CLIMATE CHANGE INDUCED-EFFECT ON BIOLOGY AND ECOLOGY OF AVOCADO INSECT PESTS ALONG ALTITUDINAL GRADIENT OF TAITA HILLS AND MOUNT KILIMANJARO

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DECLARATION

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

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This thesis is dedicated to my beloved father; the late Mwalimu Nicholas Odanga.

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GENERAL ABSTRACT

Avocado, *Persea americana* Miller (Lauraceae), is an important fruit crop cultivated by small-holder farmers along Afrotropical highlands of Taita Hills in South-eastern Kenya and Mount Kilimanjaro in Northern Tanzania. The small-holder farmers in these East African regions generate substantial food and cash from avocado fruits. However, the avocado crop is faced with challenges of infestation by insect pests some of which have detrimental effects on quality of avocado fruits that led to quarantine restrictions in the year 2008/2009 with substantial economic loss to East African region. Furthermore, biology, ecology and spatiotemporal distribution of the major pest species are not well explored in avocado cropping systems of East Africa in relation to changing climate. This study was, therefore, initiated to assess population dynamics of key avocado pests along altitudinal gradient of Taita Hills and Mount Kilimanjaro and further generate species distribution models of the major insect pest of avocado fruits using present and projected climate change scenarios that can be extrapolated to other avocado farming areas. The first specific objective of this study was to determine species diversity of arthropods associated with avocado plant and assess population changes of key insect pests of avocado fruits along altitudinal gradient of Taita Hills and Mount Kilimanjaro. This study further aimed at generating development, reproduction, longevity and mortality rates of the major avocado insect pest and its egg parasitoid at six constant temperatures (15, 20, 25, 30, 32.5 and 35°C), with a relative humidity of 75% ±5 and a L12:D12 photoperiod using phenology models. The final objective was to generate phytosanitary risk maps of the major avocado insect pest and its egg parasitoid using temperature-dependent phenology models based on present and projected climate change scenarios in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. Geographical information system environment implemented in Insect Life Cycle Modeling software (ILCYM) was used for spatial modelling of phenology data.

Findings from fruit observations, insect lures and canopy sampling for two consecutive years from August 2012 to July 2014 revealed that fifty three arthropod species were found inhabiting avocado plant in the two study areas. Observation of mature avocado fruits in lowland areas during harvesting seasons revealed that percentage damage levels was highest due to attacks by Asian invasive fruit fly (*Bactrocera invadens*) (29.3% ± 2.7; mean ± se; ground collected) followed by false codling moth (*Thaumatotibia leucotreta*) (20.3% ± 1.9; tree collected) and the least was by greenhouse thrips (*Heliothrips haemorrhoidalis*) (16.8% ± 0.8; tree collected). However, fruit infestation was extremely minimal in highlands. This findings, therefore, imply that orchards at higher elevation (>1600 masl) of Taita Hills and Mount Kilimanjaro can be considered as areas of low pest (*Bactrocera invadens*) prevalence from which avocado fruits can be exported to international market. *Bactrocera invadens* (Diptera: Tephritidae) was highly abundant in altitudes below 1500m a.s.l where temperatures were warmer throughout the year whereas *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) was evenly distributed across all elevation ranges. *Heliothrips haemorrhoidalis* (Thysanoptera: Thripidae) was more abundant in elevations above 1200m a.s.l where productive avocado trees were plentiful. The three key insect pests were recorded throughout the year in the two transects but their population fluctuated drastically depending on weather seasonality. Field data further revealed that temperature was significant ($p < 0.0001$) environmental parameter that best explained change in abundance of the major pest species (*Bactrocera invadens*) of avocado fruits. Consequently, a constant temperature-dependent phenology (life-table) experiment was carried out on *Bactrocera invadens* and its egg parasitoid using colonies established from a population that were captured during field sampling. The laboratory based phenology experiments established that optimum developmental rate of *Bactrocera invadens* was 32.5ºC and the rate declined drastically at temperatures above 35ºC, with 38.8 ºC being maximum temperature where no development took place. Temperature gradient below 15°C was unfavorable to the development of *Bactrocera invadens* and *Fopius arisanus*. The study further revealed that infestation of *Bactrocera invadens* eggs by its specific parasitoid *Fopius arisanus* was high in temperatures between 25 and 30ºC. Species distribution maps generated from the phenological models and field environmental data using projected climate change scenario of 2055 revealed that *Bactrocera invadens* and it egg parasitoid are lowland species whose potential distribution range was predicted to expand towards higher elevations in the next 42 years due to global warming. Establishment and generation indices of *Bactrocera invadens* were high enough indicating that it will remain a serious insect pest of avocado fruits in Taita Hills and Mount Kilimanjaro. Species distribution maps generated based on the establishment and generation indices can be used in designing effective bio-control strategies of *Bactrocera invadens* by pin-pointing exact location in the two study areas for release of the egg parasitoid (*Fopius arisanus*) of Asian invasive fruit fly since their preferred current and future projected habitat conditions are interrelated.

Key words: Avocado, pests, altitudinal gradient, Insect Life Cycle Modeling software (ILCYM), climate change, risk maps, Taita Hills, Mount Kilimanjaro, East Africa.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

Avocado (*Persea americana* Miller, Lauraceae) is one of the key horticultural crops in the world that face stress emanating from climate change (UNEP, 2009; Puri & Ramamurthy, 2009; Yesuf *et al*., 2008; IPCC, 2007; NEMA, 2007; World Wide Fund for Nature, 2006; IPCC 1996; World Commission on Environment and Development, 1987). This is because avocado plants thrives well in agro-ecosystems with relatively high altitude between 1000m a.s.l and 2600m a.s.l that receive average annual precipitation ranging from 120mm to 160mm and average temperature of 21^0C (Griesbach, 2005; Wasilwa *et al*., 2004; Bergh, 1992). Such ecosystems are located near indigenous habitats with favourable agricultural conditions as exemplified by Taita Hills and Mount Kilimanjaro in East Africa. The land area available for avocado farming in Afrotropical highlands of Taita Hills in South-Eastern Kenya and Mount Kilimanjaro in Northern Tanzania is limited and the potential farmland is shrinking as a result of ecological degradation. Furthermore, high yielding varieties of crops such as maize, beans, vegetables, coffee, onions and tomatoes are being grown on the same land to increase agricultural output. However, the high yielding crops require continued supply of large quantities of water and fertilizer. The more fertilizer used means extra emissions of green house gases (IPCC, 2007). Factors mentioned above in conjunction with wanton deforestation may contribute to gradual increase in regional temperature and hence climate change in the East African montane highlands of Taita Hills in Kenya and Mount Kilimanjaro, Tanzania. Climate change has had detrimental effect on general biota in Afrotropical region and more so on insect pests resulting in higher infestation of crops (Puri & Ramamurthy, 2009).

Climate change influences population dynamics and status of avocado insect pests (Cannon, 1998; Porter *et al*., 1991). These effects can be direct, through influence that weather may have on biology and behavior of insects (Samways, 2005; Birch, 1957; Birch, 1953) or can be mediated by host plants, competitors and natural enemies of pest species (Bale *et al.,* 2002; Harrington *et al*., 2001; Cammell & Knight, 1992). Furthermore, the increase in temperature, moisture and carbon dioxide ($CO_{2)}$ are the major predicted climate change scenarios that have impact on both insect pests and their host plants (Wolstenholme, 2002; Coakley *et al*., 1999; IPCC 1996). This is because insects cannot internally regulate their own temperature and their development depends on the environment which they are exposed to (World Wide Fund for Nature, 2006). Several mathematical models are explored by biologists to unravel the potential relations between natural phenomena and insect pests or parasitoids, and further understand their relationship with changing environmental variables such as temperature and precipitation (Elith & Leathwick, 2009; Phillips *et al*., 2006). Predicted scenarios can provide vital information on management of avocado pests aimed at minimizing fruit infestation and thereby ensuring availability of quality fruits for export and domestic market (MEA, 2005; Brotons *et al*., 2004). Hence, understanding current and future climate-induced effects on agriculture can play a key role in improving farming of fruit crops including avocado (Slegers & De Graaff, 2008).

The upsurge of avocado pests in Sub-Saharan Africa over the last decade is because they are highly mobile and polyphagous insects (Mohamed *et al*., 2008; Porter *et al*., 1991). This could be attributed to many factors including climate change, introduction of new avocado crop varieties that are susceptible to insect pests and lack of quality phytosanitary protocols. There has been an upsurge of thrips (Thysonoptera: Thripidae), fruit flies (Diptera: Tephritidae) and moths

(Lepidoptera: Tortricidae) population (Prinsloo & Uys, 2015; Ware *et al.*, 2012; CPC, 2007). Other notable avocado pests include bugs (Hemiptera), scales insects (Hemiptera), whiteflies (Hemiptera), weevils (Coleoptera) (CPC, 2007; Wysoki *et al.*, 1981; Du Toit *et al.,* 1979; McKenzie, 1935). The most noticeable insect pests include thrips, fruit flies and false codling moths which infests, accelerate rotting or leave marks on pericarps of avocado fruits making them undesirable for marketing (Prinsloo & Uys, 2015; Cammell & Knight, 1992). These pests impact negatively on the livelihood of many small-holder avocado farmers in Africa.

