CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background information

Over the last two decades diversification into high value horticultural crop production has been pushed as an economic development strategy for sub-Saharan Africa (Ekesi, 2010). Horticulture offers one of the most important opportunities for employment creation, affording access to education and health care and providing women with economic opportunities in rural economies where the highest production of fruit and vegetable crops takes place (Ekesi, 2010). In 2009, horticultural exports from Kenya alone were worth $1 billion, making it the biggest foreign exchange earner and contributing roughly one-fifth to the economy of the country (McConnell, 2010). Fruit production constitutes an important source of income generation for both small and large-scale farmers in Kenya and several African countries providing food and nutritional security, creating job opportunities and improving health by providing essential micronutrients and vitamins (FAO, 2004; FPEAK, 2005).

Globally, mangoes (*Mangifera indica* L.) dominated world production of tropical fruits at 31.5 million metric tons in 2009, comprising 40% of global tropical fruit output (TAPP, 2010). In tropical regions, mango is a particularly important fruit and an important source of income for a large number of small scale farmers especially in Africa (Vayssie`res *et al*., 2008). Export of mangoes and mangosteens from the continent was valued at over $35 million in 2008 (FAO, 2009). In Africa, over 80% of the produce comes from smallholders for both domestic and urban export markets of which the European Union is the major export destination (ICIPE, 2006).

However, fruit production in sub-Saharan Africa (SSA) is limited by many abiotic and biotic constraints. Ranking high amongst the biotic factors is the heavy infestation by a range of insect pests of which the fruit fly species (Diptera: Tephritidae) are considered the most important (White and Elson-Harris, 1992).

Fruit flies are recognized worldwide as the most important threat to the horticultural industry (Lux *et al*., 2003; Rwomushana, 2008; Ekesi, 2010), and cause damage worth millions of dollars to fruits annually (NAQS, 2007). Due to the phytophagous nature, many species in the family Tephritidae inflict heavy losses on fruit and vegetable crops (White & Elson-Harris, 1992) with losses ranging from 40–100% from small-scale to large area farming systems (Ekesi *et al*., 2006; Ekesi and Billah, 2007; Van Mele *et. al.,* 2007; Chang, 2008; Cugala *et al*., 2009; Ekesi, 2010). They are also one of the greatest impediments to fresh produce exports worldwide due to quarantine restrictions attributed to the insects (Chang, 2008). Sub-Saharan Africa (SSA) is the aboriginal home to 915 fruit fly species from 148 genera, out of which 299 species develop in either wild or cultivated fruits. They belong mainly to four genera: *Bactrocera*, *Ceratitis*, *Dacus,* and *Trirhithrum* (Ekesi, 2010). On mango, the results of surveys across Eastern and Sothern Africa (ESA) shows the crop is attacked several native fruit fly species such *Ceratitis cosyra* (Walker), *C. fasciventris* (Karsch), *C. rosa* (Karsch), *C. anonae* and *C. Capitata* (Wiedemann).

With the intensification of fruit trade, the African continent has become highly vulnerable to introduction of alien invasive fruit fly species, further threatening the exploitation of foreign markets and jeopardizing the lucrative trade in fresh fruits and vegetables from the region (Ekesi *et al*., 2010; Mwatawala *et al*., 2009). Examples include the introduction of *Bactrocera zonata* into Egypt in 1997, the detection of *B. invadens* in Kenya in 2003 and the Solanum fruit fly *B.*

latifrons in Tanzania in 2003. The melon fly *B. cucurbitae* has also been in Africa for years without a clear date of introduction (Ekesi *et al*., 2010).

Among the invasive fruit fly species, *B. invadens* is thought to be responsible for causing extensive economic losses to horticultural crops throughout Africa since its first detection in the continent in 2003 (Ekesi *et al*., 2010). The pest has now spread across most of SSA (Ekesi and Billah 2007) and has been reported from 28 different countries including Angola, Benin, Burkina Faso, Cameroon, Comoros Island, Democratic Republic of Congo, Equatorial Guinea, Ghana, Guinea, Ivory Coast, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo and Uganda (Drew *et al.* 2005; Vayssie`res *et al*., 2005; Ekesi *et al*., 2006; Ekesi and Billah, 2007). *Bactrocera invadens* has been reported from over 30 plant species but the most preferred cultivated host plant is mango, *M. indica* L. (Anacardiaceae), while Marula, *Sclerocarya birrea* (A.Rich) Hochst. (Anacardiaceae) and tropical almond, *Terminalia catappa* L. (Combretaceae) are the most infested non-cultivated plants (Ekesi *et al.,* 2006; Mwatawala *et al*., 2006; Mohamed *et al*., 2010). Consequently, this invasive fruit fly pest represents a new major threat to Africa's huge potential for commercial horticulture necessary for both the export and domestic markets.

1.2 Justification

Exotic insect pests typically arrive in new areas without their natural enemies (Mohamed *et al.,* 2006). This enables the insect pest to cause extensive damage to crops in the new region, as there are no co-evolved natural enemies to control its population. In over 6,000 kg of fruits collected across East Africa, no parasitoid was reported attacking *B. invadens* (Ekesi *et al*., 2009). The lack of natural enemies to suppress *B. invadens* in its invaded range is contributing to the high

pest status of the insect, causing huge damage to horticultural crops and most especially the mango industry (Lux *et al*., 2003; Drew *et al.*, 2005; Ekesi *et al*., 2006). As an invasive species, it lends itself appropriately to classical biological control through the importation of natural enemies from its aboriginal home (Mohamed *et al.*, 2006).

Fopius arisanus (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) have been introduced and released as classical biological control agents of fruit flies in many regions of the world (Wharton & Gilstrap, 1983; Messing, *et al.*, 1993; Ovruski, *et al.*, 2000). *Fopius caudatus* (Szépligeti) and *Diachasmimorpha fullawayi* (Silvestri) have also been identified as potential indigenous parasitoids against mango fruit flies (Vayssières *et al.*, 2004).

Owing to the successful use of *F. arisanus* and *D. longicaudata* as effective biocontrol agents against fruit flies, ICIPE through its African Fruit Fly Programme (AFFP) imported these two Braconid parasitoids from Hawaii in 2006 for evaluation and field releases against *B. invadens* and other indigenous fruit fly pests. Results from pre-release bioassays have shown that *F. arisanus* has the potential to effectively control *B. invadens* (Mohamed *et al*., 2010) while *D. longicaudata* is effective against some native *Ceratitis* species (Mohamed *et al*., 2008).

The introduction of parasitoids into new areas is facing more stringent regulatory hurdles than earlier biological control programs (Kroder and Messing, 2010) and although *F. arisanus* and *D. longicaudata* have been shown to be effective biocontrol agents against *B. invadens* (Mohamed *et al.*, 2008), research must also address the possibility of indirect effects, including interaction between the introduced parasitoids and indigenous natural enemies (Kroder and Messing, 2010). A first step in achieving this is to identify the indigenous parasitoid fauna present in localities

where the parasitoids will be released and in the case of *B. invadens* in Kenya, emphasis should be in major mango producing areas of the country. Temperature is often the most important abiotic factor in the acclimatization of introduced natural enemies (Loni, 1997). It is therefore necessary that the effect of temperature on the development, longevity and parasitism rates of the introduced parasitoids reared on *B. invadens* is assessed. There is also no information on the performance of *F. arisanus* and *D. longicaudata* when evaluated in a tritrophic system, involving different host fruits with *B. invadens* as the target pest. As visual and chemical cues greatly influence host location by Braconid parasitoids (Liquido *et al.*, 1991, Bautista and Harris, 1996; Bautista *et al.*, 2004) as well as parasitism rates (Altuzar *et al.* 2004), it is imperative that this study is conducted. Lastly, the African Weaver ants, *Oecophylla longinoda* abound in mango plantations on the continent and have recently been touted as effective biocontrol agents against fruit flies through the release of chemical cues that deter oviposition by fruit flies and larval predation (Van Mele *et al;* 2007; 2009). However, no information exists on the interaction between *O. longinoda* and the introduced parasitoids and the subsequent effect of the interaction on parasitoid performance.

Therefore, this study aimed at gaining insight into the indigenous tephritid fauna present in the major mango growing areas in Kenya as well as further understanding the effect of specific biotic and abiotic factors and their interactions on the overall performance of *F. arisanus* and *D. longicaudata*, as part of the wider strategy to develop sustainable management strategies for *B. invadens*.

1.3 Objectives of the study

The overall objective of this study was to evaluate the performance of two introduced parasitoids (*D. longicaudata* and *F. arisanus*) against *B. invadens* and their interaction with indigenous natural enemies. The specific objectives were to:

1. Determine the indigenous tephritid parasitoids in the Coast and Eastern provinces of Kenya
2. Evaluate the performance of the introduced parasitoids (*F. arisanus and D. longicaudata*) in a tritrophic system involving the host fruit, fruit fly and parasitoid.
3. Determine the effect of temperature on parasitism rates, developmental time and longevity of the introduced parasitoids.
4. Assess the level of interaction between *F. arisanus* and the predatory ants, *O. longinoda* and its subsequent effect on parasitoid performance.

1.4 Hypotheses

The following hypotheses were tested;

a) Indigenous tephritid parasitoids exist in the major mango growing areas in Kenya.
b) Temperature influences parasitism rates, developmental time and adult longevity of the introduced parasitoids.
c) Host fruit substrate affects the performance of the introduced parasitoids when reared from *B. invadens.*
d) Interactions between *F. arisanus* and the predatory ants, *O. longinoda* impacts negatively on the performance of the parasitoid.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MANGO

2.1.1 Production and trade

Mangoes (*Mangifera indica* L.) belong to the genus *Mangifera*, consisting of numerous species of tropical fruiting trees in the flowering plant family Anacardiaceae (Bally, 2006). The genus *Mangifera* originates in tropical Asia, with the greatest number of species found in Borneo, Java, Sumatra, and the Malay Peninsula (Bally, 2006). Mango is now cultivated throughout the tropical and subtropical world for commercial fruit production, as a garden tree, and as a shade tree for stock (Bally, 2006). Nearly half of the world's mangoes are cultivated in India alone (Jedele *et al*., 2003)

World production of tropical fruits was estimated at over 82.7 million tonnes in 2008 (FAO, 2009). Mangoes dominated world production of tropical fruit at 31.5 million metric tons, comprising 40% of global tropical fruit output (FAO, 2009). Asia is by far the largest producing region for tropical fruits, followed by Latin America and the Caribbean, Africa and Oceania. In 2008, Asia was the largest producer of mangoes, accounting for 74% of world production. Latin America and the Caribbean had a share of 16%, Africa 10%, and the balance was produced in Oceania (FAO, 2009). India is the world's largest producer of mangoes and accounted for 13.6 of the total 34.9 million tons of mango fruits produced in 2008 (FAO, 2009). It has been estimated that there are over 1000 commercial varieties in India, where mangoes are often called the "king of fruits" (HASS, 1992). Other major producing countries are China, Thailand, Indonesia, Mexico, Pakistan and Brazil (Table 2.1). The aggregate production of the top 10 countries is

responsible for roughly 80% of worldwide production. Though India is the largest producer of mangoes in the world, it accounts for less than one percent of the global mango trade; consuming most of its own output (USAID, 2006). The United States and European Union together accounted for 75% of world mango imports in 2008 (FAO, 2009). In 2009, based on growing demand, the United States Department of Agriculture (USDA) predicted that US mango imports would grow nearly 7% to 450,000 tons by 2010 (TAPP, 2010).

In Africa, Nigeria produces the largest amount of mangoes, with approximately 730,000 metric tonnes annually (Yusuf and Salau, 2007). Other major mango producing countries on the continent include Sudan, Egypt, Madagascar and Tanzania (Yusuf and Salau, 2007). Mango exports from Africa are estimated between 35,000 - 40,000 metric tonnes and worth over USD 35 million annually (FAO, 2009). The European Union is the largest destination market for mangoes exported from Africa, followed by the Middle East (Lux *et al*., 2003).

The importance and contribution of mango to the economies of producing and exporting countries cannot be overemphasized. In 2009, India, the leading exporter of mangoes and mangosteens earned approximately US$210,556,000 from exports (FAO, 2010). Mexico, The Netherlands, Brazil and Thailand, (the four major exporters of mangoes and mangosteens following India) earned US$136,942,000, US$124,575,000, US$97,686,000 and US$71,410,000 respectively in 2009 (FAO, 2010).

Table 2.1 Top mango producing countries of the world

COUNTRY	Production (Metric tonnes)
India	13557100
China	4140290
Thailand	2469810
Indonesia	2243440
Pakistan	1728000
Mexico	1509270
Brazil	1197690
Nigeria	831489
Bangladesh	828161
Philippines	771441

Source: FAO stat (2010)

2.1.2 Uses

The different parts of the mango tree can be put into several uses. The fruit flesh of a ripe mango is very sweet, with a unique taste that nevertheless varies from variety to variety. The texture of the flesh varies between cultivars, some having a soft, pulpy texture similar to an over-ripe plum, while others have firmer flesh like a cantaloupe or avocado. In some cultivars, the flesh has a fibrous texture. Mango is an excellent nutritional source, containing many vitamins, minerals, and antioxidants, as well as enzymes such as magneferin and lactase which aid in digestion and intestinal health. It is rich in a variety of phytochemicals and nutrients that qualify it as a model "superfruit", a term used to highlight the potential health value of certain edible fruits. Mango

peel contains pigments that may have antioxidant properties (Berardini *et al*., 2005; Ajila and Prasada, 2008) including carotenoids, such as the provitamin, beta-carotene, lutein and alpha-carotene (Gouado *et al*., 2007), polyphenols such as quercetin, kaempferol, gallic acid, caffeic acid, catechins, tannins, and the unique mango xanthone, mangiferin (Singh *et al*., 2004; Andreu *et al*., 2005; Mahattanatawee *et al*., 2006). These polyphenols may counteract free radicals in various disease mechanisms (Percival *et al*., 2006; Rodríguez *et al*., 2006). Mango is also used to make juices, both in ripe and unripe form. Dwarf or semi-dwarf varieties serve as ornamental plants and can be grown in containers. The bark of the mango tree is used for medicinal purposes and the leaves are used to feed livestock in most parts of Africa. It is also utilized as a 'shade tree' in most parts of the world.

2.2 FRUIT FLIES

2.2.1 Classification and description

There are over 4000 tephritid fruit fly species distributed throughout the tropical, sub-tropical and temperate regions of the world (White and Elson-Harris, 1992). The family Tephritidae includes 4,448 recognized species and subspecies of fruit flies worldwide, grouped in 484 genera. The actual number of species is much higher, as many remain undescribed (Norrborn, 2004). Within the order Diptera, the family Tephritidae belongs to the suborder Brachycera, infraorder Muscomorpha (= Cyclorrhapha), section Schizophora, and superfamily Tephritoidea (McAlpine, 1989). Taxonomic classification of tephritids into subfamilies has been controversial from old to date (White, 2000). The five subfamilies recognised and described are the Toxotrypaninae, Trypetinae, Ceratitinae, Tephritinae and Dacinae. Trypetinae includes *Rhagoletis* Loew, *Carpomyia* Costa and *Pliorecepta* Korneyev. The Toxotrypaninae includes

Anastrepha Schiner and *Toxotrypana* Gerstacker species (Rwomushana, 2008). The Ceratitinae are the commonest pests in Africa and include the genera *Ceratitis* Macleay and *Trirhithrum* Bezzi. Pest species under Ceratitinae include Trirhithrum coffeae Bezzi which mostly attacks coffee as well as *Ceratitis cosyra* (Walker) and *C. capitata* (Wiedemann). The Tephritinae are not known to attack horticultural crops and some species have potential for use as biological control agents against obnoxious weeds (White and Elson-Harris, 1992). Two genera are described in the subfamily Dacinae; *Dacus* (Fabricus) and *Bactrocera*. The species of *Bactrocera* are of Asian/Pacific origin, except for a few African species (White and Hancock, 1997). Tephritid fruit flies are one of the most economically important groups of insects (Ekesi and Billah, 2006) and species diversity is greatest in the tropics (Norrborn, 2010). They are also among the most attractive and biologically interesting Diptera, having patterned wings and often brightly coloured patterned bodies, which may be used in mimicry of jumping spiders or wasps and in elaborate courtship and other behaviours (Norrborn, 2004). Plate 2.1 shows some of the major species of fruit flies that attack horticultural crops in Africa.

Ceratitis fasciventris (Karsch)
© R.S. Copeland

Ceratitis capitata (Wiedemann)
© Scott Bauer

Bactrocera cucurbitae (Coquillett)

© USDA

Bactrocera invadens (Drew *et al*.)

© R.S. Copeland

Ceratitis rosa (Karsch)

© outdoorphoto.co.za

Ceratitis cosyra (Walker)

© G. Goergen

Plate 2.1 Some major species of fruit flies attacking horticultural crops in Africa

2.2.2 Biology of Dacinae fruit flies

Adult females lay their eggs beneath the surface of ripening fruits or ripened fruits, depending on the host plant attacked (Rwomushana, 2008) (Plate 2.2). They settle on the surface of the fruit and use their sharp ovipositors to pierce into the fruit to a depth of about 2-5 mm and deposit their eggs (Ekesi and Billah, 2006). The time spent by females during oviposition varies (Rwomushana, 2008). For example, *B. tyroni* (Froggatt) takes about 1-3 min per oviposition with a maximum daily oviposition rate of 80 eggs/female/ day (Yonow *et al*., 2004) while *B. jarvisi* (Tryon) which lays more eggs per clutch takes about 4-6 min (Fitt, 1984). The duration of oviposition of *B. invadens* is still not clear (Rwomushana, 2008) although the average daily clutch size for this species has been estimated at 18.2 eggs (Ekesi *et al*., 2006). The eggs are laid singly or in clusters and egg size and structure show some variation from species to species. There is, however, a correlation between egg size and body size or ovariole number (Fitt, 1984). There is no evidence that Dacine fruit flies deposit an epideitic oviposition-deterring pheromone after egg laying (Fitt, 1984).

Depending on the temperature conditions, the eggs hatch within 3-12 days into tiny white maggots (Ekesi and Billah, 2006) (Plate 2.2). The larvae are typical acephalic cyclorrhaphan maggots with an involuted head, three thoracic segments and eight abdominal segments (Fletcher, 1987). Larvae feed on the yeast organisms and fungi growing in the fruit and vegetable materials, and through their feeding efforts, they soon turn their food into a semi-liquid mess. When fully grown larvae are ready to pupate, they leave the rotten fruit for drier areas, and usually burrow several centimetres into the soil (Fitt, 1981; Dimou *et al*., 2003). The puparia are found buried in the soil, 2-5 cm beneath the host plant (Plate 2.2). The duration of the pupal stage can be 10-20 days depending on climatic conditions (Ekesi and Billah, 2006). When

pupation is complete, a winged fly emerges and crawls to the soil surface. The time required to complete one life cycle is mainly dependent on the temperature of the growth medium and surrounding air, with developmental time shortening with increasing temperature.

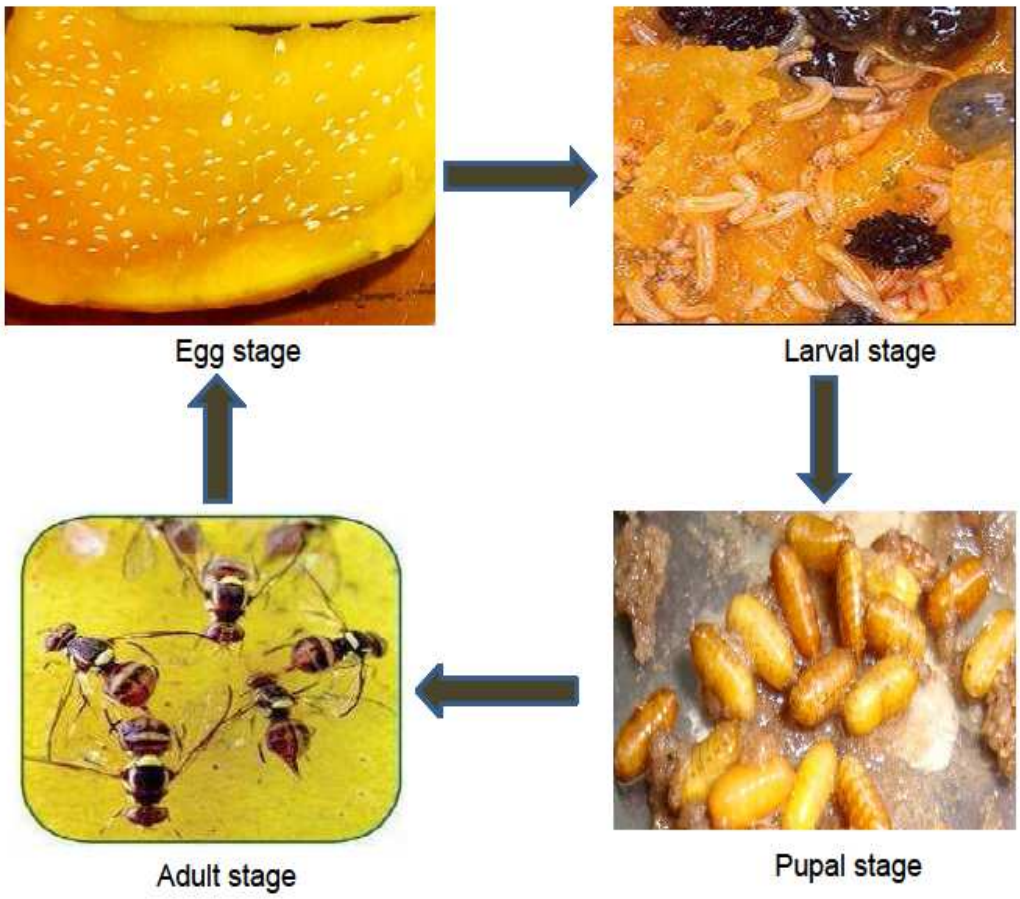

Plate 2.2 Biology of a typical fruit fly species (Diptera: Tephritidae)

Newly emerged adults require a carbohydrate source and water in order to survive. They also search for a protein source for egg maturation and to enhance their reproductive potential (Christenson and Foote, 1960; Fletcher, 1987). Dacine fruit flies normally mate at dusk under low light intensity (Arakakai *et al*., 1984) although mating behaviour has rarely been observed in

the field (Rwomushana, 2008). Tropical species of the genera *Bactrocera* and *Dacus* are multivoltine, producing several generations per year. These species may produce up to six overlapping generations per season (Bateman, 1972), thus potential for heavy fruit losses is very high.

2.2.3 Host plants of fruit flies

Most fruit-infesting flies are highly polyphagous, infesting a wide range of cultivated and wild host fruits. Members of the genus *Ceratitis* attack a wide variety of commercial indigenous and exotic fruits (Liquido *et al*., 1991; De Meyer *et al*., 2002). *Ceratitis capitata* (Wiedemann) has been reared from over 55 plant species (Copeland *et al*., 2002). *Ceratitis rosa* Karsch and *C. cosyra* (Walker) also have a relatively wide host range in Africa, although *C. cosyra* is less polyphagous and primarily considered a mango pest (Mukiama and Muraya, 1994; Lux *et al*., 2003). In Kenya, *C. cosyra* and *C. rosa* have been recorded from 9 and 28 plant species respectively, particularly the plant family Annonaceae (Copeland *et al*., 2006). *Ceratitis anonae* Graham attacks several plants in the Annonaceae, Moraceae and Sapotaceae families (Copeland *et al*., 2006). Hosts fruits of *C. fasciventris* Bezzi are similar to those of *C. rosa* (De Meyer *et al*., 1996; 1998).

Fruit flies of the genus *Bactrocera*, particularly the *B. dorsalis* complex, are also known to have wide host plant ranges (Clarke *et al*., 2005). The only exception is the olive fruit fly, *B. oleae* (Gmelin) which feeds on only one plant: the wild or commercially cultivated olive, *Olea europaea* L. and has the capacity to ruin 100% of an olive crop by damaging the fruit (Wikipedia, 2010). The key plant families containing the *B. dorsalis* complex hosts include Rutaceae, Sapotaceae, Solanaceae, Annonaceae, Anacardiaceae, Clusiaceae, Lauraceae,

Moraceae and Myrtaceae (Tsuruta *et al*., 1997; Clarke *et al*., 2005). Three species within the *B. dorsalis* complex are known for their extreme polyphagy: *Bactrocera papayae* Drew & Hancock, with 209 recorded larval hosts across 51 plant families, *B. dorsalis* with 124 host species across 42 families and *B. carambolae* Drew & Hancock with 77 host fruit species across 27 families (Hollingsworth *et al*., 2003; Clarke *et al*., 2005). *Bactrocera invadens*, which is believed to be a member of the *B. dorsalis* complex, has been reported from over 30 plant species (Ekesi *et al.* 2006; Mwatawala *et al.*, 2006; Rwomushana *et al.*, 2008).

2.2.4 Economic importance

Tephritid fruit flies cause devastating direct losses to many fresh fruit and vegetable crops (IAEA, 2003; Ekesi and Billah, 2006). In addition, few insects have greater impact on international marketing and world trade in agricultural produce than tephritid fruit flies. With expanding international trade, fruit flies as major quarantine pests of fruit and vegetable crops have taken on added importance (IAEA, 2003). Various species of fruit fly cause damage to fruit and other plant crops. The genus *Bactrocera* especially is of worldwide notoriety for its destructive impact on agriculture. Other important genera include *Ceratitis*, *Anastrepha* and *Rhagoletis*.

Fruit flies are a threat to the horticultural industry wherever they occur. In California, fruit flies are considered a major threat to the state's $26.8 billion agricultural industry (CDFA, 2007). A permanent infestation of the Mediterranean fruit fly, *Ceratitis capitata* would cost California agriculture an estimated $1 billion each year in reduced crop yields, export sanctions and eradication costs (CDFA, 2007). In 1990, the California Department of Food and Agriculture (CDFA) identified 35 commodities as possible ‘Med fly’ hosts. If accurate, the ‘Med fly’ could

have potentially affected $6.5 billion worth of horticultural produce, of which $1.71 billion was to be exported (Siebert and Pradhan, 1991). The impact on the value of production and export markets, therefore, was definitely significant. The total field costs, including the cost of pesticides and their application, were estimated to range from $349.6 million to $731.9 million (Siebert and Pradhan, 1991). In Australia, total horticulture exports (including fresh fruit, vegetable, nuts and plants including flowers) was valued at $751million in 2008 (HAL, 2010) and losses due to fruit flies is estimated at $300 million annually (Drew, 2002).

Infestation by fruit flies is also a major constraint to local fruit production in Africa, causing losses of between 30 and 70% in mangoes in East Africa (Ekesi, 2010). The damage to fleshy fruits is mainly caused by a limited number of highly polyphagous species, belonging to the genus *Bactrocera, Ceratitis* and *Dacus*. In 2005, fruit flies ruined up to 40% of Africa's two-million tonnes mango harvest (IRIN, 2008). Substantial impact on the income of producers has also been reported with grave consequences on local trade, food security and export potential (CIRAD, 2007). In South Africa, the Natal fruit fly, *C. rosa* Karsch ranks second in importance only to the Mediterranean fruit fly, *C. capitata*, and at times it is an even more serious pest. For example, 50-100% of plums were reportedly infested in a South African locality despite control measures that were applied (CDFA, 2007). The deciduous fruit export industry is of great economic importance to South Africa and almost 90 million cartons are exported annually, with total earnings of approximately US $1 billion per annum (OABS, 2005). The Western Cape in South Africa is the most important region for the production of deciduous fruits, with approximately 58,000 hectares under cultivation (OABS, 2005). The region is host to two species of Tephritid fruit flies of economic importance, the Mediterranean fruit fly, *C. capitata*, and the Natal fruit fly, *C. rosa* which attack a wide variety of subtropical, tropical, and deciduous

fruits (Annecke & Moran, 1982). Both species are international quarantine pests with the potential to restrict international fruit trade with South Africa. It has been estimated that crop losses and control costs due to fruit flies in the Western Cape alone exceed US$3.2 million per annum (Mumford and Tween, 1997). While the economic impact of Tephritid fruit flies countrywide has not been determined, the impact on the South African export fruit industry of a quarantine embargo on South African fruits due to the presence of fruit flies would be devastating (Barnes *et al*., 2007). The economic importance of the fruit flies cannot be evaluated entirely from the standpoint of the actual damage to the various crops affected (Mau and Kessing, 1992). It must also be considered from the standpoint of quarantine as quarantine laws aimed at preventing the entry and establishment of flies in new areas have been established and are vigorously enforced (Mau and Kessing, 1992).

2.2.5 *Bactrocera invadens*

In 2003, a new *Bactrocera* species was detected in Kenya coast (Lux *et al.,* 2003) and it was reported from Tanzania shortly afterwards (Mwatawala *et al.,* 2004). Taxonomic study showed that it was an unknown exotic species later described as *Bactrocera invadens* by Drew *et al.* (2005), probably introduced from Asia (plate 2.3). Within 2 years of its detection in East Africa, it was reported from 16 countries throughout the African continent (Drew *et al.,* 2005). It has been reported by Ekesi *et al*., (2009) that *B. invadens* was rapidly displacing indigenous *Ceratitis* species. The pest has now spread across most of the SSA countries and now reported from 28 countries on the continent including Angola, Benin, Burkina Faso, Cameroon, Comoros Island, Democratic Republic of Congo, Equatorial Guinea, Ghana, Guinea, Ivory Coast, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo and Uganda (Drew *et al*., 2005; Vayssie`res *et al.,* 2005; Ekesi and Billah, 2007) (Table 2.2).

Bactrocera invadens has been reported from over 30 plant species but the most preferred cultivated host plant is mango, *M. indica* L., while Marula, *S. birrea* (A. Rich) Hochst. (Anacardiaceae) and tropical almond, *T. catappa* L. (Combretaceae) are the most infested non-cultivated plants (Ekesi *et al.* 2006; Mwatawala *et al.*, 2006; Rwomushana *et al.*, 2008). The invasion by *B. invadens* has compounded the existing fruit fly problem that was largely due to *Ceratitis* species (CIRAD, 2007). It has seriously compromised potential African fruit exports to Europe, as well as production for local consumption and regional markets. Plate 2.4 and 2.5 show healthy mangoes and one damaged by *B. invadens* infestation. Heavy losses are being incurred by exporters whose fruit shipments infested with this quarantine pest are intercepted and destroyed at the entry of the EU markets (CIRAD, 2007). Kenya is currently not able to export its mangoes and avocados to several countries. Less Ugandan bananas are exported than before. Ghana's citrus and avocados face the same fate (IRIN, 2008).

Bactrocera invadens has been described by the Inter-African Phytosanitary Council as a 'devastating quarantine pest' (Drew *et al.*, 2005) and damage on mango has increased to between 40–80% with higher losses occurring in lowland areas where *B. invadens* is now the dominant fruit fly pest (Ekesi *et al.*, 2006). Since *B. invadens* is classified as a quarantine pest, the situation is particularly critical for mango exports from West Africa to the EU (CIRAD, 2007). When goods are intercepted, the economic losses are severe (over € 30,000 for the destruction of a container of mangoes). A single seizure can totally destroy the efforts of an entire export season (CIRAD, 2007).

Plate 2.3 Adult *B. invadens* flies

Plate 2.4 Healthy mango fruits

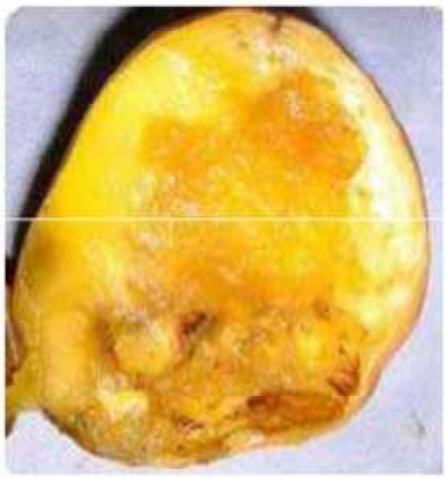

Plate 2.5 Mango damaged by *B. invadens*

Table 2.2 African countries invaded by *B. invadens* and years of detection

Date of invasion	**Country**
2003	Kenya, Tanzania, Guinea, Guinea Bissau
2004	West Africa, reported by IITA in Benin
2004	Cameroon, Angola, Benin
	Senegal, Burkina Faso, Chad
	Sudan , Congo, DR. Congo
	Uganda , Togo
2005	Ghana, Niger, Nigeria
2007	Mozambique, Liberia, Mali
2009	Burundi
2010	South Africa

2.2.6 Management of fruit flies

Several control methods have been employed in the management of fruit flies and new technologies continue to be developed. These include monitoring and trapping of fruit fly populations, the use chemical and bio-pesticides as well as protein baits, cultural methods such as orchard sanitation and fruit bagging, biological control involving the use of parasitoids and predators and the Sterile Insect Technique (SIT), and the Male Annihilation Technique (MAT). In recent times, Integrated Pest Management (IPM), in which a combination of the most suitable control methods is adopted for the management of fruit fly pests, is seen as the most effective technique for reducing fruit fly populations.

2.2.6.1 Monitoring of fruit flies

2.2.6.1.1 Monitoring through trapping

Trapping is usually used for detection and monitoring of fruit fly populations, although some level of control is achieved when properly implemented. Traps used for fruit fly monitoring are usually dependent upon the nature of the attractant (IAEA, 2003). Some of the commonly used traps include the Lynfield trap, Jackson trap, Steiner trap, McPhail trap, Tephri trap and the Multilure trap (Plate 2.6). Studies have been carried out over the last decade to evaluate a range of trap types to establish the best trap/attractant combination for the management of different species of fruit flies (Heath *et al*., 1997; Cornelius *et al*., 1999; Katsoyannos *et al*., 2000). The use of traps for monitoring and/or reduction of fruit fly populations have been widely reported (Nilakhe *et al*., 1993; Penrose, 1993; Midgarden *et al*., 2004). The drawback with traps is their susceptibility to environmental factors. Temperature, rainfall, wind speed and direction influence attractant release (from lures) and insect flight. Many insects fly and respond to semiochemicals during specific times of the day (dawn, midday, dusk, night, etc.), and only if temperatures at

that time exceed a minimum level (Weinzierl *et al*., 2005). Wind speed and direction also determine the extent of insect movement from surrounding areas to traps within a field or orchard (Weinzierl *et al*., 2005). To be effective, traps must be used in combination with good orchard sanitation.

Trimedlure baited sticky trap

McPhail trap

Jackson trap

Plate 2.6 Some traps used for monitoring of fruit flies

2.2.6.1.2 Monitoring through fruit collection

Fruit fly monitoring can also be done by collecting wild and cultivated fruits from orchards, forests, etc. and identifying the fruit flies that emerge from puparia collected from these fruits. Since most of the parapheromones used in fruit fly trapping attract only female flies, the rearing of fruit flies from field-collected fruit is currently the only monitoring technique that provides an indication of the presence of female flies (DEEDI, 2010). These fruit collections provide data on the location of breeding populations of fruit flies, which can then be targeted for eradication flies (DEEDI, 2010).

2.2.6.2 Fruit bagging

One of the most effective mechanical control methods is bagging the fruit to prevent oviposition by female fruit flies (Mau and Kessing, 1992) (Plate 2.7). This results in fruit fly and pesticide free fruits with good cosmetic appeal (Rwomushana *et al*., 2008). Notwithstanding the presence of fruit flies in the orchard, wrapping or bagging individual fruits with paper bags to exclude adult fruit flies from laying eggs on the fruits is a way of producing fruits that are free from fruit fly attack (Ekesi and Billah, 2006). To be effective, the fruits must be wrapped or bagged well before fruit fly attack, that is, at least one month before harvest (Ekesi and Billah, 2006). Although it is labour intensive, given the large number of fruits to be bagged and the huge size of mango orchards, it is an effective method for high value fruit produced for export or fruits produced in backyard gardens for family use (Ekesi and Billah, 2006).

Plate 2.7 Bagging of mango fruits in an orchard to prevent fruit fly infestation

© M.K. Billah

2.2.6.3 Orchard sanitation

The principal cultural control method used for controlling fruit flies is orchard sanitation (Ekesi and Billah, 2006). Breeding of fruit flies in unwanted fruits is probably one of the biggest sources of damaging populations (Liquido, 1991). Orchard sanitation, which entails the collection and destruction of all unmarketable and infested fruits found on the tree and fallen fruits containing fruit fly maggots on the ground, can contribute significantly to the reduction of fruit fly populations in orchards (Ekesi and Billah, 2006). Infested fruits should be buried deep under the soil surface (Dhillon *et al*., 2005) with an addition of sufficient lime (Mau and Kessing, 1992) to kill larvae. Larvae have been known to burrow to the surface in loose soil and therefore infested fruits should be buried at least 50 cm deep (Mau and Kessing, 1992; Ekesi and

Billah, 2006). Fallen fruits may also be placed in plastic bags and bags exposed to the sun to kill any emerging larvae (Plate 2.8). In areas where individual orchards are in close proximity to each other, it is important that all orchards observe crop hygiene if results are to be achieved. Early and regular harvesting of fruits also reduces food sources from which large populations may develop by keeping the quantity of ripe fruits on the trees to a minimum (Mau and Kessing, 1992).

Plate 2.8 Destruction of fallen fruits infested by fruit flies by placing them plastic bags, tying the bags and exposing them to the sun © M.K. Billah

2.2.6.4 Use of protein baits

Chemical sprays have not been completely effective in protecting fruits from fruit fly attack (Mau and Kessing, 1992). Egg laying requires only a few minutes and chemical residues do not kill adults within this time frame. Proteinaceous liquid attractants in insecticide sprays is a recommended method of controlling adult fruit fly populations in the vicinity of crops. Certain

protein hydrolysates are now known to contain the nutritive elements required by fruit flies to mature their eggs and protein baits work on this premise (Christenson and Fotte, 1960; Fletcher, 1987). The bait insecticide sprays are applied to broad leaf plants that serve as refuge for fruit fly adults. Baits serve to encourage the adults (especially females) to feed on the spray residue and get killed (Mau and Kessing, 1992). Protein baits may also be used as components of traps which are used to monitor and suppress fruit fly populations (Rwomushana, 2008). The important element of bait sprays is that it involves 'spot spraying' and overall coverage of the plants is not required. This saves time, labour and materials which all translate into considerable spot savings and can reduce the amount of insecticide applied to the crop thereby limiting non-target effects. Bait spray components include the insecticides maldison or chlorpyrifos and yeast autolysate as the attractant (Dominiak, 2007). GF-120 NF Naturalyte, a spinosad-based fruit fly bait (Dow AgroSciences LLC, Indianapolis, IN), is one of the most effective bait sprays on the market and was developed as a replacement for malathion-based fruit fly baits. GF-120 is NF is the bait, incorporated with Spinosad, a mixture of spinosyns A and D derived from the naturally occurring soil actinomycete Saccharopolyspora spinosa Mertz and Yao (Sparks *et al*., 1998) It has been classified as an environmentally and toxicologically reduced-risk insecticide (Cleveland *et al.,* 2001; Copping and Menn, 2000). Bait sprays, however, suffer from reduced effectiveness during periods of heavy rains and high fruit fly pressure. Also, BAT works well if large areas are treated as in area-wide control programmes and community baiting schemes.

2.2.6.5 Male Annihilation Technique

The Male Annihilation Technique (MAT) is a fruit fly control method that removes male insects thus reducing the male population, by distributing an appropriate amount of male attractant combined with a killing agent in the entire target area continuously for a given length of time

(Mirani, 2007; Ghanim *et. al*., 2010). Fibreboard squares that have been soaked in lure-toxicant, a mixture of an attractant (usually methyl-eugenol) and an insecticide are distributed manually in targeted areas (Mirani, 2007). This adversely affects the male: female ratio and reduces the insect's chances of mating and females produce fewer progeny (Ghanim *et. al*., 2010). As a result, the wild population in the target area declines and the insects are/may be eradicated in the end (Cunningham, 1989; Zaheeruddin, 2007).

One of the first applications of MAT involved successful eradication of a heavy infestation of oriental fruit fly, *B. dorsalis*, from the island of Rota, Mariana Islands. This was achieved through the use of cane-fibre squares saturated with a solution of methyl eugenol which were either suspended on trees or dropped from the air (Steiner *et al.*, 1965). MAT was also used to completely eradicate *B. dorsalis* from all parts of Japan by 1986 under an eradication programme initiated in 1968 on Kikai Island (Shiga, 1992). Fiberboard blocks impregnated with methyl eugenol and various insecticides (e.g., Naled, Malathion and Fipronil) had also been used to successfully eradicate the Asian papaya fruit fly, *B. papayae* (Drew & Hancock) in Australia (Cantrell *et al.*, 2002) and *Bactrocera* species in Nauru (Allwood *et al.*, 2002). While attempts at MAT on isolated islands where immigration of flies is not a problem could be successful, such attempts in non-isolated situations appear to be ineffective (Rwomushana, 2008).

2.2.6.6 Use of chemical pesticides

Insecticides are widely used for fruit fly management because of their effectiveness, rapid curative action, simplicity of application and adaptability to most situations but the practice is complicated by several human and environmental hazards (Rwomushana, 2008). In fruit fly management, cover sprays of both the fruits and foliage is practised (Rwomushana, 2008). Adult

flies are killed when they come into contact with the insecticide or residues which are left on the fruit and foliage. Some insecticides have systemic action and are absorbed into fruits to kill larvae and eggs that may be present (Heather *et al*., 1987). Maximum residue level problems no longer permit this kind of practise in the modern age. In orchards that have no control of breeding populations of flies in the general area, cover sprays such as Trichlorfon, Fenthion, and Dimethoate provide control against invasions by gravid females from invasions from surrounding areas (Dominiak, 2007; Fletcher and Bateman, 1982). However, the effect of insecticides on non-target organisms, beneficial insects and residues in the harvested fruits are major limitations to their use (Rwomushana, 2008). Consumers worldwide are also increasingly becoming conscious of chemical residues in fruits. This is a challenge to most growers because most tropical fruits are susceptible to fruit flies and require high protection at maturity stage (Rwomushana, 2008). In many African countries where fruit quality is rarely examined at domestic markets, consumers are often exposed to excessive chemical residues in fruits to the detriment of their health (Rwomushana, 2008).

