BIOECOLOGY OF THE NEW INVASIVE FRUIT FLY *Bactrocera invadens* (DIPTERA: TEPHRITIDAE) IN KENYA AND ITS INTERACTION WITH INDIGENOUS MANGO-INFESTING FRUIT FLY SPECIES

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DECLARATION BY CANDIDATE

The work presented in this thesis is a result of my own research and has not been presented for a degree in any other University.

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DEDICATION

This thesis is dedicated to the memory of my beloved late parents Mr. David.M.Bujara (RIP) and Mrs. Teopista N.Bujara (RIP) whose love, support and guidance before their demise made me what I am today. And to the Bujara family who have stood together amidst life's tough hurdles.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFFP	African Fruit Fly Programme
ANOVA	Analysis of Variance
ARCU	*icipe*-Animal Rearing and Containment Unit
BAT	Bait Application Technique
BSU	icipe-Biosystematics Research and Information Unit
DD	Day-Degrees
Df	Degrees of freedom
EUREP	European Retailers Program for Good Agricultural Practice
FAO	Food and Agriculture Organisation
FPEAK	Fresh Produce Exporters Association of Kenya
GPS	Global Positioning System
HSD	Tukey Honestly Significant Difference test
icipe	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
KEPHIS	Kenya Plant Health Inspection Service
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries, Uganda
MAT	Male Annihilation Technique
ME	Methyl Eugenol
OEPP/EPPO	European and Mediterranean Plant Protection Organization
SAS	Statistical Analysis System software
SE	Standard Error of mean
SIT	Sterile Insect Technique
SNK	Student Newman-Keuls multiple range test

ABSTRACT

Bactrocera invadens Drew, Tsuruta & White (Diptera: Tephritidae), an alien invasive fruit fly species of Asian origin was first detected in Kenya in 2003. This pest has rapidly spread across sub-Saharan Africa and is currently reported from at least 24 countries. Because of its novelty status, there was no information on its biology and ecology that could aid development of management efforts. There was also evidence that *B. invadens* co-infested the same fruits with native fruit fly species and it was speculated that this could result in competitive displacement of native fruit flies. This study, therefore, was initiated to establish the bioecology of *B. invadens* in Kenya and its interaction with indigenous mango-infesting fruit fly species. The first step was to identify the most suitable temperature range for development and survival of immature stages of this pest. Studies were conducted in the laboratory at four constant temperatures of 15, 20, 25, 30 and 35°C. The longest development period occurred at 15°C (75.74 days) and was shortest at 30°C (17.76 days). The optimal temperature for survival was found to be 25°C while 35°C was the most lethal temperature. Countrywide surveys were then initiated to establish the host plants of this pest. The survey revealed that *B. invadens* infested fourteen plant species particularly *Mangifera indica* L., *Musa* sp. *AAA* and citrus [*C. limon* (L.) Burm. f., *C. sinesis* (L.) Osbeck and *C. reticulata* Blanco.] and the wild plants *Sclerocarya birrea* (A. Rich) Hochst. and *Terminalia catappa* L. In laboratory host preference studies, *M. indica* and *Musa* sp. were found to be the most preferred host plants among the nine cultivated plant species tested. The spatial and temporal population dynamics of this pest was also studied and revealed that three fruit fly species infested mango namely *B. invadens*, *Ceratitis capitata* (Wiedemann) and *Ceratitis cosyra* (Walker). The relative abundance index (RAI) of these pests in infested fruit was in the order *B. invadens* > *C. cosyra* > *C. capitata* which was similar to the indices of adult population obtained by trapping. Percent fruit fly infestation and *B. invadens* fruit fly density was found to be always higher in mango on the ground than on the trees, demonstrating that mango fruits that fall to the ground serve as a major breeding site and may be a reservoir of non-immigrant *B. invadens* population in mango orchards in Kenya. The efficacy of the Easy, Multilure and Lynfield traps all baited with Nulure, Torula Yeast, Corn steepwater and a locally produced yeast product for trapping *B. invadens* was also evaluated. The multilure trap baited with torula yeast or nulure was the most attractive trap-bait combination and captured 19.7-30.3 *B. invadens*/trap/day and 10.54 -22.97 flies/trap/day respectively. In interspecific competition studies, there were significant differences in the larval developmental time, weight of puparia and number of adults that emerged of *B. invadens*, *C. capitata* and *C. cosyra* when the insects were sequentially co-infested on rearing medium at constant temperatures. When *B. invadens* was introduced into whole fruit before *C. cosyra*, the number of emergent adults of the latter was greatly suppressed. Higher number of *C. capitata* adults was, however, recovered when in cross infestation with *B. invadens* particularly in the treatments where *C. capitata* had a two or three days head start. This study demonstrated that the mechanisms contributing to the displacement of *C. cosyra* by *B. invadens* may be associated with intricate interactions between resource pre-emption and fluctuations in temperature in mango agroecosystems.

CHAPTER ONE

1 GENERAL INTRODUCTION

1.1 Background information

Fruit production constitutes an important source of income generation for both small and large-scale farmers in Kenya, creating job opportunities and improving diet by providing essential micronutrients and vitamins (FAO, 2004; FPEAK, 2005). Of the fruits grown in Kenya, mango *Mangifera indica* L. (Anacardiaceae) is among the most widely grown and a major candidate for both local and export markets. However, production in tropical sub-Saharan Africa is limited by many biotic and abiotic constraints. The major abiotic constraints are limited access to markets, unavailability of planting materials, poor infrastructure and high cost of inputs (Wessel, 1997). Most importantly, however, are the biotic factors particularly heavy infestations by a range of pests of which fruit fly species (Diptera: Tephritidae) are considered the most important (White & Elson-Harris, 1992). Infested fruits are unacceptable in the European Union (EU) markets as a result of strict quarantine restrictions (EUREP, 2003). Consignments with fruit fly damage are destroyed at the expense of the exporter resulting in serious economic loss to both the exporter and country. In addition to native fruit fly pests, alien invasive fruit flies on the continent further pose serious threat to the exploitation of foreign markets and jeopardise the lucrative trade in fresh fruits from the region.

A new invasive fruit fly species, *Bactrocera invadens* (Drew, Tsuruta & White) (Diptera: Tephritidae) was detected in East Africa in 2003 (Lux *et al.*, 2003a; Mwatawala *et al.*,

2004; Drew *et al.*, 2005). This pest is now established in 24 other countries in sub-Saharan Africa (Drew *et al.*, 2005; Vayssierès *et al.*, 2005; Francois-Xavier *et al.*, 2008; Umeh *et al.*, 2008). The pest is believed to be native to Sri Lanka, Asia (Drew *et al.*, 2005) where most *Bactrocera* (Diptera: Tephritidae) species are known to be endemic. *Bactrocera invadens* is an emerging polyphagous pest infesting a wide range of cultivated fruits particularly mango (Mwatawala *et al.*, 2004; Ekesi *et al.*, 2006) and other wild fruits (Drew *et al.*, 2005; Vayssierès *et al.*, 2005; Mwatawala *et al.*, 2006a). Consequently, it represents a new major threat to Africa's huge potential for commercial horticulture necessary for both the export and domestic markets.

Worldwide, fruit flies constitute one of the major threats to horticultural production, causing heavy pre-harvest and post-harvest losses and curtailing expansion of both domestic and international trade of fruits (Clausen, 1978). In the tropics, the problem is aggravated by the prevailing warm weather, which is conducive for overlapping fruiting patterns, resulting in overlapping generations of several fruit flies and the potential for year round infestation. In Kenya, yield losses of up to 30–80% due to fruit flies have been reported (Lux *et al.*, 1998). The arrival of the alien *B. invadens* on the continent has further aggravated this problem and Ekesi *et al.* (2006) found that infestation levels of 97.2 flies/kg of mango fruit due to *B. invadens* is common in some parts of Kenya. Evidently, increasing horticultural production and export in Africa will become unsustainable without due attention to the management of fruit flies on horticulture.

1.2 Justification

Since the first report of *B. invadens* in Kenya, the insect has become established in at least 24 other African countries including Angola, Benin, Burkina Faso, Cameroon, Chad, Comoros Island, Congo, Cote d'Ivoire, DR Congo, Equatorial Guinea, Gabon, Gambia, Ghana, Guinea, Mali, Mozambique, Namibia, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo and Uganda (Mwatawala *et al.*, 2004; Drew *et al.*, 2005; French, 2005; Vayssières *et al.*, 2005; Ekesi, 2006; Francois-Xavier *et al.*, 2008; Umeh *et al.*, 2008; Hanna, unpublished data). Given the fact that most tephritids have the ability to fly long distances in search of food and/or oviposition sites and the expanding problem of global warming, there is a great likelihood that the pest may spread further. The informal fruit movement between very porous African borders and general lack of strict quarantine regulations may also facilitate the spread of this pest to new areas. The recent regional integration of several African countries into Customs Unions allows free movement of all kinds of agricultural commodities that further increases the risks of spread. For example, in Papua New Guinea (PNG), of the total number of flight passengers surveyed, 38.9% were carrying fresh commodities with 34 known fruit-fly hosts from PNG or other countries (Clarke *et al.*, 2004; Putulan *et al.*, 2004).

Bactrocera invadens is a pest that is completely new to science and has only been recently described in 2005 (Drew *et al.*, 2005). There is currently a paucity of information on various aspects of its bioecology that might aid in the management of the pest. In addition, Lux *et al.* (2003a) have demonstrated that *B. invadens* can co-exist with the native *Ceratitis cosyra* (Walker) and the possibility of competitive displacement of

indigenous species is high. Indeed reviews of several cases of exotic pest invasions by Duyck *et al.* (2004a) show that in all the cases of confrontation between two genera, a species of an invasive *Bactrocera* has ultimately dominated numerically one or more species of the indigenous *Ceratitis* species (Diptera: Tephritidae), while the reverse was never observed (Debach, 1966; Keiser *et al.*, 1974; White *et al.*, 2000; Reitz & Trumble, 2002). It is not known whether introduction of this exotic pest in Kenya will result in coexistence with the indigenous pests or displacement. Thus this study aims to establish such competitive interaction.

Eradication of *Bactrocera* spp is technically possible as demonstrated in the United States (U.S) and Australia though at a tremendous cost (Weems *et al.*, 1999). However, despite the resources and strong quarantine facilities, interceptions are common at ports of entry into the U.S suggesting potential for outbreak. Weems *et al.* (1999) also points out that eradication of *B. cucurbitae* was achieved in Japan over a period of 18 years in an extensive program involving insecticides, male attractants and sterile insect technique. However, the scale of distribution of *B. invadens* at present, poor quarantine and phytosanitary management, limited knowledge of potential cultivated and wild hosts and lack of resources in Africa suggests that eradication may be difficult, if not impossible. The most practical strategy, therefore, is the development of appropriate management measures that are suited to the local conditions in Africa and sustainable. Therefore, as part of the ongoing efforts to manage this pest, the African Fruit Fly Program of *icipe* is developing and testing a range of IPM technologies that are adaptable to the region. However, such strategies can best be utilized if the bioecology of the pest is known.

Therefore, this study aims at understanding the bioecology of *B. invadens* as part of a wider strategy to develop sustainable management strategies for the pest. The host plants range of *B. invadens* is documented and the tolerance of this insect to temperature as relevant to the development of mass rearing procedures and field bioclimatic potential has been established. Population dynamics studies have been undertaken and the role of field sanitation in the management of the pest has been established. Both commercial and the International Centre of Insect Physiology and Ecology (*icipe*) developed attractants have been tested for effectiveness in the management of this pest. Lastly, the interaction of the pest with indigenous mango fruit fly species on mango agro ecosystems has been determined.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this study was to investigate the bioecology of the invasive fruit fly, *Bactrocera invadens* and its interaction with the indigenous mango-infesting fruit fly species.

1.3.2 Specific objectives

The following specific objectives were addressed;

a. To determine the effect of temperature on development and survival of immature stages of *B. invadens*.

b. To identify and document the host plants of *B. invadens* in Kenya and establish its host preference.

c. To determine the seasonal and annual dynamics and establish the abundance of *B. invadens* at a mango ecosystem area of Kenya.

d. To establish the effect of fallen ground fruits on tree fruit infestation and density of resident *B. invadens* in an orchard.

e. To determine the efficacy of commercially and locally developed food attractants and traps for their attractiveness to *B. invadens*.

f. To determine the interspecific interaction between *B. invadens* and the indigenous mango infesting fruit flies *Ceratitis capitata* (Wiedemann) and *C. cosyra* in Kenya.

1.4 Hypotheses

The following hypotheses were tested;

a. Temperature has no effect on the development and survival of immature life stages of *B. invadens*.

b. *Bactrocera invadens* does not have a wide host's plant range in Kenya and its host's plants preference is narrow.

c. The seasonal and annual population dynamics and abundance of *B. invadens* in cultivated mango orchards in Kenya is similar year round.

d. Fallen ground fruits do not have any effect on tree fruit infestation levels and resident *B. invadens* density in a mango orchard.

e. *Bactrocera invadens* adults are attracted equally to food attractants and different trap types.

f. There is no interspecific competition between *B. invadens* and the indigenous mango infesting fruit flies *C. capitata* and *C. cosyra* in Kenya.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Taxonomic classification of *Bactrocera* and *Ceratitis* species

There are over 4000 tephritid fruit fly species distributed throughout the tropical, sub-tropical and temperate regions of the world (White & Elson-Harris 1992). Taxonomic classification of the tephritids into subfamilies has always been controversial and remains so todate (White, 2000). The five subfamilies recognised and described are: Toxotrypaninae which includes *Anastrepha* Schiner and *Toxotrypana* Gerstacker species. The Trypetinae includes *Rhagoletis* Loew, *Carpomyia* Costa and *Pliorecepta* Korneyev. The Ceratitinae are the commonest pests in Africa and include the genera *Ceratitis* MacLeay and *Trirhithrum* Bezzi. Two tribes are placed under this subfamily namely: Ceratitini (mostly African) which breed in fruits or flower buds and Gastrozonini (mostly Southeast Asian) breeding in bamboo shoots or grass stems. White & Elson-Harris (1992) describe in detail the taxonomic features of members of this group. Pest species under Ceratitini include, *Trirhithrum coffeae* Bezzi attacking coffee and two species investigated in this thesis i.e., *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 & Elson-Harris, 1992). The last subfamily is the Dacinae. Two genera are described in this family namely *Dacus* (Fabricius) and *Bactrocera*. The species of *Bactrocera* are of Asian / Pacific origin, except for a few African species (White & Hancock, 1997). They differ from the *Dacus* sp. by having abdominal terga that are not fused. *Bactrocera invadens* is similar to *Bactrocera* (*Bactrocera*) *dorsalis* (Hendel), from Southeast Asia, and *Bactrocera*

(*Bactrocera*) *kandiensis* (Drew and Hancock), from Sri Lanka, in possessing a very narrow costal band and anal streak, black scutum, parallel-sided lateral postsutural vittae and abdominal tergites III–V with a dark 'T' pattern and narrow dark lateral markings on all three terga. It differs from both species in having the scutum base colour dark orange-brown with a dark fuscous to black lanceolate pattern, from *B. dorsalis* in having a longer aedeagus and narrow lateral postsutural vittae, and from *B. kandiensis* in having femora entirely fulvous. However, the colour patterns of the scutum and abdomen of *B. invadens* are remarkably variable compared to other species in the *B. dorsalis* species complex, sometimes almost inseparable from *B. dorsalis,* while most other times clearly differentiated. The full description of *B. invadens* using morphometric and molecular tools is found in Drew *et al.* (2005) and Drew *et al.* (2008).

2.2 Tephritid fruit fly pests in Kenya

A range of fruit fly species infesting commercial fruit have previously been reported in Kenya, the commonest being the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), mango fruit fly *C. cosyra*, Natal fruit fly *C. rosa* Karsch, *C. fasciventris* (Bezzi), *C. anonae* Graham, melon fly *Bactrocera cucurbitae* (Coquillett) and the olive fly *B. oleae* (Gmelin) (White & Elson-Harris, 1992; White, 2000; Copeland *et al.*, 2002; Copeland *et al.*, 2004; Copeland *et al.*, 2006; Ekesi, 2006). The genus *Ceratitis* is native to tropical Africa, while the genus *Bactrocera* is of Indo-Australian origin (Silvestri, 1914). *Ceratitis cosyra* has historically been the most important fruit fly pest of mango in Kenya with *C. anonae*, *C. rosa* and *C. fasciventris* occurring at low frequency (Lux *et al.*, 2003b). *Ceratitis capitata* is thought to originate from West Africa (Silvestri, 1914) and has become widely distributed

as a result of fruit trade. It is now established in most of the countries surrounding the Mediterranean Sea, Central and South America, Western Australia and Hawaii (Clausen, 1978). *Ceratitis rosa* is native to South and Eastern Africa (Clausen, 1978), although it's now found on the Indian Ocean islands of Mauritius and Réunion (Etienne, 1972). *Ceratitis fasciventris* and *C. anonae* are so far restricted to the Africa main land. Presently, the invasive *B. invadens* (Plate 1) is fast becoming the most damaging pest of mangoes (Plates 2.1 - 2.4) in the region.

Plate 2.1. *Bactrocera invadens*

Plate 2.2. Healthy mango

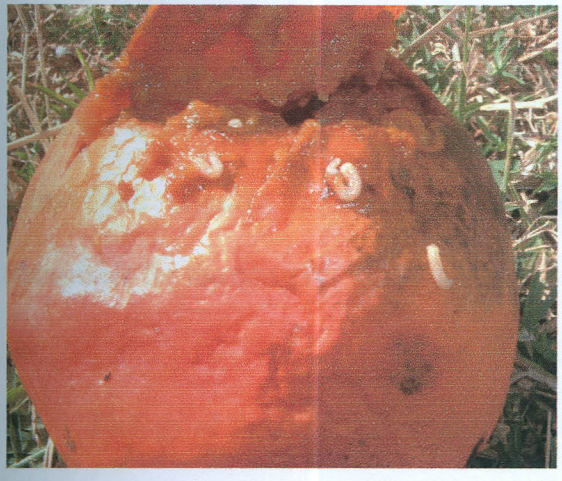

Plate 2.3. Fruit fly larvae

Plate 2.4. Damage to mango by fruit flies

2.3 *Bactrocera* species in Africa

The genus *Bactrocera* has approximately 350 to 376 species with some undescribed species remaining in collections (Lawson *et al.*, 2003). The *Bactrocera dorsalis* Hendel complex of tropical fruit flies contains 75 described species, most endemic to Asia (Weems *et al.*, 1999; Clarke *et al.*, 2005). Previous *Bactrocera* species found in Kenya included *B. cucurbitae*, *B. oleae*, *B. bigulatta* (Bezzi) and *B. munroi* White (Copeland *et al.*, 2004). Of these species, *B. cucurbitae* was the most important in Kenya infesting a wide range of cucurbits. The new *B. invadens* fruit fly has now become widely distributed in Eastern, Central and Western Africa and is regarded a quarantine pest of economic importance. *Bactrocera invadens* belongs to the *Bactrocera dorsalis* Hendel complex of tropical fruit flies which group is arguably the most destructive to fruits worldwide (White & Elson-Harris, 1992; Drew & Hancock 1994; Clarke *et al.* 2005). *Bactrocera* fruit fly invasions are not new to Africa. Egypt, Mauritius and Rèunion suffered an invasion of the peach fruit fly *Bactrocera zonata* (Saunders) which has caused significant losses to the fresh fruit industry. For example, in Egypt alone, current annual cost of damage due to this pest is estimated at 190 million Euros (OEPP/EPPO, 2005). The cost and logistics of an eradication strategy for these pests to African countries is not practical and economically feasible. Management of *B. invadens* in Kenya will require development of strategies that are feasible, economical and sustainable for the country.

2.4 Biology of Dacinae fruit flies

Adult females lay their eggs beneath the skin of suitable hosts, especially in physiologically

mature, ripening or ripe fruits, depending on the fruit fly species and the host plant attacked. The eggs are laid singly or in clusters. Egg size and structure show some variation from species to species, but there is correlation between egg size and body size or ovariole number (Fitt, 1984). Clutch size is correlated to some extent with the size of host fruits. Species that infest small hosts normally lay the fewest eggs per clutch (Fletcher, 1987). Females of some species e.g. *Bactrocera tryoni* (Froggatt) and *B. dorsalis* adjust clutch size depending on the size of the fruit, laying fewer eggs in small fruit. Length of host deprivation, suitability of the host for larval development and even the effort required to puncture the fruit skin can also influence the clutch size (Matanmi, 1975). There is no evidence that any Dacinae deposits an epideictic oviposition-deterring pheromone after egg laying (Fitt, 1984).

The larvae of Dacines are typical acephalic cyclorrhaphan maggots with an involuted head, three thoracic segments and eight abdominal segments (Fletcher, 1987). The most important features are the mouth hooks and anterior and posterior spiracles, which change during each instar (Anderson, 1963). The larval first and second instars feed on fruit pulp and grow to the third instar, which emerge from the fruit to pupate. Presence of larvae in fruit has a deterrent effect on ovipositing females of *B. oleae*, *B. cucurbitae*, *B. tryoni* and *Bactrocera jarvisi* (Tryon) (Fitt, 1984; Prokopy & Koyama, 1982). Girolami *et al.* (1981) reported a deterrent effect in fruit containing second and third instar larvae of *B. oleae*. The larvae of most fruit flies can jump along the ground and find suitable sites for pupation by burrowing several centimetres into the soil (Fitt, 1981; Neuenschwander *et al.*, 1981; Dimou *et al.*, 2003). Larvae may also pupate within the fruit (Fletcher, 1987). At the completion of the

third instar, the larval skin hardens to form a puparia with inactive fourth-instar larvae inside (Christenson & Foote, 1960) from which an adult emerges.

The newly emerged adults require a carbohydrate energy source and water in order to survive. In addition, they search for a protein source for egg maturation and their own reproductive potential (Christenson & Foote, 1960; Fletcher, 1987). The majority of dacine fruit flies mate at dusk under low light intensity (Arakakai *et al.*, 1984). Mating behaviour has rarely been observed in the field. However it seems that mating occurs predominantly on the foliage of host plants underneath the leaves and after a pre-oviposition period, which varies with species. Field cage studies indicate that males of *B. dorsalis* (Shelly, 2001), *B. cucurbitae* (Kuba & Koyama, 1985) and *B. tryoni* (Tychsen, 1977) engage in lekking behaviour. As light intensity drops near dusk, they aggregate on specific parts of the tree and take up individual territories on leaves which they aggressively defend from incursions by other males. Precopulatory courtship is apparently absent in *B. dorsalis*, and males mount approaching females without performing any obvious behavioural displays (Shelly, 2001). Females respond to these leks and have been more frequently sighted at the larger leks. After copulation, the female starts laying eggs to begin a new cycle. Time spent by females during oviposition varies. For instance *B. tryoni* takes about 1-3 min per oviposition with a maximum daily oviposition rate of 80 eggs/female per day (Yonow *et al.*, 2004) while *B. jarvisi* which lays more eggs per clutch takes about 4-6 min (Fitt, 1984). The duration of oviposition of *B. invadens* is not yet clear. However, the average daily clutch size for this species is 18.2

eggs (Ekesi *et al.*, 2006). The duration of different development stages vary with fruit fly species, host plant and climatic conditions.

Tropical species of the genera *Bactrocera* and *Dacus* are multivoltine, having several generations per year. These species may produce up to six overlapping generations in a single season (Bateman, 1972), thus potential for heavy fruit losses is very high. Damage is caused by oviposition punctures of the adults, and subsequent development of larvae which feed on the flesh of the fruits, creating tunnels and macerating the tissue. This usually causes premature fruit drop, and infested fruits that remain on the tree decay, thus resulting in both quantitative and qualitative losses. Due to low tolerance for blemished fruit by the export market, chemical control measures are often applied. However, treatment can be very expensive and may affect the quality of the product in addition to the possible risks of high pesticide levels in the fruit. Due to strict quarantine regulations recently imposed by importing countries (EUREP, 2003), fruit exports can be severely hampered if infestation is detected or when pesticide residue levels exceed the approved limits.

2.5 Influence of temperature on the biology of Dacines

Temperature not only has a direct effect on the demography of Dacines but also has indirect influence particularly on its hosts (Fletcher, 1987). One major influence of temperature on multivoltine species is in determination of development times and thus the number of generations per year. Studies on the different life stages of *B. cucurbitae* (Messenger & Flitters, 1958; Carey *et al.*, 1985), *B. dorsalis* (Messenger & Flitters, 1958; Wasti & Mitchell, 1971; Yang *et al.*, 1994; Vargas *et al.*, 1996), *B. oleae* (Tsitsipis, 1980), *B.*

umbrosa (Ibrahim, 1996) and *B. zonata* (Qureshi *et al.*, 1993; Duyck *et al.*, 2004b) at a series of constant temperatures have indicated that the temperature-development rate curve has the same general shape in all stages and species. Above a low temperature development threshold, which for the immature stages lies between 6°C and 9°C, the relationship is sigmoidal up to a maximum between 26°C and 30°C, above which development rates start to decrease again. Temperature-development rate relationships for the different life stages based either on the linear summation of day-degrees above the lower development threshold or on some nonlinear development-rate model have also been calculated for several pest species (Messenger & Flitters, 1958; Tsitsipis, 1980; Duyck *et al.*, 2004b).

Development times are fairly similar for all species; eggs take 1-2 days, larvae 7-8 days, and pupae 10-11 days to complete development at 25°C. Comparison of predictions with field observations in *B. tryoni* suggests that generation times in the field are largely determined by temperature (O'Loughlin *et al.*, 1984). However, other factors, including moisture content and ripeness of fruit in the case of eggs (Tsitsipis & Abatzis, 1980) and ripeness, fruit variety, and degree of crowding in the case of larvae (Carey *et al.*, 1985), can have significant influence on development rates. Ovarian maturation is similarly influenced by temperature, except that the lower threshold is about 12-13°C (Pritchard, 1970). Other factors that affect ovarian maturation include availability of hosts, water relationships, availability of dietary protein, and presence of males (Pritchard, 1970; Fletcher *et al.*, 1978; Fletcher & Kapatos, 1983).Temperature also acts as a major mortality factor. Outside the optimum temperature range of approximately 18-27°C

mortality increases, and there are upper and lower lethal thresholds beyond which no individuals survive long enough to complete development (Meats, 1984). Although the immature stages of Dacines can survive short periods of high temperature (>30°C) or low temperature (<5°C), adults are normally unable to survive prolonged periods at such temperatures. The stress caused by suboptimal humidity can also prolong development rates of the immature stages and inhibit the maturation of adults (Fletcher *et al.*, 1978).

2.6 Host plants of fruit flies

Most fruit-infesting tephritids are highly polyphagous infesting a wide range of fruits and wild plant species. The genus *Ceratitis* attacks a wide variety of commercial exotic and indigenous wild fruits (Liquido *et al.*, 1991; De Meyer *et al.*, 2002). In Kenya, *C. capitata* has been reared from 55 plant species (Copeland *et al.*, 2002). Other *Ceratitis* species, *C. cosyra* and *C. rosa* also have varied host range in Africa, although *C. cosyra* is primarily considered to be a pest of mango (Mukiama & Muraya, 1994; Lux *et al.*, 2003b). In Kenya, *C. cosyra* and *C. rosa* have been recorded from 9 and 28 plant species, respectively particularly the Annonaceae (Copeland *et al.*, 2006). *Ceratitis anonae* has been recorded from at least 14 plant species in Kenya including *Psidium guajava* L. (Myrtaceae) and several plants in the Annonaceae, Moraceae and Sapotaceae families (Copeland *et al.*, 2006) with a few older host records on other crops not clearly verified (De Meyer *et al.*, 2002). Host records of *C. fasciventris* are similar to those of *C. rosa* (De Meyer, 1996; 1998). Copeland *et al.* (2006) documented 20 plant species whose fruits are hosts to this insect in Kenya, of which the cultivated ones include *P. guajava* and *Zizyphus abyssinica* A. Rich (Rhamnaceae).

Fruit flies of the genus *Bactrocera*, particularly the *B. dorsalis* complex, are also known to have wide host plant ranges (Clarke *et al.*, 2005). Larvae of the *B. dorsalis* complex can complete development in most of the cultivated and several wild host plants (Drew, 1989; Drew & Hancock, 1994; Tsuruta *et al.*, 1997; Hollingsworth *et al.*, 2003). The key plant families containing *B. dorsalis* complex hosts include Anacardiaceae, Annonaceae, Clusiaceae, Lauraceae, Moraceae, Myrtaceae, Rutaceae, Sapotaceae and Solanaceae (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 species across 27 families (Clarke *et al.*, 2005). The host range of *B. cucurbitae* is primarily cucurbits, but it has been recorded from a few non-cucurbit hosts (White & Elson-Harris, 1992). *Bactrocera invadens*, which is believed to be a member of the *B. dorsalis* complex, has previously been reared from mango and two wild hosts *Strychnos mellodora* S. Moore and *Dracaena steudneri* Engl. (Copeland, unpublished data) in Kenya.

2.7 Management of fruit flies

2.7.1 Natural enemies

The eggs, larvae and puparia of fruit flies are attacked by a number of parasitic hymenoptera, particularly by species of Opiinae, belonging to the family Braconidae (Christenson & Foote, 1960; Wharton & Gilstrap, 1983). In Kenya, for instance, the Opiine braconid parasitoid *Psyttalia cosyrae* (Wilkinson) is an important natural enemy of *C. cosyra* (Mohamed *et al.*, 2003). *Psyttalia concolor* (Szèpligeti) has been reared from *B. oleae* and *C. capitata* (Kimani-Njogu *et al.*, 2001; Copeland *et al.*, 2004) while

Psyttalia lounsburyi (Silvetsri) and *Utetes africanus* (Szèpligeti) have been reared from *B. oleae* with parasitization rates estimated at 5% (Copeland *et al.*, 2004). Other studies have evaluated the biological performance and potential of the African egg-larval parasitoid *Fopius ceratitivorus* Wharton for *C. capitata* and *Bactrocera* species with some degree of success (Lopez *et al.*, 2003; Bokonon-Ganta *et al.*, 2005). Mohamed *et al.* (2006) demonstrated that the gregarious eulophid endoparasitoid *Tetrastichus giffardi* Silvestri could attack and successfully develop in the larvae of several fruit fly species in Kenya. Several species of parasitoids have indeed been collected from their native areas and introduced to areas where fruit fly pests occur in classical biological control programs (Baranowski *et al.*, 1993).

Striking examples of some success with biological control is the parasitoid *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), a wasp introduced in Hawaii in 1950 from the Malay peninsula against *B. dorsalis* (van den Bosch and Haramoto, 1951). Since its establishment, *F. arisanus* has resulted in a dramatic reduction in infestation of fruit in Hawaii through a high level of *B. dorsalis* parasitism (65-70%), and it has remained the dominant parasitoid species (Vargas *et al.*, 2007). Given its success in Hawaii, *F. arisanus* is the candidate of choice for biological control of *Bactrocera* spp. worldwide. Furthermore, releases of large numbers of the larval-pupal Opiine braconid *Diachasmimorpha longicaudata* (Ashmead) in 21 countries throughout central and southern Florida in 1972 resulted in significantly reduced numbers of fruit fly populations (Baranowski *et al.*, 1993; Sivinski *et al.*, 1996). Augmentative releases of *Diachasmimorpha tryoni* (Cameron) in Maui, Hawaii was also shown to be effective in

suppressing native populations of medflies (Wong *et al.*, 1991). However, the actual success achieved by some biological control programs can often be difficult to assess. In Malaysia, Vijaysegaran (1983) recorded high parasitism levels in carambola over an 18 month study period but still ended up with 90% damage to fruits in this same period.

Other natural enemies such as predators including birds and rodents have been recorded to feed readily on fruit fly larvae in fallen fruits (Drew, 1987). In Hawaii, the Argentine ant *Linepithema humile* (Mayr) has been shown to predate on the medfly larvae in fallen fruit and pupae in soil (Wong *et al.*, 1984). In Benin, the weaver ant *Oecophylla longinoda* (Latreille) is known to reduce fruit fly infestation on mango by preventing oviposition by fruit flies (van Mele *et al.*, 2007). However, while the effect of natural enemies in nature might be significant particularly for indigenous species, great challenges are faced when dealing with exotic pests like *B. invadens* for which no natural enemies have been reported in Kenya. Besides, for quarantine purposes, a few or even a single larva in fruit is enough to have it rejected by the more stringent export market. It is therefore unrealistic to expect natural enemies to provide a sole control measure and while their presence should be encouraged in orchards, supplementary methods that are not deleterious to them should be adopted.

2.7.2 Crop hygiene

The tropical climate in the fruit growing areas of Kenya combined with an abundance of host plants can enable uninterrupted breeding of fruit flies throughout the year. For instance, *B. invadens* has a short life cycle with rapid rates of increase (Ekesi *et al.*, 2006)

and can multiply to high numbers when host fruits are abundant. Breeding of fruit flies in unwanted fruits is probably one of the biggest sources of damaging populations (Liquido, 1991). Consequently, it is important to prevent breeding of these flies by removing and destroying all unwanted or fallen fruit. Such fruit can be destroyed by burning or burying at least 6 inches deep in the soil (Seewooruthun *et al.*, 2000; Dhillon *et al.*, 2005). In areas where individual orchards are in close proximity to each other, it is important for all orchards to observe crop hygiene. Crop hygiene generally has to be integrated into the overall management of the orchard.

2.7.3 Male lures and male annihilation technique (MAT)

The males of some species of fruit flies are strongly attracted to certain chemical compounds some of which occur in nature and generally referred to as parapheromones. These parapheromones are often utilised in MAT programmes (Steiner *et al.*, 1970). In such programmes, high density baiting stations involving a male lure laced with an insecticide (usually malathion) are utilised to reduce male population to such a low level that mating does not occur. These are suspended in traps or nailed on trees as mats, or on large scale may be distributed by air. Methyl Eugenol (ME) is perhaps one of the best known male lures and is a constituent of many plant species. Male of thirty species belonging to the *B. dorsalis* complex are known to respond to ME including the two invasive *B. invadens* and *B. zonata* species now present in Africa (Lux *et al.*, 2003a; Clarke *et al.*, 2005; Drew *et al.*, 2005; OEPP/EPPO, 2005). 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. Despite MAT attaining extremely high reduction in male populations, much lower reduction is achieved in relation to fruit infestation (Cunningham & Suda, 1986). When

used alone for protection of individual orchards, male lures appear to be of little value. Thus, while trap catches may be impressive, this has not been proven sufficient to disrupt mating. Large numbers of gravid females unaffected by the lure are often present both as resident populations and in surrounding areas to oviposit and damage fruits. MAT could be more effective in monophagous species predominantly found in cultivated areas. Simultaneous use of male lures and protein baits would appear to be a more effective strategy than use of either method alone.

