1986) in Hawaii, the authors treated a 62-ha papaya orchard with ME and malathion at the rate of nine fibreboard blocks/ha and reported that the male *B. dorsalis* population was reduced by more than 99 % relative to untreated control orchards located 2.3 km away from the MAT-treated orchard.

Our findings are also consistent with results of the eradication campaign aimed at the African population of *B. dorsalis* in the Limpopo province of South Africa (Manrakhan *et al.,* 2011). The authors reported a drop in FTD from 7.0 to 1.0 within one week of the combined application of MAT (using ME and toxicant mixture impregnated fibreboard blocks) and protein bait in a campaign that marked the first successful eradication of *B. dorsalis* in sub-Saharan Africa (SSA) after its detection in ME traps along the South Africa–Zimbabwe border. The sharp decline in *B. dorsalis* population in the MAT-treated plots compared to that in the control reported in this study, resulted in a considerable reduction in fruit fly infestation both in terms of the percentage of infested fruit and the number of puparia/kg of mango fruit. For example, the percentage of infested fruit was 25 and 18 times lower in MAT-treated plots compared to the control for seasons 1 and 2, respectively, while the mean number of puparia/kg of mango fruit was 17 and 24 times lower in MAT-treated orchards compared to the control for the two consecutive seasons. Similar findings were reported by Hanna *et al*., (2008) in a study carried out in northern Benin on the use of MAT for suppression of *B. dorsalis.* The authors reported a reduction in infestations on mango by 39.8% and 46.8% for Eldon and Kent varieties, respectively. In the same study, the number of pupae/kg of fruit was reduced by up to 60% when MAT was used for suppression of this pest. In Hawaii, comparable results were also reported by Vargas *et al*., (2010b) who found that overall reduction in fruit infestation by *B. dorsalis* (using methyl eugenol) in treatment compared to control orchards declined by 60.7%. In addition to this, they reported that fruit infestation averaged 1.89% even after the cessation of MAT. In Malaysia, Ibrahim *et al*., (1979) used methyl eugenol traps in a carambola (*Averrhoa carambola*) (Oxalidales: Oxalidaceae*)* orchard of 0.5 ha and obtained a 20% reduction in fruit infestation by *B. dorsalis.*

Balasubramaniam *et al*., (1972) used 20–100 methyl eugenol traps in a 5 ha plum orchard over a 36-month period and reduced infestation by *B. dorsalis* from 20% in a control orchard to 3% in the treated orchard.

Recently a novel and environmentally friendly MAT formulation based on Specialized Pheromone and Lure Application Technology in combination with ME and spinosad (SPLAT-MAT Spinosad ME®) was jointly developed by Dow AgroSciences and ISCA Technologies (Hsu *et al*., 2010; Gomez *et al*., 2010). The formulation confers benefits such as reduced application costs, elimination of insecticide residues on fruit, and most importantly substantial reduction of infestation (Gomez *et al*., 2010). Therefore it represents a promising component for the management of *B. dorsalis* (Vargas *et al*., 2008, 2009; Hsu *et al*., 2010). SPLAT-ME is not yet widely used in Africa but the fact that the new formulation ensures that both the lure and the toxicant are protected against ultra violet light and rain (Hsu *et al*., 2010), both of which can be of high intensity in SSA, means that the product holds high potential for use in Africa compared with the conventional liquid and toxicant on cotton wick as used in our experiment.

Although our results are in agreement with findings of other similar studies involving the use of MAT in population suppression of *B. dorsalis* (Cunningham, 1989; Vargas *et al*., 2010b; Suckling *et al.,* 2014), we do not encourage the sole use of MAT for *B. dorsalis* suppression under the SSA setting. This is primarily because of the fragmented nature of the production system, coupled with year round availability of a wide array of wild alternative fruit fly host plants that serve as refuge for and a source of re-infestation by these pests. Instead, we recommend that MAT be used within a holistic IPM approach targeting all developmental stages of fruit flies and preferably in an area-wide approach (Ekesi and Billah, 2007; Ekesi *et al*., 2014).

Indeed, the use of the area-wide approach using different management strategies such as bait-sprays, MAT, releases of sterilised flies and parasitoids and orchard sanitation have been credited with the most outstanding success of *B. dorsalis* suppression in Hawaii (Vargas *et al*., 2000; Vargas *et al.,* 2008; Vargas *et al.,* 2010b; Vargas *et al*., 2015). To be able to access quarantine-sensitive export markets, this approach must be compliant with appropriate post-harvest disinfestation measures. Parameters for cold disinfestation of *B. dorsalis* have been developed for citrus and avocado (Grout *et al*., 2011; Ware *et al*., 2012), while a similar study based on hot water disinfestation treatment for mango is under way.

CHAPTER FOUR

Release, establishment and dispersal of the parasitoids *Fopius arisanus and Diachasmimorpha longicaudata* for the management of *Bactrocera dorsalis* in Kenya

4.1 Abstract

Exotic invasive pests represent serious challenges to pest management because they usually arrive in invaded territories without their associated specialised natural enemies that co-evolved with them in their aborginal home. An example is that of *Bactrocera dorsalis* which invaded Africa in 2003 from Asia causing huge direct and indirect losses mainly through damage to fruits as well as loss of export markets. Chemical control proved unsustainable thus biological control through the use of parasitoids to complement other existing Integrated Pest Management (IPM) measures was implemented. The egg parasitoid *Fopius arisanus* and the larval pupal parasitoid *Diachasmimorpha longicaudata* were imported from Hawaii to complement each other in classical biological control of *B. dorsalis* in the mango agro-ecosystem. Following their introduction, the two parasitoid species were reared at the International Centre of Insect Physiology and Ecology and released in the Coastal and Eastern parts of Kenya at a rate of 1500 wasps ha^{-1} at a ratio of 2: 1, ♀:♂ for management of *B. dorsalis*. Fruits were then sampled for parasitoids recovery. The highest percent parasitism recorded in the Coast and Embu were 33 and 8% for *F. arisanus*; 14 and 16% for *D. longicaudata* respectively. Altitude had an effect on prasitoid establishment, with *F. arisanus* being more recovered at low elevations and warmer temperatures of the Coast and *D. longicaudata* performing well in the medium-high altitudes and cooler conditions of Embu in Eastern Kenya.

Both parasitoids were recovered 8 km from the central release point and *B. dorsalis* was more abundant as the host compared to the native *Ceratitis cosyra* as had been confirmed by the pre-release survey. The study report successful release, establishment and subsequent dispersal of *F. arisanus* and *D. longicaudata* in the mango agro-ecosystem in Kenya.

4.2 Introduction

True fruit flies (Diptera: Tephritidae) are cosmopolitan and constitute serious quarantine pests of fruits and vegetables (White and Elson-Harris, 1992). The invasive oriental fruit fly species, *Bactrocera dorsalis* (Hendel) and the indigenous *Ceratitis cosyra* (Walker) are two tephritids of economic importance in Kenya, where they cause a combined fruit loss of up to 80% on mango depending on season, locality and mango variety (Ekesi *et al*., 2006). Upon its first detection in Africa (Coastal Kenya) in 2003 (Lux *et al*., 2003b), the oriental fruit fly was thought to be a new species and accordingly described as *B. invadens* Drew, Tsuruta & White (Drew *et al*., 2005). However, recent evidence on integrative morphological, molecular, cytogenetic, behavioural and chemoecological research has led to the synonymisation of *B. invadens* with *B. dorsalis* (Bo *et al*., 2014; Schutze *et al*., 2014a; Schutze *et al*., 2014b). Since 2003, *B. dorsalis* continues to spread, causing enormous losses of fruits and vegetables as well as lucrative markets worldwide (Ekesi *et al*., 2006; USDA-APHIS, 2008; Guichard, 2009; Otieno, 2011).

Invasive species pose the greatest danger in their invasion path mainly because they arrive in new regions without their associated specialized natural enemies that co-evolved with them in their regions of origin (Torchin *et al*., 2003; Strayer *et al*., 2006). Thus they multiply unchecked and cause huge losses in revenue in addition to other financial losses as result of costs incurred through uncoordinated management strategies.

In the case of *B. dorsalis*, widespread use of pesticides soon became unsustainable following the enactment of the harmonized maximum pesticide residue levels (MRLs) stipulating the least amount of pesticide tolerated in export fruit (EU, 2005). The United States of America through the U.S. Federal Order also made the situation worse for fruit exporters by banning importation of most cultivated vegetables and fruits from African countries where *B. dorsalis* had been reported (USDA-APHIS, 2008). This was a major blow to most countries exporting fruits and vegetables to European and American markets.

In Kenya, efforts were therefore directed to identifying indigenous natural enemies capable of controlling the invasive *B. dorsalis*. Three native parasitoids namely *Psyttalia cosyrae* (Wilkinson) (Hymenoptera: Braconidae), *P. phaeostigma* (Wilkinson) (Hymenoptera: Braconidae) and *Tetrastichus giffardii* Silvestri (Hymenoptera: Eulophidae) were evaluated for possible control of *B. dorsalis* (Mohamed *et al*., 2006). Unfortunately the parasitoids were unable to complete development in the larvae of the pest due to encapsulation (Mohamed *et al*., 2006; Mohamed *et al*., 2010). Extensive fruit sampling exercises in Kenya also yielded no possible native natural enemy capable of controlling the invasive pest (Rwomushana *et al*., 2008).

Thus in the absence of any promising natural enemy locally, the first step of exploring Sri Lanka the putative aboriginal home of *B. dorsalis* (Khamis *et al*., 2009) for co-evolved biological agents was initiated. The exploration was undertaken by scientists from the International Centre of Insect Physiology and Ecology (*icipe*) and the International Institute of Tropical Agriculture (IITA), in collaboration with The Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Peradeniya, Sri Lank.

The exploration yielded four Braconids: *Diachasmimorpha longicaudata* (Ashmead), *Psyttalia incisi* (Silvestri), *Fopius arisanus* (Sonan), *Fopius* sp; one Eulophid, *Tetrastichus* sp; one Pteromalid i.e *Spalangia* sp; one Diapriid i.e *Trichopria* sp; three predators including one Staphylinid species, one unidentified beetle and another unidentified mite (Billah *et al*., 2008). Sadly none of these natural biological agents were imported into Kenya due to stringent import logistical issues pertaining to restrictions imposed by the 1993 Convention on Biological Diversity (CBD) on the conservation, sustainable use, fair and equitable distribution of biological diversity (Ekesi and Mohamed, 2010).

Following this dead end, focus shifted to identifying institutions rearing natural enemies which could be used to control *B. dorsalis.* Such an institution was the USDA-ARS facility and University of Hawaii both at Manoa, Honolulu, where *F. arisanus* and *D. longicaudata* were being reared and released for the control of the oriental fruit fly, *B. dorsalis.* Relevant statutory processes of importing the two natural enemies into Kenya were initiated. For example, applications were submitted to the Kenya Plant Health Inspectorate Service (KEPHIS), Service de Protection des Végétaux, Ministry of Agriculture of Benin and Tanzania Ministry of Agriculture and Cooperatives. Approval for importation of *F. arisanus* and *D. longicaudata* was granted to *icipe* by the Kenya Standing Technical Committee on Imports and Exports (KSTCIE) in 2006. Following the necessary laboratory assessment of the target host and other key fruit flies species, under strict quarantine conditions, an expertmenal release permit was granted to be carried out at in Nguruman (E036°04'18.4"; S01°48'75.0"; 726 m. a.s.l) under the supervision of and collaboration with KEPHIS. Thereafter, approval for country-wide releases of the two parasitoids was granted by the same committee (KSTCIE), paving way for more releases in other mango producing locations in Kenya.

Therefore, the objective of this study was to initiate a classical biological control programme against *B. dorsalis* by releasing the egg parasitoid *F. arisanus* as well as the complementary larval parasitoid *D. longicaudata* in the Coastal and Eastern provinces of Kenya and monitor for the establishment and dispersal in the mango agro ecosystem.

4.3 Material and Methods

Host and parasitoid rearing, packaging and transporting parasitoids, parasitoid releases and sampling fruits to determine parasitism were done as outlined in the following sections.

4.3.1 Host rearing

Bactrocera dorsalis was reared at the International Centre of Insect Physiology and Ecology (*icipe*) mass rearing quarantine facility in Nairobi, Kenya following the procedures described by Mohamed *et al*., 2003, Ekesi *et al*., 2007c and Ekesi and Mohamed, 2011. Flies were kept in perspex cages (80 by 80 by 80cm) and maintained at 26-28°C, 60-70 RH% and photoperiod of L12: D12. They were fed on a mixture of artificial diet consisting of ground sugar and enzymatic yeast hydrolysate ultrapure (USB Corporation, Cleveland, Ohio, USA) in the ratio 3:1 by volume and provided with water in a petri dish (8.6 cm in diameter) with a layer of pumice granules. Flies from wild populations were periodically added to the mass reared stock flies every 6-12 months to revive genetic vigour. Eggs were collected using mango domes i.e. mango fruit, cut into half, contents scooped out and offered to adult fruit flies to oviposit.

Small pieces of mango peels containing eggs were offered to *F. arisanus* to parasitise while some were placed on a carrot based diet (24.2 g), sugar (16.2 g), brewer's yeast (8.1 g), citric acid (0.6 g), methyl p-hydroxbenzoate (0.2 g), and water (50.7 ml) (Mohamed *et al*., 2003;

Mohamed *et al*., 2008) to develop into second instar larvae which were then offered to *D. longicaudata* to parasitise.

4.3.2 Parasitoid rearing

Fopius arisanus and *D. longicaudata* used in this study were, repectively, reared on eggs and second instar larvae of *B. dorsalis*. Parasitoid wasps were maintained at 25-27°C, 60-70 RH% and photoperiod of L12: D12 in perspex cages (40 × 40 × 40 cm) and provided with fine drops of pure honey streaked on the topside of the cages and water on moist cotton wool balls (5-6 cm diameter) *ad libitum*.

For *F. arisanus* colony maintenance, mango peels with host eggs were placed on small moist sponge material in modified petri dishes (oviposition units, 9 cm in diameter and 0.8 cm deep). The units were then covered using tight fitting organza material to allow female parasitoid to insert their ovipositors to parasitise the eggs. After 24 h of exposure, mango peels with the parasitized host eggs were placed into plastic bowl (12 cm in diameter and 9 cm deep) containing fresh carrot diet for larval feeding. These basins were then placed in 4 L ice cream containers (20 cm by 18 cm × 18 cm) with a hole cut in the lid (10 cm × 10 cm) and replaced with mesh material. A thin layer of sterilized sand (2 cm deep) was added into the ice cream containers to serve as medium into which mature parasitized larvae would pop into to pupate. Mature puparia were then sieved periodically and placed in perspex cages (40 × 40 × 40 cm) to allow adult parasitoids to emerge.

Diachasmimorpha longicaudata colony was maintained by offering the mature mated parasitoids approximately 500-650 second instar larvae of *B. dorsalis* in oviposition units (same as described for *F. arisanus*) containing carrot based diet.

Then the ovipostion units were covered using tight fitting lids covered with organza material through which the wasps would insert their long ovipositors to parasitise the larvae (Wong and Ramadan, 1992; Mohamed *et al*., 2003; Mohamed *et al*., 2008). The units with carrot diet and *B. dorsalis* larvae were exposed to the parsitoids for 24hrs. Thereafter, the contents of the units were emptied into plastic bowls (12 cm in diameter and 9 cm deep) with extra fresh carrot diet for the parasitized larvae to develop. The parasitized larvae were then handled in the same way as described for *F. arisanus*.

4.3.3 Packaging and transporting parasitoids

Putatively mated, 7-10 day old wasps of *F. arisanus* and *D. longicaudata* were aspirated into Perspex cages (30 × 30 × 30 cm). Each cage contained 1500 wasps (Hopper and Roush (1993) recommend release of at least 1500 individuals to achieve successful establishment) at a ratio of 2: 1, ♀:♂. The cages could be opened from the top and two of the sides for easy release. They were provided with honey streaked on the top most part of the cage plus water on moist cotton wool. Parasitoids were transported by road from the rearing facility in Nairobi to parts of the country where they were to be released.

4.3.4 Parasitoid releases

Parasitoids were released (Fig 4.1) during the mango fruiting seasons of 2011/12, 2012/13 and 2013/14 in mango orchards of at least 1 ha in size until the end of the mango production cycle.

Figure 4.1: A farmer (Joseph Maramba: foreground) releases parasitoids from a perspex cage into a mango Orchard in Kilifi (North Coast) under the guidance of a technician.

Releases were done in the Coast, E039°55'15.6"; S04°31'15.3"; 27m a. s. l (average annual rainfall is over 1,000 mm and daytime temperatures average 28-31°C) and Embu E037°58'16.0"; S00°48'21.7"; 1339 m a. s. l. (annual rainfall around 1200 mm to1500 mm and daytime temperatures average 19-22°C). They were done either in the morning (before 10 am) or late in the afternoon (after 4pm) to evade the high temperatures characteristic of the period between 10 am and 4 pm. Table 4.1 summarizes the parasitoid releases done in the Coast and Embu from December 2011 to 2014. Before releasing parasitoids in the successive year, fruits were sampled in release areas to establish if parasitoids were still in the system.

Table 4.1: Summary of *D. longicaudata* and *F. arisanus* parasitoid releases in the Coast and Embu between 2011 and 2014

Mango fruiting season	Location	No. of releases†	Total No. of wasps released	
			D. longicaudata	*F. arisanus*
2011/2012	N. Coast	9	13500	13500
	Embu	8	12000	12000
2012/13	N. Coast	2	3000	3000
	Embu	3	4500	4500
2013/14	N. Coast	3	4500	4500
	Embu	3	4500	4500

†Parasitoid releases were done during the months of January, February, March and April

4.3.5 Sampling fruits to determine parasitism

Following the releases, wind fallen mango fruits (*D. longicaudata* prefers foraging on infested fruits on the ground (Haramoto and Bess, 1970; Sivinski *et al*., 1997) as well as those still in the trees (Fruits on the ground are less attractive to *F. arisanus* than fruits still on the tree (Purcell *et al*., 1994; Eitam and Vargas, 2007)) but showing signs of fruit fly infestation were sampled after every two weeks. The objective was to collect at least a minimum of 100 fruits per site per sampling date, but sometimes was not feasible to do so due to the fact that in some locations, fallen fruits were being consumed by monkeys and baboons and in some instances individuals picked fallen mangoes for juice making.