2.2.6.7 Biological Control

Biological control has become an integral part of Integrated Pest Management (IPM); the most recommended control strategy for reducing yield losses by fruit flies (White and Elson-Harris 1992; Allwood and Drew, 1997). Biological control of fruit flies usually involves the use of entomopathogens, parasitoids and predators.

2.2.6.7.1 The use of entomopathogenic fungi

The use of entomopathogenic fungi as an important component of the fruit fly IPM strategy targeting pupariating larvae, puparia and adults is a relatively new technique for this group of

insects, but it is receiving increasing attention worldwide (Ekesi *et al*., 2002, 2003; Ekesi *et al*., 2005; Ekesi *et al*., 2007; Lacey and Shapiro 2007; Quesada-Moraga *et al*., 2008; Sookar *et al*., 2008; Dimbi *et al*., 2009). To target pupariating larvae and puparia, the fungus is usually applied by hand along the drip line of the fruit tree canopy and gently raked into the soil (Ekesi *et al*., 2011). A significant reduction in adult emergence of different fruit fly species following soil inoculation with *Metarhizium anisopliae* (Metch) Sorok has been reported both in laboratory and field cage conditions (Ekesi *et al*., 2003, 2005, 2007; Ouna 2010). Ekesi *et al*., (2011) also reported significant reduction in the population of *B.* invadens in mango orchards when *M. anisopliae* and GF-120 spinosad bait spray were applied simultaneously. *Metarhizium anisopliae* has also been observed not to have any major adverse effect on non-target fruit fly parasitoids and was able to persist in the soil for more than one year (Ekesi *et al*., 2005).

2.2.6.7.2 Use of parasitoids

Fopius arisanus and *Diachasmimorpha longicaudata* are a few of the parasitoids used in classical biological control of fruit flies in several parts of the world (Wharton & Gilstrap, 1983; Messing, *et al.*, 1993; Ovruski, *et al.*, 2000) (Plates 2.9 and 2.10). *Fopius caudatus* (Szépligeti), *F. ceratitivourous* Wharton and *D. fullawayi* (Silvestri) have also been reported as potential indigenous parasitoids against fruit flies (Vayssières *et al.*, 2004). *Fopius arisanus* (Hymenopetera: Braconidae) is an egg-pupal parasitoid of tephritid fruit flies (Rousse *et al.*, 2005). It oviposits in eggs of tephritid fruit flies and emerges from the puparia of its host, killing the host in the process. *Fopius arisanus* was first introduced into Hawaii in the late 1940's from Malaysia (Van den Bosch and Haramoto, 1951) and subsequently became the dominant fruit fly parasitoid in Hawaii (Haramoto and Bess, 1970; Wong *et al.*, 1984), causing substantial reduction in fruit fly populations (Vargas *et al.*, 1993). Haramoto and Bess (1970) reported that

the mean number of fruit fly pupae (*B. dorsalis* and *C. capitata*) collected from coffee berries in Kona, Hawaii, decreased from 23.6 pupae per 100 fruits (8.7% parasitized) in 1949 to 5.2 pupae (66.8% parasitized) in 1969. Purchell *et al*., (1998) reported that *F. arisanus* accounted for 90% of all parasitoids recovered from *B. dorsalis* in the field in Hawaii. The interaction of *F. arisanus* with other components of IPM programmes has also been documented. In particular, it has been reported that it was not responsive to protein baits (Vargas *et al*., 2002). Hence, the application of bait sprays containing spinosad or Phloxine B for *C. capitata* population suppression has little harmful effect on the parasitoid (Vargas *et al.,* 2001), highlighting the need for IPM in fruit fly suppression. Despite its potential effectiveness, *F. arisanus* has been rarely used in other parts of the world as a biological control agent against tephritid pests (Rousse *et al*., 2005).

Fopius ceratitivorus is a newly discovered species (Wharton, 1999). Unlike other parasitoids previously used in Medfly biological control, *F. ceratitivorus* was originally collected from "Medfly" in its purported aboriginal home of East Africa (Lopez *et al.,* 2003). Shipments of *Ceratitis* spp. puparia from Kenya to a newly constructed quarantine facility in Guatemala yielded both *F. ceratitivorus* and its congener, *F. caudatus* (Szepligeti) (Lopez *et al.,* 2003). Quarantine tests showed that *F. ceratitivorus* has the potential to contribute to biological control of the Mediterranean fruit fly, *C. capitata* (Lopez *et al.,* 2003). *Fopius caudatus* was recorded as a parasitoid of *C. capitata* (Wharton, 1999), but little is known about the biology of this species.

Species of the genus *Diachasmimorpha* have been introduced for the classical biological control of fruit fly pests in several parts of the world (Wharton, 1989). In Hawaii, species such as *D. longicaudata* (Ashmead) and *D. tyroni* (Cameron) successfully established during the early 1950's (Bess *et al.,* 1961; Clausen *et al.,* 1965; Wong *et al.,* 1984). *Diachasmimorpha*

longicaudata is a solitary fruit fly endoparasitoid from the Indo-Australian region, where it parasitizes at least 14 species of fruit flies in the genus *Bactrocera* (Wharton and Gilstrap, 1983). It oviposits in the larvae of its hosts and emerges from the puparia, killing the host in the process. Following introductions into different countries, *D. longicaudata* has been reported parasitizing *Anastrepha* spp., *C. capitata*, and *B. dorsalis* (Wharton *et al*., 1981; Wong *et al*., 1984; Aluja *et al*., 1990). Augmentative biological control, the mass release of parasitoids at appropriate times and places, has been proposed as a new approach for fruit fly suppression (Knipling, 1992). *Diachasmimorpha longicaudata* is considered a good candidate for this kind of control, since it is already established and methods for its mass production and release have been developed in different parts of the world (Sivinski, 1996; Burns *et al*., 1996). Sivinski *et al.,* (1996) reported a substantial reduction in mean trap capture of *Anastrepha suspensa* (Loew) in Florida, in areas where *D. longicaudata* was released through augmentative biological control *Diachasmimorpha fullawayi*, a West African Braconid egg-larval parasitoid from *Ceratitis* spp, is effective on Medfly in Hawaii (Clausen, 1956; Wharton and Gilstrap, 1983). It has also been identified as potential indigenous parasitoid against fruit flies in Africa (Vayssières *et al*., 2004).

Plate 2.9 *F. arisanus* females ovipositing in *B. invadens* eggs in a mango fruit

Plate 2.10 *D. longicaudata* females ovipositing in *B. invadens* larvae in artificial diet medium

2.2.6.7.3 Use of predatory ants

Only two species of *Oecophylla* (Hymenoptera: Formicidae) exist, namely the Asian weaver ant *Oecophylla smaragdina* (Fabricius) and the African weaver ant, *Oecophylla longinoda* (Latreille) (Van Mele *et al*., 2009). The dominant arboreal *Oecophylla* colonizes a wide range of trees and effectively controls tree pests (Van Mele and Cuc, 2000; Van Mele, 2008). The highly organized predatory behavior of weaver ants, their extensive foraging throughout the area occupied by a colony, and their potential to expand into new areas explain their success in killing or deterring many potential pests including several leaf-eating hemipteran, lepidopteran and coleopteran pests of citrus, mango, litchi, coconut and cashew (Haung and Yang, 1987, Way and Khoo 1992; Khoo *et al*., 1993; Peng *et al*., 1995). The ant workers hunt diurnally in groups; and preys that are detected visually from a relatively long distance are seized by an appendage and immobilized. This behaviour permits the ants to capture small and large insects and even other animals (Hölldobler and Wilson, 1990).

Research has shown that the Asian *Oecophylla* species can deter insect herbivores or plant eaters through info-chemical action (Vayssieres *et al.,* 2009). In Africa, *O. longinoda* has being used as a biocontrol agent against agricultural pests such as mirids on cocoa and cashew (Van Wijngaarden *et al*., 2007; Dwomoh *et al*., 2008). Due to their pronounced territoriality, permanent surveillance (all year round, day and night), and very efficient recruitment, *O. longinoda* responds quickly to any increase in prey numbers (Vayssieres *et al.,* 2009). Apart from direct control mechanisms, including the predation on or deterrence of insect pests during direct encounters, indirect mechanisms have recently been discovered involving the detection of the territories of enemy ants (Vayssieres *et al.,* 2009). Results from a study carried out by Vayssieres *et al.,* (2009) showed that the presence of weaver ants in mango trees reduced the

damage caused by the fruit fly family Tephritidae through predation of adult fruit flies (rare), predation of third-stage larvae (quite frequent) (Plate 2.11), and especially, the effect of pheromones left by the ants on the fruit so that fruit flies are repelled and are discouraged from egg-laying. The presence of weaver ants resulted in significant reduction in fruit damage. Since fruit fly population dynamics and fitness are influenced greatly by sexual, feeding and oviposition behaviours, which mainly take place in tree canopies, it is to be expected that arboreal ant species will directly and indirectly influence these behaviours (Van Mele *et al.*, 2009). The use of *O. longinoda* colonies is suitable for perennial cropping systems in SSA because they are efficient against fruit fly pests, one of the widespread threats, constantly present in tropical agricultural systems (Van Mele *et. al.*, 2009). To this end, smallholder mango farmers in West Africa are being encouraged to undertake activities that increase the population build up of *O. longinoda* in their orchards.

Pupation of economically important fruit flies takes place in the soil, after final-instar larvae have left the infested fruit. Wong *et al.*, (1984) reported that Argentine ground-nesting ants *Linepithema humile* (Hymenoptera: Formicidae) cause a mortality rate of approximately 39% of puparia and teneral adult Mediterranean fruit flies (i.e. newly-emerged adults). They concluded that ant predation can be considerable but inadequate to regulate fruit fly populations.

Plate 2.11 *Oecophylla* ants preying on fruit fly larvae

2.2.6.8 Sterile Insect Technique (SIT)

The sterile insect technique (SIT) is arguably the most ecologically-compatible means of pest control in existence (Dyck *et al*., 2005). It is not a stand-alone technology, but should be integrated with other pest management techniques in an area-wide programme (Dyck *et al*., 2005; Agricultural Research Council, 2008). The technique was invented by Knipling and colleagues to eradicate the screwworm, *Cochliomyia hominivorax* (Coquerel) in the United States (Knipling, 1959). It is a method that has been used successfully for area wide population suppression and eradication of fruit flies (Gilmore, 1989). The SIT is a genetic method of control. Sterility is induced in the sperm without affecting sperm function and capacity to compete with other sperm in a lethality described as the dominant lethal mutations (Robinson, 2002a). The primary lesion that is responsible for dominant lethal mutation is a break in

chromosome that is induced by irradiation. A break in chromosome in mature sperm remains until after the sperm has entered an egg. Following fusion, nuclear division begins and the break in chromosome drastically affects viability of the embryo as development proceeds (Robinson 2002a).

The result of a sexual encounter of sterile with wild insects is that no progeny are produced, consequently reducing the population to extinction (Dyck *et al*., 2005). The method is usually effective after the fly population has been greatly reduced by other means, because it only takes one fertile male fly to inseminate a number of female flies (Gilmore, 1989). Early examples of SIT application against fruit flies was with the melon fly on Mariana Islands (Steiner *et al.*, 1965). Current management strategies to minimize the threat of invasion of *C. capitata* include release of sterile male flies as part of preventative SIT programs which are operational in California, Florida, and Texas (Hendrichs, 1996). In the western cape of South Africa, the release of sterile 'Med flies' now protects some 18,000 ha of commercial fruit in three production areas (Barnes and Venter, 2006).

The SIT is also used to control wild Mediterranean fruit fly introductions in California and Florida in the U.S. (Barry *et al.*, 2003). A successful programme to control Mediterranean fruit fly (Medfly) involving Israel, Jordan and the Palestinian Authority resulted in a 50-fold increase in export revenue from horticultural crops (FAO, 2005). A 1997 study showed that total annual losses from 'Medfly' damage to fruit and vegetables in the region amounted to nearly US $300 million (FAO, 2005). By integrating SIT with other suppression methods, reduction in fruit infestation and insecticide use has been significant. For example, Israeli exports of Medfly-free produce have increased from less than US$1 million in 1998 to US$50 million in 2005 (FAO,

2005). The use of SIT is a complicated procedure requiring sophisticated skills, high degree of technical expertise and funding (Dyck *et al*., 2005). The process of implementing SIT requires seven components; suppression of density, mass rearing, sterilization, shipment, release, evaluation and quality control.

2.3 Response of parasitoids to synomones from host fruits

Efficient host searching by hymenopterous parasitoids to control plant pests is an important component of the biocontrol augmentation paradigm (Lewis and Martin, 1990) which involves environmental clues, physiological states of both the parasitoid and the host, and genetic plasticity in host recognition and behavior (Jang *et al*., 2000). Opiine parasitoids of Tephritidae respond to synomones from host fruits (Rousse *et al*., 2007). Ripe, infested, and/or decomposing fruit is attractive to female braconids and olfactory cues are an important component of that attraction (Jang *et al*., 2000).

Field studies suggest *F. arisanus* females respond positively to synomones of many botanical families (Rousse *et al*., 2007). Similarly, *D. longicaudata* was reported to be attracted to odours of various plant species (Greany *et al.* 1977; Messing and Jang, 1992). Messing and Jang (1992) conducted field cage experiments in which the interactive effect of fruit color and odor on *D. longicaudata* captures were also reported. Various studies have also shown a greater attractiveness for opiine parasitoids of plants infested by their hosts compared with uninfested ones (Rousse *et al*., 2007). For example, Cheng *et al.* (1992) demonstrated that *D. longicaudata* in a wind tunnel would orient more strongly to guava fruit infested by fruit fly larvae, *Bactrocera dorsalis* (Hendel), than uninfested guava. Eben *et al*., (2000) also observed that *D. longicaudata*

prefers fruits infested by its hosts and Messing *et al*., (1996) reported the same phenomenon for *P. fletcheri*.

2.4 Effect of climate on the success of classical biological control

In ecosystems, the tritrophic interactions between plants, herbivorous insects, and their natural enemies (predators, parasitoids, and pathogens) result from a long coevolutionary process specific to a particular environment and relatively stable climatic conditions (Hance *et al*., 2007). Therefore, changes in climate affects tritrophic interactions in diverse ways.

Climatic adaptation has been listed among the criteria for selecting potential biocontrol agents (van Lenteren, 1986). The failure of exotic parasitoids to establish during biological control programmes may be attributed to many factors, but among the most important is the lack of adaptation of a species to new climatic conditions (DeBach, 1958; 1965). The effects of climate are sometimes clearly seen when a particular natural enemy species simply does not establish beyond clearly identifiable climatic zones (Samways *et al*., 1999). Climate may also reduce the potential for population growth of a natural enemy within the area of its geographical distribution. This idea has been tested especially with regard to the impacts of environmental factors (Samways *et al*., 1999). Parasitoids depend on a series of adaptations to the ecology and physiology of their hosts and host plants for survival and are thus likely highly susceptible to changes in environmental conditions (Hance *et al*., 2007). Thus, the successful establishment of natural enemies in a new geographic area depends on several factors, including their adaptability to the new environment. Of the several climatic factors influencing the success of biological control programmes, temperature is the most important.

2.4.1 Temperature

Temperature is the single most important environmental factor influencing insect behaviour, distribution, development, survival, and reproduction (Petzoldt and Seaman, 2007). Some researchers even believe that the effect of temperature on insects largely overwhelms the effects of other environmental factors (Bale *et al*., 2002). Temperature is a physical factor as well as a stimulus for insects. While insects normally develop faster at higher temperatures (Wagner *et al*., 1984), optima, maxima, and minima differ among species and an understanding of these traits has important consequences for establishing natural enemies in new environments (Mohamed *et al*., 2006).

Temperature is the most important abiotic factor in the acclimatization of introduced natural enemies (Loni 1997). Generally, low temperature has been used as a criterion to evaluate the safety of the introduction of natural enemies into new geographic areas (Hayes *et al*., 2005). Temperature influences the establishment of parasitoids in several parts of the world. The Australian distribution of the opiine fruit fly parasitoid, *F. arisanus*, is limited by low winter temperature (Snowball and Lukins 1964). Another opiine, *Doryctobracon crawfordi* (Viereck) (Hymenoptera: Braconidae), has been shown to be more sensitive to high temperatures than its host, *Anastrepha ludens* (Loew) (Diptera: Tephritidae) (Darby 1933). Additionally, releases of both wild and mass-reared *Psyttalia concolor* (Sze´pligeti) in some parts of Italy have failed (Raspi & Loni 1994), possibly due to poor temperature adaptability. Temperature may therefore affect the establishment and success of introduced parasitoids by influencing parasitism rates, developmental period and adult longevity.

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS

3.1 Rearing of *B. invadens*

The initial cohort of *B. invadens* flies originated from a natural population of infested mango fruits collected from a local market in Nairobi, Kenya in 2003. The larvae were subsequently reared on a solid carrot-based artificial diet in the laboratory according to the procedure described by Ekesi *et al*., (2007). In 2008, the larvae were successfully transferred from the carrot-based solid diet onto the fruit fly liquid diet developed by Chang *et al.* (2004; 2006) (Plate 3.1). The colony has been maintained for over 100 generations at the Animal Rearing and Containment Unit (ARCU) at *icipe,* Nairobi-Kenya. The colony was rejuvenated every 6-12 months by the incorporation of wild populations. Flies were provided with water on pumice granules and fed on a diet containing enzymatic yeast hydrolysate powder and sugar at the ratio of 3:1 (Mohamed *et al.*, 2008). Rearing conditions were maintained at 28 ± 1°C, 50 ± 8% RH and photoperiod of L12: D12.

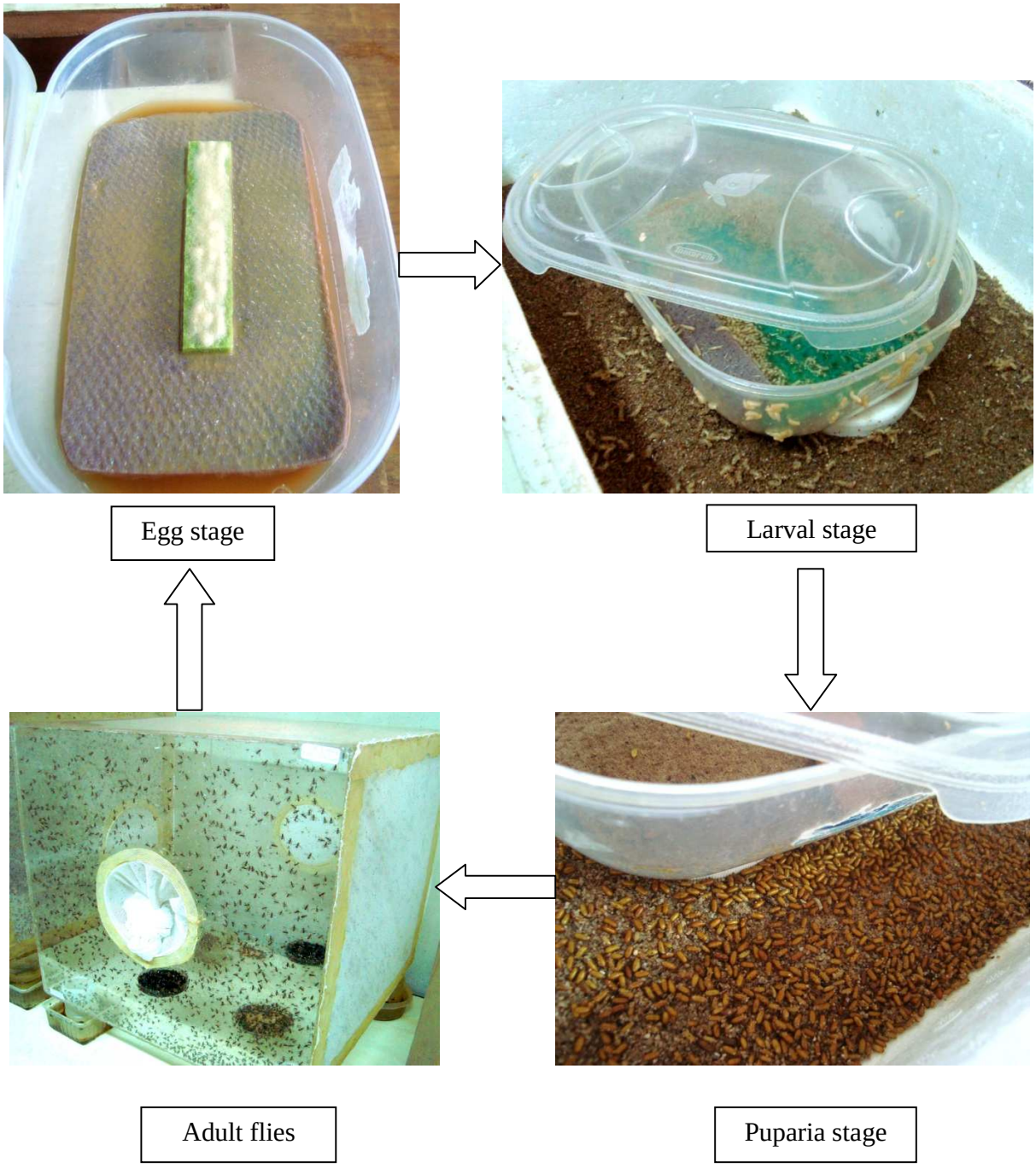

Plate 3.1 Rearing of *B. invadens* using artificial liquid diet

3.2 Rearing of parasitoids

The initial cohort of *D. longicaudata* was obtained from the University of Hawaii at Manoa in Honolulu, Hawaii (USA), where they were reared on *B. dorsalis*. The wasps were kept in quarantine at *icipe* at room temperature (25–26 °C) and were reared on the larvae of *B. invadens* using a procedure described by Mohamed *et al.,* (2008). Early third instar larvae of *B. invadens* were placed in an oviposition unit consisting of a modified Petri dish (diameter 9 cm, depth 0.5 cm), with a tightly fitting organza lid. The oviposition units were offered to wasps kept in a rearing cage (14 × 14 × 20 cm) for 24 h (Plate 3.2). The number of wasps in the cage ranged between 30 and 50. Thereafter, host larvae were transferred to Petri dishes (8.6 cm diameter) and provided with fresh carrot diet as earlier described. The Petri dishes were then placed in a plastic bowl (10.3 cm diameter, 6 cm depth) with a layer of sand at the bottom to serve as a substrate for pupation. The sand was kept moist by gently spraying water on it using a small hand sprayer for a few seconds to prevent pupal desiccation. When the larvae attained maturation they popped into the sand to pupariate, and those which failed to jump into the sand were assisted using a pair of soft forceps. A hole of 10 cm diameter was cut in the lid of the bowl and covered with a very fine net. On the seventh day after pupation, the puparia were collected from the sand and placed in Petri dishes (8.6 cm diameter) for emergence of adult flies and parasitoids. The emerging parasitoids were added to the parasitoid colony (Plate 3.3). Parasitoids were maintained at a photoperiod of 12: 12 h (L: D) and were provided with fine drops of pure honey and water in wet cotton wool.

The initial cohort of *F. arisanus* was also obtained from the University of Hawaii at Manoa, Honolulu, Hawaii, where they have been maintained on *B. dorsalis* for over 200 generations. The wasps were kept under quarantine at the International Centre of Insect Physiology and Ecology

(*icipe*), and were reared on 5-20 hour old *B. invadens* eggs using a procedure described in Mohamed *et al*, (2010). Mango domes (mango fruit skin that has the seed and pulp scooped out) were offered to *B. invadens* for egg laying in the evening of the day preceding their exposure to the parasitoids. Pieces of the mango dome (5 × 4 cm) were placed on double layers of sponge pieces (Spontex make, Nairobi, Kenya) of similar dimensions to that of the mango peel pieces and 3 mm height each, placed in oviposition units (9×0.5 cm, diameter × depth), and covered with a tight-fitting organza lid. The oviposition units were then exposed for 8 h to *F. arisanus* wasps held in a cubical cage (35 cm3). Thereafter, the oviposition units were removed and the mango peel pieces with the eggs were placed on larval diet in a plastic bowl (10.3 × 6 cm, diameter × depth). The diet was kept moist and replenished as necessary. When the larvae attained their full size, the diet was washed out through a sieve. The mature larvae were then placed back in the plastic bowl with a layer of sand on the bottom to serve as the pupation medium. A hole of 10 cm diameter was cut in the lid of the bowl and covered with a fine net for ventilation. The sand was kept moist to prevent pupal desiccation until emergence of adult flies and parasitoids. The parasitoids and flies that emerged were released into a rearing cage (35 cm^3) and the flies were later killed in 70% ethanol. The parasitoids were maintained at room temperature of 25-26 °C and photoperiod of 12 h L: 12 h D, and provided with fine drops of pure honey and water in wet cotton wool.

Plate 3.2 *B. invadens* eggs exposed to *F. arisanus* females using oviposition units

Plate 3.3 *F.arisanus* adults emerging from puparia placed in a Perspex cage

3.3 Collection of *B. invadens* eggs

Eggs of *B. invadens* used for the various experiments were collected using a similar procedure as described in Rwomushana *et al*. (2008). Mature *B. invadens* females were offered a ripe mango dome. The domes were placed over a 9 cm diameter Petri dish lined with moistened filter paper. Domes were maintained in 30 x 30 x 30 cm Perspex cages at 28 ± 1°C, 50 ± 8% RH. Several perforations were made on the outside of the dome using an entomological pin (38 mm long, 0.3 mm diameter) to facilitate oviposition. The domes were exposed to *B. invadens* adults at about 16:00 GMT on the day preceding the bioassay (Plate 3.5). The eggs (less than 18 hrs old) were collected and used for experiments immediately the next morning. For the experiments on the effect of host fruit substrates on parasitoid performance, adult *B. invadens* flies were offered perforated plastic containers lined with paper towel soaked in fruit juice. The plastic containers were inverted and placed in Perspex cages containing adult *B. invadens* flies (Plate 3.4). Subsequent procedures were the same as described above. Plastic bottles were used instead of mango domes since mango was one of the host fruit substrates tested.

Plate 3.4 *B. invadens* females ovipositing in plastic bottles lined with moist paper towel

Plate 3.5 *B. invadens* females ovipositing in a perforated mango dome

CHAPTER FOUR

4.0 INVENTORY OF HYMENOPTERAN PARASITOIDS ASSOCIATED WITH TEPHRITID INFESTING FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN THE COAST AND EASTERN PROVINCE OF KENYA

4.1 INTRODUCTION

Tephritid fruit flies pose an enormous threat to fruit and vegetable crops throughout the world (Purcell *et al*., 1998, White and Elson-Harris 1992), causing heavy pre and post-harvest losses that negatively impact on the economy and expansion of both domestic and international trade of fruits (Clausen, 1978). The problem is aggravated in the tropics by the prevailing warm weather conditions, which is conducive for overlapping fruiting patterns, resulting in several generations of economically important fruit flies and the potential for year round infestation (Rwomushana *et al*., 2008). In Africa, the damage caused by fruit flies is felt at all levels of the production chain. The situation is particularly worse for smallholder farmers who produce the bulk of fruit and vegetable crops and cannot afford expensive control measures (Mohamed *et al.*, 2008).

Before 2003, indigenous fruit flies especially *C. cosyra* destroyed an average of 40% (~ 1.9Mt) of total mangoes produced yearly in Africa (Lux *et al*., 2003). Although *C. cosyra* was the dominant fruit fly pest of mango, *C. fasciventris*, *C. rosa*, *C. anonae* and *C. capitata* also contributed to the damage. The arrival of the invasive fruit fly species, *B. invadens* on the African continent in 2003 has further aggravated the fruit fly problem in the sub-region (Drew *et al*., 2005). *Bactrocera invadens*, (initially thought to be *Bactrocera dorsalis* (Hendel) although a member of the dorsalis complex of fruit flies), was first recorded in Africa from the Kenyan coast in 2003 (Lux *et al.,* 2003). This new invasive species has spread rapidly across the sub-

Saharan region and has been reported from over 28 other countries (Drew *et al*., 2005; Ekesi *et al*., 2006; Umeh *et al*., 2004). It is multivoltine and highly fecund (Ekesi *et al*., 2006) and may be partly responsible for the displacement of indigenous mango fruit fly species (Manrakhan and Lux, 2006, Ekesi *et al*., 2009). Being an invasive species, *B. invadens* lends itself to classical biological control and this resulted in the importation of two co-evolved parasitoids, *F. arisanus* and *D. longicaudata* from Hawaii by the ICIPE-led African Fruit Fly Programme for evaluation and final release against the pest in Africa.).

Although several explorations and surveys have been carried out in East Africa on the diversity of tephritid parasitoids (Bianchi and Krauss, 1937; Greathead, 1972; Wharton *et al*., 2000), there is still very little published information on the diversity of fruit fly parasitoids in the region. Also, parasitoids from Africa including several species of *Psyttalia* and *Fopius*, have and continue to attract much interest for use as biological control agents in other parts of the world (Silvestri 1913; Messing 1999; Wharton *et al*., 2000; Lopez *et al*., 2003; Sime *et al*., 2006). Therefore, the need for further surveys and information on the diversity of tephritid parasitoids in the region cannot be overemphasized.

Many ecologists view the intentional introductions of alien species into complex biological communities as a threat to their structure and dynamics (Sime *et al*., 2008). Major concerns include the irreversibility of introductions, potential for host switching, dispersal into nonagricultural habitats, lack of research on both efficacy and ecological impacts, possibility of evolutionary adaptation to new hosts, and difficulty of predicting interaction outcomes in complex systems (Howarth, 1983; Howarth, 1991; Louda *et al*., 1997, Louda and Stiling, 2004;

Lynch and Thomas, 2000). Although *F. arisanus* and *D. longicaudata* have been shown to be effective biocontrol agents against *B. invadens* (Mohamed *et al.*, 2008), research must also address the possibility of indirect effects, including interaction between the introduced parasitoids and indigenous natural enemies. The first step to filling this knowledge gap is to establish the indigenous parasitoid guild, especially in mango growing areas in Kenya, where the introduced parasitoids would be first released.

The objective of this preliminary study therefore, was to conduct an inventory of Hymenopteran parasitoids in the major mango growing provinces of Kenya to provide baseline data for future biological control efforts. These include the introduction of *F. arisanus* and *D. longicaudata*, redistribution and augmentative release of native parasitoids into favorable environment and the possibility of exporting fruit fly parasitoids to other countries affected by the fruit fly species attacked by the native parasitoids.

4.2 MATERIALS AND METHODS

4.2.1 Sampling Sites

Host fruit survey was carried out from January, 2009 to May, 2010 in the Kwale district of the Coast province and the Embu and Meru districts of the Eastern province. Eastern and Coast provinces are the two major mango growing areas in Kenya, accounting for 54 and 22% of total mango production in the country in 2003 (FAO, 2006).

The Coast Province comprises the Indian Ocean coastal strip with the capital city at Mombasa. It is inhabited by the Mijikenda and Swahili, among others. The province covers an area of 83,603 km². It has a warm and humid climate and Mombasa, the capital city, is approximately 17m above sea level. Average annual temperatures vary from a minimum of 22.4 °C to a maximum of 30.3 °C. Mango production in the province is dominated by local varieties, namely, Ngowe (70%), Boribo, Batawi and a few minor ones. The main exotic variety grown is Apple, which is mainly cultivated in Lamu, Malindi and Kilifi districts. There are two distinct harvesting seasons. In the Malindi and Kilifi districts of Coast Province, the two harvests yield an almost equal quantity. In the whole of Coast Province, every district reported some mango harvesting for at least seven months in a year (MOSPANS, 2011).

The Eastern Province of Kenya is one of the seven provinces of Kenya. Its northern boundary is with Ethiopia; the North Eastern Province and Coast Province lie to the east and south; and the remainder of Kenya's provinces, including Central Province, run along its western border. The provincial capital is Embu. The province is principally inhabited by the Meru and Kamba and several pastoralist communities. In terms of area, it is the second largest province (159,891 km²). The province has a semi-arid to arid climate. Both local and exotic varieties of mango are grown

in the Eastern Province. The local varieties are Ngowe, Dodo, Boribo and Batawi. The exotic varieties include Apple, Kent, Keit, Tommy Atkins, Van Dyke, Haden, Sensation, Sabre, Sabine, Pafin, Maya, Kenston and Gesine. The districts with higher percentage of improved mango varieties are Thika, Embu, Mbeere, Meru Central, Makueni, Machakos and Meru South, while Mwingi and Kitui have very small areas cultivated with improved varieties (FAO, 2006). In the two provinces, mango production is primarily rain fed. Where water is available, new orchards under irrigation have been established for production of exotic varieties for export (MOSPANS, 2011).

In selection of sampling sites, priority was particularly given to locations within the provinces which had a large diversity of fruit species. Fruit availability and diversity were the main factors that influenced the selection of sites. As many locations that met the above criteria within the province were selected. At the Coast Province, sampling locations included forested areas on the fringes of the Indian Ocean and Shimba hills. In the Eastern Province (representing the highland region of the country), sampling locations were varied up to the fringes of Mount Kenya forest (Fig 4.1). At each location, approximate latitude, longitude and altitude were taken using a global positioning system device.

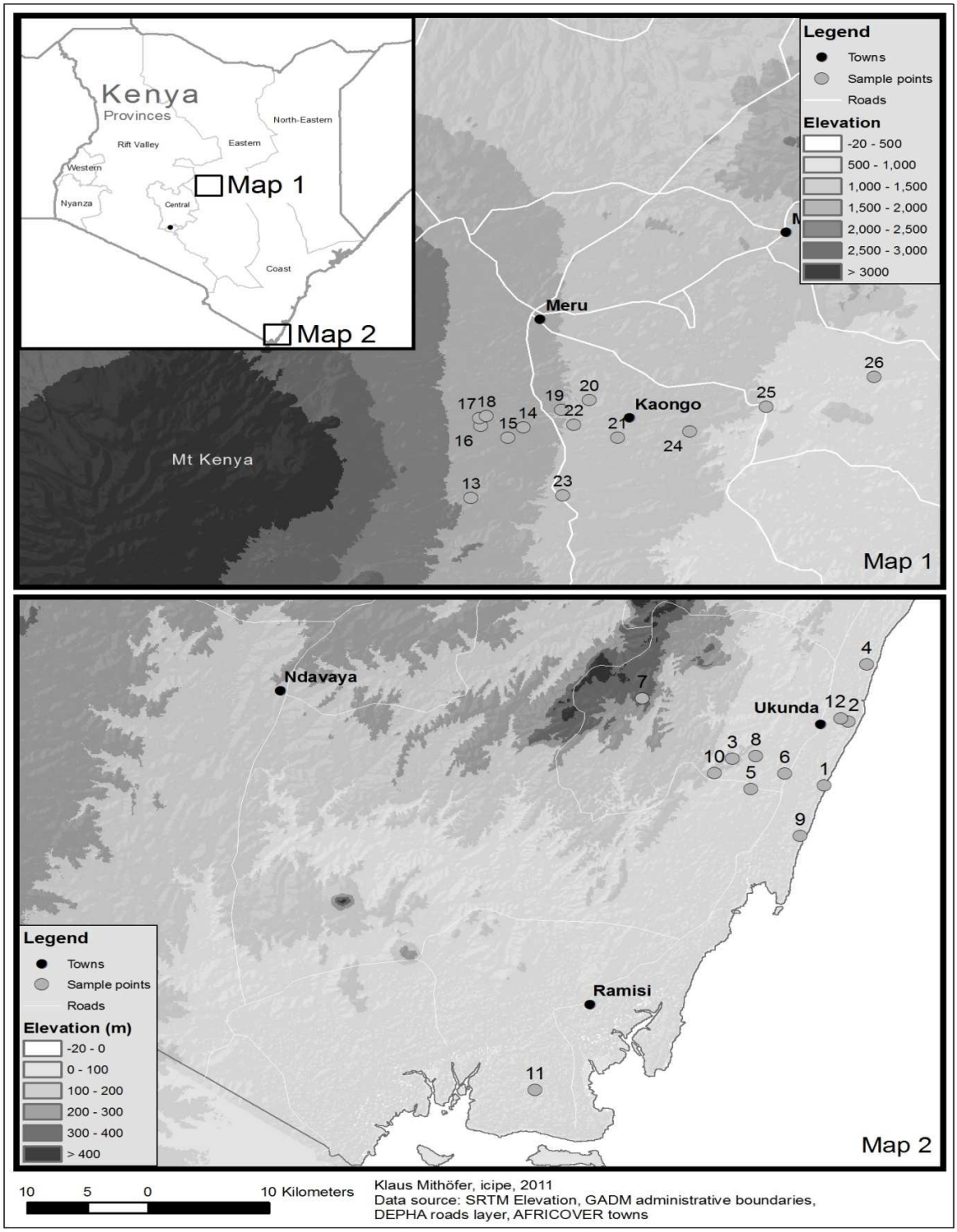

Fig 4.1 Map showing localities where fruits were sampled from in the Coast and Eastern Provinces of Kenya

4.2.2 Fruit collection, Handling and Processing

Fruits were collected, handled and processed using a methodology similar to that described by Rwomushana *et al*., (2008). Fruits were collected from backyard gardens, cultivated fields, woodlands, forested areas, roadside shrubs, and protected reserves. Some fruits not encountered at the sampling sites were purchased from roadside markets, and attempts were made to establish their places of origin. Fruit samples included ripe to overripe fruits, and those with visible symptoms of fruit fly damage both from the tree and from the ground as "windfalls." Although attempts were made to sample large quantities of each fruit species, it was not possible in some cases due to unavailability of fruits. However, efforts were made to collect a minimum of 10 fruits per plant species. Plate 4.1 shows some of the fruits sampled during the survey at the Coast province. Fruits collected from the different plant species were separately placed in perforated polyethylene bags in the field and transported to the nearest rearing facility. The rearing facilities were located in each ecozone where fruits were collected and included the International Centre of Insect Physiology and Ecology (*icipe*)-Muhaka field station for the Coast Province and the *icipe* Headquarters, in Nairobi for Eastern Province samples.

At the rearing facility, the fruits were counted, weighed, and secured in well-aerated rectangular plastic containers. Small fruits (<5 cm in diameter) were held together in 1.5-liter rectangular transparent plastic containers (20 × 12.5 × 8 cm) (Kenpoly, Nairobi, Kenya). Larger fruist (>5 cm in diameter) were held in groups of two or three in 3-liter rectangular plastic containers (20 × 12.5 × 15 cm). Fruits larger than10 cm diameter in were held in cylindrical plastic buckets (25 × 30 cm). The rim of the containers was covered with a fine netting material held in place by the perforated cover of the containers that was capable of retaining tephritid flies and associated parasitoids. The fruit containers were placed on larger ones having 40–60 mm of moistened

sterilized sand at the bottom. The sand served both as the pupation medium for the larvae that exited the fruit in addition to soaking up fruit juices. Fruits were held at ambient conditions for 4–6 weeks, depending on the fruit species. Rearing cages were checked daily, and puparia were picked from the sand with a pair of soft forceps, counted and placed in petri dishes with moistened filter paper. In some situations, pupation occurred inside the fruit, and in this case decaying fruits were dissected to completely recover all puparia. The petri dishes with puparia were held in ventilated, transparent Perspex cages (20 × 20 × 20 cm) until eclosion.

Emerging tephritids were provided with an artificial diet that consisted of a volumetric mixture of 1:3 enzymatic yeast hydrolysate and sugar, and water was provided in pumice granules. Flies were allowed to feed for 4 days until full adult development and body colorations were attained. They were then killed by placing them in a freezer and later preserved in 70% alcohol. Emerged parasitoids that emerged were kept alive by providing honey drops at the top of the Perspex cages and cotton wool soaked in water served as the water source. Fruit fly specimens and samples of parasitoids were sent to the *icipe* Biosystematics unit for identification and another lot of parasitoid specimens were shipped to the Biosystematics Unit of the Zoology Department, University of Ghana for identification by Dr. Maxwell K. Billah. The collected plant samples were identified using the keys of Kenya trees, shrubs, and lianas (Beentje, 1994). Photographs were also taken of each plant or fruit sampled to aid in plant identification. Plant nomenclature used conformed to the International Plant Names Index database (IPNI 2004).

Coccinia spp

Anona senegalensis

Solanum incanum

Chassalia curviflora

Plate 4.1 Fruits of some plant species sampled during the survey at the Coast Province.

4.3 Data handling

Percent parasitism was calculated as a/(a + b) x100, where a = number of recovered parasitoids and b = number of emerged adult flies in each sample (Steck *et al*., 1986). Infestation rate was calculated as the number of pupae per kg fruit (Vayssieres *et al*., 2010). Host/parasitoid associations were based on assumptions that parasitoids reared from a fruit sample were attacking only hosts that were also reared as adults from that sample (Vayssieres *et al*., 2010). We considered these associations only as an approximation, as many factors, such as the ability of parasitoids to select preferred hosts and encapsulation by unsuitable hosts, increase the level of uncertainty in this regard (Vayssieres *et al*., 2010). Discriptive statitistics was mainly used to show the main features of the data collected during the survey.

4.4 Results

A total of 27,095 fruits from 29 plant species and 17 plant families were sampled during the survey period. The numbers of sampled fruits per plant species during the survey are shown in Table 4.1. During the survey, fruit fly parasitoids belonging to four families of Hymenoptera were recovered: Braconidae [*Psytallia cosyrae*, *Psytallia c.f. concolor*, *P. perproxima* (Silvestri), *Diachasmimorpha fullawayi* (Silvestri) and other *Psytallia* spp (could only be identified up to genus level)], Eulophidae (*Tetrastichus giffardianus* Silvestri), Chalcididae (chalcid wasps) and Ichneumonidae (ichneumonids).

Table 4.1 Plant species and total number of fruits sampled in Coast and Eastern Province from January 2009 – May 2010.