False codling moth (*Thaumatotibia leucotreta*; Tortricidae) has been reported in many African countries (Prinsloo & Uys, 2015; CPC, 2007). It is widespread in East Africa possibly due to favorable climate and abundance of food resources. Its larvae are known to cause serious damage to avocado, citrus, cotton, macadamia, mangoes, and many others (Erichsen & Schoeman, 1994; Van der Geest *et al.*, 1991; Reed, 1974). False codling moth females occasionally oviposit on avocado fruit but the larvae are not able to mature to adult. The larva burrows tunnels into the fruit just beneath the fruit skin forming a dark lesion. Some of the major pests of fruits in Africa are Tephritid fruit flies (Correia *et al.*, 2008; Ekesi & Billah, 2006; Ekesi *et al.*, 2006). They include the Mediterranean fruit fly, *Ceratitis capitata*, which is one of the world's most destructive fruit pests that is widespread in sub-Sahara Africa (Papadopoulos, 2008). The *Natal* fruit fly, *Ceratitis rosa* is highly polyphagous causing damage to a wide range of fruit crops and tends to displace *Ceratitis capitata* in some areas where both species occur because their biology, ecology and distribution patterns are presumably similar (Hancock, 1989). The mango fruit fly, *Ceratitis cosyra*, is a pest in smallholder and commercial mango orchards across sub-Saharan Africa, where it is very destructive in some countries (Vayssières *et al.*, 2009;

Mohamed, 2003; Lux *et al*., 1998) and also infests avocado fruits (Prinsloo & Uys, 2015). The invasive fruit fly species *Bactrocera invadens* (*Bactrocera dorsalis*) has been spreading rapidly across Africa since its first encounter (Drew *et al*., 2005) and poses a significant threat to many economically important fruit crops such as avocado (Schutze *et al*., 2015a & 2b; Mohamed *et al.,* 2008; Mwatawala *et al*., 2004 & 2006). Most fruit fly species thrive well in warmer environment (Mwatawala *et al*., 2009). Several Thysanopterans attack avocado crop with the greenhouse thrips (*Heliothrips haemorrhoidalis*) being the most reported species that infest leaves and fruits (Hoddle, 2002a & 2002b; Stevens *et al*., 1999). *Heliothrips haemorrhoidalis* feed largely on the leaves and fruit-lets resulting in mature avocado fruits that have visible patches of rough brownish pericarp. Key to control and proper management of avocado insect pests is not only to rely on harmful chemicals but to enhance use of biological parasitoids, biopesticides and increasing awareness among local farming communities (Mohamed *et al.,* 2008; Mohamed *et al*., 2007; Ekesi & Billah, 2006; Ekesi *et al*., 2006). Furthermore, knowledge of avocado plant phenological events is vital in assisting scientists by providing accurate calendar month for timely biological pest control (Chmielewski *et al*., 2004; Stevens *et al*., 1999).

Mathematical models have become important analytical tools for predicting of the dynamics of insect populations in agro ecosystems and hence assessments of pests' phytosanitary risks. Predicted climate change scenarios can be simulated using species distribution models such as general linear models (GLMs) generalized additive models (GAMs), climate response surface models (CRS), artificial neural networks (ANNs), genetic algorithms (GAs), maximum entropy models (MaxEnt) and Insect Life Cycle Model (ILYCM) (Sporleder, *et al*., 2009; Peterson, 2003). Species distribution models are statistical patterns that relate surveyed data on species distribution with the associated environmental and geographical characteristics of the surveyed locations (Elith & Leathwick, 2009). These models permit analysis of a wide variety of biodiversity phenomena including current geographic distributions (Elith & Burgman, 2002; Chen & Peterson, 2002), future potential distributions under scenarios of climate change (Thomas *et al*., 2004), species' invasions (Peterson, 2003) and damage by pest organisms (Sánchez-Cordero & Martínez-Meyer, 2000). The models have an extension that can be linked with geographical information systems (GIS) for spatial visualization.

Temperature-dependent Insect Life Cycle Modeling Software (ILCYM) can be used to predict time of events during a development period of a pest species (Sporleder, *et al*., 2009). Evaluation using ILCYM requires input of laboratory-based life-cycle (phenological) data of a species because variation in daily weather patterns in orchards is not a good basis for assessing phytosanitary risks of pests. Measuring the amount of heat accumulated over time in laboratory provides a physiological time scale of a pest that is biologically more accurate than calendar days. Insect pests require a certain amount of heat to develop from one point to another in their life-cycle; from eggs-pupae-adult and during reproduction. Thus phenology models for insect species based on temperature such as ILCYM are important analytical tools for predicting, evaluating, and understanding the dynamics of pest populations in ecosystems (Nietschke, *et al*., 2007). ILCYM model is also important in predicting impact of climate change on insect species, phytosanitary risk assessments and hence generating integrated pest management (IPM) protocols. These include identification of potential release sites for parasitoids, simulation of field performance of bio-pesticides and their application frequencies. Efficient bio-control strategies are core to success in reduction of avocado insect pests.

1.2 Problem statement

Impact of climate change on the distribution of pest species may be more profound in mountainous ranges than the lowland areas of Sub-Sahara Africa. This is largely because mountains have steep elevations that allow species to track changing environment over a short distances. The Afrotropical mountainous ecosystems are characterized by steep elevation gradients along habitats; lowland plains, montane forests and heathland communities at high altitudes. Each elevational zone has an associated weather patterns or environmental parameters. In East Africa, the highlands of Mount Kilimanjaro in Tanzania and Taita Hills in Kenya provide important ecosystem service values arising from the water towers they provide for the low lying areas, flourishing avocado orchards, nutrient recycling and important biodiversity habitats of beneficial insects. Consequently, a gradual change in temperature and relative humidity along the highlands may enhance outbreaks of avocado insect pests in areas or altitudinal zones that have never experienced it. Therefore, understanding current and future impacts of climate change on key avocado insect pests and their enemies can assist in designing preventive integrated pest management strategies in East Africa. However, there is limited data available for Taita Hills, Kenya and Mount Kilimanjaro, Tanzania that can be used to infer the effect of climate change on avocado insect pests.

1.3 Justification of the study

Avocado is grown in over 18,500 ha yielding 203,000 tons annually and is thus important for ensuring food security in Ethiopia, Kenya and Tanzania. However, the major challenge to avocado farmers in Eastern Africa is the insect pests that result in direct damage to fruits leading to economic losses. In addition to direct fruit infestation, several indirect losses result from quarantine restrictions that are imposed by importing countries to prevent entry and establishment of invasive pests. Such drastic measures restrict export of avocado fruits from Eastern Africa to the lucrative overseas (European Union) market. For instance, Kenya lost 1.5 million € in avocado exports because of fruit flies quarantine restrictions in 2008 (HCDA, 2009) but there is no information on spatiotemporal population changes of the major avocado insect pests and its fruit infestation levels in Eastern Africa.

Distribution, abundance and pest status of major avocado insect pests may vary with altitudinal gradient of an agro-ecosystem, humidity and temperature. This is because turnover of biotic life zones occurs over short linear distances and with relatively small changes in elevation resulting in significant variations in temperature and relative humidity. An uphill shift of temperature in low elevation range can expose avocado pests to either suitable or harsh conditions or trigger movement of species to new hosts. Uphill range shift may also create suitable environment for increase in population of invasive avocado pest species and consequently reduce numbers of natural enemies of indigenous pests. This may enhance infestation levels of avocado by pest species. Since avocado can be grown up to 2500m a.s.l, the crop and its associated functional agro-biodiversity may serve as sensitive indicators of climatic variation. Information on the underlying mechanisms and eco-physiological responses of the target pests to climate warming is lacking and is required to adapt various pest management tools to current and future impact of climate change. Furthermore, the knowledge on the geographical distribution of avocado insect pest and parasitoid species is crucial for crop production and spatial planning. But a detailed data on distribution of avocado pests based on species modelling is not available in eastern Africa and collecting such data is very costly and labour intensive. This study aimed at generating predictive models based on detailed life table experiments for estimating species spatial distribution patterns of key avocado insect pest in East Africa. The study outputs on avocado insect pests in farmlands adjacent to Taita Hills, Kenya and Mount Kilimanjaro, Tanzania can be utilized by government agencies to develop efficient integrated pest management policies that are aimed at improving avocado crop farming in East Africa.

1.4 Broad objective

The broad objective of the study was to assess population dynamics of major avocado insect pests along altitudinal gradient of Taita Hills and Mount Kilimanjaro and further generate species distribution models of the key insect pest using present and projected climate change scenarios.

1.4.1 Specific objectives

i. To determine species diversity of arthropod pests associated with avocado plant and, further, assess population changes of key insect pests of avocado fruits along altitudinal gradient in Mount Kilimanjaro, Tanzania and Taita Hills, Kenya.
ii. To establish effects of different constant temperatures on life-table parameters of major avocado insect pest and its related parasitoid.
iii. To generate phenological models from temperature-dependent life table statistics for the major avocado insect pest and its associated parasitoid.
iv. To generate risk maps of the major avocado insect pest and its associated parasitoid using species distribution models based on present and projected climate change scenarios in Mount Kilimanjaro, Tanzania and Taita Hills, Kenya.

1.4.2 Research questions

i. Does the population density of key insect pests of avocado fruits increase with elevation in Mount Kilimanjaro, Tanzania and Taita Hills, Kenya?

ii. Do changes in temperature influence biology of the major avocado insect pest and its egg parasitoid?

iii. Can changes in the temperature-dependent life table parameters be modeled to establish phenological patterns of the major avocado insect pest and its egg parasitoid?

iv. Will future projected climate variability induce changes in distribution of major avocado insect pest and its egg parasitoid in Mount Kilimanjaro, Tanzania and Taita Hills, Kenya?

CHAPTER TWO

LITERATURE REVIEW

2.1 Avocado biology and ecology

Avocado (*Persea americana* Mill) is found worldwide where growing conditions are suitable although the crop is a native of Central America and the West Indies (Bergh & Ellstrand, 1986). *Persea americana* Mill belongs to the family Lauraceae and related plant species includes camphor, sassafras, cinnamon and laurels (Chen *et al*., 2008; Bergh, 1992). Avocado tree is subtropical and evergreen, though heavy leaf fall may occur during profuse blossoming and when the tree is affected by root rot. An avocado plant has dark green leaves which are spirally arranged and varies in size (11.5cm ± 1.5 by 22.5cm ± 2.5; mean ± se). Structure of the leaves varies from entire, elliptic or ovate to lanceolate (Chen *et al*., 2008) and plant growth habit varies from tall-upright to well-shaped and spreading (Whiley & Schaffer, 1994). Avocado fruit is one seeded berry with a single large seed that is composed of two cotyledons enclosing an embryo and is surrounded by a thick buttery fleshy mesocarp. The skin of an avocado fruit varies from very thin to medium to thick ~0.65cm (Griesbach, 2005).

Sandy loam soil is best for avocado cultivation but the plant cannot tolerate heavy soil with water logging situations. This is because the avocado tree requires a well aerated soil that is ideally more than one metre deep and with pH ranging from 5 to around 7 (Neuhaus, 2003). An avocado plant is very sensitive to stress from extreme temperatures and saline soil conditions that affects its productivity (Wolstenholme, 2002; Havaux & Lannoye, 1987). Therefore, avocado trees cannot tolerate soils which have pH above or below their limit. High salinity leads to leaf burn whereas flooding causes root rot (Neuhaus, 2003). Avocado roots are shallow and a prolonged dry condition during flowering or fruit set can accelerate dropping of flowers and fruitlets (Mugambi, 2002). For example, extreme temperatures coupled with less than 50% relative humidity do depress photosynthesis thus lowering avocado fruit yields (Griesbach, 2005). Prolonged cold season reduces availability of solar radiation leading to inefficient transpiration and photosynthesis. Excess winds can easily damage avocado trees because branches of *Persea americana* are brittle. Avocado plants, therefore, requires enough rainfall, moderate dry and wet seasons for effective productivity.