In some parts, farmers were harvesting their fruits early for export to Asian markets.

The effects of inconsistent mango fruiting seasons were also felt leading to poor harvest or no harvest at all. The sampled fruits were transported to the laboratory in plastic buckets covered with organza material to avoid popping out of mature larvae in infested fruits. In the laboratory the fruits were weighed, counted and incubated in screened wooden cages (60 × 60 × 60 cm). Heat sterilised sand was placed below the fruit holding vessels to allow mature larvae to fall on and pupate. Pupae were sieved after every three days, counted and held in aerated Perspex cages (30 × 30 × 30 cm) for fruit flies and parasitoid emergence. Thereafter, emerged fruit flies and parasitoids were counted, sexed and identified to species level.

4.3.6 Parasitoid dispersal

Parasitoids were released at a central point in Eastern Kenya (Kamwino farm; E037°64'36.3"; S00°42'66.4"; 1362 m. a. s. l). After every two weeks, 20 kg of mango fruits were collected from each farm in surrounding farms, in different directions and radii from the release point. Sampling was only done up to a radius of 8 km from the release point due to the heterogeneity of mango farms around the release point. Fruits were then incubated in the laboratory until all flies and parasitoids had emerged. Emerging flies and parasitoids were counted and recorded according to species (White and Elson-Harris, 1992; Drew *et al*., 2005; Wharton and Yoder, 2014).

4.3.7 Data analysis

Data on number of parasitoids emerging from fruits were converted to percent parasitism and subjected to ANOVA to test for differences in *F. arisanus* and *D. longicaudata* parasitism in areas where wasps were released for classical biological control of *B. dorsalis*.

Means were then separated using Duncan's multiple range test. The number of fruit flies *B. dorsalis* and *C. cosyra* emerging from fruits were presented as proportions. Counts of *F. arisanus* and *D. longicaudata* recovered from fruits in the dispersal studies were log transformed to normalise the data before ANOVA to test for effect of direction in dispersal. Mean separation was done using the Student Newman Kuels test. All analyses were performed using R software version 3.1.1 (R Development-Core-Team, 2014).

4.4 Results

The highest percent parasitism by *F. arisanus* recorded in the Coast and Embu were 33 and 8%, respectively, while that by *D. longicaudata* for same locations was 14 and 16%, respectively (Table 4.2). Location had a significant effect on percent parasitism ($F_{1,24} = 5.94$, $p = 0.023$), with parasitoid recoveries for *F. arisanus* being higher in the Coast compared to Embu (Table 4.2). Similarly, parasitoid recoveries for *D. longicaudata* were higher ($F_{1,24} = 4.81$, $p = 0.021$) in Embu than in the Coast (Table 4.2). There was a significant interaction between location and parasitoid species ($F_{1,24} = 5.84$, $p = 0.024$). However, the interaction between year and location ($F_{2,24} = 0.28$, $p = 0.76$); and year and parasitoid species ($F_{2,24} = 0.30$, $p = 0.74$) had no significant effect on percent parasitim by both parasitiod species. Generally, percent parasitism for both *D. longicaudata* and *F. arisanus* showed an increasing trend from 2012 to 2014 and still continue to rise (Fig 4.2).

The proportion of *B. dorsalis* emerging as adults from puparia was slightly higher than *C. cosyra* with 7, 42, and 53% *B. dorsalis* and 0, 41 and 14% *C. cosyra* in the Coast during the 2012, 2013 and 2014 mango fruiting seasons respectively (Table 4.2). The trend was similar in Embu in 2013 with 32% and 31% *B. dorsalis* and *C. cosyra* respectively.

Exceptions were in 2012 when *B. dorsalis* adults were used to rejuvenate the laboratory colony kept at *icipe* before they were counted and in 2014 (20% *B. dorsalis* and 41% *C. cosyra*) when methyl eugenol was deployed in Embu in a parallel IPM programme.

Fopius arisanus and *D. longicaudata* were both recovered at a distance of 8 km from the central release point (Table 4.3 & 4.4). There was a significant difference in the mean number of *F. arisanus* wasps recovered from fruits in different directions at 1-2 km from the release point ($F_{3,12} = 10.63$, $p = 0.001$), with a higher number being recovered from the northern direction. However, there were no significant differences in the mean number of the same, ($F_{3,12} = 3.37$, $p = 0.30$); ($F_{3,12} = 1.31$; $p = 0.32$) and ($F_{3,12} = 1.67$, $p = 0.23$), recovered in different directions at 2-4 km, 4-6 km and 6-8 km from the release point respectively. On the other hand, the number of *D. longicaudata* wasps recovered from fruits varied considerably with sampling direction from the release point at 1-2 km ($F_{3,12} = 7.61$, $p = 0.007$), 2-4 km ($F_{3,12} = 6.27$, $p = 0.008$) and 6-8 km ($F_{3,12} = 5.37$, $p = 0.014$) with higher numbers being recovered from the northern, western and southern directions in that order. At 4-6 km from the release point, wasps recoveries were comparable ($F_{3,\ 12} = 3.55$, $p = 0.05$) in all directions. Recoveries of both parasitoids three years after the initial release confirm that they have established in the Kenyan mango agrosystem.

Table 4.2: *Fopius arisanus* and *Diachasmimorpha longicaudata* recoveries following adult wasp releases in two mango growing areas in Eastern and Coastal provinces of Kenya, 2012-2014

Fruit sampling			Fruit weight (kg)	Total No. puparia	No. Fruit fly species emerging		No. parasitoid species emerging		% parasitism	
Location	Year	Month			*B. dorsalis*	*C. cosyra*	*D. longicaudata*	*F. arisanus*	*D. longicaudata*	*F. arisanus*
Embu	2012	Jan	30.2	120	0	99	0	0	0	0
		Feb	113.9	3985	5	357	135	46	3.39	1.15
		Mar	83	5658	0	0	67	37	1.18	0.65
Coast	2012	Jan	60.2	474	270	0	18	30	3.80	6.33
		Feb	80	2420	0	0	59	175	2.44	7.23
		Mar	36.2	945	0	0	20	58	2.12	6.14
Embu	2013	Jan	30.2	120	24	0	0	0	0	0
		Feb	113.9	3985	1134	232	135	46	3.39	1.15
		Mar	135	7698	2666	3509	112	59	1.45	0.77
Coast	2013	Jan	12	1259	348	451	20	62	1.59	4.92
		Feb	16	840	328	0	28	129	3.33	15.36
		Mar	8.2	920	595	0	18	82	1.96	8.91
Embu	2014	Jan	22	1340	552	371	122	30	9.10	2.24
		Feb	74	4610	1319	1348	124	0	2.69	0
		Mar	32	366	436	891	60	30	16.39	8.20
Coast	2014	Jan	28.5	680	590	22	18	48	2.65	7.06
		Feb	15	96	45	0	13	32	13.54	33.33
		Mar	16.2	649	114	181	24	9	3.70	1.39

The objective was to collect at least a minimum of 100 fruits per site per sampling date, but sometimes was not feasible to do so due to the fact that in some locations, fallen fruits were being consumed by monkeys and baboons and in some instances individuals picked fallen mangoes for juice making. In some parts, farmers were harvesting their fruits early for export to Asian markets.

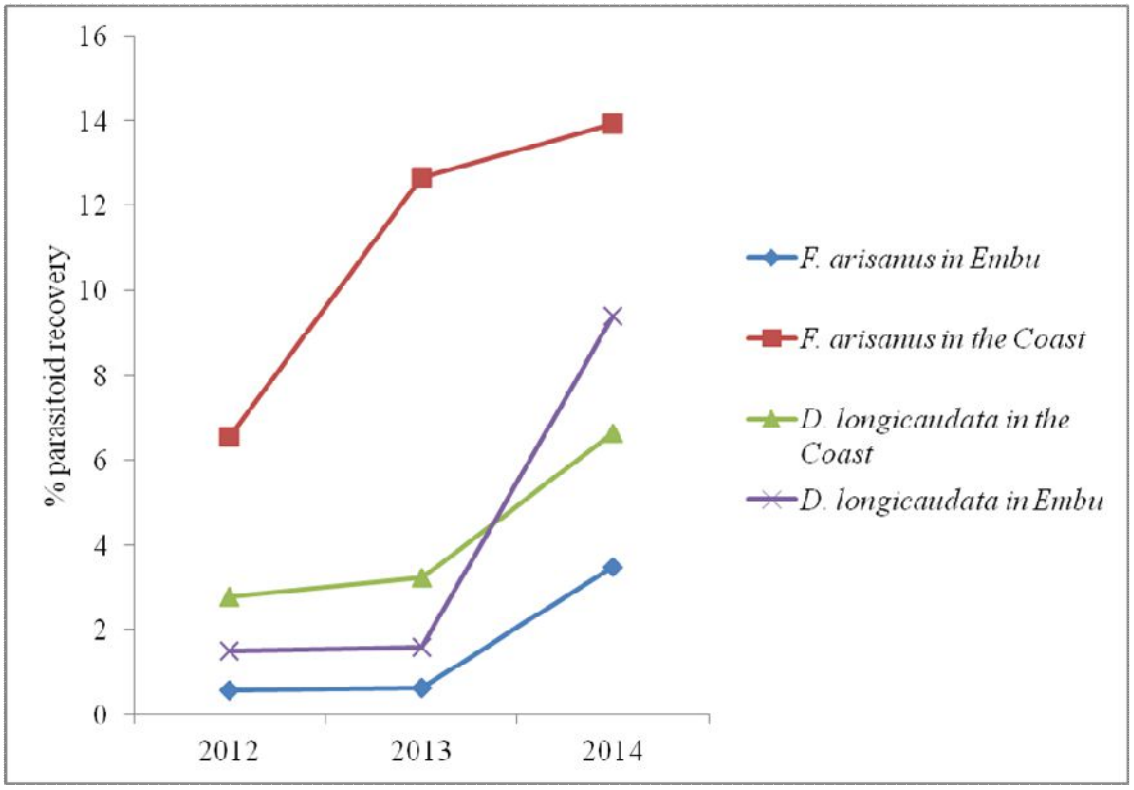

Fig 4.2: Average percent parasitoid recovery trend in Embu and the Coast from 2012-2014.

Table 4.3: Dispersal of *D. longicaudata* in Embu, in different directions from release point; 35-108 days after release.

Days after release	Direction of dispersal	Number of parasitoids emerging from 20 kg fruits at intervals from release point			
		1-2 km	2-4 km	4-6 km	6-8 km
35	N	19	1	20	7
	S	0	7	5	25
	E	2	3	0	31
	W	10	12	6	11
42	N	12	0	9	1
	S	0	7	4	39
	E	5	2	8	21
	W	19	13	24	19
79	N	17	0	14	0
	S	0	3	7	25
	E	8	7	4	9
	W	17	14	12	9
86	N	17	0	18	0
	S	0	0	0	9
	E	1	3	0	5
	W	1	2	5	16
108	N	15	0	0	6
	S	0	0	1	12
	E	9	1	0	0
	W	4	0	0	16

Table 4.5: Dispersal of *F. arisanus* in Embu in different directions from release point; 35-108 days after release.

Days after release	Direction of dispersal	Number of parasitoids emerging from 20 kg fruits at intervals from release point			
		1-2 km	2-4 km	4-6 km	6-8 km
35	N	3	1	5	5
	S	0	3	5	2
	E	2	1	1	15
	W	0	0	0	0
42	N	17	0	3	1
	S	0	1	3	6
	E	0	2	8	5
	W	4	5	9	10
79	N	19	0	4	1
	S	0	1	4	8
	E	0	2	2	2
	W	4	4	6	5
86	N	3	0	5	0
	S	0	0	8	7
	E	1	3	0	3
	W	0	5	0	11
108	N	5	2	0	1
	S	0	0	1	7
	E	0	0	0	1
	W	1	1	0	8

4.5 Discussion

The ability of a parasitoid species to establish and disperse largely affects the success of a classical biological programme. *Fopius arisanus* and *Diachasmimorpha longicaudata* were recovered in both release areas, namely Coast and Embu. Percent parasitism for *F. arisanus* was significantly higher in the Coast (33%) compared to that in Embu (8%). On the other hand *D. longicaudata* recovery was slightly higher in Embu (16%) compared to the Coast (14%). The Coast area is predominantly of low altitude (27 m a. s. l) and higher temperatures compared to Embu which is largely medium to high altitude (1339 m a. s. l) and cooler temperature. Our findings are in agreement with Wong *et al*., (1984) who reported that *F. arisanus* perfomed well at low elevations and slightly higher temperature compared to cooler higher elevations. In addition, Sivinski *et al*. (2000) reported that indeed spatial occurrence of parasitoid species follow altitudinal gradients. However these patterns in abundance are not the result of altitude in exclusivity but somewhat due to variations in environmental factors brought about by the effect of altitude (Sivinski *et al*., 2000b). In this regard it is conceivably not surprising that *F. arisanus* was more abundant in the Coast compared to Embu where *D. longicaudata* numbers were invariably higher. However levels of parasitism could actually be higher than reported in our findings especially considering that we were unable to sample as many fruits as had been planned due to the fact that some growers were harvesting their mangoes early. The situation was further compounded by the fact that the mango fruiting seasons were unreliable and sometimes fruiting was poor, presumably due to the vagaries of weather and climate change.

Some authorities have also argued that the method of parasitoid release has a bearing on levels of parasitism in the field.

For example Cameron *et al*., (1993) and Grevstad (1999) advocate for release of large numbers of parasitoids in a few or limited places in contrast to a small number in a wide array of places as advocated for by Campbell (1976) and Memmot *et al*. (1998). However the most important aspect is the minimum number required to attain establishment. Hopper and Roush (1993) recommended that successful establishment requires that at least 1000 individuals should be released at a particular site if establishment is the ultimate aim. In the present study, a total of 21 000 adult wasps of either *F. arisanus* or *D. longicaudata* were released in both Coast and Embu. Before the onset of each mango fruiting season, fruit sampling revealed the presence of *F. arisanus* and *D. longicaudata* in release areas and beyond. Parasitoids were also recovered three years after release at initial release points, which confirms establishment of the two parasitoid species in the Kenya mango agro-ecosystem. This also confirms the success of the two parasitoids in finding hosts and self perpetuation in the prevailing climatic conditions which are obviously different from their native ranges. According to DeBach and Bartlett (1964), an exotic species is considered established if adults are recovered at least one year after release at a particular location or site.

Generally, there was an increasing trend in percent parasitism for the two parasitoid species from 2012 to 2014. *Diachasmimorpha longicaudata* is known to adapt well to a number of fruit flies species and easily establishes in various habitats (Schliserman *et al*., 2003). In some instances, it is even known to form new associations with native fruit flies species (Mohamed *et al*., 2008). It is for this reason that *D. longicaudata* continues to be imported from its native origin, the Indo-Pacific region for release in many countries worldwide. Though parasitism was consistently low in Argentina (approximately 1%), the parasitoid managed to sustain itself even 40 years after its initial release (Schliserman *et al*., 2003; Ovruski and Schliserman, 2012).

The proportion of fruit flies emerging from puparia obtained from mango fruits in both the Coast and Embu were predominantly *B. dorsalis*. It would seem that *B. dorsalis* is out-competing the native *C. cosyra* be it in low lands or highlands.

These observations are in agreement with Ekesi *et al*., (2009) who reported that, four years after the *B. dorsalis* incursion in Kenya, the invasive pest had displaced *C. cosyra* to become the predominant fruit fly species in mango orchards. The same outcome was reported in Tanzania and Northern Mozambique where significantly higher numbers of *B. dorsalis* compared to the native *C. cosyra* were recovered from several hosts of economic importance (Mwatawala *et al*., 2009a; Cugala and Santos, 2013).

As has already been alluded, the propensity of a bio control agent to disperse has a positive bearing on classical biological control. Therefore, it is imperative that successful parasitoids should be efficient in searching and dispersing. Mass reared parasitoids should be able to disperse and locate their hosts under natural conditions which tend to be very different from laboratory rearing conditions (Messing *et al*., 1997). Both *F. arisanus* and *D. longicaudata* were recovered eight kilometers from the release point, 108 days after they were released. It is possible that these parasitoids could have dispersed beyond 8 km since no attempt was made in this present study to carry out fruit sampling beyond this point. This level of dispersal is impressive and brings hope in the quest to manage *B. dorsalis* through biological control. Similar results were obtained in Benin, West Africa when *F. arisanus* was released from a central position and recoveries made 6-8 km from the release point after 100 days (Gnanvossou *et al*., unpublished). We therefore, predict that as *F. arisanus* and *D. longicaudata* increase in abundance they will eventually disperse into suitable habitats throughout all mango growing regions in Kenya.

When *F. arisanus* was released in Hawaii in the 1950s, its successful establishment resulted in considerable control of the invasive *B. dorsalis* (Purcell, 1998; Wharton, 1989; Bokonon-Ganta *et al*., 2007).

In conclusion, this study has demonstrated the establishment and dispersal of two important exotic braconid parasitoid species in the quest to suppress *B. dorsalis* in the mango agro ecosystem in Kenya. Data presented here should be adequate to guide augmentative releases to boost parasitoid populations in Kenya. There is need to establish factors that impact on the successful establishment and dispersal of *F. arisanus* and *D. longicaudata* in Kenya especially with respect to climate, unreliable fruiting seasons, as well as the fragmented and heterogeneous agricultural landscape. For example wind tunnel experiments by Messing *et al*. (1997) revealed that *D. longicaudata* showed reduced flight and walking efficiency when wind speeds over 5.8 m/s prevailed in release areas. This is significant since wind currents play a pivotal role in the dispersal of insects. However, additional studies involving long term post-release sampling protocols to establish persistence of the two braconid wasps in the field is recommend. The findings of this study also advocate for the incorporation of biological control as a complementary strategy in the management of pestiferous fruit flies on top of traditional management techniques such as Male annihilation technique and baiting among others. However, biological control will only be effective if the use of pesticides is minimized over large areas.