PROVINCE	PLANT FAMILY	PLANT SPECIES	TOTAL NO. OF FRUITS SAMPLED
COAST	Oleaceae	*Jasminum fluminense*	530
	Phyllanthaceae	*Phyllanthus spp.*	4383
	Rubiaceae	*Polysphaeria parvifolia*	2761
	Thymeleaceae	*Synaptolepsis kirkii*	606
	Rutaceae	*Citrus recticulata*	316
	Anacardiaceae	*Scelocarya birrea*	375
	Rubiaceae	*Chassalia curviflora*	6727
	Anacardiaceae	*Mangifera indica*	168
	Colchicaceae	*Gloriosa superb*	7085
	Rutaceae	*Citrus sinensis*	55
	Combretaceae	*Terminalia cattapa*	194
	Anacardiaceae	*Sorindea madagascariensis*	1407
	Annonaceae	*Anona senegalensis*	117
	Solanaceae	*Solanum incanum*	576
	Anacardiaceae	*Lannea weltischii*	60
EASTERN	Solanaceae	*Solanum incanum*	57
	Solanaceae	*Solanum villosum*	199
	Lauraceae	*Persea Americana*	30
	Myrtaceae	*Psidium guajava*	50
	Rosaceae	*Robus occidentalis*	117
	Salicaceae	*Dovyalis caffra*	80
	Anacardiaceae	*Mangifera indica*	183

Table 4.1 cont'd

PROVINCE	PLANT FAMILY	PLANT SPECIES	TOTAL NO. OF FRUITS SAMPLED
	Cucurbitaceae	*Cucurbita maxima*	12
	Caricaceae	*Carica papaya*	25
	Combretaceae	*Terminalia mantaly*	68
	Cucurbitaceae	*Citrullus lanatus*	16
	Solanaceae	*Solanum lycopersicum*	79
	Cucurbitae	*Cucumis dispaceus*	73
	Rutaceae	*Citrus sinensis*	45

At the Coast Province, infestation rate varied from as low as 3.6 puparia/ kg of fruit (*Citrus reticulata*, to as high as 350 puparia/kg of fruit (*S. kirkii*). The majority of parasitoids recovered were braconids (84.7%). The most abundant of the Braconids were the *Psyttalia* spp (66.5 %), followed by *D. fullawayi* (0.8%) and the unidentified Braconids accounted for 13.1%. The family Eulophidae (*Tetrastichus giffardianus* Silvestri) contributed 3.0% of the total parasitoids recovered, 6.4 % belonged to the family Chalcidoidea (chalcid wasps), whilst the family Ichneumonidae (ichneumonids) accounted for 5.9%. These proportions are based on the total samples recovered.

The majority of parasitoids were recovered from fruits of non-cultivated plants (99.6%) *Chassalia curviflora*, Wall. accounted for 35.6%, *Phyllanthus spp* (26.7%), *Synaptolepis kirkii*, Oliv. (12.3%), *Gloriosa superba*, Linn. (11.5%), *Polysphaeria parvifolia*, Hiern. (9.3%), *Lannea welwitschii*, Hiern. and *Jasminum fluminense*, Vell. accounted for 2.1% each whilst *Terminalia cattapa*, Linn. accounted for 0.4% of total number of parasitoids recovered (Fig. 4.2).

Percent parasitism also varied across the fruit samples collected. The highest parasitism rate was recorded on *P. parviforlia* (33.3%) whilst the lowest was 2.0%, recorded on *Terminalia cattapa* (Table 4.2). Then number of parasitoids per kg of fruit also ranged from a highest of 40 recorded on *C. curviflora* to a lowest of 0.2 recorded on *T. cattapa*.

Six fruit fly species namely *T. nigerrinum, T. coffeae, C. capitata, C. cosyra, B. cucurbitae and B. invadens* were recovered from fruit samples at the Coast Province. *Bactrocera invadens* accounted for 55.8% of all fruit fly samples recovered followed by *C. cosyra* (17.1%), *T. coffae*

(14.7%), *C. capitata* (8.8%), *B. cucurbitae* (2.2%) and *T. nigerrinum* (1.3%) (Fig. 4.3). Number of fruit flies per kg of fruit was highest on *S. kirkii* (265.0) and lowest on *C. reticulata* (2.4) (Table 4.3).

At the Eastern Province, fruit infestation rate varied from a low of 1.4 puparia/ kg of fruit on *C. sinensis* (L.) Osbeck, to a high of 800.0 puparia/per kg of fruit on *R. occidentalis* (L.) (data not shown). All parasitoids recovered during the survey were *Psytallia* species with *C. arabica* (L.) contributing 95.4% of total parasitoids recovered followed by *M. indica* at 2.8%. *Cucurbita maxima* (Duchesne) and *Juglans cinerea* (L.) both yielded *Psyttalia phaeostigma* and accounted for 0.9% each of the total number of parasitoids recovered (Fig. 4.4). Parasitism rates also varied across the fruit samples collected. The highest parasitism rate was 47.9%, recorded from *Coffea arabica* whilst the lowest was 0.9%, recorded from *M. indica* (Table 4.2). The number of parasitoids per kg of fruit also ranged from a high of 22.7 (*C. arabica*) to a low of 0.5 (*Mangifera indica*) (Table 4.2).

The following seven fruit fly species in order of abundance were recovered from fruit samples at the Eastern Province; *Bactrocera invadens* (47.1%) of all fruit fly samples recovered, *C. cosyra* (24.1%), *C. capitata* (10.6%), *T. coffae* (8.1%), *Dacus spp* (6.6%), *C. rosa* (2.7%) and *C. robivora* (0.7%) (Fig. 4.5). Number of fruit flies per kg of fruit ranged from 0.2 in *C. sinensis* to 200.0 in *R. occidentalis* (Table 4.3).

Table 4.2 Host plants, parasitoid/kg of fruit and total parasitism for different parasitoid species recovered from the Coast and Eastern Province of Kenya

Province/ Locality	Plant Species	Parasitoids recovered	Parasitism (%)	No. of Parasitoids /kg of fruit
COAST				
Muhaka	*Jasminum fluminense*	*Psyttalia spp.*	11.6	3.8
	Phyllanthus spp.	*Psyttalia spp, other braconids*	12.4	6.3
	Polysphaeria parvifolia	*Psyttalia spp*	20.0	2.1
	Chassalia curviflora	*Psytallia spp, Ichneumonids*	13.1	40.0
	Gloriosa superba	*Psytallia spp*	17.2	0.7
Kibarani	*Polysphaeria parvifolia*	*Psytallia spp*	33.3	3.7
	Chassalia curviflora	*Psytallia peproxima, chalcid wasps*	16.8	8.5
	Synaptolepis kirkii	*Psytallia spp, other braconids*	26.3	20.0
	Gloriosa superba	*Psytallia concolor, other braconids*	14.9	3.5
	Phyllanthus spp.	*Psytallia spp, Ichneumonids*	28.8	11.5
Kigaleni	*Phyllanthus spp.*	*P. peproxima, Psytallia c.f. concolor*	25.0	4.0
	Gloriosa superba	*P. peproxima, Other braconids*	10.9	1.7
	Synaptolepis kirkii	*Psytallia spp*	15.9	9.2
	Chassalia curviflora	*Psytallia spp*	28.0	4.7
Kinondo	*Terminalia cattapa*	*D. fullawayi*	2.0	0.2
Mabokoni	*Chassalia curviflora*	*Psytallia spp*	17.9	3.1
Mkambani	*Chassalia curviflora*	*Psytallia spp*	17.5	2.1
Buga	*Lannea welwitschii*	*other braconids*	21.7	25.0
Shimba Hills	*Chassalia curviflora*	*Psytallia spp, Tetrastichus giffardianus*	20.8	2.6
EASTERN				
Kiamiriru	*Coffea arabica*	*Psytallia spp*	47.9	22.7
Gachanka	*Mangifera indica*	*Psytallia spp*	1.0	0.5
Kithoka	*Coffea arabica*	*Psytallia spp*	17.3	9
Muringo	*Cucurbita maxima*	*Psytallia phaeostigma*	11.1	1.3
Giaki	*Juglans cinerea*	*Psytallia phaeostigma*	2.9	0.6

Table 4.3 Host plants, infestation rate and number of flies/kg of fruit and fruit fly species recovered from fruits sampled in Coast and Eastern Province

Province / Locality	Plant species	Pupae/ Kg of fruit	Fruit flies Recovered	No. of flies /kg of fruit
COAST				
Muhaka	*Jasminum fluminense*	42.3	*T. coffeae, C. cosyra*	29.2
	Phyllanthus spp	55.5	*C. cosyra, T. coffeae*	44.3
	Polysphaeria parvifolia	14.6	*T. coffeae, C. cosyra*	8.3
	Chassalia curviflora	106.0	*T. coffeae, C. cosyra*	36.0
	Gloriosa superba	8.4	*C. capitata, T. coffae*	3.5
	Anona cherimola	25.2	*B. invadens*	15.4
	Sclerocarya birrea	76.9	*B. Invadens, C. Cosyra*	47.4
Kibarani	*Phyllanthus spp*	55.4	*C. cosyra*	36.9
	Gloriosa superba	32.5	*C. capitata, T. coffeae*	20.0
	Polysphaeria parvifolia	13.7	*T. coffeae, C. cosyra*	7.4
	Chassalia curviflora	55.1	*T. coffeae, C. capitata*	42.0
	Synaptolepis kirkii	100.0	*C. cosyra, C. capitata*	56.0
	Mangifera indica	21.9	*B. invadens*	17.3
Kigaleni	*Phyllanthus spp*	19.6	*T. coffeae, C. cosyra*	12.1
	Gloriosa superba	22.8	*T. nigerrimum, T. coffae*	13.8
	Synaptolepis kirkii	70.8	*C. cosyra, T. coffae*	48.3
	Chassalia curviflora	28.7	*T. coffee, C. cosyra*	12.0
Buga	*Mangifera indica*	18.1	*B. invadens*	14.3
	Coccinia spp	21.3	*B. curcubitae, B. invadens*	14.6
Milu farm	*Citrus sinensis*	21.2	*B. invadens, C. capitata*	9.1
	Citrus reticulata	7.7	*B. invadens, C. capitata*	5.2
	Citrus sinensis	29.7	*B. invadens, C. capitata*	20.3
	Mangifera indica	32.7	*B. invadens*	23.2
	Mangifera indica	29.2	*B. invadens*	25.3
	Citrus reticulata	3.6	*B. invadens, C. capitata*	2.4
Kinondo	*Coccinia spp*	48.9	*B. curcubitae, B. invadens*	33.3
	Synaptolepis kirkii	350.0	*T. coffee, C. cosyra*	265.0
	Terminalia cattapa	14.4	*B. invadens*	8.1
Maweni	*Sclerocarya birrea*	77.3	*B. invadens, C. Cosyra*	70.9
Mkambani	*Chassalia curviflora*	16.8	*C. cosyra*	9.7
Mkambani	*Sclerocarya birrea*	58.4	*B. invadens, C. Cosyra*	44.2

Table 4.3 cont'd

Province / Locality	Plant species	Pupae/ Kg of fruit	Fruit flies Recovered	No. of flies /kg of fruit
Mabokoni	*Annona senegalensis*	11.4	*B.invadens*	4.5
Mabokoni	*Chassalia curviflora*	26.3	*C. cosyra*	14.4
Shimba Hills	*Chassalia curviflora*	16.2	*T. coffee, C. capitata*	10.0
Diani	*Coccinia spp*	33.3	*B. cucurbitae, B. invadens*	25.6
Diani Forest	*Sorindea madagascariensis*	8.7	*B. invadens*	6.5
EASTERN				
Meru forest	*Robus occidentalis*	800.0	*C. rubivora*	200.0
Mpakone	*Dovyalis caffra*	70.5	*C. cosyra, C. capitata, C. rosa*	45.7
Kiamiriru	*Coffea Arabica*	171.0	*T. coffee*	24.7
Gachanka	*Mangifera indica*	78.8	*C. cosyra, C. rosa, B. invadens*	54.1
Muringo	*Mangifera indica*	1.9	*C. cosyra, B. invadens*	1.3
Muringo	*Mangifera indica*	110.9	*C. cosyra, B. invadens*	59.4
Muringo	*Juglans cinerea*	446.7	*Dacus spp.*	66.7
Muringo	*Cucurbita maxima*	54.7	*Dacus spp.*	10.0
Miriga	*Mangifera indica*	50.0	*C. cosyra, B. invadens*	21.3
Kithoka	*Citrullus lanatus*	67.9	*Dacus spp.*	7.9
Kithoka	*Mangifera indica*	64.0	*C. cosyra, C. rosa, B. invadens*	48.5
Kithoka	*Coffea Arabica*	121.3	*C. capitata, T. coffae*	43.0
Giaki	*Terminalia mantaly*	660.0	*C. Capitata*	140.0
Mbuuta	*Cucumis dispaceas*	9.3	*Dacus spp.*	6.2
Mbuuta	*Citrus sinensis*	1.4	*B. invadens*	0.2
Mbuuta	*Mangifera indica*	49.8	*C. cosyra, B. invadens*	43.5

Table 4.4 Fruit flies and associated parasitoid species recovered from plant species sampled at the Coast and Eastern Provinces

PROVINCE	LOCALITY	PLANT SPECIES	FRUIT FLIES	PARASITOIDS
COAST	Muhaka	*Jasminum fluminense*	*T. coffeae, C. cosyra*	*Psytallia spp*
		Phyllanthus spp	*C. cosyra, T. coffeae*	*Psytallia spp, other Braconids*
		Polysphaeria parvifolia	*T. coffeae, C. cosyra*	*Psytallia spp*
		Gloriosa superba	*C. cosyra, C. capitata*	*Psytallia spp*
		Chassalia curviflora	*T. coffeae, C. cosyra*	*Psytallia spp, Ichneumonids*
	Kibarani	*Polysphaeria parvifolia*	*T. coffeae, C. cosyra*	*Psytallia spp*
		Chassalia curviflora	*T. coffae, C. capitata*	*P. peproxima, Chalcid wasps*
		Synaptolepis kirkii	*C. cosyra, C. capitata*	*Psytallia spp,other Braconids*
		Gloriosa superba	*T. coffeae, C.capitata*	*Psytallia c.f. concolor, Braconids*
		Phyllanthus spp	*C. cosyra*	*Psytallia spp, Ichneumonids*
	Kigaleni	*Gloriosa superba*	*T. nigerrimum, T. coffeae,*	*P. peproxima,other Braconids*
		Synaptolepis kirkii	*T. coffeae, C. cosyra*	*Psytallia spp*
		Chassalia curviflora	*T. coffeae, C. cosyra*	*Psytallia spp*
		Phyllanthus spp	*T. coffeae, C. cosyra*	*P. peproxima, P. concolor,*
	Kinondo	*Synaptolepis kirkii*	*T. coffeae, C. cosyra*	*Psyttalia spp, other Braconids*
		Terminalia cattapa	*B. invadens*	*D. fullawayi*
	Mabokoni	*Chassalia curviflora*	*C. cosyra*	*Psytallia spp*
	Mkambani	*Chassalia curviflora*	*C. cosyra*	*Psytallia spp*
	Buga	*Lannea welwitschii*	*T. coffeae*	*Braconids*
	Shimba Hills	*Chassalia curviflora*	*T. coffeae,C. capitata*	*T. giffardianus, Psytallia spp.*

Table 4.4 ctd

PROVINCE	LOCALITY	PLANT SPECIES	FRUIT FLIES	PARASITOIDS
EASTERN	Kiamiriru	*Coffea arabica*	*T. coffee*	*Psytallia spp*
	Gachanka	*Mangifera indica*	*C. cosyra, C. rosa, B. invadens*	*Psytallia spp*
	Muringo	*Cucurbita maxima*	*Dacus spp*	*Psytallia phaeostigma*
	Chege	*Coffeae arabica*	*T. coffee, C. capitata*	*Psytallia spp*
	Giaki	*Juglans cinerea*	*Dacus spp*	*Psytallia phaeostigma*

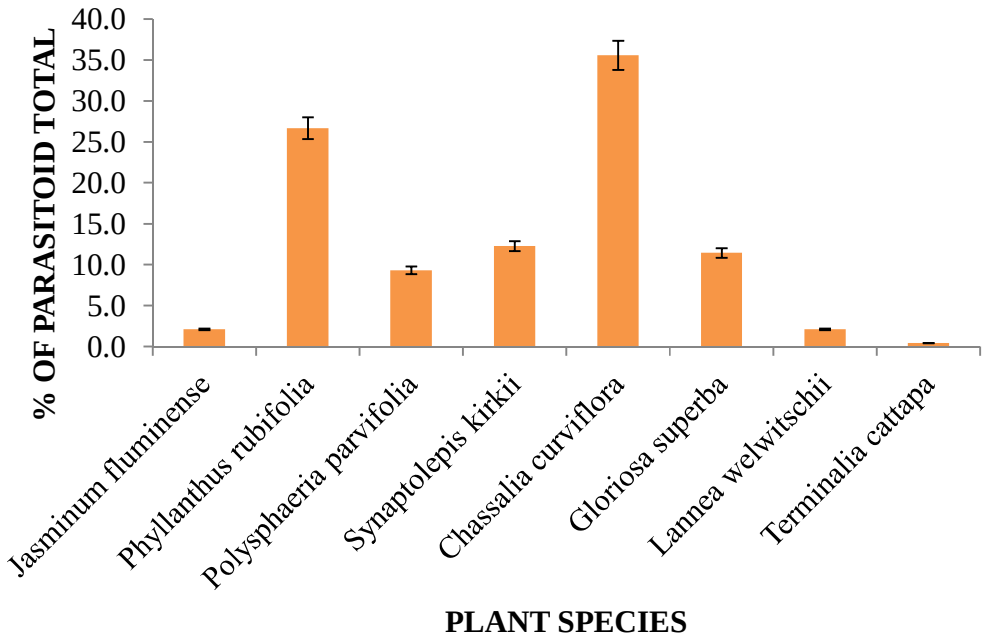

Fig. 4.2 Percentage contribution of different plant species to total number of parasitoids recovered at the Coast Province during the survey.

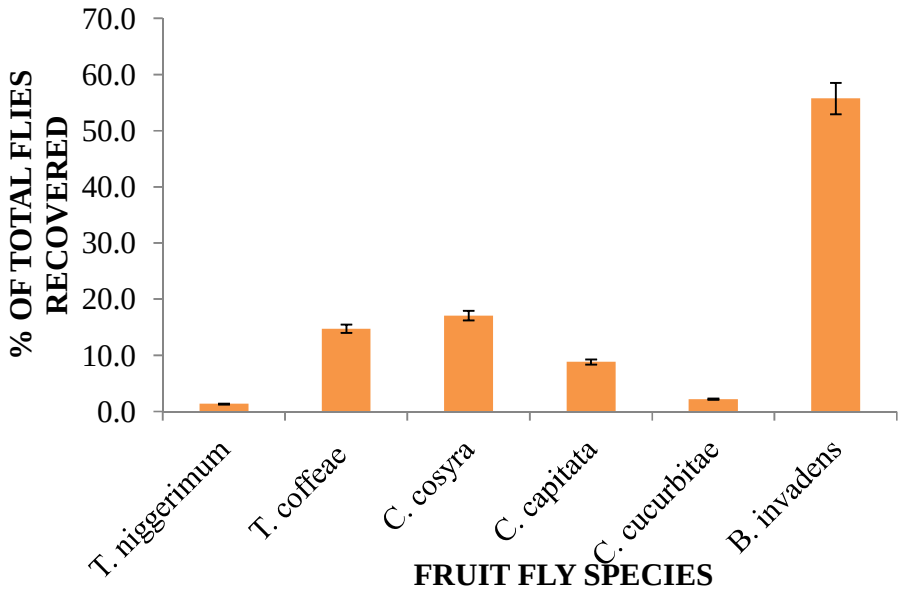

Fig. 4.3 Percentage of different fruit fly species recovered at the Coast Province during the survey

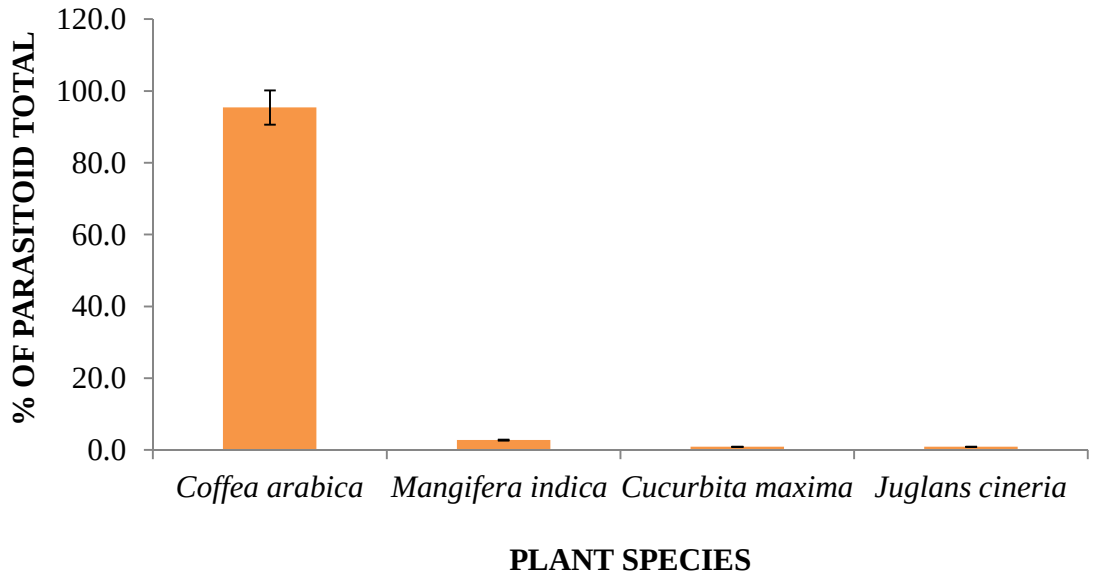

Fig. 4.4 Percentage contribution of different plant species to total number of parasitoids recovered at the Eastern Province during the survey.

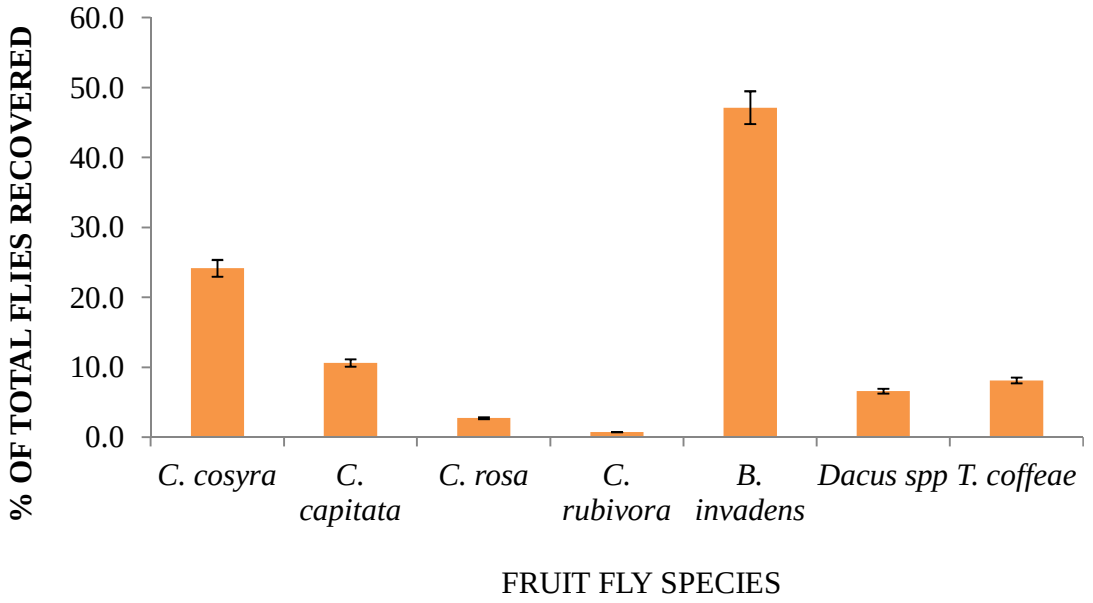

Fig 4.5 Percentage of different fruit fly species recovered at the Eastern Province during the survey

4.5 DISCUSSION

The survey yielded 345 parasitoids belonging to four different families of Hymenoptera (Braconidae, Eulophidae, Chalcidoidea and Ichneumonidae) with genus *Psyttalia* predominating in both the Coastal and Eastern Province of Kenya. Two species of *Psyttalia, P. perproxima* and *P. concolor* were identified from parasitoid samples at the Coast Province. *Psyttalia* is a fairly large, Old World genus; with about 50 described species. The highest *Psyttalia* diversity occurs in the region from Africa east through India and Southeast Asia; and there are several species of in SSA that are difficult to differentiate (Rugman-Jones *et al*., 2009). In Kenya, Copeland *et al*., (2006) sampled two fruit species (*Cordyla Africana* and *Lettowianthus stellatus*) from the Muhaka forest and Mrima Hill of Coastal province and recorded *Psytallia* spp from both fruits.

Psyttalia perproxima accounted for 11.0% of total parasitoid samples recovered from the Coast Province. *Trirhithrum nigerrimum* and *T. coffeae* were the main fruit fly species that emerged from pupae that yielded *P. perproxima*, although *C. cosyra* and *C. capitata* were also recovered. It is known that fruit fly parasitoids, in general, may develop on several tephritids but they often have a specific, preferred host (Wharton, 1989). *Psyttalia perproxima* was originally described from Benin from fruits infested by tephritids (Silvestri, 1913). Previous studies have documented attack of *P. perproxima* from different fruit fly species. In Kenya, the parasitoid was reared from *Trirhithrum teres* (Munro), *T. nigerrimum*, and *T. senex* (Munro), and in Ghana, Manrakhan *et al.,* (2010) reared it from an unknown host. In samples dominated by *T. coffeae*, Steck *et al*., (1986) also reported attack by *P. perproximus*. In other studies, *P. perproxima* has also been reported from *C. capitata* (Manrakhan *et al*., 2010), *C. cosyra* (Vayssieres *et al*., 2010) and *Dacus spp* (Wharton and Gilstrap, 1983).

Psytallia cf. concolor accounted for 6.8% of total parasitoid samples recovered from the Coast Province and it was reared from two plant species, *G. superba* and *Phyllanthus spp*. We recovered *C. cosyra*, *C. capitata* and *T. coffeae* from puparia yielding *P. concolor*. *Psyttalia concolor* has been previously reared in Kenya from coffee berries sampled from Ruiru, Koru and Rurima (Wharton *et al*., 2000). It has also been reared from *Bactrocera oleae* (Rossi) collected from *Olea europaea cuspidata* in Kenya (Copeland *et al*., 2004). Most recently, it was reared from *C. capitata* collected from coffee berries in South Africa (Manrakhan *et al*., 2010). *Psyttalia concolor* has been introduced throughout much of the world for biological control programs against various pest tephritids. It was introduced in Bolivia in 1968 to control *Ceratitis capitata* (Bennett and Squire, 1972). However, because the species of *Psyttalia* are difficult to discriminate, it is possible that more than two species of *Psyttalia* are included in those samples identified up to genus level, including either *P. cosyrae* or *P. humilis* (Silvestri).

Diachasmimorpha fullawayi was recovered from *T. cattapa* and accounted for 0.8% of total parasitoids recovered at the Coast Province. *Terminalia cattapa* also yielded only *B. invadens* flies. No fruit fly parasitoid has been reared from over 5,450 fruit samples including *T. cattapa* collected across Eastern and Southern Africa since the detection of *B. invadens* in coastal Kenya in 2003. Since only *B. invadens* was recovered from *T. catappa* in the study and only *D. fullawayi* was recovered from the host plant, it is possible that the parasitoid could have either emerged from *B. invadens* puparia or from an indigenous fruit fly species which was not recovered from the puparia obtained from the host plant. If it is true for the former scenario, this will be the first record of an indigenous parasitoid parasitizing *B. invadens* in Kenya. Additional

host fruit survey and laboratory host acceptability and suitability are warranted to ascertain the role of *D. fullawayi* in managing *B. invadens*.

Diachasmimorpha fullawayi is widespread in the Afrotropical Region, and has been recorded from Senegal to Nigeria in the West, across Congo to Kenya, and was also reported from Reunion (Silvestri 1913, 1914, Clausen *et al.*, 1965, Wharton and Gilstrap 1983, Wharton *et al.*, 2000). In Kenya, Copeland *et al.,* (2006) recorded *D. fullawayi* from several fruit fly species attacking different host plants. It readily develops in 'Medfly', which is a native host of this species in Kenya (Wharton *et al.*, 2000). Recently, *D. fullawayi* was also recovered from mango fruits that yielded *C. cosyra* and *B. invadens* in Benin (Vayssieres *et al.,* 2010). It was successfully introduced to Hawaii, where it was among the several parasitoid species providing satisfactory control of medfly on different crops (Willard and Mason, 1937).

Seven individuals of *T. giffardianus* (Hymenoptera: Eulophidae) were recovered from *Chassalia curviflora* that also yielded *T. coffeae* and *C. capitata* and accounted for 3.0% of total parasitoid samples from the Coast Province. *Tetrastichus giffardianus* is cosmopolitan in distribution, but is most commonly associated with fruit-infesting Tephritidae of African origin (LaSalle and Wharton, 2002). *Tetrastichus giffardianus* has previously been reared from tephritid flies on coffee sampled at Ruiru, Koru and Rurima in Kenya (Wharton *et al*., 2000). It was also reared from *C. anonae* and *C. fasciventris* in Kenya (Copeland *et al*., 2006). Vayssieres *et al*., (2010) and Manrakhan *et al*., (2010) also reported *T. giffardianus* from *C. capitata* in Benin and South Africa, respectively.

Fourteen ichneumon wasps (Hymenoptera: Ichneumonidae) were recovered from *C. curviflora* and *Phyllanthus spp* that also yielded *T. coffeae* and *C. cosyra* and accounted for 5.9% of total parasitoids recovered at the Coast Province. It is unclear whether the ichneumonid wasps recovered from this study parasitized tephritids or were hyperparasitoids of other Braconid wasps which emerged from the same fruits. Members of the family Braconidae and Ichneumonidae are very closely related and some species look similar. In the Ichneumons there is an extra vein on the forewing, creating a cell which cannot be found on Braconids (CSIRO, 1991). An estimated 12100 species of Ichneumonidae occur in the Afrotropical region (Africa south of the Sahara and including Madagascar) (Townes & Townes 1973), of which only 1927 have been described (Yu, 1998). Quantitative studies of ichneumonid species richness are scarce in Africa. A limited number of assessments have been conducted in Sierra Leone and Uganda (Owen & Owen 1974); Namibia (van Noort *et al.* 2000); Gabon (van Noort 2004); Central African Republic, Tanzania and South Africa (van Noort unpublished.). Very little is therefore known about the ichneumonid faunas of the majority of African countries. Ichneumonids have been used successfully as biocontrol agents and given the largely undocumented fauna there is a huge potential for their use in biocontrol programmes (Gupta 1991).

Chalcid wasps (Hymenoptera: Chalcidoidea) accounted for 9.7% of parasitoid samples from the Coast Province and were recovered from fruits of *C. curviflora*. Although *T. coffeae* and *C. capitata* were also recovered, it cannot be stated categorically that these were hosts of Chalcid wasps recovered from this study since individuals of *Psytallia cf. concolor* were also recovered from the same fruit samples. Most chalcidids are solitary, primary larval endoparasitoids, although some ectoparasitic and hyperparasitic species are known. They have a wide host range

including parasitoids of Lepidoptera, although Coleoptera and Diptera also are commonly attacked. Hyperparasitic species attack tachinid and braconid primary parasitoids. It is therefore possible that the chalcidids recovered from this study were hyperparasitoids of Braconidae recovered from the same fruits although additional studies are needed to clarify this observation.

Collections from the Eastern Province were dominated by *Psyttalia* species with berries of *C. arabica* accounting for 95.4% of total parasitoids recovered. *Trirhithrum coffeae* and *C. capitata* were the fruit fly species associated with *Psyttalia* species that emerged from coffee berries. Several *Psyttalia* spp have been previously reared in Kenya from coffee berries sampled from Ruiru, Koru and Rurima (Wharton *et al*., 2000). Two individuals of *P. phaeostigma* were reared from *Dacus* spp on *C. maxima* and *J. cineria*. In previous studies, *P. phaeostigma* were reared from *D. ciliatus* infesting cucurbits in Kenya and South Africa, and also *Dacus demmerezi* (Bezzi) in Mauritius (Wharton and Gilstrap, 1983). Although introduced and released in Hawaii and Mauritius, it failed to establish.

Bactrocera invadens was the fruit fly species mostly recovered from fruit samples, accounting for 55.8% and 47.1% of total fly samples recovered from the Coast and Eastern Province, respectively. In survey carried out by Copeland *et al*., (2006) to assess the distribution, host plants and parasitoids of African fruit flies in Muhaka and Mrima region of Coast Province, the authors reported *C. cosyra* and *C. rosa* as the predominant fruit fly pests in the region. However, in this study, although *C. cosyra* co-occur with *B. invadens* in most of the fruit samples collected, the later was by far the most abundant. This confirms the recent observation by Rwomushana *et al*., (2008) and Ekesi *et al*. (2009) that *B. invadens* has rapidly displaced indigenous fruit fly species in Kenya to become the dominant fruit fly pest. In this study, *B. invadens* was recovered

from *Coccinia spp* (Cucurbitaceae) at the Coast Province. Rwomushana *et al*. (2008) did not report fruits of the family Cucurbitaceae as hosts of the pest, and this may be the first record of *B. invadens* infesting cucurbits in Kenya. According to Rwomushana *et al*. (2008), *B. invadens* is an emerging polyphagous pest that may be capable of sustaining its population through reproduction on a range of cultivated and wild fruits.

Psyttalia parasitoids reared from mango (*Mangifera indica* L. (Anacardiaceae)) and marula (*Sclerocarya birrea* (A. Rich) (Anacardiaceae)) have been typically identified as *P. cosyrae* (Billah *et al*., 2005). The parasitoid is mostly reared from *C. cosyra* which infests these fruits. In this study, 62.1% of all flies recovered from *S. birrea* were *B. invadens*, whilst *C. cosyra* accounted for 37.9%. No parasitoid was recovered from fruits of *Sclerocarya birrea*, suggesting that the gradual displacement of *C. cosyra* by *B. invadens* is negatively affecting the reproductive potential of *P. cosyrae*, since it is unable to develop within *B. invadens* larvae.

Although classical and augmentative biological control programs have reduced tephritid fruit fly pest populations in other parts of the world (Wharton 1989; Wong *et al*., 1991; Sivinski *et al*., 1996), biological control of tephritids using parasitoids has not been applied on a commercial scale in Africa. The diversity of fruit fly parasitoids documented in this study shows that native parasitoids species hold considerable promise in contributing to the overall management of fruit flies either through augmentation and conservation approach. The huge losses being incurred by the horticultural industry on the continent as a result of the arrival of the invasive fruit fly, *B. invadens* has led to the introduction and evaluation of two co-evolved parasitoids, *F. arisanus* and *D. longicaudata* against the pest. However, their interaction with indigenous tephritid parasitoids is still unknown. Many ecologists view the intentional introductions of alien species

into complex biological communities as a threat to their structure and dynamics (Sime *et al*., 2008) and therefore knowledge of indigenous tephritid parasitoids, their abundance and parasitism rates especially in the areas where the introduced parasitoids will be first released is very important. Our results therefore provide important baseline information on the parasitoid fauna in key mango production localities in Kenya and set the scene for studies related to interaction of the exotic parasitoid species and the native species documented here before their field releases in Kenya. This study also highlights the need for future conservation effort of the indigenous tephritid parasitoids in localities and/or native host plants close to mango orchards where they can build up their population and exert impact on the fruit fly species before they move into the orchards.

CHAPTER FIVE

5.0 EFFECT OF TEMPERATURE ON DEVELOPMENTAL TIME, PARASITISM RATES AND LONGEVITY OF *FOPIUS ARISANUS* AND *DIACHASMIMORPHA LONGICAUDATA* REARED ON *BACTROCERA INVADENS*

5.1 INTRODUCTION

Tephritid fruit flies within the genus *Bactrocera* Macquart are recognized worldwide as among the most destructive insect pests of fruits (White and Elson-Harris, 1992; Clarke *et al.,* 2005). They cause enormous damage to fruits through direct feeding by the developing larvae and indirect losses are also associated with the quarantine restrictions imposed by importing countries to prevent entry and establishment of unwanted fruit flies (Rwomushana *et al.,* 2008).

In addition to the plethora of native fruit fly pests that occur in Africa, several members of the genus *Bactrocera* have also invaded the continent (Mohamed *et al.,* 2010). These include *Bactrocera cucurbitae* (Coquillett), *Bactrocera zonata* (Hashem *et al*., 2001), *Bactrocera invadens* Drew, Tsuruta and White (Lux *et al*., 2003; Drew *et al*., 2005), and most recently *Bactrocera latifrons* (Hendel) (Mwatawala *et al*., 2007).

Bactrocera invadens was first detected at the Kenyan coast in 2003 (Lux *et. al*., 2003a). Since the first record, the pest has now be reported from over 28 African countries (Drew et al. 2005; Vayssieres, Goergen, Lokossou, Dossa, and Akponon 2005; 2008, Ekesi *et al*., 2006). *B. invadens* continues to seriously threaten the mango industry in all localities where it has been reported with significant loss of export markets that is having a huge negative impact on the horticulture industry. It has a wide thermal tolerance range, with an upper developmental threshold of 35 °C and a lower developmental threshold of 8.8 °C (Rwomushana *et al*., 2008).

Exotic insect pests typically arrive in new areas without their natural enemies, raising the possibility for classical biological control through the importation of old association natural enemies from the pest's aboriginal home (Mohamed *et al.,* 2006). In 2006, the International Centre of Insect Physiology and Ecology (*icipe)* through its African Fruit Fly Programme imported two braconid parasitoids, *F. arisanus* (Sonan) (Hymenoptera: Braconidae), and *D. longicaudata* (Ashmead) (Hymenoptera: Braconidae) from Hawaii for evaluation and potential release against *B. invadens*. Both *F. arisanus* and *D. longicaudata* are koinobiont solitary parasitoids of fruit flies. They have been credited for the most successful biological control programmes ever undertaken against tephritid fruit flies (Van den Bosch *et al*., 1951; Newell and Haramoto 1968). In recent studies, Mohamed *et al*., (2010) reported over 70% parasitism of *F. arisanus* on *B. invadens* when reared on artificial diet. Earlier, Mohamed *et al*., (2008) also reported 15% parasitism of *D. longicaudata* on *B. invadens* on artificial diet.

Climatic adaptation has been listed among the criteria for selecting potential biocontrol agents (van Lenteren 1986) and temperature is often the most important factor in acclimatization of introduced natural enemies (Loni 1997). Temperature is the single most important environmental factor influencing insect behaviour, distribution, development, survival, and reproduction (Petzoldt and Seaman, 2007). Although laboratory studies has demonstrated the efficacy of both *F. arisanus* and *D. longicaudata* against *B. invadens,* and some of the major indigenous fruit fly species (Mohamed *et al*., 2008; 2010), little information exists on the effect of temperature on the development, parasitism rates and adult longevity of the two parasitoid species when reared from *B. invadens*. Such information should provide guidance on future release programme targeting different agro-ecological zones where the pest is prevalent. The overall objective of this study therefore, was to determine the effect of temperature on the parasitism rates, adult

longevity and developmental time of *F. arisanus* and *D. longicaudata* on *B. invadens* when reared on mango.

5.2 BIOASSAYS

5.2.1 Developmental time and Parasitism rates

5.2.1.1 *Fopius arisanus*

Ten mated experienced, female *F. arisanus* wasps (10-day old) were introduced into a perspex cage (12 x 12 x 12 cm). With the aid of a fine camel's hair brush, 200 eggs of *B. invadens* were counted onto a rectangular peel of mango dome (4 cm x 2 cm). Each mango peel was transferred onto an oviposition unit lined with moistened double layer of sponge of about the same dimensions as the mango peel, with the eggs facing the organza lid (Mohamed *et al*., 2010). Oviposition units were introduced into the perspex cages containing the 20 mated experienced *F. arisanus* females. The wasps were allowed to parasitize the exposed *B. invadens* eggs for 24 hours, after which the oviposition units were removed from the cages and the rectangular mango peels (with previously exposed eggs) were transferred into Petri dishes containing mango pulp (Plate 5.1). The mango peels were placed on the pulp ensuring that the side of each dome carrying the previously exposed eggs was in direct contact with the pulp. This was done to facilitate larval feeding immediately after egg hatching. Petri dishes were covered and immediately transferred to thermostatically controlled environmental chambers (MLR-153, Sanyo, Japan) set at five constant temperatures of 15 °C, 20 °C, 25 °C, 30 °C and 35 °C (±1°C) and 60 ± 8% RH, 12:12 L: D photoperiod. At egg eclosion, Petri dish covers were removed and the dishes were separately transferred into larger rectangular plastic rearing containers (20 cm x 10 cm x 10 cm) containing a thin layer of moist sterilized sand at the bottom for pupation. The

top of the plastic containers were covered with a fine netting material and fitted with a plastic lid to allow for ventilation (Plate 5.2). The containers were maintained at the same constant temperature in the environmental chambers. Mature late third instar larvae left the fruit pulps *ad libitum* and jumped into the sand in the larger containers to pupate. Those that failed to jump were assisted using a pair of soft forceps. After 7 days, the containers were observed for puparia and the puparia were thereafter separated from sand daily by sifting. Puparia were held in perspex cages (20 × 15 × 15cm) and maintained at the same five constant temperatures until eclosion. The following parameters were measured; developmental time, number of puparia recovered, total number of emerged parasitoids, and number of unemerged puparia. For each temperature, there were five replicates and each temperature was tested twice. Test temperatures were assigned randomly to various environmental units.