Avocadoes can exhibit two growth flushes per year and may flower for up to six months; the trees often exhibit an alternate bearing habit (Whiley, 2002; Robertson, 1969). Fruit can remain on the tree for several months after maturity hence both "old" and "new" fruit may be found on the same plant (Wolstenholme, 2002; Robertson, 1969). Therefore, the harvest season may be extended over several months, so growers generally pick to meet market demands. Avocados are graded based upon the size and appearance of the fruit. Fruits of avocado are unique in that they cannot ripen until harvested and may be left on the tree for some time depending on variety after reaching maturity (Griesbach, 2005). In East Africa, avocado harvest period extends over period of time with May and July being peak months. Avocado fruits are generally harvested using cherry pickers from the ground, on ladders, and in the case of very large trees by climbing. Picking clippers and picking poles are also used for harvesting avocado fruits. An avocado fruit is required to be clipped with a small amount of fruit stem remaining to slow ripening but some fruits are snapped from the tree without any fruit stem remaining. When fruits without stems are packed, they have an accelerated ripening before reaching their market and fruits pests may take advantage to attack the avocado fruits (Griesbach, 2005; Standardization Committee Report, 1924-1925).

Furthermore, fruit thinning or size picking is performed in order to allow the remaining fruit to increase in size, so that fruits of commercially desirable sizes can be harvested (Chege *et al.*, 2006; Erickson, 1964). Thinning also reduces physical contact between the fruits thereby minimizing habitats for thrips, mealybugs, omnivorous looper, leaf rollers and other insect pests (Griesbach, 2005; Standardization Committee Report, 1924-1925). Thrips do not like exposed habitat of solitary fruit, so by size-picking, growers achieve two goals at the same time (Stevens *et al.*, 1999). However, size-picking is not practiced industry-wide because it is expensive. On the other hand, if there is a shortage of, and consequent price advantage to a particular size category, some growers will concentrate on picking that particular size to meet market demand, leaving remaining fruit for a subsequent harvest. Harvest should not be done right after a rain because of the increased possibility of anthracnose; a disease that affects avocado fruits (Schaffer *et al*, 1992). The harvested fruits must be protected from the sun by covering the clean storage bins with avocado leaves or small branches while in orchards.

2.2 Avocado farming in East Africa

East Africa has natural environment for avocado production unlike in U.S.A. and Israel where farmers spend substantial resources on production (Mugambi, 2002; McKenzie, 1935). Favourable factors in Kenya and Tanzania include adequate water, good soil drainage, insignificant salinity problems, and lack of extreme weather such as frost or heat waves. In Eastern Africa, production is at subsistence scale with avocado trees per farm varying from three to twelve (Griesbach, 2005). Eight five percent of avocado in East Africa is grown mainly by small-scale growers for subsistence, local and export markets. Because of the small farms modern day agricultural technology is not utilized but proper research can resolve challenges faced in avocado productivity through use of biological control of pests. Contrary, commercial plantations of avocado specifically for export market are grown by conglomerates such as Kakuzi limited in Kenya and Afrocado company in Moshi, Tanzania. The leading importer of Eastern African avocadoes is European Union and South Africa which have imposed strict phytosanitary rules (Ekesi *et al*., 2016; Otieno, 2011).

Avocado tree grows throughout the year with the main of fruit harvesting commencing in March through September but smaller volumes are collected in minor season between October and February. Production areas in East Africa vary in altitude from 800m to 2800m with 85% production done by small-scale farmers while the remaining 15% originate from large estates (Griesbach, 2005). Avocado is, therefore, an important crop to local communities adjacent to Afromontane highlands as it is enhances the agro forestry conservation concept (Cooper *et al.*, 2003; Chege *et al*., 2006). It also plays a big role in not only improving food security but also economic livelihood in the region. In Kenya, the vast majority of avocado cultivation is concentrated in areas between Nairobi and Mount Kenya in the highlands located between 1,200 and 1,800 m above sea level (Mugambi, 2002). The volcanic soils are deep, generally well-drained, and fertile, and generally, the need for irrigation of the farmlands is minimal. Commercial companies practice supplemental irrigation especially during flowering and fruit setting. However, highlands near the coastal areas of East Africa are emerging as a favourable production region for higher quality of avocado fruits in the last few years due to favourable weather (Chege *et al*., 2006). This makes Kenya and Tanzania potential countries for significant avocado farming.

In recent years, avocado has become one of the most important tropical export fruits in the world (Whiley, 2002; Bergh, 1992). Furthermore, the hectarage under avocado having expanded, the production increased marginally while value of the fruit decreased significantly (Griesbach, 2005). This has been attributed to poor farming practices and increase in invasive insect pests as a result of impacts of climate change (Griesbach, 2005; Bale *et al.,* 2002; Clegg *et al*., 1999; Bergh, 1992; Nakasone, 1976). Majority of the East African avocadoes from small-scale farmers, therefore, cannot be marketed well because of their low quality and hence calls for further studies (Chege *et al*., 2006; Mugambi, 2002). A good knowledge of ecology and taxonomy of the key avocado insect pests and associated natural enemies may form a better knowledge on their biological control and, therefore, enhance avocado production.

2.3 Previous studies on insect pests of avocado and their natural enemies

Historical studies reveal that several insect pests have a detrimental economic impact on avocado plant (*Persea americana* Mill). The pests include thrips, scale insects, fruit flies, whiteflies and beetles (Ekesi, *et al*., 2009; Ekesi &Billah, 2006; Hoddle, 2002a; Armstrong *et al*., 1983; Du Toit *et al.,* 1979; McKenzie, 1935). Ware *et al* (2012), CPC (2007) and Dennill and Erasmus (1992) further reports that insects of high economic importance in avocado orchards in Africa are false codling moth {*Thaumatotibia (Cryptophlebia) leucotreta*; Tortricidae}, thrips and several species of fruit flies (Diptera; Tephritidae). Most of the reported findings of the studies on avocado insect pests in Sub-Sahara Africa are from South Africa and there is no comprehensive information from Eastern Africa.

True fruit flies (Diptera: Tephritidae) are some of the most devastating crop pests in the world. In Africa, there are several species that attack fruits and vegetables which belong to important genera; *Bactrocera*, *Dacus* and *Ceratitis*. The family Tephritidae includes more than 5000 species worldwide, approximately 1400 species of which develop in fleshy fruits (Norrbom *et al*., 1999; Aluja *et al*., 2004). Nearly 250 of these species are capable of achieving pest status by feeding on plants of economic importance (White and Elson-Harris, 1992). Some of the key species of economic importance in East Africa include *Ceratitis capitata*, *Ceratitis rosa*, *Ceratitis cosyra* and *Bactrocera invadens* (Ekesi & Billah, 2006; Ekesi, *et al*., 2007; Ekesi, *et al*., 2009; Norrbom *et al*., 1999). Fertilized fruit fly females usually search for a suitable host fruit to lay its eggs that hatch into larvae. The larvae feed on the flesh of the fruit causing it to decompose. When fully grown the larvae stop feeding, leave the fruit and burrow into the soil to pupate. The pupae slowly develop into adult females and males that repeat the cycle (Ekesi & Billah, 2006). Several parasitoids (Hymenoptera: Braconidae) are known to attack fruit flies and have been used as biological control of fruit flies. *Fopius arisanus* attack eggs whereas *Diachasmimorpha longicaudata* attack larvae of Tephritidae fruit flies (Rwomushana *et al*., 2008; Mohamed *et al*., 2008; Ekesi, *et al*., 2006; Ekesi & Billah, 2006; Mohamed, 2003). A study on Tephritidae fruit flies that infest avocado fruits and their related natural enemies is a prerequisite.

False codling moth, *Thaumatotibia (Cryptophlebia) leucotreta,* is an internal fruit feeding insect

(Lepidoptera: Tortricidae) of several suitable host crops. False codling moth (FCM) is a generalist with respect to host plant selection and has been recorded feeding on over 50 different plant species worldwide (CPC, 2007; CPC, 2002; Daiber, 1979). Couilloud (1988) reported that the development of false codling moth is limited by cold temperatures as it is predominantly a pest of warm regions. However, generalist feeding strategy of the false codling moth enables its survival in marginal conditions. The moth undergoes a complete metamorphosis. The adult moths emerge from cocoons located on the soil surface, mate and lay between 100 to 400 eggs at 15ºC and 25ºC (Daiber,1980). Female moths undergo a pre-oviposition period of egg maturation. Peak oviposition occurs within three days after emergence and more than 50% of the eggs are laid during the third reproductive period, which varies in length in an inverse relationship to temperature. Oviposition occurs at the highest rate in the early evening near sunset due to its nocturnal behavior (Couilloud, 1988). Eggs are laid singly on fruit and neonate larvae emerge after few days and make entry wounds. Larvae typically complete five instars of development within a fruit then exit and drop to the soil to begin construction of cocoons (Couilloud, 1988). This leaves the avocado fruit with a scar that reduces its marketability.

Several hymenopteran parasitoids and predators are known to attack FCM but their effectiveness is not well explored (Searle, 1964; Prinsloo, 1984). *Pheidole megacephala* and other ant species are known to attack FCM larvae and pupae mostly found on the ground. However, most ants are generalists and play an important role in pest suppression in agro ecosystems. Newton, (1998) reported that the key natural enemy of (*Thaumatotibia (Cryptophlebia) leucotreta*) (Tortricidae) is the egg and larval parasitoids of *Trichogrammatoidea cryptophlebia* Nagaraja (Hymenoptera: Trichogrammatidae).

2.4 Historical weather patterns of some sections of the study areas

There is no comprehensive weather data that is available for the two transects along Taita Hills, Kenya and Mount Kilimanjaro, Tanzania despite the fact that several environmental studies have been carried out in the Afromontane regions (Pellika *et al.*, 2009; Hemp, 2005). Limited data available from Kenya Meteorological Department reveals that the lowland areas of Taita Hills have witnessed increase in temperature for the last 50 years (Figure 2.1a & 2.1b). The Voi (700m a.s.l) weather station data shows that minimum temperature (Figure 2.1a; R^2=0.57) has been increasing more rapidly than maximum temperatures (Figure 2.1b; R^2=0.09) and the scenario is expected to ascent further in the next half century. Voi is within lower altitudinal range of Taita Hills study area. Although, there is no data available from Moshi, Tanzania, the lowland areas of Mount Kilimanjaro mimic the weather patterns of lowland plains of Taita Hills. Therefore, continued increase in temperature is expected in Moshi, Tanzania as envisaged in Taita Hills, Kenya (Hemp, 2005).