CHAPTER FIVE

Evaluation of the interaction between the introduced parasitoid *Diachasmimorpha longicaudata (Ashmead)* and the native parasitoid *Psyttalia cosyrae* (Wilkinson) (Hymenoptera: Braconidae) under laboratory conditions

5.1 Abstract

The exotic braconid parasitoid *Diachasmimorpha longicaudata* was imported from Hawaii for the management of the invasive tephritid, *Bactrocera dorsalis* in Kenya, where *Psyttalia cosyrae* occurs as the most abundant indigenous parasitoid controlling *Ceratitis cosyra*. Following reports that *D. longicaudata* had formed new associations with *C. cosyra*, experiments were conducted in the laboratory to investigate the interaction between the two larval-pupal endoparasitoids under three scenarios of *B. dorsalis* only, *C. cosyra* only and mixed infestation of *B. dorsalis* and *C. cosyra.* Hosts were offered to sole, sequential and simultaneous combinations of the two parasitoid species, *P. cosyrae* and *D. longicaudata. Diachasmimorpha longicaudata* was the most efficient parasitoid as shown by searching, probing and ovipositing events on all host combinations evaluated. Percent parasitism (specific) was significantly higher for *D. longicaudata* compared to *P. cosyrae* regardless of whether *B. dorsalis* or *C. cosyra* were offered to the parasitoids separately or in a mixed host scenario. For example parasitism was two times higher when *D. longicaudata* was offered *C. cosyra* as the only host compared to *P. cosyrae* on the same host. When the host was *B. dorsalis*, parasitism was 53% for *D. longicaudata* compared to zero for *P. cosyrae*. When *D. longicaudata* was offered a mixture of *B. dorsalis* and *C. cosyra* first before the same was transferred to *P. cosyrae*, percent parasitism was 11-fold more by the former parasitoid species than parasitism due to the later. In the reverse scenario, percent parasitism was slightly reduced to 10 times more than for *P. cosyrae.*

In single host scenarios, percent parasitism attributed to *D. longicaudata* was 40 times more than parasitism due to *P. cosyrae*, when the former was offered *C. cosyra* ahead of the native parasitoid. Reversing the above set up resulted in parasitism due to *D. longicaudata* being 8 times higher than that of *P. cosyrae*. Furthermore, regardless of the order in which *P. cosyrae* was offered *B. dorsalis* in separate scenarios, *P. cosyrae* was encapsulated and parasitism was zero. Offering a combination of *B. dorsalis* and *C. cosyra* simultaneously resulted in parasitism 4 fold higher for the exotic parasitoid compared to the native. Finally, percent parasitism was 37 times higher for *D. longicaudata* compared to that of *P. cosyrae* when a mixture of the two was offered *C. cosyra* as the sole host. The implications of these findings are discussed.

5.2 Introduction

The Tephritid fruit flies, have a worldwide distribution and comprises approximately 4000-4500 described species in 500 genera (White and Elson-Harris, 1992; Freidberg, 2006, Rull, 2008) in which about 1500 species are known to be associated with fruits (Zhihong *et al*., 2013). Two hundred and fifty of this number are pests of economic importance (Zhihong *et al*., 2013) causing severe direct and indirect damage resulting in yield reduction and loss of markets through quarantine restrictions imposed by importing countries (Ekesi *et al*., 2006; USDA-APHIS, 2008; Guichard, 2009; Liang, 2011; Otieno, 2011; Vargas *et al*., 2013).

In view of enormous losses incured in the horticultural agro-industry, control and management programmes aimed at suppressing or eradicating pestiferous tephritidae have been implemented worldwide, with variable success. Some of the strategies that have been used widely include chemical control (Heather *et al*., 1987; N'Depo *et al*., 2015), orchard sanitation (Pinero *et al*., 2009; Klungness *et al*., 2005), baiting technique (Steiner, 1955; Moreno *et al*., 2001; Prokorpy *et al*., 2003; Vargas *et al*., 2009), sterile insect technique

(Koyama *et al*., 2004; Wimmer, 2005; Calla *et al*., 2014; Ogaugwu, 2014); male annihilation technique (Vargas *et al*., 2000; Vargas *et al*., 2010b; Ghanim, 2013), entomopathogenic fungi (Ekesi *et al*., 2002; Toledo *et al*., 2007) and use of parasitoids (Montoya *et al*., 2000; Ovruski *et al*., 2000; Mohamed *et al*., 2008) among others. The need for cost effective, environmentally friendly and sustainable approaches to fruit fly management has greatly pushed pest management to the strengthening of strategies fitting this category or creating new prospects for tactical combinations in an IPM approach (Suckling *et al*., 2014). One such strategy is the use of parasitoids. A number of natural enemies are responsible for regulating pest populations in nature and have since been harnessed in programmes involving captive mass rearing and intentional release for the purpose of controlling target insect populations. Parasitic wasps in the order Hymenoptera, family Braconidae have been used in the management of fruit flies of economic importance such as *Ceratitis* (Ovruski *et al*., 2000), *Anastrepha* (Montoya *et al*., 2000) and *Bactrocera* (Vargas *et al* 2007, Mohamed *et al*., 2008) among others. The family is one of the largest in the Hymenoptera, comprising more than 15 000 described species with several other new species still being described (Quicke and van Achterberg, 1990; Wharton, 1993). Within the Braconidae is the subfamily Opiinae, a diverse group of koinobiont endoparasitoids of various cyclorrhaphous Diptera constituting about 1500 to 1900 species worldwide (Carmichael *et al*., 2005; Fischer and Madl, 2008; Wharton *et al*., 2012). Out of this number, more than 100 species have successfully been reared on fruit-infesting Tephritidae (Wharton and Yoder, 2014) and several have been effective in controlling most fruit flies of economic importance (Ramadan *et al*., 1989; Eben *et al*., 2000; Sivinski *et al*., 2000a; Wharton *et al*., 2000; Vargas *et al*., 2002; Bautista *et al*., 2004; Mohamed *et al*., 2008; Ovruski *et al*., 2011; Yokoyama *et al*., 2012; Bokonon-Ganta *et al*., 2013; Shariff *et al*., 2014).

Opiinae are the most preferred natural agents for fruit fly suppression because of their host specificity and high parasitism rates (Sivinski, 1996; Sivinski *et al*., 1996; Vargas *et al*., 2012).

Two tephritid fruit flies species of economic importance namely *B. dorsalis* (Hendel) and *Ceratitis cosyra* (Walker) occur in Kenya, where they cause a combined fruit loss of up to 80% on mango depending on season, locality and mango variety (Ekesi *et al*., 2006). To curb their menace, an IPM package, targeting various stages of fruit flies development, consisting of, spot application of bait spray, Male annihilation technique (MAT), biopesticide application, orchard sanitation and parasitoid release, is being promoted and implemented in different major mango growing areas (Ekesi and Billah 2007; Ekesi *et al.,*2011; Mohamed *et al.,*2012). This initiative has resulted in a drastic reduction of target fruit fly pest populations, especially *B. dorsalis* and has led to the production of high quality fruit acceptable to export markets.

Two parasitoid species, namely the indigenous *Psyttalia cosyrae* (Wilkinson) and the exotic and introduced *Diachasmimorpha longicaudata* ((Ashmead) both Hymenoptera: Braconidae)) can play a significant role in managing *C. cosyra* and *B. dorsalis* respectively in the Kenyan mango agro-ecosystem. *Psyttalia cosyrae* and *D. longicaudata* are both synovigenic, koinobiont larval-pupal parasitoid of *C. cosyra* and *B. dorsalis* respectively (Mohamed *et al*., 2003). The former parasitoid is a co evolved natural enemy of *C. cosyra* (but perhaps parasitises other fruit fly hosts) while *D. longicaudata* parasitize several fruit flies (Ovruski *et al*., 2000; Montoya *et al*., 2000; Meirelles, 2013) but has not been reported on *C. cosyra*. *Diachasmimorpha longicaudata* was introduced in 2006 in Kenya from Hawaii for the classical biological control of *B. dorsalis* but has also been reported to have formed a new association with the native fruit fly, *C. cosyra* (Mohamed *et al*., 2008).

The wasp is the most widely used control agent in biological control programs of tephritidae in North and South America as well as their associated Islands (Meirelles, 2013). It was successfully introduced into the Americas and soon became one of the most important parasitoids in the control of *Anastrepha* species and *C. capitata* (Paladino *et al*., 2010; Montoya *et al*., 2012; Suarez *et al*., 2014). Once released and established *D. longicaudata* populations become self perpetuating and persist in the system for many generations. According to Oroño and Ovruski, 2007, the parasitoid was recovered in the North western region of Argentina 40 years later after its initial release. Currently in Kenya, the parasitoid has been recovered on *B. dorsalis* as well as *C. Cosyra.* Exotic parasitoids are often introduced into new agro ecological systems with the ultimate aim of suppressing a target pest (Mills, 2003). However, they sometimes form new associations with other hosts that are usually controlled by indigenous parasitoids. In nature, a single host species is sometimes attacked by parasitoids belonging to different genera or species, resulting in interactions whose outcomes are multifaceted (Godfray, 1994; Price, 1972; Harvey *et al*., 2009). For example, one or both species might seek to exclusively use host resources (Harvey *et al*., 2013) thereby resulting in fierce competition at the level of seeking the host or among developing immature stages within the host (De Moraes *et al*., 1999). In instances where the introduced and indigenous parasitoids share the same host, competition may either affect the establishment of the former or performance of the later, resulting in the decline of reproduction and ultimate population drop of either of the two or even both depending on the severity of the contest (Boettner *et al*., 2000; Louda *et al*., 2003; Harvey *et al*., 2013). In actual sense, a host attacked by a koinobiont parasitoid, remains available to further parasitisation by other generalist parasites of different species resulting in a contest for resources (Taylor, 1988). This phenomenon is of importance in determining host-parasitoid interactions that lead to the ultimate success of the natural enemy (Taylor, 1988).

The implications of such associations are usually not fully explored, resulting in mysterious extinctions of native parasitoids at particular trophic levels. In the context of Kenya, indigenous parasitoids were unable to suppress *B. dorsalis*, due to encapsulation by the host (Mohamed *et al*., 2006; Mohamed *et al*., 2010), necessitating the importation and release of an exotic parasitoid to control *B. dorsalis*. The introduced parasitoid showed promising results in controlling the target pest and was able to successfully parasitise and complete its life cycle in the indigenous *C. cosyra*. Therefore the objective of this study was to evaluate the interaction between the introduced parasitoid *D. longicaudata* and the indigenous *P. cosyrae* at various parasitoid-host combinations involving *B. dorsalis* and *C. cosyra*.

5.3 Material and Methods

5.3.4 Hosts and Parasitoids

The fruit flies *B. dorsalis* and *C. cosyra* (Fig 5.1), as well as parasitoids *D. longicaudata* and *P. cosyrae* (Fig 5.2) used in this study were reared at the International Centre of Insect Physiology and Ecology (*icipe*) mass rearing quarantine facility in Nairobi, Kenya.

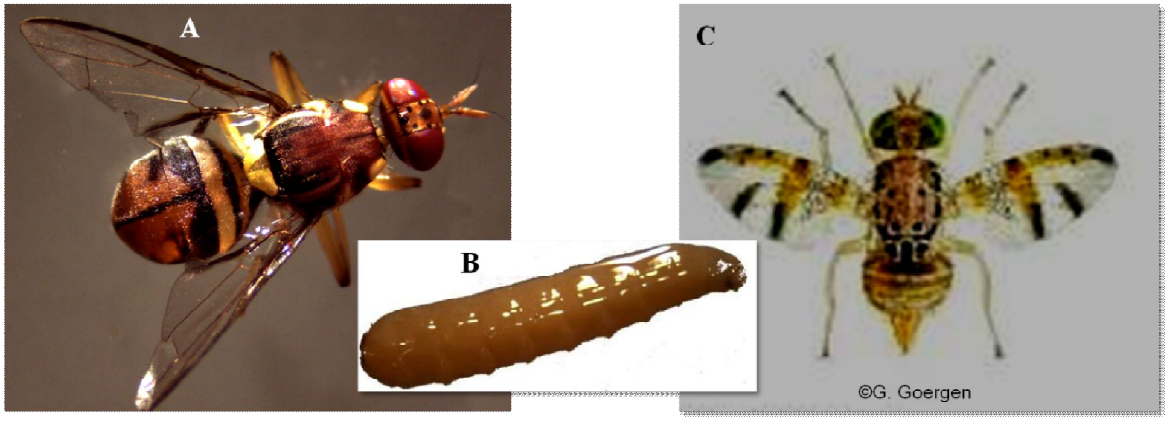

Figure 5.1: Adult fruit flies and mature larva. (A) *B. dorsalis*, (B) larva of *B. dorsalis* (superficially similar to *C. cosyra* larva (C) *C. cosyra*.

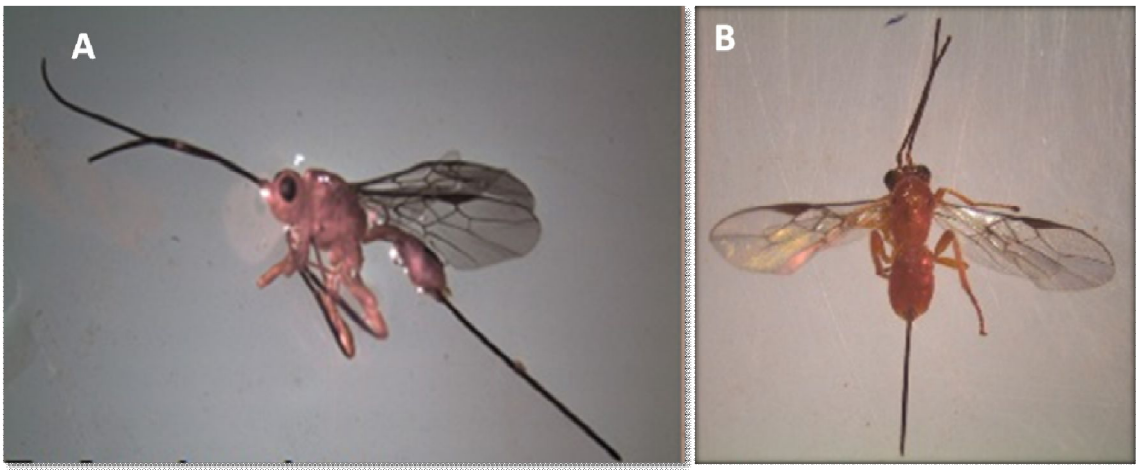

Figure 5.2: Adult parasitoids used in the interaction study. (A) *D. longicaudata* and (B) *P. cosyrae.*

The flies were reared following the procedures described by Mohamed *et al.* (2003), Ekesi *et al*. (2007) and Ekesi and Mohamed (2011). They were kept in perspex cages (80 × 80 × 80 cm) and maintained at 26-28°C, 60-70 RH% and photoperiod of L12: D12. Adult flies were fed on a mixture of artificial diet consisting of ground sugar and enzymatic yeast hydrolysate ultrapure (USB Corporation, Cleveland, Ohio, USA) in the ratio 3:1 by volume and provided with water in a Petri dish (8.6 cm in diameter) with a layer of pumice granules. Flies from wild populations were periodically added to the mass reared stock flies every 6-12 months to revive genetic vigour. *Diachasmimorpha longicaudata* was reared on late second instar larvae of *B. dorsalis* while *P. cosyrae* was raised on *C. cosyra* larvae of the same age following a procedure similar to that described by Wong and Ramadan, 1992, Mohamed *et al*., 2003. Parasitoids were maintained at 25-27°C, 60-70 RH% and photoperiod of L12: D12 in perspex cages (40 × 40 × 40 cm) and provided with fine drops of pure honey streaked on the topside of the cages and water on moist cotton wool balls (5-6 cm diameter).

5.3.5 Experimental procedure

Interaction of the two parasitoids was determined under three scenarios of *B. dorsalis* only, *C. cosyra* only and mixed infestation of *B. dorsalis* and *C. cosyra* at a ratio of 1:1. Naive mated females of *D. longicaudata* (7-9 day old) and *P. cosyrae* (13-15 day old) (choice of age of parasitoids was a function of maturity of the two species) were used in all cases outlined below. For *B. dorsalis* as host, five different set ups were evaluated as follows: (a) 100 late 2nd instar larvae (first instars are too small to support the developing parasitoid embryo and third instars have fully developed immune systems which allow them to encapsulate the parasitoid embryo) of *B. dorsalis* were placed in an oviposition unit (Fig 5.3) consisting of a customised Petri dish (9 cm diameter and 0.3 cm depth) with a tightly fitting organza lid.

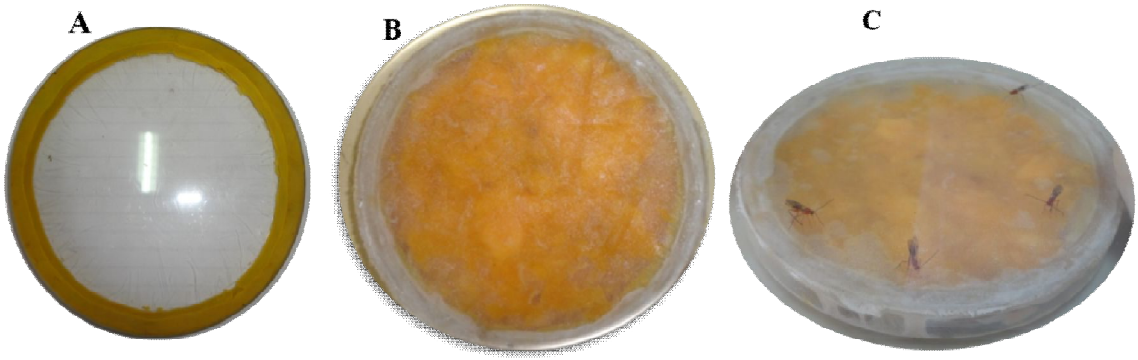

Figure 5.3 Oviposition unit made of modified petri dish. (A) Lined with molding clay so as to ensure that larvae do not hide in the walls (B) with larval diet and tightly fitted with organza material (C) with parasitoids foraging on top of the organza material.