5.2.1.2 *Diachasmimorpha longicaudata*

For *D. longicaudata*, 50 late second instar larvae (reared on artificial liquid diet) were counted into mango pulps in oviposition units, which were covered with organza lids. Larvae were transferred into the mango pulp using a blunt forceps to minimize damage. Thereafter, the oviposition units were introduced into perspex cages containing five mated experienced females of *D. longicaudata*. After exposure time of 24 hrs, the oviposition units were removed and the larvae transferred into Petri dishes containing fresh amount of mango pulp. Petri dishes were covered and immediately transferred to thermostatically controlled environmental chambers (MLR-153, Sanyo, Japan) set at five constant temperatures of 15 °C, 20 °C, 25 °C, 30 °C and 35 °C (±1°C) and 60 ± 8% RH, 12:12 L: D photoperiod. Subsequent procedures were the same as described for *F. arisanus* above. Parameters measured were the same as for *F. arisanus*.

To establish the lower development thresholds, the developmental time (i.e. the time required for 50% of adult parasitoids to emerge after eggs were exposed to wasps) was determined at the series of constant temperatures and the developmental rate estimated (i.e., 1/developmental time) (Dent and Walton, 1997; Samira *et al*., 2006). Percent parasitism was calculated by dividing the total number of emerged parasitoids by the total number of puparia recovered and multiplying by 100.

Plate 5.1 Mango pulp in petri dishes and sterilized sand at the bottom of plastic containers served as larval food and pupation medium respectively.

Plate 5.2 Pupation container inside one of the environmental chambers set at 25°C.

5.2.2 Adult longevity

To determine the longevity of *F. arisanus* and *D. longicaudata* reared on *B. invadens*, 50 newly emerged adults of each parasitoid (25 of each sex) were placed in 5 separate perspex cages (15×15×15cm). The perspex cages were immediately transferred into thermostatically controlled environmental chambers (MLR-153, Sanyo, Japan) set at five constant temperatures of 15 °C, 20 °C, 25 °C, 30 °C and 35 °C (±1°C) and 60 ± 8% RH, 12:12 L: D photoperiod. Parasitoids were provided with honey streaks and cotton wool soaked in water served as the water source. Male and female mortality for both parasitoids was recorded on a daily basis. The total length of time (days) taken for all parasitoids of each sex to die under the prevailing temperature was also recorded. This length of time (days) was assumed as the adult longevity of each sex of the two parasitoid species at a specific temperature. For each constant temperature tested, the experiment was replicated 3 times.

In all experiments, the Completely Randomized Design (CRD) was used.

5.3 Data analysis

Regression analysis was used to estimate lower development thresholds for the parasitoids and to establish the strength of the relationship between constant temperature, puparia recovered, parasitism rates and adult longevity. The average number of degree-days required to complete pre-imaginal development of the parasitoids was estimated as 1/slope and the lower temperature threshold for development was estimated as the x-intercept (Dent & Walton 1997). To examine the effects of temperature on life history parameters, data for developmental time, puparia recovered, parasitism rates and adult longevity was subjected to one-way analysis of variance (ANOVA) using the General Linear Models procedure of SAS (SAS Institute 2000). Log_{10}±0.5

and arcsine square root transformation were used respectively, on counts and percentages before statistical analyses (Sokal and Rohlf, 1981). When treatment effects were significant (i.e., P <0.05), means were separated using Student-Newman-Keul's (SNK) test.

5.4 RESULTS

5.4.1 Effect of temperature on developmental time

Both *F. arisanus* and *D. longicaudata* were able to complete development at all the temperatures tested except at 35 °C, at which no puparia was recovered. Temperature had a significant effect on developmental time of *F. arisanus*, decreasing significantly as temperature increased (F =4054.0; df = 3, 36; P </0.0001 and F =3824.0; df = 3, 36; P </0.0001) for males and females, respectively. Mean developmental time for *F. arisanus* males ranged between 67.40 ± 0.4 days at 15 °C and 18.40 ± 0.2 at 30 °C. Female mean developmental time was between 71.60 ± 0.4 days at 15 °C and 20.2 ± 0.2 days at 30 °C (Table 5.1).

Temperature also had a significant effect on the developmental time of *D. longicaudata*, decreasing significantly with increasing temperature (F =3173.0; df = 3, 36; P </0.0001 and F =4691.0; df = 3, 36; P </0.0001) for males and females respectively. Mean developmental time for males ranged from 62.70 ± 0.6 days at 15 °C to 14.70 ± 0.2 days at 30 °C and 66.20 ± 0.4 days at 15 °C to 16.50 ± 0.17 days at 30°C for females (Table 5.1).

The linear regression model showed a strong positive linear relationship between temperature and development rate for *F. arisanus* (R^2= 0.998) (fig. 5.1) with a lower development threshold of 13.5 °C. It required 500 DD for *F. arisanus* to complete development. For *D. longicaudata*, the linear regression model also showed a strong positive linear relationship between temperature

and development rate (R^2= 0.993) (fig. 5.2) with a lower development threshold of 12.0 °C. It required 333.3 DD for *D. longicaudata* to complete development.

Table 5.1 Effect of temperature on developmental time of *F. arisanus* and *D. longicaudata* reared from *B. invadens*.

	F. arisanus		*D. longicaudata*	
Temperature (±1°C)	Males	Females	Males	Females
15	67.40±0.4a	71.6±0.4a	62.7±0.6a	66.2±0.4a
20	34.9±0.4b	37.0±0.4b	29.7±0.3b	32.7±0.3b
25	23.4±0.3c	25.2±0.2c	16.5±0.2c	18.7±0.2c
30	18.4±0.2d	20.2±0.2d	14.7±0.2d	16.5±0.2d
35	0	0	0	0

* Means followed by the same letter in the same column are not significantly different (P <0.05), Student-Newman-Keul's (SNK) test.

5.4.2 Effect of temperature on puparia recovered

For both parasitoid species, puparia were recovered at all temperatures tested except at 35 °C. Temperature had a significant effect on the number of puparia recovered (F =48.0; df =3, 36; P <0.0001 and for *F. arisanus.* Temperature also had a significant effect on number of puparia recovered for *D. longicaudata* (F =46.9; df =3, 36; P < 0.0001)

For *F. arisanus*, the highest number of puparia was recovered at 25 °C (136.7 ± 3.08) and the lowest at 15 °C (92.1 ± 2.91). The number of puparia recovered at 20 and 30 °C were not significantly different but the numbers differed significantly with that recovered at 15 and 25 °C (Table 5.2). The highest percentage of unemerged puparia for *F. arisanus* was recorded at 15 °C whilst the lowest percentage was recorded at 25 °C (Table 5.2). Polynomial regression model

showed a strong positive relationship between temperature and percent puparia recovered for *F. arisanus* (R^2= 0.90) (Fig 5.3).

The number of puparia recovered was significantly different across all four temperatures tested for *D. longicaudata* and was highest at 25 °C (44.60 ± 0.86) and lowest at 15°C (31.80 ± 1.03) (Table 5.3). The polynomial regression model also showed a strong positive relationship between temperature and percent puparia recovered (R^2= 0.96) (Fig 4.3). The highest percentage of unemerged puparia for *D. longicaudata* was recorded for at 15° C whilst the lowest percentages were recorded at 25 °C (Table 5.3).

Percent puparia recovered was also significantly different across the temperatures tested: *F. arisanus* (F =46.0; df =3, 36; P <0.0001) and *D. longicaudata*: (F =47.9; df =3, 36; P < 0.0001). Percent puparia recovered was highest at 25°C (68.35 ± 1.54 and 89.20 ± 1.72) and lowest at 15°C (46.05 ± 1.46 and 63.60 ± 2.06) for *F. arisanus* and *D. longicaudata* respectively (Table 5.2 and 5.3). Temperature had a significant effect on the number of unemerged puparia across the temperatures tested as clearly shown by analysis of percent unemerged puparia values (F =30.22; df =3, 36; P <0.001and F =15.35; df =3, 36; P <0.001) for *F. arisanus* and *D. longicaudata,* respectively.

TABLE 5.2 Effect of temperature (Mean ± SE) on puparia recovered, parasitism rates and percent unemerged puparia of *Fopius arisanus* on *B. invadens*

Temperature (± 1.0˚C)	Puparia recovered (%)	Total Parasitoid number	Parasitism rate (%)	Sex ratio (Proportion of females)	Unemerged puparia (%)
15	46.05 ± 1.46a	42.60 ± 1.54a	46.42 ± 1.43a	0.55 ± 0.02a	26.44 ± 0.51a
20	59.50 ± 1.37b	75.50 ± 3.06b	63.33 ± 1.74b	0.45 ± 0.03b	18.29 ± 0.88b
25	68.35 ± 1.54c	97.50 ± 4.73c	71.08 ± 2.46c	0.55 ± 0.02a	15.16 ± 0.97c
30	63.05 ± 1.10b	69.30 ± 2.61b	55.06 ± 2.18d	0.57 ± 0.02a	22.55 ± 1.03d
35	0	0	0	0	0

Means followed by the same letter in the same column are not significantly different (P </0.05) Student-Newman-Keul's (SNK) test.

TABLE 5.3 Effect of temperature (Mean ± SE) on puparia recovered, parasitism rates and percent unemerged puparia of *Diachasmimorpha longicaudata* on *B. invadens*

Temperature (± 1.0˚C)	Puparia recovered (%)	Total Parasitoid number	Parasitism rate (%)	Sex ratio (Proportion of females)	Unemerged puparia (%)
15	63.60 ± 2.06a	8.70 ± 0.70a	27.74 ± 2.53a	0.53 ± 0.03a	36.60 ± 1.26a
20	80.20 ± 0.96b	20.90 ± 1.62b	52.15 ± 3.99b	0.52 ± 0.04a	25.92 ± 1.69b
25	89.20 ± 1.72c	21.80 ± 1.20b	48.85 ± 2.37b	0.59 ± 0.03a	25.80 ± 1.16b
30	69.40 ± 1.43d	13.40 ± 0.64c	38.67 ± 1.71c	0.53 ± 0.02a	31.08 ± 0.85c
35	0	0	0	0	0

* Means followed by the same letter in the same column are not significantly different (P <0.05) Student-Newman-Keul's (SNK) test.

5.4.3 Effect of temperature on parasitism rates

With the exception of 35 °C, adult parasitoids were obtained from the other four temperatures tested. Temperature had a significant effect on the number of parasitoids that emerged (*F. arisanus*: F =67.97; df =3, 36; P <0.0001), (*D. longicaudata*: F =42.97; df =3, 36; P < 0.0001). The highest number of parasitoids was obtained at 25 °C (97.50 ± 4.73 and 21.8 ± 1.2) for *F. arisanus* and *D. longicaudata* respectively whilst the lowest parasitoid numbers were recorded at 15 °C (*F. arisanus*: 42.6 ± 1.54), (*D. longicaudata*: 8.7 ± 0.7) (Tables 5.2 and 5.3). The total number of parasitoids that emerged was not significantly different at 20 and 30°C for *F. arisanus* and at 20 and 25°C for *D. longicaudata*. The polynomial regression model showed a strong positive relationship between temperature and percent parasitism (R^2 =0.97 and R^2 =0.99) for *F. arisanus* and *D. longicaudata* respectively (Fig. 5.4).

Percent parasitism was significantly different across the temperatures tested (F =27.60; df =3, 36; P < 0.0001 and F =16.03; df =3, 36; P < 0.0001) for *F. arisanus* and *D. longicaudata* respectively. Percent parasitism was highest at 25°C (71.08 ± 2.46) for *F. arisanus* and at 20°C (52.15 ± 3.99) for *D. longicaudata* (Tables 5.2 and 5.3). It was lowest at 15°C for both parasitoids. Percent parasitism was significantly different across all the five temperatures tested for *F. arisanus* but was not significantly different between 20 and 25°C for *D. longicaudata* (Table 5.2 and 5.3).

5.4.4 Effect of temperature on adult longevity

Adult longevity of *F. arisanus* was significantly influenced by temperature (F =571.8; df =4, 10; P <0.0001 and F =326.6; df =4, 10; P <0.0001 for males and females respectively. There was no significant difference in adult longevity of *F. arisanus* (both males and females) at 15 and 20°C.

Longevity at 15 and 20°C was however significantly different from that obtained at 25, 30 and 35°C (Table 5.4). Longevity of *F. arisanus* adults was shortest at 35°C (7.7±0.3 and 11.4±0.7 for males and females respectively) and longest at 15°C (110.0±1.2 and131.0±3.8 for males and females, respectively) (Table 5.4).

Temperature significantly affected the lifespan of *D. longicaudata* adults ($F = 646.6$; df =4, 10; $P < 0.0001$ and $F = 1553.0$; df =4, 10; $P < 0.0001$ for males and females respectively). Similar to results obtained for *F. arisanus*, there was no significant difference in adult longevity of *D. longicaudata* (both males and females) at 15 and 20 °C. However, longevity at 15 and 20 °C significantly differed from that obtained at 25, 30 and 35°C (Table 5.4). Adult longevity for *D. longicaudata* was shortest at 35 °C (5.7±0.7 and 8.3±0.3 for males and females respectively) and longest at 15 °C (119.0±2.1 and142.7±2.3 for males and females respectively) (Table 5.4).

The linear regression model further confirmed a strong relationship between temperature and adult longevity for both parasitoids as shown by the r^2 values (*F. arisanus*= 0.93) and (*D. longicaudata*= 0.94) (figs. 5.5 and 5.6). For both parasitoids, females lived significantly longer than males at all temperatures tested (Table 5.4).

Table 5.4 Mean ± S.E. adult longevity of *F. arisanus* and *D. longicaudata* reared on *B. invadens* at five constant temperatures.

Temperature	*F. arisanus*		*D. longicaudata*	
	Male	Female	Male	Female
15	110.0±1.2Aa	131.0±3.8Ab	119.0±2.1Aa	142.7±2.3Ab
20	106.3±2.4Aa	127.7±4.3Ab	114.0±3.6Aa	140.0±1.2Ab
25	77.3±2.9Ba	102.0±3.1Bb	71.7±0.9Ba	92.7±1.8Bb
30	28.0±1.7Ca	38.0±1.5Cb	22.3±1.5Ca	32.3±1.5Cb
35	7.7±0.3Da	11.4±0.7Db	5.7±0.7Da	8.3±0.3Cb

* Means followed by the same capital letter in the same column are not significantly different. Means followed by the same small letter in the same row are not significantly different ($P < 0.05$) Student-Newman-Keul's (SNK) test.

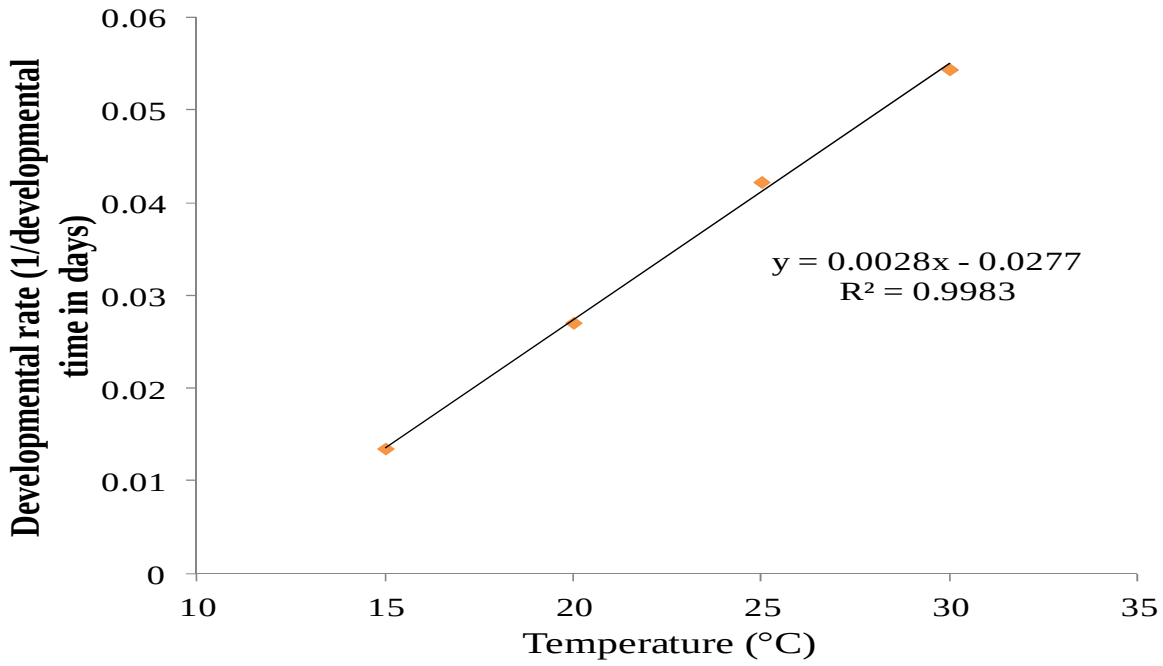

Fig 5.1 Effect of constant temperature on developmental rate of *Fopius arisanus* on *B. invadens*

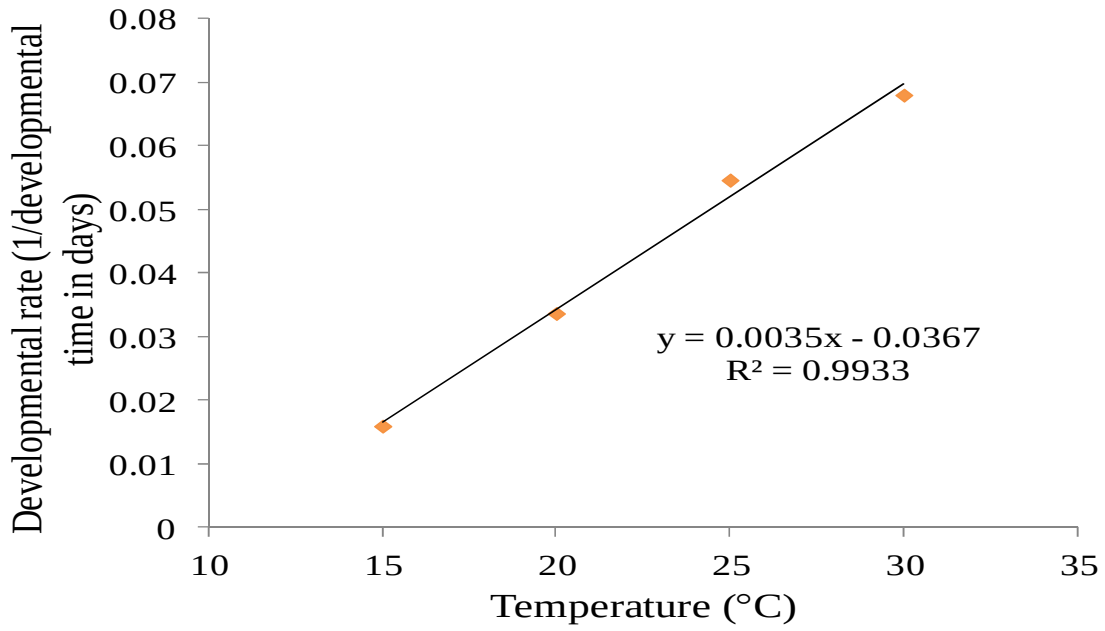

Fig 5.2 Effect of constant temperature on developmental rate of *D. longicaudata* on *B. invadens*.

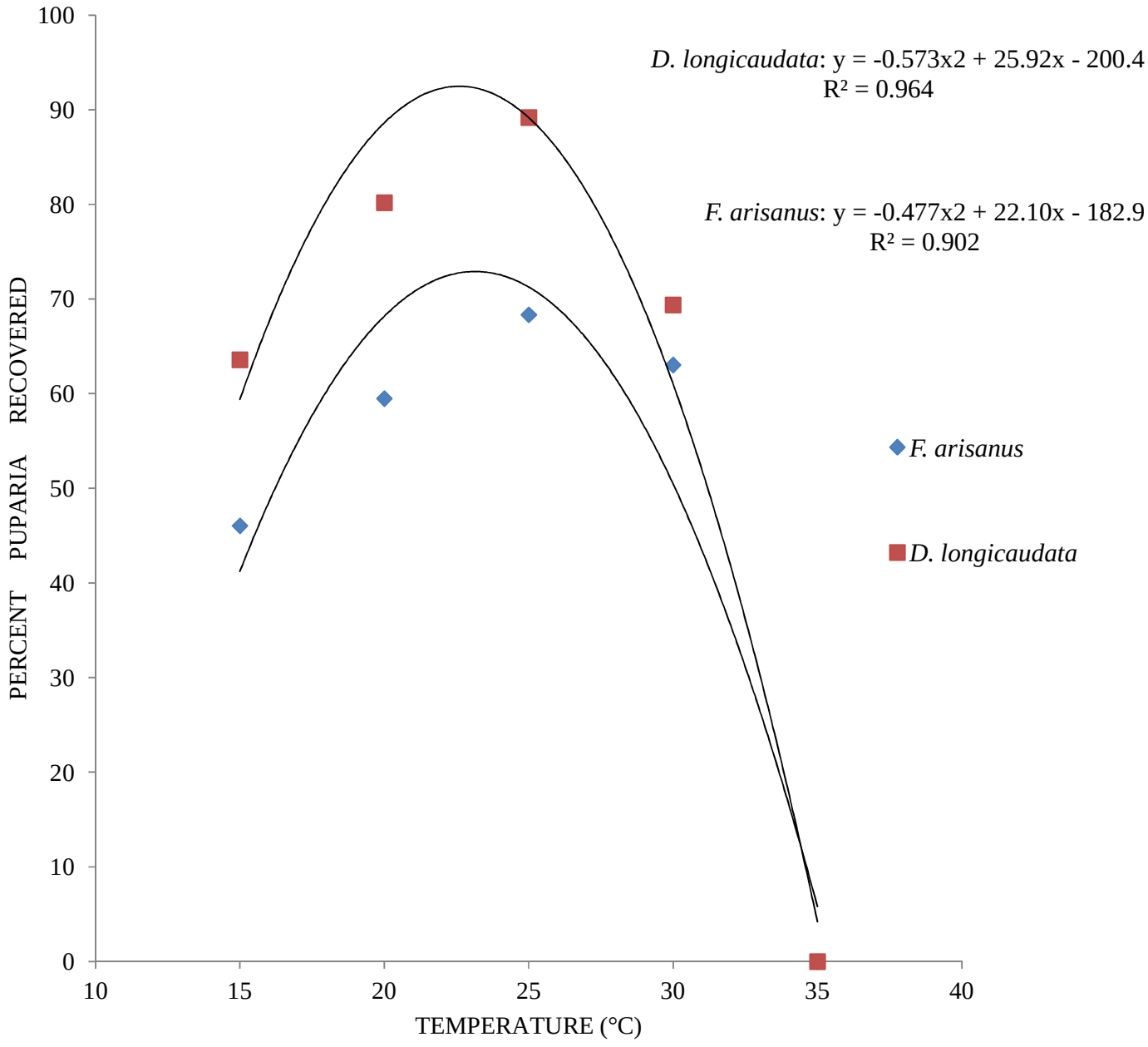

Fig. 5.3 Effect of constant temperature on percentage of puparia recovered for *F. arisanus* and *D. longicaudata* on *B. invadens.*

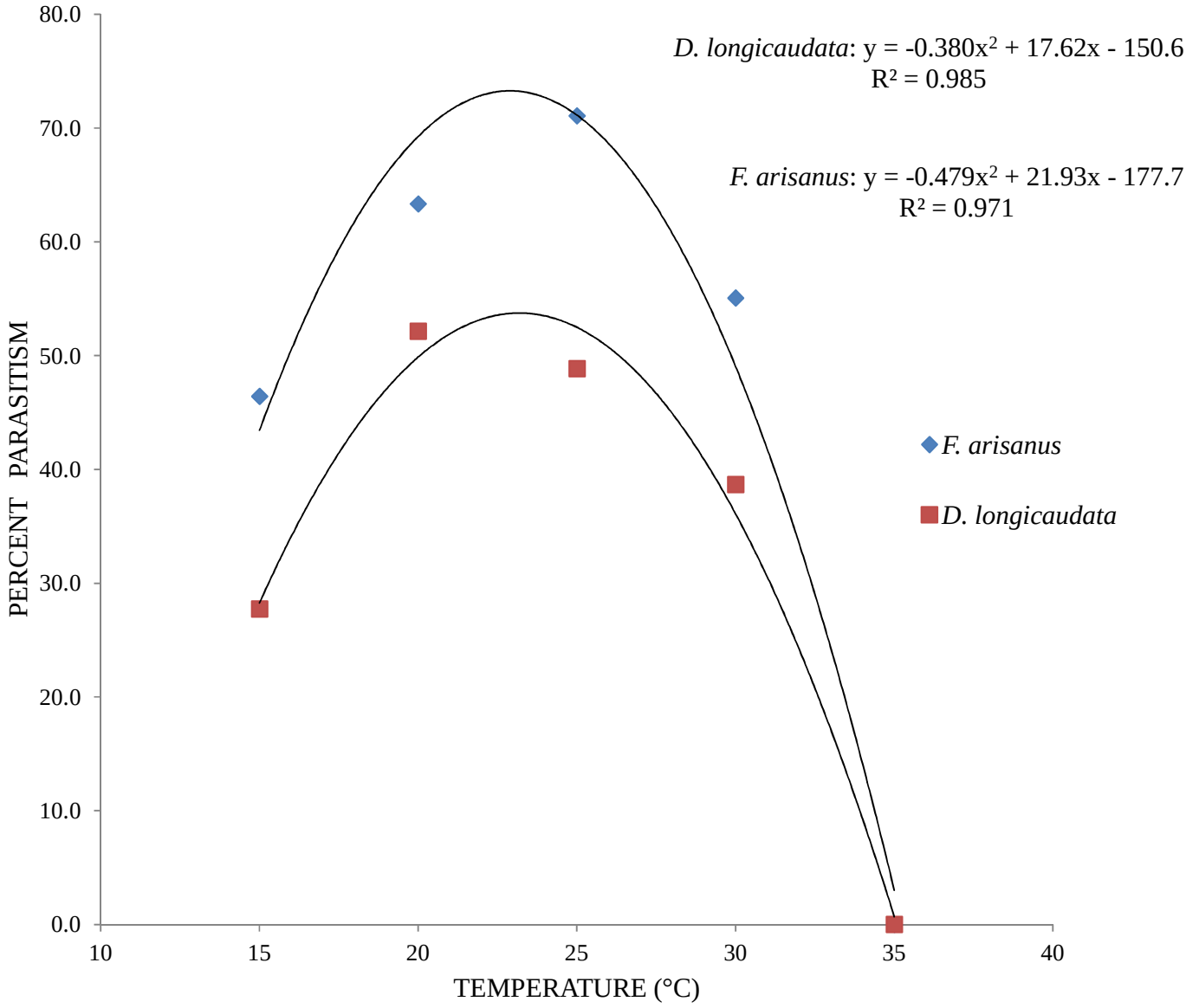

Fig 5.4 Effect of constant temperature on percent parasitism of *F. arisanus* and *D. longicaudata* on *B. invadens*

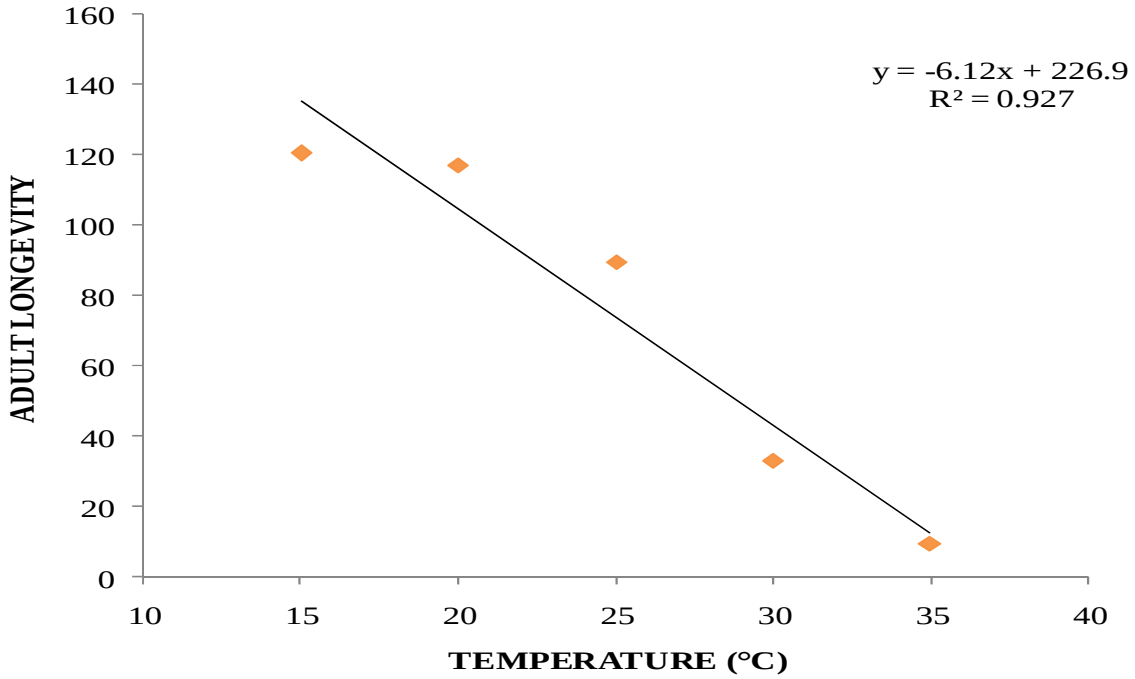

Fig 5.5 Effect of temperature on adult longevity of *F. arisanus*

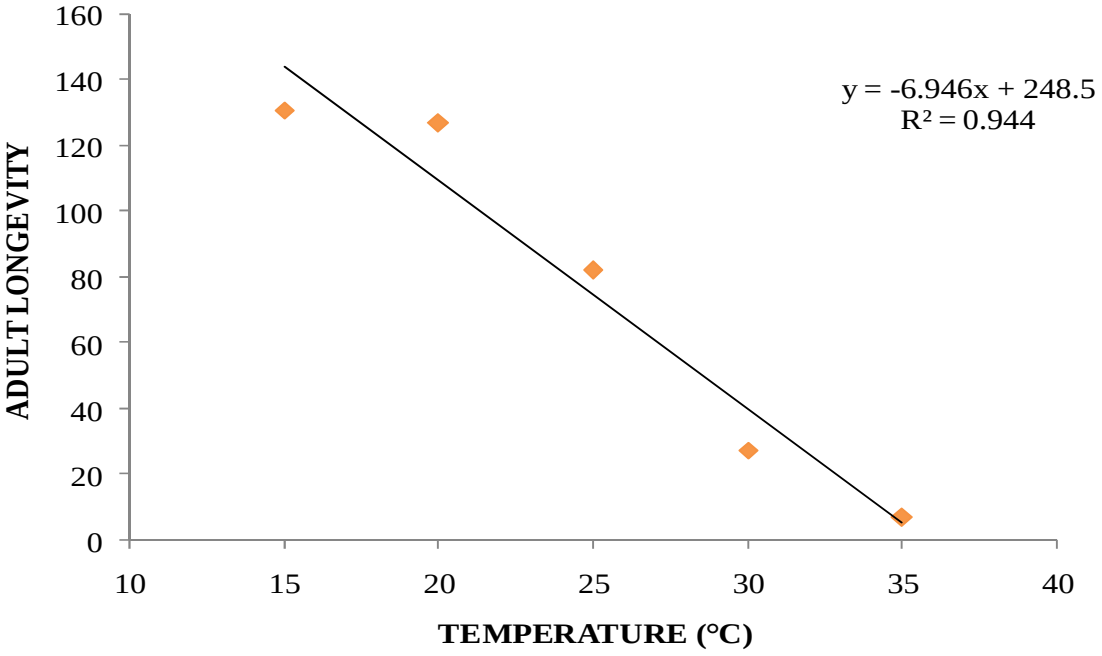

Fig 5.6 Effect of temperature on adult longevity of *D. longicaudata*

5.5 DISCUSSION

Results from this study showed that temperature significantly influences parasitism rates and developmental time of *F. arisanus* and *D. longicaudata*. This is consistent with similar studies carried out for most tephritid fruit fly parasitoids (Hurtrel *et al*., 2001; Samira *et al*., 2006; Daane *et al*., 2008; Rousse *et al*., 2009; Kroder and Messing, 2010). From this study, a temperature range of 20 to 30 °C was found to be most suitable for the development of *F. arisanus*. In previous studies, Rwomushana *et al*., (2008) reported that larval development and survival of *B. invadens* was highest within this temperature range. *Fopius arisanus* is therefore expected to effectively parasitize and successfully develop from *B. invadens* since its optimal temperature for development lies exactly within that of its target host. The emergence of *F. arisanus* adults was significantly lower at 15 °C and no emergence occurred at 35 °C from this study and the results are consistent with observations from its target host, *B. invadens* (Rwomushana *et al*., 2008).

A lower developmental threshold of 13.5 °C was obtained for *F. arisanus* in this study. This is higher than the lower threshold of 8.8°C established for the egg stage of *B. invadens* (Rwomushana *et. al*., 2008). This implies that the pest will be able to successfully develop at lower temperatures than the parasitoid. This is not particularly surprising as *F. arisanus* tends to be less abundant at cooler, high elevation sites (Wong *et al*., 1983; 1984) which could be partly attributed to its inability to achieve successful development at lower temperatures. Temperature sensitivity was also reported in mass-reared *F. arisanus*, in which cohorts chilled for 1 hour and destined for aerial release techniques showed lower survival than those that were not chilled prior to release (Baeza-Larios *et al*., 2002). *Fopius ceratitivorous*, (originally collected from >1600 m elevation in central Kenya: Wharton *et al*., 2000) has been reported to have a broader temperature tolerance than *F. arisanus* (Kroder and Messing, 2010).

Based on a combination of shorter developmental times and high parasitism rates, the optimal temperature for development of *F. arisanus* from this study was found to be at 25 ºC. This is consistent with the optimal temperature stated for the laboratory rearing of this parasitoid from other tephritid pests. For example, Montoya *et al*. (2009) reared *F. arisanus* on *Anastrepha ludens* (Loew) at a temperature of 22 ± 2 °C, whilst Rousse *et al*., (2006) reared it on *Bactrocera zonata* (Saunders) at a temperature of 25 ± 2 °C, both of which are very close to the optimum of 25 ± 1 °C, obtained from this study when *F. arisanus* was reared on *B. invadens*.

Temperature had a significant influence on parasitism rates of *F. arisanus* on *B. invadens* (46-71%). Rousse *et al*., (2009) reported that temperature was strongly correlated with parasitism rates of *F. arisanus*. They further concluded that the foraging motivation of *F. arisanus* females seems to be determined by temperature, though humidity played a role as well. Ambient temperature has also been reported to have a significant impact on the wasps' interest in infested fruits, with increasing activity at higher temperatures (Kroder and Messing, 2010). Kroder and Messing also reported higher parasitism rates at 25 and 30 °C for *F. arisanus*. In this study however, significantly higher parasitism rates were recorded at 20 and 25 °C for *F. arisanus*. This study also showed significantly higher parasitism rates at 25 ºC compared to 30 °C, compared to that reported by Kroder and Messing (2010), where parasitism rates at 25 ºC and 30 °C temperatures were not significantly different. There was barely any parasitism at 18 °C in the study conducted by Kroder and Messing (2010). In this study, *F. arisanus* was able to achieve up to 46.4% parasitism on *B. invadens* at 15 °C. These differences may be attributed to the different methodologies employed in both studies. Whilst parasitoids were allowed to parasitize *B. invadens* eggs before the eggs were introduced into environmental chambers set at the different temperatures in this study, parasitism by *F. arisanus* females took place within the

environmental chambers set at the different temperatures in the studies by Kroder and Messing (2010). Also, *F. arisanus* was reared on *C. capitata* in the studies by Kroder and Messing whilst the parasitoid was reared on *B. invadens* in this study. The different host insects on which the parasitoid was reared on in the separate studies may have further accounted for the differences in results observed at temperatures below 20 °C. The suitable temperature range 20-30 °C which resulted in higher parasitism rates is consistent with studies carried out for other Braconid parasitoids. For example, Garcia-Martin *et al*., 2008 reported that optimum parasitism was realized for *Chelonus oculator* (F.), an egg-larval parasitoid of noctuid lepidopteran species at 20 and 30 °C.

At 20 °C, the pre-imago developmental period of *F. arisanus* males and females at was 35 and 37 days respectively. These values were close to that reported by Calvitti *et al*., (2002) at the same temperature (33±1.7 and 35±1.0 days) for *F. arisanus* males and females respectively reared on *B. oleae*. Pre-imago developmental period of *F. arisanus* at 30 °C (18.4 ± 0.2 and 20.2 ± 0.2 days) for males and females respectively did not differ much from the 20.0±0.4 and 21.8 ± 1.1 days reported for males and females respectively of its congener, *F. ceratitivorous* reared on *C. capitata* at 28 ± 2 °C (Bokonon-Ganta *et al*., 2005).

The most suitable temperature range for development and optimum parasitism of *D. longicaudata* on *B. invadens* was found to be at 20 – 25 ° C. Results are consistent with those reported for its congener, *D. tyroni* (Hurtrel *et al*., 2001).

Parasitism rates were significantly higher at 20 and 25 °C, compared to 30 °C. Although the highest parasitism rate was realized at 20 °C, developmental time at this temperature was significantly longer than at 25 °C. Based on a combination of shorter developmental times and high parasitism rates, the optimal temperature for development of *D. longicaudata* from this study was 25 ºC. Cancino and Montoya (2006) rearing *D. longicaudata* on *C. capitata* argued that a temperature of 26 °C in the emergence room leads to a shorter wait for adult emergence, thus reducing the need for space and keeping the conditions suitable for activities such as mating and feeding during the initial emergence period. They further state that when immature stages are developed at 26 °C, adults emerge in the required time, facilitating the coordination of shipments for mass releases. It confirms findings from this study which showed no significant difference in parasitism rates at 20 and 25 °C but a significantly longer developmental time at 20 °C compared to 25 °C. The optimum temperature of 25 °C also falls within the range of 24 – 27 °C reported by Lawrence *et al*. (1976) as suitable for the development of *D.longicaudata* eggs into adults on *Anastrepha suspense* (Loew).

Findings from this study are also consistent with that reported by Sime *et al*., (2006) that moderate temperatures (22 – 25 °C) are optimal for the development of both *D. longicaudata* and its congener, *D. kraussi on B. oleae*. The developmental time of 16.5 ± 0.2 and 18.7 ± 0.2 days for males and females respectively recorded at 25°C did not differ much from that reported by Sime *et al*., (2006) of 17.0 ± 0.7 and 20.8 ± 0.9 days for males and females respectively. It also did not deviate much from that reported by Paladino *et al*., 2010 of 16.0 days (both sexes pooled) for *D. longicaudata* reared on *C. capitata* at 25 °C.

D. longicaudata was able to complete development and emerged from puparia at 30°C. This is contrary to the findings of Sime *et al*. (2006) who reported that *D. longicaudata* was unable to complete development at this temperature on *B. oleae*. Hurtrel *et al*., (2001) also reported that no parasitoids emerged at 29 and 30 °C when *D. tyroni* was reared on *Ceratitis capitata*. The different results obtained may be due to differences in host insects and methodology.

The lower threshold for development of *D. longicaudata* in this study was found to be at 12.0°C. This is higher than the 9.19 °C found for *D. tyroni* on *C. capitata* (Hurtrel *et al*., 2001). The value is however comparable to that recorded for other Braconid larval-pupal parasitoids. For example, Mohamed *et al*., (2006) recorded a lower developmental threshold of 11.9 °C when *Psytallia cosyrae* was reared on *C. cosyrae* and Loni (1997) established a lower threshold of 11.7 °C for *P. concolor*. The lower temperature threshold of 12.0 °C for *D. longicaudata* is however slightly higher than the 9.4 °C, found for the larval stage of its target pest, *B. invadens*, suggesting that the target pest will be able to develop at relatively lower temperatures compared to the parasitoid. The degree days required by *D. longicaudata* (333.3) to complete development also did not deviate greatly from that recorded for *D. tyroni* (322.6) (Hurtrel *et al*., 2001).

Adult longevity of both *F. arisanus* and *D. longicaudata* was significantly influenced by temperature, with longevity significantly shorter at higher temperatures (30 and 35 °C) and longest at lower temperatures (15 and 20 °C). The negative effect of higher temperatures on the survival or longevity of Braconid parasitoids is well documented. For example, Mohamed *et al*., (2006) reported that longevity of *Psyttalia cosyrae* was greater at 25 °C than at 27 and 30 °C for both males and females. In this study, adult females of both *F. arisanus* and *D. longicaudata* lived significantly longer compared to males at all temperatures tested. This is consistent with

results reported by several authors for hymenopteran parasitoids (Ramadan *et al*., 1989; Bokonon-Ganta *et al*., 2005; Mohamed *et al*., 2006). Some opiine parasitoids may reabsorb mature eggs when deprived of hosts, which may result in increased longevity of non-ovipositing females (Ramadan *et al*., 1989). For example, Willard (1920) observed that actively ovipositing females of *P. fletcheri* lived about 1 month, which was far shorter than the 2-3-month life span of those kept from ovipositing. The adult longevity recorded in the present study was, in general, higher than that reported for other Braconid parasitoids. The different environmental conditions and host species used can mainly explain the differences between our results and the ones obtained by other authors. Furthermore, parasitoids used in this study were well fed and watered on a daily basis, compared to other studies where parasitoids were starved. Also, female parasitoids used in this study were non-ovipositing, a factor that has been reported to increase longevity due to the re-absorption of matured eggs (Ramadan *et al*., 1989). All these factors could have contributed to the increased adult longevity observed for parasitoids in this study.