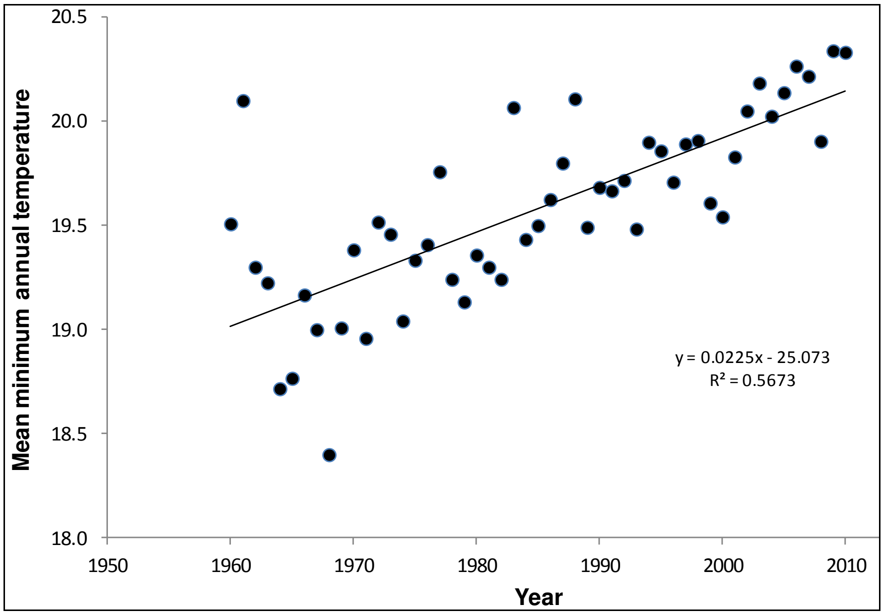


Figure 2.1a: Linear relationship between historical minimum annual temperature and change in years at elevation of 700m a.s.l in Voi, Kenya. Voi is within lower altitudinal zone of Taita Hills study area. Source of raw data: Kenya Meteorological Department.

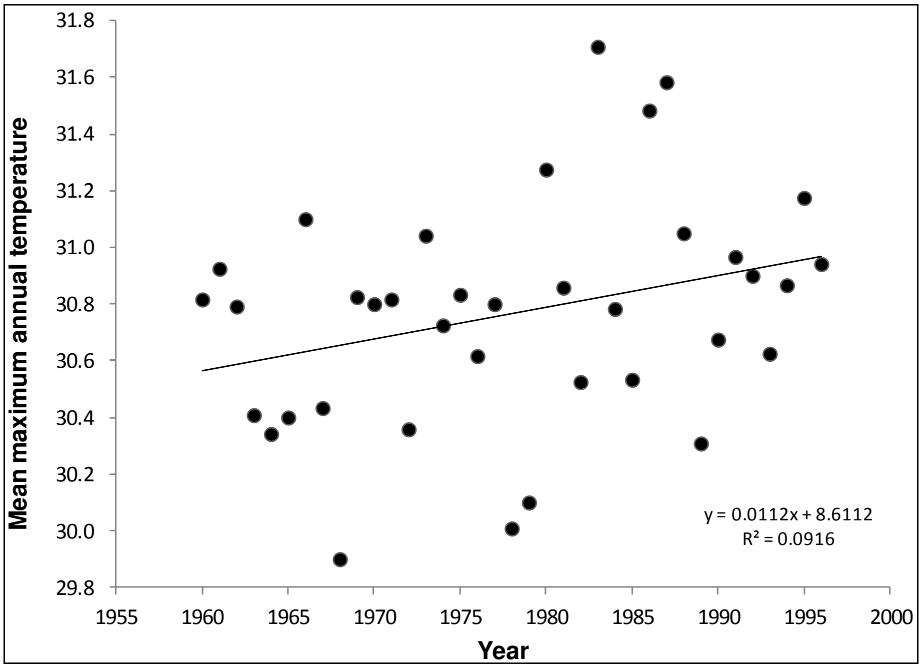


Figure 2.1b: Linear relationship between historical maximum annual temperature and change in years at elevation of 700m a.s.l in Voi, Kenya. Voi is within lower altitudinal zone of Taita Hills study area. Source of raw data: Kenya Meteorological Department.

Gradual increase in temperatures has been witnessed globally over decades. Pellikka *et al.*, (2009) approximated that half of the cloud forests in Taita hills have been cleared for agricultural use for the period ranging from year 1955 to 2004. Such long-term changes have an effect on variations in precipitation and temperature patterns thereby facilitating regional warming of Afromontane highlands. This has a negative impact on the sustainability of agricultural systems especially to small-scale avocado farmers.

Natural and human activities such as induced fires have been cited as being responsible for the upward shift of indigenous forests at Mount Kilimanjaro in the last three decades (Pellika *et al.*, 2009). The Glaciers on the summit of Mount Kilimanjaro have decreased in area by 80 per cent since the early 20th century (Hemp, 2005). While glacial retreat globally has been linked with rising air temperatures, there is evidence that the decline of Kilimanjaro glaciers, along with changes in the boundaries of vegetation zones on the mountain, may be due in large part to a more local trend of decreasing precipitation that began in the 1880s (Hemp, 2005). The changes are attributed to human activities and if this trend continues, Mt. Kilimanjaro summit will continue to be warmer in future (Hemp, 2005).

2.5 Climate change and agriculture

Major research publications report that agriculture is vulnerable to extreme temperatures, droughts, rainfall and floods which reduce crop productivity (UNEP, 2009; IPCC 1996; IPCC 1990). The current status of insect pests of horticultural crops and the general economic cost of agriculture in developing countries are projected to increase due to effects of climate change (WWF, 2006; IPCC 2001). Countries in Sub-Sahara Africa are vulnerable because most farmers practice small-scale horticulture with traditional technologies dominating the sector and agriculture is rain-fed. The agricultural system is a low input and low output farming practice. The effects of climate change make crops and stored produce susceptible to damage by emerging pest species. Furthermore, there has been a shift in the status of several insect pests in the current scenario of climate change, after the introduction of high-yielding varieties of crops through developments in agricultural technology and modifications of farming practices (Dhaliwal & Koul, 2010). In addition, the use of chemicals has lead to problems of pesticide resistance, resurgence and contamination of different components of the environment (Gupta *et al.*, 2009). This has led to build-up of crop pests, with the result that intensity of several pests has increased and many minor pests have assumed the status of major pests. Several new pest problems have appeared in certain regions for the first time due to creation of favourable environment whereas there has been drastic reduction of related natural enemies (Vennila, 2008). A good example is the emergence of *Bactrocera invadens* as a key invasive fruit fly pest species that attacks most fruit crops (Ekesi *et al*., 2009). In most African states, the impacts of climate change are projected to be worse due to heavy dependence on rain-fed agriculture making food security vulnerable.

Agricultural activities are the main agents of fragmentation of Afromontane native forests in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania (Pellika *et al*., 2009; Hemp, 2005). Together with natural phenomenon, these man-made factors impacts on natural ecosystem services such as the global carbon cycle. In this case; carbon released when trees are cut, burned, or as they decompose enter the atmosphere as CO^2 leading to global warming and climate change (IPCC 2001). Key effect of climate change in Sub-Sahara Africa is demonstrated by extreme weather patterns which affect farming. Horticultural crops such as avocado will be highly affected by short-term intense rainfall amounts with irregular patterns and increased temperatures. The projected scenario in East African countries is increased temperatures and decreased mean annual precipitation due to shortened seasons of extreme rainfall (IFPRI, 2004; Richard & Dannele, 2008). The result is reduction of efficiency of natural ecosystems core function of moderating humidity and temperature in adjacent farmlands leading to changes in species diversity (Kokwaro, 1988; Bishop, 1993). Economically important insects are some of the most affected animals. Migration and upsurge of insect pests, most of them being alien species are also expected to accelerate with climate change (Bleher *et al*., 2006). Such changes have an impact on the productivity of avocado crop production resulting in poor fruit quality and quantity leading to economic loss. In this regard, species distribution models are useful tools for estimating the potential for crop pest to occur in a specific agro-ecological zone over time.

2.6 Species distribution models

Species distribution models use spatial information on species occurrences and relate them to environmental data to estimate potential or suitable habitat for species using environmental GIS predictors. There are a number of analytical approaches such as general linear models (GLMs) generalized additive models (GAMs), logistic regression, resource selection function, neural networks, MaxEnt model, classification and boosted regression trees (Thomas *et al*., 2004; Chen & Peterson, 2002). Most scientists employ user-friendly software application called MaxEnt, which stands for Maximum Entropy (Phillips *et al*., 2006; Phillips & Dudík, 2004). This software is designed for estimating species distribution using 'presence-only' data where information is only known about species locations (presences) and not their absences (Phillips *et al*. 2006). The data format may not be the best desirable relative to models using presence-absence data. Historical or secondary sources of data such as museum and herbarium records have location data of surveyed species but it does not provide occurrence information where there was no sampling of the species. Hence, if no other information is available, presence-only models such as MaxEnt are the best choices in generating current and estimating future or past species distribution or ecological patterns. To overcome weakness of presence only species distribution models, the Insect Life Cycle Modeling software (ILCYM) (Sporleder, *et al*, 2007) was designed to use detailed experimental phenology (life-cycle) data sets that are generated under different constant temperatures which simulates field environment. The advantages of ILCYM software is explained in detail below.

2.6.1 Insect life cycle software (ILCYM)

The International Potato center (CIP) initially developed a temperature-driven phenology model (ILCYM) for the potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechidae). Insect Life Cycle Modeling software (ILCYM) model predicted well life-table parameters for different agro ecological zones; the model was validated through field and laboratory data (Sporleder *et al*., 2007). It was used to predict the establishment risk and potential pest activity in specific agro ecologies according to temperature records. Linked with geographic information systems (GIS) and atmospheric temperature the model allows simulating these risk indices on a worldwide scale or using it to predict future changes of these indices due to global warming. Past and future avocado insect pests' and parasitoids' survival scenarios are simulated based on life stages; egg, larvae, pupae and adult (phenological events). Future projected climate data used are acquired from WorldClim database (Nietschke *et al*., 2007). These predictions can be validated using data from temperature dependent survival laboratory experiment. Model validation tool in ILCYM allows the user to evaluate the ability of the developed phenology model to reproduce the insect species behavior under fluctuating temperature conditions.