The larvae were provided with a diet containing carrot powder (24.2 g), sugar (16.2 g), brewer's yeast (8.1 g), citric acid (0.6 g), methyl p-hydroxybenzoate (0.2 g), and water (50.7 ml) (Mohamed *et al*., 2003; Mohamed *et al*., 2008). Oviposition units were placed in ventilated perspex cages (12 × 12 × 12 cm) and 20 females of *D. longicaudata* released inside to forage and oviposit for 6 hrs. (b) Twenty female parasitoids of *P. cosyrae* were released as

in (a) above. (c) Ten females of *D. longicaudata* and another 10 of *P. cosyrae* were released simultaneously and allowed to oviposit. Furthermore, in the fourth set up, (d) Ten females of *D. longicaudata* were released first and allowed to oviposit for 3 hours, after which they were removed and the same number of *P. cosyrae* were also released and allowed to oviposit for the same period of time. (e) The fifth scenario was the same as scenario (d), except that *P. cosyrae* was released first. In all cases, the control constituted of host larvae in which no parasitoids were introduced to determine natural host mortality as well as emergence rates. The experiments comprised 5 separate cages which were observed at uniform inetrvals. These were repeated 11 times to represent 11replicates and procedures (a)-(e) repeated on *C. cosyra* as host. The number of parasitoids, landing, searching and ovipositing were recorded at 30mins intervals for 3hrs, only in scenarios where the two parasitoids were not mixed. Each observation lasted for 5 seconds. After the exposure period of 6 hr, the contents of the oviposition units were transferred to fresh carrot diet to allow host larvae to develop at ambient conditions. The recovered puparia were kept under the same conditions to allow parasitoids and flies to emerge. Thereafter, the emerging parasitoids and flies of each species were counted and sexed, while uneclosed puparia, were dissected to reveal pharate adults which were then handled in the same way as above.

5.3.6 Data analysis

Percent parasitoids searching, probing and ovipositing over time when *D. longicaudata* and *P. cosyrae* were offered hosts (*B. dorsalis* and *C. cosyra*) separately or mixed were arcsine transformed to normalise the data. These were then subjected to ANOVA to test for effect of host on parasitoid activity (searching, probing, ovipositing over time). Percent total and specific parasitism resulting from *D. longicaudata* and *P. cosyrae* were also transformed and analysed in the same way to test for effect of host when offered to parasitoids separately, mixed or sequentially under different host-parasitoid scenarios. Separation of means was

done using the Tukey's test. All analyses were performed using R software version 3.1.1 (R Development- Core-Team, 2014).

5.4 Results

Diachasmimorpha longicaudata wasps were faster than *Psyttalia cosyrae* in landing on the host ($F_{6,1344}$ = 288.31, p < 0.001), probing ($F_{6,1344}$ = 288.31, p < 0.001) and initiating oviposition ($F_{6,1344}$ = 288.31, p < 0.001), (Figure 5.4, 5.5 and 5.6) in all parasitoid host combinations tested. In addition, there was a rapid increase in landing, probing and ovipositing within the first 30 minutes for *D. longicaudata,* with these activities levelling off and becoming constant over the entire 180 minutes (Figure 5.4, 5.5 and 5.6). On the other hand, landing, probing and ovipositing started off slowly for *P. cosyrae,* and increased gradually at a steady slow pace in all parasitoid: host combinations evaluated (Figure 5.4, 5.5 and 5.6). Furthermore, percent *D. longicaudata* searching, probing and ovipositing ($F_{5,\ 1344}$ = 937.64, p < 0.001; $F_{5,\ 1344}$ = 534.12, p < 0.001; $F_{5,\ 1344}$ = 228.21, p < 0.001) was significantly higher compared to *P. cosyrae* in all parasitoid-host combinations. Searching events for *D. longicaudata* were comparable regardless of whether the host was solely *B. dorsalis*, *C. cosyra* or a mixture of the two (Figure 5.7). However probing and ovipositing events were significantly lower when *B. dorsalis* and *C. cosyra* were mixed compared to a scenario when the hosts were offered separately (Figure 5.7). With respect to *P. cosyrae,* searching events there were significant differences in all the three host combinations, being higher when *C. cosyra* was the sole host, followed by *B. dorsalis* as the sole host and finally being lowest when the hosts were mixed (Figure 5.7). The trend was similar for probing events being higher when *C. cosyra* was offered as the sole host, followed by *B. dorsalis* as the only host and finally being lowest when the hosts were mixed (Figure 5.7).

Percent parasitism (specific) was significantly higher for *D. longicaudata* compared to *P. cosyrae* regardless of whether *B. dorsalis* or *C. cosyra* were offered to the parasitoids separately or in a mixed host scenario (Table 5.1). Parasitism was two times higher when *D. longicaudata* was offered *C. cosyra* as the only host compared to *P. cosyrae* on the same host. When the host was *B. dorsalis*, parasitism was 53% for *D. longicaudata* compared to zero for *P. cosyrae.* Dissecting *B. dorsalis* parasitized by *P. cosyrae* wasps revealed 100% encapsulation and failure of *P. cosyrae* to complete development in *B. dorsalis* as a host. Mixing *B. dorsalis* and *C. cosyra* had no effect on parasitism due to *P. cosyrae* but had a significant effect on parasitism resulting from the exotic *D. longicaudata* (Table 5.1).

When *D. longicaudata* was offered a mixture of *B. dorsalis* and *C. cosyra* first before the same was transferred to *P. cosyrae*, percent parasitism was 11-fold more than parasitism due to the later. When the reverse was done, percent parasitism was slightly reduced to 10 times more than for *P. cosyrae.* In single host scenarios, percent parasitism attributed to *D. longicaudata* was 40 times more than parasitism due to *P. cosyrae* when the former was offered *C. cosyra* ahead of the native parasitoid. In the reverse of the above set up, where the native parasitoid was offered *C. cosyra* first, parasitism due to *D. longicaudata* was only 8 times higher than that of *P. cosyrae.* Furthermore, regardless of the order in which the two parasitoids were offered *B. dorsalis* in separate scenarios, *P. cosyrae* was encapsulated and parasitism was zero.

When a mixture of the two wasps was offered a combination of *B. dorsalis* and *C. cosyra* simultaneously, percent parasitism attributed to the exotic parasitoid was 4-fold higher than that of the native parasitoid. Furthermore, parasitism was 37 times higher for *D. longicaudata* compared to that of *P. cosyrae* when a mixture of the two was offered *C. cosyra* as the sole host. In addition, in the scenario involving both parasitoids being offered *C. cosyra,* percent

parasitism was zero for *P. cosyrae* and 51% for *D. longicaudata.* Finally, there were significant differences in total parasitism among all host-parasitoid combinations (Table 5.1), being highest when *D. longicaudata* was offered *C. cosyra* as the only host, followed by the scenario when the two parasitoids were mixed and the hosts offered simultaneously.

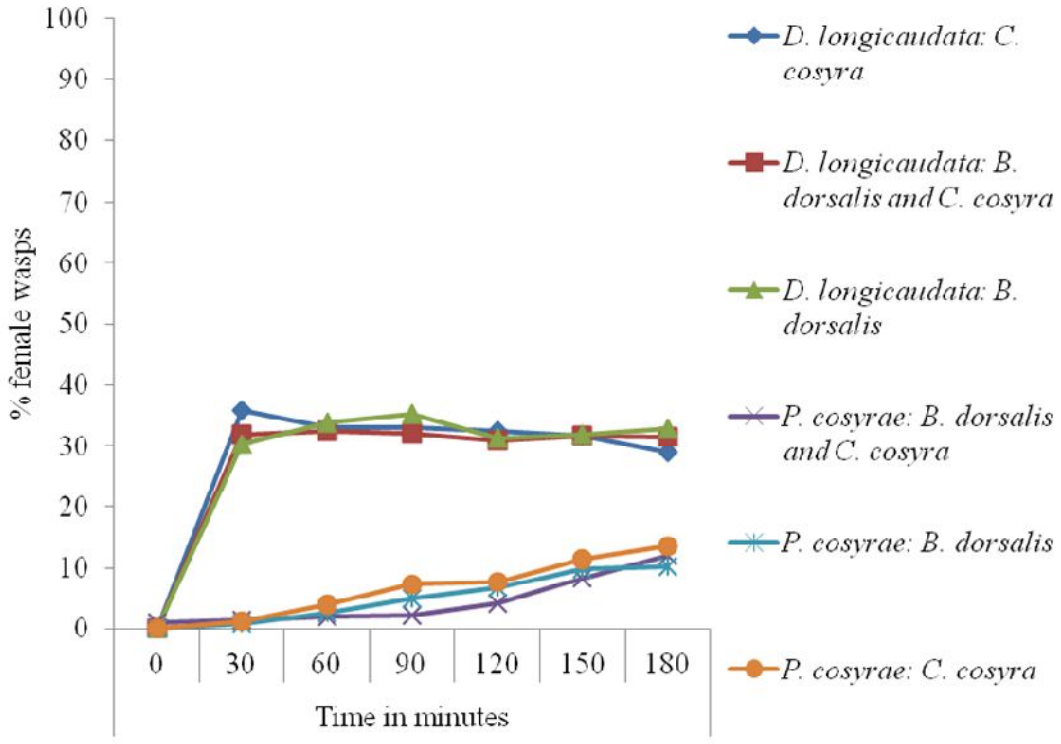

Figure 5.4: Landing activity trend of parasitoids on the oviposition unit over time, when exposed to various combinations of fruit fly hosts.

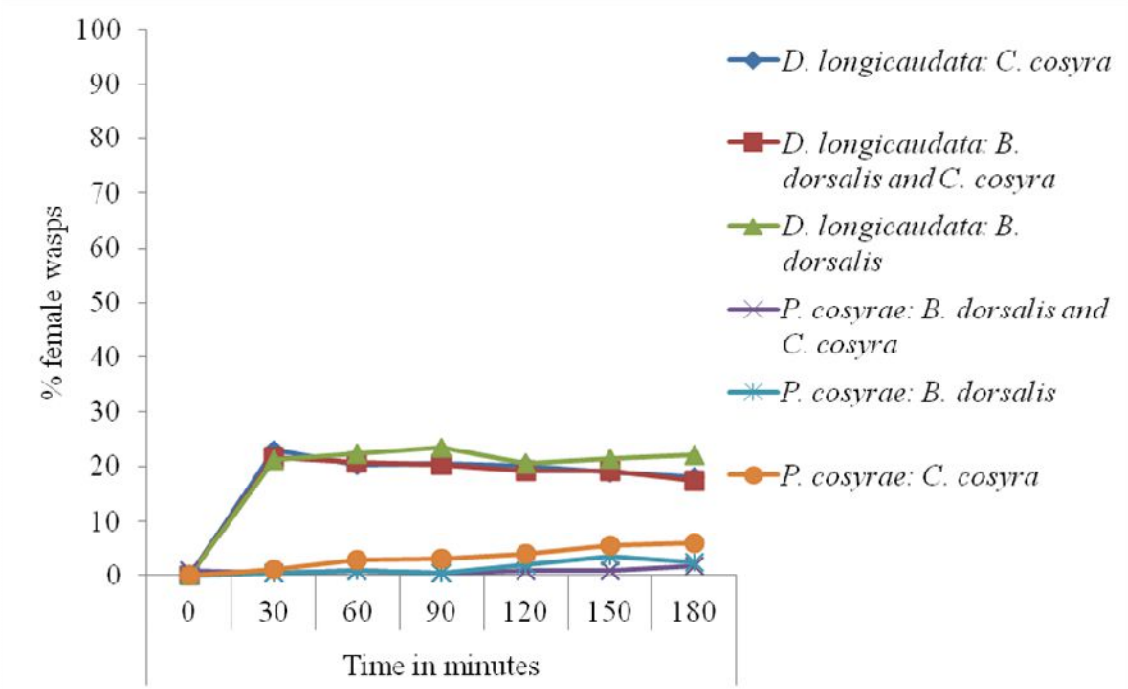

Figure 5.5: Probing activity trend of parasitoids landing on the oviposition unit over time, when exposed to various combinations of fruit fly hosts.

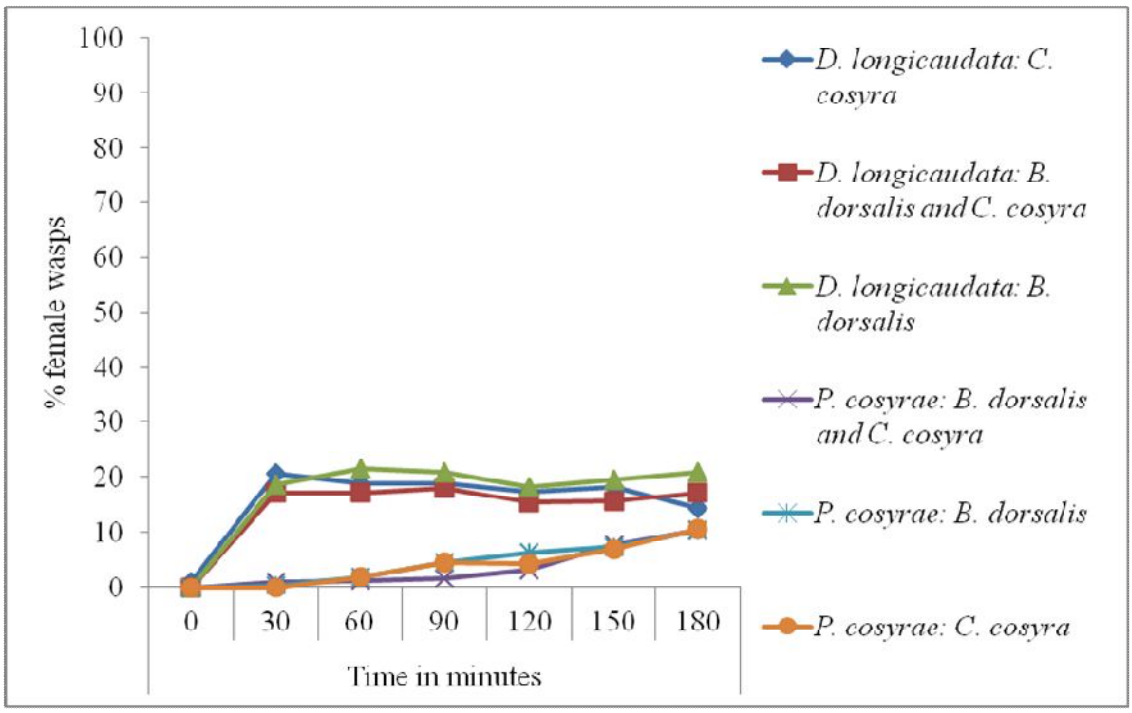

Figure 5.6: Ovipositing activity trend of parasitoids landing on the oviposition unit over time, when exposed to various combinations of fruit fly hosts

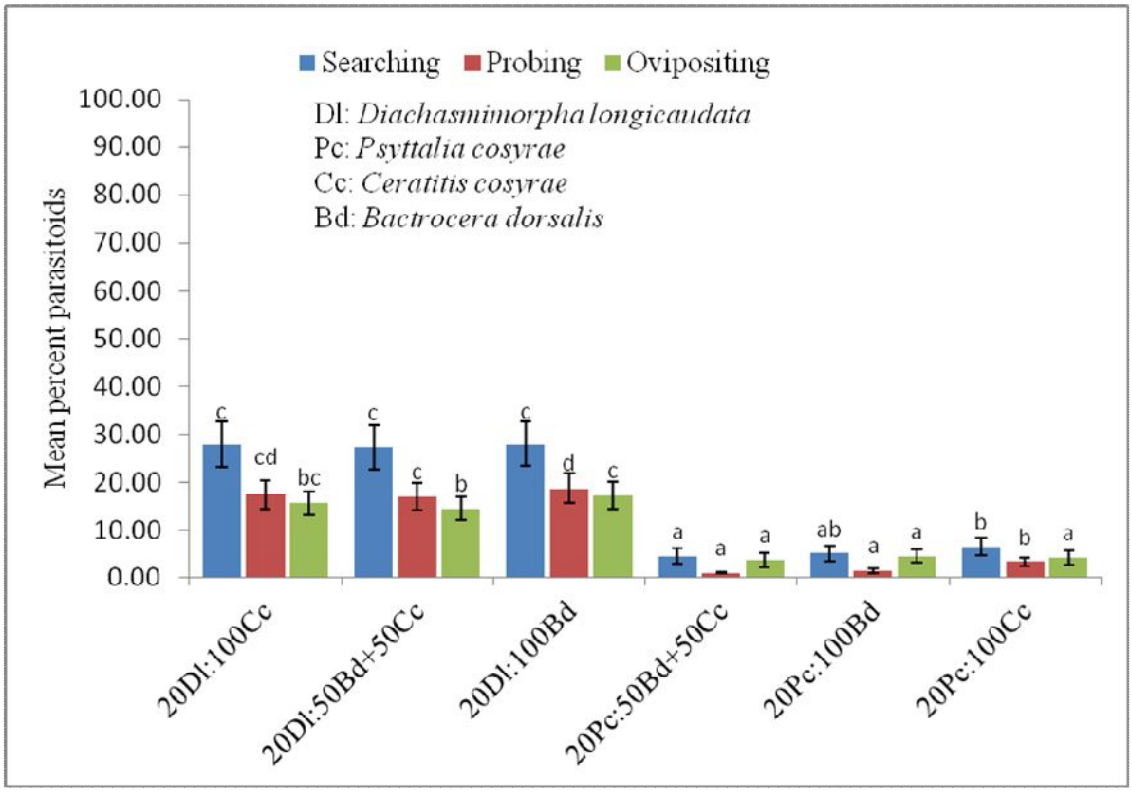

Figure 5.7: Mean percent parasitoids searching, probing and ovipositing over a time period of 180 minutes.