2.7.4 Female lures and bait sprays

The earliest food baits for fruit flies consisted of carbohydrates and fermenting sugars, molasses and syrups. Subsequent improvements incorporated hydrolysed protein (Steiner, 1952). The use of protein-based attractants was a behavioural manipulation of the flies based on the obligatory protein diet essential for egg maturation, hence, the greater attraction of females than males. Certain protein hydrolysates are now known to contain the nutritive elements required by the fruit flies and protein baits work on this premise (Christenson & Foote, 1960; Fletcher, 1987). Protein hydrolysate is produced by hydrolysing a plant protein with hydrochloric acid followed by addition of sodium hydroxide to neutralise excess acid. In the recent years, yeast autolysate, a superior protein product was developed through heating and enzymatic proteolysis of yeast cells. Examples of enzymatically-hydrolysed proteins include: Yeast Protein Autolysates [Mauri's Pinnacle Insect Protein Lure (MPIPL)] and Promur in Malaysia. The acid hydrolysed proteins include products such as Protein hydrolysate (AgriSense, UK) and Nulure (Miller Chemical and Fertilizer, Hanover, PA). During field application, traps baited with these proteins are used to monitor and suppress fruit fly populations. In the

past decade, a wide range of baits have been tested for management of *Anastrepha*, *Bactrocera* and *Ceratitis* species (Epsky *et al.*, 1995; Cornelius *et al.*, 1999; Katsoyannos *et al.*, 1999ab; Miranda *et al.*, 2001; Fabre *et al.*, 2003). Related studies have also been carried out to evaluate a range of trap types to establish the best trap/attractant combination for management of different species of fruit fly (Heath *et al.*, 1996; Cornelius *et al.*, 1999; Katsoyannos *et al.*, 2000).

Protein baits can also be utilised as bait sprays in bait application programmes (BAT). The beauty of the bait sprays is that it involves 'spot spraying' and overall coverage of plants is not required. This saves time, labour and materials which all translate into considerable cost savings and can reduce the amount of insecticide applied to the crop thereby limiting non-target effects (Sonoo *et al.*, 1996). BAT has proved to be very effective and aerial application of bait suppressed medfly in Hawaii (Steiner, 1952). 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. The efforts of one grower will be of limited value if the neighbours did nothing on their farms. Presently in Kenya, bait sprays are being evaluated for management of *B. invadens* (Ekesi *et al.*, unpublished data) and it is envisaged that this technique will be widely adopted in the country. *Icipe* has also embarked on production of protein baits locally such as the conversion of waste brewery yeast into an attractive fruit fly bait product.

2.7.5 Fruit wrapping/bagging

Wrapping or bagging of individual fruits, to prevent egg oviposition, can be employed to produce fruit fly and pesticide free fruits. This technique is effective and enables production of fruits with good cosmetic appeal. Individual fruits are protected against fruit flies by bagging them in newspaper bags or waxed paper. The bags provide a continuous barrier from the time of bagging to harvest thus preventing female flies from laying eggs in fruit. It is also simple to apply and has no side effects to the environment. In Malaysia, bagging is extensively used in carambola production and for mango in the Philippines (Hapitan & Castilo, 1976). Some constraints, however, prevail for wide application of this technique. Generally, trees have to be at a manageable height, thus the old taller mango trees as observed in orchards of many areas of Coast and Rift valley provinces in Kenya could pose a challenge to treat. The labour intensiveness, given the huge number of fruits to be bagged and the size of the orchards severely restricts application of this technique. This technique, however, may easily be applied where orchard sizes are small and where the objective is production of high quality fruits for export.

2.7.6 Pesticides

Pesticides are widely used for fruit fly management because of their effectiveness, rapid curative action, simplicity of application and adaptability to most situations. In fruit fly management, cover sprays of both the fruits and foliage is practised. Adult flies are killed when they come into direct contact with the pesticide or residues which are left on the fruit and foliage. Some pesticides have systemic action and are absorbed into fruits to kill larvae and eggs that may be present (Heather *et al.*, 1987). In orchards that have no

control of breeding populations of flies in the general area, cover sprays such as Fenthion and Dimethoate provide control against invasions by gravid females from invasions from surrounding areas (Fletcher & Bateman, 1982). However, the effect of pesticides on non-target organisms, beneficial insects and residues in the harvested fruits are major limitations to use of pesticides in cover sprays. Consumers worldwide are also increasingly becoming conscious of chemical residues in food (EUREP, 2003). This is a challenge to most growers because most tropical fruits are susceptible to fruit flies and require high protection at maturity stage. Ironically, the most effective chemicals are those that have the highest residual activity in fruits. In many countries in Africa where fruit quality is rarely examined at domestic markets, consumers are often exposed to excessive chemical residues in fruit to the detriment of their health.

2.7.7 Sterile Insect Technique

The Sterile Insect Technique (SIT) was invented by Knipling and colleagues to eradicate the screwworm *Cochliomyia hominivorax* (Coquerel) in the United States (Knipling, 1955) and was successfully implemented in Florida and some southwestern states of the United States (Knipling, 1959). In this technique, sterile male insects are released repeatedly in large numbers to increase their chances of mating with the wild females to produce infertile eggs consequently reducing the population to extinction. Sterile males are obtained by irradiation treatment of x-rays or gamma rays emitted by Cobalt-60 or Cesium-137. Sterilisation should not seriously alter the behaviour and fitness of the insects. This is essential, as the success of SIT will depend on mating competitiveness of the released males in comparison with the wild males.

Early examples of SIT application against fruit flies was with the melon fly on Mariana Islands (Steiner *et al.*, 1965a; 1965b) and Kume Island of Japan (Iwahasi, 1977). The use of SIT is a complicated procedure requiring sophisticated skills, high degree of technical expertise and funding. The process of implementing SIT requires seven components: suppression of density, mass rearing, sterilization, shipment, release, evaluation, and quality control. The number of sterile insects released must surpass that of wild insects for successful control (Knipling, 1955). In the SIT program in Japan, the density of wild melon flies was suppressed by two other control methods to reduce the number of sterile flies required. One method involved distributing cotton strings soaked with cue-lure, an attractant of the male melon fly, and Naled®, an insecticide, in the target area (Taniguchi *et al.*, 1988). The density of male melon flies was reduced to less than 10% of previous density (Taniguchi *et al.*, 1988) though this method alone was insufficient to eradicate the melon fly. Although it is initially expensive, one main advantage of SIT is that it is species-specific and has no side effects to the environment. However, in a country with competing species complexes, its application on one species could lead to resurgence in other species utilizing the same ecological niche. Besides SIT is normally useful immediately a new species is detected and its application to the current situation in Kenya may be difficult.

2.8 Interspecific competition and displacement among tephritids

Once an exotic species is introduced, its population rapidly increases to damaging levels often largely due to lack of natural enemies (Simberloff & Wilson, 1970) and the competitive abilities of the invaders (Byers, 2000). A classical dichotomy was introduced

by MacArthur & Wilson (1967) between the so called r- and K-selected species. In their view, r species display a suite of traits that favour rapid population growth and colonisation of new habitats (mature early, have small size, high growth rates, little resistance to competitors, and efficient dispersal) while K species are adapted to competition in saturated habitats (long lifespan, low fecundity, large sizes, and little mobility). A modern coexistence theory reminiscent of r and K strategists states that coexistence of several species can be promoted in a structured habitat by (i) a perturbation regime that constantly regenerates new empty sites, and (ii) competition–colonisation trade-offs among different species (Tilman, 1994). The bad competitors must be good colonisers because their maintenance depends on their being first in colonising empty sites. Using life-history or demographic traits to characterize the competitor (or K) strategy implies that competition is mainly related to exploitation of resources.

In hierarchical competition, the superior competitor always dominates and triggered either by niche differentiation or by a colonisation–competition trade-off, will displace the inferior, be they native or exotic themselves (Reitz & Trumble, 2002). Duyck *et al.* (2004a) has reviewed several cases where displacement occurred in interspecific interactions among tephritids fruit flies. For instance, in Australia, *C. capitata* was introduced from Europe in 1897 or thereabouts (Hooper & Drew, 1989; Vera *et al.*, 2002). It was then gradually displaced around the Sydney area by *B. tryoni* which invaded Australia from the north in the early 20th century (Debach, 1966). A similar phenomenon occurred in Hawaii in 1945, when *B. dorsalis* largely displaced *C. capitata*

from coastal zones. *Ceratitis capitata* had itself been introduced in 1910 and had become a major pest throughout Hawaii (Debach, 1966). Since the invasion by *B. dorsalis*, *C. capitata* has generally been restricted to cooler climates at high altitudes, where *B. dorsalis* is not found. However, the ecological segregation between the two species is modulated by the host fruit: *C. capitata* is found at low altitudes on coffee, to which it seems to be more suited than *B. dorsalis* while it is only rarely found on guava and mango, although they were by far its preferred hosts before the establishment of *B. dorsalis* (Debach, 1966; Keiser *et al.*, 1974; Reitz & Trumble, 2002).

On the Mascarene Islands, the Mascarene fruit fly *Ceratitis catoirii* Guérin-Méneville was an indigenous species on both Réunion and Mauritius islands. *Ceratitis capitata* became established in Réunion in 1939 and Mauritius in 1942 (Orian & Moutia, 1960; Etienne, 1972). A further invasion by *C. rosa* was seen in Mauritius in 1953 and Réunion island in 1955 (Orian & Moutia, 1960; Etienne, 1972). More recently, *B. zonata* was reported in Mauritius in 1987 and Réunion in 1991 (White *et al.*, 2000, Quilici & Jeuffrault, 2001). In Réunion, *C. catoirii* is now only found in small numbers on the east and south coast of the island, while it seems to have disappeared completely from Mauritius (White *et al.*, 2000). *Ceratitis rosa* is generally dominant at high altitudes in Réunion, however, *B. zonata* is continuing to spread and has already colonised a large proportion of the niches used by the other three species at low altitudes. Although invasions seem to support the hierarchical mode of competition, complete competitive exclusion usually does not occur. Indeed, among all the documented cases, competitive displacements and niche shifts are more frequent than exclusions. Therefore, in view of

these reported cases, it's probable that potential for displacement of native *Ceratitis* species in East Africa by *B. invadens* may occur.

CHAPTER THREE

3 EFFECT OF TEMPERATURE ON THE DEVELOPMENT AND SURVIVAL OF IMMATURE STAGES OF *BACTROCERA INVADENS* (DIPTERA: TEPHRITIDAE)

3.1 Introduction

Tephritid fruit flies within the genus *Bactrocera* Macquart are recognised worldwide as among the most destructive insect pests of fruits (White & 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 quarantine restrictions imposed by importing countries to prevent entry and establishment of unwanted fruit flies. Although the genus *Bactrocera* are known to be largely endemic to Asia and the Pacific, six species namely *B. cucurbitae* (Coquillett) (Diptera: Tephritidae), *B. dorsalis* Hendel, *B. invadens* Drew, Tsuruta & White, *B. latifrons* (Hendel), *B. oleae* (Gmelin) and *B. zonata* (Saunders) have successfully invaded other regions of the world and have become established (White & Elson-Harris, 1992; Quilici & Jeuffrault, 2001; Clarke *et al.*, 2005; Ekesi *et al.*, 2006).

Bactrocera invadens was first detected at the Kenya coast in 2003 (Lux *et al.*, 2003a). This pest has now rapidly spread across most of the sub-Saharan African region and currently has been reported from 24 countries including the Comoros Island (Drew *et al.*, 2005; Vayssierès *et al.*, 2005; Francois-Xavier *et al.*, 2008; Umeh *et al.*, 2008). It has been recovered from over 30 host plants species including cultivated and wild hosts

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although mango appears to be the most preferred cultivated plant (Drew *et al.*, 2005; Vayssières *et al.*, 2005; Ekesi *et al.*, 2006; Mwatawala *et al.*, 2006a; Rwomushana *et al.*, 2008). *Bactrocera invadens* is also rapidly displacing the indigenous fruit fly species on mango. For example in Kenya, 82% of the flies emerging from mango during 2003 season was *C. cosyra* and 18% was *B. invadens*. In 2004, 23% of the flies emerging from mango was *C. cosyra* and 76% was *B. invadens* (Ekesi *et al.*, unpublished data). By 2005, 92% of the fruit flies emerging from mango were *B. invadens* (Ekesi *et al.*, 2006). This insect like several other *Bactrocera* species is multivoltine and highly fecund, laying over 1000 eggs per female (Ekesi *et al.*, 2006) and may partly be responsible for gradually displacing the native mango fruit fly species that lay about 300 eggs (Manrakhan & Lux, 2006). Although the basic biological studies of *B. invadens* was reported by Ekesi *et al.* (2006) and artificial diet for mass rearing of the insect has been developed (Ekesi *et al.*, 2007a), the thermal requirements for development of the insect have not been fully described.

Many biotic factors affect insect growth and development and temperature is probably the single most important environmental factor affecting the development of poikilothermic organisms. Two fundamental thermal parameters that express how the rate of development of ectotherms depend on temperature are the lower threshold temperature for development (T_{min}: temperature below which no measurable development takes place) and the thermal constant, K (number of degree days above temperature T_{min} for completion of development) (Higley *et al.*, 1986). These parameters reflect the process of heat accumulation and use the linear portion of the rate versus temperature development

curve (Higley *et al.*, 1986; Hanula *et al.*, 1987; Herrera *et al.*, 2005). Temperatures have been reported to be the main abiotic factors affecting survival and development of many tephritid species (Fletcher, 1987; Vargas *et al.*, 1997; Brévault & Quilici, 2000; Duyck & Quilici, 2002). Since no report exists on the effect of this important variable on *B. invadens*, this work aimed to study the effects of different constant temperatures on the survival and development of the insect. This information will be relevant in the optimization of laboratory rearing conditions for mass rearing of the insect and its parasitoids for experimental purposes, understanding temporal and geographical patterns of abundance and application of suppression methods, development of population models and understanding intra- and inter-specific relations of *B. invadens* with other native fruit fly species.

3.2 Materials and Methods

3.2.1 *Insect culture*

The initial stock culture of *B. invadens* originated from a natural population from infested mango fruits collected at a local market in Nairobi, Kenya in 2003 and the larvae were subsequently reared on a yeast-carrot-based artificial diet (hereafter referred to as diet) in the laboratory. The colony has been maintained for more than 100 generations at the Animal Rearing and Containment Unit (ARCU) of *icipe*, Nairobi, Kenya. Rearing conditions were maintained at 28 ± 1°C, 50 ± 8% RH and photoperiod of L12: D12.

3.2.2 *Egg collection*

Eggs of *B. invadens* were collected from the stock colony by offering to mature female

flies a ripe mango dome (fruit skin that has the seed and pulp scooped out). 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 cage at 28 ± 1°C , 50 ± 8% RH. Each dome was pierced with an entomological pin (38 mm long, 0.3 mm diameter) to facilitate oviposition. Eggs were collected within 2 h of oviposition using a moistened fine camel's hair brush.

3.2.3 *Effect of temperature on development and survival of Bactrocera invadens*

Fifty eggs were counted and carefully lined on a rectangular piece of sterilized black cloth in a Petri dish and placed on top of 50g of diet. The composition and insect performance on this diet is reported in Ekesi *et al.* (2007a). The Petri dishes were immediately transferred to thermostatically controlled environmental chambers (MLR-153, Sanyo, Japan) set at 5 constant temperatures of 15, 20, 25, 30 and 35°C (± 1°C) and 50 ± 8% RH, 12:12 L:D photoperiod. Duration of egg stage was observed at 8-hourly intervals under a binocular microscope for determination of egg hatch.

At egg eclosion, Petri dish covers were removed and the dishes were separately transferred into larger rectangular plastic rearing containers (7 x 7 x 5 cm) containing a thin layer (~ 0.5 cm) of moist sterilized sand at the bottom for pupation. The top of the plastic containers were screened with light cloth netting material for ventilation. The containers were then maintained at the same constant temperature in the environmental chambers. Mature late third instar larvae leave the Petri dishes containing the artificial diets *ad libitum* and jump into the sand in the larger containers to pupate. After 7 days,

the containers were observed for puparia and the puparia were thereafter separated from sand daily by sifting.

The puparia were held in smaller-ventilated transparent cylindrical plastic cages (5.5 x12.5 cm) (J-12, GP plastics, Kenya) and maintained at the same five constant temperatures until eclosion. Records were kept of the duration and developmental rate of the different stages and mortality of egg, larvae and puparia. Developmental duration was estimated as the observed time when 50% of the stages either hatched, formed puparia or emerged as adult (Vargas *et al.*, 1984). Stage-specific survival rates were determined by dividing the number of individuals alive at the end of each stage by the initial number (Vargas *et al.*, 1984). The final number of emerged adults per 50 eggs was calculated as the product of survival rates in the different stages from egg to adult.

3.2.4 *Temperature thresholds and thermal constants*

Regression analysis was used to estimate lower development thresholds for egg, larvae and puparia (Liu *et al.*, 1995; Liu & Meng, 1999). To establish this relationship, the developmental time of individual life stages (i.e. the time required for 50% of individuals to complete a given biological stage) was determined at the series of constant temperatures and the developmental rate estimated (i.e. 100/developmental time) and then plotted against temperature (Brevault & Quilici, 2000). In this regression model, the development rate is $V(T) = a + bT$, where a and b are regression parameters fitted to the data of individual insects that develop to adult (Liu & Meng, 1999). The lower development threshold t (i.e. the temperature at which the development rate is zero) was

estimated by solving the regression equation for the x-intercept, which represented the estimate of the development threshold (Price, 1984). The thermal constant (degree-day (DD) above the lower threshold required to complete development) was calculated by the formula K = n(T - t) where, K = thermal constant, n = duration of development (days), T = average temperature of the period (°C), and t = threshold temperature (°C), with the corresponding data of the five thermal levels, for each stage and for the stage development, averaging the corresponding data (Pruess, 1983; Vargas *et al.*, 1996; Urra & Apablaza, 2005). The range of variation in developmental time for each immature stage was determined from: r.v = max developmental time – min developmental time. The coefficient of variation which estimates the degree of variation in development between individuals for each stage was calculated as c.v = (100 x r.v.) / developmental time (Brevault & Quilici, 2000).

3.2.5 Data analyses

For each temperature, there were five replicates consisting of 50 eggs in a Petri dish containing artificial diet and each temperature was tested thrice over time such that there were 15 Petri dishes for each temperature. Each developmental of the insect stage was studied using a completely randomized block design, considering the various replicates as multiple observations at each temperature. Standard analyses of variance (ANOVA) were used to test the effect of the various treatments on development time and survival. Means were compared, where appropriate, by the Student Newman-Keuls (SNK) multiple range tests ($P = 0.05$) (SAS Institute, 2001).

3.3 Results

3.3.1 *Effect of temperature on stage development*

The time required for eggs to hatch ranged from 5.71 days at 15°C and decreased to 1.24 days at 35°C ($F = 544.2$, d.f = 4, 15, $P = 0.0001$) (Table 3.2). The highest mean range of variation (m.r.v) for egg was at 15°C ($F = 4.0$, d.f = 4, 15, $P = 0.0001$) (Table 3.2). Mean coefficient of variation (m.c.v) for egg development varied from 39% at 15°C to 81% at 35°C ($F = 3.6$, d.f = 4, 15, $P = 0.0001$) (Table 3.2). The linear regression model showed a strong positive linear relationship between temperature and egg development rate ($R^2 = 0.97$; $P = 0.0001$) (Fig 3.1a) with a lower development threshold of 8.8°C for this stage. The egg stage required 31 degree-days (DD) to complete development (Table 3.1).

Table 3.1 Temperature thresholds (t) and degree-day (DD) requirements for the development of immature stages of *Bactrocera invadens*

Stage	t (^{0}C)	DD*
Egg	8.8	31
Larva	9.4	168
Pupae	8.7	177
Total		376

*DD, Day Degrees

At larval stage, the trend was similar as with egg, with development periods decreasing from 35.95 days at 15°C to 6.64 days at 35°C ($F = 694.6$, d.f = 4, 15, $P = 0.0001$). Mean range of variation (m.r.v) was highest (10.1 days) at 15°C, decreasing to 2 days at 20°C and fairly uniform across 25-35 days ($F = 168.4$, d.f = 4, 15, $P = 0.0001$). The m.c.v varied from 11% to 28% among the various temperatures tested ($F = 9.4$, d.f = 4, 15, $P = 0.0001$). The linear regression between temperature and development rate for this stage

was positive (R^2 = 0.97; P = 0.0001) (Fig 3.1b). *Bactrocera invadens* required 168 DD above development threshold of 9.4°C to complete development from larval stage to the pupal stage.

Temperature had a significant effect on development of puparia (F = 548.6, d.f = 4, 15, P = 0.0001). The longest duration occurred at 15°C (34.1 days) and it took 8.5 days to reach eclosion at 30°C. There was no eclosion at 35°C. As with the other stages, m.r.v was highest at 15°C (F = 391.9, d.f = 4, 15, P = 0.0001) and c.v ranged from 11% to 35% across the temperatures (F = 41.0, d.f = 4, 15, P = 0.0001). The linear regression between temperature and development rate for this stage was strongly positive (R^2 = 0.96; P = 0.0001) (Fig 3.1c) with a lower development threshold of 8.7°C. The pupa required 178 DD to complete development. Total developmental duration was longest at 15°C (75.74 days) at 15°C and shortest at 30°C (17.76 days) (Table 3.2).

3.3.2 *Survival rates*

Survival rates at the tested temperatures varied significantly for the immature stage development (Table 3.3). Overall, the highest survival occurred between 20-30°C (Table 3.3). At egg stage, survival ranged between 87% at 35°C to 95% at 20°C (F = 2.5, d.f = 4, 15, P = 0.0078) but did not differ significantly between the temperatures tested. Survivorship at the larval stage ranged between 84% to 99% at 35°C and 25°C, respectively (F = 2.8, d.f = 4, 15, P = 0.0001) but did not differ significantly between the upper and lower temperature limits tested and at 20°C. At the pupal stage, survival was 0% at 35°C and 96% at 25°C (F = 34.0, d.f = 4, 15, P = 0.0001).

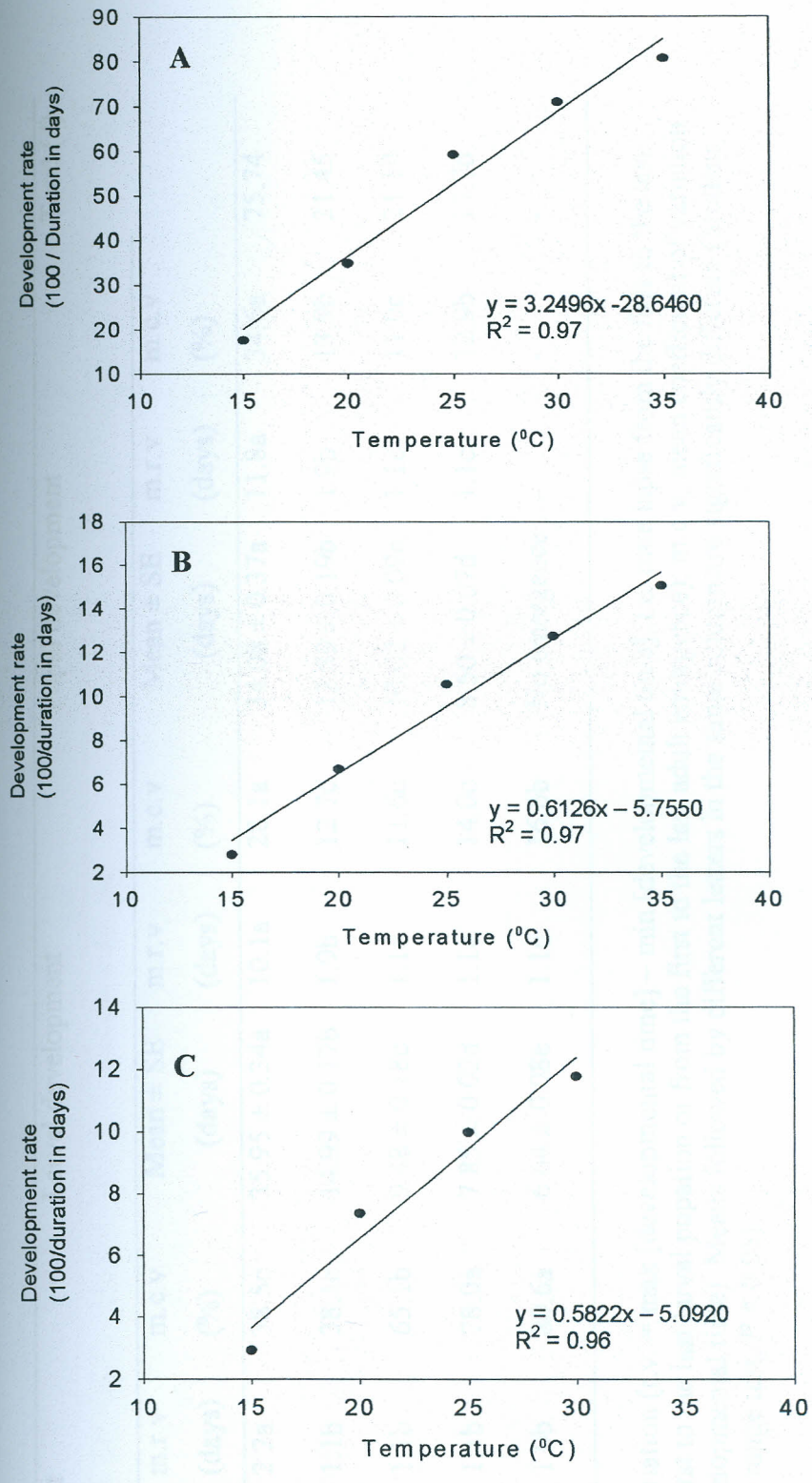

Fig. 3.1 Effect of constant temperature on development rates (100/duration in days) of different life stages of *Bactrocera invadens*, A) egg; B) larva; C) pupa

Table 3.2 Mean developmental time (days ± SE), range of variation and coefficient of variation of immature stages of *Bactrocera invadens* at five constant temperatures

Temp (°C)	Egg development			Larval development			Pupal development			Total (d)
	Mean ± SE (days)	m.r.v (days)	m.c.v (%)	Mean ± SE (days)	m.r.v (days)	m.c.v (%)	Mean ± SE (days)	m.r.v (days)	m.c.v (%)	
15	5.71 ± 0.04a	2.2a	38.5c	35.95 ± 0.34a	10.1a	28.1a	34.08 ± 0.37a	11.8a	34.6a	75.74
20	2.88 ± 0.04b	1.1b	38.1c	14.99 ± 0.17b	1.9b	12.7c	13.59 ± 0.19b	1.9b	13.9b	31.45
25	1.69 ± 0.02c	1.1b	65.1b	9.48 ± 0.18c	1.1c	11.6c	10.02 ± 0.09c	1.1c	11.0c	21.19
30	1.41 ± 0.02d	1.1b	78.0a	7.85 ± 0.02d	1.1c	14.0c	8.50 ± 0.07d	1.1c	12.9b	17.76
35	1.24 ± 0.03e	1.0b	80.6a	6.64 ± 0.08e	1.1c	16.6b	No emergence	-	-	-

m.r.v., mean range of variation (r.v. = max [developmental time] – min [developmental time], i.e. time lapse from the first to the last egg eclosion, from the first to the last larval pupation or from the first to the last adult emergence); m.c.v, mean coefficient of variation (c.v. = [100 × r.v.] / developmental time). Means followed by different letters in the same column are significantly different (Student Newman-Keuls multiple range test, $P < 0.05$).

Table 3.3 Mean survivorship (% ±SE), pupae weight and number of adults that emerged from the immature stages of *Bactrocera invadens* at five constant temperatures

Temp (^{0}C)	Egg survival	Larval survival	Pupal survival	Mean adults/50 eggs
	Mean ± SE (%)	Mean ± SE (%)	Mean ± SE (%)	
15	90.67 ± 1.83ab	83.54 ± 3.13b	72.16 ± 2.47b	27.01 ± 1.08b
20	94.80 ± 1.67a	90.29 ± 2.85ab	92.91 ± 3.79a	39.73 ±2.17a
25	93.47 ± 1.44a	98.61 ±0.57a	95.51 ± 1.30a	44.00 ± 0.90a
30	93.60 ± 1.48a	93.31 ± 1.70a	95.40 ± 0.90a	41.80 ± 1.38a
35	87.47 ± 1.58b	84.52 ± 3.18b	0.00 ± 0.00c	0.00 ± 0.00c

Means followed by different letters in the same column are significantly different (Student Newman-Keuls multiple range test, $P < 0.05$).

3.4 Discussion

The developmental time of immature stages of *B. invadens* was affected by temperature with the duration of each stage decreasing as temperature increased. The results are consistent with that of earlier workers who have reported similar trends with different species of Tephritid fruit flies (Carey *et al.*, 1985; Vargas *et al.*, 1996; Brevault & Quilici, 2000; Duyck & Quilici, 2002; Duyck *et al.*, 2004b). In this study, development was prolonged at 15 and 20°C in all developmental stages. Generally, the linear effect of temperature on development rate falls off at average daily temperatures than those experienced normally in the field (Howe, 1967), implying that there is an intermediate 'optimum' temperature for development. In this study, optimum temperature for development was found to be between 25-30°C. In *B. cucurbitae*, *B. dorsalis* and *B. oleae*, optimum temperatures for development have been reported to lie between 26°C to 30°C (Messenger & Flitters, 1958; Tsitsipis, 1980). Ekesi *et al.* (2006) have previously shown that *B. invadens* successfully completed development at 28°C which lie within the optimum range of 20 - 30°C reported in the present study. At 35°C, the duration of development for egg and larva was low and pupa suffered the highest mortality. This suggests that the upper developmental threshold for *B. invadens* lies between 30°C and 35°C. However, the damaging and irreversible effect of temperature of 35°C on development may be dependent on the length of exposure. Indeed, conditions of high temperatures do not occur for extended periods of time during a given day although this also depends on the locality. Indeed, all fruit fly developmental stages are sheltered from extremes of temperature (eggs and larvae) in fruits and puparia occur in the soil under

tree canopies (Fletcher, 1987). Therefore, extrapolation of these findings into field conditions must be done with caution.

The regression coefficients were significant and close to one, indicating a strong linearity of the model between 15°C and 35°C for egg and larvae and 15°C and 30°C for puparia. The linearity of this relationship was consistent with previous findings with Tephritidae (Vargas *et al.*, 1996; Brevault & Quilici, 2000; Duyck & Quilici, 2002; Duyck *et al.*, 2004a). Because of the difficulties associated with data generation across the full range of temperatures, thermal constants or degree-days (DD) are often employed to account for differences in development rate due to temperature (Wagner *et al.*, 1984). In this study, lower temperature threshold for *B. invadens* was 8.8, 9.4 and 8.7°C for the egg, larva and puparium, respectively with corresponding thermal constants of 31, 168 and 177 DD. In *B. zonata*, developmental thresholds for egg, larva and puparium were estimated to be 12.7, 12.6 and 12.8°C with thermal constants of 25, 68 and 131 DD (Duyck *et al.*, 2004a). In *B. dorsalis*, Vargas *et al.* (1996) estimated lower temperature thresholds and thermal constants of 11.8, 5.6, 9.3°C and 21, 161, 176 DD for egg, larva and puparium, respectively. The values reported here are lower in comparison with those of *B. zonata* but within the range reported for *B. dorsalis*. This is perhaps not surprising given that *B. invadens* is believed to be a member of the *B. dorsalis* complex (Drew *et al.*, 2005).

Bactrocera invadens has been described as a devastating quarantine pest (French, 2005). In assessing the risk posed by this insect to horticultural industries outside its current range of distribution, one critical component should include determination of the

likelihood of eggs hatching as commodities travel along pathways from the field to their final destination. Degree-days (DD) and developmental threshold becomes an important tool in such risk assessment (Sharpe *et al.*, 1976; Thomas, 1997). For example, in the event that any commodity is harvested soon after eggs of *B. invadens* are deposited in fruits, using the DD and lower temperatures established for this study, it implies that a 9.1 DD (egg DD + larva DD/2) is accumulated each day before egg hatch. It therefore means that untreated commodities with *B. invadens* eggs would need to be either utilized or destroyed within 8 days since it takes 31 DD for eggs to hatch. However, constant temperature studies underestimate developmental thresholds and actual thresholds tend to be lower than those obtained experimentally (Messenger, 1964; Judd & McBrien, 1994; Liu & Meng, 1999). Degree-day models are also more accurate when temperatures fall within the lower and optimal development curve. The example given above must therefore be applied with caution also taking into account the protection from extremes of temperatures offered to the developmental stages by the commodities.

Laboratory mass rearing of fruit flies is best carried out in controlled temperature conditions and high survival rates coupled with short generation time are considered important criteria (Vargas *et al.*, 1993; Kaspi *et al.*, 2002). In this study, the survival rate for all developmental stages of *B. invadens* did not differ significantly between 20 to 30°C. The total developmental time was however highest at 20°C (31.5 days) compared with 25 and 30°C (17.8-21.2 days). A suitable compromise between high survival rates and short developmental time would be to maintain the eggs, larvae and puparia at 25°C. Recent studies at *icipe* are also concentrating on mass rearing of *Fopius arisanus* (Sonan)

for classical biological control of *B. invadens* (Mohamed *et al.*, unpublished data) and the present findings have direct bearing on parasitoids rearing in the laboratory for field releases. In this case the strategy would be to allow for longer egg and larval duration of *B. invadens* to have an adequate supply of these stages. Thus holding the immature stages at temperature of 20°C would therefore be appropriate since it delays onset of subsequent development stages while retaining high survivorship.

The m.r.v between the first and the last individual to complete each stage was variable with temperature and development duration particularly at 15°C. However, it was less variable at the higher temperatures. The m.c.v which estimates the degree of variation in development between individuals at each stage compared with the mean development duration was also variable with temperature and for the different immature stages. A lower m.c.v indicates that more individuals complete the stage development nearer to the mean development duration for each stage. Higher variations were observed with the egg development stage than with larva or pupa. These parameters of variation could be important in laboratory mass rearing procedures for quality control purposes by quickly predicting fertility of a cohort.

The extent to which an invasive species can extend its range or an existing species to respond to climate change is largely related to climatic factors such as temperature. Understanding the effect of temperature on survival of an insect which ultimately influences abundance and dispersal is fundamental to the study of insect ecology (Andrewartha & Birch, 1954). In this study, survival of *B. invadens* was reduced at the

temperature of 15°C. This may have probably contributed to limiting spread of the insect in highland areas of Kenya (Ekesi *et al.*, 2006). In Australia, the bioclimatic potential of *Bactrocera tryoni* (Froggatt) is related to thermal restrictions. Its altitude limits in the cooler southern parts are set by lethally low minimum winter temperatures (Meats, 1981). In related thermotolerance studies, there was no ovarian maturation of some adult tephritids reared at 15°C (Duyck & Quilici, 2002; Duyck *et al.*, 2004a). The flies were not maintained beyond emergence from puparia, thus the effect of low temperatures on ovarian maturation was not determined, but several morphotypes of *B. invadens* are known (Drew *et al.*, 2005) and the possible existence of cold-hardening ecotypes of the insect cannot be ruled out. Huey *et al.* (1991) postulated that the relationship between development rate and temperature can be considered as the result of natural selection because this relationship changes when insects are exposed to different temperature regimes for many generations. Gilbert & Raworth (1996) claimed that insects are selected for slow development in spring but fast development in summer. The entire physiological processes therefore have adaptive and ecological implications.