2.6.2 Advantages of using insect life cycle modeling software (ILCYM)

ILCYM can be used to describe temperature dependence of insect mean developmental rates based on various nonlinear models; exponential, third-order polynomial, Stinnerfunction (Stinner *et al*., 1974), Logan-function (Logan *et al*., 1976), Sharpe & DeMichele model (Sharpe & DeMichele 1977; Schoolfield *et al*., 1981). The Modified Sharpe & DeMichele-model has previously been used because of its biological significance (Sporleder *et al*., 2007). This model postulates that the development of a poikilotherm organism is driven by a rate-determining enzyme, which has three basic reversible energy states (inactive at cold temperature, active at optimum temperatures and inactive at extreme hot temperatures). Furthermore, ILCYM is a process based phenology models or simplified mathematical models, which describe the basic physiological principles of the insect species' growth; its development, survival and reproduction (Sporleder *et al.*, 2009). This is based on detailed laboratory assessments that produces life-table parameters and allow the simulation of populations according to real or interpolated temperature data for a given region and time (Sporleder *et al.*, 2007; Nietschke *et al.*, 2007). The ILCYM software was employed in the study to generate phenological models and risk maps of insect pest and parasitoid using data from life-cycle experiment.

CHAPTER THREE

DIVERSITY AND ABUNDANCE OF ARTHROPOD PESTS ASSOCIATED WITH AVOCADO CROP IN FARMLANDS AT TAITA HILLS AND MOUNT KILIMANJARO

Abstract

Avocado, *Persea americana* Miller, is the main fruit crop cultivated along altitudinal gradient of Taita Hills and Mount Kilimanjaro that are located in East African initial highlands from the Indian Ocean. Local small-holder farmers depend on avocado fruits as source of their livelihood but the crop is faced with challenges of infestation by insect pests. A study was carried out to assess distribution and damage levels by key insect pests in farmlands along slopes of Mount Kilimanjaro in northern Tanzania and Taita Hills in South-eastern Kenya. The broad objective of this study was to determine species diversity of arthropod pests associated with avocado plant and further assess population changes of key insect pests of avocado fruits along altitudinal gradient of the Mount Kilimanjaro region. Study findings revealed that fifty three arthropod species were found inhabiting on the avocado crop. Observation of mature avocado fruits during harvesting seasons revealed that percentage damage levels were highest due to attacks by Asian invasive fruit fly (*Bactrocera invadens*) followed by false codling moth (*Thaumatotibia leucotreta*) and the least was by green house thrips (*Heliothrips haemorrhoidalis*). The three species were the most abundant along altitudinal gradient of the study areas. *Bactrocera invadens* (Diptera: Tephritidae) was highly abundant in lowlands below 1200m a.s.l where temperatures were warmer throughout the year ($p<0.0001$) whereas *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) was evenly distributed across all elevation ranges. *Heliothrips haemorrhoidalis* (Thysanoptera: Thripidae) was more abundant in highlands above 1200m a.s.l where productive avocado trees were plentiful. The three key insect pests were recorded throughout the year along the two transects but their population differed significantly ($p<0.0001$) depending on weather seasonality. The findings proved that the Asian invasive fruit fly (*Bactrocera invadens*) prefers infesting ripened fruits which confirms that it is a serious post-harvest insect pest of avocado fruits. This information can be utilized by policy makers in East Africa to design effective integrated pest management strategies aimed at minimizing infestation of avocado fruits by the key insect pests.

3.1 Introduction

Avocado plant is the most important fruit crop along the agro-ecological zones of Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. The crop supports livelihood of local farmers as food and also from sale of its fruits as raw materials to international markets (Cammell & Knight, 1992; Bergh, 1992). Major importers; European Union and South African, have strict phytosanitary requirements on avocado exports from East Africa due to direct damage to the fruits by insect pest species and economic loss that can occur in their countries. Furthermore, the temporary quarantine restriction by South African government in the year 2009 as a result of fruit damage by insect pests led to severe economic hardship to East African avocado farmers. Chege *et al* (2006) and Mugambi (2002) reported that majority of avocadoes from small-scale farmers in East Africa cannot be marketed well because they are of low quality. This is attributed to inferior farming practices, poor implementation of phytosanitary protocols and increase in avocado insect pests (Dennill & Erasmus, 1992).

Different parts of the avocado crop are infested by numerous insect pests at diverse phases of the plant growth. These pests include; the spiraling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), scale insects, thrips, false codling moth (*Thaumatotibia leucotreta*) and Tephritidae fruit flies (Prinsloo & Uys, 2015; Ware *et al*., 2012; CPC, 2007; Dennill & Erasmus, 1992). Heavy infestation is mostly by larval stages of insect pests of economic importance. Ware *et al* (2012) and Prinsloo & Uys (2015) further reports that Asian invasive fruit fly (*Bactrocera invadens*), false codling moth (*Thaumatotibia leucotreta*) and green house thrips (*Heliothrips haemorrhoidalis*) are the key insect pests of avocado fruits. Larvae of false codling moths and green house thrips infest immature avocado fruits but the Asian invasive fruit fly attacks mostly ripe ones. However, there is no published information listing arthropod pests associated with the avocado crop in East Africa. Establishing a checklist and spatiotemporal distribution status of key avocado insect species can assist in designing pest control strategies aimed at improving avocado farming in eastern Africa.

Spatiotemporal changes in population density of pests and related infestation status are dependent on variability of environmental conditions of a region (Geurts *et al*., 2014; Slegers & De Graaff, 2008; Nyankanga *et al*., 2007). Local altitudinal gradient, monthly temperatures, humidity, rainfall and host availability are some factors that may influence population fluctuations of key avocado insect pests. However, information on spatiotemporal population changes of key insect pests and how they relate with the environment is lacking in avocado farming areas in Tanzania and Kenya. Such critical data is unavailable despite Kenya being the leading producer of avocado fruits (186,292metric tones) in Africa and Tanzania witnessing the highest avocado production growths (20%) between 2005 and 2012 (FAO, 2013; Griesbach, 2005). Moreover, the two countries lie closer to the equator with seasonal tropical conditions that create a challenge of insect pests. This chapter, therefore, describes general diversity of the arthropod pests inhabiting avocado trees and further details the spatiotemporal distribution of key insect pest species that infests avocado fruits at Mount Kilimanjaro and Taita Hills. This chapter focused on the following specific objectives: (1) To establish arthropod species associated with avocado crop in farmlands along slopes of Mount Kilimanjaro and Taita Hills; (2) To identify the key insect pest species of avocado fruits in the two study areas; (3) To assess percentage damage levels on mature avocado fruits by the key insect pests in the two study areas; (4) To describe spatiotemporal population changes of the key insect pests of avocado fruits along altitudinal gradient of the two study areas and (5) To determine how environmental variables influence changes in population density of the major insect pest of avocado fruits in the two study areas.

3.2 Materials and methods

3.2.1 Study areas

The study was carried out along increasing altitudinal gradient from 800 to 1800m a.s.l. in avocado farmlands within Taita Hills, South-Eastern Kenya and South-Eastern slopes of Mt. Kilimanjaro in Northern Tanzania (Figures 3.1a & 3.1b). The highlands in Taita Hills and Mount Kilimanjaro are the first uppermost elevated montane forms inland from the Indian Ocean and are important catchment areas for surrounding lowland areas of Voi and Moshi in Kenya and Tanzania respectively. The study sites in the two East African countries were selected based on the criteria that they represents steep elevation gradient with no large estate avocado farming that would affect the land use patterns and interactions between the lowland and highland environment. Localized form of Hass variety is the dominant avocado crop that is grown in Taita Hills and Mount Kilimanjaro. Elevation was key to division of study transects in Taita Hills and Mount Kilimanjaro into three agro-ecological regions (Table 3.1; Figure 3.1c); lowland (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane zone (1500-1799m a.s.l). This was based on the fact that each of the altitudinal zones had a unique microclimate regime (Hemp, 2006a).

Table 3.1: Annual average ± se environmental data along agro-ecological zones in Taita Hills and Mount Kilimanjaro transects. The mean data was averaged from information that was collected for a period of two years between August 2012 and July 2014.

Elevation range (m a.s.l)	Agro-ecological zone	Mean elevation (m a.s.l)	Temperature (ºC)			Relative humidity (%)			Mean Rainfall (mm)	Peak avocado harvesting months
			Tmean	Tmax	Tmin	RHmean	RHmax	RHmin		
1500-1799	**Montane**	1687 ± 8.1	17.6 ± 0.2	25.1 ± 0.4	13.3 ± .02	84.4 ± 0.9	98.4 ± 0.4	53.1 ± 1.4	218.7 ± 38.7	June & July
1200-1499	**Sub-montane**	1381.4 ± 10.4	19.9 ± 0.2	27.8 ± 0.3	15.2 ± 0.2	81.1 ± 0.8	97.1 ± 0.3	44.3 ± 0.4	93.6 ± 16.0	May & June
800-1199	**Lowland**	1085.7 ± 9.5	22 ± 0.2	30.2 ± 0.3	16.5 ± 1.1	75.2 ± 0.8	94.4 ± 0.4	41.5 ± 1.1	52.2 ± 11.3	April & May
800- 1799	**All zones**	1384.9 ± 19.3	19.9 ± 0.2	27.7 ± 0.3	15.1 ± 0.2	80.2 ± 0.6	97.4 ± 0.2	46.3 ± 1.45	121.5 ± 15.8	May & June

Tmean = mean temperature, Tmax = maximum temperature, Tmin = minimum temperature, RHmean = mean relative humidity, RHmax = maximum relative humidity and RHmin = minimum relative humidity.