Failure of exotic parasitoids to establish during biological control programmes may be attributed to many factors, but among the most important is the lack of adaptation of a species to new climatic conditions (DeBach, 1958, 1965). Temperature is regarded as one of the most important factors in acclimatization of introduced natural enemies (Loni, 1997). While insects normally develop faster at higher temperatures (Wagner *et al*., 1984), optima, maxima, and minima differ among species and an understanding of these traits has important consequences for establishing natural enemies in new environments. Our results demonstrate that both *F. arisanus* and *D. longicaudata* are able to complete development, survive and achieve high levels of parasitism on *B. invadens* under the temperature conditions as prevalent in agro-ecological zones suitable for horticultural production and should be able to contribute to the overall suppression of this

invasive pest species. *Fopius arisanus* is capable of adapting to the warm lowland regions whilst *D. longicaudata* is likely to adapt to the cooler highland regions of the sub region. However, the extrapolation of these findings into field condition must be done with caution. Indeed, extremes of temperatures are unlikely to occur for extended periods of time and all fruit fly developmental stages are sheltered from excessive of temperature (Fletcher, 1987). Overall, findings from this study provide some guidance for future mass rearing, field releases and modeling the impact of *F. arisanus* and *D. longicaudata* on *B. invadens*. It must be stated that other climatic factors such as humidity, rainfall and windspeed will also affect the adaptability of these parasitoids to the various regions and their effects need to be evaluated as well.

CHAPTER SIX

6.0 EFFECT OF HOST FRUIT SUBSTRATE ON THE PREFERENCE AND PERFORMANCE OF TWO INTRODUCED PARASITOIDS, *FOPIUS ARISANUS* AND *DIACHASMIMORPHA LONGICAUDATA* ON *BACTROCERA INVADENS*

6.1 INTRODUCTION

Tephritid fruit flies cause devastating losses to fresh fruit and vegetable crops and with expanding international trade have taken on added importance as major quarantine pests (White and Elson-Harris 1992; IAEA 2003). Of the 1.9 million tonnes of mangos produced in Africa annually, about 40% is lost due to damage caused by fruit flies (Lux *et al.*, 2003). In addition to the native fruit fly pest complex (mainly *Ceratitis* and *Dacus* species), which African farmers have struggled to control, four Asian species of the genus *Bactrocera* Macquart have invaded the continent, with two species appearing within a span of 3 years, further compounding the problem (Mohamed *et al.*, 2008). *Bactrocera invadens*, the most devastating of the four invasive species infests over 39 plant species but mango, *Mangifera indica* L. (Anacardiaceae) is the most preferred host (Ekesi e*t al*., 2006). In Africa, farmers normally resort to the use of conventional insecticides to help reduce losses caused by fruit flies with limited effect (Van Mele and Vayssieres, 2007).

Following the invasion of the continent by *B. invadens* and the lack of efficient indigenous parasitoids species to suppress the invasive pest (Mohamed *et al*., 2008; 2010), two Braconid parasitoids, *F. arisanus* and *D. longicaudata* were imported from Hawaii by the icipe-led African Fruit Fly Programme for evaluation and subsequent release against *B. invadens* in Africa. *Fopius arisanus* and *D. longicaudata* are two parasitoids that have been used in classical biological

control of different species of fruit flies in several parts of the world (Wharton & Gilstrap, 1983; Messing, *et al.*, 1993; Ovruski, *et al.*, 2000).

Tritrophic level interactions among the parasitoid, the host, and the host plant influence both search and recognition behaviors (Lewis *et al*., 1990; Tumlinson *et al*., 1993). The use of volatiles emanating from host fruits for host location behavior has been demonstrated in several parasitoid species that attack late instars of several species of tephritid flies (Greany *et al*., 1977; Eben *et al*., 2000; Jang *et al*., 2000; Henneman *et al*., 2002; Altuzar *et al*., 2004).

Results from laboratory experiments carried out by Mohamed *et al*., (2008; 2010) have shown that the introduced parasitoids (*F. arisanus* and *D. longicaudata*) are effective against *B. invadens* and other indigenous fruit flies. Although information abounds on the effect of tritrophic interactions on the performance of *F. arisanus* and *D. longicaudata* on several fruit fly species, no such information exists for *B. invadens*. Since host fruit volatiles play an important role in parasitoid host location and parasitism, it is necessary to determine the effect of different host fruits on the overall performance of the introduced parasitoids on *B. invadens* in a tritrophic system. The objective of this study was therefore to evaluate the effect of tritrophic interactions on the performance of *F. arisanus* and *D. longicaudata*, with *B. invadens* as the target pest.

6.2 MATERIALS AND METHODS

6.2.1 Host fruits

The following host fruits were utilized for the host fruit preference studies: mango (*M. indica)*, *Pawpaw (C. papaya), Guava (P. guajava),* Sweet orange (*C. sinensis*), Marula (*S. birrea*) and Tropical almond (*T. cattapa*). To determine the effect of host fruit on parasitoid performance, four cultivated fruits (mango, *Pawpaw*, *Guava*, Sweet orange and a wild host fruit, Custard apple (*Annona reticulata*) were used. Due to methodological problems (i.e. scarcity of fruits and difficulty of obtaining sufficient quantity of fruit pulp for the experiments (see below), *S. birrea* and *T. catappa* were not included in the parasitoid performance trials.

6.2.2 Egg and larval collection

Eggs of *B. invadens* were collected from the stock colony by providing inverted plastic cups to mature female flies. The inner parts of the plastic cups were lined with filter paper sprayed with mango juice and covered at the top with aluminium foil. They were placed on 9-cm diameter Petri dishes lined with moistened filter paper. Each plastic cup was pierced several times with an entomological pin (38 mm long, 0.3 mm diameter) to facilitate oviposition. The cups were exposed to the ovipositing female flies at about 16:00 h on the day preceding the test. Eggs were collected from the cups the morning after; using a moistened fine camel's hair brush. Larvae of *B. invadens* exposed to *D. longicaudata* were directly picked from artificial liquid diet (on which they were being raised) using a soft forceps.

6.2.3 Bioassays

6.2.3.1 Effect of host fruit substrate on parasitoid preference

This experiment was conducted using a 'choice' set up. Fruit peels (4 x 3 cm) from mango, pawpaw, citrus, guava, marula and tropical almond were cut and placed on a double layer of sponge pieces (Spontex make, Nairobi, Kenya) and transferred to an oviposition unit (Mohamed *et al*., 2010). Using a camel's hairbrush, 20 eggs of *B. invadens* (5-20 hour old) were counted onto each fruit peel in the oviposition unit and the units were thereafter covered with tight-fitting organza lids. Thirty mated, experienced female wasps (7–10 days old) of *F. arisanus* were transferred into a Perspex rearing cage (60 cm x 30 cm x 15 cm).

The oviposition units were inverted and then introduced into the rearing cage containing the parasitoids to avoid differential attraction by female wasps to different oviposition units based on the sequence of their introduction in the experimental cage (Mohamed *et al*., 2008). Thereafter, the oviposition units were re-inverted after all units had been introduced into the cage. Oviposition units (each with a different fruit peel) were arranged in two rows of three across the length of the perspex cage.

Observations on parasitoid behaviour were initiated 5 min later by recording the number of female wasps searching and/or ovipositing in *B. invadens* on each fruit peel at 15-min intervals for 3 hours. New fruit peels and a new cohort of parasitoids was used in each replicate. A separate bioassay (involving all the fruits with the exception of mango) was also conducted. This was carried out to ascertain the effect of mango on parasitoid preference in the first bioassay as it

was the fruit on which *B. invadens* eggs had been exposed to *F. arisanus* in the laboratory rearing of the parasitoid over several generations.

For *D. longicaudata*, 20 late 2nd instar larvae were counted into small Petri dishes (3.7 cm diameter by 0.5 cm depth) containing the pulp of each fruit (mango, pawpaw, guava, citrus, marula). Tropical almond was excluded because its pulp was usually very fibrous and dry, and therefore difficult to obtain a juicy fruit pulp out of it. Each fruit pulp was then covered with a flattened peel of the same fruit (3.5 cm diameter). The peels were perforated several times using an entomological pin to aid oviposition. The Petri dishes were then introduced into a Perspex cage (60 cm x 30cm x 15cm) containing 30 mated, experienced *D. longicaudata* females. Observations on parasitoid behaviour were initiated 5 min later and the number of searching and/or ovipositing female wasps was recorded at 15-min intervals for 3 h. Experiments were replicated 10 times in a completely randomized design.

6.2.3.2 Effect of host fruit on puparia recovery and parasitism rates

Cut peels (4 x 3 cm) of mango, pawpaw, citrus, guava and custard apple were placed on double layers of sponge pieces (Spontex make, Nairobi, Kenya) and placed in the oviposition units. Using a camel's hairbrush, 200 eggs of *B. invadens* were counted onto each fruit peel and the oviposition units thereafter covered with tight-fitting organza lids. Ten mated, experienced female wasps of *F. arisanus* (7–10 day old) were introduced into 5 different Perspex cages (12 cm x 12 cm x 12 cm). Each oviposition unit was introduced into one of these perspex cages and 10 *F. arisanus* females were allowed foraging and parasitizing of *B. invadens* eggs for 24 hours. The oviposition units were removed thereafter and the individual fruit peels with the eggs were

placed on pulps of the same fruit in Petri dishes. For example, the citrus peel with parasitized *B. invadens* eggs was placed on citrus pulp.

The Petri dishes were placed in plastic containers with (12 x 9 x 5.5 cm) with a layer of sand at the bottom to aid larval pupation. The plastic containers were covered with a fine net and a lid. As the quantity of pulp depleted as result of larval feeding, it was replenished with fresh fruit pulp as necessary. When the larvae attained their full size, they jumped *ad libitum* into the sand provided for pupation. Those larvae that failed to jump were aided with the help of a soft forceps. Puparia were sieved from the sand and held in Perspex cages (12 cm x 12 cm x 12 cm) for fly and/or parasitoid emergence. The total number of wasps (males and females) as well as the developmental time (number of days from exposure to parasitoids until 50% of parasitoids emerged) was recorded against each fruit. The experiment was conducted in a Completely Randomized Design and replicated 10 times.

For *D. longicaudata*, 50 late 2nd instar larvae were counted into small Petri dishes (3.7 cm diameter 0.5 cm depth), each containing fruit pulp (mango, pawpaw, guava, citrus, marula). Each fruit pulp was then covered with a peel of the same fruit (3.5 cm diameter). The peels were perforated several times using an entomological pin to aid oviposition. The Petri dishes were then introduced into individual Perspex cages (12cm x 12cm x 12cm) containing 5 mated experienced *D. longicaudata* females for 24 hours. Subsequent procedures were the same as described for *F. arisanus* above.

6.2.4 Data collection and analysis

The mean number of female parasitoids searching and/or ovipositing across the different host fruits at each 15 minute interval over the 3 hour observation period was calculated. The observational means were then divided by the mean number of female parasitoids used in the bioassay and multiplied by 100 to obtain the Mean Percentage. Percent parasitism was obtained by dividing the total number of adult parasitoids produced for each replicate by the total number of puparia recovered and multiplying by 100. Sex ratio (proportion of females) was calculated by dividing the number of emerged female parasitoids by the total number of emerged parasitoids.

Log10 (x + 0.5) and arcsine square root transformation were used on counts and percentages, respectively (Sokal and Rohlf 1981) before being subjected to one-way analysis of variance. When treatment effects were significant (i.e. $P < 0.05$), treatment mean values were separated using Student–Newman–Keuls (SNK) test.

6.3 RESULTS

6.3.1 Effect of host fruit substrate on parasitoid behaviour

All the fruits tested were attractive to the females of *F. arisanus* and *D. longicaudata*. Figures 6.1, 6.2 and 6.3 depict the mean percentage of female wasps searching and/or ovipositing in *B. invadens* eggs on the different host fruits over a three (3) hour exposure period. Host fruit had a significant effect on the total number of *F. arisanus* females searching and/or ovipositing in eggs of *B. invadens* (F= 65.9, df= 5, 66, P= 0.0001(mango included) and F= 31.8, df= 4, 55 and P= 0.0001 (mango excluded) (Table 6.1). The highest number of females searching and/or ovipositing was recorded on mango (50.7±3.5) and the lowest on marula (10.3±1.0) and tropical

almond (8.2±0.7), respectively. When mango was excluded from the bioassay, the highest number of *F. arisanus* females searching and/or ovipositing was recorded on pawpaw (36.7±2.8) and the lowest on tropical almond (13.3±0.9) and citrus (12.7±1.0), respectively (Table 6.1). Host fruit also had a significant effect on the total number of *D. longicaudata* females searching and/or ovipositing in larvae of *B. invadens* (F= 46.3, df= 4, 55 and P= 0.0001) (Table 6.1). The highest number of females searching and/or ovipositing was recorded on mango (31.8±0.9) and the lowest on marula (16.2±1.1) and guava (18.5±0.5) (Table 6.1).

6.3.2 Effect of host fruit on puparia recovery, parasitism rate and developmental time

Puparia and adult parasitoids were recovered from each of the host fruits tested in the study. Host fruit substrate had a significant effect on puparia recovered (F =77.1, df =4, 45, *P*=0.0001) and parasitism rate (F =139.6, df =4, 45, *P*=0.0001) of *F. arisanus*. It also had a significant effect on developmental time (F =18.4, df =4, 45, *P*=0.0001). Pawpaw and mango yielded the highest numbers of puparia (130.7 ± 4.8 and 128.2 ± 4.0) whilst the lowest number of puparia was recovered from custard apple (90.2 ± 3.8) (Table 6.2). Parasitism rate was also highest in pawpaw and mango (63.2 ± 1.7 and 62.3 ± 2.2, respectively) and lowest in custard apple (11.2 ± 1.4). Developmental time was significantly longer in custard apple and citrus (24.1 ± 0.3 and 24.0 ± 0.3 days, respectively) compared to mango, pawpaw and guava (Table 6.2).

For *D. longicaudata*, host fruit substrate did not have a significant effect on puparia recovered (F =1.98, df =4, 45, *P* =0.113)**.** It, however, had a significant effect on parasitism rate (F =12.0, df =4, 45, *P*=0.0001) and developmental time (F =5.0, df =4, 45, *P*=0.002). Parasitism rate was significantly higher in mango and pawpaw (41.2 ± 2.3 and 34.4 ± 3.6) compared to guava, citrus and custard apple (Table 6.3). Developmental time was comparable among all the host fruits

tested but longest in custard apple (19.4 ± 0.2 days) and shortest in pawpaw (18.0 ± 0.3 days) (Table 6.3).

Table 6.1. Mean number of female parasitoid respondents (searching and/or ovipositing) on *B. invadens* eggs and larvae on six different host fruits

	F. arisanus		*D. longicaudata*
Fruit	+ Mango	- Mango	
Mango	50.7 ± 3.5a		22.3 ± 0.9b
Pawpaw	25.7 ± 1.5b	36.7 ± 2.8a	22.9 ± 0.9b
Guava	16.3 ± 1.9c	24.3 ± 1.7b	18.5 ± 0.5c
Citrus	16.7 ± 1.5c	12.7 ± 1.0c	31.8 ± 0.9a
Marula	10.3 ± 1.0cd	27.2 ± 1.8b	16.2 ± 1.1c
Tropical almond	8.2 ± 0.7d	13.3 ± 0.9c	

Means followed by the same letter in the same column are not significantly different (P <0.05), Student Newman-Keul's test.

Table 6.2. Mean puparia recovered, parasitism rate and developmental time of *F. arisanus* reared on different host fruit substrate following exposure to eggs of *B. invadens*

Host fruit	Puparia recovered	% puparia recovered	Total parasitoid no.	Parasitism rate (%)	Developmental time (days)
Mango	128.2 ± 4.0a	64.1 ± 2.0a	80.4 ± 5.0a	62.3 ± 2.2a	21.6 ± 0.3a
Pawpaw	130.7 ± 4.8a	65.4 ± 2.4a	82.5 ± 3.4a	63.2 ± 1.7a	21.7 ± 0.3a
Citrus	54.4 ± 2.2b	27.2 ± 1.1b	15.4 ± 1.2b	28.6 ± 2.4b	24.0 ± 0.3b
Guava	111.8 ± 2.7c	55.9 ± 1.4b	63.3 ± 2.5c	56.6 ± 1.7a	22.5 ± 0.3a
Custard apple	90.2 ± 3.8d	45.1 ± 1.9b	10.0 ± 1.2d	11.2 ± 1.4c	24.1 ± 0.3b

Means followed by the same letter in the same column are not significantly different (P <0.05), Student-Newman-Keul's test.

Table 6.3 Mean puparia recovered, parasitism rate and developmental time of *D. longicaudata* reared on different host fruits following exposure to larvae of *B. invadens*

Host fruit	Puparia recovered	% puparia recovered	Total parasitoid no.	Parasitism rate (%)	Developmental time (days)
Mango	40.3 ± 0.8a	80.6 ± 1.6ab	16.6 ± 0.9a	41.2 ± 2.3a	18.5 ± 0.2bc
Pawpaw	41.0 ± 0.9a	82.0 ± 1.8ab	14.0 ±1.4a	34.4 ± 3.6a	18.0 ± 0.3c
Guava	38.1 ± 1.4a	76.2 ± 2.8ab	9.0 ± 0.8b	24.0 ± 2.4b	18.4 ± 0.3bc
Citrus	41.3 ± 1.8a	82.6 ± 3.5a	8.9 ± 1.1b	21.5 ± 2.3b	19.2 ± 0.3ab
Custard apple	36.7 ± 0.8a	73.4 ± 1.5b	7.0 ± 0.8b	19.3 ± 2.4b	19.4 ± 0.2a

Means followed by the same letter in the same column are not significantly different (P <0.05), Student-Newman-Keul's test.

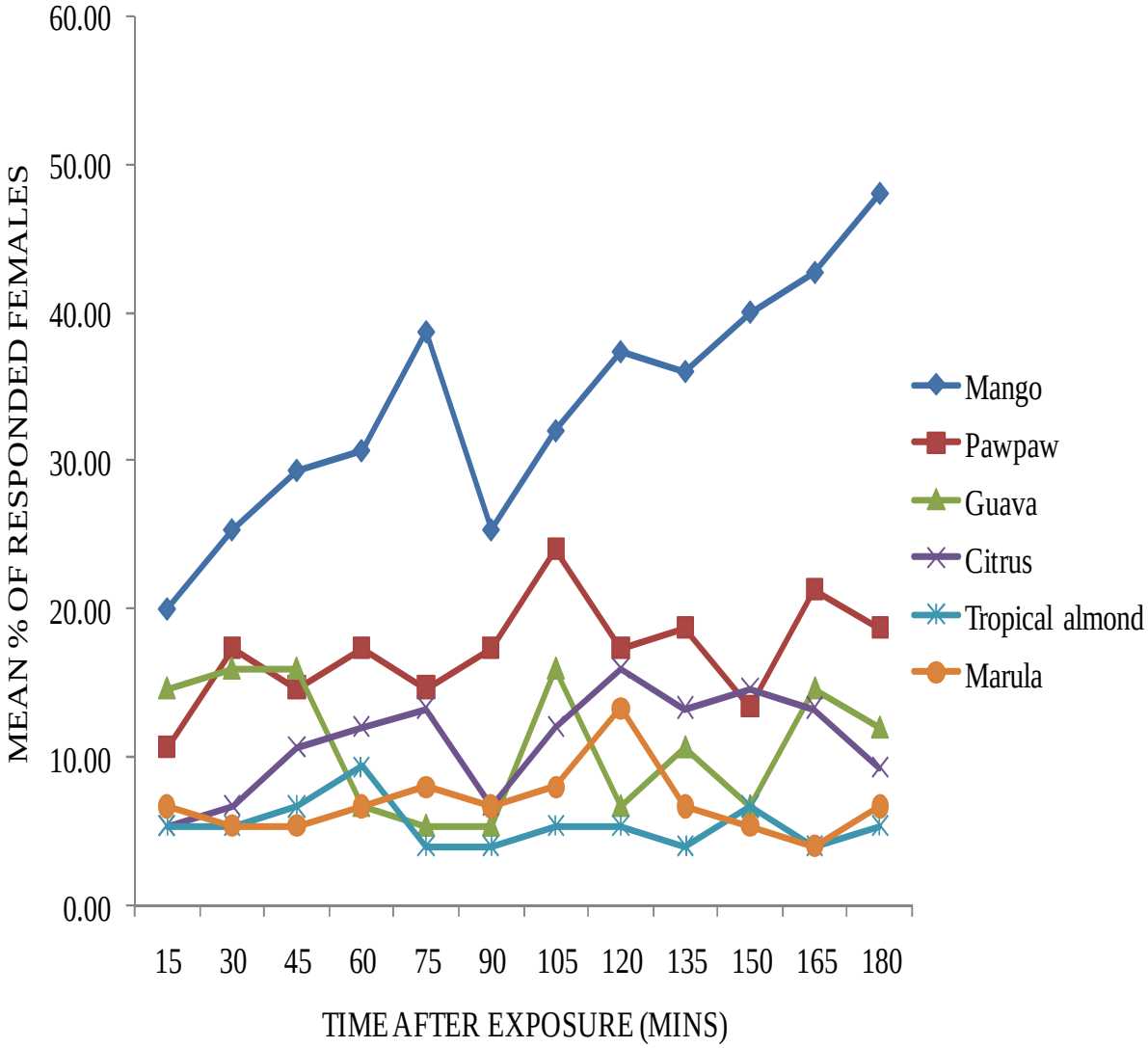

Fig 6.1 Temporal response of *F. arisanus* females (searching and/or ovipositing) to *B. invadens* eggs on six different host fruits (mango included) over a 3-hour exposure period.

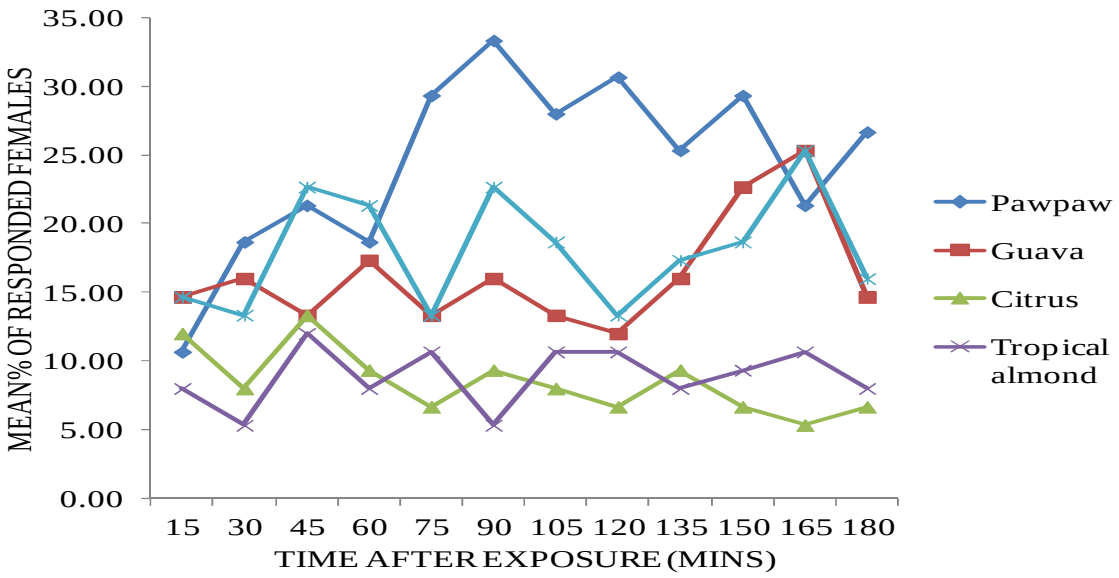

Fig. 6.2 Temporal response of *F. arisanus* females (searching and/or ovipositing) to *B. invadens* eggs on six different host fruits (mango excluded) over a 3-hour exposure period.

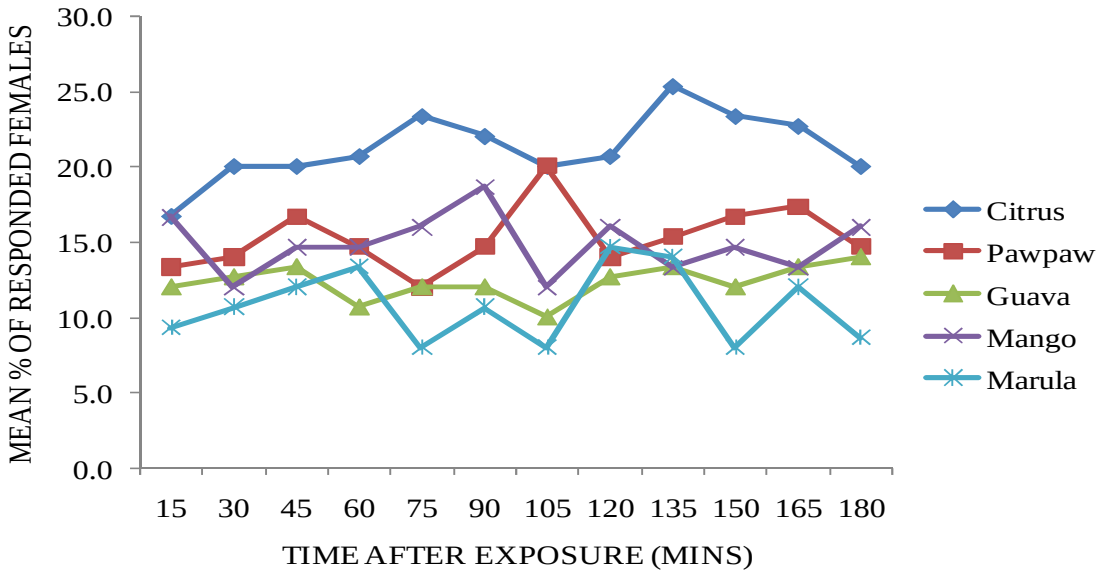

Fig 6.3 Temporal response of *D. longicaudata* females (searching and/or ovipositing) to *B. invadens* larvae on six different host fruits (mango excluded) over a 3-hour period.

6.4 DISCUSSION

Results from this study showed a significant effect of host fruits on preference and performance of females of *F. arisanus* and *D. longicaudata*. The differential attraction of female parasitoids to fruit volatiles, observed in the behavioural experiment, indicates that both parasitoid females have an innate response toward olfactory cues and use fruit volatiles during host location process. Our findings support results of earlier studies which demonstrate that Braconid parasitoids use fruit volatiles during host location behaviour (Leyva *et al.*, 1991; Liquido, 1991; Messing and Wong 1992; Eben *et al.*, 2000; Bautista *et al*., 2004; Altuzar *et al*., 2004; Rousse *et al*., 2007). Rousse *et al*. (2007) reported that observed preferences to different host fruits by parasitoids may be as a result of variations in concentrations of volatiles between the fruit species (Rousse *et al*., 2007).

Mango was the most preferred host fruit by *F. arisanus* in this study, followed by pawpaw and to a lesser extent guava and citrus. Rousse *et al*., (2007) reported that *F. arisanus* females respond positively to synomones from infested mango fruits, but the response was not significantly different to synomones from guava. Altuzar *et al.* (2004) also reported that significantly more *F. arisanus* females flew upwind and landed on guava compared to citrus fruits in a 'choice situation'. On the other hand Bautista *et al*., (2004) observed no significant difference in the preference of *F. arisanus* to five host fruits infested with eggs of the melon fly, *B. cucurbitae*.

The significantly high number of *F. arisanus* females that searched and/or oviposited in *B. invadens* eggs on mango in our study may be explained by the fact that mango had been used in the rearing of the parasitoid over several generations in the laboratory. However, when mango was excluded from the various host plants species tested, pawpaw recorded significantly higher

number of searching/ovipositing females than guava. It is probable that the presence of mango (on which *B. invadens* eggs have been exposed to the wasps for several generations) may have probably masked the effect of volatiles emanating from the other fruits. Overall, the contrasting results could be attributed to either host plant species, volatiles concentration emitted from host fruits peels during the experimental period as well as methodological differences in the various studies. *Terminalia cattapa* recorded the lowest number by *F. arisanus* searchings and/or ovipositions in this study. This is perhaps surprising given that high parasitism rates by *F. arisanus* on *B. dorsalis* and *B. zonata* infesting *T. cattapa* fruits has been reported in Hawaii and Reunion islands respectively (Eitam and Vargas, 2007; Quilici *et al*., 2008). However, given that host fruit preference behavioural studies were carried out under a 'choice experimental set up', the presence of the other five host fruits may have influenced the number of *F. arisanus* females attracted to *T. cattapa*. Unfortunately, *T. cattapa* fruits were not included in our 'no-choice' parasitism experiment; hence no parasitism rates were obtained for this fruit in the present study.

Diachasmimorpha longicaudata females were attracted to larvae of *B. invadens* in all the fruits peels tested although citrus was the most preferred followed by mango and pawpaw and marula was the least preferred. Leyva *et al*., (1991) reported that citrus volatiles attracted almost twice as many *D. longicaudata* female parasitoids as volatiles of mango, or peach. On the contrary Eben *et al*., (2000) reported no preference for infested mangoes or grapefruits when the volatiles of both fruits were presented simultaneously to *D. longicaudata* females. In another study, Jang *et al*., (2000) reported that *D. longicaudata* females were five times more likely to land on yellow plastic spheres emitting the odor of ripe guava fruit than to spheres emitting clean air. Jang *et al*., (2000) further stated that *D. longicaudata* females may be instinctively attracted to foliage and to

fruit odor but that landing (arrestment) and oviposition are influenced more by odor than by the appearance of fruit or foliage. It would appear that cues related to both visual (yellow citrus peels) in addition to olfaction contributed to the higher preference of *D. longicaudata* for citrus over and above the other fruits tested in our study. In a related larval parasitoid, *P. fletcheri*, female parasitoids showed a higher preference for cucumber and zucchini compared to tomato and egg plant (Baustita *et al*., 2004). In general, our findings on the attraction of *D. longicaudata* to fruit volatiles from mango, pawpaw, citrus and guava therefore agree with those of previous authors.

Several reports also indicate that larval parasitoids are more attracted to infested fruits compared to uninfested ones. For example, Messing *et al.*, (1996) demonstrated using an olfactometer that the odour of decomposing foliage and fruit were primary stimuli for the host-searching behaviour of *P. fletcheri*. Other studies have corroborated the relatively high preference for infested compared to uninfested fruits by Braconid parasitoids (Eben *et al*., 2000; Carrasco *et al*., 2005; Rousse *et al*., 2007). Though it is impossible to confirm this from the current study (since all the fruits tested were infested), it makes sense for parasitoids to orient more towards fruits infested by their hosts as this is more likely to result in the propagation of successive generations.

Fruit substrate had a significant effect on puparia recovered for *F. arisanus*. Significantly higher number of puparia was recovered from mango and pawpaw compared to the other fruits. Citrus yielded the least number of puparia. Bautista and Harris (1996) also reported significant effect of fruit substrate on pupal recovery when *F. arisanus* was reared from *B. dorsalis* and *C. capitata*. Chemicals in citrus peel are toxic to eggs and larvae of tephritid fruit flies (Bautista and Harris,

1996). This may have resulted in the poor development of most *B. invadens* eggs, and subsequently the very low number of puparia recovered from citrus for *F. arisanus*.

Mean number of puparia recovered for *D. longicaudata* was comparable among all fruit substrates tested and mean percent puparia recovered was above 70% for all fruits. The relatively high number of puparia recovered for *D. longicaudata* across all fruits may be attributed to the low susceptibility of *B. invadens* larvae to mortality compared to eggs. The relatively low number of puparia recovered in *F. arisanus* experiments compared to *D. longicaudata* may also be due to the fact that *B. invadens* were exposed to *D. longicaudata* in citrus pulp as late 2nd instar larvae whilst eggs were exposed to *F. arisanus* on citrus peels, which Bautista and Harris (1996) described as toxic to fruit fly eggs. Mortality at the early developmental stages may have been overcome through the provision of later developmental stages for *B. invadens* parasitism by *D. longicaudata*.

Fruit substrate also had a significant influence on parasitism rates of *F. arisanus* and *D. longicaudata*. Parasitism rates by *F. arisanus* were higher in pawpaw and mango compared to guava, citrus and custard apple. Bautista & Harris (1996) reported that parasitization of *B. dorsalis* by *F. arisanus* was influenced by fruit substrate; percent parasitism was significantly higher when host was reared on *Musa sapientum* Linn, comparable when reared on *Carica papaya* Linn, *T. cattapa* and *M. indica* but significantly lower when reared on *Citrus aurantifolia* Christm. Our results are consistent with the observations of these authors. Bautista & Harris (1996) also suggested that chemicals in citrus peel are toxic to eggs and larvae of tephritid fruit flies which could probably be the reason for the low parasitization of *B. invadens* by *F. arisanus* in citrus fruits, as observed in this study. In another study, Bautista *et al.*, (2004) reported that

eggs of *B. cucurbitae* in zucchini, *Cucurbita pepo* L. were more heavily parasitized compared to four other host fruits.

Differences in parasitism rates for different fruits under field conditions have also been reported. For example, Eitam and Vargas (2007) reported significantly lower parasitism rates for *F. arisanus* in commercial papaya compared to common guava and other fruits for both tree and ground collected samples. Similar studies on *D. longicaudata* confirm that parasitization of tephritid hosts is greatly influenced by fruit substrate. For example, Eben *et al*., (2000) reported that *D. longicaudata* females reacted more strongly to the odour of mangoes and parasitism rates were higher in *Anastrepha ludens* (Loew) larvae reared in mango than in citrus. This is consistent with results obtained in this study where parasitism rate was significantly higher in *B. invadens* reared on mango compared to citrus.

Leyva *et al*., (1991), however reported no significant difference in percentage parasitism of *D. longicaudata* when *A. ludens* was reared on peach, mango and citrus, although percentage parasitism was generally higher in mango compared to citrus. Rodriguez (1991) also found under semi-natural and laboratory conditions that a higher number of *D. longicaudata* females were attracted to mangoes infested by *A. obliqua* than on oranges infested by *A. ludens*. However, parasitism rates in the respective fruits were not significantly different. Effect of fruit substrates on parasitization by other larval-pupal Braconids has also been documented. For example, Bautista *et al*., (2004) found that larvae of *B. cucurbitae* in zucchini were heavily parasitized by *P. fletcheri* compared to those infesting other fruit types.

In conclusion, the findings of this study confirm the fact that fruit substrates play a significant role in the host location behaviour of *F. arisanus* and *D. longicaudata*, and this greatly influences the parasitization of *B. invadens*. Parasitoid preference for certain fruits may not be fully understood but it could be an important factor to consider during mass rearing and releases of parasitoids in the natural habitat (Bautista *et al*., 2004). The potential effects, exerted by the food substrate of fruit fly larvae on the quality of mass reared and field released parasitoids might be of considerable importance for the success of biological control programs (Eben *et al*., 2000). The information generated from this study could serve as a basis for future mass rearing and field releases of *F. arisanus* and *D. longicaudata* to suppress populations of *B. invadens*.

CHAPTER SEVEN

7.0 INTERACTIONS INVOLVING *FOPIUS ARISANUS*, THE AFRICAN WEAVER ANT, *OECOPHYLLA LONGINODA* AND *BACTROCERA INVADENS*

7.1 INTRODUCTION

Frugivorous tephritid fruit flies are major threat to fruit and vegetable production and export wherever they occur (White and Elson-Harris 1992; Purcell 1998). Most tephritid pest species of economic importance belong to the genera *Anastrepha* Schiner, *Bactrocera* Macquart, *Ceratitis* MacLeay, *Dacus* Fabricius and *Rhagoletis* Loew (White and Elson-Harris 1992). In Africa, the native *Ceratitis* genus, and invasive *Bactrocera* are the most economically important, causing substantial losses to fruits and vegetables as well as limiting exports due to quarantine restrictions. The presence of the invasive fruit fly, *Bactrocera invadens* on the African continent has major economic impacts (De Meyer *et al*., 2009), severely limiting the export of fruits to large lucrative markets in South Africa, Europe, the Middle East, Japan and USA, due to quarantine restrictions on fruit fly-infested fruits (Ekesi, 2010).

Being an alien pest, *B. invadens* lends itself particularly to classical biological control. Since native parasitoid species are incapable of counteracting its immune system, the ideal alternative is to identify a co-evolved parasitoid of this pest and reunite the parasitoid with the invasive fruit fly species in Africa (Mohamed *et al*., 2008). In 2006, *F. arisanus*, an egg parasitoid credited with outstanding successes of classical biological control of fruit flies in several parts of the world (van den Bosch and Haramato 1951; Clausen *et al*., 1965; Vargas *et al*., 1993) was imported from Hawaii for testing and potential field releases against *B. invadens*. In preliminary

laboratory studies, Mohamed *et al*., (2010) reported parasitism rates above 70% on *B. invadens* and concluded that *F. arisanus* could be a potent biocontrol agent against the pest.

Currently IITA in close collaboration with *icipe* are in the process commencing large-scale field releases of *F. arisanus* in several African countries threatened by *B. invadens* invasion. The success of a classical biological control agent depends upon a number of factors and interaction with resident species in the introduced range is considered to be crucial. If resident organisms are detrimental to the classical biological control agent, i.e. are causing biotic interference, then the biocontrol agent's establishment and/or efficacy can be inhibited (Goeden and Louda, 1976). Biotic interference has been shown to reduce biological control efficacy in many situations and recent reviews have implicated biotic interference as causing close to 20% of classical biological control failures (Kimberling, 2004). Determining whether biotic interference is likely to occur on an introduced species should therefore increase the predictability of biological control. Although biotic interference can occur via trophic interactions, competition and/or through interactions with protection mutualists, predation against arthropod biological control agents is the most common form (Chacón *et al*., 2008).

There are numerous examples of predatory ants deterring predators and parasites of different insect pests (Cudjoe *et al*., 1993; Wimp and Whitham, 2001; Jahn et al, 2003). *Oecophylla longinoda* is reported not to attack parasites of their attended hemipterans but others can be severely hampered (Buckley and Gullan, 1991; Way and Khoo, 1992). Although *O. longinoda* and *F. arisanus* have been experimentally proven to provide a good control of *B. invadens*, the application of both control agents must not result in biotic interference among them. While most of the adverse effects of predatory ants are associated with trophobiotic relationships between the

ants and their attended hemipteran pests, preliminary observations have shown significant aggression of *O. longinoda* to *F. arisanus* especially at high densities (Ekesi *et al*., unpublished data).

When an herbivore is abundant and or affects other species and trophic levels, community structure may be altered (Dickson and Whitham, 1996) and since *O. longinoda* forage at different levels of the ecosystem, there is the need for a systematic study to assess interaction between *F. arisanus* and *O. longinoda* before recommendation on the combined use of both biocontrol agents. The objective of this study therefore, was to evaluate possible biotic interference by *O. longinoda* in the overall performance of *F. arisanus* as a biological control agent against *B. invadens*. The mechanisms underlying the control of *B. invadens* as a result of the predatory and deterrent activities of *O. longinoda* was also investigated.

7.2 MATERIALS AND METHODS

7.2.1 Establishment and maintenance of *O. longinoda* colony

Nests of *O. longinoda* (queen as well as worker nests) were obtained from Muhaka, in the Coast Province, Kenya. The nests were carried in plastic containers (45 x 30 x 15 cm), with openings at the side and on the lid which were covered with fine netting material to allow for ventilation. They were transported from Muhaka to *icipe's* headquarters in Nairobi, Kenya. The nests were removed from the plastic containers and carefully placed on branches of *Ficus benjamina*, L. (Moraceae) grown in plastic bags in a greenhouse. When leaves of the nests brought from the field started drying up, the ants weaved new nests using fresh leaves of *Ficus benjamina* on which they had been placed. The edges of the table on which the potted plants were placed were lined with sticky material (Tangle trap paste) to prevent other insects (especially the black

predatory ants, *Pheidole megacephala*) from climbing onto the *Ficus* seedlings and preying on the weaver ants. The table stands were also placed in containers filled with soapy water to further deter other predatory insects from getting access to the ants. The water in the containers was replenished as necessary.

Ants were fed thrice a week with adult stem borer moths, *Chilo partellus* S. by holding slightly paralyzed moths very close to nests and allowing worker ants to grasp them firmly and finally, transporting them into the nests. Additional food source were provided using cotton wool soaked in saturated sugar solution and mounted on Petri dishes around nests to mimic honeydew in the field. This was replaced after every 48 hrs, when the soaked cotton wool dried up. The branches and leaves of the seedlings were regularly cleared of spider nests and other unnecessary debris when necessary. Seedlings were watered regularly and the soil anchoring their roots fertilized every three months. Pruning of the plants was also done regularly to prevent branches and leaves from touching the edges of the greenhouse to minimize ants escape access by other predatory fauna.

7.2.2 Bioassays

7.2.2.1 Effect of varying densities of *O. longinoda* on *B. invadens* oviposition

To ascertain if the presence of O. longinoda deter oviposition by *B. invadens*, mango dome (half cut mango with the seed and pulp scooped out and perforated several times using an entomological pin to facilitate *B. invadens* oviposition) was placed on a Petri dish inside a Perspex cage (12 x 12 x 12 cm). Weaver ants were then introduced into the cages at varying densities of 2, 4, 6, 8 and 10 per mango dome. Thereafter, five, experience *B. invadens* females (10-14 day old) were released into the cages and observations on fly behaviour were initiated 5

min later after release. The total number of female flies probing and/or ovipositing in mango domes was recorded at 15-min intervals for 3 hrs. Similarly, the total number of times that fruit fly probing and/or oviposition was interfered with as a result of the mere presence or physical aggression by *O. longinoda* was also recorded. At termination of each replicate, total fecundity over the 3 hour of exposure was recorded across all the ant densities tested. Each ant density was replicated 6 times and a new cohort of ants and flies was used in each replicate. Two observers were involved in the collection of the behavioural data.