The data generated offers valuable information on the development and survival of *B. invadens* under laboratory conditions and provides a basis for understanding the bioecology of the pest and development of control measures. The data also provides information that may be useful for optimizing environmental conditions necessary for mass rearing of *B. invadens* for experimental purposes. The range of thermal parameters generated should help in the making of informed decisions regarding the quarantine risk associated with the insect. The data reported should also allow for development of or

improvement of models to better understand the bioclimatic potential of *B. invadens* and consequently its distributional limits and abundance. It is most likely that this information will become increasingly important as *B. invadens* continues to colonize new geographical areas.

CHAPTER FOUR

4 HOST PLANTS AND HOST PLANT PREFERENCE STUDIES FOR *BACTROCERA INVADENS* (DIPTERA: TEPHRITIDAE) IN KENYA, A NEW INVASIVE FRUIT FLY SPECIES IN AFRICA

4.1 Introduction

In March 2003, an invasive species of fruit fly from the genus *Bactrocera* Macquart was detected in Kenya (Lux *et al.*, 2003a) and most recently described as *Bactrocera invadens* Drew Tsuruta & White. (Drew *et al.*, 2005). Since the first report in Costal Kenya, the insect has rapidly spread across the African continent and it is now known from 24 other countries (Drew *et al.*, 2005; French, 2005; Vayssières *et al.*, 2005; Ekesi *et al.*, 2006; R. Hanna, unpublished data). *Bactrocera invadens* is believed to have invaded Africa from the Indian subcontinent and was discovered in Sri Lanka after it was first reported from Africa (Drew *et al.*, 2005), where it has become a significant pest of quarantine and economic importance (Mwatawala *et al.*, 2004; Vayssières *et al.*, 2005; Ekesi, 2006; Ekesi *et al.*, 2006).

Bactrocera invadens belongs to the *Bactrocera dorsalis* Hendel complex of tropical fruit flies (French, 2005), which comprises more than 75 species largely endemic to South-East Asia (Drew & Hancock, 1994; Tsuruta & White, 2001; Clarke *et al.*, 2004; Clarke *et al.*, 2005) with undescribed species remaining in collections (Lawson *et al.*, 2003). The group is arguably regarded as one of the most destructive to fruits worldwide (White & Elson-Harris, 1992; Clarke *et al.*, 2005; Drew *et al.*, 2005). Most recently *B. dorsalis* was

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accidentally introduced into French Polynesia where it has displaced two other tephritid species, spread to five different Society Islands, and infests 29 different host fruits so far (Vargas *et al.*, 2007). Previously documented species of *Bactrocera* in Kenya includes melon fly *Bactrocera cucurbitae* (Coquillett), olive fruit fly *B. oleae* (Gmelin), *B. biguttula* (Bezzi) and *B. munroi* White (White & Elson-Harris, 1992; Copeland *et al.*, 2004). Among the four species, *B. cucurbitae* was the most prevalent and destructive, attacking both cultivated and wild cucurbit plants (Ekesi, 2006). Other *Bactrocera* species within the *B. dorsalis* complex in Africa include *Bactrocera zonata* (Saunders) which is also an invasive species now established in Egypt (Anonymous, 2005) and known to be highly destructive, attacking 13 plant species in Mauritius and Réunion (Quilici & Jeuffrault, 2001). The arrival of *B. invadens* in Kenya adds to the list of *Bactrocera* species on the continent and neighbouring Islands and compounds the fruit fly problems in the region.

Most frugivorous tephritids within the *B. dorsalis* complex are known to attack a wide range of fruits and wild plant species. For example, three species within *B. dorsalis* complex: *B. papayae* (Drew & Hancock) has 209 recorded hosts across 51 plant families; *B. dorsalis* has 124 host species across 42 families in tropical Asia; and *B. carambolae* (Drew & Hancock) has 77 host species across 27 families (Drew, 1989; Hollingsworth *et al.*, 2003; Clarke *et al.*, 2005). Of the *Bactrocera* species reported in Kenya, the host range of *B. cucurbitae* are primarily cucurbits, but it has been recorded from a few non-cucurbit hosts (White & Elson-Harris, 1992). The olive fly, *B. oleae* exclusively infests fruits of *Olea europaea* L., *B. munroi* infests *O. europaea* and *O. welwitschii* Gilg &

Schnellenberg and *B. biguttula* the coastal olive, *O. woodiana* Knobl. (Copeland *et al.*, 2004). In Benin, Vayssières *et al.* (2005) reported 10 plant species as hosts of *B. invadens*. In Tanzania, Mwatawala *et al.* (2006a) identified 15 fruit species as hosts to *B. invadens*. The only published host record from Kenya is its attack on *Strychnos mellodora* S. Moore and mango (*Mangifera indica* L.) (Lux *et al.*, 2003a; Drew *et al.*, 2005; Ekesi *et al.*, 2006). Host status is a dynamic phenomenon and this list is by no means exhaustive and given that the *B. dorsalis* complex, to which *B. invadens* belongs, attack several host plant species, it is envisaged that this list is likely to increase.

Because of the "novelty status" of *B. invadens*, very little is known about the ecology of this pest and the need to document the host plants of this important quarantine pest becomes crucial. The objective of this study, therefore, was to catalogue the host plants of *B. invadens* in Kenya, given its importance as a major quarantine pest in order to provide necessary information that may be useful for management of the pest. Host preference studies were also conducted in the laboratory in choice and no-choice tests that included nine of the major export fruits that were either infested or not infested in the field survey to ascertain the most preferred host plants of the insect.

4.2 Materials and Methods

4.2.1 *Field survey*

Host fruits survey was carried out from December 2004 to April 2006 in three provinces in Kenya where *B. invadens* had been previously confirmed with ME baited traps to be in high abundance (Lux *et al.*, 2003a; Ekesi *et al.*, unpublished data). Priority was

particularly given to locations with large diversity of fruits, spread across the Coast, Eastern and Rift Valley Provinces of the country (Fig. 4.1). In the Rift Valley Province, surveys were concentrated at Nguruman division. In the Coast Province, sampling locations included forested areas on the fringes of the Indian Ocean and high altitude areas in Taita hills. In Eastern Province (representing the highland region of the country), sampling locations were varied up to the fringes of Mt Kenya forest. At each location, approximate latitude, longitude and altitude were taken using a GPS device (Table 4.1).

4.2.2 *Fruit collection, handling and processing*

Fruits were collected from cultivated fields, backyard gardens, woodlands, roadside shrubs, forested areas and protected reserves. Often, a few fruits not encountered from sampling sites were purchased from roadside markets and whenever possible attempts were made to establish the place of origin. Fruit samples collected included ripe to overripe fruits, including those with visible symptoms of fruit fly damage both from the tree and from the ground as "windfalls." Attempts were made to sample large quantity of fruits with a minimum of 15 fruits per fruiting species although in some cases this sample size could not be maintained due to unavailability of fruits. Fruit collections of the different plant species were separately placed in perforated polyethylene bags in the field for transport to the rearing facility. The rearing facilities were located in each ecozone where fruits were collected and included the *icipe* - Muhaka field station for Coast, *icipe* field station at Nguruman for Rift Valley and *icipe* headquarters in Nairobi for Eastern Province samples.

At the rearing facility, fruits were counted, weighed and secured in well-aerated rectangular plastic containers. Small fruits (<5 cm diameter) were held together in 1.5 litre rectangular transparent plastic containers (20 x 12.5 x 8 cm) (Kenpoly®, Kenya). Larger fruits (>5 cm diameter) were held in groups of two or three in 3 litre rectangular plastic containers (20 x 12.5 x 15 cm) (Kenpoly®, Kenya).

Fruits >10 cm diameter were held in cylindrical plastic buckets (25 x 30 cm) (No.20, Nairobi Plastics Limited, Kenya). 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 adult tephritids. The fruits were placed on 40-60 mm of moistened sterilized sand at the bottom of the rearing containers. The sand served both as the pupation medium for the larvae that exited the fruits in addition to soaking up fruit juices (Woods *et al.*, 2005). 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 large juicy fruits, pupation occurred inside the fruit and in this case rotting fruits were also dissected to completely recover all remaining puparia. The petri dishes with puparia were then held in small-ventilated transparent cylindrical plastic cages (5.5 x 12.5 cm) (No. J-12, GP plastics, Kenya) until eclosion. 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.

Table 4.1 Fruit sampling sites with approximate geo-referenced positions and altitude

Province	District	Locality	Approximate longitude	Approximate latitude	Approximate altitude (m)
Coast	Kwale	Diani Forest	04° 20' 03 S	39° 34' 10 E	30
	Kwale	Kibarani	04° 19' 47 S	39° 31' 03 E	350
	Kwale	Mkambani	04° 12' 35 S	39° 37' 03 E	44
	Kwale	Muhaka area	04° 16' 35 S	39° 33' 36 E	44
	Kwale	Muhaka forest	04° 19' 27 S	39° 32' 27 E	46
	Kwale	Shimba Hills	04° 13' 21 S	39° 22' 09 E	380
	Malindi	Malindi	03° 11' 40 S	40° 05' 20 E	32
	Kilifi	Kilifi	03° 47' 15 S	39° 51' 56 E	167
	Mombasa	Fort Jesus	04°.02' 36 S	39° 35' 37 E	20
	Mombasa	Mombasa	04° 03' 25 S	39° 39' 32 E	40
	Taita Taveta	Taita hills	03° 24' 51 S	39° 35' 20 E	1405
Eastern	Embu	Rwika	00° 37' 43 S	37° 30' 03 E	1213
	Embu	Manyatta	00° 23' 39 S	37° 30' 07 E	1600
	Embu	Nthagaiya	00° 29' 24 S	37° 35' 31 E	1328
	Embu	Mt. Kenya forest	00° 20' 51 S	37° 28' 55 E	2004
	Embu	Nguruka	00° 22' 28 S	37° 32' 46 E	1183
	Embu	Rukuriri	00° 21' 52 S	37° 31' 49 E	1732
	Embu	Runyenjes	00° 25' 23 S	37° 34' 09 E	1532
	Embu	Thingingi	00° 30' 49 S	37° 38' 07 E	1850
	Mbeere	Mbeere	00° 33' 42 S	37° 38' 49 E	1200
Rift Valley	Kajiado	Nguruman	01° 48' 31 S	36° 03' 34 E	760

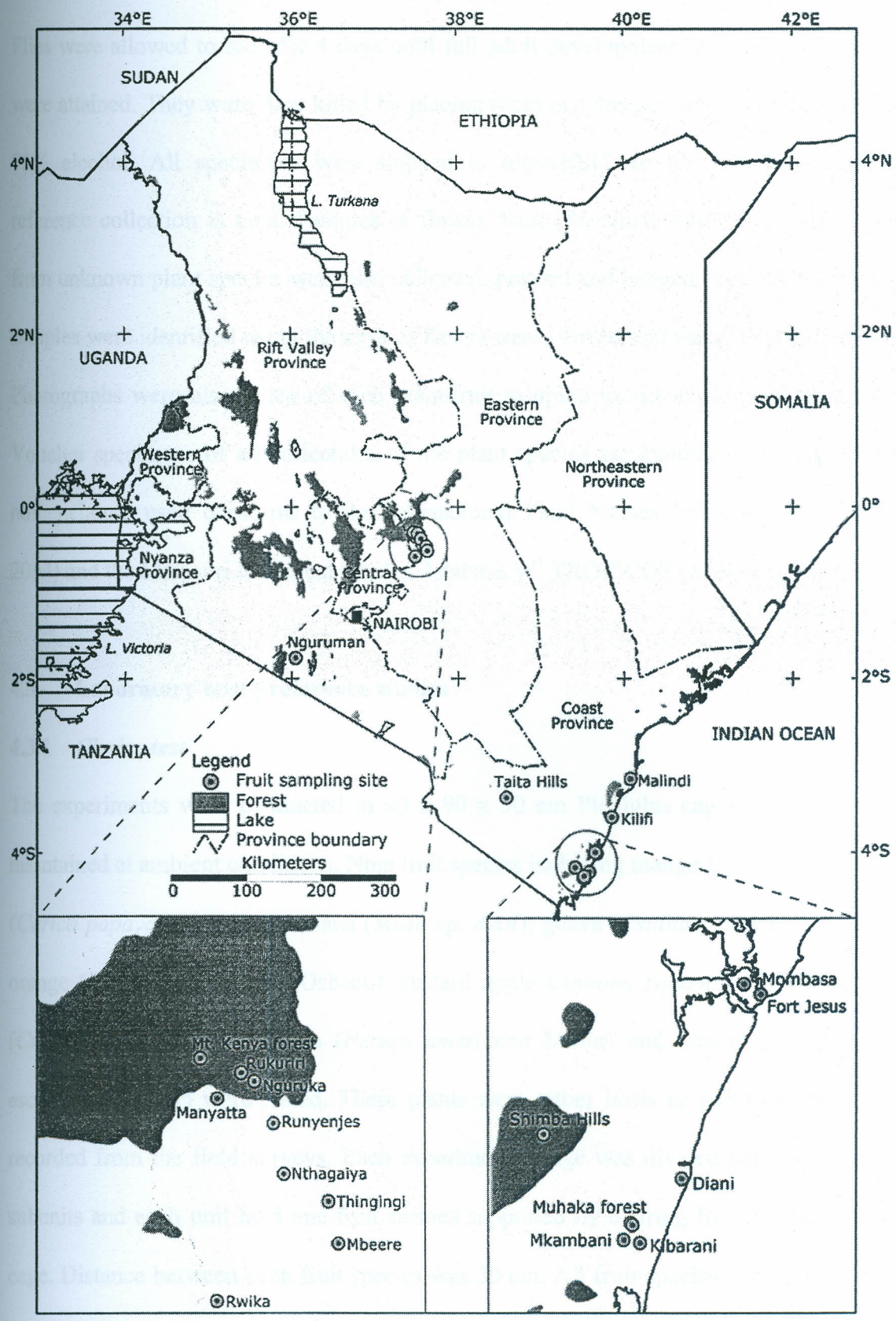

Fig. 4.1 Map of Kenya showing fruit collection sites

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. All specimens were shipped to *icipe*-BSU for identification where a reference collection is kept. Samples of flower, fruit (for small fruit), leaf and/or twig from unknown plant species were also collected, pressed and bagged. The collected plant samples were identified using the keys of Kenya trees, shrubs and lianas (Beenjte, 1994). Photographs were also taken of each plant/fruit sampled to aid in plant identification. Voucher specimens of all collections of the plant species are maintained at *icipe*. Plant nomenclature used conforms to the International Plant Names Index database (IPNI, 2004) and the Missouri Botanical Garden database W^3 TROPICOS (MBOT, 2006).

4.3 Laboratory host preference studies

4.3.1 *Choice test*

The experiments were conducted in 90 x 90 x 90 cm Plexiglas cages in a laboratory maintained at ambient conditions. Nine fruit species including mango (*M. indica*), papaya (*Carica papaya*. L.), sweet banana (*Musa* sp. *AAA*), guava (*Psidium guajava* L.), sweet orange (*Citrus sinensis* (L.) Osbeck), custard apple (*Annona squamosa* L.), cucumber (*Cucumis sativus* L.), avocado (*Persea americana* Miller) and tomato (*Lycopersicon esculentum* Miller) were tested. These plants were either hosts or non-hosts that were recorded from the field surveys. Each experimental cage was divided equally into nine subunits and each unit held one fruit species supported by a string from the roof of the cage. Distance between each fruit species was 30 cm. All fruit species were tested when they were either fully ripe (mango, papaya, banana, orange, guava, tomato, custard apple)

or at mature green stage (avocado and cucumber). It is generally well established that host plants are most susceptible to fruit flies at these stages. One hundred adult *B. invadens* (consisting of 50 females and 50 males) at 2-3 weeks old were then released inside the cages for a period of 24 hrs. Flies were fed on 1:3 volumetric mixture of enzymatic yeast hydrolysate and sugar and water was provided in pumice granules. After 24 h, all fruit species were removed and incubated individually as described for field surveys. Records were kept for pupal recovery and percentage of adult emergence from the total puparia recovered. Four replicated cages were maintained and the experiment was repeated twice.

4.3.2 *No choice test*

Two sets of experiments were conducted under the no-choice test. In the first experiment, the female fecundity on fruit domes of the nine plant species for a period of ten days measured. Fruit domes were made by scooping the pulp and seeds out of the fruits. Fruit peel thickness varied slightly among the fruit species and ranged from 2.2 to 2.5 mm in diameter. Each fruit dome was then transferred into a 20 x 20 x 20 cm Plexiglas cage and a pair of adult *B. invadens* released inside the cage. Records were kept of the number of eggs laid daily on the dome by washing the eggs off the underside of the domes. Flies were fed as previously described. To record hatch rate, the eggs were transferred onto strips of moist blotting paper and hatch rate was determined by observing for eclosion under a binocular microscope after 2 days. The cages were arranged in a complete randomized design with 3 replications.

In the second experiment, whole fruits were exposed to flies in 25 x 25 x 25 cm Plexiglas cages. A single fruit sample from each of the nine plant species listed above was transferred into each cage. Forty 2-3 weeks old adult flies (20 females and 20 males) were released in each cage for a period of 24 h. Flies were fed as in previous experiment. At the end of the exposure period, the fruit was removed and processed as previously described. Five replicates were maintained and the experiment was repeated twice.

4.4 Statistical analyses

Data for field surveys are presented according to plant species, family, location, number of fruits collected and weight, number of infested fruit and number of adults. Calculation of levels of infestation by *B. invadens* followed the methodology of Cowley *et al.* (1992) and was calculated as the ratio of the number of adults/kg of fruit collected (Infestation index). In the laboratory experiments, data were tested for normality and homogeneity of variance by transforming to natural logarithms (pupal recovery and fecundity) and angular transformation (% egg hatch and adult emergence) before subjecting to analyses. Since the experimental design in the choice experiment did not support the assumption of sample independence for analysis of variance, the non-parametric equivalent, Kruskal-Wallis and chi-square tests, were used to analyse the data. In the no-choice experiment, data were subjected to analysis of variance using the generalized linear model (Proc GLM) and means were separated by Tukey (HSD) test (P=0.05). All analyses were performed using the SAS package (2001).

4.5 Results

4.5.1 *Field survey*

Bactrocera invadens was reared from a total collection of 3913 fruits from a range of habitats that comprised 14 plant species and 8 families from surveys carried out at the Coast, Eastern and Rift Valley Provinces of Kenya (Tables 4.1 and 4.2). Fruit species positive for *B. invadens* included both cultivated and wild host plants (Table 4.2). The majority of *B. invadens* infested samples were from commercial fruits. Ten of the host plants are new records for *B. invadens* in Kenya. During the survey period, a collection of 4630 fruits comprising 76 other plant species from 32 families did not yield *B. invadens*. The data indicated that *B. invadens* was capable of infesting fruits over an altitudinal range of 20–1335 metres above sea level (masl) with infestation varying from 1.1 to 652.8 flies/kg of fruit (Tables 4.1 and 4.2).

Among the plant species sampled, *B. invadens* infestation was recorded from *Annona cherimola* L. (cherimolia), *A. muricata* L. (soursop), *A. squamosa* L. Engl. (custard apple) [Annonaceae], *Citrus limon* (L.) Burm.f. (lemon), *C. reticulata* Blanco (tangerine), *C. sinensis* L. (sweet orange) [Rutaceae], *Cordia myxa* [Boraginaceae], *L. esculentum* (tomato) [Solanaceae], *M. indica* (mango) [Anacardiaceae], *Musa* sp. *AAA* (banana) [Musaceae], *P. guajava* (guava) [Myrtaceae], *Sclerocarya birrea* (A.Rich.) Hochst. (marula), *Sorindeia madagascariensis* L., [Anacardiaceae] and *Terminalia catappa* L. (tropical almond) (Combretaceae) (Table 4.2). The families Anacardiaceae, Annonaceae and Rutaceae had the highest number of species infested, with *B. invadens* reared from three species sampled in each family.

The most heavily infested Anacardiaceae was *M. indica* with infestation reaching 130.3 flies/kg fruit at Muhaka, Coast Province while in the Annonaceae, the wild species, *A. cherimola* sampled at Nthagaiya, Eastern Province recorded the highest number of *B. invadens* (85.0 flies/kg fruit) (Table 4.2). Among the Rutaceae, *C. limon* was the most infested at Nguruman, Rift Valley Province (32.3 flies/kg fruit). In the other Provinces, members of the Rutaceae sampled were less infested and *C. sinensis* and *C. reticulata* had the highest level of infestation (2.0 to 5.6 flies/kg fruit).

Of the wild host fruits sampled, the highest level of infestation was recorded on *T. catappa* (652.8 flies/kg fruit) sampled from Nguruman, Rift Valley Province and *S. birrea* (238.8 flies/kg fruit) sampled from Muhaka, Coast Province (Table 4.2). Generally fruit infestations were higher at low elevations than at the highland areas (Tables 4.1 and 4.2). For example, mango fruit infestation varied from 39.2 to 130.3 flies/kg fruit at the low elevation locales in the Coast Province and Rift Valley compared with 0 to 29.4 flies/kg fruit in the high elevation areas of the Eastern Province (Table 4.2).

Other Tephritid species encountered during the survey period included *B. cucurbitae* *Ceratitis anonae* (Graham), *C. capitata* (Wiedemann), *C. cosyra* (Walker), *C. rosa* Karsch, *C. pedestris* (Bezzi), *C. pinax* Munro, *Dacus frontalis* Becker, *Dacus vertebratus* (Bezzi), *Trirhithrum nigerrimum* (Bezzi) and *T. senex* Munro. The host plants data collected for these fruit flies is listed in Appendix 1.

4.5.2 Laboratory host preference studies

4.5.2.1 *Choice test*

There was a significant difference in the number of puparia recovered (Kruskal-Wallis, H = 56.2, df = 8, P = 0.0001) and adult emergence (Kruskal-Wallis, H = 36.2, df = 8, P = 0.0001) from the nine fruit species exposed to *B. invadens* (Fig. 4.2A). The highest number of puparia was recovered from mango, papaya and banana while the lowest recovery was observed in tomato, cucumber and custard apple (Fig. 4.2A). Percentage adult emergence ranged from 60 to 86% (Fig. 4.2A).

4.5.2.2 *No-choice test*

There was a significant difference among the fruit species in total fecundity over ten days (F = 12.6, df = 8, 18, P = 0.0034) and fertility (F = 24.5, df = 8, 18, P = 0.0001) (Fig. 4.2B). The highest number of eggs was recovered from mango, papaya, banana and cucumber domes compared with the other five fruit species (Fig. 4.2B). Egg fertility ranged from as low as 21% in tomato to 82% in mango (Fig. 4.2B).

In the second set of experiments in the no-choice test, pupal recovery and adult emergence were also significantly different among the fruit species, F = 33.4, df = 8, 81, P = 0.0001 and F = 24.0, df = 8, 81, P = 0.0001, respectively (Fig. 4.2C). Mango, papaya and banana recorded the highest number of puparia while the lowest number occurred on cucumber (Fig. 4.2C). Percentage adult emergence varied from 31 to 86% (Fig. 4.2C).

Table 4.2 Host fruit infestation indices for *Bactrocera invadens* in 3 provinces of Kenya during December 2004-April 2006

Province/ Locality	Plant species	Plant family	No. of fruits	Fruit wt. (kg)	% fruit infested	No. *B. invadens* adults	*B. invadens*/ kg fruits
Coast Province							
Malindi	*Mangifera indica* L.	Anacardiaceae	206	65.9	64.4	6012	91.2
	Citrus sinensis (L.) Osbeck	Rutaceae	114	10.4	28.5	23	2.2
	Psidium guajava L.	Myrtaceae	84	9.8	31.3	41	4.2
Kilifi	*Musa* sp. *AAA*	Musaceae	262	5.2	36.4	66	12.7
	Mangifera indica L.	Anacardiaceae	43	13.2	50.8	1204	91.2
	Citrus sinensis (L.) Osbeck	Rutaceae	31	2.6	12.3	12	4.6
	Terminalia catappa L.	Combretaceae	121	3.6	35.2	443	123.1
Muhaka	*Annona cherimola* Mill.	Annonaceae	35	0.7	31.4	21	30.1
	Citrus limon (L.) Burm.f	Rutaceae	32	2.6	0.0	0	0.0
	Citrus reticulata Blanco	Rutaceae	40	4.3	12.5	24	5.6
	Citrus sinensis (L.) Osbeck	Rutaceae	15	1.9	12.5	7	3.7
	Cordia sp. cf *myxa*	Boraginaceae	33	0.6	6.1	10	17.1

Table 4.2 continues. Host fruit infestation indices for *Bactrocera invadens* in 3 provinces of Kenya during December 2004-April 2006

Province/ Locality	Plant species	Plant family	No. of fruits	Fruit wt. (kg)	% fruit infested	No. *B. invadens* adults	*B. invadens*/ kg fruits
	Mangifera indica L.	Anacardiaceae	119	38.4	59.7	5004	130.3
	Musa sp. *AAA*	Musaceae	24	0.2	0.0	0	0.0
	Psidium guajava L.	Myrtaceae	32	3.6	34.4	61	17.0
	Sclerocarya birrea (A, R.) H.	Anacardiaceae	127	2.5	36.2	597	238.8
	Sorindeia madagascariensis B.	Anacardiaceae	108	0.1	1	1	10.0
Mombasa	*Annona muricata* L.	Annonaceae	16	6.5	6.3	9	1.4
	Terminalia catappa L.	Combretaceae	16	0.4	18.8	7	17.5
Eastern Province							
Mbeere	*Annona cherimola* Mill.	Annonaceae	40	1.1	15.0	59	53.6
	Mangifera indica L.	Anacardiaceae	112	29.5	40.2	296	10.0
	Musa sp. *AAA*	Musaceae	52	3.3	0.0	0	0.0
	Citrus sinensis (L.) Osbeck	Rutaceae	101	9.8	12.6	11	1.1

Table 4.2 continues. Host fruit infestation indices for *Bactrocera invadens* in 3 provinces of Kenya during December 2004-April 2006

Province/ Locality	Plant species	Plant family	No. of fruits	Fruit wt. (kg)	% fruit infested	No. *B. invadens* adults	*B. invadens*/ kg fruits
Nthagaiya	*Annona cherimola* Mill.	Annonaceae	6	0.6	50.0	51	85.0
	Mangifera indica L.	Anacardiaceae	145	40.0	31.2	257	6.4
	Musa sp. *AAA*	Musaceae	145	9.3	12.1	55	5.9
	Psidium guajava L.	Myrtaceae	31	3.1	12.0	36	11.6
	Citrus limon (L.) Burm.f	Rutaceae	30	2.7	0.0	0	0.0
Rwika	*Annona cherimola* Mill.	Annonaceae	86	2.2	23.5	62	28.2
	Citrus reticulata Blanco	Rutaceae	46	4.8	10.3	18	3.8
	Citrus sinensis (L.) Osbeck	Rutaceae	224	25.6	12.7	49	2.0
	Mangifera indica L.	Anacardiaceae	204	72.9	40.2	2141	29.4
	Musa sp. *AAA*	Musaceae	132	2.8	10.2	21	7.5
Rukuriri	*Annona muricata* L.	Annonaceae	5	0.5	20.0	0	0.0
	Lycopersicon esculentum Mill.	Solanaceae	48	4.7	0.0	0	0.0

Table 4.2 continues. Host fruit infestation indices for *Bactrocera invadens* in 3 provinces of Kenya during December 2004-April 2006

Province/ Locality	Plant species	Plant family	No. of fruits	Fruit wt. (kg)	% fruit infested	No. *B. invadens* adults	*B. invadens*/ kg fruits
	Mangifera indica L.	Anacardiaceae	158	19.4	1.3	0	0.0
	Psidium guajava L.	Myrtaceae	65	2.4	17.5	0	0.0
Rift Valley Province							
Nguruman	*Annona squamosa* L.	Annonaceae	58	13.2	54.1	33	2.5
	Citrus limon (L.) Burm.f	Rutaceae	21	3.0	28.6	97	32.3
	Citrus sinensis (L.) Osbeck	Rutaceae	14	1.5	0.0	0	0.0
	Lycopersicon esculentum Mill.	Solanaceae	23	1.2	8.7	2	1.7
	Mangifera indica L.	Anacardiaceae	454	148.6	57.5	5830	39.2
	Musa sp. *AAA*	Musaceae	9	0.9	33.3	123	129.9
	Sclerocarya birrea (A, R.) H.	Anacardiaceae	154	3.1	41.3	123	39.7
	Terminalia catappa L.	Combretaceae	92	3.2	83.9	2089	652.8

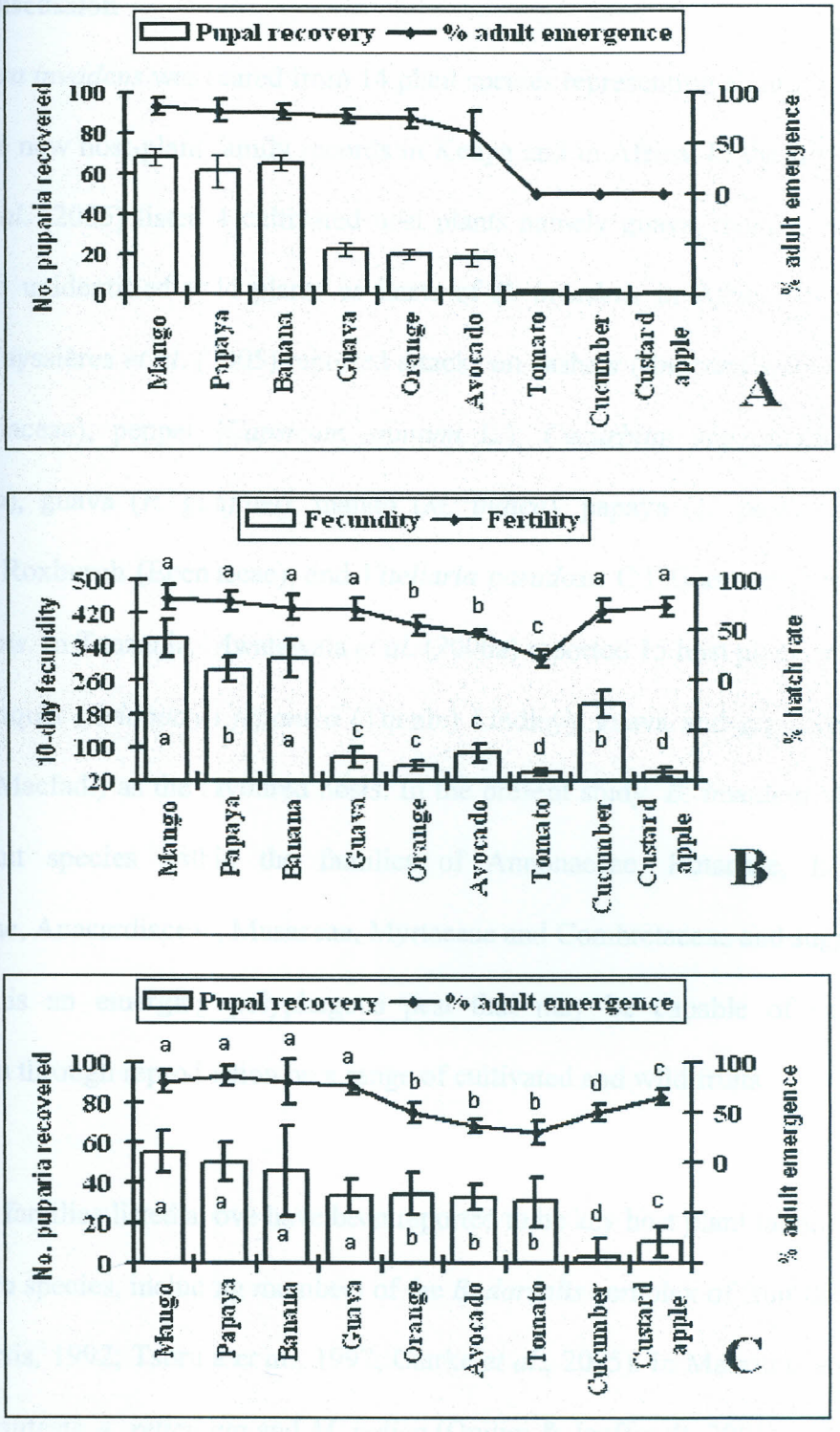

Fig. 4.2 Host preference and performance of *Bactrocera invadens* in terms of pupal recovery, adult emergence, fecundity and fertility on nine cultivated plant species in a choice (A) and no-choice (B, C) tests in the laboratory. Bars and data points on line with same letter do not differ significantly by Tukey (HSD) test (P=0.05). Error bars denote SE.

4.6 Discussion

Bactrocera invadens was reared from 14 plant species representing eight families most of which are new host-plant family records in Kenya and in Africa. In the first description, Drew *et al.* (2005) listed 4 cultivated host plants namely guava, mango, citrus, papaya and some unidentified wild plants as hosts of *B. invadens* in Africa. In Benin, West Africa, Vayssières *et al.* (2005) reported attacks on cashew (*Anacardium occidentale* L.) (Anacardiaceae), pepper (*Capsicum annuum* L.), *Cucurbita.* spp, custard apple (*A. squamosa*), guava (*P. guajava*), mango (*M. indica*), papaya (*C. papaya*), *Diospyros montana* Roxburgh (Ebenaceae), and *Vitellaria paradoxa* C.F.Gaertner (Sapotaceae) by *B. invadens*. In Tanzania, Mwatawala *et al.* (2006a) reported 15 host plants and identified mango, loquat (*Eriobotrya japonica* (Thunb.) Lindley), guava and grapefruit (*Citrus* x *paradisi* Macfad.) as the favoured hosts. In the present study, *B. invadens* was found to infest fruit species within the families of Annonaceae, Rutaceae, Boraginaceae, Solanaceae, Anacardiaceae, Musaceae, Myrtaceae and Combretaceae and suggests that *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.

The plant families listed above have been reported to be key host plant families of several *Bactrocera* species, including members of the *B. dorsalis* complex of fruit flies (White & Elson-Harris, 1992; Tsuruta *et al.*, 1997; Clarke *et al.*, 2005). In Mauritius and Réunion, *B. zonata* infests *A. reticulata* and *M. indica* (Quilici & Jeuffrault, 2001) and the relative importance of these plants as hosts of *B. invadens* is also confirmed by these results. *Bactrocera dorsalis* infests *Annona* spp., *Citrus* spp., *M. indica*, *Musa* spp. and *P.*

guajava (Armstrong, 1983; Allwood *et al.*, 1999; Clarke *et al.*, 2005) and this is consistent with findings for *B. invadens* in this study.

Mangifera indica was the most important host of *B. invadens* among the fruits sampled within the Anacardiaceae. The data reveals mango infestation in mid to high elevation areas of Eastern Province of Kenya. In previous studies by Ekesi *et al.* (2006), no *B. invadens* was recovered from mango in this locality. The current record of *B. invadens* in this locality clearly indicates that the pest is gradually expanding its range and exploiting host fruit at higher elevation areas of the country. *Sclerocarya birrea* is also an important reservoir host for *B. invadens.* High infestation levels were recorded at the Coast and Rift Valley Provinces (238.8 *B. invadens*/kg and 39.7 *B. invadens*/kg respectively). This plant generally fruits sporadically throughout the year (Jøker & Erdey, 2003) and may be an important off-season host for *B. invadens* in the absence of the primary cultivated host plants.