3.2.2 Taita Hills study site

The Taita Hills transect is located in south-Eastern Kenya, 25km west of Voi town in the Taita-Taveta County between 03^{0} 481$^{'}$S, 38^{0} 378$^{'}$E and 03^{0} 402$^{'}$S, 38^{0} 296$^{'}$E (Figure 3.1a). Taita Hills study area is situated 90km on the north-East of Mount Kilimanjaro and about 150km inland from Indian Ocean town of Mombasa. The surrounding semi-arid lowlands plains of Taita Hills have altitude of 600 to 900m a.s.l. rise abruptly to a series of ridges reaching Vuria peak at 2208m a.s.l. Taita Hills form the northern part of the Eastern Arc Mountains which are part of the 25 globally recognized biodiversity hotspots qualified by virtue of its high biodiversity, endemic species and a severe degree of threat (Newmark, 2002; Myers *et al*., 2000; Bennun & Njoroge, 1999). The Eastern Arc Mountains are a series of isolated and geologically ancient mountains, stretching from the Udzungwa range in Eastern Tanzania to the Taita Hills in Kenya (Bennun & Njoroge, 1999). The steepness of the Taita Hill rocks is due to the hardness of a quartzite cap overlying soft metamorphic rocks (Conte, 2010). Due to the elevation levels and proximity to the Indian Ocean influencing atmospheric moisture, the hills support moist tropical forests with unique agro-biodiversity (Brooks *et al*., 1998; Beentje, 1988). The avocado comprises majority of crop trees within farmlands of Taita Hills that are intertwined between a few relicts of the original cloud forests forming a strong agro-ecosystem along the study area.

The climate of Taita Hills is under influence of the inter-tropical convergence zone with bimodal rainfall. Taita Hills has two rainy seasons; long rains from March to May and short rains from October to December. This is due to proximity to the Indian Ocean that has an effect on the weather. Taita Hills form the first barrier to moisture-laden wind from Indian Ocean leading to formation of continuous heavy cloud that provides a stable climate more so in higher altitudes (Bytebier, 2001; Bennun & Njoroge, 1999). However, due to changing climate, these weather patterns have become unpredictable. The lowlands surrounding Taita Hills are very dry contrasting sharply with the cloudy forests located in higher altitude. Although due to mist and clouds in the hills, precipitation is a year-round phenomenon in higher altitudinal zones above 1500m a.s.l. The altitude for study area ranged from 900 to1800m a.s.l. with mean elevation was 1397.02±22.31 (mean ± se). The average annual rainfall in Taita Hills transect was 135.19mm±18.7 with a mean annual humidity was 81.46%±0.5 and mean annual temperature was 19.56ºC±0.19. Avocado trees thrive well in the windward South-Eastern slopes because on this section of Taita Hills has ambient temperature and adequate rainfall than the leeward side.

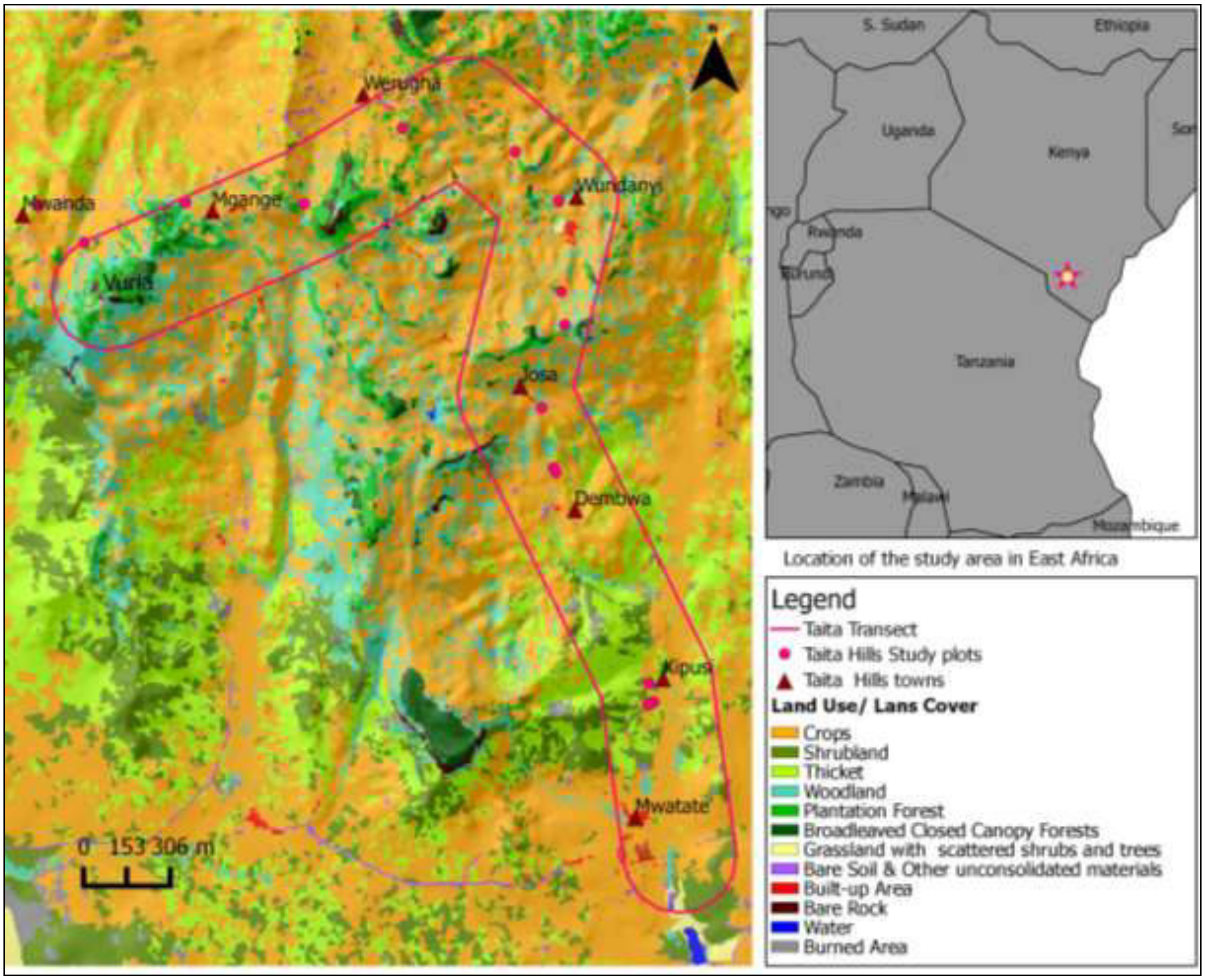


Figure 3.1a: Map of study transect along South-Eastern altitudinal gradient of Taita Hills, Kenya. The transect run from Mwatate (800m a.s.l) through Wundanyi (1500m a.s.l) up to higher elevated Vuria area (1800m a.s.l).

3.2.3 Mount Kilimanjaro study area

Mount Kilimanjaro study area is located between 03^{0} 378$^{'}$S, 37^{0} 450$^{'}$E and 03^{0} 481$^{'}$S, 37^{0} 456$^{'}$E (Figure 3.1b). The study transect ran from the forest edge (1800m a.s.l) through mid-altitude farmlands to the lowland savanna fragments (900m a.s.l) on the southern slopes of Mount Kilimanjaro. The study area consisted of Kirua Vunjo in Moshi Rural District. There are three distinct agro-ecological zones along the southern slopes of the mountain; the lowland crop farming belt, midland transition zone and the highland region with conspicuous *Chagga* home garden area consisting of coffee, banana, and avocado. The scarcely populated lowlands extend below 900m a.s.l. Midlands are the transition zone between the distinct *Chagga* home garden area and the lowlands rising up to 1500m a.s.l. The extremely densely populated home garden zone covers all the southern slopes of the mountain between 1200 m a.s.l. and 1800 m a.s.l. (Zongolo *et al*., 2000). Avocado trees dominate the homogenous agro-ecosystem of the *Chagga* people along the Mount Kilimanjaro study transect that ran up to the forest edge at 1800m a.s.l.

Mount Kilimanjaro region has a bimodal rainfall pattern; the short season from October to December and long rains from March to May. The southern slopes of Mount Kilimanjaro have higher precipitation because it receives most of the moist dominant wind from the Indian Ocean than northern leeward side (Hemp 2006a; Hemp 2006b). The study plots were located in the southern-eastern windward slopes of Mount Kilimanjaro with elevation of ranging from 900 - 1800m a.s.l. The mean altitude of transect was 1372.69m ± 16.34 (mean ± se). The average annual rainfall in Mount Kilimanjaro transect was 107.83mm± 12.79 with a mean annual humidity was 78.97%± 0.66 and mean annual temperature was 20.14°C± 0.66.