7.2.2.2 Effect of varying densities of *O. longinoda* on oviposition and parasitism by *F. arisanus*

Weaver ants at densities of 2, 4, 6, 8 and 10 were released into a Perspex cage (12 x 12 x 12 cm). Ten holes perforated with an entomological pin were made on the surface of one fully ripe whole mango fruit (cultivar Apple). Five eggs of *B. invadens* (5-20 hour old) were counted and gently transferred into each hole on the mangoes (total number of eggs per fruit = making a total of 50 eggs). Each mango fruit (infested with *B. invadens* eggs) was then placed in separate Perspex cage. Thereafter, ten experienced *F. arisanus* females were aspirated from the rearing cages into the Perspex cage and observations on parasitoid behaviour initiated 5 min later. Neither the parasitoids nor their parents had any prior experience with weaver ants. Parameters taken included: the total number of parasitoids searching and/or ovipositing at 15-min intervals for 3 hrs, total number of times searching and/or oviposition by parasitoids was interfered with as a result of the mere presence and/or predation by ants, and total parasitoid mortality (due to weaver ant predation) at each ant density tested. Each ant density was replicated 6 times and a new cohort of ants and parasitoids was used in each replicate. Two observers were involved in the collection of the behavioural data.

At the end of the 3 hrs of exposure, mangoes were removed from the cages and transferred into separate plastic containers (20 x 15 x 15 cm). The bottom of the containers was covered with sterilized sand to aid larval pupation. The top of the containers were covered with a fine netting material and a tight fitting hollow lid to aid ventilation. Containers were drained of mango juice from time to time to prevent the sand from becoming soggy. When larvae reached full maturity, they jumped *ad libitum* into the sand provided at the bottom of the containers for pupariation. Mangoes were split opened and larvae that failed to jump were assisted using a soft forceps. Puparia were later collected and held in Perspex cages for emergence of flies and/or parasitoids. The total number of emerged parasitoids and/or flies was recorded for each ant density tested.

7.2.2.3 Effect of cues deposited by *O. longinoda* on mango fruit on oviposition and parasitism by *F. arisanus*

Weaver ants could be induced to deposit their pheromones on substrates (Jander and Jander, 1979). Since ant cues have been reported to deter oviposition by fruit flies (Van Mele *et al*., (2009), this study examined the role of ant cues in preventing oviposition and parasitism by *F. arisanus*. The methodology employed was similar to that described in Van Mele *et al*., (2009). Four mango fruits were perforated as described above. Two of the mango fruits were then transported to the greenhouse where *O. longinoda* were maintained. In the greenhouse, the two mango fruits were put in a plastic container (20 x 15 x 15cm). Two nests (each with approximately 250 weaver ants) were placed in the same container. The top of the container was covered with a fine mesh (22 × 18 cm) and tightly closed to confine the ants to expose the mango fruits to the ant pheromones. Ants were provided with sugar and water as source of food and exposure was completed after 48 h. These mangoes were referred to as ‘ant-exposed’ and the

remaining two mangoes termed ‘ant-unexposed’. At the end of the exposure period, the ant-exposed mangoes were carefully removed from the plastic container with a large forceps to minimize interference with the fruit surface and each hole on the mango was inoculated with five eggs of *B. invadens* (50 eggs per fruit). Mangoes from the control (unexposed) and treated (ant exposed) were then placed in separate Perspex cages (12 × 12 × 12cm). Thereafter, ten experienced *F. arisanus* females were aspirated from rearing cages and transferred into the Perspex cage and observations on parasitoid behaviour initiated 5 min later. The number of *F. arisanus* females searching and/or ovipositing on ant-exposed and ant-unexposed mangoes was recorded over a three hour period. Neither the parasitoids nor their parents had any prior experience with weaver ants. The experiment was replicated 6 times with a new cohort of parasitoids used in each replicate. Two observers were involved in the collection of the behavioural data. All experiments were replicated six times and the Completely Randomized Design (CRD) was used.

7.2.3 Data analysis

Means on the total number of female wasps searching and/or ovipositing on ant-exposed and ant-unexposed mangoes were subjected to Student's T-test to test for significant difference in parasitoid behaviour in relation to the two treatments.

For the total number of eggs deposited in mango domes by *B. invadens* females across the different ant densities, Kruskal-Wallis one way ANOVA on ranks with pair wise multiple comparisons based on Student-Neuman-Keul's test was carried out. This analysis does not require that the data sets be normal and their variances homogenous. All other data was Log10 (x+1) transformed to stabilize the variance and normalize the data. Analysis of variance was then performed using the general linear model procedure and mean separations were undertaken using the Student-Newman-Keul's test. Liner regression analysis was done to establish the strength of the association between ant density and the number of *B. invadens* eggs oviposited. The same was done for the effect of ant density on parasitism rates of *F. arisanus.*

7.3 RESULTS

7.3.1 Effect of varying densities of *O. longinoda* on *B. invadens* oviposition

Females of *Bactrocera invadens* searched and oviposited in all mango domes placed in the Perspex cages with different ant densities. Ant density had a significant effect on number of *B. invadens* females searching and/or ovipositing (F =12.2, df =5, 30, P=0.0001). Significantly higher number of *B. invadens* females searched and/or oviposited in control experiments (37.7±4.4) compared to the other treatments with ants presence (Fig. 7.1). The least number of *B. invadens* females searching and/or ovipositing was recorded when ant densities reached 8 and 10 (16.5±1.8 and 13.8±2.8, respectively) (Fig. 7.1).

Weaver ants also interfered with searching and/or oviposition by *B. invadens* females through deterrence (observed frequently) and predation (observed occasionally). Ant density had a significant effect on the level of interference observed (F =37.2, df =4, 25, P=0.0001). The level of interference was significantly higher at the higher ant densities of 6, 8 and 10 compared to the lower ant densities of 2 and 4 (Fig. 7.2).

Ant density also had a significant effect on the total number of eggs laid by *B. invadens* females (F =14.6, df =5, 30, P=0.0001). Number of eggs laid by *B. invadens* females was significantly higher in controls with no ants (98.5 ± 5.4) and at ant density of 2 (95.8 ± 6.2) compared to the other treatments (Table 7.1). The lowest number of eggs laid was recorded when ant density reached a maximum of 8 and 10 (Table 7.1). The linear regression model showed a strong relationship between ant density and the total number of eggs laid by *B. invadens* females (r^2 =0.923) (Fig. 7.3).

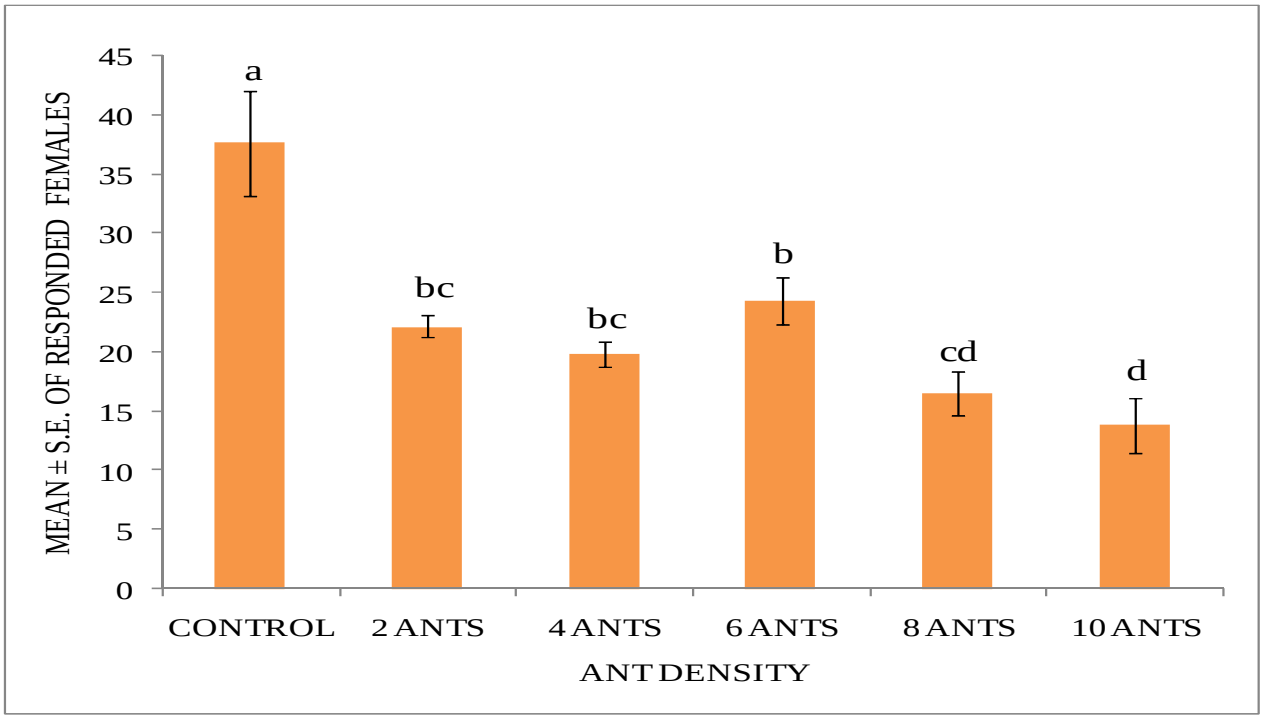

Fig 7.1 Mean ± (S.E.) number of *B. invadens* females searching and/or ovipositing across the different ant densities tested.

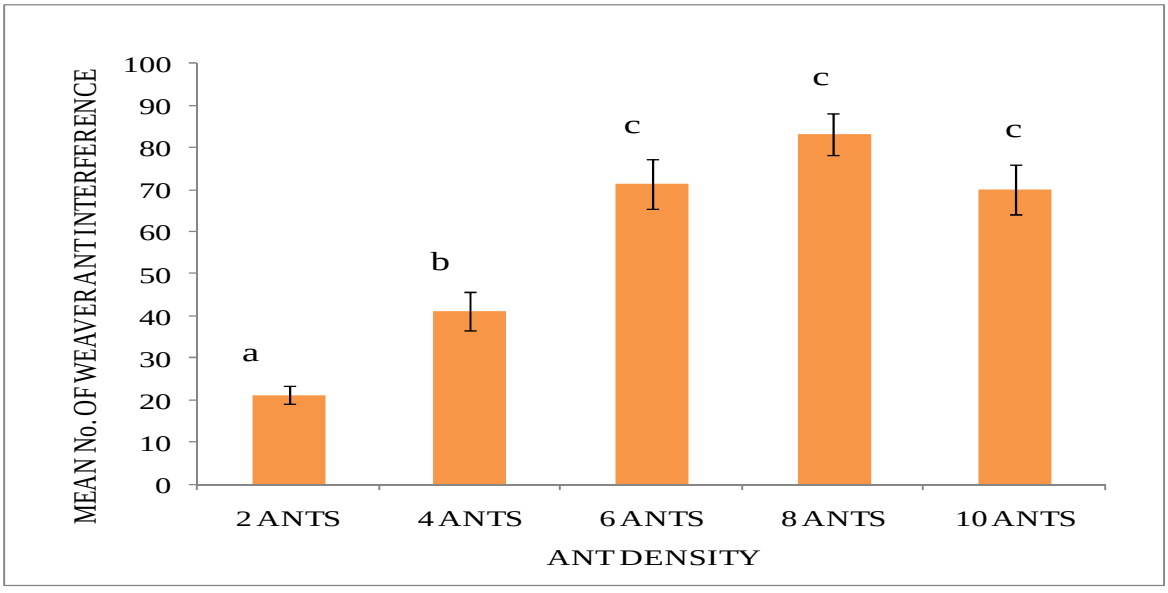

Fig 7.2 Mean ± (S.E.) number of times weaver ants interfered with searching and/or oviposition by *B. invadens* females through deterrence (mostly) and predation (less frequently)

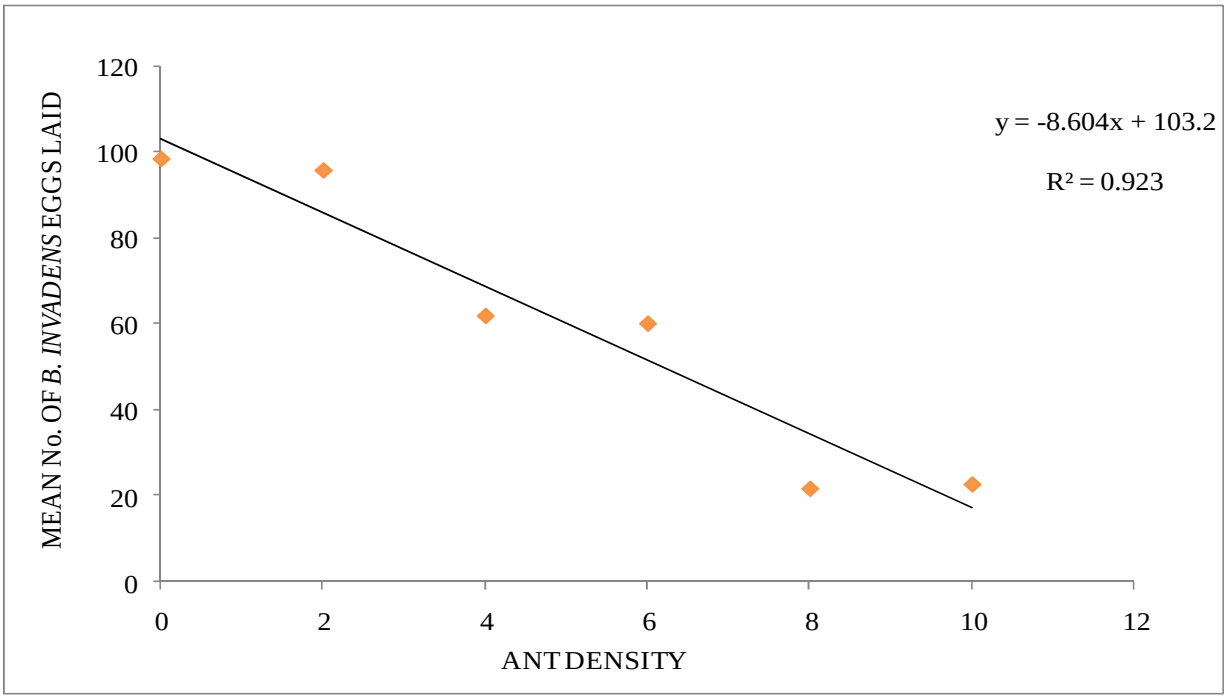

Fig 7.3 Linear regression showing the effect of ant density on the total number of eggs laid by *B. invadens* after 3 hour of exposure

Table 7.1 Mean ± (S.E.) total number of eggs deposited in mango domes by *B. invadens* females at different ant densities tested after 3 hours of exposure

Treatment	Number of eggs oviposited
Control (*B. invadens* only)	98.5 ± 5.4a
2 ants	95.8 ± 6.2a
4 ants	62.0 ± 13.3b
6 ants	60.2 ± 4.8b
8 ants	21.8 ± 10.7c
10 ants	22.8 ± 8.9c

Means followed by the same letter in the same column are not significantly different (P <0.05), Student-Newman-Keul's test.

7.3.2 Effect of varying densities of *O. longinoda* on oviposition and parasitism by *F. arisanus*

Females of *F. arisanus* searched and oviposited in all mango domes placed in Perspex cages with varying numbers of ant densities. Ant density had a significant effect on the number of *F. arisanus* females searching and/or ovipositing (F =12.2, df =5, 30, P=0.0001). The number of *F. arisanus* females that searched and/or oviposited in control experiments without ants (66.8±8.9) and at the ant density of 2 (66.2±6.6) was significantly higher compared to the other treatments (Fig 7.4). The least number of *F. arisanus* females searching and/or ovipositing was recorded when ant densities reached 8 and 10 (24.8±4.7 and 24.7±2.4 respectively) (Fig. 7.4).

Oecophylla longinoda also interfered with searching and/or oviposition by *F. arisanus* females through deterrence and predation (both parameters observed frequently). Ant density had a significant effect on the level of interference observed (F =22.7, df =4, 25, P=0.0001). The level of interference was significantly higher at the higher ant densities of 6, 8 and 10 compared to the lower ant densities of 2 and 4 (Fig. 7.5).

Ant density also had a significant effect on the total number of *F. arisanus* adults that emerged from puparia collected from the different treatments (F =62.2, df =5, 30, P=0.0001). Total number of emerged parasitoids was significantly higher in controls without ants (18.0 ± 3.2) compared to the significantly low number of parasitoids that emerged at the higher ant densities of 6, 8 and 10 (Table 7.2). The linear regression model showed a strong relationship between ant density and the total number of emerged *F. arisanus* adults (r^2 =0.728) (Fig. 7.6).

Predation of *F. arisanus* females by weaver ants was frequently observed during bioassays. This resulted in varying levels of mortality of *F. arisanus* females across the different ant densities tested. Mortality was significantly influenced by ant density (F =53.7, df =5, 30, P=0.0001). Significantly high mortality levels were observed at ant densities of 8 and 10 whilst there was no mortality at the lowest ant densities of 0 (control), 2 and 4 (Table 7.3).

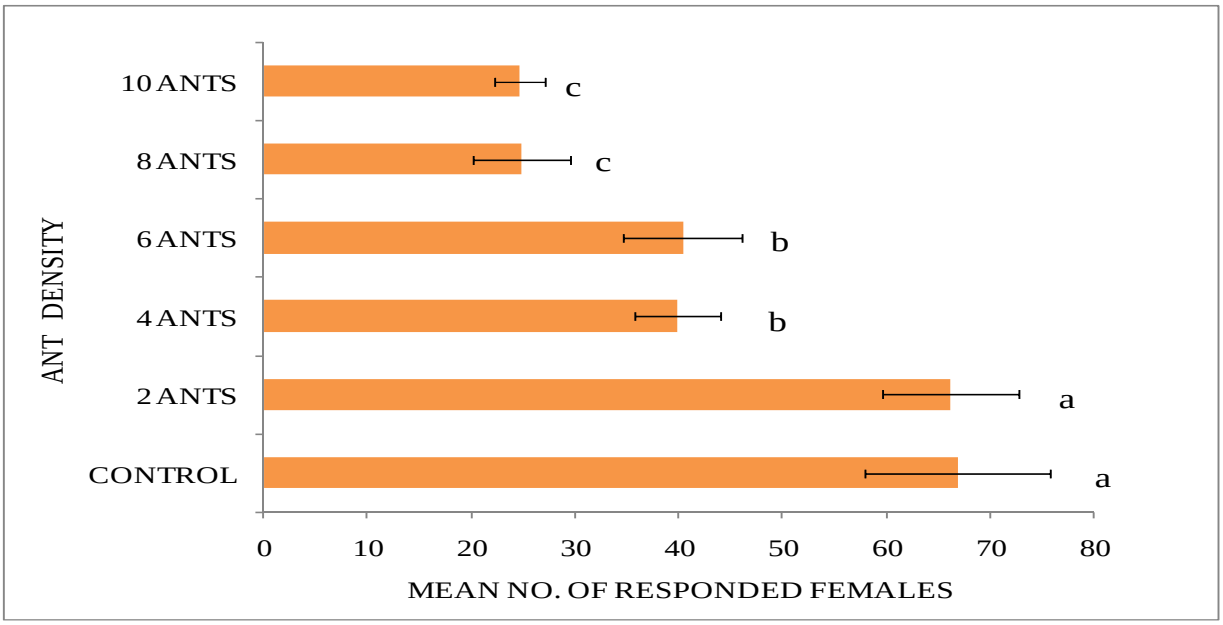

Fig 7.4 Mean ± (S.E.) number of *F. arisanus* females searching and/or ovipositing across the different ant densities tested.

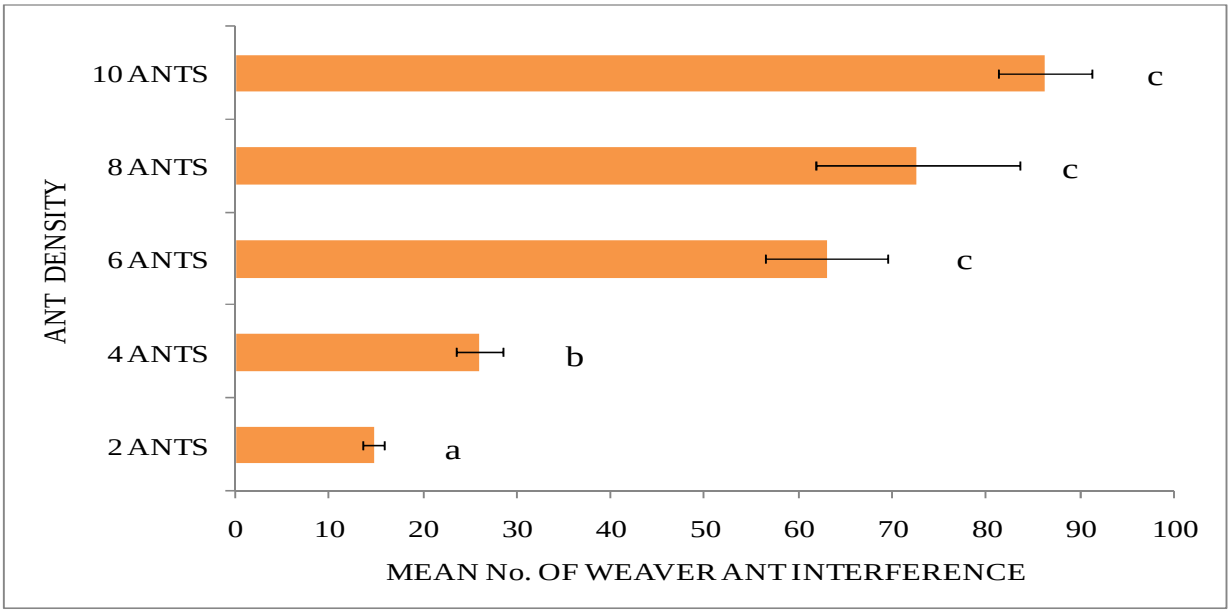

Fig 7.5 Mean ± (S.E.) number of times weaver ants interfered with searching and/or oviposition by *F. arisanus* females through deterrence and predation

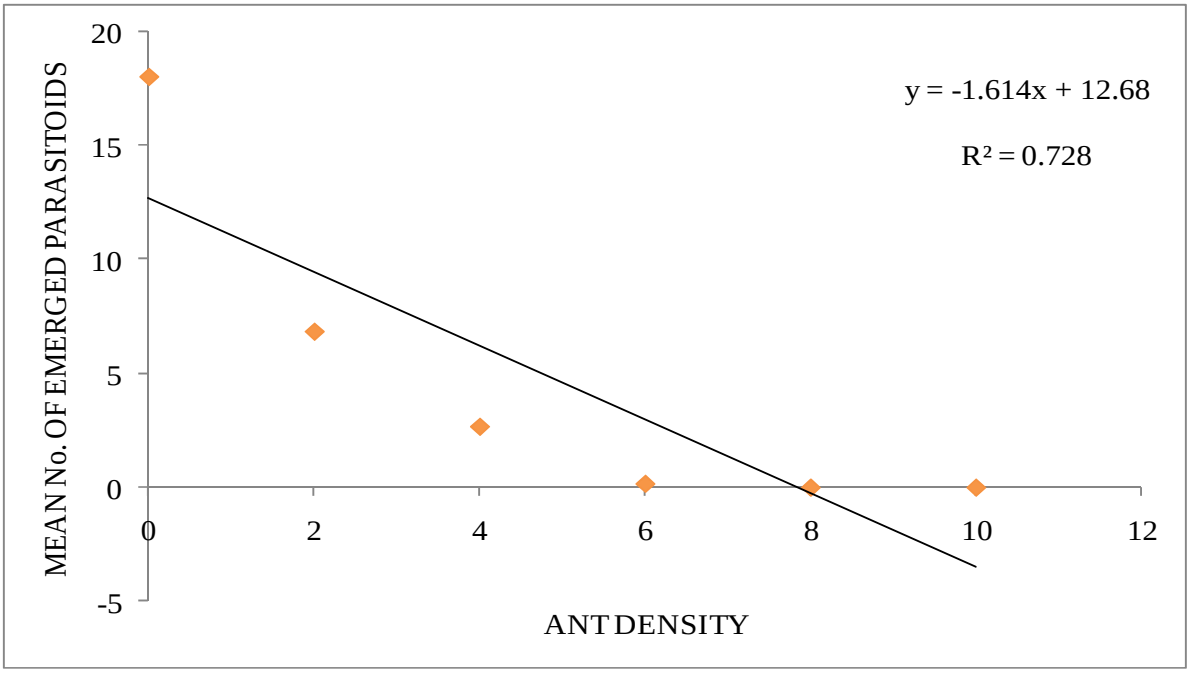

Fig 7.6 Linear regression showing the effect of ant density on the total number of emerged *F. arisanus* adults.

Table 7.2 Mean ± (S.E.) total number of emerged *F. arisanus* adults following exposure to *B. invadens* eggs at different ant densities tested

Treatment	Number of emerged parasitoids
Control (*F. arisanus* only)	18.0 ± 3.2a
2 ants	6.8 ± 0.9b
4 ants	2.7 ± 1.0c
6 ants	0.2 ± 0.2d
8 ants	0.0 ± 0.0d
10 ants	0.0 ± 0.0d

Means followed by the same letter in the same column are not significantly different (P <0.05), Student-Newman-Keul's test.

Table 7.3 Mean ± (S.E.) mortality of *F. arisanus* at different ant densities tested as a result of predation

Treatment	Mortality
Control (*F. arisanus* only)	0.0 ± 0.0a
2 ants	0.0 ± 0.0a
4 ants	0.0 ± 0.0a
6 ants	13.3 ± 0.2b
8 ants	23.3 ± 0.2c
10 ants	28.3 ± 0.3c

Means followed by the same letter in the same column are not significantly different (P <0.05), Student-Newman-Keul's test.

7.3.3 Effect of cues deposited by *O. longinoda* on mango fruit on oviposition and parasitism by *F. arisanus*

Fopius arisanus females searched and/or oviposited on both ant-exposed and unexposed mangoes. However, the number of parasitoids that searched and/or oviposited in ant-unexposed mangoes was significantly higher compared to ant-exposed mangoes ($t = 44.1$, $df = 143$, $P < 0.0001$). Mean number of parasitoids that searched and/or oviposited in both ant-exposed and ant-unexposed mangoes over the three-hour observation period is shown in Fig 7.7.

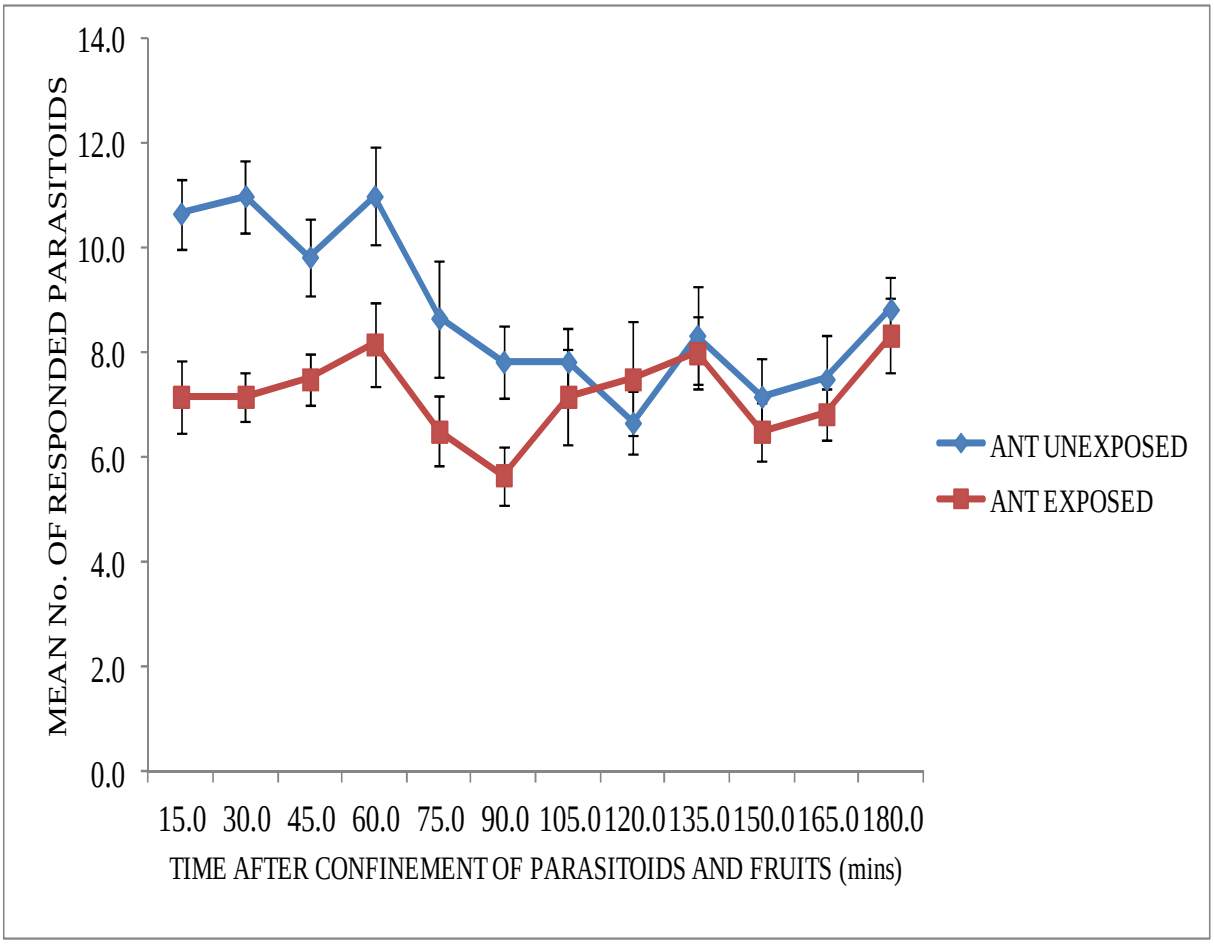

Fig 7.7 Mean ± S.E. *F. arisanus* females searching and/or ovipositing in ant-exposed and unexposed mangoes over a three hour observation period.

7.4 DISCUSSION

7.4.1 Ant/*B. invadens* interaction

The effectiveness of weaver ants in suppressing populations of several insect pests has been widely reported (Varela, 1992; Peng *et al*., 2004; Peng and Christian, 2005; van Wijngaarden *et al*., 2007**;** Dwomoh *et al*., 2008; Van Mele *et al*., 2007, 2009 Vayssieres *et al*., 2009). Results from the current study confirm the above. In the current study, significantly low number of eggs were laid by *B. invadens* females in the presence of *O. longinoda* compared to controls (no ants present). The number of eggs laid by *B. invadens* females also reduced significantly with increasing ant density, indicating that ant density on the trees should be an important factor in the management of fruit flies by *O. longinoda*.

Predation of adult *B. invadens* was rarely observed in this study compared to physical deterrence, which was observed most frequent. Physical disturbance by the weaver ants during oviposition by *B. invadens* is therefore largely responsible for the significantly low number of eggs laid by *B. invadens* especially at higher ant densities. Dicke (2000) and Offenberg *et al*., (2004) reported that the direct mechanisms underlying weaver ant protection of plants against herbivores and frugivores include predation on, or deterrence of, the organisms during direct encounters. The results of this study are consistent with that reported by Abel (2010) to the effect that direct interaction between *B. invadens* and *O. longinoda* under field conditions primarily consists of disturbance, as predation is hardly observed. Vayssie'res *et al*., (2009) reported that predation of adult fruit flies by *O. longinoda* is rare. Nielson (2010) simplifies the phenomenon by saying that "Since it takes several minutes for fruit flies to lay eggs, when an ant comes along, the fly will give up, or it will be eaten by the ant". In Australia and Southeast Asia, the use of weaver ants as biocontrol agents has especially been effective for fruit agriculture (Van Mele *et al*., 2001; Peng

and Christian, 2007). Fruit trees harboring weaver ants produce higher quality fruits, show less leaf damage by herbivores, and require fewer applications of synthetic pesticides (Van Mele *et al*., 2007; Peng and Christian, 2007; 2008). The results of this study confirm findings of several others authors and if integrated into an IPM strategy, weaver ants in tandem with other control measures have the potential to suppress fruit fly populations.

7.4.1 Ant/*F.arisanus* interaction

Findings from this study also show that *O. longinoda* interferes with the searching and oviposition behaviour of *F. arisanus* through predation on, or deterrence of, parasitoids during direct encounters. Predation by *O. longinoda* was a very common occurrence in the interaction between ants and *F. arisanus* females, compared to ant-fruit fly interactions where physical deterrence was the more the frequent parameter observed. It is probable that size may have played a very important role in the predator-prey relationship given the relatively smaller size of the parasitoids compared to fruit flies. Also, parasitoids take relatively longer time in host searching and oviposition compared to fruit flies and the risk of predation by weaver ants is likely to be higher.

Biotic interference by the weaver ants also resulted in significantly lower number of *F. arisanus* adults emerging from fruit fly puparia at 2 and 4 ants per mango and no parasitoids emerged at higher ant densities of 6, 8 and 10 and clearly provide evidence that interference by ants could have negative effect on oviposition by parasitoids, and subsequently the number of emerged parasitoids.

Predation by *O. longinoda* also resulted in significant parasitoid mortality; especially at higher ant densities. Although high levels of mortality was observed in our study, extrapolation of the results to field conditions must be done with caution. The experiments were conducted in small sized Perspex cages that gave parasitoids very little room to escape from weaver ant attacks. Also, direct ant-parasitoid encounters are likely to be less frequent under field conditions compared to results obtained from laboratory assays conducted in enclosed Perspex cages. However, in fig-fig wasp system, a behaviorally mediated indirect effect caused by the ant, *Crematogaster scutellaris,* resulted in significant preying on pollinator wasps (Schatz and Hossaert-McKey, 2003). In rambutan fruit orchard, the presence of O. smaragdina nests on the trees significantly lowered the flower-visiting rate of the major pollinator *Trigona minangkabau* but other arboreal *Crematogaster* ants had no effect on the flower visitors (Tsuji *et al*., 2004). The observed effect was attributed to *Oecophylla*'s characteristic behavior associated with strong territorial defence and aggression toward other animals (Hölldobler 1983; Hölldobler & Wilson 1990) which could be similar in *O. longinoda-F. arisanus* interactions. Suffice to say that, the role of habitat complexity in modifying parasitoid competitive success and consequent coexistence warrants further investigations.

The presence of ant cues on mango fruits had a significant effect on the searching and oviposition behaviour of parasitoids, as significantly higher number of *F. arisanus* females searched and oviposited in ant-unexposed mangoes compared to ant-exposed mangoes. The parasitoids used in the ant cue experiments were never exposed to the physical presence of weaver ants. Therefore enemy avoidance by parasitoids is not attributable to learning, but is the result of cues that trigger responses to potential predator risk (Van Mele *et al*., 2009).

The negative effect of ant pheromones on the behaviour of other insects, particularly fruit flies has been reported. Van Mele *et al*., (2009) found that ant cues have a suppressing effect on fly landings and time spent on fruits by *C. cosyra* and *B. invadens* but their study did not investigate the effect of ant cues on oviposition by *B. invadens* females. However Dicke (2000) suggests that territorial ant pheromones may present reliable cues of ant presence and predation risk, and therefore can be exploited by potential prey. Offenberg *et al.* (2004) also propose that the repellent effect on herbivores in Thai mangrove systems was as a result of *Oecophylla* pheromones.

In fruit fly-parasitoid system, field studies suggest that *F. arisanus* females use chemical cues to orient toward eggs of fruit flies (Liquido 1991). The significantly low number of *F. arisanus* females searching and/or ovipositing in ant-exposed mangoes could therefore be attributed to *Oecophylla* pheromones interfering or even masking the effect of chemical cues used by *F. arisanus* to orient towards fruit fly eggs, thereby making infested ant–exposed fruits less attractive to the parasitoids. The direct and indirect ways through which *O. longinoda* interferes with parasitoid searching and oviposition is likely to have a negative impact on the overall performance of *F. arisanus* in the management of *B. invadens*. Indeed under field conditions *Oecophylla* ants have been documented to have negative effects on beneficial fauna (Thomas, 1988; Tsuji *et al*., 2004) and the use of *O. longinoda* and *F. arisanus* could result in biotic interference and potential failure of *F. arisanus* to establish in agro-ecosystems where the ants are predominant.

Fopius arisanus and *O. longinoda* are natural enemies with the potential to effectively suppress populations of *B. invadens* and other indigenous fruit flies. Although findings from this study and several others suggest that *O. longinoda* is an effective biocontrol agent for the suppression of fruit flies, it interferes with the searching and oviposition behaviour of *F. arisanus*. This negatively affects parasitism, as clearly documented in this study. *Oecophylla longinoda* also preys on fruit fly larvae (Vayssieres *et al*., 2009), and this may include parasitized larvae in addition to *F. arisanus* adults as demonstrated in this study. Oviposition deterrence and predation by *O. longinoda* could possibly have a detrimental effect on the establishment and population build up of *F. arisanus*, and could render the parasitoid ineffective but additional field cage studies are needed to clarify the effect. In some parasitoid-ant systems, not all interactions between the foragers and antagonist are necessarily fatal and some parasitoids may benefit from learning the extent of the danger of such interactions that could lead to co-existence (Hubner and Volkl, 1996). Further studies with regard to experiential learning in *O. longinoda-F. arisanus* interactions are therefore necessary to inform researchers on whether or not both biocontrol agents can be accommodated in an IPM strategy for the field suppression of fruit flies.

CHAPTER EIGHT

8.0 SUMMARY OF RESULTS, CONCLUSIONS AND RECOMMENDATIONS

8.1 SUMMARY OF RESULTS

Bactrocera invadens, an invasive and devastating fruit fly pest of Asian origin was first detected in Kenya in 2003. Since its detection in 2003 along the coastline of Kenya, *B. invadens* has spread to over 28 other countries across the African continent. It has been recorded from over 40 plant species but mango appears to be the most preferred host plant.

Since *B. invadens* is an invasive pest, it lends itself to classical biological. In 2006, the African Fruit Fly Programme (AFFP) imported two Braconid parasitoids, *F. arisanus* and *D. longicaudata* from Hawaii for evaluation against *B. invadens* and other indigenous fruit fly pests. Results from laboratory studies have shown that both parasitoids are effective biological control agents for the control of *B. invadens* and some indigenous fruit fly pests. For field releases to be approved however, the interaction between the introduced parasitoids and indigenous natural enemies, the thermotolerance levels of the introduced parasitoids, as well as their overall performance across the major host fruits attacked by *B. invadens* needs to be evaluated. Consequently, the study documents the indigenous parasitoid fauna present in the major mango growing areas in Kenya where the introduced parasitoids are to be released and later documented the effect of temperature, as well as host fruit substrate on the overall performance of *F. arisanus* and *D. longicaudata* and to investigate possible interaction between the introduced parasitoids and indigenous natural enemies. It also documents research related to the performance of the introduced parasitoids on *B. invadens* across major host fruits of the pest, as well as the effect of temperature on the development, longevity and parasitism rates of the introduced parasitoids.

Finally, bioassays were carried out to investigate the interaction between *F. arisanus* and *O. longinoda*, an indigenous natural enemy that abounds in major mango growing areas on the continent and the effect of such an interaction on the overall performance of the parasitoid.

A survey of the indigenous parasitoid fauna in the major mango growing areas of Kenya (the Coast and Eastern Provinces) was carried out as a first step to assessing the interaction between the introduced and indigenous parasitoid fauna. The major mango growing areas were targeted because these localities would be the first point of parasitoid releases, as mango is the most preferred host plant of *B. invadens*. A total of 345 parasitoids belonging to four different families of Hymenoptera (Braconidae, Eulophidae, Chalcidoidea and Ichneumonidae) were recovered during the survey with the genus *Psytallia* predominating in both the Coastal and Eastern Province of Kenya. A wide range of tephritid fruit fly pests were also recovered from fruits surveyed with *B. invadens* predominating in both the Coastal and Eastern Province. No parasitoid emerged from fruits that yielded *B. invadens*, confirming earlier studies suggesting that indigenous parasitoid fauna are unable to successfully complete development in *B. invadens* due to their inability to overcome the immune system of the pest. The greater majority of parasitoids were recovered from fruits of non-cultivated plant species, with the exception of *C. arabica*. Parasitism rates were also very low in most cases. Clearly, indigenous tephritid parasitoids are present in the major mango areas in Kenya as reported in this study and several others. But the relative concentration of most indigenous tephritid parasitoids on wild host plants, their relatively low parasitism rates, as well as their inability to successfully develop and emerge out of the most devastating fruit fly pest on the continent, *B. invadens* makes them relatively inefficient in most of the mango growing areas in Kenya. Therefore, the introduction

and subsequent release of a co-evolved parasitoid of *B. invadens* is clearly warranted. This study therefore provides important baseline information on the parasitoid fauna in key mango production localities in Kenya and sets the scene for studies related to interaction of the exotic parasitoid species and the documented native species before their field releases in Kenya. The study also highlights the need for future conservation efforts of the indigenous tephritid parasitoids in localities and/or native host plants close to mango orchards where they can build up their population and exert impact on the fruit fly species before they move into the orchards.

The effect of temperature on the development, longevity and parasitism rates of *F. arisanus* and *D. longicaudata* was evaluated in the laboratory to gain firsthand knowledge on the adaptability and subsequent establishment of the introduced parasitoids to the varying temperatures across the continent. Developmental time of both parasitoids was found to be greatly influenced by temperature with the duration decreasing as temperature increased. The longest total development period for both parasitoids occurred at 15 °C and was shortest at 30 °C. This is not surprising given that low temperature has been shown to slow development of many insects including parasitoids. A linear regression model provided a reliable fit of development rate versus temperature with lower development thresholds of 13.5 °C and 12.0 °C for *F. arisanus* and *D. longicaudata* respectively. Parasitism rates were also significantly affected by temperature. For *F. arisanus*, the highest parasitism rate was realized at 25°C whilst the highest parasitism rate for *D. longicaudata* was realized at both 20 and 25 °C. The lowest parasitism rate was recorded at 15 °C. This is consistent with several studies that have demonstrated the significant effect of temperature on the behaviour and performance of several Hymenopteran parasitoids. Percent parasitism was optimal between 20 and 30 °C for both parasitoids, the same

temperature range that favours the development of its target pest, *B. invadens*. Temperatures above 30 °C were found to be detrimental to parasitism rates. Based on a combination of shorter developmental time and high parasitism rates, the optimal temperature for the rearing of *F. arisanus* and *D. longicaudata* on *B. invadens* is 25 °C. This study demonstrates that both *F. arisanus* and *D. longicaudata* have the potential to develop and achieve high levels of parasitism on *B. invadens* under the temperature conditions as prevalent in agro-ecological zones suitable for horticultural production and should be able to contribute to the overall suppression of this invasive pest species.