Among the Annonaceae, *B. invadens* was reared from *A. squamosa*, *A. muricata* and *A. cherimola*. Mwatawala *et al.* (2006a) showed that *A. muricata* was a major host of *B. invadens* in Tanzania. Studies elsewhere have shown that other species of *Bactrocera* such as *B. carambolae*, *B. correcta* (Bezzi), *B. dorsalis*, *B. kandiensis* Drew and Hancock, *B. frauenfeldi* (Schiner) and *B. papayae* are frugivorous on this family (Tsuruta *et al.*, 1997; Hadwen *et al.*, 1998; Hollingsworth *et al.*, 2003; Clarke *et al.*, 2005). *Bactrocera invadens* evidently utilizes these wild and planted cultivars of Annonaceae

and management activities directed at *B. invadens* should take into account the importance of these plants as hosts of the insect.

Citrus limon was the most infested of the Rutaceae by *B. invadens* compared to *C. reticulata* and *C. sinesis*. In Tanzania, *B. invadens* was not found to attack *C. limon* although infestations were observed on *C. reticulata* and *C. sinensis* (Mwatawala *et al.*, 2006a). Among the *B. dorsalis* complex, *C. sinensis* appears to be a less preferred host plant. For example in Surinam and South America, Clarke *et al.* (2005) reported that infestation rates by *B. dorsalis* on *C. sinensis* was 1.2% compared to 16.3% for *M. indica* and 11.3% for *P. guajava*. In this study, it was observed an infestation rate of up to 4.6 and 5.6 *B. invadens*/kg on *C. sinensis* and *C. reticulata,* respectively. The observed high levels of infestation in *C. limon* was indeed remarkable given the acidic nature of this plant. Vayssières *et al.* (2005) reported high infestation of *B. invadens* in a similarly acidic host plant (*A. occidentale)* suggesting that the pest may be adapted to a wide range of fruit characteristics.

The Combretaceae, *T. catappa* is generally known to harbour a complex of fruit fly species of the *B. dorsalis* complex including *B. zonata, B. correcta*, *B. dorsalis*, *B. kandiensis*, *B. papayae*, *B. zonata* and *Bactrocera* sp. near *nigrotibialis* (taxon A) (Tsuruta *et al.*, 1997; Quilici & Jeuffrault, 2001; Hollingsworth *et al.*, 2003; Clarke *et al.*, 2005; Quilici *et al.*, 2005). The high infestation levels recorded in this study (652.8/kg *B. invadens*) confirm the status of *T. catappa* as an important host plant of *B. invadens*. This is perhaps not surprising given that the plant species is native to Asia (Styger *et al.*, 1999;

Thomson & Evans, 2006). *Terminalia catappa* can flower up to 3 times a year producing fruits almost throughout the year (Thomson & Evans, 2006; S. Ekesi, unpublished data) and probably harbours successive generations of this pest which infest orchards when fruiting begins. In Kenya, *T. catappa* thrives as an ornamental tree, mostly utilized as shade trees around the homesteads and sometimes in close proximity to mango orchards. Under such systems, management strategies for *B. invadens* must also take cognizance of the presence of this important wild host in addition to the cultivated plants.

Bactrocera invadens was reared from banana (Musaceae) which is known to be a major host of *Bactrocera* species, the most important being *B. musae* (Tryon), and *B. papayae* (White & Elson-Harris, 1992; Clarke *et al.*, 2005). *Bactrocera invadens* can infest green banana both in the laboratory and field (S. Ekesi, unpublished data). Because this fruit is largely exported around the world at the mature green stage, it's likely that a strategy exploiting avoidance of *B. invadens* by harvesting and shipping banana at maturity may be inappropriate for evading infestation by this pest.

The Myrtaceae, *P. guajava* (guava) is known to host a variety of fruit fly species worldwide including several species of *Bactrocera* (White & Elson-Harris, 1992; De Meyer *et al.*, 2002; Clarke *et al.*, 2005). *Bactrocera invadens* was reared from this plant confirming the status of guava as a major host plant of fruit flies. In West and Central Africa, Vayssières *et al.* (2005) reported this plant as a major host of *B. invadens*. Similarly in Tanzania, Mwatawala *et al.* (2006a) showed that guava was highly favoured by *B. invadens*.

Among the Solanaceae sampled in the current survey, *B. invadens* was recorded from tomato. In other regions of the world, *L. esculentum* is attacked by other *Bactrocera* species such as *B. carambolae* (Clarke *et al.*, 2005), *B. papayae* (Hadwen *et al.*, 1998; Clarke *et al.*, 2005), *B. tryoni* (Froggatt) (Balagawi *et al.*, 2005) and *B. latifrons* (Hendel) (Liquido *et al.*, 1994). In West and Central Africa, *B. invadens* has been reported from tomato (Hanna, personal communication). However, Mwatawala *et al.* (2006a) did not find infestation on this plant in Tanzania. In the surveys, tomato samples from which *B. invadens* was reared were collected from a backyard garden. No flies have yet been detected from fruit collections at commercial scale where pesticides are regularly used for management of fruit worms and tetranychid mites.

Bactrocera invadens was also reared from the Boraginaceae, *C. myxa* collected from Coast Province. Fruit fly records for *Cordia* species in Africa are scanty, nevertheless the Boraginaceae are reported to be hosts to some *Ceratitis* species (Quilici & Jeuffrault, 2001; De Meyer *et al.*, 2002). No tephritids were reared from fruits of eight *Cordia* species collected in Kenya from 1999-2001 (Copeland *et al.*, 2002). This record from Kenya is the first with regard to infestation by *Bactrocera* species.

Among all the infested plant species, the highest infestation rates were recorded from low elevation areas of the Coast and Rift Valley Provinces compared to the higher elevation areas of Eastern Province. In a recent study on mangoes in Kenya, Ekesi *et al.* (2006) showed a significant inverse relationship between the numbers of *B. invadens* infestation per kg of mango fruits and elevation from which fruits were collected and the authors

concluded that *B. invadens* appeared to be a lowland resident pest. Most fruit flies from the genus *Bactrocera* are considered to be lowland residents. Vargas *et al.*(1983) demonstrated that fruit infestation by *B. dorsalis* in native and exotic forests on Kauai Island (Hawaii) was moderate at middle (579-800 masl) elevation and low at high (>800 masl) elevation. Generally, elevation by itself does not determine fruit fly distribution but associated factors such as temperature, rainfall and host plants at such elevation play a significant role (Nishida *et al.*, 1980).

In the laboratory host preference studies, results of the choice experiment showed that mango and papaya were the most preferred host plants of *B. invadens* followed by banana. These laboratory results agree with the results obtained from the field on mango and banana but sharply contrast field survey results for papaya where no infestation was recorded. The reason for lack of infestation of papaya in the field is not clear given that large quantity of papaya (42.3 kg) was sampled from localities that were heavily infested by *B. invadens*. In Tanzania, Mwatawala *et al.* (2006a) did not record any infestation on papaya by *B. invadens* in the Morogoro region. However, in the Mikocheni region of the country, *B. invadens* infestation on papaya have been observed in field samples as low as 2 kg of fruits in a locality with a lower prevalence of *B. invadens* than Nguruman, Kenya (S. Seguni & W. Mwaiko, unpublished data). This observation largely highlights the need for continuous field survey in different localities in Kenya.

In the no-choice fecundity and fertility studies, higher numbers of eggs were laid on mango, papaya, banana and cucumber domes than the other plant species tested. Pupal

recovery from mango, papaya and banana was consistent with the results from fecundity and fertility tests, but the level of pupal recovery from cucumber was the lowest among all the fruits tested. In general, fecundity and fertility results for papaya and mango support pupal recovery but this was not the case with cucumber. The reason for the contrasting results is unclear and warrants further investigation.

Within the *B. dorsalis* complex, to which *B. invadens* belongs, some insects are specialist host range species while others are general polyphagous species. These results suggest that *B. invadens* may be an emerging polyphagous species. In general, the host list generated in the current study is unlikely to be exhaustive and periodic surveys especially for the *B. invadens* negative plant species would be necessary. It is also acknowledged that the sample size for some of the fruit species collected in the current study may be low but results presented here may still be useful in making some phytosanitary and pest management decisions.

Host plant preference studies clearly demonstrated that mango and banana are the most preferred host plants of *B. invadens* in Kenya thus the pest is likely to jeopardize lucrative export of these crops from this region. Indeed some countries have already banned the importation of these fruits from Kenya and Uganda due to the threat posed by *B. invadens* (S. Muchemi, KEPHIS, personal communication; E. Niyibigira, MAAIF, Uganda, personal communication).

CHAPTER FIVE

5 POPULATION DYNAMICS OF *BACTROCERA INVADENS* (DIPTERA: TEPHRITIDAE) AND OTHER MANGO-INFESTING FRUIT FLIES AT NGURUMAN, KENYA AND THE ROLE OF FALLEN FRUITS AS RESERVOIR HOSTS

5.1 Introduction

Bactrocera invadens (Diptera: Tephritidae) Drew, Tsuruta & White is a destructive pest of several tropical and subtropical fruits including cultivated fruits such as mango (*Mangifera indica* L). (Anacardiaceae), banana (*Musa* sp. *AAA*) (Musaceae), guava (*Psidium guajava* L.) (Myrtaceae), *Annona* spp (custard apple, soursop and sugar apple) (Annonaceae), tomato (*Lycopersicon esculentum* Mill.) (Solanaceae) and citrus (lemon, orange and tangerine) (Rutaceae) (Drew *et al.*, 2005; Rwomushana *et al.*, 2008). Major wild host plants include marula *Sclerocarya birrea* (A. Rich) Hochst. (Anacardiaceae) and tropical almond *Terminalia catappa* L. (Combretaceae) (Vayssiéres *et al.*, 2005; Mwatawala *et al.*, 2006a; Rwomushana *et al.*, 2008). *Bactrocera invadens* belongs to the *B. dorsalis* complex of fruit flies that includes other economically important fruit fly species such as *B. carambolae*, *B. papayae* and *B. kandiensis* (Drew & Hancock, 1994). Recent studies have shown a significant congruence between the morphological and biological species boundaries of these species and *B. invadens* (Drew *et al.*, 2008).

Prior to the invasion of *B. invadens*, important indigenous fruit flies on mango included the mango fruit fly *Ceratitis cosyra* (Walker), Mediterranean fruit fly *Ceratitis capitata*

(Wiedemann), Natal fruit fly *C. rosa* Karsch, *C. fasciventris* (Bezzi) and *C. anonae* Graham (Lux et al., 2003b). These species are confined to the Afrotropical bio-geographical region, including some of the Indian Ocean islands (De Meyer, 2000). Although *C. cosyra* is distributed over a wide geographic area in Kenya, it has a restricted host range (Mukiama & Muraya, 1994; De Meyer *et al.*, 2002; Copeland *et al.*, 2006) but has traditionally been considered the main fruit fly pest of mangoes in Kenya among the various *Ceratitis* species and accounts for the major losses in mango production (Mukiama & Muraya, 1994; Lux *et al.*, 2003b). *Ceratitis capitata* is thought to originate from West Africa (Silvestri, 1914) and is perhaps the most widely known pest fruit fly, having invaded several continents as a result of travels and fruit trade (White & Elson-Harris, 1992). It is established in most of the countries surrounding the Mediterranean Sea, Central and South America, Western Australia and Hawaii (Clausen, 1978). *Ceratitis rosa* is native to South and Eastern Africa (Clausen, 1978), but has spread to the Indian Ocean islands of Mauritius and Réunion (Etienne, 1972). *Ceratitis fasciventris* and *C. anonae* are so far restricted to the Africa main land (White & Elson-Harris, 1992).

The arrival of *B. invadens* in Kenya and other African countries is likely to impact on the population abundance and distribution of native mango pests. In assessing the level of damage of *B. invadens* on mango, Ekesi *et al.* (2006) observed unusually high densities of the invasive species over the indigenous *C. cosyra* which has been known to be the main fruit fly pest of mango. Trapping with standard protein baits in a study to evaluate several attractants also showed co-occurrence of *B. invadens* with the native fruit fly species in mango agroecosystems with the exotic pest in higher abundance (Section 6.0).

However, despite this emerging trend, the population dynamics and temporal interaction of *B. invadens* with other species of fruit fly occurring on mango in Kenya has not been studied.

Generally, population dynamics studies on tephritids in many parts of the world have concentrated on adult trapping (Vargas *et al.*, 1989; Israely *et al.*, 2005), although some studies have also examined the effect of host fruits or both (Harris *et al.*, 1993; Aluja *et al.*, 1996; Katsoyannos *et al.*, 1998; Papadopoulos *et al.*, 2001). From these studies, host fruit availability and abundance can particularly have a significant impact on population dynamics of fruit fly species. Indeed, from previous observations at Nguruman, fruit fly populations are observed to increase when mango is fruiting (Ekesi *et al.*, unpublished data). However, the impact of host fruit availability on the population dynamics of *B. invadens* and native fruit flies has not been quantified. Additionally, there is little organized effort to remove infested and overripe fruit that fall to the ground (Plate 5.1). The lack of field sanitation practises could potentially serve as an important breeding source particularly for resident populations of *B. invadens* and other fruit flies in mango orchards and influence the seasonal population dynamics of these pests. But presently, there are no studies relating the impact of fallen fruit on field *B. invadens* densities.

The objectives of the present study therefore were: (i) to establish the temporal seasonal and annual population trends of mango infesting fruit flies at Nguruman, Kenya and (ii) examine the role of fallen mango fruits on the population build-up of these fruit fly pests. Results from this study should be useful in the development of intervention strategies and

also provide important baseline information on measuring the success of a planned fruit fly suppression program through classical biological control.

5.2 Materials and methods

5.2.1 *Study area and climate*

The studies were conducted from October 2005 to September 2007 at Nguruman, Kajiado district in Rift Valley Province of Kenya. The chosen study sites had previous history of abundance of native fruit fly pests (Ekesi *et al.*, unpublished data) and recently recorded high population levels of *B. invadens* (Ekesi *et al.*, 2006). Several fruit fly hosts in the area such as mango, guava, marula, tropical almond, custard apple, banana and lemon (De Meyer *et al.*, 2002; Copeland *et al.*, 2006; Rwomushana *et al.*, 2008) are planted in the mango orchards and in patches adjacent to or in close proximity to the study sites (Table 5.1). The fruiting period of these plants is variable but begins in late August and ends in January. However, other host plants such as marula and tropical almond, fruit several times throughout the year while a few local mango varieties fruit outside the main mango season (ie. April - June) (Fig. 5.1). The climate of this area is warm and dry with average temperatures and humidity of (20°C - 38°C) and (60% - 70%), respectively. Rainfall is received once a year from April to June although some showers occur during November-December. Fruit production in this area however occurs all year round with irrigation water from a nearby escarpment. The following mango orchards were selected for the experiments; (1) Orchard A (latitude 01°48.5' S, longitude 036°03.5' E, 1.062 ha); (2) Orchard B (latitude 01°48.7' S, longitude 036°03.2' E, 3.605 ha); (3) Orchard C (latitude 01°48.7' S, longitude 036° 04.3' E, 1.155 ha) and Orchard D

(latitude 01°48.1' S, longitude 036°03.6' E, 2.152 ha). All orchards were approximately 760 m.a.s.l and within 500 m from each other. The mango trees are more than 15 years old, between 8-10 metres tall and are planted at the recommended spacing. The varieties of mango grown on the orchards are indicated in Fig 5.1. No form of fruit fly management practices is carried out in the orchards and sanitation was never observed.

Table 5.1 Host plants of *Bactrocera invadens, Ceratitis capitata* and *Ceratitis cosyra* at Nguruman

Host Plant Family	Host Scientific Name	Host Common Name	Source of data
Anacardiaceae	*Mangifera indica* L.	Mango	1,2,3,4,5
	Sclerocarya birrea (A. Rich.) Hochst.	Marula	1,2, 4
Annonaceae	*Annona squamosa* L.	Custard apple	4,5
	Annona muricata L.	Custard apple	1,5,6
	Annona reticulata L.	Soursop	1,5
Combretaceae	*Terminalia catappa* L.	Tropical almond	1,2,5
Myrtaceae	*Psidium guajava* L.	Guava	1,5,6
Rutaceae	*Citrus* sp	Sweet orange	1,5

Sources of data: [1] De Meyer *et al.* (2002), [2] PW. Nderitu (AFFI data, unpublished), [3] Mukiama & Muraya (1994), [4] Copeland *et al.* (2006), [5] Rwomushana *et al.* (2008), [6] Mwatawala *et al.* (2006a)

Plate 5.1: Fallen mango fruits at Nguruman

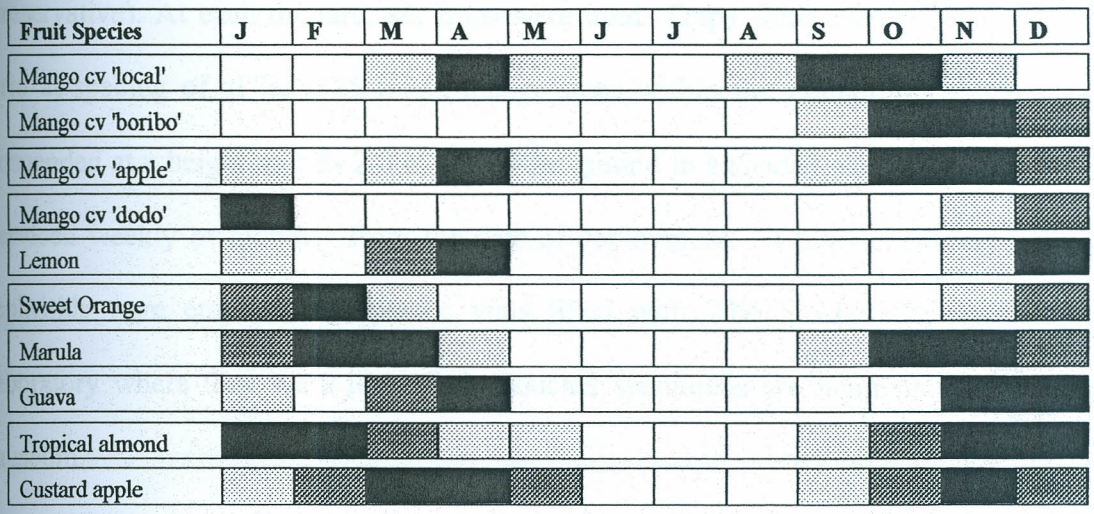

Fig. 5.1 Typical yearly fruiting phenology of the host plants common to *Bactrocera invadens*, *Ceratitis capitata* and *Ceratitis cosyra* at Nguruman, Kenya in 2006.

5.2.2 Seasonal and annual population monitoring

5.2.2.1 *Adult population monitoring*

Seasonal monitoring of adult fruit fly populations started on 27 October 2005 to 15 January 2006 and was repeated from 27 October 2006 to 15 January 2007 in orchards A and B. During the main mango season, data on number of adults was collected weekly beginning at the onset of fruiting until the very end of harvest. For the annual population trends of the adult flies, trapping was conducted monthly from October 2005 to September 2006, and thereafter carried out weekly from October 2006 to September 2007, such that each month of the year had at least one week data collection points. The indices of population abundance were obtained by trapping using Multilure® Trap (Better World, USA) baited with 300 ml of an aqueous solution of the food based attractant, 2% NuLure® (Miller Chemical and Fertilizer, Hanover, PA) + 1% borax (as a

preservative). At each orchard, six traps were used. Traps were evenly distributed in a grid at spacing of at least 25 m on mango trees within the orchards. The traps were suspended at a height of 1.5- 2.0 m above the ground in a shaded part of the canopy and serviced weekly or monthly from the date of deployment. At trap service, all captured tephritids were emptied into plastic vials filled with 70% alcohol and taken to the laboratory where they were identified. Voucher specimens are maintained at the *icipe* museum.

5.2.2.2 *Fruit sampling*

Seasonal pattern of fruit infestation by the different fruit flies in the study area was carried out by random sampling of mango fruits at weekly intervals for the two mango fruiting seasons in orchards A and B. The sampling procedure consisted of identification of a spot in the centre of the study orchards and then four transects were developed from the spot towards the North, South, East and West of the field. Ten trees were then selected at random at each direction of the transect for fruit collection. Fifty mature mango fruits were collected from the trees and another 50 from the ground (two per tree) at each site on every sampling occasion (100 fruits) and taken to the laboratory for processing to establish infestation levels using the procedure described in section 4.2.2.

5.2.3 Assessment of the role of fallen mango fruits as reservoir hosts

5.2.3.1 *Adult population monitoring*

Monitoring for adult density was conducted concurrently with fruit collection at orchards C and D. Multilure traps baited with 2% NuLure + 1% borax were used for the adult

monitoring. At each orchard, four traps were used at similar spacing and height placement as described in section 5.2.2.1. Traps were serviced weekly and all captured tephritids emptied into plastic vials filled with 70% alcohol for counting and identification. A voucher collection of all captured specimens is kept at the *icipe* museum.

5.2.3.2 *Fruit sampling for role of fallen mango fruits as reservoir hosts*

To assess the role of fallen fruits on the ground as a possible reservoir for flies in the orchard, 50 fruits were sampled from trees and the same number from the ground (either as 'windfalls' or harvest rejects). Fruits were collected on a weekly basis from orchards C and D and taken to the laboratory for processing following the method described in 5.2.4 below. The fruits collected from the ground and tree were held separately during processing

5.2.4 Fruit processing

In the laboratory, fruits were weighed and then transferred individually to 3 litre rectangular plastic containers (20 cm x 12.5 cm x 15 cm) (Kenpoly®, Kenya) containing a dry sand layer (3-5 mm deep) and held at ambient conditions in the laboratory at the *icipe*- Nguruman field facility. When the sand was soaked with fruit juices, the fruits were transferred to fresh containers to prevent larvae from drowning in the juices (Woods *et al.*, 2005). 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 adult tephritids. Fruit samples were held for three weeks, enough time for most immature

stages in the fruits to complete development. Because pupation often occurred inside the flesh, rotting fruits were also dissected to recover all remaining puparia and mature larvae found in the fruits to pre-empt them to pupariate. Sand in the containers was sieved daily to recover puparia which were counted and held in smaller-ventilated transparent cylindrical plastic cages (5.5 x 12.5 cm) (No. J-12, GP plastics, Kenya) until they eclosed. Emerged tephritids were provided with an artificial diet that consisted of a sugar-water solution in cotton wool. Flies were fed for 4 days until full adult development and body colorations were attained. They were then killed by immersion in 70% alcohol and preserved for later counting and identification. A reference collection of the recovered insects is kept at *icipe* museum.

5.3 Data analyses

For orchards A and B, the number of fruit flies of each species captured per trap per week was determined, which was then converted to flies per day using the formula: flies/trap/day = total number of fruit flies/(number of traps x 7days). For orchards C and D, the trap data is presented as flies/trap/week correlating with the weekly fruit collections. The estimates of relative abundance index (RAI) for the fruit fly species reared from mango from orchards A and B was adopted from Segura *et al.* (2006) while the measure of infestation in ground and tree fruits (infestation index) and % infestation was estimated according to Cowley *et al.* (1992) on pooled data from orchards C and D. The two sample *t* test was used to compare infestation of fruits from the ground and tree at each sampling date. Correlation analysis was used to ascertain the relationship between the number of flies found in fruits collected from the ground, those directly from the tree

fruits and density of adults in the orchard using the Pearson's product moment (SAS. Institute, 2001).

5.4 Results

5.4.1 Seasonal and annual population trends

5.4.1.1 *Trap catches*

During the period of fly activity, the temporal pattern of trap captures for both *B. invadens* and *C. cosyra* was closely associated with the seasonal maturation of mango, mainly from October to January. Increased fly activity was also detected during the shorter mango fruiting period in April-June 2007. Generally, adult population of *B. invadens* and *C. cosyra* was low at the onset of fruit maturity in October, peaking in December when fruit ripening occurred and began to decline in January which corresponded to the completion of the mango harvest (Fig. 5.2). The trend for the two year annual population dynamics from October 2005 to September 2007 distinctly showed peak abundance of both fruit flies to be from October to December. However, overall abundance during October to December 2006 was significantly lower than that from October to December 2005.

Data from this study showed that *B. invadens* dominated in the trap catches compared to *C. cosyra* and *C. capitata* in the mango orchards (figures 5.2 and 5.3). During the 2005 season, abundance of *B. invadens* captures at orchard A was 29.8 ± 2.84 flies/trap/day compared to 2.46 ± 0.3 and 0.2 ± 0.03 for *C. cosyra* and *C. capitata,* respectively (Fig. 5.3a). In orchard B, the captures were 13.9 ± 1.8, 0.24 ± 0.04 and 0.04 ± 0.01 for *B.*

invadens, *C. cosyra* and *C. capitata,* respectively (Fig. 5.3b). This represents 12 and 58 fold more *B. invadens* compared to *C. cosyra* present at orchards A and B, respectively. During the subsequent fruiting season, fruit fly captures at orchard A stood at 13.8 ± 1.2, 0.44 ± 0.06 and 0.009 ± 0.004 flies/trap/day for *B. invadens*, *C. cosyra* and *C. capitata,* respectively (Fig. 5.3c). At orchard B, captures were 8.69 ± 1.1, 0.14 ± 0.03 and 0.01 ± 0.004 flies/trap/day for *B. invadens*, *C. cosyra* and *C. capitata,* respectively (Fig. 5.3d) representing 31 and 62 fold more *B. invadens* compared to *C. cosyra* captured at orchards A and B respectively.

5.4.1.2 *Mango infestation patterns*

During the two seasons of the study, a total of 2150 mango fruits were collected from the two orchards. From these fruits, a total of 5624 *B. invadens* (83.1%), 1141 *C. cosyra* (16.8%) and 9 *C. capitata* (0.1%) adults emerged. The relative abundance index (RAI) at both orchards was in the order *B. invadens* > *C. cosyra* > *C. capitata*. The RAI for *B. invadens* at orchard A over both seasons ranged from 0.70-1.00 and at orchard B from 0.73-1.00 (Table 5.2). The value for *C. cosyra* ranged from 0.16-0.30 at orchard A and from 0.04 -0.28 at orchard B while that for *C. capitata* it ranged from 0.00-0.01 at orchard A and from 0.00-0.02 at orchard B (Table 5.2). No *C. capitata* was recovered from mango during the November 2006 to January 2007 fruiting season.

5.4.2 Assessment of the role of fallen fruits as reservoir hosts

5.4.2.1 *Trap catches*

Trap catches of *B. invadens* were generally low when infestation in fruits from the tree

and ground was low, peaking during December which coincided with the peak infestation of mango from ground and tree fruits. For instance, during 2005 season, 4.6 *B. invadens* flies/trap/week were captured in October peaking at 127.9 flies/trap/week in December (Table 5.3). During the 2006/2007 season, 89.4 flies/trap/week were captured in early November, peaking to 139.8 flies/trap/week from late November to mid December (113.8 flies/trap/week) and declining to 19.3 flies/trap/week in January (Table 5.3).

5.4.2.2 *Mango infestation on ground and tree fruit*

From the fruit collections of the 2005/2006 season, density of *B. invadens* in the fruits collected from the ground and tree increased gradually at the onset of fruit maturity in October from 2.42 flies/kg fruit and 0 flies/kg fruit, respectively with a peak in November at 13.8 flies/kg for ground fruits and 10.43 flies/kg fruit for tree collected fruits ($t = 6.82$, $P = 0.0001$). Later in the mango season, higher density of adults was recovered from fruits on the tree (8.66 flies/kg fruit) than from the ground (7.74 flies/kg fruit) during the December sampling (Fig. 5.4A) ($t = 8.50$, $P = 0.0001$). In the 2006/2007 fruiting season, peak infestation in fruits occurred in November in fruits from the ground (35.24 flies/kg fruit) as well as those from the tree (21.15 flies/kg fruit) ($t = 11.47$, $P = 0.0001$). Similarly, higher density of adults was recovered from tree fruits (6.23 flies/kg fruit) than from ground fruits (1.16 flies/kg fruit) in fruit samples collected at the end of the 2006/2007 season (Fig. 5.4B) ($t = 8.62$, $P = 0.0001$). The density of *B. invadens* in ground fruits correlated significantly with density of the fly from tree fruits ($r = 0.53$, $P = 0.0001$) but not with the density of the adults trapped in the orchards (Table 5.4).

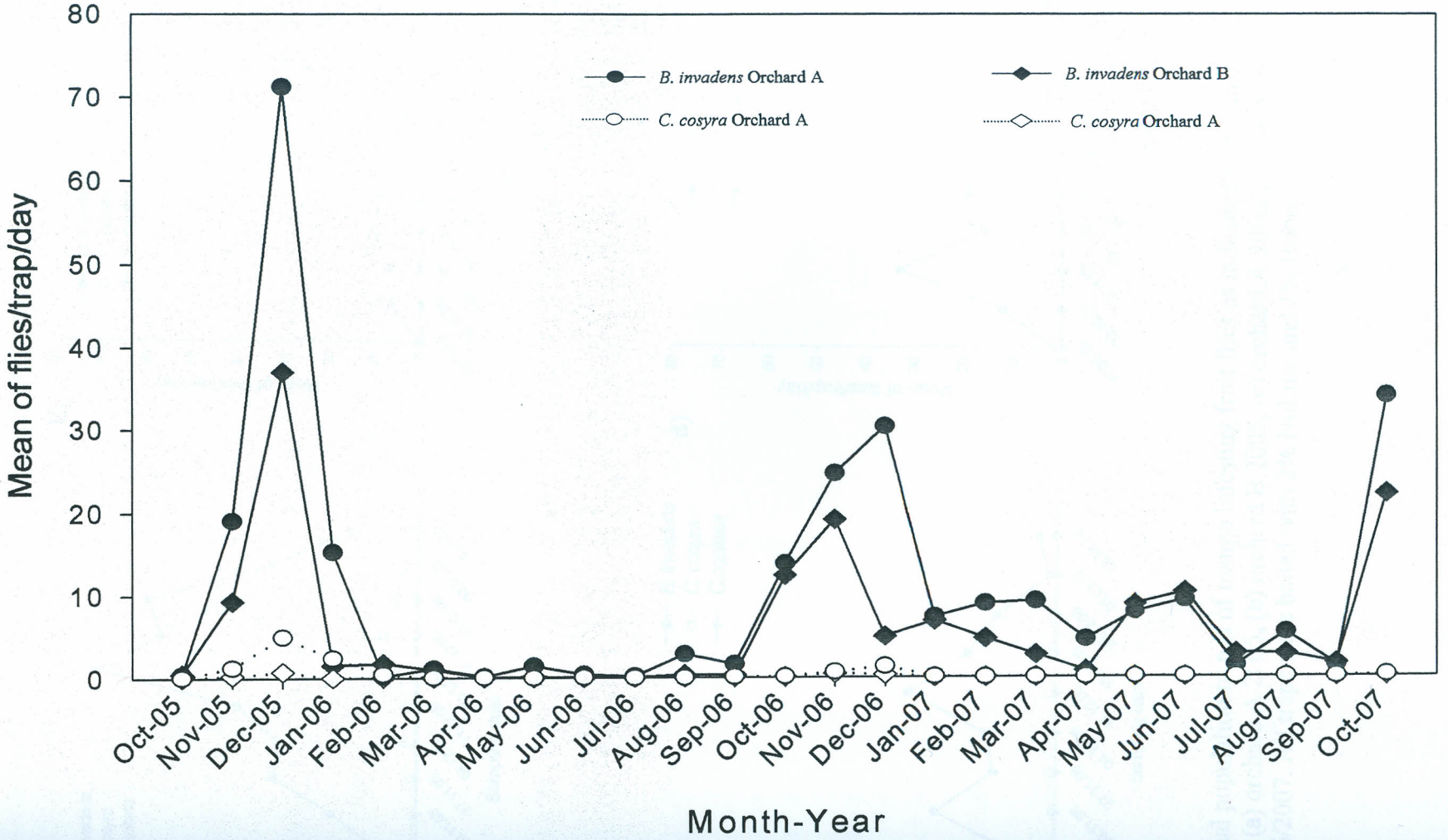

Fig. 5.2 Annual population trends of mango-infesting fruit flies at Orchard A and Orchard B, Nguruman, Kenya

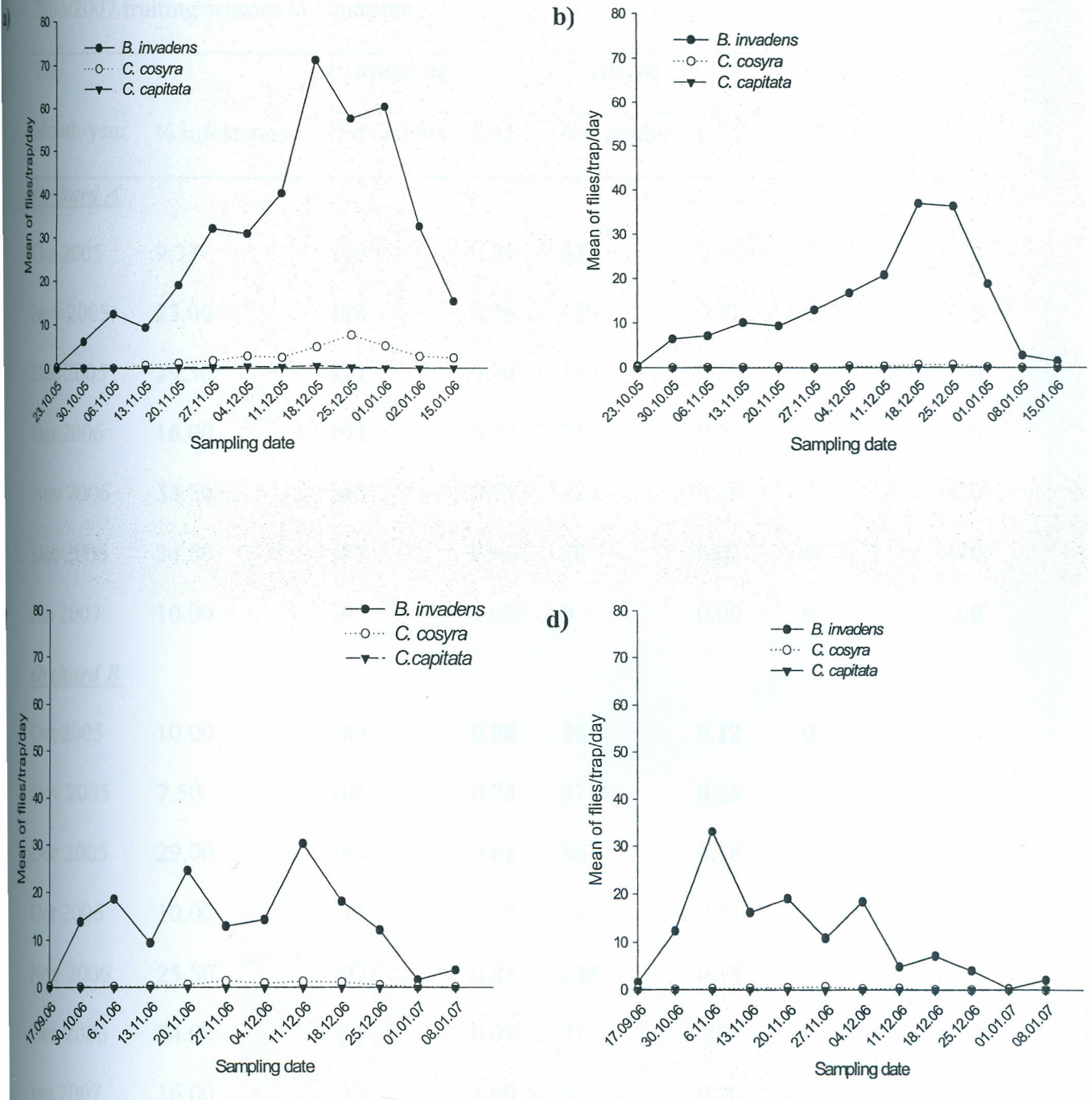

Fig. 5.3 Seasonal population trends of mango infesting fruit flies as indicated by mean of flies/trap/week (a) orchard A 2005, (b) orchard B 2005, (c) orchard A 2006/2007 and (d) orchard B 2006/2007. All traps were baited with 2% NuLure and 1% Borax