Error bars denote standard error of the mean (S.E.). For each parameter, means superscripted by the same letter do not differ significantly at $p = 0.05$ (Tukey's test)

Table 5.1: Mean percent parasitism of *B. dorsalis* and *C. cosyra* by *D. longicaudata* and *P. cosyrae* in different parasitoid: host ratios and scenarios

Treatment	Host combination		% parasitism		
				Specific parasitism	
	B. dorsalis	*C. cosyra*	Total parasitism	*D. longicaudata*	*P. cosyrae*
10 *D. longicaudata* then 10 *P. cosyrae*	50	50	46.19 ± 0.78 c	45.45 ± 0.89 aB	4.27 ± 0.87 bcA
10 *P. cosyrae* then 10 *D. longicaudata*	50	50	48.31 ± 0.77 dcd	47.50 ± 0.75 abB	4.79 ± 0.85 cA
10 *D. longicaudata* then 10 *P. cosyrae*	100	0	52.30 ± 0.84 de	52.30 ± 0.84 bcdB	0 ± 0 aB
10 *D. longicaudata* then 10 *P. cosyrae*	0	100	51.17 ± 0.91 cde	51.02 ± 0.72 bcdB	1.29 ± 0.44 abA
10 *D. longicaudata* and 10 *P. cosyrae*	50	50	59.94 ± 1.71 fg	55.12 ± 1.60 dB	15.03 ± 0.63 dA
10 *D. longicaudata* and 10 *P. cosyrae*	100	0	51.37 ± 1.5 cde	51.37 ± 1.51 bcdB	0 ± 0 aB
10 *D. longicaudata* and 10 *P. cosyrae*	0	100	53.22 ± 1.32 de	53.01 ± 1.30 cdB	1.42 ± 0.50 abA
10 *P. cosyrae* then 10 *D. longicaudata*	100	0	49.22 ± 0.91 cd	49.22 ± 0.91 abcB	0 ± 0 aA
10 *P. cosyrae* then 10 *D. longicaudata*	0	100	56.69 ± 0.85 ef	54.31 ± 0.79 cdB	6.96 ± 0.87 cA
20 *D. longicaudata*	0	100	62.59 ± 1.09 g	62.59 ± 1.09 e	-
20 *D. longicaudata*	50	50	52.49 ± 1.34 de	52.49 ± 1.34 bcd	-
20 *D. longicaudata*	100	0	53.49 ± 0.99 de	53.49 ± 0.99 cd	-
20 *P. cosyrae*	50	50	24.69 ± 0.95 b	-	24.69 ± 0.95 e
20 *P. cosyrae*	100	0	0 ± 0 a	-	0 ± 0a
20 *P. cosyrae*	0	100	25.89 ± 1.42 b	-	25.89 ± 1.42 e
F value			224.09	14.86	182.53
df			14, 480	11, 384	11,384
p			< 0.001	< 0.001	< 0.001

Means within a column, followed by the same lowercase letter are not significantly different at $p = 0.05$ (Tukey's test).

Means within the same row (for specific parasitism), followed by the same uppercase letter are not significantly different at $p = 0.05$ (Tukey's test).

5.5 Discussion

In terms of finding the host, searching for a suitable oviposition site, probing and eventually ovipositing, *D. longicaudata* was more efficient compared to the native *P. cosyrae.* These are quality attributes for an efficient natural enemy and conveys a superior competitive advantage over its competitor. Effective biological control relies on parasitoids that are highly efficient in foraging for a host to minimize time and energy expended in such exercise (Godfray, 1994; Montoya *et al.,* 2012).

Our findings indicate that the exotic and introduced parasitoid was able to parasitise both *B. doraslis* and *C. cosyra* and produce viable progeny. This is in contrast to *P. cosyrae,* which was unable to develop in *B. dorsalis* due to encapsulation. Encapsulation is a reaction by the host to an invasion by foreign material such as the parasitoid egg being laid into the haemocoel of the insect, thus prompting haemocytes to gather around the foreign body, surround it and arrest its development (Rizki and Rizki, 1990; Blumberg, 1997; Reed *et al*., 2007). This is often the case with parasitoid- host relations where the two evolved independently of each other and are only brought together by chance. When *B. dorsalis* was reported for the first time in Africa, efforts were channelled towards identifying a potential indigenous natural enemy capable of controlling the invasive pest without expending resources in foreign exploration trips for natural enemies to release in classical biological programmes. These efforts failed to bear fruit as both *Psyttalia phaeostigma* and *Testrastichus giffardii* which occur as parasitoids of *Dacus ciliatus* and *D. bivittatus cucumaris* in most cultivated Cucurbitaceae in Kenya were encapsulated (Mohamed *et al.,* 2006; Mohamed *et al.,* 2010). Although various host fruits were sampled extensively in Kenya, no parasitoid was found to be effective against the pest (Rwomushana *et al.,* 2008).

Percent parasitism for *D. longicaudata* on *C. cosyra* was much higher compared to *B. dorsalis*, though the introduced parasitoid and the invasive pest are considered to have co evolved in Asia. This trend is similar to the findings of Mohamed *et al*., 2008 but different in the sense that realized parasitism on *B. dorsalis* has appreciated 4 fold from a mere 13% to 53%. This is a positive response as earlier recommendations were already geared towards finding an alternate parasitoid to control *B. dorsalis* as *D. longicaudata* parasitism was unacceptably low in the initial pre release studies conducted in Kenya. Furthermore, *C. cosyra* parasitism by the exotic wasp has also doubled to 63%. This is encouraging, as *D. longicaudata* shows great promise in controlling both *B. dorsalis* and *C. cosyra* and possibly contribute in a positive way to overall parasitism of the later for which percent parasitism by *P. cosyrae* is unacceptably low (Copeland *et al*., 2006; Mohamed *et al*., 2008).

In thie current study, percent parasitism by *P. cosyrae* on *C. cosyra* as the only host was 26%. This may be considered relatively low especially in high infestation areas. In situations where *C. cosyra* was offered to the parasitoids with either of the two being given first preference to forage, the results were encouraging as specific parasitism attributed to each species was additive to give a relatively higher overall percent parasitism. The order of exposure did not affect the outcome of percent parasitism by either of the parasitoids. This effectively rules out interference competition or exclusive utilisation of the host by the parasitoid reaching the host first.

We anticipate as alleged by Mohamed *et al*. (2008) that *D. longicaudata* will attack late instar larvae of *C. cosyra* and in this case amount to sufficient niche overlap, allowing *P. cosyrae* to attack exclusively second instar larvae. This sounds plausible considering that *D. longicaudata* has a relatively longer ovipositor compared to *P. cosyrae,* thus is able to attack older larvae foraging deep inside the fruit pulp for example in larger fruits such as the mango.

Furthermore, coexistence in opine has been shown to occur through resource partitioning and various foraging behaviours resulting in divergence of niches (Sivinski and Aluja, 2012). For example García-Medel *et al*. (2007) reported that *Doryctobracon crawfordi* (Viereck) preferred foraging on infested fruits fallen to the ground compared to the majority of other neotropical Opiinae parasitizing a wide range of fruit flies.

We note that the only worrying point is the failure by *P. cosyrae* to effectively discriminate the two available hosts and channel its efforts to parasitizing *C. cosyra* its co-evolved host. Continually attacking *B. dorsalis* represents misplaced efforts as such amounts to a reproductive sink with detrimental long term effects on populations of *P. cosyrae*. However, the possibility for co-existence cannot be ruled out as evolutionary adjustments occur over time. According to the optimal oviposition theory, female parasitoids should be able to choose hosts that confer a great amount of fitness to the offspring (Pöykkö, 2006). *Psyttalia cosyrae* has managed to maintain its populations in Africa even at low *C. cosyra* densities (Copeland *et al*., 2006) thus might be able to utilize narrow pest population niches where the aggressive and much active *D. longicaudata* fails. Such a scenario has been observed to occur in *Doryctobracon areolatus* and *Opius hirtus* (Fischer), parasitoids of *Anastrepha* spp which have perfected their foraging behaviour in locating hosts at extremely low densities, thus are able to sustain their populations where most parasitoids are unable to do so (García-Medel *et al*., 2007).

Biological control through the use of parasitoids is slowly gaining momentum in Africa, where exotic natural enemies are being imported for the control of invasive pests which in most cases invade new territories leaving behind their co-evolved natural enemies.

The ultimate dynamics of introduced as well as indigenous parasitoids are often less explored as long as the introduced natural enemy shows acceptable control of the target pest. Interactions between the introduced and the native assemblage of parasitoids where there is a potential of niche overlap are not so well studied especially in African agro ecosystems. Though imported specifically for the management of *B. dorsalis*, *D. longicaudata* was able to form a new association with the indigenous *C. cosyra* (Mohamed *et al*., 2008). The scenario created by *D. longicaudata* in expanding its host range, did create a substantial interaction between the native and the introduced parasitoids. It is undeniable that such interactions are key in shaping future structures of parasitoid population dynamics (Godfray, 1994).

In conclusion, our findings indicating that *D. longicaudata* and *P. cosyrae* were able to share *C. cosyra* and contribute in their small ways to a relatively higher overall percent parasitism gives us hope that the two will be able to partition their niches both in space and time thereby contributing positively to the biological control of the two pests. Thus, the findings of this study should form the basis of a series of future investigations of interactions between the two parasitoids over several generations and field conditions where options are much wider for both parasitoids. At such levels where the hosts are not limited and choices are wide open, we anticipate a sustained and effective co-existence of the two.

CHAPTER SIX

Post-harvest disinfestation of *Bactrocera dorsalis* on mango using hot water treatments[2]

6.1 Abstract

Mango, *Mangifera indica* L. (Anacardiaceae), is one of the most important fruits in Africa, providing household nutrition and economic development opportunities for millions of growers across the continent. In Kenya, over 80% of mango production is carried out by smallholders who produce this crop for both the domestic and the export markets. Despite its importance mango production is hampered by several constraints, including infestation by fruit flies, especially the exotic *Bactrocera dorsalis*. In addition to its direct damage to fruits, the high quarantine status of the pest restricts the export of fruits and limits access to lucrative markets, impacting negatively on export earnings. To facilitate access to export markets, postharvest management measures such as hot water treatment, are required to ensure quarantine security. Internationally this level has been set as either 99.99% (Probit 8.7) or 99.9968% (Probit 9). In developing a protocol, the development of immature life stages of *B. dorsalis* in "Apple" mango was established. Using this information, infested mangoes proven to be harbouring the different immature life stages were subjected to a hot water treatment of 46.1°C for 8, 23, 38, 53 and 68 minutes, and the egg and larval mortality determined. The third instar life stage was the most heat-tolerant, followed by second and first instar larvae and the egg stage respectively. The immersion time of 81.47 minutes (95% CL 75.77-87.18) was established as the time required to achieve 99.99% security level.

[2] Published as: Ndlela, S., Ekesi, S., Ndegwa, P. N., Ong'amo, G. O., & Mohamed, S. (2017). Postharvest disinfestation of *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae) in mango using hot water treatments. *Journal of applied Entomology*. 2017:00:1-12. https://DOI:.org/ 10.1111/jen.12404

In the validation experiment, there were no survivors from the 51,101 third instar individuals treated in "*Apple*" mango weighing 400-500 g. Furthermore there were no survivors from the 44,651 third instars exposed to 46.1°C for 68 minutes. These results provide sound evidence that the treatment is an effective postharvest disinfestation treatment against *B. dorsalis,* and should facilitate access to export markets for mango fruits from Africa.

6.2 Introduction

The genus *Bactrocera* contains over 500 described species in the family Tephritidae with many undescribed species yet to be incorporated into current knowledge (Drew and Hancock, 2000; Mwatawala *et al*., 2004). Lately the genus has been the subject of taxonomical revisions with new species being described (Drew and Raghu, 2002). This has complicated fruit fly management and protocols with quarantine restrictions having to be modified. Taxonomical changes have been made to the *Bactrocera dorsalis* (Hendel) complex (Schutze *et al*., 2014a, 2014b; Bo *et al*., 2014). The complex comprises over 90 sibling species and has undergone many revisions in the past with more likely to occur in the near future, judging from ongoing debate relating to current taxonomical knowledge (Augustinos *et al*., 2014). Globally, several members of the genus *Bactrocera* are notorious pests of horticulture, causing major direct losses from fruit damage and significant indirect export losses due to quarantine restrictions (White and Elson-Harris, 1992; Abd-El-Samie and El –Fiky, 2011).

Bactrocera dorsalis was recorded in Africa in 2003 and initial opinion suggested that the exotic fruit fly was a morphological variant of *B. dorsalis* (Lux *et al*., 2003b). Two years later, Drew *et al*., (2005) described the Kenyan specimens as a new invasive fruit fly species, *Bactrocera invadens* Drew, Tsuruta and White. However, based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological findings, Schutze *et*

al., 2014b concluded that *B. invadens* together with *B. philippinensis* and *B. papayae* Drew and Hancock, were synonymous with *B. dorsalis.* This development, though a great milestone for science, has far-reaching consequences (Augustinos *et al*., 2014). Several questions arising from that status require answers and assurances to policymakers on quarantine, postharvest treatment requirements and harmonisation of pest management strategies that are currently based on knowledge that *B. dorsalis* and *B. invadens* are indeed distinct taxonomical entities. For example, following the early invasion of *B. dorsalis* into Africa, the United States of America through a U.S. Federal Order responded by banning the importation of most cultivated vegetables and fruits from African countries where *B. dorsalis* had been reported (USDA-APHIS, 2008). The European Union had earlier responded to the increased usage of pesticides in affected regions by enacting import tolerance legislation, regulating pesticide residue in fruits and vegetables through harmonised maximum pesticide residue levels to ensure a high level of consumer protection for their citizens (EU, 2005). These events have been major setbacks for most African economies that rely on export earnings from horticulture especially with such large trading blocks such as the EU and the USA.

One of the major requirements of many importing countries is that fresh produce that are known hosts of particular quarantine fruit fly species be subjected to a standard postharvest disinfestation treatment also called quarantine treatment that satisfies specific requirements. Quarantine treatments seek to ensure that the risk of introducing unwanted pests is minimised or totally eliminated (Landolt *et al*., 1984; Haack *et al*., 2011). The International Standards of Phytosanitary Measures as outlined in ISPM No. 28 (IPPC, 2007) requires that Probit 9 efficacy (99.9968% mortality at a confidence of 95%) be demonstrated for the target pests before export can be granted (IPPC, 2007).

This level of efficacy is based on the rationale by Baker (1939) whose objective was to assure no survival of fruit flies in whatever commodity subjected to some form of treatment to kill immature life stages. This is achieved when a treatment results in no survivors in 94,587 treated test individuals (Couey and Chew, 1986; Schortemeyer *et al*., 2011). A number of countries including the United States, recognize quarantine treatments for a wide array of pests based on the concept of Probit 9 (Follett and Neven, 2006; Haack *et al*., 2011). However, though there is no official communication from the USDA, regarding acceptance of phytosanitary treatments falling short of Probit 9, some treatments such as the irradiation treatment protocol for *Sternochetus mangiferae* have been accepted on the basis that the pest poses minimum economic and environmental impact (USDA-APHIS, 2002; Follett *et al*., 2007). Other countries which are important export destinations for Africa such as New Zealand, Australia and Japan, require exporting countries to demonstrate quarantine efficacy of 99.99% at a confidence of 95% (Couey and Chew, 1986; Follett and Neven, 2006; Schortemeyer *et al*., 2011). The latter requirement, translates to 29.956 test insects with no survivors at the 95% confidence level (Couey and Chew, 1986; Schortemeyer *et al*., 2011).

Mango is one of the commodities that require quarantine treatment especially against fruit flies before it can be exported. The mango fruit is grown in the tropical and subtropical lowlands of the world and is fast becoming a significant foreign currency earner for many countries (Asif *et al*., 2011). For example, in 2010, Kenya exported only 2% of their total mango production but earned in excess of US$10.1 million worth of foreign exchange (Kenya Ministry of Agriculture, 2012). However, authorities in export market destinations have become more strict as a result of infestations by *B. dorsalis*.

In order to circumvent quarantine restrictions from export markets, several postharvest treatments are available, among them hot water disinfestation for certain commodities (Sharp *et al.,* 1989a; Sharp and Picho-Martinez, 1990; Sharp, 1993; Mitcham and Yahia, 2008; Verghese *et al*., 2011). Hot water treatment protocols (USDA-APHIS, 2016) have been developed for fruit flies such as *Ceratitis capitata* (Wiedemann) and *Anastrepha serpentina* on "*Ataulfo*" mangoes (Wiedemann)(Sharp *et al.,* 1989b) as well as *B. dorsalis* (Verghese *et al*., 2006; Armstrong and Follett, 2007; Verghese *et al*., 2011; Hernández *et al*., 2012) in mango and litchi, *Litchi chinensis* Sonn (Sapindales:Sapindaceae).

On mango, most hot water treatment procedures have been shown to be effective at temperatures of 46.1°C over specified periods for specific pests and varieties (USDA-APHIS, 2009) and justify the need to test the responses of various fruit fly species and mango varieties to this temperature. The effective time of immersion also varies depending on the fruit shape and weight. Submerging most perishable fruits in hot water at temperatures between 43.0 and 46.7°C for 35-90 min is known to be an effective quarantine treatment for most insect pests and diseases (McGuire, 1991; De La Cruz Medina and Garcia, 2002). For example, hot water treatment at 46.1°C for 35min resulted in 99.9968% mortality of the Caribbean fruit fly (*Anastrepha suspensa* (Loew) in guavas (*Psidium guajava* L.) (Gould, 1994). In Mexico, the authorised hot water quarantine treatment against *A. ludens* and *A. obliqua* in mango involves dipping fruits in hot water at 46.1°C for 65-90 min depending on fruit weight (Yahia *et al*., 1999). However, protocols are in constant revision by the USDA, in order to reflect current knowledge and industry expectations.