Studies on the effect of host fruit substrate on the behaviour and performance of *F. arisanus* and *D. longicaudata* on *B. invadens* was also carried out. It has been widely reported that host fruit substrate has a great influence on parasitoid behaviour and performance. It was therefore imperative that the performance of the parasitoids on mango, the most preferred host plant of *B. invadens*, as well as other alternative host plants of the pest be evaluated. Host fruit substrate had a significant effect on preference and parasitism rates of both parasitoids. Mango was the most preferred fruit by *F. arisanus* compared to pawpaw, guava, citrus, tropical almond and marula. This was to be expected as mango was the host fruit substrate used in the rearing of *F. arisanus* for several generations in the laboratory. In subsequent experiments when mango was excluded from the bioassay, pawpaw was the most preferred fruit, followed by marula, which was one of the least preferred fruits when mango was included in the first bioassay. It is probable that the presence of mango (on which *B. invadens* eggs have been exposed to the wasps for several generations) may have probably masked the effect of volatiles emanating from the other fruits. *D. longicaudata* females had a strong preference for citrus, followed by mango and pawpaw.

Parasitism rates for both *F. arisanus* and *D. longicaudata* were also significantly high on mango and pawpaw compared to other fruit substrates tested. The differential attraction of female parasitoids to fruit odours, observed in the behavioural experiment, indicates that both parasitoid females have an innate response toward olfactory cues and use fruit volatiles during host location process. The observed preferences to different host fruits by the two parasitoids may be as a result of variations in concentrations of volatiles between the fruit species. This subsequently influenced parasitism rates and overall performance of the parasitoid. The effect of host fruit substrate on the preference and performance of several Hymenopteran parasitoids is widely documented. This study further confirms the fact that fruit substrates play a significant role in the host location behaviour of *F. arisanus* and *D. longicaudata*, and this greatly influences the parasitization of *B. invadens*. Parasitoid preference for certain fruits may not be fully understood but it could be an important factor to consider during releases of parasitoids in the natural habitat.

The predatory African weaver ants, *O. longinoda* have been used as biological control agents for the management of some insect pests attacking major fruit trees in Africa. Recently, studies have shown that the presence of *O. longinoda* in mango orchards resulted in better quality fruits as a result of a reduction in fruit fly damage, a consequence of the predatory and deterring behaviour of the ants. The interaction between *O. longinoda* and the introduced parasitoids and the subsequent impact on the overall performance of the parasitoids is however unknown. To this end, studies on the interaction between *O. longinoda* and the egg-pupal parasitoid, *F. arisanus* and the implications of such an interaction on the performance of the parasitoids was carried out in the laboratory using Perspex cages.

Also, the mechanisms underlying the management of *B. invadens* by *O. longinoda* was further investigated. Ant density had a significant influence on the number of *B. invadens* females searching and/or ovipositing in mango domes. It also had a significant effect on the number of eggs laid by *B. invadens* females in mango domes. Significantly high number of female flies searched and oviposited in controls (ant absent), and at lower ant densities but the number of respondents significantly reduced at the highest ant density of 10. This in turn, greatly influenced the number of eggs deposited by *B. invadens* females, with significantly low number of eggs laid in mango domes at the higher ant densities of 8 and 10 and significantly high numbers in the controls and the lower ant density of 2. *Oecophylla longinoda* significantly reduced the number of eggs deposited by *B. invadens* females through disturbance and/or deterrence. Predation of *B. invadens* by *O. longinoda* was rarely observed. This study shows that the presence of *O. longinoda* deters *B. invadens* from oviposition. However ant density plays a very important role in the level of control likely to be achieved using *O. longinoda* as a biocontrol agent against *B. invadens*. A high population build up of weaver ants in mango orchards is more likely to result in considerable control of fruit flies due to increased deterrence, deterrence and predation. Findings from this study should however be interpreted with caution as the level of influence exerted by *O. longinoda* on the behaviour of *B. invadens* may not be necessarily replicated in the field due to a reduction in direct ant-fly interactions, especially on fruits.

Findings from this study also show that *O. longinoda* interferes with the searching and oviposition behaviour of *F. arisanus* through predation on, or deterrence of, parasitoids during direct encounters. Predation by *O. longinoda* was a very common occurrence in the interaction between weaver ants and *F. arisanus* females, compared to ant-fruit fly interactions where

physical deterrence was the more frequent parameter observed. It is probable that size may have played a very important role in the predator-prey relationship given the relatively smaller size of the parasitoids compared to fruit flies. Also, parasitoids take relatively longer time in host searching and oviposition compared to fruit flies and the risk of predation by weaver ants is therefore likely to be higher. Biotic interference by the weaver ants also resulted in significantly lower number of *F. arisanus* adults emerging from fruit fly puparia, with no parasitoids emerging from experiments involving the higher ant densities of 6, 8 and 10 but significantly high number of parasitoids emerging from controls (ants absent) ant at the lower ant densities of 2 and 4. Predation by *O. longinoda* also resulted in significant parasitoid mortality; especially at higher ant densities. This may not necessarily be the case in field conditions where parasitoids have a higher chance of escaping *O. longinoda* attacks compared to experiments carried out in perspex cages. Also, direct interactions between *O. longinoda* and *F. arisanus* females in the field may not be as frequent as observed in laboratory bioassays conducted in perspex cages. Therefore, extrapolation of results from this study into field conditions should be done with caution. This study clearly shows that deterrence and predation by *O. longinoda*, which negatively affects parasitism rates, is more likely to have a detrimental effect on the establishment and population build up of *F. arisanus*, and could render the parasitoid ineffective.

8.2 CONCLUSIONS

1) Indigenous tephritid parasitoids abound in the major mango growing areas of Kenya. However, parasitism rates are very low, and occur mainly on fruit fly species of the genus *Ceratitis,* compared to other genera and are also concentrated on infested wild host plants compared to cultivated ones. *Bactrocera invadens*, currently the most devastating fruit fly in Kenya, is rarely parasitized. These factors render indigenous parasitoids relatively ineffective in controlling fruit fly populations, emphasizing the need for introduced parasitoids.

2) The developmental time and parasitism rates of *F. arisanus* and *D. longicaudata* were greatly affected by temperature with the total development duration decreasing as temperature increased. Temperature also had a significant influence on puparia recovered for both parasitoids. Parasitism rates were optimum when parasitoids were reared within the temperature range of 20-30 °C. Temperatures above 30 °C and below 15 °C were detrimental to the development of both parasitoids.

3) Host fruit substrate had a significant effect on the preference and performance of *F. arisanus* and *D. longicaudata*. Host fruit substrate also had a significant influence on puparia recovered for both parasitoids. In choice experiments, mango was the most preferred fruit by *F. arisanus* whilst *D. longicaudata* preferred citrus the most. Parasitism rates for both parasitoids were significantly higher on mango and pawpaw compared to the other fruits tested.

4) The activities of the weaver ants, *O. longinoda* interfered with the behaviour of *B. invadens*, resulting in significantly less number of eggs being oviposited by the pest in mangoes. Although weaver ant activity was beneficial in deterring fruit flies from ovipositing, it also interfered negatively with the behaviour of *F. arisanus* and subsequently its overall performance. Deterrence and predation by *O. longinoda*, which negatively affected parasitism rates, is more

likely to have a detrimental effect on the establishment and population build up of *F. arisanus*, and could render the parasitoid ineffective.

8.3 RECOMMENDATIONS FOR FUTURE STUDIES

1) This study provides important baseline information on the parasitoid fauna in the major mango provinces of Kenya. Additional survey is needed for other localities. Subsequent studies related to interaction of the exotic parasitoid species and the native species documented in this study before their field releases in Kenya needs to be carried out. This study also highlights the need for future conservation effort of the indigenous tephritid parasitoids in localities and /or native host plants close to mango orchards where they can build up their population and exert impact on the fruit fly species before they move into the orchards.

2. The temperature range required for the optimum development *F. arisanus* and *D. longicaudata* on *B. invadens* have been established. This information should guide researchers in the mass rearing of both parasitoids for field releases. Also, the thermotolerance studies provide a baseline data for the development of models for predicting regions where *F. arisanus* and *D. longicaudata* can potentially establish and impact on the population of the fruit flies on the African continent.

3. Information generated on the effect of host fruit substrate on the performance of *F. arisanus* and *D. longicaudata* should serve as a basis for future field releases of the parasitoids. In addition to the preferred host in which the parasitoids thrive (i.e. mango and pawpaw), alternative host plants such as guava and citrus should be able to sustain parasitoid population during time of the year when mango and pawpaw are off. This will help in the initial build up of the parasitoid population, which is essential for its establishment and eventual success on

important horticultural crops. Since tritrophic experiments were conducted in the laboratory, it would be necessary to replicate it under field cage conditions that simulate the wild environment, using whole fruits.

4. Although *O. longinoda* impacted negatively on the exotic parasitoid species, in some parasitoid-ant systems not all interactions between the foragers and antagonist are necessarily fatal. Some parasitoids may benefit from learning the extent of the danger of such interactions that could lead to co-existence. Further studies with regard to experiential learning in *O. longinoda- F. arisanus* interactions will inform researchers on whether or not both biocontrol agents can be accommodated in an IPM strategy for the field suppression of fruit flies.

5. In Africa, smallholder farmers usually resort to the use of pesticides for fruit fly control in order to reduce fruit damage. With the introduction and potential releases of the introduced parasitoids, farmers should be educated on the need to significantly reduce the use of pesticides, especially broad spectrum ones which are likely to cause significant parasitoid mortality.

6. Biological control using parasitoids is not likely to be a 'stand alone' management strategy against fruit flies. In addition to the release of parasitoids, growers should be educated on other components of the IPM strategy including orchard sanitation, fruit bagging, male annihilation technique, application of spot bait sprays, the use of entomopathogenic fungi and application of the Augmentorium.

REFERENCES

Abel, C. (2010). Interactions between African weaver ants (*Oecophylla longinoda*) and the invasive fruit fly *Bactrocera invadens* on Senegalese mango. *PhD abstract*, University of Aarhus, 175 pp.

Agricultural Research Council (2008). Evolution of SIT for fruit pests in South Africa. Sourced online at http://www.arc.agric.za.

Ajila, C.M. and Prasada Rao, U.J. (2008). Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by *Mangifera indica* L. peel extract. *Food and Chemical Toxicology* **46** (1): 303–9.

Allwood, A. J., Vueti, E. T., Leblanc, L. and Bull, R. (2002). Eradication of introduced *Bactrocera* species (Diptera:Tephritidae) in Nauru using male annihilation and protein bait application techniques. In C. R. Veitch and M. N.Clout [eds.], Turning the tide: the eradication of invasive species. *Proceedings of the International Conference on Eradication of Island Invasives*. IUCN Publications Services Unit, Cambridge, United Kingdom, pp.19-25.

Allwood, A.J. and Drew, R.A.I. (1997). "Management of Fruit Flies in the Pacific, A Regional Symposium, Nadi, Fiji". *ACIAR Proceedings, No. 76.*

Altuzar , A., Montoya, P. and Rojas, J. (2004). Response of *Fopius arisanus* (Hymenoptera: Braconidae) to fruit volatiles in a wind tunnel. *Florida Entomologist* **87:** 616 – 618

Aluja, M., Guillén J., Liedo P., Cabrera M., Ríos E., De la Rosa, G. and Celedonio, H. (1990). Fruit infesting tephritids (Diptera: Tephritidae) and associated parasitoids in Chiapas, México. *Entomophaga* **35**: 39-48.

Andreu, G.L., Delgado, R., Velho, J.A., Curti, C. and Vercesi, A.E. (2005). Mangiferin, a natural occurring glucosyl xanthone, increases susceptibility of rat liver mitochondria to calcium-induced permeability transition. *Archives of Biochemistry and Biophysics* **439 (2)**: 184–93.

Annecke, D. P. and Moran, V.C. (1982). Insects and Mites of Cultivated Plants in South Africa. Butterworths & Co., Durban, South Africa, 383 pp.

Arakaki, N., Kuba, H. and Soemori, H. (1984). Mating behavior of the oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae). *Applied Entomology Zoology* **19**: 42-51.

Baeza-Larios, G., Sivinski, J., Holler, T. and Aluja, M., (2002). The effects of chilling on the fecundity and life span of mass-reared parasitoids (Hymenoptera: Braconidae) of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann) (Diptera:Tephritidae). *Biocontrol, Science and Technology* **12**: 205–215.

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., Good, J.E.G., Harrington, R., Hartley, S., Jones. T.H., Lindroth, R.L., Press, M.C., Symrnioudis, I., Watt, A.D. and Whittaker J.B. (2002). Herbivory in global climate change research: direct effects of rising temperatures on insect herbivores. *Global Change Biology* **8**:1-16.

Bally, I.S.E. (2006). Species profiles for Pacific Island Agro-forestry. 25 pp. Sourced online at http://www.agroforestry.net/tti/Mangifera-mango.pdf

Barnes, B., Rosenberg, S., Arnolds, L. and Johnson J. (2007) Production and quality assurance in the SIT Africa Mediterranean fruit fly (Diptera: Tephritidae) rearing facility in South Africa. *Florida Entomologist* **90 (1)**: 41-52.

Barnes, B.N. and Venter, J. (2006). The South African fruit fly action plan – Area-wide suppression and exotic species surveillance. *Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance*, Salvador, Brazil, pp. 271-283.

Barry, J. D., Shelly, T.E., McInnis, D.O., and Morse, J.G. (2003). Potential for reducing overflooding ratios of sterile Mediterranean fruit flies (Diptera: Tephritidae) with the use of ginger root oil**.** *The Florida Entomologist*, **86 (1)**: 29-33.

Bateman, M.A. (1972). The ecology of fruit flies. *Annual Review of Entomology* **17**: 493-518.

Bautista , R.C., Harris , E.J., Vargas , R.I. and Jang, E.B . (2004). Parasitization of melon fly (Diptera: Tephritidae) by *Fopius arisanus* and *Psyttalia fletcheri* (Hymenoptera: Braconidae) and the effect of fruit substrates on host preference by parasitoids. *Biological Control* **30**: 156 – 164.

Bautista, R. C. and Harris, E. J. (1996). Effect of fruit substrate on parasitization of the tephritid fruit flies (Diptera) by the parasitoid *Biosteres arisanus* (Hymenoptera: Braconidae). *Environmental Entomology* **25**: 470-475.

Beentje, H.J. (1994). Kenya trees, shrubs and lianas. National museums of Kenya, Nairobi.

Bennett, F.D. and Squire, F.A. (1972). Investigations on the biological control of some insect pests in Bolivia. *Pest Articles and News Summaries, London* **18 (4)**: 459-467

Berardini, N., Fezer, R., Conrad, J., Beifuss, U., Carle, R. and Schieber, A. (2005) Screening of mango (*Mangifera indica* L.) cultivars for their contents of flavonol *O* – and xanthone *C*-glycosides, anthocyanins, and pectin. *Journal of Agricultural and Food Chemistry* **53 (5)**: 1563–1570.

Bess, H.A., Van den Bosch, R. and Haramoto, F. (1961). Fruit fly parasites and their activities in Hawaii. *Proceedings of the Hawaiian Entomological Society* **17**: 367-378.

Bianchi, F.A. and Krauss, N.H. (1937). Fruit fly investigations in East Africa. *Hawaiian Planters' Record* **41:** 299–306.

Billah, M., Kimani-Njogu, S., Overholt, W.A., Wharton, R.A., Wilson, D.D. and Cobblah, M.A. (2005). The effect of host larvae on three *Psyttalia* species (Hymenoptera: Braconidae), parasitoids of fruit-infesting flies (Diptera: Tephritidae). *International Journal of Tropical Insect Science* **25**: 168–175.

Bokonon-Ganta, A.H., Ramadan, M.M., Wang, X.G. and Messing, R.H. (2005). Biological performance and potential of *Fopius ceratitivorus* (Hymenoptera: Braconidae), an egg-larval parasitoid of tephritid fruit flies newly imported to Hawaii. *Biological Control* **33**: 238–247.

Burns, E.D., Diaz, J. and Holler, T.C. (1996). Inundative release of the parasitoid *Diachasmimorpha longicaudata* for the control of the Carribean fruit fly, *Anastrepha suspense* (Loew). *In* "Fruit Fly Pests. A World Assessment of Their Biology and Management" (B.A. McPheron, and G.F. Steck, Eds.), St. Lucie Press. Delray Beach. FL, pp 377-381.

California Department of Food and Agriculture (CDFA) (2007). Mediterranean fruit fly pest profile. Sourced online at http://www.cdfa.ca.gov.

Calvitti , M., Antonelli, M., Moretti, R. and Bautista, R.C. (2002). Oviposition response and development of the egg-pupal parasitoid *Fopius arisanus* on *Bactrocera oleae* , a tephritid fruit fly pest of olive in the Mediterranean basin. *Entomologia Experimentalis et Applicata* **102**: 65-73.

Cancino, J. and Montoya, P. (2006). Advances and perspectives in the mass rearing of fruit fly parasitoids in Mexico. *Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance*, pp. 133-142.

Cantrell, B. K., Chadwick, B. and Cahill, A. (2002). Fruit fly fighters: eradication of papaya fruit fly. Commonwealth Scientific and Industrial Research Organization Publishing, Collingwood, VIC, Australia.

Carrasco, M., Montoya, P., Cruz-lopez, L. and Rojas, J.C. (2005). Response of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) to mango fruit volatiles. *Environmental Entomology* **34 (3)**:576-583.

Chacon, J., Landis, D. and Heimpel, G. (2008). Potential for biotic interference of a classical biological control agent of the soybean aphid *Biological control* **46 (2)**: 216-225

Chang, C.L., Caceres, C. and Jang, E.B. (2004). A novel liquid larval diet and its rearing system for melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). *Annals of the Entomological Society of America* **97**: 524–528.

Chang, C.L., Vargas, R.I., Jang, E.B., Caceres, C. and Cho, I.K. (2006). Development and assessment of a liquid larval diet for *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Annals of the Entomological Society of America* **99**: 1191–1198.

Chang, C.L. (2008). Fruit fly liquid larval diet technology transfer and update. *Journal of Applied Entomology* **33 (3):** 164-173.

Cheng, C. C., Yao, A. L., Lee, L.W. Y. and Chang, J. C. (1992). Olfactory responses of *Diachasmimorpha longicaudata* and *Opius incisi* to animal plant host related volatile sources. *Bulletin of the Institute of Zoology Academia Sinica* **31:** 131–135.

Christenson, L.D. and Foote, R.H.(1960). Biology of fruit flies. *Annual Review of Entomology* **5**:171-192.

CIRAD (2007). Fighting Fruit and Vegetable Flies Regionally in Western Africa. *Information letter* **7**, 4 pp.

Clarke, A.R., Armstrong, K.F., Carmichael, A.E., Milne, J.R., Raghu, S., Roderick, G.K., Yeates, D.K. (2005). Invasive phytophagous pests arising through a recent tropical evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology* **50**: 293-319.

Clausen, C.P. (1978). Introduced parasites and predators of arthropod pests and weeds: a world review. Washington, DC: United States Department of Agriculture, Agriculture Handbook No. 480.

Clausen, C.P., Clancy, D.W. and Chock, Q.C. (1965). Biological control of the Oriental fruit fly (*Dacus dorsalis* Hendel) and other fruit flies in Hawaii. *USDA Technical Bulletin 1322*.

Clausen, C.P. (1956). Biological control of fruit flies. *Journal of Economic Entomology*, **49** (2): 176-178.

Cleveland, C.B., Mayes, M.A. and Cryer, S.A. (2001). An ecological risk assessment for spinosad use on cotton. *Pest Management Science* **58**: 70–84.

Copeland, R.S., Wharton, R.A., Luke, Q., De Meyer, M., Lux, S., Zenz, N., Machera, P. and Okumu, M. (2006). Geographic distribution, host fruit, and parasitoids of African fruit fly pests *Ceratitis anonae*, *Ceratitis cosyra*, *Ceratitis fasciventris*, and *Ceratitis rosa* (Diptera: Tephritidae) in Kenya. *Annals of the Entomological Society of America*, **99 (2)**: 261-278.

Copeland, R.S., White, I.M., Okumu, M., Machera, P., and Wharton, R.A. (2004). 'Insects associated with fruits of the Oleaceae (Asteridae, Lamiales) in Kenya, with special reference to the Tephritidae (Diptera).' *Bishop Museum Bulletin in Entomology*, **12**: 135-164.

Copeland, R.S., Wharton, R.A., Luke, Q. and De Meyer, M. (2002). Indigenous hosts of *Ceratitis capitata* (Diptera: Tephritidae) in Kenya. *Annals of the Entomological Society of America* **95 (6)**: 672–694.

Copping, L.G. and Menn, J.J. (2000). Biopesticides: a review of their action, application and efficacy. *Pest Management Science* **56**: 651–676.

Cornelius, M. L., Duan, J. J. and Messing, R. H. (1999). Visual stimuli and the response of female oriental fruit flies (Diptera: Tephritidae) to fruit-mimicking traps. *Journal of Economic Entomology* 92: 121-129.

CSIRO (1991) Insects of Australia. Division of Entomology, Melbourne University Press, 2nd Edition, 943 pp.

Cudjoe, A.R., Neuenschwander, P. and Copland, M.J.W. (1993). Interference by ants in biological control of the cassava mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) in Ghana. *Bulletin of Entomological Research* **83**: 15-22.

Cugala, D., José L., Mahumane, C. and Mangana, S. (2009). Fruit flies pest status, with emphasis on the occurrence of the invasive fruit fly, *Bactrocera invadens* (Diptera:Tephritidae) in Mozambique. Paper presented at the African Crop Science Society Conference, 28 September to 1 October 2009, Cape Town, South Africa.

Cunningham, R.T. (1989). Male annihilation. In: Robinson, A.S., Cooper, G. (eds.), Fruit flies: their biology, natural enemies and control. Elsevier World Crop Pest 3B, pp. 345-351.

Daane, K.M., Sime, K.R., Wang, X.G., Nadel, H., Johnson, M.W., Walton, V.M., Kirk A. and Pickett, C.H. (2008). *Psyttalia lounsburyi* (Hymenoptera: Braconidae), potential biological control agent for the olive fruit fly in California. *Biological Control* **44**: 79 – 89.

Darby, H. 1933. Insects and micro-climate. *Nature* **131**: 839.

DeBach, P. (1965) Weather and the success of parasites in population regulation. *The Canadian Entomologist*, **97**:848-863.

DeBach, P. (1958). Selective breeding to improve adaptations of parasitic insects. Proceedings *of the tenth International Congress of Entomology* **4**:759-768.

De Meyer, M., Robertson, M. P., Mansell, M. W., Ekesi, S., Tsuruta, K., Mwaiko, Vayssieres, J-F. and Peterson, A. T. (2009). Ecological niche and potential geographic distribution of the invasive fruit fly *Bactrocera invadens* (Diptera, Tephritidae), *Bulletin of Entomological Research* **100**: 35–48

De Meyer, M., Copeland, R.S., Lux, S., Mansell, M., Wharton, R., White, I.M. & Zenz, N. (2002). Annotated check list of host plants for Afrotropical fruit flies (Diptera: Tephritidae) of the genus Ceratitis. *Zoologische Documentatie Koninklijk Museum voor Midden Afrika*, **27**: 1–92.

De Meyer, M. (1998). Revision of the subgenus *Ceratitis* (*Ceratalaspis*) Hancock (Diptera: Tephritidae). *Bulletin of Entomological Research* **88**: 257-290.

De Meyer, M. (1996). Revision of the subgenus *Ceratitis* (Pardalaspis) Bezzi, 1918 (Diptera: Tephritidae: Ceratitini). *Systematic Entomology* **21**: 15–26.

DEEDI, (2010). Department of Employment, Economic Development and Innovation, the State of Queensland, Australia. Winning the war against the Papaya fruit fly. Available at http://www2.dpi.gld.gov.au/health/4639.html.

Dent, D.R. and Walton, M.P. (1997). Methods in Ecological and Agriculture Entomology. CAB International, UK

Dhillon, M.K., Singh, R., Naresh, J.S. and Sharma, H.C. (2005). The melon fruit fly, *Bactrocera cucurbitae*: A review of its biology and management. *Journal of Insect Science* **5**:**40**, 16pp.

Dicke, M. (2000). Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochemical Systematics and Ecology*, **28:** 601-617.

Dickson, L. L. and Whitham, T. G. (1996). Genetically-based plant resistance traits affect arthropods, fungi, and birds. *Oecologia* **106**: 400-406.

Dimbi, S., Maniania, N.K. and Ekesi, S. (2009). Effect of *Metarhizium anisopliae* inoculation on the mating behavior of three species of African Tephritid fruit flies', *Ceratitis capitata, Ceratitis cosyra* and *Ceratitis fasciventris*. *Biological Control*, **50**: 111-116.

Dimou, I., Koutsikopoulos, C., Economopoulos, A.P. and Lykakis, J. (2003) Depth of pupation of the wild olive fruit fly *Bactrocera (Dacus) oleae* (Gmel.) (Diptera, Trypetidae). *Journal of Applied Entomology* **127(1)**: 12-17.

Dominiak B. (2007). Control of Queensland fruit fly in New South Wales. Primefact 518. Sourced online at www.dpi.nsw.gov.au/primefacts

Drew, R.A.I., Tsuruta, K. and White, I.M. (2005). A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. *African Entomology*, **13**: 149-154.

Drew, R.D. (2002). The fruit fly man. Available online at http://www.abc.net .au.

Duan, J.J. and Messing, R.H. (1997). Effect of two opiine parasitoids (Hymenoptera: Braconidae) introduced for fruit fly control on a native Hawaiian tephritid, *Trupanea dubautiae* (Diptera: Tephritidae). *Biological Control* **8**:177-184.

Dwomoh, E.A., Afun, J.V.K., Ackonor, J.B. and Agene, V.N. (2008). Investigations on *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae) as a biocontrol agent in the protection of cashew plantations. *Pest Management Science* **65**: 41–46.

Dyck, V. A., J. Hendrichs, and A. S. Robinson. (2005). Sterile insect technique, principles and practice in area-wide integrated pest management. Springer, Dordrecht, The Netherlands.

Eben, A., Benrey, B., Sivinski, J. and Aluja, M. (2000). Host species and host plant effects on preference and performance of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). *Environmental Entomology* **29**:87–94.

Eitam, A. and Vargas, R.I. (2007). Host habitat preference of *Fopius arisanus* (Hymenoptera: Braconidae), a parasitoid of fruit flies. *Annals of the Entomological Society of America* **100 (4)**: 603-608.

Ekesi, S., Maniania, N.K. and Mohamed, S.A. (2011). Efficacy of soil application of *Metarhizium anisopliae* and the use of GF-120 spinosad bait spray for suppression of *Bactrocera invadens* (Diptera: Tephritidae) in mango orchards. *Biocontrol Science and Technology*, **21 (3)**: 299-316.

Ekesi, S. (2010). Combating fruit flies in Eastern and Southern Africa (COFESA): Elements of a Strategy and Action Plan for a Regional Cooperation Program. *Fruit Fly Issue Paper*-World Bank, 24 pp.

Ekesi, S. (2010). What can farmers do against fruit flies? *The Organic Farmer*, **59**: 2-6.

Ekesi, S., Billah, M.K., Nderitu, P.W., Lux, S.A. and Rwomushana, I. (2009). Evidence for competitive displacement of the mango fruit fly, *Ceratitis cosyra* by the invasive fruit fly, *Bactrocera invadens* (Diptera: Tephritidae) on mango and mechanisms contributing to the displacement. *Journal of Economic Entomology* **102(3):** 981-991.

Ekesi, S., Nderitu, P.W. and Chang, C.L. (2007). Adaptation to and small-scale rearing of the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) on Artificial Diet. *Annals of the Entomological Society of America* **100**: 562-567.

Ekesi, S. and M. K. Billah [eds.] (2006). A field guide to the management of economically important tephritid fruit flies in Africa. ICIPE, Nairobi, Kenya.

Ekesi, S., Nderitu, P.W. and Rwomushana, I. (2006). Field infestation, life history and demographic parameters of the fruit fly, *Bactrocera invadens* (Diptera: Tephritidae) in Africa. *Bulletin of Entomological Research* **96**: 279-386.

Ekesi, S., Maniania, N.K., Mohamed, S.A., and Lux, S.A. (2005) Effect of soil application of different formulations of *Metarhizium anisopliae* on African Tephritid fruit flies and their associated endoparasitoids. *Biological Control* **35**: 83-91.

Ekesi, S., Maniania, N.K., and Lux, S.A. (2003) Effect of soil temperature and moisture on survival and infectivity of *Metarhizium anisopliae* to four Tephritid fruit fly puparia, *Journal of Invertebrate Pathology* **83**: 157-167.

Ekesi, S., Maniania, N.K. and Lux, S.A. (2002). Mortality in three economically important African Tephritid fruit fly puparia and adults caused by the entomopathogenic Fungi, *Metarhizium anisopliae* and *Beauveria bassiana.' Biocontrol*, *Science and Technology* **12**:7-17.

FAO (2010). Value of mango exports from Africa. Sourced online at http://www.faostat.org.

FAO (2009). Food market analysis of tropical fruits. Economic and Social Development Department, FAO. Sourced online at http://www.fao.org/docrep/012/ak341e/ak341e14.htm

FAO (2009). Value of mango exports from Africa. Sourced online at http://www.faostat.org

FAO (2006). Value chain analysis: a case study of mangoes in Kenya. Report prepared by the Sugar and Beverages Group. Raw Materials, Tropical and Horticultural Products Service Commodities and Trade Division. Food and Agriculture Organization of the United Nations, 11 pp.

FAO (2005). Medfly control in the Middle-East. Food and Agricultural Organization**.** Sourced online at http://www.fao.org.

Fitt, G.P. (1981). Pupal survival of two Northern Australian tephritid species and its relationship to soil conditions. *Australian Journal of Entomology*, **20 (2):** 139-144.

Fittt, G.P. (1984). Oviposition behaviour of two tephritid fruit flies, *D. tryoni* and *D. jarvisi*, as influenced by the presence of larvae in host fruit. *Oecologia* **62**: 37-46.

Fletcher B.S. (1987). The biology of dacinae fruit flies. *Annual Review of Entomology* **32**: 115-144.

Fletcher, B.S. and Bateman, M.A. (1982). Combating the fruit fly problems in Australia: The current and future prospects. *In* R. Cavalloro (ed.), Fruit flies of economic importance: *Proceedings of the CEC/IOBC International Symposium*, Rotterdam, Athens, Greece, pp. 553-563.

FPEAK (2005). Fresh Produce Exporters Association of Kenya. Sourced online at http://www.fpeak.org.

García-Martín, M., Gámez, M., Torres-Ruiz, A. and Cabello, T. (2008). Functional response of *Chelonus oculator* (Hymenoptera:Braconidae) to temperature, and its consequences to parasitism**.** *Community Ecology* **9 (1):** 45-51

Ghanim, N.M., Moustafa, SA., El-Metwally, M.M., Afia, Y.E., Salman, M.S. and Mostafa, M.E. (2010). Efficiency of some insecticides in male annihilation technique of peach fruit fly, *Bactrocera zonata* (Saunders) under Egyptian conditions Egypt. *Academic Journal of Biological Science* **2 (1):** 13-19.

Gilmore, J. E. (1989). Sterile Insect Technique (SIT). *In* Robinson A. S. and Hooper G. (eds.), Fruit flies, their biology, natural enemies and control. World crop pests, Vol. 3B. Amsterdam, Netherlands, Elsevier, pp. 353-363.

Goeden, R. D. and Louda, S. (1976). Biotic interference with insects imported for weed control. *Annual Review of Entomology* **21**: 325 - 42.

Gouado, I., Schweigert F.J., Ejoh R.A., Tchouanguep, M.F. and Camp, J.V. (2007). Systemic levels of carotenoids from mangoes and papaya consumed in three forms (juice, fresh and dry slice). *European Journal of Clinical Nutrition* **61 (10):** 1180–1188.

Greany, P.D., Tumlinson, J.H., Chambers, D.L. and Boush, G.M. (1977). Chemically mediated host finding by *Biosteres* (*Opius*) *longicaudatus*. A parasitoid tephritid fruit fly larvae. *Journal of Chemical Ecology* **3**: 189-195.

Greathead, D. (1972) Notes on coffee fruit-flies and their parasites at Kawanda (Uganda). *Technical Bulletin of the Commonwealth Institute of Biological Control* **15**: 11–18.

Gupta, V.K. (1991). The parasitic Hymenoptera and biological control of the African Ichneumonidae. *Insect Science and its Application* **12 (1-3):** 9-18.

HAL (2010). Horticultural Australia Limited. Value of Horticulture. Sourced online at http://www.horticulture.com.au/areas_of_Investment/Environment/Climate/value_horticulture.asp

Hance, T., van Baaren, J., Vernon, P and Boivin, G. (2007). Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology* **52**:107 - 126

Hapitan, J.C.J. and Castilo, B.S. (1976). Commercial Mango Production in the Philippines. Agriculture Publishing Corporation. pp. 35.

Haramoto, F. H. and Bess, H.A. (1970). Recent studies on the abundance of the Oriental and Mediterranean fruit flies and the status of their parasites. *Proceedings of Hawaiian Entomological Society* **20 (3):** 551-556.

Hashem, A.G., Mohamed, S.M.A. and El-Wakkad, M.F. (2001). Diversity and abundance of Mediterranean and Peach fruit flies (Diptera: Tephritidae) in different horticultural orchards. *Egyptian Journal of Applied Science* **16**: 303-314.

HASS (1992). Hawaiian Agricultural Statistics Service. General Crop Information. Sourced online at http://www.extento.hawaii.edu/kbase/crop/crops/i_mango.htm

Hayes, T., Goulet, H., Mason, P.G. and Kuhlmann, U. (2005). Does fundamental host range match ecological host range? A retrospective case study of *Lygus* plant bug parasitoid. *Biological Control* **35**:55–67

Heath, R. R., Epsky N. D., Dueben B. D., Rizzo J. and Jeronimo, F. (1997). Adding methyl-substituted ammonia derivatives to a food-based synthetic attractant on capture of the Mediterranean and Mexican fruit flies (Diptera: Tephritidae). *Journal of Economic Entomology* **90**:1584–1589.

Heather, N.W., Hargreaves, P.A., Corcoran, R.J. and Melksham, K.J. (1987) Dimethoate and fenthion as packing line treatments for tomatoes against *Dacus tryoni*. *Australian Journal of Experimental Agriculture* **27**: 465-469.

Hendrichs, J. (1996). Action programs against fruit flies of economic importance session overview. *In*: McPheron B. A. and Steck, G. J. (eds.). Fruit fly pests; a world assessment of their biology and management, St. Lucie Press, pp. 513-519.

Henneman, M.L., Dyreson, E.G., Takabayashi J. and Raguso R.A. (2002). Response to walnut olfactory and visual cues by the parasitic wasp *Diachasmimorpha juglandis*. *Journal of Chemical Ecology* **28**: 2221–2244.

Hölldobler, B. and Wilson, E., O (1990). *The Ants*, Harvard University Press, Cambridge, Massachusetts, USA.

Hölldobler, B. (1983). Territorial behavior in the green tree ant (*Oecophylla smaragdina*). *Biotropica* **15**: 241-250.

Hollingsworth, R.G., Drew, R.A., Allwood, A.J., Romig, M., Vagalo, M. and Tsatsia, F. 2003. Host plants and relative abundance of fruit fly (Diptera: Tephritidae) species in the Solomon islands. *Australian Journal of Entomology*, **42 (2):** 95-108

Howarth, F.G. (1983) Biological control: panacea or Pandora's box? *Proceedings of the Hawaiian Entomological Society* **24**: 239–244

Howarth, R. G. (1991). Environmental impacts of classical biological control. *Annual Review of Entomology* **36**: 485–509.

Huang, H.T. and Yang, P. (1987). The ancient cultured citrus ant. *Biosciences* **37**: 665–671.

Hubner, G. and Volkl, W. (1996). Behavioral strategies of aphid hyperparasitoids to escape aggression by honeydew collecting ants. *Journal of Insect Behaviour* **9**: 143–157.

Hurtrel, B., Quilici, S., Nénon, J.P. and Lelannic, J. (2001). Preimaginal developmental biology of *Diachasmimorpha tryoni* (cameron), a parasitoid of the Mediterranean fruit fly. *Insect Science and its application* **21(1):** 81-88

IAEA (2003). Trapping guidelines for Area-Wide Fruit Fly Programmes. IAEA/FAO manual (TG/FFP-2003), Vienna, Austria

ICIPE (2006). Development and implementation of a sustainable IPM program for major mango pests and opportunity for improving market and processing information in Sub-Saharan Africa. *Proposal* submitted to the German Ministry for Economic Cooperation and Development (BMZ).

IPNI (International Plant Names Index). (**2004).** [online]. Sourced online at www.ipni.org.

IRIN (2008). Integrated Regional Information Networks. Africa, Wiping out hunger – one fruit fly at a time. Sourced online at http://www.IRINnews.org

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

Jahn, G.C., Beardsley, J.W. and González-Hernández, H. (2003). A review of the association of ants with mealybug wilt disease of pineapple. *Proceedings of the Hawaian Entomological Society*, **36**: 9-28.

Jander R. and Jander, U. (1979). An exact field test for the fade-out time of the odor trials of the asian weaver ants, *Oecophylla smaragdina*. *Insects Sociaux*, **26 (3)**: 165-169.

Jang, E. B., Messing, R. H. and Carvalho, L. A. (2000). Flight tunnel responses of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) to olfactory and visual stimuli. *Journal of Insect Behaviour*. **13**: 525–538.

Jedele, S., Hau, A.M and Oppen, M.V. (2003). An analysis of the world market for mangoes and its importance for developing countries. Paper presented at the Conference on International Agricultural Research for Development, Gottingen, Germany.

Katsoyannos, B. I., Nikos, T, Papadoupopulos, T. and Stavridis, D. (2000a) Evaluation of trap types and food attractants for Rhagoletis cerasi L (Diptera: Tephritidae). *Journal of Economic Entomology* **93 (3):** 1005-1010

Khoo, K.C., Ooi, P.A.C. and Ho, C.T. (1993). Crop Pests and Their Management in Malaysia. *Tropical Press Sdn. Bhd*., Kuala Lumpur, Malaysia, 171 pp.

Kimberling, D.N. (2004). Lessons from history: predicting successes and risks of intentional introductions for arthropod biological control. *Biological Invasions* **6**: 301-318.

Knipling, E. F. (1992) Principles of insect parasitism analyzed from new perspectives. Practical implications for regulating insect populations by biological means. USDA, ARS, *Agriculture Handbook*, 693 pp.

Knipling, E. F. (1959). Screwworm eradication: Concepts and research leading to the sterile male method. Smithsonian Institution Publication 4365. Smithsonian Institution, pp. 409-418.

Kroder, S. and Messing, R.H. (2010). A new parasitoid from Kenya, *Fopius ceratitivorus* complements the extant parasitoid guild attacking Mediterranean fruit fly in Hawaii. *Biological Control* **53**: 223-229

Lacey, L.A. and Shapiro-Ilan, D.I. (2007). Microbial Control of Insect Pests in Temperate Orchard Systems: Potential for Incorporation into IPM. *Annual Review of Entomology* **53**: 121-144.

LaSalle, J. and Wharton, R. A. (2002). The identity and recognition of African *Tetrastichus* species (Hymenoptera: Eulophidae) associated with fruit flies (Diptera: Tephritidae) *African Entomology* **10**: 297-304.

Lawrence, P.O., Baranowski, R.M., Greany, P.D. and Nation J.L. (1976). Effect of host age on development of *Biosteres (Opius) longicaudatus*, a parasitoid of the Caribbean fruit fly, *Anastrepha suspensa*. *Florida Entomologist* **59**: 33-39.

Lever, R. J. A. W. (1938) Fruit flies and their control: biological and chemical. *Agriculture Journal* **9:** 19-20.

Lewis, W. J., Vet, L. E. M., Tumlinson, J. H., Van Lenteren, J. C. and Papaj, R. P. (1990). Variations in parasitoid foraging behavior essential elements of a sound biological control theory. *Environmental Entomology* **19**: 41–48.

Lewis, W.J. & Martin, W.R. Jr. (1990). Semiochemicals for use with parasitoids: status and future. *Journal of Chemical Ecology*, **16:** 3067-3089.

Leyva, J. L., Browning, H. W. and Gilstrap, F. E. (1991). Effect of host fruit species, size and color on parasitization of *Anastrepha ludens* (Diptera: Tephdtidae) by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). *Environmental Entomology* **20**: 1469-1474.

Liquido, N. J. (1991). Effect of ripeness and location of papaya fruits on the parasitization rates of oriental fruit fly and melon fly (Diptera: Tephritidae) by Braconid (Hymenoptera) parasitoids. *Environmental Entomology* **20**: 1732-1736.

Loni A. (1997). Developmental rate of Opius concolor (Hymenoptera: Braconidae) at various constant temperatures. *Entomophaga* **42**: 359-366.

Lopez, M., Sivinski, J., Rendon, P., Holler, T., Bloem, K., Copeland, R., Trostle M. and Aluja M. (2003). Colonization of *Fopius ceratitivorus*, a Newly Discovered African Egg-Pupal Parasitoid (Hymenoptera: Braconidae) of *Ceratitis capitata* (Diptera: Tephritidae). *The Florida Entomologist* **86 (1):** 53-60.

Louda, S.M. and Stiling, P. (2004) The double edge sword of biological control in conservation and restoration. *Conservation Biology* **18**: 50–53.

Louda, S.M., Kendall, D. and Simberloff, D. (1997) Ecological effects of an insect introduced for the biological control of weeds. *Science* **277**: 1088–1090.