Table 5.2 Fruit collection data showing the % infestation levels, number of fruit flies reared from mango and the Relative Abundance Index* (RAI) values during 2005 and 2006/2007 fruiting seasons at Nguruman

Site/		*B. invadens*		*C. cosyra*		*C. capitata*	
Month-year	% infestation	†No. adults	RAI	No. adults	RAI	No. adults	RAI
Orchard A							
Oct 2005	9.33	219	0.84	41	0.16	0	0.00
Nov 2005	23.00	482	0.78	129	0.21	6	0.01
Dec 2005	29.50	423	0.70	183	0.30	0	0.00
Oct 2006	16.00	194	0.72	75	0.28	0	0.00
Nov 2006	33.50	645	0.74	223	0.26	0	0.00
Dec 2006	34.50	892	0.96	38	0.04	0	0.00
Jan 2007	10.00	29	1.00	0	0.00	0	0.00
Orchard B							
Oct 2005	10.00	189	0.88	26	0.12	0	0.00
Nov 2005	7.50	106	0.73	37	0.25	3	0.02
Dec 2005	29.00	393	0.82	86	0.18	1	0.00
Oct 2006	10.00	111	0.79	29	0.21	0	0.00
Nov 2006	25.50	1071	0.82	238	0.18	0	0.00
Dec 2006	34.00	651	0.95	37	0.05	0	0.00
Jan 2007	16.00	219	1.00	0	0.00	0	0.00

*RAI = $x_i/(x_{i1}+x_{i2}+x_{i3})$ (Segura *et al.*, 2006), †Total number of adults/50 fruits

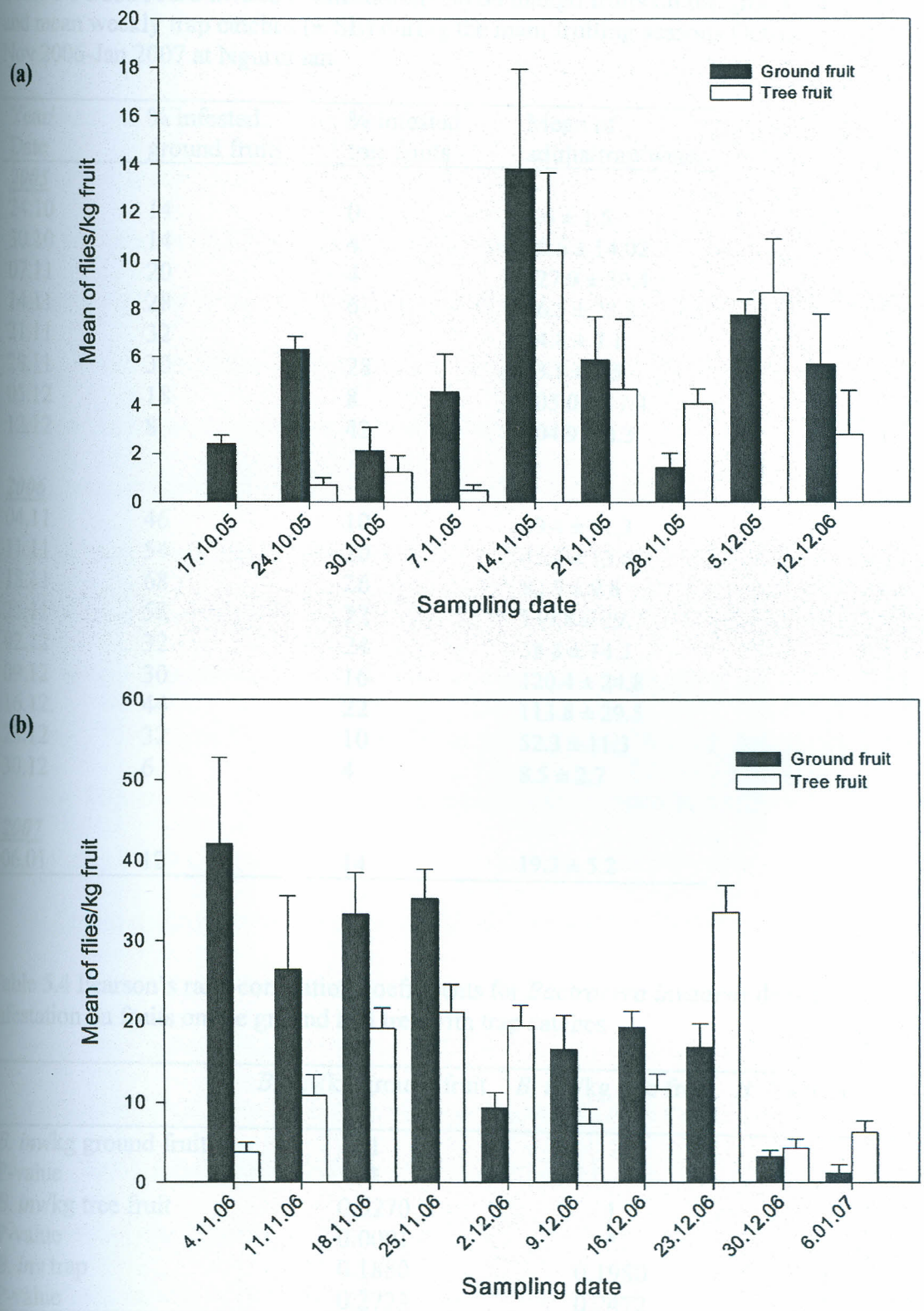

Fig 5.4 Mean density of *Bactrocera invadens* in mango fruits on the ground and on trees at Nguruman, during the fruiting seasons (a) October-December 2005 and (b) November 2006-January 2007.

Table 5.3 *Bactrocera invadens* infestation (%) on mango fruits on the ground and tree and mean weekly trap catches (± SE) during the main fruiting seasons Oct-Dec 2005 and Nov 2006-Jan 2007 at Nguruman

Year/ Date	% infested ground fruits	% infested tree fruits	Mean of adults/trap/week
2005			
24.10	14	0	4.6 ± 1.5
30.10	14	4	70.6 ± 14.02
07.11	20	4	127.9 ± 30.4
14.11	28	6	96.5 ± 23.8
21.11	32	6	64.8 ± 7.1
28.11	30	28	63.8 ± 8.5
05.12	18	8	105.0 ± 17.4
12.12	8	42	104.9 ± 8.3
2006			
04.11	46	10	89.4 ± 11.3
11.11	54	20	45.4 ± 13.6
18.11	68	26	82.9 ± 9.8
25.11	58	22	139.8 ± 29.7
02.12	32	24	58.8 ± 14.3
09.12	30	16	120.4 ± 24.8
16.12.	44	22	113.8 ± 29.5
23.12	32	10	52.3 ± 11.3
30.12	6	4	8.5 ± 2.7
2007			
06.01	12	14	19.3 ± 5.2

Table 5.4 Pearson's rank correlation coefficients for *Bactrocera invadens* density and % infestation on fruits on the ground and tree with trap catches

	B. inv/kg ground fruit	*B. inv*/kg tree fruit	*B. inv*/trap
B. inv/kg ground fruit	1		
P-value	*		
B. inv/kg tree fruit	0.5270	1	
P-value	0.0001	*	
B. inv trap	0.1880	0.1980	1
P-value	0.2723	0.2472	*

5.5 Discussion

The results of this study demonstrate a clear and distinct seasonal pattern in population fluctuations of mango infesting fruit flies at Nguruman. The population of the two most important fruit fly pests of mango, *B. invadens* and *C. cosyra* was low from March to August, increasing from September and peaking during November to December. Adult activity declined at the end of January, but did not cease completely during the year. The year round availability of other known hosts such as guava, marula plum and tropical almond in the study area may have also contributed to the recorded peaks. The seasonal and annual fluctuations were most closely associated with seasonal and annual occurrence, abundance and maturation of mango (Fig. 5.1). In general, host availability and abundance combined with the total biomass of fruits of each host species are among the factors determining the population fluctuations of *Bactrocera* species (Vargas & Carey., 1990; Tora Vueti *et al.*, 1997) as well as other fruit fly species (Harris *et al.*, 1993; Segura *et al.*, 2006), although climatic variables such as temperature and rainfall may play a role (Amice & Sales, 1997). In Tanzania, where the agro climatic conditions are similar to Kenya, Mwatawala *et al.* (2006a) showed that *B. invadens* was permanently present at low and mid-altitudes, with peak periods coinciding with the fruiting season of mango and guava. Similar studies in Benin showed an increase in population of *B. invadens* which is directly linked to the ripening of different mango cultivars (Vayssiéres *et al.*, 2005). Previous results from host range studies demonstrated that several cultivated and wild plants can harbour high infestation of *B. invadens* (Rwomushana *et al.*, 2008), which are capable of sustaining off season fruit fly populations.

Bactrocera invadens was capable of all year round breeding, which suggests that a sufficient reproductive base in terms of alternative host plants (Fig 5.1) that were found within and in proximity to the orchard which were also fruiting erratically contributed to bridging the gap in the absence of mango. In addition, adults of this pest are known to have a life expectancy of up to 75 days (Ekesi *et al.*, 2006) and given that weather conditions in this area are not highly variable, and this pest is not known to diapause, it was estimated that *B. invadens* can complete its life cycle between 3-4 weeks, potentially resulting in between 10-12 generations per year. This may partly explain the persistence of this pest in the mango agroecosystems. In addition to the plants listed in Fig 5.1, other alternative host plants that may have played a critical role in sustaining populations of *B. invadens* in the absence of mango fruits in the study location includes tomato, banana and wild *Annona* species, which all fruit outside the main mango season (Rwomushana *et al.*, 2008).

The abundance of *C. cosyra* was surpassed by that of *B. invadens* both from the trap catches and fruit collections throughout the entire study period. *Ceratitis cosyra* has previously been the primary pest of mango in Kenya. For example, damage to mango by *C. cosyra* was estimated at 60 to 70% in 1998 at the same experimental location (Lux *et al.*, 1999). During the 2003 mango fruiting season, 82% of the flies emerging from mango at Nguruman was *C. cosyra* and 18% was *B. invadens* (Ekesi *et al.*, unpublished data). However, in 2004, 23% of the flies that emerged from mango fruit was found to be *C. cosyra* and 76% was *B. invadens* (Ekesi *et al.* unpublished data). By 2005, 92% of the fruit flies emerging from mango were *B. invadens* (Ekesi *et al.*, 2006). In the current

study, the total tephritids recovered was between 34-35% in mango fruits and *C. cosyra* accounted for 16.8% compared to 83.1% by *B. invadens*. The relative abundance index of *B. invadens* from fruits in majority of the sampling dates accounted for close to 1.00 while that for *C. cosyra* fell to as low as 0.00 in January samples and never exceeded 0.30 while infestation by *C. capitata* was not significant. Trap catches with protein bait during and outside the fruiting period also consistently showed a predominance of *B. invadens* to *C. cosyra* with up to 62 times more *B. invadens* than the native pest. The current data supports the observations of Ekesi *et al.* (2006) in affirming that indeed *C. cosyra* has been displaced by *B. invadens* in mango orchards at Nguruman. The observed dominance of *B. invadens* relative to *C. cosyra* has also been reported by other authors outside of Kenya. A study by Mwatawala *et al.* (2006b) in Tanzania and Vayssières *et al.* (2005) in Benin revealed that *B. invadens* was higher in abundance compared to native fruit flies. Because these two species principally occupy the same ecological niche, it is evident that *B. invadens* is competitively displacing *C. cosyra* in mango agroecosystems in Kenya. Laboratory interspecific interaction studies for both species revealed a decline in adult numbers of *C. cosyra* when they co-infest mango fruits (Section 7). Duyck *et al.* (2004) reviewed several cases where polyphagous *Bactrocera* species had been introduced into an area already occupied by *Ceratitis* species and concluded that interspecific competition resulted in the decrease in number and niche shift of the pre-established *Ceratitis* species.

Previous studies in Kenya indicated that *C. capitata* does not infest mango fruits under field conditions (White & Elson-Harris, 1992; Mukiama & Muraya, 1994; Copeland *et*

al., 2002; De Meyer *et al.*, 2002) although the pest is normally recovered from the protein baited traps deployed in mango orchards (Section 6). These results document for the first time field infestation of mango fruits by *C. capitata* at Nguruman although at relatively low density. In laboratory interspecific competition studies between *B. invadens* and *C. capitata,* the *Ceratitis* species was always a better competitor under co-infestation of mango (Section 7.0). These laboratory results, however, did not translate into field infestation data because *B. invadens* was generally far much abundant than *C. capitata* throughout the experimental period. Although the interaction between *C. cosyra* and *C. capitata* has not been documented, it is probable that with the rapid displacement of *C. cosyra* by *B. invadens*, *C. capitata* is discovering a niche on mango and this should warrant close attention.

Apart from the late season data, it was observed that significantly more *B. invadens* emerged from fruits on the ground compared with fruits that were sampled directly from the tree, and higher adult catches were evident when plenty of fruits were lying on the ground. The density of *B. invadens* found in ground fruits also correlated with that from tree fruits, indicating that adult fly density developed and emerged mostly from fruits on the ground. Tephritid insects of the genus *Bactrocera* have an unusually high ability to move long distances in search of food and oviposition sites, but when resources are adequate, the flies may become resident in the habitat (Fletcher, 1987). However, in this study, there was no correlation between the number of flies in the traps with the density of flies in tree fruits or with fruit on the ground. It is, therefore, not very clear if *B. invadens* aggregates in habitats where the fly has adequate food sources as evidenced

with other tephritids (Harris & Lee, 1986, 1987; Katsoyannos *et al.*, 1998; Papadopoulos *et al.*, 2001). On the contrary in Hawaii, Liquido *et al* (1991) found that melon fly density in ground fruits was correlated with the density of flies in tree fruits and adult density in the field which was related to availability of food and oviposition sites. The variation in the results could be attributed to immigrant populations from important alternative host plants such as *Terminalia catappa* L. (Rwomushana *et al.*, 2008) that surrounded the experimental area but additional studies on the dispersal ability of *B. invadens* requires attention.

Breeding of fruit flies in unwanted fruit in orchards is undoubtedly the biggest source of damaging populations (Jang & Light, 1991), and this could explain in part the high levels of adult populations in the mango orchards studied. Considering the high levels of infestation in fruit samples collected from the ground, orchard sanitation is strongly recommended as a component of Integrated Pest Management (IPM) against *B. invadens* in mango orchards. This approach has been used for other fruit fly species. For example, field sanitation is recommended as a primary component for melon fly management (Dhillon *et al.*, 2005). In Surinam, papayas are exported from the country under a quarantine system that mandates field sanitation (van Sauers-Muller, 1993). The oriental fruit fly eradication program in Mauritius included a fruit sanitation component with some degree of success (Seewooruthun *et. al.*, 2000).

The results of this study have direct implications for the management of the fruit fly species on mango. First, this study suggests that the build up of *B. invadens* in mango

orchards begins in October at mango maturity and peak abundance occurs during December. Control strategies based on soil inoculation with entomopathogenic fungi (Ekesi *et al.*, 2007b) and baiting technique should be initiated preferably at the onset of fruiting (September/October). Secondly, it is evident that some refuge host plants exist that harbour small populations of fruit flies during the mango season; unfortunately such alternative host plants are usually neglected by traditional pest control efforts. It is recommended that bait sprays should target such alternative host plants in proximity to the orchards as this may minimize off season and early season build up of fly population. Alternatively, destruction of non commercial host plants within the proximity of the orchard may minimize off season breeding of the fly when mango is not fruiting. The data presented provides baseline population estimates of *B. invadens* which should be useful for evaluating the effectiveness of a classical biological control effort using *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) that is underway at the study location.

CHAPTER SIX

6 FIELD EVALUATION OF FOOD ATTRACTANTS AND TRAPS FOR *BACTROCERA INVADENS* (DIPTERA: TEPHRITIDAE) IN KENYA

6.1 Introduction

The invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) Drew, Tsuruta & White, is a notorious insect pest that was first reported in Kenya in 2003 (Lux *et al.*, 2003a). It has since become established in several parts of sub-Saharan Africa and recorded from 24 countries including the Comoros Island (Mwatawala *et al.*, 2006a, Vayssierès *et al.*, 2005; Ekesi *et al.*, 2006). The pest is believed to be native to Sri Lanka (Drew *et al.*, 2005) and recently recorded from Bhutan (De Meyer *et al.*, in press). This insect is an emerging polyphagous pest infesting both cultivated fruits particularly mango (Mwatawala *et al.*, 2004; Ekesi *et al.*, 2006) and a range of other wild fruits (Drew *et al.*, 2005; Vayssierès *et al.*, 2005; Mwatawala *et al.*, 2006b; Rwomushana *et al.*, 2008). *Bactrocera invadens* has been described as a quarantine pest of major importance (French, 2005) and represents a new major threat to commercial horticulture in tropical regions of Africa and beyond and jeopardises the lucrative trade in fresh fruits.

Bactrocera invadens is now present in most mango production areas across Kenya (Lux *et al.*, 2003a; Ekesi *et al.*, 2006). Infestation levels of up to 97.2 flies/kg of mango fruit have been reported from some parts of the country (Ekesi *et al.*, 2006). The development of suitable management strategies for this pest is thus paramount to restrict fruit losses. Because indigenous forests with fruiting trees, homestead gardens and roadside fruit trees

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suitable for the development of *B. invadens* are scattered across the fragmented agriculture in Africa, an eradication strategy is not an option for this devastating pest. Although males of *B. invadens* are strongly attracted to methyl eugenol (Lux *et al.*, 2003a, Mwatawala *et al.*, 2006ab), use of male annihilation technique (MAT) may not have a major impact on damaging female populations of the pest unless male density can be decreased by 99% (Steiner *et al.*, 1965b). The application of a generic integrated pest management (IPM) package that exploits behavioural control through targeting females that reside in or migrate to orchards using lure and kill techniques in the form of bait sprays and/or trapping systems represents a more appropriate management strategy. In the bait application technique, adults are attracted to and killed by spots of protein bait mixed with insecticidal toxicant (Roessler, 1989). Trapping devices combine visual and olfactory cues to capture tephritid fruit fly pests (Epsky *et al.*, 1995; Epsky & Heath, 1998). The bait spray and trapping strategy against *B. invadens*, however, can best be utilised when suitable attractants and traps for this pest are known.

Female fruit flies require protein for full ovarian development and egg production (Christenson & Foote, 1960; Fletcher, 1987), thus volatile chemicals released from protein sources can provide food cues to foragers. Therefore, to exploit this behaviour, a range of food and/or host odours, have been developed in the recent past for the management of a range of tephritid fruit flies particularly species of *Anastrepha* (Epsky *et al.*, 1993; Robacker *et al.*, 1996), *Bactrocera* (Cornelius *et al.*, 2000ab; Broumas *et al.*, 2002; Barry *et al.*, 2006) and *Ceratitis* (Katsoyannos *et al.*, 1999ab; Miranda *et al.*, 2001; Broughton & De Lima, 2002) with varying degrees of success. Some of these food-based

attractants such as hydrolysed proteins have been shown to be equally attractive to males (Heath *et al.*, 1994; Fabre *et al.*, 2003). Related studies have also evaluated a range of visual traps with or without adhesive in order to establish the most efficient trap/attractant combination (Gazit *et al.*, 1998; Katsoyannos *et al.*, 2000; Robacker & Czokajlo, 2005; Ros *et al.*, 2005). A strategy utilising traps with new long-lasting food-lure dispensers (Biolure) consisting of ammonium acetate, putrescine, and trimethylamine was recently evaluated in Kenya and Tanzania with moderate attractiveness to different species of fruit flies including *B. invadens* (Mwatawala *et al.*, 2006ab; Ekesi *et al.*, unpublished;). However, the cost of application of this technique is still well beyond the reach of African farmers. Liquid protein baits have the most promise for application in a bait application strategy. In Tanzania, a liquid protein bait was found to be more attractive to *B. invadens* than the Biolures (Mwatawala *et al.*, 2006b). The efficacy of widely used liquid commercial protein baits and visual traps for *B. invadens* is, however, not known. A new locally produced yeast attractant has also shown promising results from laboratory studies (Ekesi *et al.*, unpublished data). However, its potency in the field compared to the commercial lures is unknown.

This study evaluated three trap types and four food-based attractants that could be exploited in the management of *B. invadens* in mango orchards. The identification of potent attractants for *B. invadens* for potential use in bait stations or application in a bait spray program would permit production of fruits that are free from infestation, safeguard current fruit fly free zones and promote international trade in mango as well as other commercial fruits normally attacked by this pest. Importantly, the threat of introduction

of this pest into new areas of the world requires identification of potent lures for use in detection, monitoring and suppression programs.

6.2 Materials and methods

6.2.1 Site description

The study was conducted at Nguruman division located in Kajiado district, Rift Valley Province of Kenya. This is a prime agricultural area producing a wide range of fruits for local and export markets. The field trials were carried out in an unmanaged mango orchard for eight weeks from 27 October to 15 December 2006 and repeated during the subsequent mango season from 30 November 2007 to 24 January 2008. The orchard is approximately 5 ha located at latitude 01°48.8' S, longitude 036°03.1' E and altitude of 760 meters above sea level (masl). The orchard is approximately 6.5ha in size and predominantly covered with mango trees of "apple", "dodo" and "boribo" varieties. However, a few other host plants of *B. invadens* including citrus, guava, custard apple and tropical almond can be found within and in proximity to the orchard. Means of monthly prevailing weather conditions during the study period are given in table 6.1.

Table 6.1 Mean monthly temperature, rainfall and humidity at Nguruman during the experimental periods of 2006 and 2007/2008

	2006			*2007/2008*		
Parameter	Oct	Nov	Dec	Nov	Dec	Jan
Temp (°C)	29.1	27.6	31.4	26.8	25.8	28.4
*Rainfall (mm)	9.3	46.3	24.7	130.5	199.9	38.8
Humidity (%)	62.8	59.6	61.8	69.3	72.1	68.4

*Season 2007/2008 had more than average rainfall

6.2.2 Traps and attractants

The following three types of visual traps were tested: (1) the Multilure® trap (Better World, USA) (Plate 6.1), a McPhail type trap which is a plastic and cylindrical shaped trap with two interlocking parts. The upper part (13 cm high, 13 cm upper diameter, and 16 cm base diameter) is transparent with a yellow bottom part (7 cm high, 17 cm upper diameter, and 12 cm base diameter). At the center of the bottom half is a funnel-like orifice (5.5 cm diameter) that allowed the exit of the attractive odour and serves as an entrance for the flies; (2) the Easy trap® (Ros *et al.*, 2005) (Plate 6.2), is a plastic interlocking rectangular shaped trap (14 cm length, 9.5 cm wide and 5 cm high) that consists of a transparent side and a yellow coloured side. Near the top half on both ends are orifices (1 cm diameter) through which the bait is poured and serves as an entrance for the flies and; (3) a locally crafted Lynfield trap (Plate 6.3) which is a cylindrical transparent plastic trap (10 cm diameter and 10cm high) with four holes (2.5 cm diameter) evenly spaced at the top half of the trap for entry of the flies.

The following attractants were tested: (1) An aqueous solution of 9% protein Nulure (Miller Chemical and Fertilizer, Hanover, PA) (vol: vol) with 3% sodium tetraborate decahydrate (borax) (wt: vol) as preservative. Addition of borax to the protein bait reduces decomposition of the trapped flies (Lopez & Beceril, 1967). Because observation in another study showed that 2% Nulure with 1% borax (wt: vol) was equally attractive, the 9% Nulure used in the first trials was replaced with the above concentration during the second trial; (2) An aqueous solution of 10% Corn steepwater (Corn Products, Summit Argo, IL), an enzymatically hydrolyzed protein from corn processing, plus 1%

borax. (3) Torula yeast pellets (ISCA Technologies, Riverside, CA)-borax (4:5 torula yeast: borax), three pellets dissolved in 300 ml water to make an aqueous bait solution; and (4) An aqueous solution of 8% fresh waste brewer's yeast plus 9% molasses and 1% borax hereafter referred to as yeast product. The traps were baited with 250 ml solution of the food-attractants. All the lures were tested in the 4 traps resulting in a total of 12 treatments. A randomized complete block design was used in all experiments, with three replications for each trap-lure combinations in each of the three blocks.

The traps were hung in the middle exterior of the tree canopy in grids at about 1.5-2 m above the ground and at a distance of 15 m apart. Trap hangers were coated with insect adhesive (Tanglefoot, Grand Rapids, MI) to prevent ants from entering the traps and feeding on the catches. The traps were serviced (ie. adults in the traps removed) weekly and captured insects were transferred into vials containing 70% alcohol and taken to the laboratory for identification. Aqueous solutions in the traps were renewed after servicing of each trap and the traps were rinsed with tap water prior to bait renewal. After each check, the positions of the traps within blocks were re-randomized to minimise the influence of individual trapping location.

6.3 Data analyses

To compare the relative effectiveness of the products (traps and attractants), for each season, the total number of flies caught in each trap/attractant combination during each week was divided by seven days to convert the data to numbers of male, female and total (males+females) captured per day. The trap data were analysed with two way analysis of

variance (ANOVA) with first order interaction using PROC GLM (SAS Institute, 2001). Data were log transformed prior to analysis (ln [x + 1]) to meet the assumption of homogeneity, however untransformed means are presented in the results. When the F value was significant, means were separated by the Students Newmans Keuls multiple range test at 95% confidence level.

6.4 Results

During the experimental period, the weather was characterised by moderate temperatures (max 38^0C, min 20^0C) and humidity (60 - 70%). In both seasons, significant differences were observed for the trap types and attractants in the male, female and total captures of *B. invadens* (Appendix 2). Significant interactions were also observed for trap type, attractant and type x attractant for the male, female and total catches of *B. invadens* (Appendix 2). Trap efficiency was in the order Multilure >Lynfield >Easy trap (F = 29.79, df = 2, 3, P = 0.0001; F = 37.22, df = 2, 3, P = 0.0001) regardless of the attractant used, in 2006 and 2007/2008 seasons, respectively.

During the 2006 trapping season, *B. invadens* male catches varied significantly among the different trap/attractant combinations (F = 15.34, df = 11, 24, P = 0.0001) (Table 6.2). Catches were highest with the multilure trap/torula yeast [13.63 flies/trap/day (FTD)] followed by multilure trap/nulure (9.86 FTD) both of which were not significantly different from one another (Table 6.2). The least number of males was captured with easy trap/yeast product (0.76 FTD) and lynfield trap/cornsteep (0.85 FTD).

Plate 6.1: Multilure trap

Plate 6.2: Easy trap

Plate 6.3: Lynfield type trap

Corn steepwater and the yeast product did not differ significantly in attractiveness to male flies irrespective of the trap used (Table 6.2). Generally, fewer males were captured with the easy trap compared to the multilure or lynfield traps when the same attractants were used.

Female catches during this season were highest with multilure trap/torula yeast (16.67 FTD) which did not differ significantly with catches from multilure trap/nulure (13.11 FTD) (Table 6.2). The least number of females was captured with lynfield trap/cornsteep (0.73 FTD) and easy trap with the yeast product and corn steepwater (0.68 and 0.60 FTD respectively). All easy trap/attractant combinations captured lower number of female flies when compared with the respective multilure and lynfield traps and attractant combinations (Table 6.2).

The highest total *B. invadens* captures (males + females) during the 2006 trapping season were from multilure trap/torula yeast combination (30.3 FTD) (Table 6.2). Nulure similarly attracted high number of *B. invadens* flies with the multilure trap (22.97 FTD) with 9% concentration of the attractant. The least number of adults was captured with the yeast product and corn steepwater in the lynfield and easy traps (Table 6.2). The lynfield trap/torula yeast combination captured significantly more flies (16.49 FTD) than easy trap/torula yeast (9.13 FTD) compared to the other attractants using these two trap types (Table 6.2).

Table 6.2 Comparison of mean numbers (± SE) of *Bactrocera invadens* males, females, total flies and % females in multilure, lynfield and easy traps over an 8 week field trial during Oct-Dec 2006

		Mean no. fruit flies/trap/day (± SE)			
Trap type	Lure	♂♂	♀♀	Total flies	(♀♀ %)
Multilure	Yeast product	2.39 ± 0.81d	2.20 ± 0.63cde	4.59 ± 1.43cd	48.03 ± 4.56 ab
	Corn steepwater	4.96 ± 2.04cd	3.69 ± 1.30cd	8.65 ± 3.34c	42.66 ± 4.32 ab
	†Nulure	9.86 ± 1.79ab	13.11 ± 1.87ab	22.97 ± 3.56ab	57.06 ± 3.47 a
	Torula yeast	13.63 ± 2.73a	16.67 ± 2.20a	30.30 ± 4.84a	55.02 ± 1.73 a
Lynfield	Yeast product	1.11 ± 0.51d	1.26 ± 0.44de	2.37 ± 0.91d	53.39 ± 7.13 a
	Corn steepwater	0.85 ± 0.37d	0.73 ± 0.27e	1.58 ± 0.64d	46.20 ± 6.64 ab
	Nulure	2.46 ± 0.48cd	3.20 ± 0.53c	5.66 ± 1.00c	56.44 ± 3.69 a
	Torula yeast	6.90 ± 1.54b	9.58 ± 1.66b	16.48 ± 3.15b	58.10 ± 2.12 a
Easy	Yeast product	0.76 ± 0.24d	0.68 ± 0.20e	1.44 ± 0.44d	47.55 ± 7.10 ab
	Corn steepwater	1.40 ± 0.53cd	0.60 ± 0.22e	2.00 ± 0.74d	30.15 ± 6.32 b
	Nulure	2.42 ± 0.61cd	3.26 ± 0.59c	5.68 ± 1.16c	57.39 ± 4.92 a
	Torula yeast	3.70 ± 0.86c	5.43 ± 1.18c	9.13 ± 2.00c	59.47 ± 4.81 a
	F-value	15.34	27.06	21.00	
	(df =11,24; $P = 0.0001$)				

†9% NuLure +3% Borax

Means followed by the same letter in the same column are not significantly different [$P < 0.05$, Student Newmans-Keuls test on ln (x + 1) transformed data].

Table 6.3 Comparison of mean number (± SE) of *Bactrocera invadens* males, females, total flies and % females in multilure, lynfield and easy traps over an 8 week field trial during Nov 2007-Jan 2008

		Mean no. fruit flies/trap/day (± SE)			
Trap type	Lure	♂♂	♀♀	Total flies	(♀♀ %)
Multilure	Yeast product	0.77± 0.29c	0.80 ± 0.22c	1.57 ± 0.51d	50.96 ± 6.30 a
	Corn steepwater	1.10 ± 0.34c	1.35 ± 0.36c	2.45 ± 0.70d	55.10 ± 6.12 a
	†Nulure	4.54 ± 0.97b	6.00 ± 1.11b	10.54 ± 2.06b	56.93 ± 2.40 a
	Torula yeast	8.39 ± 1.44a	11.31 ± 1.46a	19.70 ± 2.84a	57.41 ± 3.08 a
Lynfield	Yeast product	0.57 ± 0.11c	0.73 ± 0.14c	1.30 ± 0.24d	56.15 ± 4.99 a
	Corn steepwater	0.33 ± 0.11c	0.24 ± 0.07c	0.57 ± 0.17d	42.11 ± 7.74 a
	Nulure	0.79 ± 0.22c	0.99 ± 0.19c	1.78 ± 0.40d	55.62 ± 5.58 a
	Torula yeast	3.06 ± 0.43b	4.73 ± 0.48b	7.79 ± 0.86bc	60.72 ± 2.14 a
Easy	Yeast product	0.47 ± 0.12c	0.55 ± 0.10c	1.02 ± 0.21d	53.92 ± 6.02 a
	Corn steepwater	0.16 ± 0.07c	0.20 ± 0.06c	0.36 ± 0.13d	55.56 ± 9.29 a
	Nulure	0.53 ± 0.18c	0.61 ± 0.21c	1.14 ± 0.37d	53.51 ± 6.90a
	Torula yeast	3.15 ± 0.49b	3.98 ± 0.51b	7.13 ± 0.96c	55.82 ± 2.80 a
	F-value	30.26	32.69	50.42	
	(df =11,24; $P = 0.0001$)				

†2% NuLure +1% Borax, Means followed by the same letter in the same column are not significantly different [$P < 0.05$, Student Newman-Keuls test on ln (x + 1) transformed data].

Table 6.4 Number of other tephritid fruit flies captured over the entire fruiting season with the tested attractants

Season	*Ceratitis capitata*	*Ceratitis cosyra*	*Ceratitis fasciventris*	*Bactrocera cucurbitae*	*Dacus* sp.
2006					
Yeast product	13 (15.4)*	82 (56.1)	9 (11.1)	26 (65.4)	1 (100)
Cornsteep	109 (58.7)	424 (68.4)	24 (62.5)	30 (60)	3 (100)
Nulure	210 (54.8)	1003 (68)	31 (58.1)	4 (75)	0 (0)
Torula yeast	281 (48)	1339 (63.5)	30 (60)	1 (100)	5 (60)
2007/2008					
Yeast product	0 (0)	145 (74.5)	30 (40)	2 (50)	1 (100)
Cornsteep	0 (0)	30 (70)	9 (22.2)	0 (0)	0 (0)
Nulure	2 (100)	444 (57.2)	11 (36.4)	26 (38.5)	4 (25)
Torula yeast	10 (50)	740 (64.7)	108 (45.4)	46 (34.8)	2 (50)

*Percentage of females in parentheses

There were significant differences in percentage of females between the treatments during the trapping season of 2006 ($F = 3.47$, df = 11, 24, $P = 0.0001$) (Table 6.2). The nulure and torula yeast attractants, irrespective of trap type, attracted higher percentage of *B. invadens* female flies compared to the males (Table 6.2). An average of 56-57% female flies was captured with nulure while 55-59% females were captured with torula yeast with the three traps. Easy trap/corn steepwater captured only 30% females (Table 6.2).

During the 2007/2008 season, the trapping trends were similar to that of the 2006 season. The highest number of males was captured with multilure trap/torula yeast (8.39 FTD) which differed significantly from the other trap/attractant combinations (Table 6.3). Similar number of males was captured with multilure trap/nulure (4.54 FTD) and torula yeast with the lynfield (3.06 FTD) and easy (3.15 FTD) traps. Corn steepwater and the yeast product captured the least number of males (0.16-1.10 and 0.47-0.77 FTD respectively) irrespective of the trap used (Table 6.3).