In addition to disinfesting fruits, heat treatment has also been reported to increase tolerance of some fruit to chilling temperature, as well as enhancing the postharvest shelf life of mangoes (Grové *et al*., 1999; Irtwange, 2006; Yimyong *et al*., 2011; Çandir *et al*., 2012).

The objective of this study was to determine hot water disinfestation treatment parameters for *B. dorsalis* in an important hybrid mango variety "*Apple*" that is widely grown for export in Kenya and most other East African countries (Olunga, 2015). The study describes how the heat tolerance of the egg and larval life stages were determined, and the process of validating the 99.99% mortality level of quarantine security to enable risk-free export of mango.

6.3 Material and methods

In Phase 1 the developmental times of the egg and first (1^{st}), second (2^{nd}) and third (3^{rd}) instar larvae of *B. dorsalis* in "*Apple"* mango were measured. Thermal tolerance is known to vary in different fruit fly species according to their various stages of development in different host fruits and varieties (Armstrong *et al*., 1989; Foliaki and Armstrong, 1997; Sales *et al*., 1997) in Phase 2 the relative heat tolerance of each of the immature stages of development on "*Apple*" mango at water temperature of 46.1^{0}C was determined. In Phase 3 large scale treatments against the most tolerant life stage of *B. dorsalis* were undertaken.

6.3.1 Experimental fruit flies

Bactrocera dorsalis used in this study were reared at the animal rearing and quarantine unit of the International Centre of Insect Physiology and Ecology (*icipe*) Nairobi, Kenya, following the procedures described by Ekesi *et al*., 2007c and Ekesi and Mohamed, 2011.

Adult flies were kept in Perspex cages (80 × 80 × 80 cm) and maintained at 26-28°C, 60-70% RH and a photoperiod of L12:D12. Adult flies were fed on an artificial diet consisting of a mixture of sugar and ultrapure grade enzymatic yeast hydrolysate (USB Corporation, Cleveland, Ohio, USA) at the ratio of 3:1 by volume, and were provided with water in a Petri dish (8.6 cm diameter) with a layer of pumice granules. Wild flies were added to the mass-reared colony at three months intervals to maintain genetic variability.

6.3.2 Experimental fruits

"*Apple*" mango fruits were obtained from two orchards; 3-5ha in size in Nairobi (S01°16′441"; E36°90′543", 1576 m above sea level) and Kilifi (S03°58′904″; E39°86′482″, 27 m above sea level). No chemicals were applied to control pests in the field. However mango trees were treated twice with a broad-spectrum non-systemic fungicide (mancozeb 750 WG, Cheminova Australia Pvt Ltd, North Ryde, NSW, Australia) at the label rate of 0.8 kg a.i./ha, at flower bud formation until fruits reached the approximate size of a chicken egg (55-60 g) and were ready to be bagged. Thereafter mango fruits required for this study were selected based on visual appearance and were bagged in brown paper bags (Fig 6.1) (20 × 14 × 40cm; Paperbags Ltd, Nairobi, Kenya) to prevent exposure to fruit flies.

Figure 6.1: Brown paper bags (© Laser Edge Graphics) used for bagging mangoes to prevent exposure of mangoes to fruit flies.

The bags used in this study, were fastened in such a way to enable free circulation of air but excluding entry of fruit flies thus did not interfere with physiological development of the fruits. The maturity stage at which mango fruits were bagged was deemed sufficient to have allowed early physiological development to occur under these conditions. Bagged fruit were harvested at the physiologically mature stage (hard and green) along with a stalk of ~3cm.

These were transported to the laboratory in plastic crates (55.8 ×36.5 × 22.4 cm; Kenpoly Manufacturers Ltd, Nairobi, Kenya). Only fruits weighing between 400 and 500 g, and free of visible signs of disease, pests and injuries, were used for the experiments.

6.3.3 Hot water treatment

An insulated stainless steel tank (Fig 6.2) (Desbro Engineering Ltd, Nairobi, Kenya) with a usable volume of 185litres, capable of treating approximately 30 kg of mango fruits, was used in the experiments The tank was fitted with two TE-10D thermo-regulators (Techne Inc, Bibby Scientific Ltd, Staffordshire, UK) with a total heating power of 2000 W, and with a water pump that could circulate 20 L per min. The thermo-regulators had a digital temperature selection, with Platinum resistance thermometer sensors with a set point accuracy of ±1°C and temperature stability of ±0.01°C. The decrease in temperature of water in the tank during fruit loading was recorded using the digital temperature sensor of the thermo-regulator and confirmed by four mercury thermometers (-10^{o}C to 110^{o}C) affixed to the tank.

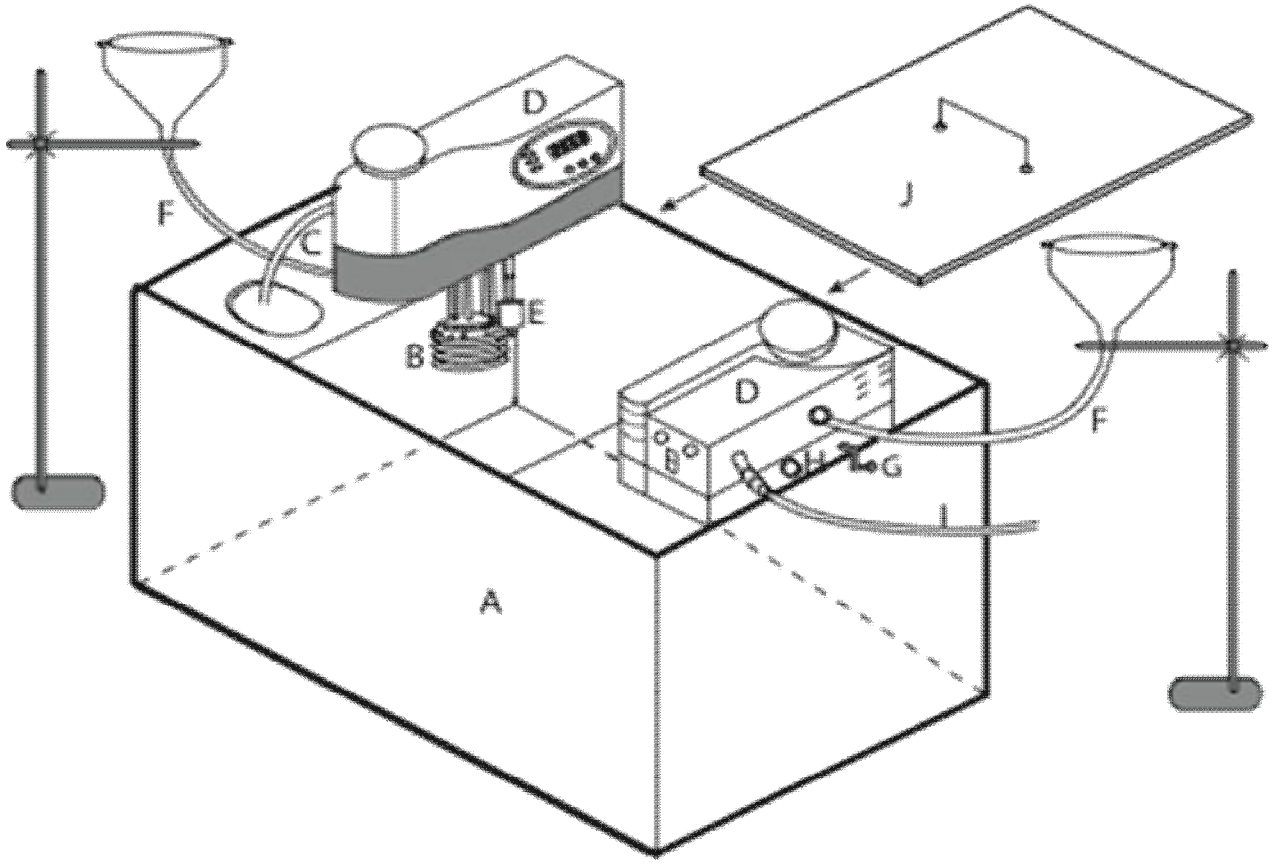

Figure 6.2 Hot water treatment tank with a volume of 185 litres used for the immersion of mangoes in disinfesting them of B. dorsalis.

(A) thick insulated wall; (B) heating element; (C) polythene pipe to help circulate water; (D) thermo-regulator with LCD screen; (E) water level gauge; (F) water funnel inlet on stand; (G) clamp; (H) water outlet; (I) power cable; (J) lid

6.3.4 Determination of developmental period for immature stages of *B. dorsalis* in "*Apple*" mango (phase 1)

A batch of 120 mango fruits were superficially cleaned using a damp cloth, and then divided for easy handling into 12 groups of 10 each.

Each group of 10 fruits was placed in a Perspex cage (80 x 80 x 80 cm) containing approximately 2000-3000 male and female *B. dorsalis* adults aged between 11 and 18 d (at 26-28°C, 60-70% RH and a photoperiod of L12:D12). Different batches of females were allowed to oviposit for 90 min before the fruits were removed and each group of 10 placed in plastic crates described above and kept in a room at ambient conditions (24-25°C, 60-70% RH). The fruits were protected from secondary infestation by placing the crates in insect-proof wooden cages whose sides were made of fine mesh material to allow free circulation of air while excluding fruit flies. A total of 15 mango fruits were picked at random each day for eight consecutive days from the batch of 120 and dissected under a stereomicroscope (Leica EZ4D digital stereomicroscope; Leica Microsystems, Heerbrug, Switzerland). All eggs and larvae of *B. dorsalis* that were found were placed in 70% alcohol. Twenty-one individuals from each stage were later examined with a scanning electron microscope (Jeol SMJ-5800LV, JEOL, Tokyo, Japan) to confirm the different stages of development. The developmental times of the eggs and three life stages was determined by examining 200 individuals of each immature stage at random. The immature life stages of development were determined using the morphological features, described by Steck and Malavasi (1988) and Carroll and Wharton (1989):

Egg: creamy white in colour, elongate, with a mean length and width of 1.17±0.0089 and 0.22±0.0029 mm respectively. The anterior end is slightly broad and rounded with a protuberant micropyle, whereas the posterior end tapers gently and has surface reticulation (Fig 6.3).

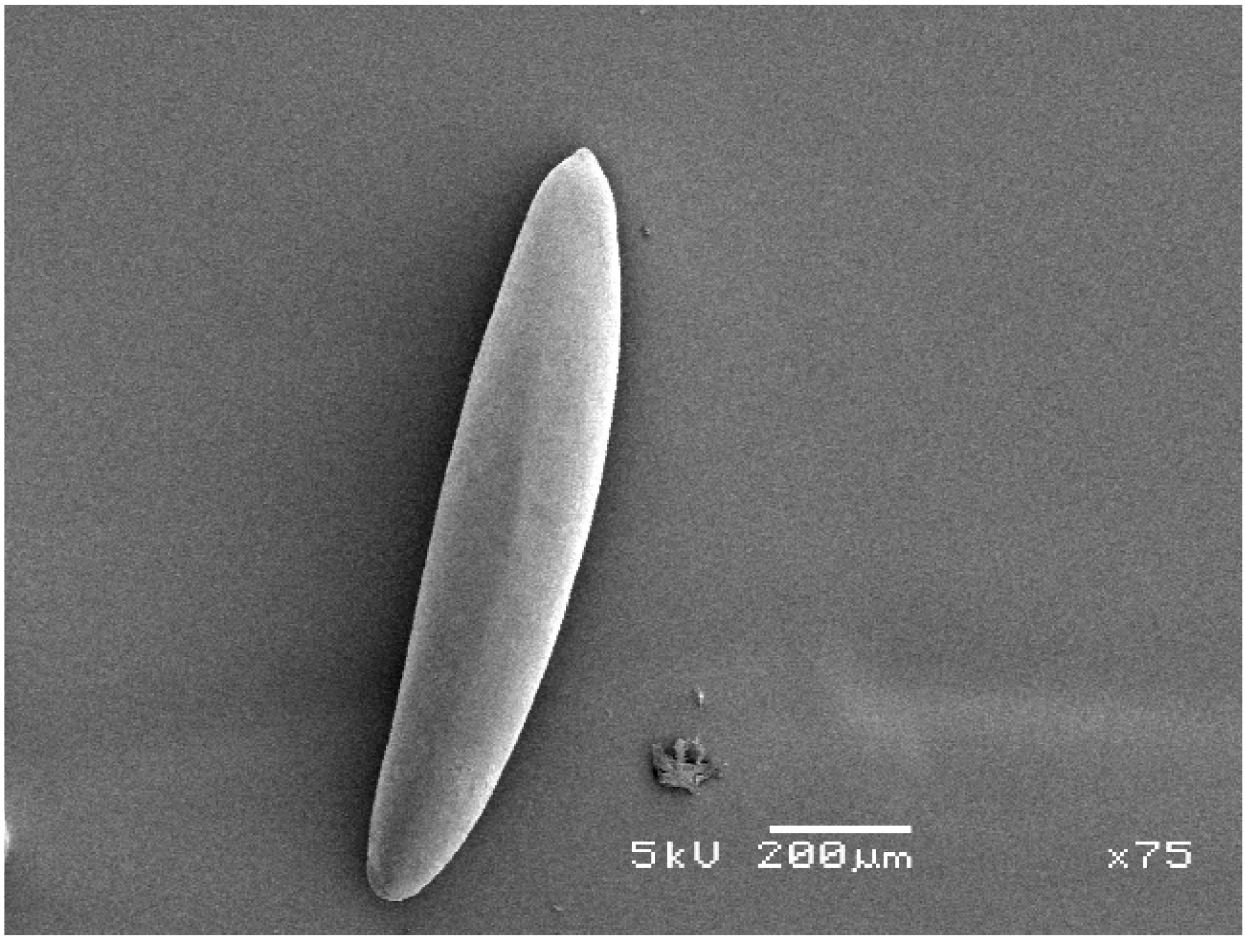

Figure 6.3: *Bactrocera dorsalis* egg as seen from the electron microscope

First instar: distinct brown curved apical tooth and dark pre-apical tooth (Fig. 6.4A); anterior sclerite conspicuously absent; mandible length 0.14±0.0037 mm; mandible base 0.077±0.0039 mm; apical tooth to ventral apodeme 0.095±0.0037 mm; cephalopharyngeal skeleton tip to notch 0.41±0.0048 mm.

*Second instar***:** mandible without pre-apical tooth, (Fig. 6.4B); anterior sclerite absent; mandibles brown with a tinge of black; sclerotisation in all other parts of cephalopharyngeal skeleton weak; mandible length 0.26±0.006 mm; mandible base 0.17±0.0048 mm; apical tooth to ventral

apodeme 0.20 ±0.0044 mm;cephalopharyngeal skeleton tip to notch 0.66±0.0.011 mm.

*Third instar***:** mandible without pre-apical tooth (Fig. 6.4B); tentopharyngeal sclerite, cornua notch and hypopharyngeal sclerite highly sclerotised and dark; mandible length 0.27±0.0046 mm; mandible base 0.17±0.0064 mm; apical tooth to ventral apodeme 0.22±0.022 mm; cephalopharyngeal skeleton tip to notch 0.69±0.0062 mm.

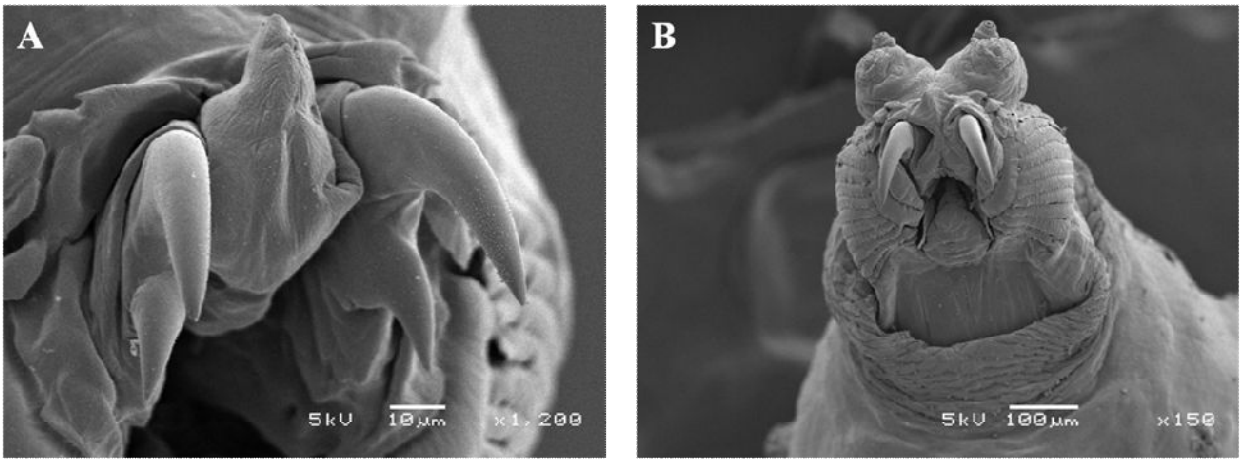

Figure 6. 4 Apical and pre-apical teeth of the first instar (A) and the apical teeth of the second and third instars (B) of *Bactrocera dorsalis*.