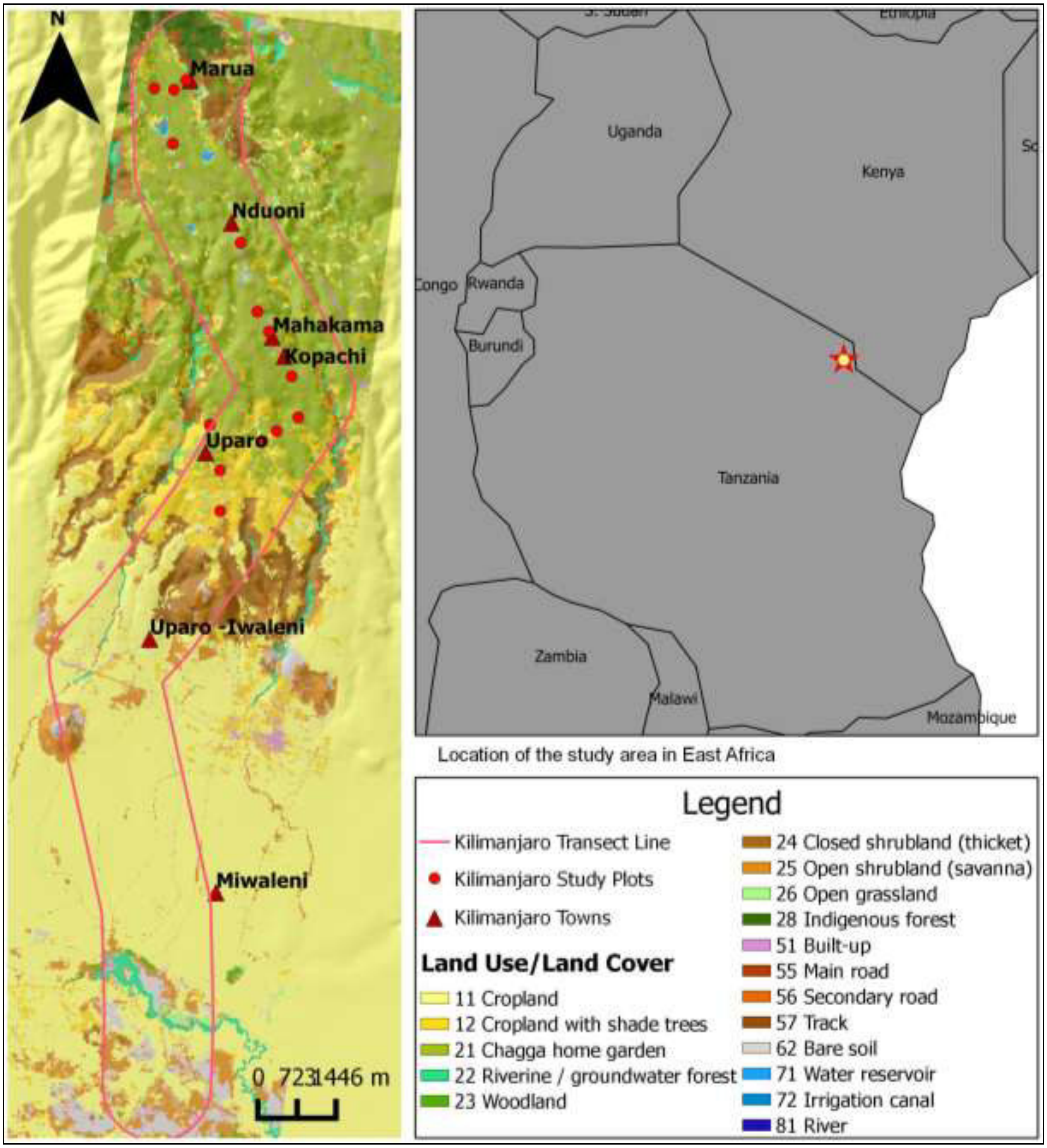


Figure 3.1b: Map of study transect along South-Eastern slopes of Mount Kilimanjaro, Tanzania. The transect run from Uparo (900m a.s.l) through Mahakama (1500m a.s.l) up to higher elevated Marua (1800m a.s.l).

3.2.4 Study design and collection of weather data

Each of the two transects in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania is located from a minimum of 900m a.s.l. to 1800m a.s.l. There were fifteen (15) blocks along each of the study transects. Each block was divided into five sub-blocks comprising of twenty avocado trees that formed the lowest sampling unit. In total, fifteen (15) sampling blocks were divided along altitudinal gradient of the two study areas. Avocado plants sampled were selected along a 350m linear transect at each of the sub-blocks. Mean distance between each avocado plant that was surveyed monthly in a sampling unit was 71.13m ± 1.09. At every sub-block, temperature (ºC) and relative humidity (%) were automatically recorded every 30 minutes for the two sampling years using iButton™ data loggers from Maxim Integrated Products, Incorporation, USA (https://www.maximintegrated.com), enhanced by Maxim's 1-Wire communications protocol. The data loggers were carefully set hanging on the lower canopy of avocado tree at a height of 1.5m above ground level with allowance of free movement of sunlight and air. Rainfall (mm) was measured using the weather station set along the study transects in Kenya and Tanzania. The geographical coordinates and elevation of each sub-block was recorded using a hand-held Germin GPS model eTrex 30.

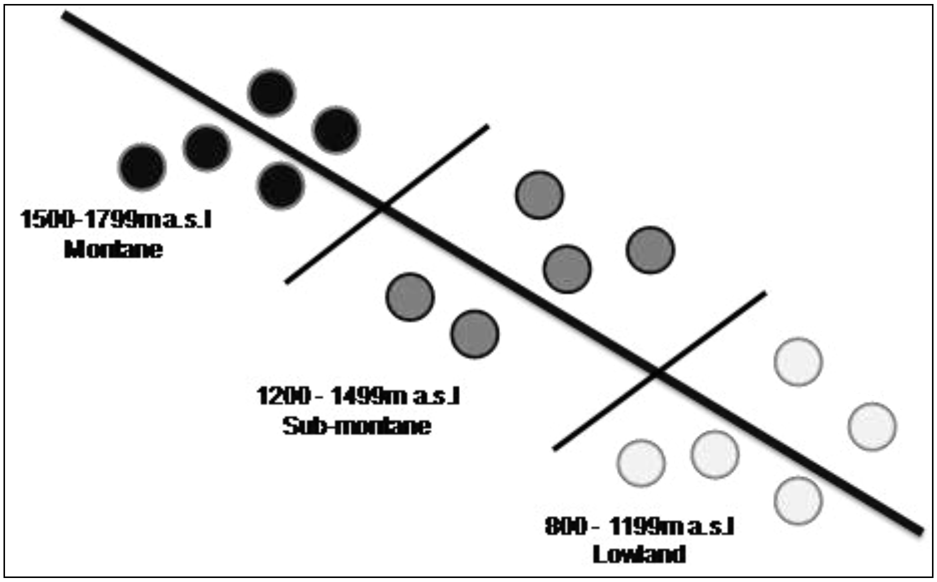


Figure 3.1c: Schematic drawing of the sampling design for avocado insect pests along agro-ecological zones (Lowland, Sub-montane and Montane) in Taita Hills and Mount Kilimanjaro. Study transects were located along South-east slopes of the study areas.

3.2.5 Sampling of arthropods inhabiting the avocado crop

Sampling of avocado insect pests was carried out weekly for two consecutive years along the two transects. The first survey commenced at onset of avocado flowering season in August 2012 and elapsed in July 2013. The second season started in August 2013 and was finalized in July 2014. The flowering season is between August and September each year. The fruiting season commences from late October, through November and December and the pre-mature fruit can be found from February and March. Avocado fruits are mainly harvested in May and June but they mature few weeks earlier in Mount Kilimanjaro than in Taita Hills. The aim of field sampling timing was to capture annual abundance data of key insect pests during avocado plant phenological events and weather seasonality. The avocado tree had abundant floral resources for the periods of flowering and fruiting and this attracts many insect pests associated with the crop.

Five avocado trees were randomly selected from the twenty sampling unit of the productive plants at each of the fifteen sub-blocks. They were sampled for arthropods every mid-month for two consecutive years. White coloured beating tray and camel brush was used for sampling arthropod species on the avocado flowers and leaves. Ten bunches of leaves from each avocado tree were sampled for two years. Furthermore, ten blooms from each avocado tree were sampled during flowering season. Each tree was visually divided into four equal quarters in order to evenly sample. Randomization of sampling units was done every month and the survey of thrips took place between 9.00am and 3pm, East African time. Timing of sampling was selected to effect standardization of replicates when insects highly active during the day.

The leaves and flowers of each avocado were shaken gently for several minutes to dislodge arthropods onto a tray. The arthropods were collected swiftly using a fine camel brush into well labeled 1.5ml clear vials containing 60% ethyl alcohol. Alcohol acts as a humane killing agent and also as a preservative for collected arthropod specimens. The specimens were taken to the National Museums of Kenya (NMK) entomology laboratory in Nairobi where they were first counted for overall abundance and later sorted into morpho-species in preparation for taxonomic identification.

For thrips, three specimens of each morpho-species sampled monthly were then prepared for mounting onto microscope slides following standard protocols for thrips by Millar *et al* (2000) and Moritz *et al* (2001). Internal soft tissues of thrips were destroyed by dipping them into 10% potassium hydroxide solution in a process called maceration. The materials were then thoroughly rinsed in distilled water containing few drops of acetic acid. To prevent distortion, a series of increasing ethanol concentration ranging from 60% to 100% was used for dehydration of specimens for fifteen minutes in each level. The materials were then cleared in clove oil since the thrip specimens were required as permanent mounts. Each prepared thrip specimen was finally mounted onto a glass slide in a drop of canada balsam and spread carefully using an entomological pin so that appendages do not obscure the body. Every specimen of thrips was then finally covered by lowering a 13mm diameter circular cover-slip onto the mount and the slide dried in oven at 37ºC for seven days. This intricate protocol is important since all thrip specimens have to be mounted so that they can be studied under a compound microscope for their taxonomic features (Palmer, 1990).

3.2.6 Sampling of the Asian invasive fruit fly and false codling moth along altitudinal gradient

Field sampling of the Asian invasive fruit fly (*Bactrocera invadens*) and false codling moth (*Thaumatotibia leucotreta*) was done along altitudinal gradient in all seasons and phenological events of avocado trees. Three *Bactrocera invadens* methyl eugenol (ME) lure traps consisting of a cotton wick immersed in one part of methyl eugenol and three parts of othothion were hanged on an avocado tree in each sub-block along the two transects in Kenya and Tanzania as described by Ekesi & Billah (2006). The traps were hanged in the lower canopy of avocado tree at a height of 1.85m from the ground. The lure traps were randomly rotated every week within the twenty reproductive avocado trees in each sub-block. In total, forty five ME-based traps set along each transect in Kenya and Tanzania that were evaluated weekly for fruit flies presence from August 2012 to July 2014.

Three false codling moth (*Thaumatotibia leucotreta*) lure traps branded, "*Yellow delta traps*", were hanged on different avocado trees in each sub-block along the two transects in Kenya and Tanzania as described by Stibick (2006). The traps were hanged in the lower canopy of avocado tree at a height of 1.85metre from the ground. The active ingredient in the male lure of False Codling Moth is dodecenyl acetate. The lure traps were randomly rotated every week within the twenty reproductive avocado trees in each sub-block. In total, there were forty five dodecenyl acetate-based lure traps that were evaluated for false codling moth presence every seventh day along each transect for two years. The lure traps for Asian invasive fruit fly and false codling moth were never hanged on same tree at single time in order to maintain independence of count data recorded.

3.2.7 Assessment of damage levels to avocado fruits by insect pests

The fruits observed for infestation by insect pests (Appendix 2a, b & c) were collected along each study transect during peak avocado harvesting seasons between May and July and experiments followed a standard protocol described below.

3.2.7.1 Asian invasive fruit fly (Bactrocera invadens)

Four avocado fruits from each five trees that were randomly selected at each sub-block were carefully collected, observed for *Bactrocera invadens* emergency (Appendix 1a & 3). Of the four avocado fruits randomly sampled; two were collected on the ground below the tree whereas the remaining two were plucked directly from the tree. Percentage infestation was quantified from the fruit observation as described by Ekesi *et al*., 2006. The avocado fruit infestation observation experiments were carried out in temporary laboratories along the study areas in warmer lowland plains (700m a.s.l) at Mwatate (Taita Hills, Kenya) and Uparo (Mount Kilimanjaro, Tanzania), during avocado harvesting months of May and July in year 2013 and 2014 as described by Ekesi *et al*., 2006. The field-based avocado fruit infestation experiment was repeated for two years using a total of 1200 avocado fruits.