Female catches during this season were highest with multilure trap/torula yeast (11.31 FTD) which differed significantly from the other trap/attractant combinations (Table 6.3). Similarly, high number of females was also captured with multilure trap/nulure (6.0 FTD) while the least numbers were captured with corn steepwater and yeast product irrespective of the trap type used. The easy trap captured lower number of female flies when compared with the respective multilure and lynfield traps baited with the four attractants (Table 6.3).

Higher number of male and female flies (19.7 FTD) was captured with multilure trap/torula yeast followed by multilure trap/nulure (10.54 FTD) (Table 6.3). In general, fewer flies were captured with the yeast product and corn steepwater irrespective of the trap used. Reducing the concentration of nulure from 9% to 2% did not result in a substantial difference in captures of *B. invadens* relative to torula yeast. However, using 9% nulure with multilure and easy trap captured proportionately fewer flies compared to 2% nulure with the same trap types. But with the lynfield trap, much higher efficacy was observed with the higher concentration of nulure.

There was no significant difference in percentage of females between the three traps and the four attractants during the fruiting season of 2007/2008 ($F = 1.82$, df =11, $P = 0.052$) (Table 6.3).

A few of other tephritid pests were captured in the study. The mango fruit fly *C. cosyra* (Walker) was the next most abundant tephritid species captured followed by; *Ceratitis capitata* (Wiedemann) > *Ceratitis fasciventris* (Bezzi) > *Bactrocera cucurbitae* (Coquillett) > *Dacus* spp. (Table 6.4). The most abundant non-target insects captured were diptera particularly *Drosophila* sp and ants (Table 6.5). These non-targets have been commonly reported in McPhail traps baited with liquid protein solution (Steyskal, 1977; Thomas, 2003). The 9% Nulure generally captured more non-target insects than the 2% Nulure or the other attractants tested.

Table 6.5 Number of non-targeted insects captured over the fruiting seasons of 2006 and 2007/2008 with the tested attractants

	Hymenoptera	Diptera	Coleoptera	Spiders	Ants	Lepidoptera	Dictyoptera	Lacewigs	Orthoptera	Others
2006										
Yeast product	4	900	1	16	576	8	1	0	0	2
Cornsteep	6	981	0	24	396	25	2	0	0	5
Nulure	3	1533	0	6	375	26	6	1	0	3
Torula yeast	1	1113	0	5	216	29	4	0	0	1
2007/2008										
Yeast product	5	333	2	12	9	54	3	0	0	0
Cornsteep	1	164	1	9	32	15	1	0	1	0
Nulure	3	889	1	33	30	17	0	1	0	1
Torula yeast	4	1289	0	45	29	39	0	2	0	1

6.5 Discussion

The results from this study confirm that *B. invadens* responds to a range of liquid protein hydrolysates but to varying degrees. Except for the yeast product, all the attractants evaluated in this study have previously been used to attract tephritid fruit flies with varying levels of success. For instance, nulure is the standard tephritid protein bait widely used to monitor *C. capitata*, oriental fly *Bactrocera dorsalis* (Hendel) and the melon fly *B. cucurbitae* and has been applied successfully in bait sprays as a field suppression attractant (Wakabayashi & Cunningham 1991; Epsky *et al.*, 1993; Miranda *et al.*, 2001; Fabre *et al.*, 2003). Corn steepwater has also been used against the Caribbean fruit fly *Anastrepha suspensa* (Loew) (Epsky *et al.*, 1994), melon fly (Fabre *et al.*, 2003) and oriental fly (Barry *et al.*, 2006). Hydrolysed torula yeast has been shown to be superior to other attractants including nulure for attracting *Anastrepha* sp. (Lopez *et al.*, 1971; Epsky *et al.*, 1993; Heath *et al.*, 1996; Thomas *et al.*, 2001; Holler *et al.*, 2006) and *Bactrocera oleae* (Gmelin) (Burrack *et al.*, 2008). This study demonstrates the effectiveness of exploiting the various food baits in attracting *B. invadens* in mango orchards.

There were differences in efficacy of the traps in capturing *B. invadens* in both seasons. Trap and lure performance for the best four combinations ranked as follows; Multilure + Torula yeast > Multilure + Nulure > Lynfield + Torula yeast > Easy trap + Torula yeast. These four best combinations were also marginally superior in attracting more females than males. Multilure trap was the best trap in the trial and in absolute terms caught the most females. A wide range of traps have been used elsewhere for capturing tephritid fruit flies with varying efficiency. For instance, Katsoyannos (1999b) and Epsky *et al.*

(1999) used multilure traps baited with food-based attractants for capture of *C. capitata*. The multilure trap captured more *B. oleae* flies than did the other four trap and lure combinations tested (Burrack *et al.*, 2008). Robacker & Czokajlo (2005) showed that the multilure trap baited with Biolures was the most efficient in monitoring the Mexican fruit fly, *Anastrepha ludens* (Loew). The multilure trap used in this study is very similar in design to the McPhail which is commonly used for monitoring several fruit fly species (Papadopoulos *et al.*, 2001; Thomas *et al.*, 2001; Broughton & De Lima, 2002; Castrejon-Gomez *et al.*, 2004). Perhaps not surprising is that it outperformed the other traps in capturing *B. invadens* in the current trial.

Visual cues can play important role in fruit fly attraction and most fruit fly species respond positively to colour, especially to yellow (Prokopy, 1968; 1972; Economopoulos, 1989). This characteristic may have contributed to the better performance of the multilure trap over the other traps although the yellow attribute did not translate to better performance of the easy trap. The trap architecture of the easy trap, which is rectangular with just 2 holes (1 cm diameter), may have had a confounding effect on the performance of the trap. The modified lynfield trap was not as efficient probably because of its transparency and the positioning of entrance holes which probably allowed for escape of some trapped flies. Suffice to say that the attractants used obviously played a significant role in the performance of the traps and it is likely that the catches may be increased with a more powerful attractant.

Torula yeast was consistently the best performing attractant irrespective of trap type. This

attractant is widely used for monitoring a wide range of tephritid fruit flies. There is not much published information on the efficacy of torula yeast for monitoring populations of *Bactrocera* species. However, Malavasi *et al.* (1990) and Epsky *et al.* (1993) showed that more *Anastrepha* fruit flies were attracted to torula yeast than culure or corn protein hydrolysate. Similarly, Thomas *et al.* (2001) showed that torula yeast caught as many *Anastrepha* fruit flies as synthetic Biolures. Burrack *et al.* (2008) showed that torula yeast was more attractive to *B. oleae* than ammonia lures. This study demonstrates that torula yeast can be a powerful attractant for monitoring *B. invadens* especially when used with multilure traps. Although not widely used as a suppression tool, the results demonstrate that this attractant can also be employed in baiting stations for field suppression of *B. invadens* on mango. However, torula yeast may not be suitable in sterile insect technique programs for *B. invadens* as it was observed to remove significant proportions of the male populations.

Nulure at the range of 9% is the recommended concentration for the monitoring of several species of fruit flies (IAEA, 2003). However, by reducing the concentration to 2% nulure, it caught 1.83 times more *B. invadens* than 9% nulure. Fabre *et al.* (2003) showed that 2% nulure caught almost equal number of *B. cucurbitae* like 10%. At concentrations between 0.5% and 2%, there was a high rise in catches. However, between 5% and 10% concentration, the incremental catches were minimal and not significant (Fabre *et al.*, 2003). The observed efficacy of lower concentration nulure for *B. invadens* should reduce rates of application of the food-bait. Fabre *et al.* (2003) also showed that 10% corn steepwater was more or equally effective as the standard nulure for capturing *B.*

cucurbitae although it was not quite effective at extremely low concentrations of 0.5%. The reason for the poor efficacy of corn steepwater in attracting *B. invadens* in the current study is unclear but highlights the need for testing and identification of species specific attractive substances in baits used for fruit fly management.

Although adult *B. invadens* responded in large numbers to the waste brewer's yeast in the laboratory (Ekesi *et al.*, unpublished), the product was not as effective in the field when compared to the other food attractants. The yeast was tested in its crude form and probably requires digestion with appropriate enzyme (e.g. papain) to release the necessary amines that are required to enhance attractivity. Lloyd & Drew (1996) showed that waste yeast slurry that had not undergone proteolysis was poorly attractive to *Bactrocera* (Froggatt). Further processing through pasteurizing and heat concentration, proteolysis by addition of papain and pH adjustment may increase the efficacy of the Kenyan yeast product. Indeed using this procedure, highly attractive bait was developed that is effective against different species of *Bactrocera* in the South Pacific (Lloyd & Drew, 1996). Similarly in Mauritius, Gopaul & Price (1999) showed that locally produced protein autolysate baits from brewers' wastes that had been digested with papain were generally as attractive for *Bactrocera zonata* (Saunders) in McPhail traps as the imported protein hydrolysate formulations.

This study has identified important food baits that could be utilised for detection, monitoring and suppression of *B. invadens*. Although torula yeast is normally not used in bait sprays for fruit fly suppression, its high efficacy in trapping *B. invadens* suggests it

could be a very useful tool in detection and monitoring of the insect both in countries that the insect has not invaded and in cultivated orchards to guide management decisions. The attractant also holds promise as an important food bait for use in baiting stations. Nulure on the other hand has been successfully used in bait sprays to control several species of fruit flies within the *Anastrepha*, *Bactrocera* and *Ceratitis* genera (Mohammad & Alianazee, 1989; Burns *et al.*, 2001; Yee, 2007). The efficacy of the product observed in this study implies that the attractant could be exploited in suppression programs for *B. invadens*. Compared to torula yeast, nulure is generally less expensive but all these products have to be imported to Africa, hence the need to pursue the development of a local food bait from waste brewer's yeast. Although the lynfield trap was not as effective as the multilure trap, this trap can be easily crafted by local artisans, and could be an important tool for monitoring and decision making in regard to timing and frequency of bait spray. The development of highly effective and selective trapping systems that targets female fruit flies provides a mechanism for behavioural control that adds to the list of biologically-based IPM technologies to suppress fruit flies. Further studies, however, are needed to identify the optimum trap density and deployment pattern to improve the efficacy.

CHAPTER SEVEN

7 INTERSPECIFIC COMPETITION BETWEEN THE MANGO INFESTING FRUIT FLIES *BACTROCERA INVADENS* WITH *CERATITIS CAPITATA* AND *CERATITIS COSYRA* (DIPTERA: TEPHRITIDAE) AT CONSTANT TEMPERATURE

7.1 Introduction

Globalization of trade in fresh fruits and increased travel has intensified the risk of inadvertent spread of alien invasive species, a leading anthropogenic disturbance with far reaching implications (Sandlund *et al.*, 1999). Invasive species are notorious for altering successional patterns, mutualistic relationships, community dynamics, ecosystem function and resource distribution (Mooney & Cleland, 2001). In addition, invasive species that cause extinction of native species will ultimately reduce local and global species diversity (Vitousek *et al.*, 1996; Collins *et al.*, 2002). Among arthropods, alien invasives have also been reported to negatively impact native species through ecological interactions such as competition (Denno *et al.*, 1995; Duyck *et al.*, 2006) that occur through many different processes that are broadly categorized as exploitative and interference (Begon *et al.*, 1986). In exploitative competition, individuals of one species acquire resources to a greater extent than individuals of another species while in the latter, members of one species limit or deny individuals of another species access to resources (Reitz & Trumble, 2002).

Among the Tephritidae, the dacine fruit flies are well documented invaders and rank high

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on quarantine lists worldwide (Clarke *et al.*, 2005). Through fruit trade, many of these fruit flies have been introduced into various countries with the resultant direct and indirect cost of their introductions running into hundreds of millions of dollars (Duyck *et al.*, 2004a; Follet & Neven, 2006). In Africa, one such invader was detected in 2003 at the Kenyan coast (Lux *et al.*, 2003a) and later described as *Bactrocera invadens* Drew, Tsuruta & White (Drew *et al.*, 2005). The pest is rapidly expanding its geographical range and is now reported from 24 African countries including the Comoros Island (Drew *et al.*, 2005; French, 2005; Vayssiéres *et al.*, 2005; Ekesi *et al.*, 2006; Mwatawala *et al.*, 2006a; Francois-Xavier *et al.*, 2008; Rwomushana *et al.*, 2008). Before the arrival of *B. invadens*, the mango fruit fly *Ceratitis cosyra* (Walker) (Plate 9), an indigenous fruit fly to Africa (White & Elson-Harris, 1992; Mukiama & Muraya, 1994; Lux *et al.*, 2003b) has traditionally been the most important pest of mango *Mangifera indica* L. (Anacardiaceae) in Kenya. Most known rearing records for fruit flies of mango in Kenya have not reported presence of *C. capitata* (Wiedemann) (Plate 10) (Mukiama & Muraya, 1994; Copeland *et al.*, 2002; De Meyer *et al.*, 2002) although it is normally captured from protein baited traps in mango orchards (Section 5.0).

In 2004, a shift in dominance between *C. cosyra* and *B. invadens* was observed in mango orchards at Nguruman, Rift Valley Province of Kenya, just one year after detection of the invasive species in the country and Ekesi *et al.* (2006) in assessing the level of damage of the new invasive species on mango speculated that competitive displacement appeared to be in progress. Similar overturn in abundance has also been noted in Tanzania and Benin between these two species (Vayssières *et al.*, 2005; Mwatawala *et al.*, 2006b). The

mechanisms that trigger competitive displacement are usually very difficult to establish and may be specific to each pair of competing species. *Bactrocera invadens*, *C. capitata* and *C. cosyra* are ecological homologues that compete for the same ecological niche. In the conventional niche theory, the primary determinant of competition is overlap in resources (or niche overlap). Indeed this overlap presents an opportunity for competitive responses (the ability of a species to withstand competition exerted by other species) and competitive effect (the negative effects of a species on other species) (Goldberg & Barton, 1992).

Factors such as superior competitive abilities, resource pre-emption, release from natural enemies, abiotic factors including temperature and anthropogenic disturbances, may permit an invasive species to become dominant. Understanding the interspecific interactions between an invader and resident should lead to better predictive ability and a more effective way of managing the invasive species (Williamson, 1996). This study was designed to assess whether competitive superiority through resource exploitation at different temperatures is the principle mechanism for the dominance of *B. invadens* over *C. cosyra* or the recent emergence of *C. capitata* as a pest of mango in the field. This study, therefore, report results in which manipulative trials were conducted at 4 constant temperatures to detect asymmetrical competition between the larvae of the invasive species *B. invadens* with the native species *C. capitata* and *C. cosyra*.

Plate 7.1: *Ceratitis cosyra*

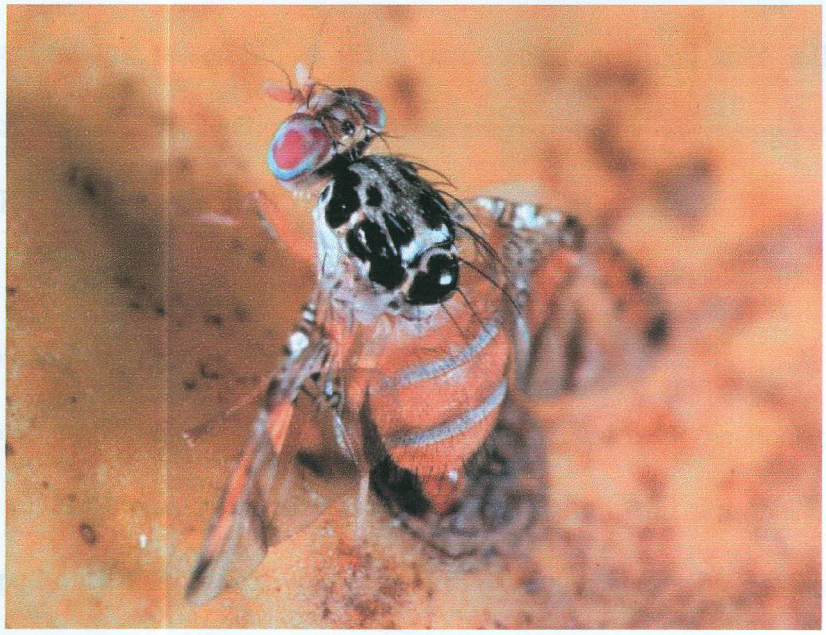

Plate 7.2: *Ceratitis capitata*

7.2 Materials and Methods

7.2.1 Insect material

The initial stock culture of *B. invadens* originated from a natural population of infested mango fruits collected at a local market in Nairobi, Kenya in 2003 and the larvae were subsequently reared on a yeast-carrot-based artificial diet in the laboratory for about 54 generations following the methodology described by Ekesi *et al.* (2007a). Prior to use in the experiments for interspecific competition studies, the insects were transferred back to mango "variety apple" and reared for a minimum of 5 generations. Adult female *C. cosyra* were obtained from a laboratory culture which has been maintained on mango "variety apple" for 108 generations following the methodology of Lux *et al.* (2005). Both fruit fly cultures are rejuvenated every 6 to 12 months by incorporation of wild populations to ensure that the cultures are genetically similar to wild populations. The *C. capitata* adults were obtained from laboratory cultures that have been maintained at *icipe* for several generations reared on a yeast-carrot-based artificial diet following the methodology of Ekesi *et al.* (2007a). The cultures are maintained in rearing rooms at 28 ± 1°C, 50 ± 8% R.H. and photoperiod of L12: D12.

7.2.2 Egg collection

Eggs of *B. invadens*, *C. cosyra* and *C. capitata* were collected from the stock colonies by offering to the mature female flies, ripe mango dome (mango fruit skin that has the seed and pulp scooped out). Each dome was pierced in several places with an entomological pin (38 mm long, 0.3 mm diameter) to facilitate oviposition. The domes were placed over a 9 cm diameter Petri dish lined with moistened filter paper and placed in 30 x 30 x 30

cm Perspex cage having adult flies of either species of fruit fly. Eggs were collected from the underside of the domes within 6 h of oviposition using a moistened fine camel's hair brush then placed on a wet filter paper in a Petri dish and held at ambient temperature to allow for hatch.

7.2.3 Larval competition experiments

The opportunity for interspecific competition largely depends on the frequency of co-infestations and density of larvae within fruits (Barker, 1983). The larval competition experiments were therefore conducted in the laboratory through pairwise combinations of newly emerged *B. invadens* and *C. cosyra* larvae. Shortly after eclosion, 20 newly emerged larvae of each species of fruit fly were collected from the dish and gently introduced with a moistened fine camel's hair brush into each of 20 (control fruit) or 40 holes (20 holes per each fruit fly species) (treated or co-infested fruit). The holes were ~1mm in diameter and 1mm depth, perforated with an entomological pin on the surface of a single large ripe mango. This density of larval infestation has previously been used in interspecific interaction studies of tephritidae (Keiser *et al.*, 1974; Fitt, 1986; Krainacker *et al.*, 1987; Qureshi *et al.*, 1987; Duyck *et al.*, 2006) and was comparable to observed adult densities from field collected mango samples (Section 5). Each hole was ~ 1 mm in diameter and 1 cm in depth.

Infestation procedure was either done on the same day or in asynchrony of 1, 2 or 3 day intervals. The treatments therefore included: (1) Fruit infested same day with larvae of both species (2) Fruit infested with *B. invadens* larvae at 1 day before introduction of *C.*

cosyra larvae (3) Fruit infested with *B. invadens* at 2 days before introduction *C. cosyra* larvae (4) Fruit infested with *B. invadens* larvae at 3 days before introduction *C. cosyra* larvae (5) Fruit infested with *C. cosyra* at 1 day before introduction of *B. invadens* (6) Fruit infested with *C. cosyra* larvae at 2 days before introduction *B. invadens* larvae (7) Fruit infested with *C. cosyra* larvae at 3 days before introduction *B. invadens* larvae (8) Controls (no co-infestation). The same infestation procedure and treatments were used for the larval competition experiments between *B. invadens* and *C. capitata*.

After larval introduction, the holes were sealed with tape to prevent larvae from boring out of the fruit. Each mango was then transferred into a 3-liter rectangular, plastic container (20 x 12.5 x 15 cm) (Kenpoly®, Kenya) containing a moistened sand layer at the bottom (3-5 mm deep). The sand held the exudate dripping from the rotting fruits and served as pupation medium for the mature larvae that left the fruits (Woods *et al.*, 2005). Fruit samples were then transferred to thermostatically controlled environmental chambers (MLR-153, Sanyo, Japan) set at 4 constant temperatures of 15, 20, 25 and 30°C (± 1°C) and 50 ± 8% RH. From the sixth day after fruit infestation, the sand was sifted daily to recover puparia and developmental duration to reach this stage recorded. Each puparium from the controls and co-infested fruits was weighed and then held individually at ambient temperature in transparent 30 ml glass vials plugged with cotton wool. By holding the puparium individually it was possible to relate the pupal mass of each vial to the identity of the fly at eclosion. The vials were observed daily and number of emerged adults was recorded. The range of variation of pupal development of the test species (i.e the lapse of time from the first to the last adult emergence) is known to vary between 1-

10 days depending on the temperature (Section 3.0) and since diapause has not been reported for these species, puparium failing to eclose at the end of 30 days were considered unviable. Each fruit served as a replicate and there were five replications per treatment.

7.3 Data analyses

All parameters recorded (duration of larval development in days, pupal mass and adult emergence) at each temperature were transformed by ln (x + 1) to reduce heteroscedasticity and then subjected to one-factor analysis of variance (ANOVA). Student Newman Keuls (SNK) tests (P = 0.05) was used to identify significant main effects for each temperature. At each temperature, a two sample t test was used to determine the effect of interspecific competition on the life history parameters between the two fruit species. All statistical tests were performed using the SAS (SAS Institute, 2001) software.

7.4 Results

7.4.1 Competition between *Bactrocera invadens* and *Ceratitis cosyra*

7.4.1.1 *Larval development*

At 15°C, there was no significant difference in larval development of *B. invadens* between the control (27.6 days) and the co-infested fruits (F = 0.96, df = 7, 32, P = 0.4745) (Table 7.1). At this temperature, *C. cosyra* took 30.8 days in the control fruits, but the larvae could not complete development to pupal stage under interspecific competition with *B. invadens* irrespective of the infestation sequence (Table 7.1).

When the mango fruits were held at 20°C, larval development of *B. invadens* in the treatment where the invasive species was infested two days before *C. cosyra* and the treatment in which *C. cosyra* was given a one day head start, was found to be significantly shorter ($P < 0.05$) (14.4 and 15.0 days respectively) compared with where there was no co-infestation (18.4 days) (Table 7.1). In *C. cosyra*, apart from the treatment in which fruits were infested on the same day, larval development was significantly shorter under interspecific competition with *B. invadens* (Table 7.1) compared with the control fruits. However, *C. cosyra* could not complete development to pupal stage under co-infestation with *B. invadens* at all instances when *B. invadens* infested mango fruits one, two and three days earlier (Table 7.1). The development of *C. cosyra* was marginally faster than *B. invadens* when it was given a one, two and three days head start although the difference was not significant (Table 7.1).

At 25°C, there was no significant difference in larval development of *B. invadens* among the co-infested fruits and the control treatment ($F = 0.90$, df = 7, 32, $P = 0.5176$) (Table 7.1). For *C. cosyra*, apart from fruit samples in which both insects were infested on the same day, asynchronous infestation of either species one, two and three days earlier resulted in significantly shorter developmental time compared with the control (Table 7.1). There was no significant difference in larval developmental time between the two species across the various co-infested treatments (Table 7.1). However, in the control, a significantly longer development time of *C. cosyra* (11.3 days) was observed compared with *B. invadens* (9.2 days) ($t = 5.31$, $P = 0.0007$) (Table 7.1).

At 30°C, *B. invadens* larvae developed at a similar rate under interspecific competition with *C. cosyra* (F = 0.66, df = 7, 32, P = 0.7048) (Table 7.1). In *C. cosyra*, larval development was however shorter under interspecific competition with *B. invadens* compared with the control (F = 1.31, df = 6, 28, P = 0.0312) (Table 7.1). The t test did not reveal any significant difference in the duration of larval development among the two species both in the co-infested and control fruits (Table 7.1).

7.4.1.2 *Pupal weight*

At 15°C, *B. invadens* pupal weight was significantly higher when fruits were co-infested on the same day (15.1 mg) and in the control (14.2 mg) (Table 7.2). No *C. cosyra* puparia was recovered at this temperature at all the co-infested treatments (Table 7.2). The pupal weight of *C. cosyra* was significantly lower (10.2 mg) compared with *B. invadens* (14.2 mg) in the control fruits (t = 6.40, P = 0.0007) (Table 7.2).

At 20°C, significantly heavier *B. invadens* puparia was recovered when both species were introduced to fruit on the same day (14.9 mg) and lower in the treatments where *C. cosyra* had a 3 day head start (12.9 mg) (F = 6.78, df = 7, 32, P = 0.0001) (Table 7.2). For *C. cosyra*, there was no significant difference in the pupal weight under co-infestation (11.3 and 13.3 mg for same day and three day head start respectively) compared with the control (12.2 mg). There was also no significant difference in pupal weight between *B. invadens* and *C. cosyra* for all the treatments (Table 7.2).

When the fruits were held at 25°C, weight of the *B. invadens* puparia varied significantly

between the co-infested fruits and the control (Table 7.2). Infestation of *B. invadens* and *C. cosyra* on same day and *B. invadens* at one day before *C. cosyra* resulted in heavier *B. invadens* puparia (15.0mg and 15.3 mg respectively) compared with the other treatments ($F = 5.62$, df = 7, 32, $P = 0.0003$) (Table 7.2). For *C. cosyra*, there was no clear pattern of differences among co-infested treatments and the control and the weight of puparia ranged from 9.9 mg to 12.9 mg. At this temperature, the *t* test revealed that *B. invadens* puparium in the control was significantly heavier (15.8 mg) than puparium of *C. cosyra* (12.3 mg) (Table 7.2). *Bactrocera invadens* pupal weight was larger even when *C. cosyra* was given a one, two or three day head start although this trend did not manifest under same day infestation and when the invasive species had a 1-3 day infestation advantage (Table 7.2).

At 30°C, there was no significant difference in weight of *B. invadens* puparia among the co-infested treatments and the control, except when *B. invadens* had a three day head start (11.2 mg) ($F = 3.24$, df = 7, 32, $P = 0.0103$). Similarly, in *C. cosyra*, apart from the same day infestation (9.6 mg) and the treatment in which the indigenous species was given a one day head start (9.0 mg), pupal weights under interspecific competition did not differ significantly from the control (11.5 mg). When the weight of the puparia of both species under control treatments was subjected to *t* test, there was no significant difference: *B. invadens* = 12.6 mg and *C. cosyra* = 11.5 mg. Under interspecific competition, puparium of *B. invadens* was significantly heavier than puparium of *C. cosyra* when the insects were infested on the same day, but this difference was not observed in the other infestation sequences (Table 7.2).

Table 7.1 Effect of interspecific competition between *Bactrocera invadens* and *Ceratitis cosyra* at different temperatures on the duration of larval development of the two species

Temp	Infestation sequence	Larva development time (days)			
°C		*B. invadens*	*C. cosyra*	*t-value*	*P*
15	Infested same day	24.8 ± 1.8 a	nd	-	-
	B. inv 1d before *C. cos*	29.4 ± 1.4 a	nd	-	-
	B. inv 2d before *C. cos*	27.6 ± 1.7 a	nd	-	-
	B. inv 3d before *C. cos*	27.2 ± 0.8 a	nd	-	-
	C. cos 1d before *B. inv*	27.3 ± 0.4 a	nd	-	-
	C. cos 2d before *B. inv*	26.8 ± 0.9 a	nd	-	-
	C. cos 3d before *B. inv*	27.2 ± 1.8 a	nd	-	-
	Controls	27.6 ± 2.3 a	30.8 ± 2.1	2.28	0.0625
		$F_{[7,32]} = 0.96$	-		
		$P = 0.4745$	-		
20	Infested same day	17.1 ± 0.5 ab	21.7 ± 0.6 a		
	B. inv 1d before *C. cos*	16.8 ± 0.9 ab	nd	-	-
	B. inv 2d before *C. cos*	14.6 ± 1.1 b	nd	-	-
	B. inv 3d before *C. cos*	15.0 ± 1.2 ab	nd	-	-
	C. cos 1d before *B. inv*	14.4 ± 0.7 b	11.2 ± 0.9 b	1.03	0.0984
	C. cos 2d before *B. inv*	15.1 ± 1.0 ab	11.8 ± 0.5 b	2.33	0.0528
	C. cos 3d before *B. inv*	16.8 ± 0.3 ab	13.1 ± 0.5 b	1.66	0.1356
	Controls	18.4 ± 0.3 a	20.3 ± 0.4 a	2.56	0.0936
		$F_{[7,32]} = 3.43$	$F_{[4,20]} = 1.07$		
		$P = 0.0078$	$P = 0.0394$		
25	Infested same day	9.2 ± 0.9 a	10.9 ± 0.8 a	0.96	0.3637
	B. inv 1d before *C. cos*	9.2 ± 0.6 a	8.1 ± 0.1 b	2.02	0.1141
	B. inv 2d before *C. cos*	9.5 ± 1.3 a	8.8 ± 0.5 b	0.68	0.5221
	B. inv 3d before *C. cos*	9.2 ± 1.1 a	8.9 ± 0.1 b	0.67	0.5371
	C. cos 1d before *B. inv*	9.9 ± 0.3 a	8.1 ± 0.4 b	1.83	0.1172
	C. cos 2d before *B. inv*	9.8 ± 0.5 a	8.0 ± 0.4 b	1.35	0.2243
	C. cos 3d before *B. inv*	9.3 ± 0.4 a	8.4 ± 0.5 b	0.62	0.5703
	Controls	9.2 ± 0.2 a	11.3 ± 0.1 a	5.31	0.0007
		$F_{[7,32]} = 0.90$	$F_{[7,32]} = 5.92$		
		$P = 0.5176$	$P = 0.0024$		
30	Infested same day	7.1 ± 0.9 a	6.6 ± 0.3 b	0.49	0.6383
	B. inv 1d before *C. cos*	6.3 ± 0.8 a	6.8 ± 0.4 b	2.19	0.0713
	B. inv 2d before *C. cos*	8.5 ± 0.7 a	6.9 ± 0.2 b	2.92	0.0781
	B. inv 3d before *C. cos*	8.5 ± 1.5 a	nd	-	-
	C. cos 1d before *B. inv*	7.0 ± 0.1 a	7.4 ± 0.3 b	0.54	0.6125
	C. cos 2d before *B. inv*	6.5 ± 0.4 a	7.3 ± 0.5 b	0.80	0.9372
	C. cos 3d before *B. inv*	8.4 ± 0.1 a	6.8 ± 0.5 b	1.88	0.1335
	Controls	6.7 ± 0.6 a	10.7 ± 0.4 a	2.21	0.0583
		$F_{[7,32]} = 0.66$	$F_{[6,28]} = 1.31$		
		$P = 0.7048$	$P = 0.0312$		

B. inv = *Bactrocera invadens*, *C. cos* = *Ceratitis cosyra*, nd = not determined, Means in same row followed by the same letter are not significantly different [Student Newman-Keuls (SNK) test, $P = 0.05$], Means in the same column are not significantly different level when P >0.05 (students t-test)

Table 7.2 Mean weight of puparium following interspecific competition between larvae of *Bactrocera invadens* and *Ceratitis cosyra* at different temperatures on mango

Temp	Infestation sequence	Pupa weight (mg)			
°C		*B. invadens*	*C. cosyra*	*t-value*	*P*
15	Infested same day	15.1 ± 0.4 a	nd	-	-
	B. inv 1d before *C. cos*	13.2 ± 0.6 ab	nd	-	-
	B. inv 2d before *C. cos*	13.7 ± 0.9 ab	nd	-	-
	B. inv 3d before *C. cos*	13.6 ± 0.7 ab	nd	-	-
	C. cos 1d before *B. inv*	12.4 ± 0.1 b	nd	-	-
	C. cos 2d before *B. inv*	12.7 ± 0.3 ab	nd	-	-
	C. cos 3d before *B. inv*	12.1 ± 0.2 b	nd	-	-
	Controls	14.2 ± 0.3 a	10.2 ± 0.3	6.40	0.0007
		$F_{[7,32]} = 1.37$			
		$P = 0.0255$			
20	Infested same day	14.9 ± 0.4 a	13.3 ± 3.3a	1.51	0.2198
	B. inv 1d before *C. cos*	12.9 ± 0.4 c	nd	-	-
	B. inv 2d before *C. cos*	14.4 ± 0.3 ab	nd	-	-
	B. inv 3d before *C. cos*	14.0 ± 0.4 b	nd	-	-
	C. cos 1d before *B. inv*	12.6 ± 0.4 c	11.3 ± 0.2a	2.35	0.2381
	C. cos 2d before *B. inv*	13.2 ± 0.4 bc	12.6 ± 0.2a	5.62	0.1500
	C. cos 3d before *B. inv*	12.9 ± 0.1 c	12.9 ± 0.1a	6.88	0.3900
	Controls	13.5 ± 0.2 bc	12.2 ± 0.1a	5.96	0.1000
		$F_{[7,32]} = 6.78$	$F_{[4,20]} = 1.68$		
		$P = 0.0001$	$P = 0.2204$		
25	Infested same day	15.0 ± 0.5 ab	12.9 ± 0.8 a	2.29	0.0513
	B. inv 1d before *C. cos*	15.3 ± 0.7 a	12.1 ± 0.1 ab	1.80	0.1463
	B. inv 2d before *C. cos*	13.2 ± 0.2 c	11.8 ± 0.5 ab	3.23	0.1780
	B. inv 3d before *C. cos*	13.0 ± 0.3 c	11.8 ± 0.1 ab	3.82	0.0880
	C. cos 1d before *B. inv*	14.0 ± 0.3 bc	10.6 ± 0.1 b	2.68	0.0367
	C. cos 2d before *B. inv*	14.0 ± 0.3 bc	11.1± 0.1 ab	2.52	0.0355
	C. cos 3d before *B. inv*	14.1 ± 0.1 bc	9.9 ± 0.1 b	12.72	0.0002
	Controls	15.8 ± 0.6 a	12.3 ± 0.1 ab	6.23	0.0033
		$F_{[7,32]} = 5.62$	$F_{[7,32]} = 1.57$		
		$P = 0.0003$	$P = 0.0233$		
30	Infested same day	12.5 ± 0.4 ab	9.6 ± 0.3 bc	4.86	0.0038
	B. inv 1d before *C. cos*	12.3 ± 0.6 ab	10.8 ± 0.4 b	1.83	0.1164
	B. inv 2d before *C. cos*	13.4 ± 0.4 ab	13.9 ± 0.2 a	2.09	0.0821
	B. inv 3d before *C. cos*	11.2 ± 1.4 b	nd	-	-
	C. cos 1d before *B. inv*	13.7 ± 0.3 ab	9.0 ± 0.1 c	5.98	0.0019
	C. cos 2d before *B. inv*	14.0 ± 0.6 a	12.4 ± 0.4 a	4.84	0.2230
	C. cos 3d before *B. inv*	14.1 ± 0.4 a	12.2 ± 0.2 a	3.21	0.3240
	Controls	12.6 ± 0.4 ab	11.5 ± 0.4 ab	1.83	0.1043
		$F_{[7,32]} = 3.24$	$F_{[6,28]} = 6.10$		
		$P = 0.0103$	$P = 0.0026$		

B. inv = *Bactrocera invadens*, *C. cos* = *Ceratitis cosyra*, nd = not determined, Means in same row followed by the same letter are not significantly different [Student Newman-Keuls (SNK) test, $P = 0.05$], Means in the same column are not significantly different level when P >0.05 (students t-test)

7.4.1.3 *Adult emergence*

Lower temperatures generally adversely affected adult emergence of both species and this was particularly more pronounced under interspecific competition than when either insect infested mango fruits alone. For example, at 15°C, out of 14 .0 *B. invadens* puparia that were obtained in the control treatment, 9.0 adults emerged while no *C. cosyra* emerged from the 6.4 puparia that were harvested from the control fruits ($F = 8.11$, df = 7, 32, $P = 0.0001$) (Table 7.3). *Bactrocera invadens* adult emergence under co-infestation was 2.2 flies when *C. cosyra* had a one or three day head start and 9.2 flies when *B. invadens* had a 3 day headstart. Although *C. cosyra* puparia were recovered from the fruit, no adult flies emerged from the puparia in both the co-infested and control fruits at 15°C (Table 7.3).