6.3.5 Determination of the most heat-tolerant immature life stage of *B. dorsalis* (phase 2)

Information obtained from Phase 1 was used to determine the most heat tolerant immature life stage of *B. dorsalis* to 46.1°C. Mango fruits were prepared and infested as described above, and infested mangoes held at ambient conditions for larval development to the 1st, 2nd and 3rd instars. Fruits with eggs were also held under the same conditions for 6 h before the hot water treatment. To confirm the number of eggs in treated infested fruit, an equal number of mangoes infested in the same way, was dissected and eggs and 1st instar larvae counted. Groups of eight mango fruits

were picked at random from the holding crates and placed in five nylon mesh bags measuring 82 × 50 cm (Leno bags; Planet Plastics, Maharashtra, India), which allowed hot water to circulate freely around the fruits. A stone weighing approximately 1 kg was added to each bag to force the bagged fruit to sink to at least 15 cm below the water surface. The experiment was replicated 18 times and a total of 720 mango fruits were treated for each stage of development. An equal number of infested mangoes were set aside as control to use as an estimation of the number of eggs or larvae in the treated fruit. Timing of the hot water treatment commenced when all fruit in a treatment batch were completely submerged in the hot water tank containing water already at 41.6°C and the lid closed. The period between loading and closing the tank was approximately 30 seconds. As the mangoes were being loaded into the tank, a pipe fed hot water at 41.6°C into the tank to offset the drop in temperature caused by the ambient temperature fruit. On the other side of the tank, an outlet pipe drained excess water to maintain a constant level in the tank. For each immature stage of *B. dorsalis*, the infested fruit was immersed in the water tank for 8, 23, 38, 53 and 68 min. After treatment fruits were stored under the pre-treatment conditions to allow them to dry and cool naturally. The following day the treated fruits were dissected and the numbers of live and dead larvae and eggs were recorded. Time-mortality relationships and the Probit 9 quarantine security level for each stage was then established by Probit analysis.

6.3.6 Large scale validation (phase 3)

Mango fruits were infested and immature stages allowed to develop to the most heat-tolerant immature stage determined and described above. Validation of the 99.9968% quarantine security level was carried out at 81.47 min (the time that is theoretically required to kill all immature stages of *B. dorsalis* in "*Apple*" mango with a net weight of up to 500 g.

A total of 1122 mango fruits infested with the third instar were used and an equal number were set aside as an untreated control. The experiment was replicated 17 times. Thereafter, the fruits were dissected and the number of dead and live larvae recorded. In addition we also investigated 68 min, which was the treatment time at which 100% mortality was obtained for all treatment stages in all treatments in Phase 2. Therefore for this validation, 990 fruit were infested and treated while the same number of fruit was infested but not subjected to hot water treatment (control). The experiment was replicated 15 times.

6.3.7 Data analysis

The number of eggs, 1st, 2nd and 3rd instar larvae out of the 200 examined daily, were scored as a percentage to estimate relative abundance of each stage over eight days. Mortality data from the heat-tolerance determination were corrected for control mortality (Abbott, 1925). The data were then analysed using the generalised linear model of regression, assuming a binomial family with a complementary log log link function to determine time required to attain 50, 90, 99 and 99.9968% mortality. The values predicted by the model were compared using 95% confidence intervals based on whether or not they overlapped. Non-overlap of confidence intervals is approximately equal to a test of significant difference at $p = 0.05$ (Savin *et al*., 1977; Khan and Morse, 1998; Liu *et al*., 2003). Data from the validation phase were expressed as % mortality. All analyses were performed using R software version 3.1.1 (R-Development-Core-Team, 2014).

6.4 Results

6.4.1 Phase 1: development of immature life stages over time

Based on the morphological characters described in the Material and methods section, on day 1 after oviposition only eggs were present (Table 6.1); all eggs had hatched by day 2.

On days 2 and 3, more than 99% of the larvae were in the first instar. By days 4 and 5, 97.1% and 99.8% of the larvae respectively were in the second instar. By days 6, 7 and 8, third instar larvae accounted for between 98.7% and 100% of larvae in the fruit, with most mature larvae leaving the fruits to pupate on day 8. On this basis the 1st, 3rd, 5th and 7th day were deemed to represent the egg, first, second and third instars respectively (Table 6.1).

Table 6.1: Relative percentages of the immature stages of *Bactrocera dorsalis* in "*Apple*" mango fruit following infestation in the laboratory.

Day	No. larvae examined	% of different developmental stages			
		Eggs	First instar	Second instar	Third instar
1	-[a]	100	NA	NA	NA
2	200	0	99.88	0.12	0
3	200	0	99.35	0.65	0
4	200	0	2.69	97.31	0
5	200	0	0.12	99.81	0.07
6	200	0	0	0.96	99.04
7	200	0	0	0.27	99.73
8	200	0	0	0	100

[a] No larvae in any examined fruit. Eggs were clearly visible and scored as 100% eggs

6.4.2 Phase 2: Determination of the most heat-tolerant immature life stage

Mortality of all immature stages of *B. dorsalis* increased as treatment time increased, with 100% mortality of all stages after 68 min of hot water treatment at 46.1°C (Table 6.2). Mean natural mortality in the untreated control for the eggs and first, second and third instar larvae was 6.1±0.44%, 5.7±0.22%, 5.6±0.42% and 4.5±0.62% respectively and ranged from 3.0% to 7.3% for all immature stages (Table 6.2).

Based on time-mortality relationships, for the time required to attain 50, 90, 99 and 99.9968% mortality, the third instar was found to be the most heat-tolerant immature stage (Table 6.3) – estimated $LT_{99.9968\%}$ mortality was 81.47 min (range 75.77-87.18min). This suggests that "*Apple*" mango fruits infested with third instar larvae of *B. dorsalis* treated at 46.1^0C for 81.47 min should ensure 99.9968% level of quarantine security. The egg stage and first and second instar larvae required less treatment time to reach the 99.9968% mortality level – 46.25 min (range 45.51-46.99 min), 73.10 min (range 71.33-74.87 min) and 75.59 min (range 74.09-77.10 min) respectively (Table 6.3). However, there was no significant difference in treatment time between the first and second instars, and the second and third instars but the third instars were chosen as the most heat tolerant stage on arithmetic basis (Table 6.3). Based on these results, large scale validation experiments were carried out on mango fruits infested with the third instar for 81.47 min at 46.1°C.

Table 6.2 Time-mortality relationship for the immature stages of *Bactrocera dorsalis* in "*Apple*" mango fruit after immersion in hot water of 46.1°C.

Stage of development	Time (min)	Hot water treatment at 46.1°C			Untreated control		
		No. treated	No. dead	% mortality	No. alive†	No. dead	% mortality
Egg	8	6636	221	3.33	6837	467	6.83
	23	3720	1357	36.48	3434	245	7.13
	38	3879	3811	98.25	3834	195	5.09
	53	3559	3558	99.97	4430	221	4.99
	68	3786	3786	100	3181	197	6.19
First instar	8	10483	1127	10.75	9313	573	6.15
	23	7740	3211	41.49	7946	484	6.09
	38	7074	4349	61.48	7841	433	5.52
	53	4678	4005	85.61	6032	298	4.94
	68	5512	5512	100	5880	348	5.92
Second instar	8	9536	968	10.15	8487	464	5.47
	23	7863	2846	36.19	8351	485	5.81
	38	9564	5538	57.90	8991	419	4.66
	53	9762	9056	92.77	12049	708	5.88
	68	7898	7898	100	9854	716	7.27
Third instar	8	10288	1413	13.73	10052	582	5.79
	23	11274	4264	37.82	10749	392	3.65
	38	13033	6693	51.35	12426	374	3.01
	53	11423	9891	86.59	11928	735	6.16
	68	11900	11900	100	9037	358	3.96

†The number of larvae presented in this table are based on actual counts following the dissection and washing of manmgo pulp to expose infesting larvae.

Table 6.3 Complementary log-log model estimates of time required to achieve 50, 90, 99 and 99.9968% mortality of different immature stages of *Bactrocera dorsalis* in "*Apple*" mango fruit after hot water treatment of 46.1°C.

Stage of development	No. fruit treated	Lethal time (95% Confidence intervals) (min)			
		LT_{50}	LT_{90}	LT_{99}	$LT_{99.9968}$
Egg	17,822[1]	24.79 (24.44-25.14)	34.32 (33.93-34.72)	39.83 (39.28-40.36)	46.25a (45.51-46.99)
First instar	17,895[2]	29.24 (28.49-29.99)	48.72 47.88-49.55)	59.96 (58.74-61.19)	73.10b (71.33-74.87)
Second instar	44,623	33.38 (32.74-34.00)	52.13 (51.38-52.87)	62.95 (61.89-64.02)	75.59b (74.09-77.10)
Third instar	57,633	34.75 (32.36-37.14)	55.5 (52.71-58.29)	67.48 (63.46-71.49)	81.47bc (75.77-87.18)

[1 and 2] Two replicates in the egg stage and three in the first larval instar were excluded from the model because they had 0 and 100% mortality extremes, hence not suitable for model assumptions. Lethal times in the last column followed by a different letter are significantly different (p =0.05; non-overlap of confidence intervals). (95% Confidence intervals in parenthesis and all values are after correcting for natural mortality).

6.4.3 Phase 3: Large scale validation trials

6.4.3.1 Treatment regime: 46.1°C for 81.47 min

There were no survivors from 51,101 third instar larvae treated at this regime. Mean natural mortality in the untreated control was 4.1% (range 1.5-7.5%), from 55, 789 larvae (Table 6.4).

Table 6.4 Mortality of third instar larvae of *Bactrocera dorsalis* subjected to hot water of 46.1°C for 81.47 min in "*Apple*" mango fruit.

	Hot water treatment				Untreated control			
Treatment No.	Total larvae treated	No. dead	No. alive	% mortality	Total larvae treated	No. dead	No. alive	% mortality
1	4795	4795	0	100	3963	139	3824	3.50
2	3680	3680	0	100	2848	161	2687	5.65
3	3586	3586	0	100	3997	248	3749	6.20
4	3777	3777	0	100	2656	136	2520	5.12
5	2643	2643	0	100	3078	161	2917	5.23
6	3579	3579	0	100	2965	98	2867	3.31
7	2902	2902	0	100	4064	60	4004	1.48
8	2253	2253	0	100	3433	145	3288	4.22
9	1704	1704	0	100	3232	197	3035	6.10
10	3174	3174	0	100	4118	153	3965	3.72
11	2554	2554	0	100	2700	65	2635	2.41
12	2501	2501	0	100	2235	108	2127	4.83
13	3332	3332	0	100	4230	121	4109	2.86
14	3837	3837	0	100	3415	104	3311	3.05
15	2717	2717	0	100	2628	70	2558	2.66
16	1891	1891	0	100	3495	262	3233	7.50
17	2176	2176	0	100	2732	43	2689	1.57
Total	51,101	51,101	0	100	55,789	2271	53,518	4.07

6.4.3.2 Treatment regime: 46.1°C for 68 min

There were no survivors from 44,651 third instar larvae treated at this regime. Mortality was therefore 100% for all eggs and larvae inside mangoes treated for 68.0 min. Mean natural mortality from 49,389 larvae was 3.9% (range 0.4-6.2%) (Table 6.5).

Table 6.5 Mortality of third instar larvae of *Bactrocera dorsalis* subjected to hot water treatment of 46.1°C for 68 min in "*Apple*" mango fruit.

	Hot water treatment				Untreated control			
Treatment No.	Total larvae treated	No. dead	No. alive	% mortality	Total larvae treated	No. dead	No. alive	% mortality
1	2902	2902	0	100	3043	69	2974	2.27
2	3775	3775	0	100	3286	168	3118	5.11
3	3192	3192	0	100	2971	115	2856	3.87
4	2831	2831	0	100	3621	158	3463	4.36
5	2737	2737	0	100	2994	68	2926	2.27
6	2462	2462	0	100	3370	78	3292	2.31
7	3470	3470	0	100	3763	113	3650	3.00
8	2250	2250	0	100	3478	103	3375	2.96
9	2702	2702	0	100	2680	180	2500	6.22
10	3249	3249	0	100	3661	192	3469	5.24
11	2533	2533	0	100	3651	224	3427	6.14
12	2779	2779	0	100	2809	10	2799	0.36
13	2197	2197	0	100	3694	223	3471	6.04
14	3485	3485	0	100	3133	61	3072	1.95
15	4087	4087	0	100	3685	169	3516	4.59
Total	44,651	44,651	0	100	49,839	1931	47,908	3.87

6.4.4 Temperature fluctuation during treatment

At the point of loading mango fruits into the hot water treatment tank, the mean drop in water temperature was 0.35±0.07°C (range 0.1-1.1°C). This required an average of 2.58min for the thermo-regulators to reset the temperature back to 46.1°C (Fig. 6 5).

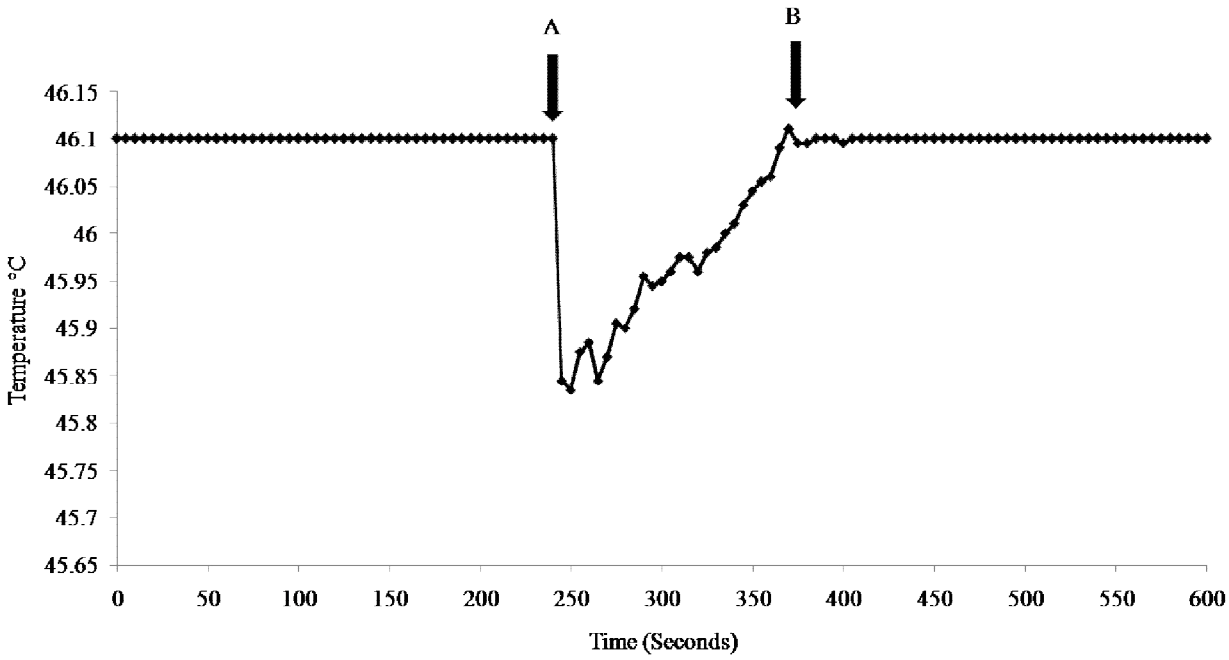

Figure 6.5 Temperature fluctuation of water in hot water treatment tank after immersion of mango fruits (A) and subsequent temperature stabilization (B).

6.5 Discussion

The incubation period of the egg stage of *B. dorsalis* in "*Apple*" mango at ambient conditions (26-28°C) was 24-48 h, which is comparable to results reported by previous workers. Ekesi *et al*., (2006) reported egg incubation time for this pest on mango of 1.20 d at 28±1°C, 50±8% RH while Gomina *et al*., (2014) reported an egg incubation period of 1.28 d at 27.5±1°C, 79.5±3% RH. Gomina *et al*., (2014) reported that the average time period of development for the first, second and third instars of *B. dorsalis* combined was 11.35±1.13 days.

In the present study, infested mangoes were subjected to hot water treatment on days three, five and seven after oviposition, time periods which fall within the above total developmental time.

In tephritid fruit flies and other insects in general, temperature is one of the major factors that influence development of the insect (Bateman, 1972; Rwomushana *et al*., 2008; Salum *et al*., 2014). Different immature life stages are known to respond differently when subjected to heat and /or host fruit species varieties (Sharp and Chew, 1987; Foliaki and Armstrong, 1997; Sales *et al*., 1997). In this study the egg stage was the least tolerant to heat, followed by the first and second instars, with the third instar being the most heat tolerant. This is in contrast to findings by some other workers. Armstrong *et al*., (1989) reported that the third instars of *B. dorsalis* and *C. capitata* were the least heat-tolerant compared to the egg and other larval stages when infested papayas were subjected to high temperature. Heard *et al*., (1991) treated infested "*Kensington*" mangoes with vapour heat at 44, 45, 46, 47, and 48°C and reported that mature eggs and third instar larvae of *Bactrocera tryoni* (Froggatt) were the most heat-tolerant developmental stages, with first and second instars being intermediate in tolerance. The difference in egg heat tolerance in their paper could be the different ages at which the eggs were treated. The difference in tolerance with regard to the egg stage when compared to our findings could be due to the age factor as we only treated young 6 h old eggs. Though the treatment method differs from ours, their findings on third instars are in agreement with our findings that third instars of *B. dorsalis* were the most heat-tolerant. In *C. capitata*, Hernández *et al*., 2012 found the first instar to be the most heat tolerant stage in infested "Ataulfo" mangoes, requiring fruit immersion for 95 min at temperatures between 46.1 and 47°C. The reasons for these variations in heat tolerance may lie in the different treatment techniques, experimental tanks and host fruit species used by the different authors.

Most studies on quarantine treatments such as Hayes *et al*., 1984, Gould and Sharp, 1990, Corcoran *et al*., 1993, Jessup, 1994, Armstrong *et al*., 1995, among others hardly mention pilot studies to determine the most heat tolerant life stages or the implications of mortality in the control (West and Hallman, 2013). This makes direct comparisons difficult as only the effective Probit is stated without reference to any particular stage of development.