Lux S.A, Copeland R.S, White I.M, Manrakhan A, Billah M.K. (2003). A new invasive fruit fly species from the *Bactrocera dorsalis* (Hendel) group detected in East Africa. *Insect Science and its Application*, **23(4):** 355-361.

Lynch L.D. and Thomas M.B. (2000) Non-target effects in the biocontrol of insects with insects, nematodes and microbial agents: the evidence. *Biocontrol News Information* **21**: 117–130.

Mahattanatawee, K., Manthey, J.A., Luzio, G., Talcott, S.T., Goodner, K. and Baldwin, E.A. (2006). Total antioxidant activity and fiber content of select Florida-grown tropical fruits. *Journal of Agriculture and Food Chemistry* **54 (19):** 7355–7363.

Manrakhan, A. and Lux, S.A. (2006) Contribution of natural food sources to reproductive behaviour, fecundity and longevity of *Ceratitis cosyra* , *C. fasciventris* and *C. capitata* (Diptera: Tephritidae) . *Bulletin of Entomological Research* **96**: 259-268.

Manrakhan, A., Nadel, H., Middleton, M.C. and Daane, K.M. (2010). Fruit fly parasitoids in coffee in Mpumalanga Province, South Africa. *Biocontrol Science and Technology* **20**: 621-624.

Mau, R. F. L., and J. L. M. Kessing (1992). *Ceratitis capitata* (Wiedemann). Crop Knowledge Master (on-line database). Sourced online at http://www.extento.hawaii.edu/kbase/crop/Type/

McAlpine, J. F. (1989). Phylogeny and classification of the Muscomorpha. In: McAlpine J.F., Wood D.M. (eds.) *Manual of Nearctic Diptera* 3. Research Branch, Agriculture Canada, *Monograph* **32**: 1397-1518.

McConnell, T. (2010). Kenya horticulture exports stopped by volcano ash cloud. *Global Post*, Sourced at http://www.globalpost.com/dispatch/kenya/100420/kenyan-horticulture-exports-stopped-volcano-ash-cloud

Messing, R. H. (1999). The impact of non-target control on the practice of biological control. In: Non-target Impact of Biological Control. Ed. by Follett, P. A.; Duan, J. J.Norwell, MA: Kluwer Academic Publishers, pp. 45–55.

Messing, R. H., Klungness, L. M., Jang, E. B. and Nishijima, K. A. 1996. Messing, R. H., Klungness, L. M., Jang, E. B. and Nishijima, K. A. (1996). Response of the melon fly parasitoid *Psytalia fletcheri* (Hymenoptera: Braconidae) to host-habitat stimuli. *Journal of Insect Behaviour*. **9:** 933–945.

Messing, R. H. and Jang, E. B. (1992). Response of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) to host-fruit stimuli. *Biological Control* **21:** 1189–1195.

Messing, R. H., Klungness, L. M., Purcell, M. and Wong, T. (1993). Quality control parameters of mass-reared opiine parasitoids used in augmentative biological control of tephritid fruit flies in Hawaii. *Biological control* **3:** 140-147.

Messing, R. H., and Wong, T.T.Y. (1992). An effective trapping method for field studies of Opiine Braconid parasitoids of tephritid fruit flies. *Entomophaga* **37**: 391-396.

Midgarden D., Ovalle, O., Epsky, N.D., Puche, H., Kendra, P.E., Rendon, P. and Heath, R. R. (2004). Capture of Mediterranean Fruit Flies (Diptera: Tephritidae) in dry traps baited with a

food-based attractant and Jackson traps baited with trimedlure during sterile male release in Guatemala. *Journal of Economic Entomology* **97 (6)**: 2137–2143.

Mirani, Z. (2007). Study of diffusion and adoption of Male Annihilation Technique. *International Journal of Education and Development using Information and Communication Technology* **3**: 89-99.

Mohamed, S. A., Sunday, E. and Hanna, R. (2010). Old and new host-parasitoid associations: parasitism of the invasive fruit fly, *Bactrocera invadens* (Diptera: Tephritidae) and five African fruit fly species by *Fopius arisanus*, an Asian opiine parasitoid. *Biocontrol Science and Technology* **20 (2)**: 183-196.

Mohamed, S. A., Sunday, E. and Hanna, R. (2008). Evaluation of the impact of *Diachasmimorpha longicaudata* on *Bactrocera invadens* and five African fruit fly species. *Journal of Applied Entomology* **132**: 789–797.

Mohamed, S.A., Overholt, W.A., Wharton, R.A. and Lux, S.A. (2006). Effect of temperature on developmental time and longevity of *Psyttalia cosyrae* (Hymenoptera: Braconidae) *Biocontrol, Science & Technology* **16 (7)**:717-726.

Montoya, P., Benrey, B., Barrera, J.F., Zenil, M., Ruiz, L. and Liedo P. (2003). Oviposition behavior and conspecific host discrimination in *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae), a fruit fly parasitoid. *Biocontrol, Science and Technology* **13**: 683-690.

Montoya, P., Sua´rez, A., Lo´pez, F. and Cancino, J. (2009). *Fopius arisanus* oviposition in four Anastrepha fruit fly species of economic importance in Mexico. *Biological Control* **54**: 437–444

MOSPANS (2011). Geography of the Eastern and Coast Provinces of Kenya. Accessed online at http://en.wikipedia.org/wiki/Eastern_Province_%28Kenya%29

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

Mumford, J. and Tween, G. (1997) Economic feasibility study for the control of the Mediterranean and Natal fruit fly in the Western Cape Province of South Africa. *Expert Mission Report RU-7135, I.A.E.A., Vienna, Austria.*

Mwatawala, M., De Meyer, M., Makundi R. and Maerere A. (2009). Host range and distribution of fruit-infesting pestiferous fruit flies (Diptera, Tephritidae) in selected areas of Central Tanzania. *Bulletin of Entomological Research* **99** (1): 1-13.

Mwatawala, M.W., De Meyer, M., Makundi, R.H. and Maerere, A.P. (2006). Seasonality and Host Utilization of the Invasive Fruit Fly, *Bactrocera invadens* (Diptera: Tephritidae) in Central Tanzania. *Journal of Applied Entomology* **130**: 530-537.

Mwatawala, M.W., De Meyer, M., White, I.M., Maerere, M., and Makundi, R.H. (2007). Detection of the Solanum Fruit Fly, *Bactrocera latifrons* (Hendel) in Tanzania (Diptera:Tephritidae). *Journal of Applied Entomology* **131**: 501-503.

Mwatawala, M.W., White, I.M., Maerere, A.P., Senkondo, F.J. & De Meyer, M. (2004). A new invasive *Bactrocera* species (Diptera: Tephritidae) in Tanzania. *African Entomology* **12**: 154–156.

NAQS, (2007). Exotic fruit flies. Northern Australia Quarantine Strategy (NAQS) fact sheet on exotic fruit flies. Available at http://www.daff.gov.au/aqis/quarantine/pests-diseases/plants-products/exotic_fruit_flies

Newell, I.M. and Haramoto, F.H. (1968). Biotic factors influencing populations of *Dacus dorsalis* in Hawaii. *Proceedings of the Hawaiian Entomological Society* **20**: 81-139.

Nielson, M.G. (2010). Weaver ants to help African farms yield fruit. Available at http://www.freshplaza.com/news_detail.asp?id=73653#SlideFrame_1

Nilakhe, I. S., Cox, H. B., Cunningham, R. T., Davidson, J. L., Bingham, R. R., Burns, R. E., Rosander, R. and Abel, W. L. (1993). Detection of exotic fruit flies in the United States, In Aluja, M. and Liedo, P. (eds.) Fruit flies biology and management. Springer New York, pp. 395-399.

Norborrn, A.L. (2004). Fruit Fly (Diptera: Tephritidae), Classification and Diversity. Sourced online at http://www.sel.barc.usda.gov/diptera/tephriti/Tephclas.htm

OABS (2005). Key deciduous fruit statistics 2005. *Optimal Agricultural Business Systems, Paarl, South Africa.*

Offenberg, J., Nielsen, M.G., Macintosh, D.J., Havanon, S. and Aksornkoae, S. (2004). Evidence that insect herbivores are deterred by ant pheromones. *Proceedings of the Royal Society of London Series B, Bio Scis*, **271:** 433-435.

Ouna, E.A. (2010). Entomopathogenicity of hyphomycetes fungi to the new invasive fruit fly species *Bactrocera invadens* and their potential for biological control on mango. MSc dissertation, Department of Zoology, Kenyatta University.

Ovruski, S., Aluja, M., Sivinski, J. and Wharton, R. A. (2000). Hymenoptera Parasitoids on fruit infesting Tephritidae (Diptera) in Latin America and the southern United States: Diversity, distribution, taxonomic status and their use in fruit fly biocontrol. *Integrated Pest Management Reviews* **5:** 81-107.

Owen, D.F. and Owen, J. (1974). Species diversity in temperate and tropical Ichneumonidae. *Nature* **249:** 583–584.

Paladino, L., Papeschi, A. and Cladera, J. (2010). Immature stages of development in the parasitoid wasp, *Diachasmimorpha longicaudata. Journal of Insect Science* **56**: 1-13.

Peng, R.K., Christian, K. and Gibb, K. (1995) The effect of the green ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae), on insect pests of cashew trees in Australia. *Bulletin of Entomological Research* **85**: 279–284.

Peng, R., Christian, K. and Gibb, K. (2001). Potential of using colonies of the green ant, *Oecophylla smaragdina* (F.), to control cashew insect pests. *Technical Bulletin of the Department of Primary Industry and Fisheries* **288**: 81-93.

Peng, R. and Christian, K. (2004). The weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae), an effective biological control agent of the red-banded thrips, *Selenothrips rubrocinctus* (Thysanoptera: Thripidae) in mango crop in the Northern Territory of Australia. *International Journal of Pest Management* **50**: 107-114.

Peng, R. and Christian, K. (2005). Integrated pest management in mango orchards in the Northern Territory Australia, using the weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae) as a key element. *International Journal of Pest Management* **51**: 149-155.

Peng, R. and Christian, K. (2007). The effect of the weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae), on the mango seed weevil, *Sternochetus mangiferae* (Coleoptera: Curculionidae), in mango orchards in the Northern Territory of Australia. *International Journal of Pest Management*, **53**, 15-24.

Peng, R. and Christian, K. (2008). The dimpling bug, *Campylomma austrina* Malipatil (Hemiptera : Miridae): the damage and its relationship with ants in mango orchards in the Northern Territory of Australia. *International Journal of Pest Management* **54**:173-179.

Penrose, R. (1993). The 1989/1990 Mediterranean fruit fly eradication program in California. In: Aluja, M. and Liedo, P. Fruit flies biology and management (eds.), Springer New York, pp. 401-406.

Percival, S.S., Talcott, S.T., Chin, S.T., Mallak, A.C., Lounds-Singleton, A. and Pettit-Moore, J. (2006). Neoplastic transformation of BALB/3T3 cells and cell cycle of HL-60 cells are inhibited by mango (*Mangifera indica* L.) juice and mango juice extracts. *Journal of Nutrition* **136 (5):** 1300–1304.

Petzoldt, C. and Seaman, A. (2007). Climate Change Effects on Insects and Pathogens. Fact Sheet. http://www.climateandfarming.org/clr-cc.php

Prosser, C. L. (1952). Compilation of Animal Physiology. Saunders Co., Philadelphia, 374 pp.

Purcell, M., Herr, J. C., Messing, R. H. and Wong, T. (1998). Interactions between augmentatively released *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) and a complex of opiine parasitoids in a commercial guava orchard. *Biocontrol Science and Technology* **8:** 139-151.

Quesada-Moraga, E., Martin-Carballo, I., Garrido-Jurado, I., and Santiago-A´ lvarez, C. (2008). Horizontal transmission of Metarhizium anisopliae among laboratory populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), *Biological Control*, **47**: 115-124.

Quilici, S., Rousse, P., Deguine, J., Simiand, C., Franck1, A., Gourdon, F., Mangine, T. and Harris, E. (2008). Successful acclimatization of the ovo-pupal parasitoid *Fopius arisanus* in Reunion island for the biological control of the peach fruit fly, *Bactrocera zonata*. *Abstract for the First Meeting of TEAM,* Palma of Mallorca.

Ramadan, M. M. (2004). Mass-rearing biology of *Fopius vandenboschi* (Hymenoptera: Braconidae). *Journal of Applied Entomology* **128:** 226-232.

Ramadan, M.M., Wong, T.T.Y. and Beardsley J.W. (1989) Survivorship, potential and realized fecundity of *Biosteres tryoni* (Hymenoptera: Braconidae) a larval parasitoid of Ceratitis capitata (Diptera: Tephritidae). *Entomophaga* **34**: 291-297.

Raspi A. and Loni A., (1994). Alcune note sull'allevamento di *Opius concolor* Szépl. (Hymenoptera Braconidae) e su recent tentativi d'introduzione della specie in Toscana ed in Liguria - *Frustula Entomologica* **17**: 135-145.

Robinson, A. S. 2002a. Mutations and their use in insect control. *Mutation Research Reviews* **511**: 113-132.

Rodríguez, R. (1991). Parasitismo y preferencia de *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) en tres especies de *Anastrepha* Schiner (Diptera: Tephritidae). Tesis de Maestría, Colegio de Postgraduados Montecillo, Mexico.

Rodríguez, J., Di Pierro, D., Gioia, M., Monaco, S., Delgado, R., Coletta, M. and Marini, S. 2006. Effects of a natural extract from *Mangifera indica* L, and its active compound, mangiferin, on energy state and lipid peroxidation of red blood cells". *Biochimica et Biophys Acta* **1760 (9)**: 1333–42.

Rousse P., Harris E.J. and Quilici, S. (2005). *Fopius arisanus*, an egg-pupal parasitoid of Tephritidae. Overview. *Biocontrol News Information* **26**: 59–69

Rousse, P., Gourdon, F. and Quilici, S. (2006). Host specificity of the egg pupal parasitoid *Fopius arisanus* (Hymenoptera: Braconidae) in La Reunion. *Biological Control* **37**: 284–290

Rousse, P., Chiroleu F., Veslot J. and Quilici S. (2007). The host- and microhabitat olfactory location by Fopius arisanus suggests a broad potential host range. *Physiological Entomology* **32(4):** 313-321.

Rousse, P., Gourdon, F., Roubaud, M., Chiroleu, F. and Quilici, S. (2009) Biotic and Abiotic Factors Affecting the Flight Activity of *Fopius arisanus*, an Egg-Pupal Parasitoid of Fruit Fly Pests. *Environmental Entomology*, **38(3):** 896-903.

Rugman-Jones P.F., Wharton, R., van Noort, T. and Stouthamer, R. (2009) Molecular differentiation of the *Psyttalia concolor* (Szepligeti) species complex (Hymenoptera: Braconidae) associated with olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), in Africa. *Biological Control* **49**: 17–26

Rwomushana, I. (2008). Bio-ecology of the new invasive fruit fly, *Bactrocera invadens* (Diptera Tephritidae) in Kenya and its interaction with indigenous mango-infesting fruit fly species. PhD thesis submitted to the Kenyatta University. Reg. no. 184/15237/2005, 200 pp.

Rwomushana, I., Ekesi, S., Gordon, I. and Ogol, C.K.P.O. (2008). Host Plants and Host Plant Preference Studies for *Bactrocera invadens* (Diptera: Tephritidae) in Kenya, a New Invasive Fruit Fly Species in Africa. *Annals of the Entomological Society of America* **101(2):** 331-340.

Samways, M.J., Osborn, R., Hastings, H. and Hattingh, V. (1999). Global climate change and accuracy of prediction of species' geographical ranges: establishment success of introduced ladybirds (Coccinellidae, *Chilocorus* spp.) worldwide. *Journal of Biogeography*, **26**: 795-812.

Shiga, M. (1992). Future prospects for the eradication of fruit flies. *Technical Bulletin of Food and Fertilizer Technology Center* **128**: 1-12.

Siebert, J. and Pradhan, V. (1991). The Potential Economic Impact of the Mediterranean fruit fly, *Ceratitis Capitata* (Wied.), upon establishment in California: "An Update". Department of Agriculture and Natural Resources, University of California. Working Paper No. 547.

Silvestri, F. (1913). Viaggio in Africa per cercare parassiti di mosche dei frutti. *Bollettino del Laboratorio di Zoologia General e Agraria, Portici* **8:** 1-164.

Silvestri, F. (1914). Report of an expedition to Africa in search of the natural enemiesof fruit flies (Trypaneidae) with descriptions, observations and biological notes. *Division of Entomology, Board of Agriculture and Forestry, Territory of Hawaii Bulletin* **3:** 1-176.

Sime K.R., Daane K.M., Wang X.G, Johnson, M.W. and Messing, R.H. (2008). Evaluation of *Fopius arisanus* as a biological control agent for the olive fruit fly in California. *Agricultural and Forest Entomology* **10**: 423–31.

Sime, K., Daane, K., Nadel, H., Funk, C., Messing, R.H., Andrews Jr, J., Johnson, M. and Pickett, C. (2006). *Diachasmimorpha longicaudata* and *Diachasmimorpha kraussii* (Hymenoptera: Braconidae), potential parasitoids of the olive fruit fly. *Biocontrol Science and Technology* **16 (2):** 169-179

Singh, U.P., Singh, D.P., Singh, M., Maurya, S., Srivastava, J.S., Singh, R.B. and Singh, S.P. (2004). Characterization of phenolic compounds in some Indian mango cultivars. *International Journal of Food Science and Nutrition* **55 (2):** 163–169.

Sivinski, J.M. (1996). The past and potential of biological control of fruit flies. *In* "Fruit Fly Pests. A World Assessment of Their Biology and Management" (McPheron, B.A. and Steck, G.F. (eds.)). St. Lucie Press. Delray Beach. FL, pp. 369-375

Sivinski, J.M., Calkins, C.O., Baranowsky, R., Harris, D., Brambila, J., Diaz, J., Burns, R.E., Holler, T. and Dodson, D. (1996). Suppression of the Caribbean fruit fly (Anastrepha suspense (Loew) Diptera: Tephritidae) population through augmented releases of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). *Biological Control* **6**:177-185.

Snowball , B.V. and Lukins , R.G . (1964b) Status of introduced parasites of Queensland fruit fly (*Strumeta tryoni*), 1960 – 1962 . *Australian Journal of Agricultural Research* , **15:** 586 –608

Sokal, R.R. and Rohlf, F.J. (1981). Biometry: The principles and practices of statistics in biological research. 2nd edition, W.H. Freeman and Co., San Francisco, 859 pp.

Sookar, P., Bhagwant, S., and Ouna, E.A. (2008) Isolation of entomopathogenic fungi from soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae), *Journal of Applied Entomology* **132**: 778-788.

Sparks, T.C., Thompson, G.D., Kirst, H.A., Hertlein, M.B., Larson, L.L., Worden, T.V. and Thibault, S.T. (1998). Biological activity of the spinosyns, new fermentation derived insect control agents, on tobacco budworm (Lepidoptera: Noctuidae) larvae. *Journal of Economic Entomology* **91**: 1277–1283.

Steck G.J., Gilstrap F.E., Wharton R.A. and Hart W.G. (1986) Braconid parasitoids of Tephritidae (Diptera) infesting coffee and other fruits in West-Central Africa. *Entomophaga* **31**: 59-67.

Steiner, L. F. (1952). Methyl eugenol as an attractant for oriental fruit fly. *Journal of Economic Entomology* **45**:241–248.

Steiner, L.F., Mitchell, W.C., Harris, E.J., Kozuma, T.T. and Fujimoto, M.S. (**1965)**. Oriental fruit fly eradication by male annihilation. *Journal of Economic Entomology* **58**: 961-964.

TAPP, (2010). Tanzania Agriculture Productivity Program. Mangoes, *Market Bulletin* 1. 5pp. Available online at www.tanzania-agric.org

Thomas, D.W. (1998). The Influence of Aggressive Ants on Fruit Removal in the Tropical Tree, *Ficus capensis* (Moraceae) *Biotropica* **20 (1):** 49-53

Townes, H. and Townes, M. (1973). A catalogue and reclassification of the Ethiopian Ichneumonidae. *Memoirs of the American Entomological Institute* **19**: 1-416.

Tsuji, K., Harlion, A.H. and Nakamura, K. (2004). Asian weaver ants, *Oecophylla smaragdina*, and their repelling of pollinators. *Ecological Research* **19**: 669–673

Tsuruta, K., White, I.M., Bandara, H.M.J., Rajapakse, H., Sundaraperuma, S.A.H., Kahawatta, S.B.M.U.C. and Rajapakse, G.B.J.P. (1997) A preliminary notes on the hosts of fruit flies of the tribe Dacini (Diptera, Tephritidae) in Sri Lanka. *Esakia*, **37**: 149-160.

Tumlinson, J. H., Lewis, W. J., and Vet, L. E. M. (1993). How parasitic wasps find their hosts. *Scientific American* 268: 101-105.

Umeh, V.C., Olaniyan, A.A., Ker, J. and Andir, J. (2004). Development of citrus fruit fly control strategies for small-holders in Nigeria. *Fruits* 59: 265-274.

USAID (2006). USAID Helps Indian Mango Farmers Access New Markets. Available online at http://www.usaid.gov/in/newsroom/press_releases/may03_6p.htm

Van den Bosch, R. and Haramoto, F.H. (1951). *Opius oophilus* Fullaway, an egg-larval parasite of the oriental fruit fly discovered in Hawaii. *Proceedings of the Hawaiian Entomological Society* **2**: 251-255.

Van Lenteren, J.C. (1986). Parasitoids in the greenhouses: Successes with seasonal inoculative release systems. In: Waage J, Greathead D, editors. Insect parasitoids. London: Academic Press, pp 341-374.

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

Van Mele, P. (2008) A historical review of research on the weaver ant, *Oecophylla* in biological control. *Agricultural and Forest Entomology*, **10**: 13–22.

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

Van Mele, P. and Cuc, N.T.T. (2000). Evolution and status of *Oecophylla smaragdina* (Fabricius) as a pest control agent in citrus in the Mekong Delta, Vietnam. *International Journal of Pest Management* **46**: 295-301.

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

Van Noort, S. (2004). Ichneumonid (Hymenoptera: Ichneumonoidea) diversity across an elevational gradient on Monts Doudou in Southwestern Gabon. *California Academy of Sciences Memoir* **28 (204):** 187-216.

Van Noort, S., G. L. Prinsloo, and S. G. Compton. (2000). Hymenoptera excluding Apoidea (Apiformes) and Formicidae (Insecta). Pp. 289–364 in Dâures—biodiversity of the Brandberg Massif, Namibia. Kirk-Spriggs, A. H. and Marais, E. (eds.), Cimbebasia Memoir 9, National Museumof Namibia, Windhoek, 389 pp.

Van Wijngaarden, P.M., Van Kessel M. & Van Huis A. (2007). *Oecophylla longinoda* (Hymenoptera: Formicidae) as a biological control agent for cocoa capsids (Hemiptera: Miridae) *Proceedings of Netherlands Entomological Society* **18**: 21-30.

Varela, A.M. (1992) Role of *Oecophylla longinoda (Formicidae)* in control of *Pseudotheraptus wayi* (*Coreidae*) on coconut in Tanzania. PhD Dissertation, University of London.

Vargas, R. I., Leblanc, L., Putoa, R. and Eitam, A. (2007). Impact of introduction of *Bactrocera dorsalis* (Diptera:Tephritidae) and classical biological control releases of *Fopius arisanus* (Hymenoptera: Braconidae) on economically important fruit flies in French Polynesia. *Journal of Economic Entomology* **100**: 670-679.

Vargas, R.I., Ramadan, M.M., Hussain, T., Mochizuki, N., Bautista, R.C. and Stark, J.D. (2002). Comparative demography of six fruit fly (Diptera: Tephritidae) parasitioids (Hymenoptera: Braconidae). *Biological control* **25**: 30-40.

Vargas, R.I., Peck, S.L., McQuate, G.T., Jackson, C.G., Stark, J.D. and Armstrong, J.W. (2001). Potential for area-wide integrated pest management of Mediterranean fruit fly (Diptera: Tephritidae) with braconid parasitoid and a novel bait spray. *Journal of Economic Entomology* **94**: 4 (817-825).

Vargas, R.I., Stark, J.D., Uchida, G.K. and Purcell, M. (**1993)** Opiine parasitoids (Hymenoptera: Braconidae) of Oriental fruit fly (Diptera: Tephritidae) on Kauai Island, Hawaii: islandwide relative abundance and parasitism rates in wild and orchard guava habitats. *Environmental Entomology* **22(1):** 246-253.

Vayssie`res, J.F.,Wharton, R. Adandonon, A. and Sinzogan, A. (2010). Preliminary inventory of parasitoids associated with fruit in Benin, *Biological Control* DOI 10.1007/s10526-010-9313-y

Vayssières , J-F., Adandonon, A., Sinzogan, A. and Van Mele, P. (2009). Allies in nature. Available at http://r4dreview.org/2009/10/allies-in-nature/

Vayssieres, J. F., Korie, S., Coulibaly, O., Temple, L. and Boueyi, S. P. (2008). The mango tree in central and northern Benin: cultivar inventory, yield assessment, infested stages and loss due to fruit flies (Diptera Tephritidae). *Fruits* **63 (6)**: 335-348.

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

Vayssières, J.F., Wharton, R., Delvare, G. and Sanogo, F. (2004). In: Barnes Brian N. (ed.) *Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, Stellenbosch, South Africa*. *Isteg Scientific Publications*, pp. 461-464.

Wagner, T.L., Wu, H., Sharpe, P.J.H., Schoolfield, R.H. and Coulson, R.N. (**1984).** Modelling insect development rates: a literature review and application of a biophysical model. *Annals of the Entomological Society of America* **77**: 208-225.

Wang XG, Messing RH. (2004) Potential interactions between pupal and egg- or larval-pupal parasitoids of tephritid fruit flies. *Environmental Entomology* **33**: 1313-1320.

Waterhouse, D. F. (1993) Pest Fruit flies in the Oceanic Pacific, in (Ed.) Biological Control: Pacific Prospects - Supplement 2, pp. 4-47

Way, M.J. (1954). Studies of the life history and ecology of the ant, *Oecophylla longinoda* Latreille. *Bulletin of Entomological Research* **45**:93–112

Way, M. J. and Khoo, K. C. (1992.) Role of ants in pest management. *Annual Review of Entomol*ogy **37**: 479-503.

Weinzierl, R., Henn, T., Koehler, P. G. and Tucker, C. L. (1995). Insect Attractants and Traps in Alternatives in Insect Management, Office of Agricultural Entomology, University of Illinois. Sourced online at http://www.edis.ifas.ufl.edu.

Wessel, G. (1997). Mango production and marketing in Kenya. Report, 40 pp.

Wharton, R. A., Trostle, M. K., Messing, R. H., Copeland, R. S., Kiman-Njogu, S. W., Lux, S., Overholt, W. A., Mohamed, S. and Sivinski, J. (2000). Parasitoids of medfly, *Ceratitis capitata*, and related tephritids in Kenyan coffee: a predominantly koinobiont assemblage. *Bulletin of Entomological Research* **90**: 517-526.

Wharton, R.A. (1999). A review of the old world genus *Fopius*, with description of two new species reared from fruit-infesting Tephritidae. *Journal of Hymenopteran Research* **8**: 48–64.

Wharton, R. A. (1989). *Classical biological control of fruit Tephritidae*. In: World Crop Pests. Fruit flies: their biology, natural enemies, and control, Vol. 3b. Amsterdam: Robinson, A. and Harper, G. *Elsevier Science* 303–313.

Wharton, R.A. and Gilstrap, F.E. (1983). Key to and status of opiine braconid (Hymenoptera) parasitoids used in biological control of *Ceratitis* and *Dacus* s.l. (Diptera: Tephritidae). *Annals of the Entomological Society of America* **76**: 721-742.

Wharton, R.A., Gilstrap, F.E., Rhode, R.H., Fische, M. and Hart, W.H. (1981). Hymenopterous egg-pupal and larval-pupal parasitoids of *Ceratitis capitata* and *Anastrepha spp* (Diptera: Tephritidae) in Costa Rica. *Entomophaga* **26**: 285-290.

White, I.M. (2000). Taxonomy of the the Dacina (Diptera:Tephritidae) of Africa and the Middle East. *Africa Entomological Memoirs* **2**: 7-156.

White, I.M. and Hancock, D.L. (1997). CABIKEY to the Dacinae of the Indo-Australasia. Windows CD-ROM. CAB International, Wallingford.

White I.M. and Elson-Harris, M.M. (1992). *Fruit flies of economic significance: their identification and bionomics.* CAB International. Oxon, UK. 601 pp.

Wikipedia, (2010). Tephritidae. Sourced online at http://en.wikipedia.org/wiki/Tephritidae

Willard, H.F. and Mason, A.C. (1937) Parasitization of the Mediterranean fruit fly in Hawaii 1914-33, *Circular of the U.S. Department of Agriculture* 439,18 pp.

Willard, H.F. (1920) *Opius fletcheri* as a parasite of the melon fly in Hawaii. *Journal of Agricultural Research* **20**: 423-438.

Wimp, G. M. and Whitham, T. G. (2001). Biodiversity consequences of predation and host plant hybridization on an aphid-ant mutualism. *Ecology* **82**: 440-452.

Wong, T. T. Y., Ramadan, M. M., Mcinnis, D. O., Mochizuki, N., Nishimoto, J. J. and Herr, J. C. (1991). Augmentative releases of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) to suppress a mediterranean fruit fly (Diptera: Tephritidae) population in Kula, Maui, Hawaii. *Biological Control* **1**: 1-7.

Wong, T.T.Y., Mochizuki, N. and Nishimoto, J.I. (1984). Seasonal abundance of parasitoids of the Mediterranean and Oriental fruit flies (Diptera:Tephritidae) in the Kula area of Maui, Hawaii. *Environmental Entomology* **13**: (1) 140-145.

Wong, T.T.Y., Nishimoto, J.I. and Mochizuki, N. (1983) Infestation patterns of Mediterranean fruit fly and the oriental fruit fly (Diptera, Tephritidae) in the Kula area of Maui, Hawaii. *Environmental Entomology* **12**: 1031–1039.

Yonow, T., Zalucki, M.P., Sutherst, R.W., Dominiak, B.C., Maywald, G.F., Maelzer D.A. and Kriticos, D.J. (2004). Modelling the population dynamics of the Queensland fruit fly, *Bactrocera (Dacus) tryoni*: a cohort-based approach incorporating the effects of weather. *Ecological Modelling* **173**:9-30.

YU, D. S. (1998). *Interactive Catalogue of World Ichneumonidae 1998. CD-ROM. Taxapad. Vancouver,Canada*

Yusuf, S.A. and Salau, A.S. (2007). Forecasting Mango and Citrus Production in Nigeria: A Trend analysis (Unpublished).

Zaheeruddin, M. (2007). Study of diffusion and adoption of Male Annihilation Technique. *International Journal Education and Development using Information and Communication Technology* **3 (2)**: 89-99

APPENDICES

Appendix 1. Fruit sampling sites with approximate geo-referenced position and altitude

PROVINCE	LOCALITY	APPROXIMATE LONGITUDE	APPROXIMATE LATITUDE	APPROXIMATE ALTITUDE (m)
	Diani forest	04° 20’ 03 S	39° 34’ 10 E	25
COAST	Diani area	04° 16’ 40 S	39° 35’ 14 E	8
	Kibarani	04° 18’ 38 S	39° 30’ 05 E	338
	Mkambani	04° 13’ 36 S	39° 36’ 04 E	48
	Muhaka area	04° 20’ 15 S	39° 30’ 55 E	52
	Muhaka forest	04° 19’ 25 S	39° 32’ 25 E	46
	Shimba Hills	04° 15’ 26 S	39° 26’ 05 E	385
	Mabokoni	04° 18’ 29 S	39° 31’ 07 E	87
	Miilu farm	04° 23’ 10 S	39° 33’ 42 E	55
	Buga	04° 19’ 23 S	39° 29’ 18 E	92
	Kigaleni	04° 36’ 16 S	39° 21’ 18 E	48
	Kinondo	04° 16’ 29 S	39° 34’ 54 E	6

CONT'D

PROVINCE	LOCALITY	LONGITUDE	LATITUDE	ALTITUDE (m)
EASTERN	Rocky hill	00° 06' 31 S	37° 35' 55 E	1860
	Kinoru	00° 02' 46 S	37° 38' 16 E	1710
	Meru forest	00° 03' 18 S	37°37' 34 E	1776
	Mpakone	00° 02' 41 S	37° 36' 22 E	1888
	Mpuri	00° 02' 16 S	37° 36' 19 E	1892
	Kiamiriru	00° 02' 09 S	37° 36' 37 E	1845
	Gachanka	00° 01' 49 S	37° 39' 57 E	1508
	Muringo	00° 01' 18 S	37° 41' 12 E	1390
	Miriga	00° 03' 18 S	37° 42' 27 E	1280
	Muthangari	00° 02' 38 S	37° 40' 31 E	1441
	Kithoka	00° 06' 23 S	37° 40' 1 E	1509
	Giaki	00° 02' 58 S	37° 45' 40 E	1152
	Kimburini	00° 01' 40 S	37° 49' 3 E	1016
	Mbuuta	00° 00' 04 S	37° 53' 51 E	891

Appendix 2. Parasitoid composition from the survey at the Coast Province.

LOCALITY	PLANT SPECIES	*Psyttalia* spp	*P. peproxima*	*P. concolor*	*D. Fullawayi*
Muhaka	*Jasminum fluminense*	5			
Muhaka	*Phyllanthus rubifolia*	8			
Muhaka	*Polysphaeria parvifolia*	5			
Kinondo	*Synaptolepis kirkii*	8			
Kibarani	*Polysphaeria parvifolia*	17			
Kibarani	*Chassalia curviflora*	24	11		
Kibarani	*Synaptolepis kirkii*	2			
Kigaleni	*Phyllanthus rubifolia*	1	10	12	
Shimba hills	*Chassalia curviflora*	3			1
Kibarani	*Gloriosa superba*	1		4	
Muhaka	*Chassalia curviflora*	4			
Buga	*Lannea welwitschii*				
Muhaka	*Gloriosa superba*	5			
Kigaleni	*Gloriosa superba*	1	5		
Kigaleni	*Synaptolepis kirkii*	11			
Mkambani	*Chassalia curviflora*	7			
Kinondo	*Terminalia cattapa*				1
Kigaleni	*Chassalia curviflora*	7			
Mabokoni	*Chassalia curviflora*	5			
Kibarani	*Phyllanthus rubifolia*	1			

Appendix 2 CONTD. Parasitoid composition from survey at the Coast Province CONT'D

LOCALITY	PLANT SPECIES	Braconids	*T. giffardianus*	Ichneumonids	Chalcid wasps
Muhaka	*Jasminum fluminense*				
Muhaka	*Phyllanthus rubifolia*	17			
Muhaka	*Polysphaeria parvifolia*				
Kinondo	*Synaptolepis kirkii*				
Kibarani	*Polysphaeria parvifolia*				
Kibarani	*Chassalia curviflora*				15
Kibarani	*Synaptolepis kirkii*	8			
Kigaleni	*Phyllanthus rubifolia*				
Shimba hills	*Chassalia curviflora*		7		
Kibarani	*Gloriosa superba*	2			
Muhaka	*Chassalia curviflora*				
Buga	*Lannea welwitschii*	5			
Muhaka	*Gloriosa superba*				
Kigaleni	*Gloriosa superba*	9			
Kigaleni	*Synaptolepis kirkii*				
Mkambani	*Chassalia curviflora*				
Kinondo	*Terminalia cattapa*				
Kigaleni	*Chassalia curviflora*				
Mabokoni	*Chassalia curviflora*				
Kibarani	*Phyllanthus rubifolia*			14	

Appendix 3. Fruit fly composition from survey at the Coast Province

LOCALITY	FRUIT NAME	*T. niggerimum*	*T. coffeae*	*C. capitata*
Muhaka	*Jasminum fluminense*		25	
Muhaka	*Phyllanthus rubifolia*		59	
Muhaka	*Polysphaeria parvifolia*		8	
Kinondo	*Synaptolepis kirkii*		53	
Milu farm	*Citrus reticulate*			20
Maweni	*Sclerocarya birrea*			
Kibarani	*Polysphaeria parvifolia*		21	
Kibarani	*Chassalia curviflora*		121	127
Kibarani	*Synaptolepis kirkii*		13	7
Buga	*Mangifera indica*			
Kigaleni	*Phyllanthus rubifolia*			
Diani	*Coccinia spp*			
Buga	*Citrus reticulata*			13
Shimba Hills	*Chassalia curviflora*		42	
Kibarani	*Gloriosa superba*		24	16
Muhaka	*Chassalia curviflora*		11	
Milu farm	*Citrus sinensis*			9
Milu farm	*Citrus reticulata*			30
Milu farm	*Citrus sinensis*			15
Milu farm	*Mangifera indica*			

Appendix 3. CONT'D

LOCALITY	FRUIT NAME	*T. niggerimum*	*T. coffeae*	*C. Capitata*
Kibarani	*Mangifera indica*			
Muhaka	*Gloriosa superba*		10	14
Buga	*Mangifera indica*			
Kigaleni	*Gloriosa superba*	*44*	41	38
Kigaleni	*Synaptolepis kirkii*		28	
Mkambani	*Chassalia curviflora*			
Kinondo	*Terminalia cattapa*			
Buga	*Coccinia spp*			
Muhaka	*Sclerocarya birrea*			
Kigaleni	*Chassalia curviflora*		8	
Mabokoni	*Chassalia curviflora*			
Diani Forest	*Sorindea madagascariensis*			
Mkambani	*Sclerocarya birrea*			
Mabokoni	*Annona senegalensis*			
Kibarani	*Phyllanthus rubifolia*			
Muhaka	*Anona cherimola*			
Buga	*Lannea welwitschii*		18	

Appendix 3. Fruit fly composition from survey at the Coast Province CONT'D

LOCALITY	FRUIT NAME	*C. cosyrae*	*B. invadens*	*B. cucurbitae*
Muhaka	*Jasminum fluminense*	13		
Muhaka	*Phyllanthus rubifolia*	118		
Muhaka	*Polysphaeria parvifolia*	12		
Kinondo	*Synaptolepis kirkii*			
Milu farm	*Citrus reticulate*		33	
Maweni	*Sclerocarya birrea*	26	52	
Kibarani	*Polysphaeria parvifolia*	13		
Kibarani	*Chassalia curviflora*			
Kibarani	*Synaptolepis kirkii*	8		
Buga	*Mangifera indica*		594	
Kigaleni	*Phyllanthus rubifolia*	69		
Diani	*Coccinia spp*		10	13
Buga	*Citrus reticulate*		7	
Shimba Hills	*Chassalia curviflora*			
Kibarani	*Gloriosa superb*			
Muhaka	*Chassalia curviflora*	7		
Milu farm	*Citrus sinensis*		6	
Milu farm	*Citrus reticulate*		12	
Milu farm	*Citrus sinensis*		13	
Milu farm	*Mangifera indica*		165	
Milu farm	*Mangifera indica*		286	

Appendix 3. CONT'D

LOCALITY	FRUIT NAME	*C. cosyrae*	*B. invadens*	*B. cucurbitae*
Kibarani	*Mangifera indica*		140	
Muhaka	*Gloriosa superb*			
Buga	*Mangifera indica*		130	
Kigaleni	*Gloriosa superb*			
Kigaleni	*Synaptolepis kirkii*	30		
Mkambani	*Chassalia curviflora*	33		
Kinondo	*Terminalia cattapa*		50	
Buga	*Coccinia spp*		24	33
Muhaka	*Sclerocarya birrea*	63	122	
Kigaleni	*Chassalia curviflora*	10		
Mabokoni	*Chassalia curviflora*	23		
Diani Forest	*Sorindea madagascariensis*		15	
Mkambani	*Sclerocarya birrea*	86	113	
Mabokoni	*Annona senegalensis*		10	
Kibarani	*Phyllanthus rubifolia*	48		
Muhaka	*Anona cherimola*		22	

Appendix 4. Fruit fly composition from survey at the Eastern Province

LOCATION	**PLANT SPECIES**	***C. cosyrae***	***C. Capitata***	***C. rosa***
Mpakone	*Dovyalis caffra*	15	3	6
Kiamiriru	*Coffeae arabica*			
Gachanka	*Mangifera indica*	231		1
Muringo Mbugi	*Mangifera indica*	4		
Muringo Mbugi	*Mangifera indica*	27		
Muringo Mbugi	*Juglans cinerea*			
Muringo Mbugi	*Cucurbita maxima*			
Miriga mieru	*Mangifera indica*	14		1
Muthangari	*Coffeae arabica*			
Kithoka	*Citrullus lanatus*			
Kithoka	*Mangifera indica*	30		29
Kithoka	*Coffeae arabica*		135	
Giaki	*Terminalia mantaly*		7	
Mbuuta	*Cucumis dispaceas*			
Mbuuta	*Citrus sinensis*			
Mbuuta	*Mangifera indica*	9		

Appendix 4. Fruit fly composition from survey at the Eastern Province CONT'D

LOCATION	PLANT SPECIES	*B. invadens*	*Dacus spp*	*T. coffeae*
Mpakone	*Dovyalis caffra*			
Kiamiriru	*Coffeae arabica*			74
Gachanka	*Mangifera indica*	71		
Muringo Mbugi	*Mangifera indica*	3		
Muringo Mbugi	*Mangifera indica*	25		
Muringo Mbugi	*Juglans cinerea*		10	
Muringo Mbugi	*Cucurbita maxima*		8	
Miriga mieru	*Mangifera indica*	17		
Muthangari	*Coffeae arabica*			
Kithoka	*Citrullus lanatus*		25	
Kithoka	*Mangifera indica*	38		
Kithoka	*Coffeae arabica*			37
Giaki	*Terminalia mantaly*			
Mbuuta	*Cucumis dispaceas*		14	
Mbuuta	*Citrus sinensis*	1		
Mbuuta	*Mangifera indica*	489		