When the fruits were held at 20°C, a mean of 13.3 *B. invadens* adult flies emerged from the control fruits which did not differ significantly ($P < 0.05$) from the number of *B. invadens* adults that were recovered when the invasive species infested the fruit at two and three days before *C. cosyra* (13.0 and 11.4 flies respectively) (Table 7.3). The number of *C. cosyra* adults was completely suppressed when this species was introduced to the fruit at 1-3 days after *B. invadens* and less than two adults emerged when both insects were infested on the same day ($F = 7.940$, df = 4, 20, $P = 0.0001$) (Table 7.3). Significantly ($P < 0.05$) higher number of *C. cosyra* was, however, recovered when the native species infested the mango fruit before the invasive species, and it generally out-competed *B. invadens* at 20°C in all cases when it was given a head start (Table 7.3).

Table 7.3 Mean adult emergence following interspecific competition between larvae of *Bactrocera invadens* and *Ceratitis cosyra* at different temperatures on mango

Temp	Infestation sequence	Total	No. of adults			
°C		puparia	*B. invadens*	*C. cosyra*	*t-value*	*P*
15	Infested same day	22.0	3.8 ± 0.4 b	no emergence	-	-
	B. inv 1d before *C. cos*	16.0	8.4 ± 0.7 a	no emergence	-	-
	B. inv 2d before *C. cos*	18.3	6.2 ± 0.6 ab	no emergence	-	-
	B. inv 3d before *C. cos*	15.0	9.2 ± 1.2 a	no emergence	-	-
	C. cos 1d before *B. inv*	16.0	2.2 ± 0.6 b	no emergence	-	-
	C. cos 2d before *B. inv*	17.6	3.4 ± 1.2 b	no emergence	-	-
	C. cos 3d before *B. inv*	8.0	2.2 ± 0.8 b	no emergence	-	-
	Controls	14.0/6.4[k]	9.0 ± 0.3 a	no emergence	-	-
			$F_{[7,32]} = 8.11$			
			$P = 0.0001$			
20	Infested same day	26.0	8.0 ± 1.4 b	1.3 ± 0.3 c	5.73	0.0023
	B. inv 1d before *C. cos*	16.5	7.8 ± 1.2 b	no emergence	-	-
	B. inv 2d before *C. cos*	19.8	13.0 ±1.7 a	no emergence	-	-
	B. inv 3d before *C. cos*	16.4	11.4 ± 1.0 a	no emergence	-	-
	C. cos 1d before *B. inv*	25.3	8.2 ± 1.7 b	15.0 ± 1.6 a	6.95	0.0010
	C. cos 2d before *B. inv*	24.6	9.8 ± 1.2 b	12.6 ± 2.4 b	11.14	<0.0001
	C. cos 3d before *B. inv*	16.4	2.5 ± 0.7 c	13.0 ± 1.0 b	4.34	0.0025
	Controls	14.2/16.6	13.3 ± 1.1 a	15.5 ± 0.7 a	0.38	0.7122
			$F_{[7,32]} = 6.41$	$F_{[4,20]} = 7.940$		
			$P = 0.0001$	$P = 0.0001$		
25	Infested same day	27.0	12.6 ± 0.9 c	2.8 ± 0.8 c	5.75	0.0004
	B. inv 1d before *C. cos*	19.3	11.2 ± 0.4 c	1.0 ± 0.1 c	23.74	<0.0001
	B. inv 2d before *C. cos*	27.3	14.2 ± 1.3 b	1.0 ± 0.1 c	16.51	<0.0001
	B. inv 3d before *C. cos*	20.0	12.2 ± 1.3 c	1.0 ± 0.1 c	7.85	0.0014
	C. cos 1d before *B. inv*	30.6	15.0 ± 0.7 ab	14.2 ± 1.6 a	4.45	0.0735
	C. cos 2d before *B. inv*	25.0	15.6 ± 0.2 ab	7.4 ± 1.2 b	6.63	0.0006
	C. cos 3d before *B. inv*	26.5	16.6 ± 1.0 a	8.6 ± 1.1 b	6.04	0.0038
	Controls	18.5/17.2	16.2 ± 0.7 a	15.2 ± 0.6 a	1.02	0.3362
			$F_{[7,32]} = 2.91$	$F_{[7,32]} = 13.95$		
			$P = 0.0180$	$P = 0.0001$		
30	Infested same day	16.3	6.8 ± 0.5 c	1.3 ± 0.3 b	9.49	<0.0001
	B. inv 1d before *C. cos*	21.6	10.2 ± 2.1 b	2.7 ± 1.2 b	3.62	<0.0001
	B. inv 2d before *C. cos*	17.2	11.0 ± 1.3 b	1.5 ± 0.3 b	3.16	0.0203
	B. inv 3d before *C. cos*	16.6	10.6 ± 1.2 b	1.0 ± 0.1 b	9.51	0.0002
	C. cos 1d before *B. inv*	18.6	7.6 ± 1.5 c	2.0 ± 1.2 b	2.98	0.0023
	C. cos 2d before *B. inv*	19.4	10.2 ± 1.0 b	3.4 ± 0.1 b	7.26	<0.0001
	C. cos 3d before *B. inv*	17.8	10.4 ± 0.7 b	3.8 ± 0.6 b	11.19	0.0004
	Controls	14.0/12.3	13.8 ± 1.9 a	6.0 ± 0.3 a	2.90	<0.0001
			$F_{[7,32]} = 1.54$	$F_{[7,32]} = 6.54$		
			$P = 0.0190$	$P = 0.0019$		

B. inv = *Bactrocera invadens*, *C. cos* = *Ceratitis cosyra*, Means in same row followed by the same letter are not significantly different [Student Newman-Keuls (SNK) test, $P = 0.05$], Means in the same column are not significantly different level when P >0.05 (students t-test), [K] = values denote *B. invadens* control/*C. cosyra* control.

At 25°C, significantly fewer *B. invadens* adults emerged under interspecific competition compared with both the control treatment (16.2 flies) and the treatments in which *C. cosyra* was given a 1, 2 and 3 days head start (15.0, 15.6 and 16.6 flies respectively) (F =2.91, df = 7, 32, P = 0.0180) (Table 7.3). Significantly ($P < 0.05$) lower number of *C. cosyra* (< 2.8 flies) emerged when *C. cosyra* and *B. invadens* co-infested fruits on the same day and when *B. invadens* had a head start irrespective of the duration of asynchrony. But when *C. cosyra* was given the head start, the number of emergent adults did not differ at 1 day interval (14.2 flies) compared with the control (15.2 flies) but was lower at 2 and3 days infestation interval (7.4 and 8.6 flies respectively) (Table 7.3). At this temperature, both insects equally tolerated 25°C in the control fruits (*B. invadens* = 16.2 flies; *C. cosyra* = 15.2 flies) (t = 1.02, P = 0.3362) (Table 7.3).

At 30°C, the trend in adult emergence between the treatments was similar to when the fruits were held at 25°C. Significantly ($P < 0.05$) more *B. invadens* emerged in the control fruits (13.8 flies) compared with the co-infested fruits (F = 1.54, df = 7, 2, P = 0.0190) (Table 7.3). Similarly, a significantly higher number of *C. cosyra* (6.0 flies) was recovered from the control fruits than the co-infested fruits (F = 6.54, df = 7, 32, P = 0.0019). Two sample t test analysis showed that the invasive species out-competed *C. cosyra* in terms of the number of adults recovered under interspecific competition but temperature may have played a significant role in the interaction because a similar trend was also observed in the control (t = 2.90, P = 0.0001) (Table 7.3).

7.4.2 Competition between *Bactrocera invadens* and *Ceratitis capitata*

7.4.2.1 *Larval development*

At 15°C, there was no significant difference in larval development of *B. invadens* among the infestation sequences and the control (27.8 days) except for the treatment where *C. capitata* had a 3 day head start (25.3 days) (Table 7.4). At the same temperature, *C. capitata* larval development took between 20.5 to 26.7 days, which did not differ significantly among the infestation sequences except when *C. capitata* had a 3 days head start (18.5 days) and with the control (16.9 days). Larvae of *C. capitata* developed significantly ($P < 0.05$) faster when both species were co-infested on the same day and when *C. capitata* had a head start (Table 7.4).

When the larvae were held at 20°C, development of *B. invadens* did not vary significantly with the different infestation sequences and with the control (~14 days) except when it was given a 2 day head start (15.0 days) (Table 7.4). Larval development time of *C. capitata* was longer when *B. invadens* had a 1, 2 and 3 days head start (12.0, 12.0 and 12.8 days respectively) but was significantly shorter when *C. capitata* was introduced first (9.5, 9.1 and 9.9 days at 1, 2 and 3 days head start respectively). Except for 3 days head start of *B. invadens* ($t = 1.54$, $P = 0.1628$), all treatments had a significantly shorter larval developmental time for *C. capitata* under interspecific interaction with *B. invadens*.

At 25°C, there was no significant difference in larval developmental time of *B. invadens* among the infestation sequences and the control ($F = 0.51$, df = 7, 32, $P = 0.238$) (Table 7.4). For *C. capitata*, there was no difference in larval developmental time between the

control and the treatments, except when *B. invadens* had a 1 and 3 day head start (8.6 and 8.9 days respectively). Apart from the infestation sequence where *B. invadens* had a 3 day head start, the *t* test revealed a significantly shorter larval developmental times of *C. capitata* than that of *B. invadens* at the respective infestation sequences (Table 7.4).

At 30°C, *B. invadens* larvae took longer to complete development when introduced to fruit first (8.5. 8.8 and 9.4 days at 1, 2 and 3 days *B. invadens* head start respectively) than when *C. capitata* had the head start (6.0, 5.9 and 6.2 days at 1, 2 and 3 days *C. capitata* head start respectively) (Table 7.4). For *C. capitata*, larvae developed at the same rate under interspecific competition as the control (F = 1.86, df = 7, 32, P = 0.1087). The two sample *t* test revealed significantly shorter larval developmental periods of *C. capitata* at the infestation sequences where *B. invadens* had a head start (Table 7.4).

7.4.2.2 *Pupal weight*

Across all temperatures, the pupa weight of *B. invadens* differed among the infestation sequences only when the larvae were held at 20°C (F = 7.60, df = 7, 32, P = 0.0001) and 30°C (F = 5.85, df = 7, 32, P = 0.0002) (Table 7.5). For *C. capitata*, the pupal weights differed among the infestation sequences for each temperature. At 15°C, *C capitata* pupae were lighter when larvae were introduced on the same day (7.5 mg) and heavier when *C. capitata* had a 1 day head start and *B. invadens* had a 2 day head start (8.3 mg). At 20°C, lighter pupae were harvested when *C. capitata* had a 3 days head start (7.1 mg) and were heavier when larvae were introduced on the same day (8.7 mg). Holding the larvae at 25°C resulted in heavier pupae when larvae of both insects were introduced on

the same day (8.4 mg), but lighter pupae were harvested at all remaining treatments and the control (7.8 mg). At 30°C, heavier *C. capitata* pupae weighing 8.3, 8.2 and 8.1 mg were recovered when *B. invadens* had a 1, 2 and 3 day head start respectively. In the control and at all treatments when *C. capitata* had a head start, pupae harvested weighed ≤7.5 mg. The pupa weight of *B. invadens* (12.1-14.6 mg) was always significantly higher than for *C. capitata* (7.2-8.7 mg) irrespective of the infestation sequence (Table 7.5).

7.4.2.3 *Adult emergence*

At 15°C, higher number of *B. invadens* adults emerged when *B. invadens* had a 1 day head start (10.4 flies) and in the control (11.0 adults) (Table 7.6). For *C. capitata*, higher adult emergence occurred when it was introduced 3 days before *B. invadens* and the control (11.4 flies). When *B. invadens* was introduced 3 days after *C. capitata*, only 3.2 flies of the former species emerged. Significantly higher *C. capitata* adults (11.8 flies) than of *B. invadens* (3.2 flies) emerged when *C. capitata* had a 3 days head start ($t = 4.86$, $P = 0.0013$).

At 20°C, significantly higher number of *B. invadens* adults emerged when mango was infested with *B. invadens* 2 and 3 days before *C. capitata* (9.8 and10.8 flies respectively) and in the control (11.6 flies) than when *C. capitata* was introduced 2 or 3 days before (3.8 and 5.2 flies respectively) (Table 7.6). For *C. capitata*, the number of adults when both insects were co-infested on the same day did not differ significantly with the control (15.6 flies) and when *B. invadens* and *C. capitata* were introduced on the same day and or when *B. invadens* had a one day head start (14.2 and 15.0 flies respectively). But when

either *B. invadens* or *C. capitata* had a 2 or 3 days head start, the number of *C. capitata* flies emerging reduced (12.6 and 13.0 flies respectively). At all infestation sequences and the control, higher number of *C. capitata* than *B. invadens* emerged (Table 7.6).

At 25°C, significantly higher number of *B. invadens* adults was recovered when *B. invadens* was co-infested on the same day with *C. capitata* and when given a 1 or 3 day head start which did not differ with the control ($F = 16.07$, df = 7, 32, $P = 0.0001$) (Table 7.6). At the infestation sequences where *C. capitata* had 2 and 3 days headstart, the number of emergent *B. invadens* decreased drastically (5.8 and 4.4 flies respectively). For *C. capitata*, lower number of adults emerged when *B. invadens* had a 2 and 3 days head start (7.4 and 8.6 flies respectively) compared with the control (17.8 flies) and at all infestation sequences when it had a head start. More *B. invadens* and *C. capitata* emerged when either insect had a 2 and 3 days head start than the competitor (Table 7.6).

When the insects were held at 30°C, the number of recovered *B. invadens* adults was higher in the control (14.8 flies) than among all the infestation sequences ($F = 5.00$, df = 7, 32, $P = 0.0007$). Fewer adults emerged when *C. capitata* had a 2 or 3 days head start (5.5 and 4.6 flies respectively) (Table 7.6). For *C. capitata*, higher number of adults was obtained when it had a 2 and 3 days head start (16.0 and 17.0 flies respectively) and when insects were co-infested on the same day (15.0 flies). Giving *B. invadens* a 2 to 3 days head start significantly ($P < 0.05$) reduced the number of *C. capitata* adults (Table 7.6). Similarly, a *C. capitata* head start, irrespective of the asynchrony, resulted in reduced number of *B.invadens* adults (Table 7.6).

Table 7.4 Effect of interspecific competition between *Bactrocera invadens* and *Ceratitis capitata* at different temperatures on duration of larval development of the two species

Temp °C	Infestation sequence	Larva development time (days)			
		B. invadens	*C. capitata*	*t-value*	*P*
15	Infested same day	27.1 ± 0.8 ab	20.5 ± 2.4 ab	3.59	0.0071
	B. inv 1d before *C. cap*	27.5 ± 0.4 ab	22.1 ± 1.0 ab	2.11	0.0683
	B. inv 2d before *C. cap*	26.4 ± 1.1 ab	23.4 ± 1.8 ab	1.28	0.2351
	B. inv 3d before *C. cap*	27.3 ± 0.4 ab	26.6 ± 1.1 a	0.53	0.6183
	C. cap 1d before *B. inv*	29.6 ± 0.4 a	24.8 ± 2.2 ab	2.18	0.0611
	C. cap 2d before *B. inv*	27.4 ± 0.2 ab	22.1 ± 2.0 ab	2.50	0.0369
	C. cap 3d before *B. inv*	25.3 ± 1.2 b	18.5 ± 1.3 b	3.95	0.0042
	Controls	27.8 ± 0.2 ab	16.9 ± 1.9 b	9.85	<0.0001
		$F_{[7,32]} = 3.37$	$F_{[7,32]} = 3.58$		
		$P = 0.0083$	$P = 0.0059$		
20	Infested same day	14.4 ± 0.7 b	10.3 ± 0.2 b	5.69	0.0005
	B. inv 1d before *C. cap*	14.3 ± 0.9 b	12.0 ± 0.6 ab	2.75	0.0249
	B. inv 2d before *C. cap*	15.0 ± 1.3 a	12.0 ± 0.5 ab	2.87	0.0207
	B. inv 3d before *C. cap*	14.1 ± 1.9 b	12.8 ± 0.3 a	1.54	0.1628
	C. cap 1d before *B. inv*	14.1 ± 0.5 b	9.5 ± 0.5 bc	6.50	0.0002
	C. cap 2d before *B. inv*	14.2 ± 1.1 b	9.1 ± 0.2 c	4.48	0.0021
	C. cap 3d before *B. inv*	14.3 ± 0.4 b	9.9 ± 0.4 bc	11.28	<0.0001
	Controls	14.3 ± 0.4 b	9.6 ± 0.6 bc	9.46	<0.0001
		$F_{[7,32]} = 2.98$	$F_{[7,32]} = 6.98$		
		$P = 0.016$	$P = 0.0001$		
25	Infested same day	9.9 ± 0.3 a	6.4 ± 0.2 b	7.8	<0.0001
	B. inv 1d before *C. cap*	10.0 ± 0.4 a	8.6 ± 0.5 a	3.32	0.0105
	B. inv 2d before *C. cap*	9.7 ± 0.6 a	7.8 ± 0.4 ab	2.66	0.0290
	B. inv 3d before *C. cap*	9.4 ± 0.4 a	8.9 ± 0.8 a	1.07	0.3142
	C. cap 1d before *B. inv*	10.4 ± 0.7 a	7.1 ± 0.4 ab	4.13	0.0033
	C. cap 2d before *B. inv*	9.9 ± 0.7 a	7.3 ± 0.3 ab	3.32	0.0202
	C. cap 3d before *B. inv*	10.6 ± 1.0 a	6.8 ± 0.3 b	3.81	0.0052
	Controls	10.0 ± 0.1 a	6.3 ± 0.6 b	7.40	<0.0001
		$F_{[7,32]} = 0.51$	$F_{[7,32]} = 3.78$		
		$P = 0.8238$	$P = 0.0043$		
30	Infested same day	6.5 ± 0.3 c	6.2 ± 0.1 a	0.33	0.7438
	B. inv 1d before *C. cap*	8.8 ± 0.2 ab	7.7 ± 0.3 a	4.71	0.0156
	B. inv 2d before *C. cap*	8.5 ± 0.6 b	6.7 ± 0.3 a	1.84	0.0103
	B. inv 3d before *C. cap*	9.4 ± 0.2 a	7.6 ± 0.2 a	4.15	0.0325
	C. cap 1d before *B. inv*	6.0 ± 0.4 c	6.8 ± 0.1 a	2.05	0.0741
	C. cap 2d before *B. inv*	6.2 ± 0.1 c	6.8 ± 0.1 a	3.73	0.0831
	C. cap 3d before *B. inv*	5.9 ± 0.1 c	6.4 ± 0.1 a	4.92	0.0713
	Controls	6.1 ± 0.1 c	6.3 ± 0.1 a	0.57	0.5071
		$F_{[7,32]} = 38.27$	$F_{[7,32]} = 1.86$		
		$P = 0.0001$	$P = 0.1087$		

B. inv = *Bactrocera invadens*, *C. cap* = *Ceratitis capitata*, Means in same column followed by the same letter are not significantly different [Student Newman-Keuls (SNK) test, $P = 0.05$].

Table 7.5 Mean weight of puparium following interspecific competition between larvae of *Bactrocera invadens* and *Ceratitis capitata* at different temperatures on mango

Temp °C	Infestation sequence	Pupa weight (mg)			
		B. invadens	*C. capitata*	*t-value*	*P*
15	Infested same day	13.1 ± 0.4 a	7.5 ± 0.1 b	12.21	<0.0001
	B. inv 1d before *C. cap*	12.4 ± 0.1 a	8.1 ± 0.2 ab	21.32	<0.0001
	B. inv 2d before *C. cap*	12.7 ± 0.3 a	8.3 ± 0.2 a	13.36	<0.0001
	B. inv 3d before *C. cap*	12.1 ± 0.2 a	8.2 ± 0.2 ab	16.50	<0.0001
	C. cap 1d before *B. inv*	12.2 ± 0.1 a	8.3 ± 0.2 a	17.33	<0.0001
	C. cap 2d before *B. inv*	12.4 ± 0.6 a	8.2 ± 0.1 ab	6.80	<0.0001
	C. cap 3d before *B. inv*	12.1 ± 0.2 a	7.9 ± 0.1 ab	20.08	<0.0001
	Controls	13.1 ± 0.2 a	8.0 ± 0.2 ab	17.27	<0.0001
		$F_{[7,32]} = 1.88$	$F_{[7,32]} = 2.53$		
		$P = 0.1058$	$P = 0.0345$		
20	Infested same day	13.2 ± 0.2 ab	8.7 ± 0.3 a	12.41	<0.0001
	B. inv 1d before *C. cap*	12.6 ± 0.4 b	7.9 ± 0.1abc	12.25	<0.0001
	B. inv 2d before *C. cap*	13.2 ± 0.4 ab	7.8 ± 0.2 abc	13.01	<0.0001
	B. inv 3d before *C. cap*	12.8 ± 0.2 ab	8.5 ± 0.3 a	10.26	<0.0001
	C. cap 1d before *B. inv*	13.5 ± 0.1 ab	8.2 ± 0.2 ab	19.46	<0.0001
	C. cap 2d before *B. inv*	13.0 ± 0.4 ab	7.3 ± 0.3 bc	10.93	<0.0001
	C. cap 3d before *B. inv*	13.6 ± 0.2 ab	7.1 ± 0.1 c	25.11	<0.0001
	Controls	13.8 ± 0.3 a	7.9 ± 0.4 abc	10.59	<0.0001
		$F_{[7,32]} = 7.60$	$F_{[7,32]} = 5.31$		
		$P = 0.0001$	$P = 0.0004$		
25	Infested same day	13.0 ± 0.4 abc	8.4 ± 0.1 a	11.37	<0.0001
	B. inv 1d before *C. cap*	14.0 ± 0.3 a	8.0 ± 0.1 abc	21.32	<0.0001
	B. inv 2d before *C. cap*	14.0 ± 0.3 a	7.9 ± 0.1 bc	19.76	<0.0001
	B. inv 3d before *C. cap*	14.1 ± 0.2 a	7.7 ± 0.1 bc	32.96	<0.0001
	C. cap 1d before *B. inv*	12.6 ± 0.4 bc	8.2 ± 0.1 abc	9.87	<0.0001
	C. cap 2d before *B. inv*	12.0 ± 0.3 c	8.2 ± 0.1 abc	13.51	<0.0001
	C. cap 3d before *B. inv*	13.2 ± 0.3 ab	8.2 ± 0.1 abc	17.08	<0.0001
	Controls	13.3 ± 0.3 ab	7.8 ± 0.2 bc	16.96	<0.0001
		$F_{[7,32]} = 5.85$	$F_{[7,32]} = 4.02$		
		$P = 0.0002$	$P = 0.0029$		
30	Infested same day	13.7 ± 0.3 a	7.8 ± 0.3 ab	13.84	<0.0001
	B. inv 1d before *C. cap*	13.7 ± 0.2 a	8.3 ± 0.1 a	20.48	<0.0001
	B. inv 2d before *C. cap*	14.0 ± 0.6 a	8.2 ± 0.4 a	8.39	<0.0001
	B. inv 3d before *C. cap*	14.1 ± 0.5 a	8.1 ± 0.2 a	13.34	<0.0001
	C. cap 1d before *B. inv*	13.3 ± 0.3 a	7.5 ± 0.1 ab	17.97	<0.0001
	C. cap 2d before *B. inv*	13.5 ± 0.5 a	7.5 ± 0.5 ab	11.59	<0.0001
	C. cap 3d before *B. inv*	14.6 ± 0.1 a	7.2 ± 0.1 b	49.13	<0.0001
	Controls	13.5 ± 0.1 a	7.2 ± 0.1 b	37.36	<0.0001
		$F_{[7,32]} = 1.12$	$F_{[7,32]} = 5.58$		
		$P = 0.3769$	$P = 0.0003$		

B. inv = *Bactrocera invadens*, *C. cap* = *Ceratitis capitata*, Means in same column followed by the same letter are not significantly different [Student Newman-Keuls (SNK) test, $P = 0.05$].

Table 7.6 Mean adult emergence following interspecific competition between larvae of *Bactrocera invadens* and *Ceratitis capitata* at different temperatures on mango

Temp °C	Infestation sequence	Total Puparia	Number of adults			
			B. invadens	*C. capitata*	*t-value*	*P*
15	Infested same day	37.4	8.8 ± 1.7 ab	6.4 ± 1.0 c	1.20	0.2660
	B. inv 1d before *C. cap*	36.4	10.4 ± 0.7 a	11.4 ± 0.7 ab	0.99	0.3511
	B. inv 2d before *C. cap*	29.2	6.2 ± 0.6 b	7.4 ± 1.1 bc	0.98	0.3641
	B. inv 3d before *C. cap*	30.4	9.2 ± 1.2 ab	8.8 ± 1.1 bc	0.20	0.8467
	C. cap 1d before *B. inv*	29.8	8.4 ± 1.2 ab	6.8 ± 0.9 b	1.02	0.3390
	C. cap 2d before *B. inv*	29.4	5.6 ± 0.7 bc	8.0 ± 1.1 bc	1.78	0.1136
	C. cap 3d before *B. inv*	22.6	3.2 ± 0.8 c	11.8 ± 1.5 a	4.86	0.0013
	Controls	18.2/15.8[k]	11.0 ± 0.3 a	11.4 ± 1.2 ab	0.80	0.9416
			$F_{[7,32]} = 8.11$	$F_{[7,32]} = 3.81$		
			$P = 0.0001$	$P = 0.0041$		
20	Infested same day	25.2	7.0 ± 1.1 b	14.2 ± 1.5 ab	3.88	0.0047
	B. inv 1d before *C. cap*	25.8	8.2 ± 1.8 b	15.0 ± 1.6 a	2.59	0.0227
	B. inv 2d before *C. cap*	27.8	9.8 ±1.2 ab	12.6 ± 2.4 b	0.83	0.0431
	B. inv 3d before *C. cap*	15.8	10.8 ± 0.8 a	13.0 ± 1.0 b	6.61	0.0002
	C. cap 1d before *B. inv*	25.8	7.8 ± 0.7 b	14.2 ± 1.0 ab	5.51	0.0006
	C. cap 2d before *B. inv*	18.0	3.8 ± 1.1 c	13.0 ± 0.6 b	5.10	0.0009
	C. cap 3d before *B. inv*	19.6	5.2 ± 1.0 c	12.8 ± 0.6 b	4.43	0.0022
	Controls	16.8/17.8	11.6 ± 0.6 a	15.6 ± 1.0 a	3.65	0.0069
			$F_{[7,32]} = 12.63$	$F_{[7,32]} = 0.75$		
			$P = 0.0001$	$P = 0.0063$		
25	Infested same day	36.4	14.6 ± 1.0 a	15.2 ± 1.6 a	0.24	0.8152
	B. inv 1d before *C. cap*	34.0	15.0 ± 0.7 a	14.2 ± 1.6 a	0.59	0.5739
	B. inv 2d before *C. cap*	26.4	15.6 ± 0.2 a	7.4 ± 1.2 b	5.50	0.0006
	B. inv 3d before *C. cap*	28.8	16.6 ± 1.0 a	8.6 ± 1.1 b	5.16	0.0009
	C. cap 1d before *B. inv*	34.2	13.2 ± 1.6 a	15.4 ± 1.0 a	1.26	0.2446
	C. cap 2d before *B. inv*	28.4	5.8 ± 1.0 b	18.2 ± 0.8 a	3.32	0.0105
	C. cap 3d before *B. inv*	24.2	4.4 ± 1.2 b	16.5 ± 0.5 a	4.68	0.0016
	Controls	16.9/18.5	16.5 ± 0.5 a	17.8 ± 0.5 a	0.55	0.5951
			$F_{[7,32]} = 16.07$	$F_{[7,32]} = 12.62$		
			$P = 0.0001$	$P = 0.0001$		
30	Infested same day	26.4	7.4 ± 1.2 bc	15.0 ± 1.3 ab	3.49	0.0082
	B. inv 1d before *C. cap*	31.6	10.4 ± 1.5 b	13.2 ± 1.2 b	1.61	0.1450
	B. inv 2d before *C. cap*	14.4	10.4 ± 1.0 b	3.4 ± 0.4 c	6.84	0.0001
	B. inv 3d before *C. cap*	18.6	8.0 ± 0.7 bc	3.8 ± 0.6 c	4.20	0.0030
	C. cap 1d before *B. inv*	25.2	8.0 ± 1.8 bc	12.6 ± 1.2 b	1.99	0.0081
	C. cap 2d before *B. inv*	26.0	5.4 ± 1.6 bc	17.0 ± 0.4 a	3.91	0.0045
	C. cap 3d before *B. inv*	23.6	4.6 ± 0.2 c	16.0 ± 0.7 a	18.15	<0.0001
	Controls	16.0/16.9	14.8 ± 0.5 a	16.5 ± 0.5 b	3.04	0.1666
			$F_{[7,32]} = 5.00$	$F_{[7,32]} = 47.87$		
			$P = 0.0007$	$P = 0.0001$		

B. inv = *Bactrocera invadens*, *C. cap* = *Ceratitis capitata*, Means in same column followed by the same letter are not significantly different [Student Newman-Keuls (SNK) test, $P = 0.05$]. [K] = values denotes *B. invadens* control/*C. cosyra* control.

7.5 Discussion

Interspecific competition has long been considered as one of the primary factors that influence community assembly (Elton, 1946; Schoener, 1974; Chase & Leibold, 2003). Because the mechanisms governing community assembly and biotic invasions are conceptually similar (Tilman, 2004), it is reasonable to test whether superior competitive ability is the primary mechanism by which some invasive species become dominant and, in turn, reduce the abundance and species richness of native species (Holway, 1999; Bruno *et al.*, 2005). In this study, interspecific competition between *B. invadens* and *C. cosyra* on mango at different temperatures was found to reduce larval survival, pupal mass and adult emergence and at most of the insect/temperature combinations, *C. cosyra* was clearly the inferior competitor. Differential temperature tolerance by insects is one of the critical factors that mediate interspecific competition (Denno *et al.*, 1995), and in this study, temperature indeed played a significant role in the outcome of the competitive interaction between the two species. *Ceratitis cosyra* was more affected by temperature change in the face of interspecific competition: it went into extinction (larvae could not develop to pupal stage) at all infestation sequences at 15°C as well as when *B. invadens* was given a head start at 20°C. It, however, co-existed with *B. invadens* at 25 and 30°C and particularly out-competed the invasive species in terms of adult emergence when it was given a head start at 20°C. It is recognized that species tend to co-exist at intermediate temperatures and competitive extinction or dominance occurs at extreme temperatures (Park, 1954; Wilson *et al.*, 1984; Phillips *et al.*, 1995; Davis *et al.*, 1998ab). The upper temperature limit for the current study did not exceed 30°C and since the fitness of both insects was negatively correlated with temperature, and perhaps negatively

impacted *C. cosyra* more than *B. invadens*, the relative abundance of *C. cosyra* would be expected to decrease with increasing levels of the unfavourable temperature extreme, to a point where its reduced fitness would result in competitive displacement by *B. invadens* especially at temperatures within the range of 15°C and higher than 30°C.

Interspecific competition may also be more likely to affect species responses to environmental change in communities characterized by diffuse competition i.e. competitive interactions in which species are affected more or less equally in the face of environment change (MacArthur, 1972). This was manifested at the temperatures of 25 and 30°C where *C. cosyra* evaded extinction due to competitive interaction with *B. invadens*. It was observed that at the various co-infestation treatments, temperature also altered the strength of competitive interactions between the species in that a significant number of *C. cosyra* emerged despite the competitive dominance of *B. invadens*, and particularly when it was given a head start. Higher temperature means individuals of each species must take up more resources to meet their higher metabolic needs exerting higher per capita competitive intensity upon each other. Although *B. invadens* exerted a higher competitive dominance over *C. cosyra* at higher temperatures, the lack of extinction indicates a slightly higher per capita competitive strength of C. *cosyra* at higher temperature compared to the lower temperatures.

The results also showed that when competition takes place between *B. invadens* and *C. cosyra*, an asynchrony of 1 to 3 days was sufficient to change the relative competitive ability of either species, and this was particularly noticeable at 20°C. An earlier head start

by *B. invadens* drives *C. cosyra* to extinction at 20°C but with the reverse, higher levels of *C. cosyra* are recovered from mango. Thus temporal variation in egg laying of higher magnitude and egg laying tactics at a given temperature regime and consequently a relatively high number of offspring of *B. invadens* (Ekesi *et al.*, 2006) may give competitive advantage to *B. invadens* at the cost of *C. cosyra* under field conditions. Similar results have been reported, albeit, intraspecifically in *Rhagoletis pomonella* (Walsh) (Averill & Prokopy, 1987).

It is difficult to say unambiguously the factors that lead to shorter developmental period of the larvae and reduced emergence under co-infestation of the two species. It has however been noted by Duyck *et al.* (2006) that shorter larval development time of *Bactrocera zonata* (Saunders) compared with *Ceratitis catoirii* Guérin-Mèneville, *C. capitata* (Wiedemann) and *C. rosa* Karsch conferred superior competitive ability on *B. zonata* than the *Ceratitis* species. Krijger *et al.* (2001) also showed that shorter developmental times between *Drosophila* species were associated with superior competitive ability. The present results concur with Krijger *et al.* (2001) at 20°C where shorter development time of *C. cosyra* when it was given a head start resulted in higher adult emergence of the indigenous species under interspecific interaction. This narrow window of infestation asynchrony and competitive temperature advantage of 20°C may be contributing to the co-existence of small populations of *C. cosyra* with *B. invadens* on mango in the field. In this regard, this study also provides direct evidence for another mechanism: that differential temperature tolerance can lead to coexistence of fruit fly competitors. Among the different species of fruit fly, abiotic factors such as temperature

have been demonstrated to promote co-existence (Duyck *et al.*, 2006). Between *B. invadens* and *C. cosyra*, field observations also show that co-existence appears to occur within microhabitat scale with *C. cosyra* having a highly specialized host searching ability on mango (S. Ekesi, unpublished data). This, perhaps, is because it has a narrow host range (Copeland *et al.*, 2006) and more closely linked to mango in its aboriginal home of Africa compared with *B. invadens* that have a wider host range (Rwomushana *et al.*, 2008) and still exploring the new environment. The interaction between temperature and specialized foraging abilities may therefore support co-existence among the 2 species.