In this study, 99.99% mortality corresponding to Probit 8.72 for the most heat-tolerant immature stage of *B. dorsalis* (third instar) was achieved at a water temperature of 46.1°C for 81.47 min (CL 75.77-87.1 min). However, 100% mortality in 44,651 third instar larvae was demonstrated after 68 min at 46.1°C, nearly 14 minutes before the calculated efficacy level of above. Hoover *et al*., (2010) reported that modelling mortality response data sometimes results in overestimating the dose above that obtained experimentally, leading to increased treatment costs and detrimental effects on treated commodities. Thus, the use of confidence intervals becomes necessary when choosing effective treatment time period. The study reports effective treatment time of 75 to 87 min which could be adopted for 400-500 g "*Apple*" mango. From the author's experience, "*Apple*" mangoes with a net weight corresponding to this category are preferred for export, while those smaller or larger than this are consumed locally or simply used in juice making. Nevertheless, values reported in this study fall within the range of treatment parameters used for other tephritid fruit fly species. For example, at 46.1°C Sharp (1988) estimated Probit 9 quarantine security for the mango varieties "*Tommy Atkins*", "*Kent*", "*Haden*", "*Keitt*" to be 71.4 and 64.5 min for *Anastrepha ludens* (Loew) and *A. serpentina* (Wiedemann) respectively. In similar studies 66.8 and 83.6 min were estimated to be effective for laboratory and wild strains of *A. obliqua* (Marquart) infesting "*Kent*" mangoes (Sharp *et al*., 1989a).

The "*Apple*" mango variety tested in our study is largely exported from Kenya to the European Union (EU) and Asia, with United States of America as an emerging market (Olunga, 2015). The development of a protocol for "*Apple*" mango fruit of up to 500 g net weight to hot water at 46.1°C for 81.47 min (CL 75.77-87.1 min) falls within the range of findings from other studies. For example, the EU recommends a quarantine security level of 65 to 90 min at 46°C for *B. dorsalis* for mango, depending on the shape and size of the fruits (EPPO, 2005). The United States Department of Agriculture approved a treatment time of 75 to 110 min at 46.1ºC as the requirement for rounded mango varieties such as "*Tommy Atkins"*, "*Kent"*, "*Haden"* and "*Keitt"*, depending on the weight and shape of the fruit (Yahia and Pedro Campos 2000; Follett and Neven 2006). In India, Verghese *et al*., (2011) showed that hot water treatment of different varieties of mango for 60 min at 48°C resulted in no survivors of *B. dorsalis* though the number of test insects were small as seen in their methodology. Despite the fact that the protocol of Verghese *et al*., (2011) is different to the one reported in this study, there are points of convergence with respect to the second and third instars having thermal responses which are not significantly different to each other. The estimated quarantine parameters are within the range of findings reported in other similar studies for Kenyan mango export. It is expected that this protocol offers a lesser stringent approach to the requirements for Probit 9, which in the strictest sense was developed for highly infested fruit (Follett and McQuate, 2001), but due to systems approach and area wide campaigns especially for fruit flies, infestation levels have drastically been reduced in recent years. Thus alternate treatment efficacy approaches should be adopted in order to save on time and resources required in developing quarantine treatment protocols such as those requiring the strict Probit 9 standard which requires treatment of 94.587 test insects or more.

The different exposure times for the immature life stages of *B. dorsalis* in this study could be attributed to a number of factors, one of which could be described as "positional effects". The variable response of the various stages of development could be due to the position they occupied within the fruit at the time of treatment. For example, eggs are oviposited just below the exocarp of the mango fruit and are immobile. A heat treatment will therefore affect this stage of development sooner and more effectively than other mobile stages that may be deeper in the fruit. However, the age of the eggs at the time of treatment has also been shown to affect susceptibility to heat. Although this parameter was not measured, very young eggs of *B. dorsalis* and other fruit fly species have been found to be highly susceptible to heat compared to later developmental stages (Foliaki and Armstrong, 1997; Sales *et al*., 1997; Dohino *et al*., 2014). The eggs used in this study (6 h old: choice of egg age based on the fact that oviposition holes left by fruit flies as they laid eggs would have closed sufficiently for the requirements of the experiment) were considerably young, which made them highly susceptible to heat treatment. First instar larvae are small and are mostly restricted to the area immediately under the exocarp; very few are able to tunnel deeper into the innermost part of the mesocarp. These larvae are therefore killed by the heat earlier in the treatment than the second and third larval instars. By contrast, second instar larvae are able to tunnel into the innermost part of the mesorcap and are thus more protected from the immediate effects of the heat. Third instar larvae are found mostly in the very innermost part of the mesorcap, have a more mature integument than earlier larval stages, and are very mobile. These attributes may have allowed third instar larvae to escape lethal temperatures for a period of time before succumbing to the heat as the heat penetrated deeper down into the fruit and there was no cooler place to hide.

Besides hot water immersion treatment, other phytosanitary treatments such as vapour heat, controlled atmosphere, and irradiation can be used for *B. dorsalis* disinfestation (Burikam *et al*., 1992; Armstrong *et al*., 1995; EPPO, 2005; IPPC, 2009b). Irradiation disinfestation commodities are usually not readily accepted by consumers for fear of perceived radio active substances, thus in the EU, "…*legislation requests that any irradiated food or food ingredient, even if it is present in trace amounts in a non-irradiated compound food, has to be labelled as irradiated or treated with ionizing radiation, in order to allow consumers an informed choice*" (EU, 1999). Controlled atmosphere disinfestation is usually used for both fresh and dried fruit but set up costs are usually prohibitive (Sen *et al*., 2010). A physical treatment, such as hot water immersion, is regarded as the most appropriate and cost-effective option for insect disinfestation of mango in Kenya and other East African countries. In contrast to hot air quarantine treatments, immersion of fruits in hot water is also considered to be a more efficient method of heat transfer into the fruit when the temperature of the water bath is properly maintained (Shellie and Mangan, 1994). Literature available on hot water treatments, as well as our initial findings (S. Ndlela, unpublished data), suggested that a hot water immersion treatment does not adversely affect either the physical or biochemical properties of mango fruits. If recommended harvesting and fruit handling procedures are followed prior to the treatment, the physical characteristics (marketability) and biochemical characteristics of fruits are not altered (Mansour *et al*., 2006; Djioua *et al*., 2009; Kumah *et al*., 2011; Verghese *et al*., 2011).

The condition of mango fruit prior to hot water treatment has a significant bearing on the quality of the fruit after treatment. The application of Mancozeb at flowering and fruit set ensured that mangoes used in the experiment were disease free (Ndlela *et al*., 2016).

It was felt that black spots, blackening of the fruit surface and cracks on the mango which are typical symptoms of fungal infection would affect the fruit during hot water treatment process, thus it was necessary to ensure that experimental fruits were healthy. The author chose to bag fruit prior to the anticipated fruit flies population build up which usually occur when young aborted fruits (less than 60 g) begin to fall. *Bactrocera dorsalis* known to infest all stages of mango development including premature fruit (Rattanapun *et al*., 2009; Diatta *et al*., 2013). Fruit flies populations have also been shown to increase significantly as a result of infestation of aborted fruit (Diatta *et al*., 2013). Pre-harvest factors negatively influencing the effect of a hot water treatment include harvesting the fruits before they reach the physiological maturity stage, use of improper harvesting techniques, and sap-burn injury at harvest, improper packaging and transportation among others. In the present study, fruits were harvested by hand or long-handled pruners, and were washed under tap water immediately after de-stemming to remove sap and avoid sap-burn injury. In other studies, sap-burn injury was avoided by de-stemming mango fruits under a calcium hydroxide solution (Amin *et al*., 2008; Maqbool and Malik, 2008).

In conclusion, results showed that the required 99.99% quarantine security level which corresponds to Probit 8.72 at the 95% confidence level was achieved for mango of the "*Apple*" variety at a water temperature of 46.1°C in 81.47 min (Couey and Chew, 1986; Schortemeyer *et al*., 2011). Although this conforms to the requirements of certain importing countries such as Australia, New Zealand and Japan, who allow quarantine treatment efficacy at the level reported in this study, (Follett and Neven, 2006), it was observed that a shorter exposure time of 68 min at 46.1°C was sufficient to result in no survivors in 46.651 tested insects causing 99.99% mortality in the most heat-tolerant immature stage (third instar). Extrapolation of these findings to other varieties and sizes should be made with caution.

Efforts are underway to develop protocols for mango varieties of different shapes and weight. If implemented, current results provide sufficient evidence to allow National Plant Protection Organisations and trading partners to open market access previously blocked due to the presence of *B. dorsalis*.

CHAPTER SEVEN

General discussion, conclusions and recommendations

Fruit flies are serious pests of economic importance causing devastating losses to fruits as well as impacting negatively on international trade due to quarantine restrictions imposed by importing countries. Thus, it has become a priority to suppress fruit flies populations to levels below the economic thresholds as well as reducing the widespread and wanton use of insecticides. Results of this study have demonstrated the suppression of *B. dorsalis* using the MAT, and consequently leading to the reduction in fruit damage by the invasive pest. The main advantage of this technique being the reduced amount of insecticide used compared to full cover sprays. The insecticide is not directly applied to the fruit, but on cotton dispensers that are relatively easy to make at farm level by smallholders, thus results in no chemical residues on the fruit. The product is also commerically available as polymeric plug or where both the lure and insecticide are blended together ready to use. Furthermore, farmers usually have inadequate knowledge of pesticide safety and insufficient means to procure proper protective equipment, predisposing them to toxic and hazardous substances. Implementing MAT reduces this risk to a substantial level. This technique has also proved to be of popular choice by farmers as they can see the captured dead flies in the lynfield trap, thus this is sufficient evidence of the efficacy of the method.

The successful release, establishment and subsequent dispersal of *F. arisanus* and *D. longicaudata*, provides hope for biological control of *B. dorsalis* and *C. cosyra* in the mango agro-ecosystem in Kenya. However there is need to increase release points in all mango producing districts and attempt to sample different cultivated as well as wild fruits which are hosts to *B. dorsalis* and *C. cosyra.*

Parasitoid recovery and dispersal data reported in this study could possibly be an underestimate as wild fruits and other fruits besides mango were not considered. Due to limitations, dispersal data could not be collected beyond 8 km, thus parasitoids could have dispersed beyond this point. Though the exotic *D. longicaudata* also parasitises the indigenous *C. cosyra*, released parasitods pose no detrimental effects to the native *P. cosyrae*, a natural enemy of *C. cosyra*. This is not suprising as the later (parasitoid and fruit fly) co-evolved, thus co-existence of the introduced and native parasitoid can not be ruled out. There is however need to pursue interaction studies at field level in order to understand the intricate dynamic associations of the introduced parasitoids, host, and indigenous natural enemies and predators.

Pre-harvest management measures are seldom 100% effective, thus post-harvest measures such as HWT are necessary. Results of the HWT presented here provide adequate evidence for effective post-harvest disinfestations treatment against *B. dorsalis,* and may facilitate access to export markets for mango fruits from Africa. The treatment did not in any way affect the physical appearance, taste and biochemical properties of the treated fruit. There is need to replicate the HWT studies in different mango varieties since size, shape and biochemical properties may affect the treatment duration required to impart effectiveness.

Based on overall findings of this study, it is therefore recommended that MAT, biological control using parasitoids and HWT be packaged together with other management techniques such as protein bait sprays, entomopathogenic fungi, and field sanitation among others, into an environmentally friendly, affordable and sustainable package that can be implemented in Integrated Pest Management (IPM) in the mango agro-system. Further work should also be done to speed up uptake of these techniques especially at smallholder level.

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Appendices

Appendix 1: Journal publication based on objective 1 (Chapter 3)

Ndlela, S., Mohamed, S., Ndegwa, P.N., Ong'Amo, G.O., & Ekesi, S. (2016). Male annihilation technique using methyl eugenol for field suppression of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) on mango in Kenya. *African Entomology*, 24 (2), 437-447.

Male annihilation technique using methyl eugenol for field suppression of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) on mango in Kenya

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The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), is one of the most devastating tephritid fruit flies of horticulture worldwide. Field trials were carried out for two seasons on mango (*Mangifera indica* L.) to evaluate the use of male annihilation technique (MAT) using methyl eugenol laced with deltamethrin instead of the commonly used malathion for the suppression of the pest on mango in coastal Kenya. Prior to application of the MAT, mean total numbers of *B. dorsalis* flies per trap per day (FTD) in pre-suppression monitoring data were comparable in orchards assigned to MAT treatment (FTD = 3.5) and those assigned to the control (FTD = 3.5) in season 1 and 12.4 and 10.5 FTD, respectively, in season 2. Following the application of MAT systems, total FTD were significantly lower in MAT-treated orchards (0.1 and 2.7 FTD, for seasons 1 and 2, respectively) compared to that in the control (18.6 and 21.5 FTD, for seasons 1 and 2, respectively) at 49 days after deployment of the control measures. This represented a reduction in the *B. dorsalis* population of 99.5 % in both seasons, resulting in a significant reduction of fruit infestation in the MAT-treated orchards compared to the control. The percentage of infested fruit was 25 and 18 times lower in MAT-treated orchards compared to the control for the first and second season, respectively. The number of puparia/kg of mango fruit was 17 and 24 fold lower in MAT-treated orchards compared to the control for the two consecutive seasons. These findings demonstrate the suppression of *B. dorsalis* using the MAT, and subsequent reduction in fruit damage by the pest. It is therefore recommended that MAT be adopted within a holistic Integrated Pest Management (IPM) approach in the mango agro-system, preferably covering large areas.

Key words: FTD, infestation, male annihilation technique, ME, monitoring, reduction.

INTRODUCTION

Globally, Tephritidae fruit flies such as *Ceratitis capitata* (Wiedemann), *Bactrocera cucurbitae* (Coquillett) and *Dacus* sp. represent a serious threat to the horticulture industry (White & Elson-Harris 1994; Vargas *et al.* 2013). In the tropics, the fruit fly problem is further compounded by predominantly conducive weather conditions and the availability of host fruits throughout the year (Purcell 1998). Some species of this family have of late attracted much attention due to their ability to invade new areas, where they are relatively unknown, and causing significant damage before control measures can be initiated. The best example of these is a member of the genus *Bactrocera*, that was first detected in Africa in 2003 (Lux *et al.* 2003). Two years later, the pest was described as *B. invadens* Drew, Tsuruta & White (Drew *et al.* 2005). However, recently *B. invadens* was synonymised with the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) following several years of intense integrative morphological, molecular, cytogenetic, behavioural and chemoecological research (Bo *et al.* 2014; Schutze *et al.* 2014a; Schutze *et al.* 2014b). Since its first detection in Africa, *B. dorsalis* continues to cause widespread damage to various commercially-grown fruit varieties, thereby compelling importing countries to enforce quarantine restrictions on fruit originating from regions where the pest has been reported (Ekesi *et al.* 2006; USDA-APHIS 2008; Guichard 2009; Otieno 2011). For example in Kenya, direct mango fruit losses due to fruit flies infestation have doubled (from approximately 40 % to 80 %) following the invasion by *B. dorsalis* (Ekesi *et al.* 2006; Rwomushana *et al.* 2008), in addition to the indirect losses incurred at the regional and international markets.

Various management strategies such as the use of food baits, parasitoids, pathogens, field sanitation, fruit bagging and male annihilation technique

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Appendix 2: Journal publication based on objective 4 (Chapter 6)

Ndlela S, Ekesi S, Ndegwa PN, Ong'amo GO, Mohamed SA. Post-harvest disinfestation of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) in mango using hot-water treatments. *Journal of Appllied Entomology*. 2017; 00:1–12. https://doi.org/10.1111/jen.12404.

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Post-harvest disinfestation of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) in mango using hot-water treatments

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Abstract

Mango, *Mangifera indica* L. (Anacardiaceae), is one of the most important fruits in Africa, providing household nutrition and economic development opportunities for millions of growers across the continent. In Kenya, over 80% of mango production is carried out by smallholders who produce this crop for both the domestic and the export markets. Despite its importance, mango production is hampered by several constraints, including infestation by fruit flies, especially the exotic *Bactrocera dorsalis*. In addition to its direct damage to fruits, the high quarantine status of the pest restricts the export of fruits and limits access to lucrative markets, impacting negatively on export earnings. To facilitate access to export markets, post-harvest management measures such as hot-water treatment are required to ensure quarantine security. Internationally, this level has been set as either 99.99% (Probit 8.7) or 99.9968% (Probit 9). In developing a protocol, the development of immature life stages of *B. dorsalis* in 'Apple' mango was established. Using this information, infested mangoes harbouring the different immature life stages were subjected to a hot-water treatment of 46.1°C for four different times, and the egg mortality and larval mortality were determined. The third-instar life stage was the most heat tolerant, followed by second- and first-instar larvae and the egg stage, respectively. The immersion time of 81.47 min (95% CL 75.77–87.18) was established as the time required to achieve 99.99% security level. In the validation experiment, there were no survivors from the 51,101 third-instar individuals treated in '*Apple*' mango weighing 400–500 g. Furthermore, there were no survivors from the 44,651 third instars exposed to 46.1°C for 68 min. These results provide sound evidence that the shorter treatment duration is an effective post-harvest disinfestations treatment against *B. dorsalis*, and should facilitate access to export markets for mango fruits from Africa.

KEYWORDS

export markets, *Mangifera indica*, oriental fruit fly, quarantine security

1 | INTRODUCTION

The genus *Bactrocera* contains over 500 described species in the family Tephritidae with many undescribed species yet to be incorporated into current knowledge (Drew & Hancock, 2000; Mwatawala, White, Maerere, Senkondo, & De Meyer, 2004). Lately the genus has been the subject of taxonomical revisions with new species being described (Drew & Raghu, 2002). This has complicated fruit fly management and protocols with quarantine restrictions having to be modified. Taxonomical changes have been made to the *Bactrocera dorsalis* (Hendel) complex (Bo et al., 2014; Schutze, Mahmood, et al., 2014; Schutze, Aketarawong, et al., 2014). The complex comprises over 90