The observed pattern in this study also suggests a competitive pre-emption of resources among species i.e. the first larvae to develop benefit from more resources than the later ones (Qureshi *et al.*, 1987; Blanckenhorn, 1999; Krijger *et al.*, 2001; Duyck *et al.*, 2006). When two groups of differently sized and aged juvenile insects are reared together, the smaller and younger cohort suffers from increased mortality and reduced size (Averill & Prokopy, 1987; Edgerly & Livdahl, 1992; Dukas *et al.*, 2001; Cameron *et al.*, 2007). Another crucial factor in the case of fruit flies may be resource degradation arising from variation in nutritional quality inside the mango fruit, and it is likely that more of the lower quality resources are consumed by the inferior competitor. For example, in *C. capitata*, it is known that larvae are sensitive to variation in the nutritional quality of food and able to select the best among the available alternatives (Zucoloto, 1987). It has also been reported that chemical changes that reduce larval growth may be accelerated with increased competition (Fitt, 1989).

The success of many invasive species in their new environment is believed to result primarily from their superior competitive abilities relative to native species (Juliano, 1998; Bruno *et al.*, 2005). In a series of Tephritids invasions on La Reunion, Duyck *et al.* (2006) demonstrated that the invasive species *B. zonata*, tends to have higher ranks than the previously established invasive (*C. rosa* and *C. capitata*) and native (*C. catoirii*) species in the hierarchy. In their study, *B. zonata* which was the most recently established species was the dominant in both forms of competition (scramble and interference) which the authors attributed to large body size and shorter developmental period. Although not many studies have addressed competitive interaction between tephritids of different genera, the results agree with that of Duyck *et al.* (2006) that *Bactrocera* species tend to have superior competitive ability over *Ceratitis* species over a range of temperatures and infestation asynchrony.

For the interspecific competition between *B. invadens* and *C. capitata,* this study demonstrated that when they interact interspecifically, the better competitor was *C. capitata*. It also showed that the outcome of interspecific competition could also be predicted from the differences in larval developmental time of *B. invadens* and *C. capitata*: the species with the longer development time suffered more from interspecific competition. At most temperatures, *C. capitata* was the better competitor in all interactions and particularly when it was given a head start. In general, when species differ in the reaction of development time to temperature, one species will be the 'fastest' at one temperature, but the 'slowest' at another, inducing a switch in competitive rank. Larval growth rates will be higher in 'fast' species, resulting in a faster growth of total

larval biomass. Indeed, *C. capitata* was able to achieve its total biomass faster than *B. invadens*. Thus in some cases, species that are considered competitively strong may be less resistant against abiotic stresses such as temperature, creating a relative advantage for weak competitors in stressful environments.

These results also showed that an asynchrony of one, two and three days was sufficient to change the relative competitive ability of *B. invadens* and *C. capitata*. Christenson & Foote (1960) reported that when comparable numbers of *B. dorsalis* larvae newly hatched from eggs were placed over newly hatched *C. capitata* larvae on carrot medium, there was little or no evidence of competition. However, depression of the C. *capitata* population in the rearing medium and inhibition of development in fruits were especially pronounced when *B. dorsalis* hatch preceded *C. capitata* hatch by 12 to 48 hr. But when the *C. capitata* larvae were at least two days old before *B. dorsalis* eggs hatched, there was no significant inhibition of *C. capitata* development. This observation closely resembles the interaction between *B. invadens* and *C. capitata* in this study. Insects exploiting the same resource are frequently faced with rapidly exhausted patches, creating a time constraint on food acquisition (Hanski, 1989, Wertheim *et al.*, 2000). The faster developing species are therefore expected to consume a disproportional share of the resource while suffering less from competition.

Understanding the factors that govern the spread and success of invasive species is a critical step towards reducing their impact (Williamson, 1996). Under natural conditions, several other mechanisms not studied here may combine to play a role in interspecific

competition between these two species. In general, these include release from natural enemies (S. Ekesi, unpublished data), one species having greater realized fecundity than the competitor (which mechanism applies not just to numbers of offspring but also to the ability to produce proportionately more females from the same resources) and interference competition through behavioural displacement of one by the more aggressive invader (S. Ekesi, unpublished data). This study has shown that resource pre-emption and the capacity to tolerate a wide range of temperatures are among the factors contributing to the displacement of *C. cosyra* by *B. invadens* on mango. The results also stress the importance of interspecific competition in shaping the distribution of tephritids and explain, at least partly, the observed shift in dominance between *B. invadens* and *C. cosyra* on mango in many parts of Africa.

CHAPTER EIGHT

8 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General discussion

An invasive and devastating quarantine fruit fly pest of Asian origin was first detected in March 2003 in Kenya and later described as *Bactrocera invadens* because of its rapid spread over large geographical areas. This pest has subsequently been reported from 24 other countries across the African continent including Angola, Benin, Burkina Faso, Cameroon, Chad, Comoros Island, Congo, Cote d'Ivoire, DR Congo, Equatorial Guinea, Gabon, Gambia, Ghana, Guinea, Mali, Mozambique, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo and Uganda. Sri Lanka is believed to be the putative aboriginal home of this pest but the insect has also been reported from Bhutan.

Due to the novelty status of *B. invadens* there had been little known about the biology and ecology of this pest that would inform development and implementation of any management strategies. Preliminary field observations had indicated that the numbers of the previously important native mango pest, *C. cosyra* was also on the decline in mango fruit collections while those of the exotic pest continued to increase with a presumption that competitive displacement of the native species was in progress. Consequently this study was initiated to study the bioecology of *B. invadens* and its interspecific competition mechanisms with the indigenous species *C. cosyra* and *C. capitata*. The study documents research related to the response of the insect to temperature and assessed field ecology with relation to host plants as well as seasonal and annual

dynamics of the pests in Kenya and evaluated key food attractants and traps that could be utilized for management of the pest on mango. Finally, laboratory studies were carried out to unravel the mechanisms contributing to the displacement of the indigenous species by the exotic *B. invadens* in mango agroecosystems.

The temperature limits for survival and development of this pest was carried out in the laboratory to help in identifying regions where it could establish and aid in improving the efficiency of mass rearing in the laboratory. The developmental time of immature stages of *B. invadens* was found to be greatly affected by temperature with the duration of each stage decreasing as temperature increased. The longest total development period occurred at 15°C (75.74 days) and was shortest at 30°C (17.76 days) perhaps not surprising given that low temperature has been shown to slow development of many insects including tephritids. Survival closely correlated with temperature, being highest at 25°C and lowest at 15°C while no survival was observed at 35°C. A linear regression model provided a reliable fit of development rates versus temperature for the immature stages with lower developmental thresholds for the egg, larva and pupa stages at 8.8, 9.4 and 8.7 °C respectively. This data corroborates earlier field observations that revealed that *B. invadens* was largely a lowland resident pest in most parts of Kenya. The range of thermal parameters described in this study should help in the making of informed decisions regarding the quarantine risk associated with the insect in countries where *B. invadens* has not invaded. The data reported here should also allow for development or improvement of models to better understand the bioclimatic potential of *B. invadens* and consequently its distributional limits and abundance. It is most likely that this information

will become increasingly important as *B. invadens* continues to colonize new geographical areas.

Host plants survey for *B. invadens* was then carried out in Kenya spreading across the Coast, Eastern and Rift Valley Provinces of the country where *B. invadens* had previously been confirmed to be in high abundance and where a large diversity of fruits existed. Sampling locations included forested areas, coastal areas near the Indian Ocean, mid altitude areas of Rift Valley and the high altitude areas in the Taita hills and the fringes of Mt Kenya forest. Altogether, fruits were collected from 90 plant species representing 40 families. Fourteen plants species, among them cultivated and wild fruiting species were found to be hosts of *B. invadens*. Fruits of mango, banana and citrus (lemon, orange and tangerine) were among the cultivated species heavily infested by *B. invadens*. Marula and tropical almond were found to be the most infested non-cultivated plants. These wild plants evidently ensured that sufficient reproductive bases existed for *B. invadens* during the off-season when the cultivated hosts were not in fruiting. Laboratory host preference studies were also conducted on the most economically important cultivated fruits grown in Kenya. These included mango, papaya, sweet banana, guava, sweet orange, custard apple, cucumber, avocado and tomato, which were hosts and non-hosts records from the field surveys and tested in choice and no choice situations. Mango and banana were found to be the most preferred host plants. These results suggested that *B. invadens* is an emerging polyphagous species capable of surviving in a wide range of hosts and can jeopardize the lucrative export of these crops

from this region. Indeed, some countries have already banned the importation of these fruit from Kenya and Uganda.

Studies related to the spatial and temporal population dynamics of *B. invadens* and other mango infesting fruit flies and the underlying influencing factors was also carried out. This was achieved using Multilure traps baited with Nulure combined with regular fruit collections. Three fruit fly species were reared from mango including *B. invadens*, *C. capitata* and *C. cosyra*. Using the number of emerged adults of each species, the relative abundance index (RAI) of these pests in infested fruit was found to be in the order *B. invadens* > *C. cosyra* > *C. capitata*. For *B. invadens* RAI ranged from 0.70-1.00 and that for *C. cosyra* from 0.04-0.30 while that for *C. capitata* was negligible. The findings provide strong evidence for competitive displacement of the native mango fruit fly *C. cosyra* by *B. invadens* in Kenyan lowlands. The study also demonstrated that mango fruiting and maturity in the field was the most important factor for rapid increase in *B. invadens* and *C. cosyra* populations. *Ceratitis capitata* had previously not been recognised as a pest of mango in Kenya but it was found for the first time infesting field collected fruits. Although the interaction between *C. cosyra* and *C. capitata* has not been documented, it is probable that with the rapid displacement of *C. cosyra* by *B. invadens*, *C. capitata* is discovering a niche on mango and this warrants close attention.

The removal of infested fruit from the environment is a widely recognized practice and fruit stripping and disposal is part of a regular strategy when the presence of an infestation of a quarantine fruit fly is detected. In Kenya, however, very little organized

effort is made to remove infested fruit from the cropping systems. There was no data that would form the basis for educating farmers on the importance of this practice as the first line of defence against *B. invadens*. Therefore, infestation rates of mango fruits on the tree and on the ground by this pest were investigated at an orchard in Kenya. The infestation rates were then correlated to the density of resident flies trapped from the orchard. More *B. invadens* emerged from fruits on the ground compared with fruits that were sampled directly from the tree and higher adult catches were evident when plenty of fruits were lying on the ground. The density of *B. invadens* found in ground fruits also correlated with that from tree fruits indicating that adult fly density developed and emerged mostly from fruits on the ground. Considering the high levels of infestation in fruit samples collected from the ground, orchard sanitation is strongly recommended as a component of Integrated Pest Management (IPM) against *B. invadens* in mango orchards.

Baiting technique is one of the recommended control strategies for the management of fruit flies worldwide. The pests are attracted to the lures that contain specific toxicant and are killed when they feed on the food source and can contribute to the suppression of the pest population. In this regard, the efficacy of three commercial and one locally produced attractant for capture of *B. invadens* was evaluated on a farmer's field. The attractants were tested with three trap types including the Easy, Multilure and Lynfield traps all baited with nulure, torula yeast, corn steepwater and a local yeast attractant that is based on waste brewer's yeast. Multilure traps baited with torula yeast or nulure were the most attractive trap-bait combinations. All attractants caught more females than males except for corn steepwater. Although torula yeast is normally not used in bait sprays for fruit fly

suppression, its high efficacy in trapping *B. invadens* suggests that it could be a very useful tool in detection and monitoring of the insect both in countries that the insect has not invaded and in cultivated orchards to guide management decisions. Nulure on the other hand has been successfully used in bait sprays elsewhere and its efficacy as documented in this study showed that it can be exploited in field suppression programs as a bait spray for *B. invadens*. However, both these products have to be imported to Africa and the need to further improve the local food bait from waste brewer's yeast requires urgent attention.

Host plant surveys and seasonal dynamics studies in Kenya and other related studies in Benin and Tanzania have all demonstrated that since the invasion of *B. invadens* into Africa, there has been an overturn in abundance of the pest over the usual native fruit flies that were originally found in the mango agroecosystems. Because immatures of the native *C. cosyra* and the exotic *B. invadens* share the same ecological niche (the fruit), there is every likelihood of interspecific competition and possible displacement of one of the species. In a competitive interaction study, the mechanisms contributing to the competitive success of *B. invadens* over the indigenous fruit flies, *C. cosyra* and *C. capitata* in mango fruits under different temperatures was tested in laboratory. Temperature was found to play a significant role in mediating both the competitive response and competitive effect of both species. When *B. invadens* was in competitive interaction with *C. cosyra*, there was clear competitive advantage in favour of *B. invadens* at all the tested temperatures. For instance, when the larvae were held at 20°C, *C. cosyra* was driven to extinction (no adult emergence) when *B. invadens* infested

mango fruits 1-3 days earlier. The number of emergent adults when *B. invadens* was in competitive interaction with *C. capitata* was highly variable suggesting that this species can equally co-exist with *B. invadens* in this fruit. The results also suggest that the mechanisms contributing to the displacement of *C. cosyra* by *B. invadens* may be associated with intricate interactions between resource pre-emption and fluctuations in temperature in mango agroecosystems.

8.2 Conclusions

1) The developmental time of immature stages of *B. invadens* was greatly affected by temperature with the duration of each stage decreasing as temperature increased. Survival closely correlated with temperature but at 35°C, no adults emerged. A linear regression model provided a reliable fit of development rates versus temperature for the immature stages.
2) Fourteen plants species were hosts of *B. invadens*. Mango, banana and citrus (lemon, orange and tangerine) were among the cultivated species heavily infested by *B. invadens* while marula and tropical almond were the most infested non-cultivated plants. From the laboratory host preference studies, mango and banana were the most preferred host plants.
3) Three fruit fly species were reared from mango including *B. invadens*, *C. capitata* and *C. cosyra* with the RAI in the order *B. invadens* > *C. cosyra* > *C. capitata*. The study also demonstrated that mango fruiting and maturity in the field was the most important factor for rapid increase in *B. invadens* and *C. cosyra* populations.

Ceratitis capitata had previously not been recognised as a pest of mango in Kenya but it was found for the first time infesting field collected fruits.

4) More *B. invadens* emerged from fruits on the ground compared with fruits sampled directly from the tree and higher adult catches were evident when plenty of fruits were lying on the ground. There was also a strong correlation between the density of *B. invadens* found in ground fruits with that from tree fruits.

5) The multilure trap baited with torula yeast or nulure were the most attractive trap-bait combinations for *B. invadens* and caught more females than males except for corn steepwater.

6) Temperature played a significant role in mediating both the competitive response and competitive effect of *B. invadens* with *C. cosyra* and *C. capitata*. There was clear competitive advantage in favour of *B. invadens* against *C. cosyra* across a range of temperatures. However, *C. capitata* was able co-exist with *B. invadens*.

8.3 Recommendations for application and future study

1) Host range of invasive phytophagous insects is a dynamic phenomenon particularly as a result of climate change. It is very likely that the host list presented here may not be conclusive data for *B. invadens* and may change over time. Further survey activity is therefore strongly recommended.

2) The food baits tested in this study and found to be effective against *B. invadens* Torula yeast and Nulure) are not registered for use by the relevant authorities in Kenya. As growers await approval there is an urgent need to continue doing

research on the development of local baits from waste brewer's yeast for field suppression programs.

3) Because *B. invadens* apparently occurs in low abundance in its native range of Sri Lanka, it indicates that the pest is either under biological control and/or direct and indirect competition by other native (or alien) fruit fly species, making the insect a 'classical' candidate for classical biological control in Kenya. The parasitoid, *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), resulted in a dramatic reduction in infestation of fruit in Hawaii (Vargas *et al.*, 2007) through a high level of *B. dorsalis* parasitism (65-70%) and has shown high efficacy for *B. invadens* in laboratory bioassays in Kenya making it a prime candidate for release.
4) Field suppression activities at the production level may not be a panacea to the *B. invadens* problems in Africa especially if fruits have to be exported to quarantine sensitive markets. There is therefore the need to address post harvest treatment research for *B. invadens*.
5) The thermotolerance seasonal/annual population studies provide a baseline data for development of geospatial models for predicting areas where *B. invadens* can potentially establish. There is therefore need for the development of an early warning system especially for regions where the pest has not invaded.
6) Awareness campaigns and education of the growers about the importance of fruit flies in general and availability of management practices will be crucial for dealing with all the fruit fly pests. Farmer field days and field demonstration

activities of available *icipe* IPM packages should form an integral part of the fruit fly management programs.

7) Private sector initiatives particularly by fruit exporters and processing industries that can compel farmers to implement minimum management strategies are necessary. These can include certifying farms that implement recommended management practises, purchasing fruit from pest free zones and setting fruit quality standards.

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APPENDICES

Appendix 1 *Bactrocera invadens* negative fruits collected during December 2004-April 2006

Plant species	Plant family	No. fruits	Fruit wt (g)	% fruit infested	No. tephritid flies/kg	Tephritid species
Allophylus rubifolius Engl.	Sapindaceae	32	7	0	0	na
Anacardium occidentale L.	Anacardiaceae	28	1965	0	0	na
Ananas comosus (L.) Merr.	Bromeliaceae	8	5000	0	0	na
Antidesma venosum E.Mey.	Eurphobiaceae	111	16	0	0	na
Apodytes dimidiata E.Mey.	Icacinaceae	133	40	0	0	na
Averrhoa bilimbi L.	Oxalidaceae	17	420	0	0	na
Averrhoea carambola L.	Oxalidaceae	6	6	0	0	na
Azadirachta indica A. Juss.	Meliaceae	670	445	0	0	na
Bourreria sp. cf *litt*?? B.Br.	Boraginaceae	223	35	0	0	na
Capsicum annuum L.	Solanaceae	16	495	6.25	2.02	*C. cosyra*
Capsicum frutescens L.	Solanaceae	31	80	0	0	na
Carica papaya L.	Caricaceae	19	6611	10.53	0.30	*C. fasciventris*

Appendix 1 (Continued). *Bactrocera invadens* negative fruits collected during December 2004-April 2006

Plant species	Plant family	No. fruits	Fruit wt (g)	% fruit infested	No. tephritid flies/kg	Tephritid species
Carissa edulis Vahl	Rosaceae	3	50	0	0	na
Casimiroa edulis S.Watson	Rutaceae	20	320	0	0	na
Catunaregam nilotica (Stapf)	Rubiaceae	41	6	0	0	na
Chasalia umbraticola Vatke	Rubiaceae	148	38	48.65	368.42	*T. nigerrimum*
Cissampelos pareira L.	Memispermaceae	22	2	9.09	500	*T. nigerrimum*
Cissus rotundifolia??Blume	Vitaceae	32	13	0	0	na
Citrullus lanatus (Thunb.)	Cucurbitaceae	23	3160	8.70	18.35	*D. frontalis*
Citrus aurantifolia Swingle	Rutaceae	36	1195	0	0	na
Citrus x paradisi Macfad	Rutaceae	12	1920	0	0	na
Coccinia grandis (L.) Voigt	Cucurbitaceae	61	493	50.82	62.88	*B. cucurbitae*
						D. frontalis
						B. cucurbitae

Appendix 1 (Continued). *Bactrocera invadens* negative fruits collected during December 2004-April 2006

Plant species	Plant family	No. fruits	Fruit wt (g)	% fruit infested	No. tephritid flies/kg	Tephritid species
Commiphora sp.	Burseraceae	42	180	0	0	na
Ctenolepis cerasiformis C.B.Clarke	Cucurbitaceae	52	40	0	0	na
Cucumis dipsaceus Spach	Cucurbitaceae	13	220	38.46	168.18	*D. vertebratus*
Cucumis sp.	Cucurbitaceae	13	360	0	0	na
Cucurbita sp.	Cucurbitaceae	6	630	33.33	33.33	*D. vertebratus*
Cucurbita maxima Duchesne ex Lam.	Cucurbitaceae	2	35	0	0	na
Cucurbita pepo L.	Cucurbitaceae	3	140	66.67	57.14	*B. cucurbitae*
Cyphomandra betaceae (Cav.) Sendtner	Solanaceae	21	1010	0	0	na
Dioscorea sp.	Dioscoreaceae	3	0.3	0	0	na
Dovyalis caffra Sim	Flacourtiaceae	9	320	22.22	0	no adults
Eugenia uniflora L.	Myrtaceae	14	60	0	0	na
Ficus sp.	Moraceae	126	3135	0	0	na

Appendix 1 (Continued). *Bactrocera invadens* negative fruits collected during December 2004-April 2006

Plant species	Plant family	No. fruits	Fruit wt (g)	% fruit infested	No. tephritid flies/kg	Tephritid species
Flacourtia indica (Burman f.) Merr.	Flacourtiaceae	59	110	0	0	na
Flagellaria guineensis Schum.	Flagellariaceae	342	173	38.30	531.79	*C. capitata*, *T. nigerrimum*, *C. pinax*
Garcinia sp.	Clusiaceae	5	55	0	0	na
Harrisonia abyssinica Oliver	Simaroubaceae	157	60	0	0	na
Jasminum sp.	Oleaceae	80	46	0	0	na
Lantana camara L.	Verbenaceae	50	9	0	0	na
Macadamia sp.	Proteaceae	14	80	0	0	na
[a]Milan palm	Palmae	144	870	0	0	na
Momordica charantia L.	Cucurbitaceae	21	1250	0	0	na
Oncoba sp.	Flacourtiaceae	60	50	0	0	na

Appendix 1 (Continued). *Bactrocera invadens* negative fruits collected during December 2004-April 2006

Plant species	Plant family	No. fruits	Fruit wt (g)	% fruit infested	No. tephritid flies/kg	Tephritid species
[a]Oncocalyx-like, yellow fruits	Loranthaceae	89	200	0	0	na
Opuntia sp.	Cactaceae	5	590	0	0	na
Opuntia ficus-indica (L.) Mill.	Cactaceae	100	1230	0	0	na
Passiflora edulis Sims	Passifloraceae	33	1895	0	0	na
Paulinia pinnata?? Gled.	Sapindaceae	17	5	0	0	na
Persea americana Mill.	Lauraceae	67	9176	0	0	na
Phyllanthus acidus (L.) Skeels	Euphorbiaceae	40	200	0	0	na
Polysphaeria parvifolia Hiern	Rubiaceae	182	45	39.01	755.56	*T. nigerrimum*
Punica granatum L.	Punicaeae	5	815	0	0	na
Rosaceae	Rosaceae	51	50	0	0	na
Rubus sp.	Rosaceae	320	160	19.38	50	*C. anonae*
Saba comorensis (Bojer) Pichon	Apocynaceae	24	2005	0	0	na

Appendix 1 (Continued). *Bactrocera invadens* negative fruits collected during December 2004-April 2006

Plant species	Plant family	No. fruits	Fruit wt (g)	% fruit infested	No. tephritid flies/kg	Tephritid species
Scadoxus multiflorus Raf.	Amaryllidaceae	54	100	0	0	na
[a]Small red fruit	Unidentified	60	120	11.67	66.67	*T. nigerrimum*
Solanum aculeastrum Dun.	Solanaceae	19	1300	0	0	na
Solanum incanum L.	Solanaceae	160	1170	0	0	na
Solanum melongena L.	Solanaceae	10	360	0	0	na
Spondias cytherea Sonn.	Anacardiaceae	21	1280	0	0	na
Spondias mombin L.	Anacardiaceae	14	100	0	0	na
[b]*Strychnos madagascariensis* Poir.	Loganiaceae	1	320	100	na	*Mussidia* sp
Strychnos sp.	Loganiaceae	8	1500	0	0	na
Strychnos sp.	Loganiaceae	15	2347	26.67	29.40	*C. pedestris*
Syzygium cumini (L.) Skeels	Myrtaceae	78	410	1.28	2.44	*C. rosa*
Tabernaemontana elegans Stapf	Apocynaceae	17	985	0	0	na

Appendix 1 (Continued). *Bactrocera invadens* negative fruits collected during December 2004-April 2006

Plant species	Plant family	No. fruits	Fruit wt (g)	% fruit infested	No. tephritid flies/kg	Tephritid species
Vitex sp. cf *payos*	Verbenaceae	56	310	0	0	na
Vitis vinifera L.	Vitaceae	46	120	0	0	na
Ximenia americana L.	Olacaceae	40	1150	0	0	na
Ziziphus sp. cf *mauritania* Lamarck	Rhamnaceae	170	638	0	0	na

na = not applicable, [a]Only common names/ fruit description and family known, [b]Lepidopteran pest recovered
Other tephritids reared from *B. invadens* positive samples listed in table 4.2 include; *C. cosyra* from (*A. cherimola*, *C. limon*, *M. indica*, *S. birrec* and *P. guajava*); *C. capitata*, *C. cosyra*, *C. rosa*, *C. fasciventris* from *M. indica*, and *C. fasciventris* from *P. guajava*

Appendix 2 ANOVA parameters for main effects and associated interactions of trap catches of *Bactrocera invadens* females, males and totals for the two fruiting seasons

Parameter		2006 season		2007/2008 season	
	df	*F*	*P*	*F*	*P*
Females					
Trap	2	35.97	<0.0001	43.87	<0.0001
Lure	3	44.30	<0.0001	70.15	<0.0001
Trap x lure	6	5.60	<0.0001	10.23	<0.0001
Males					
Trap	2	22.60	<0.0001	28.14	<0.0001
Lure	3	15.98	<0.0001	39.47	<0.0001
Trap x lure	6	2.47	0.0242	6.21	<0.0001
Total flies					
Trap	2	29.79	<0.0001	37.22	<0.0001
Lure	3	28.67	<0.0001	56.33	<0.0001
Trap x lure	6	3.90	0.0009	8.45	<0.0001

Appendix 3 Effect of interspecific competition between *Bactrocera invadens* and *Ceratitis cosyra* at different temperature on the duration of pupal development of the two species

Temp	Infestation sequence	Pupa development time			
		B. invadens	*C. cosyra*	*t-value*	*P*
15	Infested same day	38.5 ± 1.4 a	nd	-	-
	B. inv 1d before *C. cos*	35.5 ± 1.0 a	nd	-	-
	B. inv 2d before *C. cos*	38.7 ± 3.5 a	nd	-	-
	B. inv 3d before *C. cos*	33.4 ± 1.0 a	nd	-	-
	C. cos 1d before *B. inv*	32.6 ± 0.4 a	nd	-	-
	C. cos 2d before *B. inv*	33.5 ± 0.4 a	nd	-	-
	C. cos 3d before *B. inv*	34.9 ± 3.3 a	nd	-	-
	Controls	34.1 ± 0.4 a	34.8 ± 1.4	0.39	0.7527
		$F_{[7,32]} = 2.21$			
		$P = 0.0597$			
20	Infested same day	18.6 ± 0.8 a	18.0 ± 0.3 a	0.71	0.5079
	B. inv 1d before *C. cos*	19.4 ± 0.4 a	nd	-	-
	B. inv 2d before *C. cos*	18.7 ± 0.4 a	nd	-	-
	B. inv 3d before *C. cos*	18.9 ± 0.3 a	nd	-	-
	C. cos 1d before *B. inv*	19.8 ± 0.1 a	17.7 ± 0.4 a	1.87	0.095
	C. cos 2d before *B. inv*	19.1 ± 0.8 a	17.5 ± 0.6 a	1.31	0.2306
	C. cos 3d before *B. inv*	19.5 ± 1.1 a	18.3 ± 0.6 a	0.13	0.8944
	Controls	18.6 ± 0.9 a	16.4 ± 1.6 b	2.12	0.0677
		$F_{[7,32]} = 0.45$	$F_{[4,20]} = 3.68$		
		$P = 0.8631$	$P = 0.0407$		
25	Infested same day	11.0 ± 0.8 a	10.9 ± 1.0 a	0.35	0.7328
	B. inv 1d before *C. cos*	11.2 ± 0.5 a	10.0 ± 0.1 a	1.66	0.1724
	B. inv 2d before *C. cos*	9.7 ± 0.3 b	10.7 ± 0.7 a	0.67	0.5644
	B. inv 3d before *C. cos*	9.6 ± 0.6 b	10.0 ± 0.2 a	1.73	0.1584
	C. cos 1d before *B. inv*	11.4 ± 0.4 a	10.0 ± 0.1 a	1.98	0.0957
	C. cos 2d before *B. inv*	11.6 ± 0.3 a	9.7± 0.2 ab	0.50	0.0634
	C. cos 3d before *B. inv*	11.3 ± 0.2 a	10.1 ± 0.3 a	1.55	0.1957
	Controls	11.6 ± 0.4 a	8.8 ± 0.6 b	4.57	0.0027
		$F_{[7,32]} = 2.77$	$F_{[7,32]} = 1.04$		
		$P = 0.0227$	$P = 0.0445$		
30	Infested same day	8.5 ± 0.3 a	8.3± 0.9 bc	1.86	0.1115
	B. inv 1d before *C. cos*	7.3 ± 0.4 a	8.0 ± 0.4 bc	1.90	0.1066
	B. inv 2d before *C. cos*	7.2 ± 0.7 a	6.5 ± 0.2 c	1.95	0.1133
	B. inv 3d before *C. cos*	7.0 ± 1.0 a	nd	-	-
	C. cos 1d before *B. inv*	8.0 ± 0.1 a	6.3 ± 0.5 c	1.06	0.3385
	C. cos 2d before *B. inv*	8.0 ± 0.5 a	8.5 ± 0.5 bc	0.37	0.7283
	C. cos 3d before *B. inv*	8.4 ± 0.3 a	8.1 ± 0.5 bc	1.06	0.3506
	Controls	8.2 ± 0.6 a	10.7 ± 0.1 a	0.89	0.3970
		$F_{[7,32]} = 1.35$	$F_{[6,28]} = 3.41$		
		$P = 0.2598$	$P = 0.0274$		

B. inv = *Bactrocera invadens*, *C. cos* = *Ceratitis cosyra*, nd = not determined, Means (± SE) in same column followed by the same letter are not significantly different [Student Newman-Keuls (SNK) test, $P = 0.05$].

Appendix 4 Effect of interspecific competition between *Bactrocera invadens* and *Ceratitis capitata* at different temperature on the duration of pupal development of the two species

Temp	Infestation sequence	Pupa development time			
		B. invadens	*C. capitata*	*t-value*	*P*
15	Infested same day	33.3 ± 0.9 ab	36.2 ± 2.2 a	1.22	0.2590
	B. inv 1d before *C. cap*	32.6 ± 0.4 ab	34.5 ± 2.4 ab	0.77	0.4639
	B. inv 2d before *C. cap*	33.5 ± 0.4 ab	33.6 ± 0.8 ab	0.14	0.8944
	B. inv 3d before *C. cap*	34.9 ± 3.3 ab	36.4 ± 2.7 a	0.34	0.7426
	C. cap 1d before *B. inv*	27.7 ± 1.8 b	33.3 ± 2.3 ab	1.93	0.0903
	C. cap 2d before *B. inv*	32.6 ± 1.8 ab	34.0 ± 2.5 ab	0.47	0.6517
	C. cap 3d before *B. inv*	36.5 ± 1.9 a	30.4 ± 1.8 b	2.29	0.0510
	Controls	33.5 ± 0.4 ab	33.4 ± 0.5 ab	0.12	0.9100
		$F_{[7,32]} = 2.30$	$F_{[7,32]} = 0.91$		
		$P = 0.0214$	$P = 0.0311$		
20	Infested same day	20.7 ± 0.5 a	16.4 ± 0.5 ab	6.45	0.0002
	B. inv 1d before *C. cap*	19.8 ± 1.0 a	17.7 ± 0.4 a	1.93	0.0296
	B. inv 2d before *C. cap*	19.1 ± 0.8 ab	17.5 ± 0.6 a	1.72	0.1234
	B. inv 3d before *C. cap*	18.2 ± 0.5 ab	18.3 ± 0.6 a	2.16	0.0627
	C. cap 1d before *B. inv*	15.2 ± 1.2 b	15.6 ± 0.3 b	0.33	0.7507
	C. cap 2d before *B. inv*	16.3 ± 0.3 ab	15.3 ± 0.6 b	1.57	0.1556
	C. cap 3d before *B. inv*	15.8 ± 1.0 b	15.5 ± 0.4 b	0.29	0.7763
	Controls	18.5 ± 0.6 ab	16.4 ± 0.4 ab	2.88	0.0204
		$F_{[7,32]} = 5.07$	$F_{[7,32]} = 10.49$		
		$P = 0.0006$	$P = 0.0001$		
25	Infested same day	12.9 ± 0.6 a	13.2 ± 0.7 a	0.50	0.6282
	B. inv 1d before *C. cap*	11.4 ± 0.4 ab	10.0 ± 0.1 b	3.83	0.0150
	B. inv 2d before *C. cap*	11.6 ± 0.3 ab	9.7 ± 0.3 b	4.88	0.0012
	B. inv 3d before *C. cap*	11.3 ± 0.2 ab	10.1 ± 0.3 b	3.11	0.0144
	C. cap 1d before *B. inv*	11.1 ± 0.2 ab	13.1 ± 0.7 a	2.70	0.0272
	C. cap 2d before *B. inv*	11.8 ± 0.6 ab	11.1 ± 1.0 ab	0.64	0.5395
	C. cap 3d before *B. inv*	11.0 ± 0.3 b	10.4 ± 0.5 b	1.01	0.3429
	Controls	12.3 ± 0.4 ab	10.2 ± 0.3 b	4.56	0.0018
		$F_{[7,32]} = 2.54$	$F_{[7,32]} = 6.62$		
		$P = 0.0336$	$P = 0.0001$		
30	Infested same day	9.8 ± 0.4 ab	8.7 ± 0.1 ab	2.39	0.0439
	B. inv 1d before *C. cap*	8.0 ± 0.1 c	6.3 ± 0.5 c	3.49	0.0082
	B. inv 2d before *C. cap*	8.0 ± 0.5 c	8.5 ± 0.2 ab	0.95	0.3688
	B. inv 3d before *C. cap*	8.4 ± 0.3 bc	8.1 ± 0.4 b	0.76	0.4665
	C. cap 1d before *B. inv*	10.2 ± 0.3 a	8.7 ± 0.1 ab	4.04	0.0038
	C. cap 2d before *B. inv*	9.6 ± 0.3 abc	8.6 ± 0.1 ab	3.15	0.0737
	C. cap 3d before *B. inv*	8.8 ± 0.7 abc	8.8 ± 0.1 ab	0.55	0.9576
	Controls	9.3 ± 0.2 abc	9.3 ± 0.1 a	0.12	0.9109
		$F_{[7,32]} = 4.40$	$F_{[7,32]} = 14.19$		
		$P = 0.0016$	$P = 0.0001$		

B. inv = *Bactrocera invadens*, *C. cap* = *Ceratitis capitata*, Means (± SE) in same column followed by the same letter are not significantly different [Student Newman-Keuls (SNK) test, $P = 0.05$].