3.2.7.2 False codling moth (*Thaumatotibia leucotreta*)

Observation for avocado fruit infestation by *Thaumatotibia leucotreta* (false codling moth) followed a protocol different from that of fruit fly. This is because larvae of false codling moths do not mature to adult within avocado fruit. Therefore, percentage infestation by false codling moth was recorded from on tree mature avocado fruits during harvesting season that run between May and June in years 2013 and 2014 along the two transects. Three hundred avocado fruits were observed annually for the typical triangle black scars on the exocarp of avocado fruit caused by the false codling moth (Appendix 2a & 1b; Whiley, 2002; Du Toit *et al.*, 1979).

3.2.7.3 Green house thrips (*Heliothrips haemorrhoidalis*)

Percentage infestation by *Heliothrips haemorrhoidalis* was recorded from mature avocado fruits at the harvesting season that run between May and June in two transects. Six hundred avocado fruits were observed annually for browning scaring of skin caused by *Heliothrips haemorrhoidalis* (Appendix 2c & 1c) during harvesting season in years 2013 and 2014. Typical surface lesions on avocado fruit caused by *Heliothrips haemorrhoidalis* is well described in Whiley (2002) and Du Toit *et al* (1979). The authors further describe the green house thrips, *Heliothrips haemorrhoidalis*, as an important avocado pest in Afrotropical region.

3.2.8 Taxonomic verification of the pest species

3.2.8.1 Fruit fly species and false codling moth

The false codling moth (*Thaumatotibia leucotreta*) specimens were kept in entomological envelopes and stored as dry samples. Fruit fly specimens collected were stored in vials containing 60% ethyl alcohol. Alcohol acts as a humane killing agent and also as a preservative for wet insect specimens. The samples were then taken to the National Museums of Kenya (NMK) entomology laboratory in Nairobi for taxonomic verification. Confirmation of taxonomically identified fruit fly species was done at International Centre of Insect Physiology and Ecology (ICIPE) using Ekesi & Billah (2006) whereas the identified false codling moth specimens were verified using entomological collection at the National Museums of Kenya and ICIPE.

3.2.8.2 Thrips and other arthropods

The mounted thrips specimens were taxonomically identified at the NMK entomology laboratory using manuals including Mound (2010), Moritz *et al* (2001) and Palmer *et al* (1992). Confirmation of identified thrip species was done by comparing with the slide-mounted Thysanopteran collection at NMK, Nairobi. The thrips species were also verified by Professor Laurence Mound, a world authority on thrips and an honorary research fellow at Australian National Insect Collection, CSIRO entomology, Canberra. Generic and species placement of the identified thrips followed the classification by Odanga *et al* (2014) and Mound (2010). Other arthropods were taxonomically identified at entomology laboratory of National Museums of Kenya. The fully identified and confirmed thrip species have been deposited in zoological collection at the National Museums of Kenya as voucher specimens

3.2.9 Statistical analysis

Sampling information for the two consecutive years of the insect pests was averaged into a single data set comprising of one avocado season (twelve months) that was used for data analysis. To compare species richness with increasing sampling effort between the two habitats and in order to check for the completeness of sampling, species-accumulation curves and estimated total species richness were constructed for each habitat using non-parametric species richness estimators: Chao, Jackknife 1, Jacknife 2, and Bootstrap. The species accumulation curve was generated for a given number of sampled transects by repeatedly (500 times) calculating the cumulative number of species for a random selection of transects and calculating the average of these random samples (Crawley, 2007; Crawley, 2005). Rank abundance test was used to estimate most important arthropod species based on their population density using Biodiversity-

R software (R Development Core Team, 2012). Kruskal-Wallis Chi-square test was used to test differences between proportional infestation levels of avocado fruits by key insect pests in agro-ecological zones.

Abundance data of the key avocado insect pests did not have a normal distribution and variances were not homogenous; thus several non-parametric tests were used. Spearman's rank correlation analyses, Wilcoxon Signed Rank and Kruskal-Wallis tests were performed in R 2.15.1 (R Development Core Team, 2012). For Spearman's rank correlations absolute abundances were used and for Kruskal-Wallis tests abundance of a species were log+1 transformed to improve normality. Wilcoxon signed rank test was employed to test differences between pairs of non-normal datasets whereas Kruskal-Wallis Chi-square test was used to test differences between three or more groups of variables that were not normally distributed. Tukey's HSD post hoc test was used to pinpoint what exact sub-sets within a group that had significant differences from each other. Simple hypothesis tests were employed using methods, assumptions and notation outlined by Crawley (2007). Linear trends were tested using simple linear regression (Crawley, 2005; Verzan, 2005). For cases where data were non-normal even after transformation, Spearman's rank correlation was used. Spatial maps of the key avocado pests were generated using kriging method by interpolating mean monthly abundance of pest species along the two study transects using QGIS version 1.8.0.

Linear mixed effect models (LME) was used to determine environmental variables that best explained change in population density of the key avocado insect pest along altitudinal gradient of the Mount Kilimanjaro region (R Development Core Team, 2012). All possible models were constructed based on sets of sampled explanatory variables and model evaluation was done using

Akaike Information Criterion (AIC). The environmental variables used in analysis were; mean temperature, average relative humidity, mean annual rainfall, elevation and micro-habitats or agro-ecological zones of the two study areas. The best model with environmental variables that best explained change in abundance of a pest had the lowest AIC and highest R^2 values (R Development Core Team, 2012; R Development Core Team, 2008; Crawley, 2007; Verzan, 2005; Burnham & Anderson, 2004). Mixed-effect models were used instead of other models due to spatial nearness of data collected. Abundance of insects in adjacent agro-ecological zones is never independent and therefore, the use of linear mixed effect models controlled for this type of non-independence (Burnham & Anderson, 2004). Consequently, the study plots were recorded as a random variable in the model whereas mean temperature and agro-ecological zone were used as fixed effects in some models.

3.3 Results

3.3.1 Arthropod species associated with avocado crop in Taita Hills and Mount Kilimanjaro

A combined total of 860,882 arthropod individuals represented by 53 species were sampled on avocado plants in farmlands adjacent to forests within Taita Hills in South-eastern Kenya and Mount Kilimanjaro in North-eastern Tanzania. Findings revealed that 99.3% of the overall total abundance of arthropod species associated with avocado crop recorded in avocado orchards of Taita Hills and Mount Kilimanjaro consisted of Asian invasive fruit fly (*Bactrocera invadens*; 90.3%) and false codling moth *(Thaumatotibia leucotreta*, 9%) (Table 3.2; Appendix 1a, b, c & d). Thrip species; *Frankliniella schultzei* and *Heliothrips haemorrhoidalis* (Thysanoptera: Thripidae) ranked third and fourth respectively but both Thysanopteran avocado pests accounted for a gross of only 0.3% (Table 3.2). The remaining arthropod species was represented by 0.4% of the overall abundance of recorded.

Table 3.2: Arthropod species recorded on avocado crop in the two study areas of Taita Hills and Mount Kilimanjaro for two years between August 2012 and July 2014.

Rank	**Scientific name**	**Common name**	**Status**	**% abundance**	**Log abundance**	**Order**	**Habitat sampled**
1	*Bactrocera invadens*	Asian fruit fly	Ripening fruit pest	90.3	5.9	Diptera	T & K
2	*Thaumatotibia leucotreta*	False codling moth	Immature fruit pest	9.0	4.9	Lepidoptera	T & K
3	*Frankliniella schultzei*	common blossom thrips	Flower pest	0.2	3.2	Thysanoptera	T & K
4	*Heliothrips haemorrhoidalis*	Greenhouse thrips	Fruit & leave pest	0.1	2.9	Thysanoptera	T & K
5	*Megalirothrips sjostedti*	Cowpea flower thrips	Flower pest	0.1	2.8	Thysanoptera	T & K
6	*Thrips austarlis*	Western flower thrips	Flower pest	*	2.6	Thysanoptera	T & K
7	*Thrips pusillus*	Thrips	_	*	2.5	Thysanoptera	T & K
8	*Aleyrodicus dispersus*	Spiralling whitefly	_	*	2.4	Hemiptera	T & K
9	*Haplothrips gowdeyi*	Thrips	_	*	2.4	Thysanoptera	T & K
10	*Cheilomenes sulphurea*	Ladybird beetle	Predator of aphids	*	2.3	Coleoptera	T & K
11	*Pheidole megacephala*	Sugar ant	pest	*	2.2	Hymenoptera	T & K
12	*Rhinocoris* sp.	Assassin bug	_	*	2.1	Hemiptera	T & K
13	*Trialeurodes vapørariorum*	Greenhouse whitefly	_	*	2.0	Hemiptera	T & K
14	*Tetranychus* sp.	Red spider mite	_	*	1.8	Trombidiformes	T & K
15	*Bactrothrips* sp.	Thrips	_	*	1.7	Thysanoptera	T & K
16	*Nezara viridula*	southern green stink bug	_	*	1.7	Hemiptera	T & K
17	*Helopeltis schoutedeni*	Mirid (plant bugs)	_	*	1.7	Hemiptera	T & K
18	*Cheilomenes lunata*	Ladybird beetle	Predator of aphids	*	1.7	Coleoptera	T & K
19	*Thrips abyssiniae*	Thrips	_	*	1.7	Thysanoptera	T & K
20	*Franklinothrips* sp.	Thrips	_	*	1.6	Thysanoptera	T & K
21	*Proboscidocoris fuliginosus*	Bugs	_	*	1.6	Hemiptera	T & K
22	*Franklinothrips megalops*	Predatory thrips & mimics ant	Predator of other thrips	*	1.5	Thysanoptera	T & K
23	*Dolicholepta jeanneli*	Thrips	_	*	1.4	Thysanoptera	T & K
24	*Gynaikothrips*sp	Thrips	_	*	1.3	Thysanoptera	T & K
25	*Chilothrips frontalis*	Thrips	_	*	1.3	Thysanoptera	T & K
26	*Dendrothrips* sp	Thrips	_	*	1.3	Thysanoptera	T & K
27	*Scirtothrips dorsalis*	Chilli thrips or yellow tea thrips	_	*	1.3	Thysanoptera	T & K
28	*Apterygothrips sp*	Thrips	_	*	1.2	Thysanoptera	T & K
29	*Scirtothrips* sp	Thrips	_	*	1.2	Thysanoptera	T & K
30	*Frankliniella occidentalis*	Thrips	_	*	1.2	Thysanoptera	T & K
31	*Haplothrips* Sp	Thrips	_	*	1.0	Thysanoptera	K
32	*Gigantothrips*sp	Thrips	_	*	1.0	Thysanoptera	T & K
33	*Microcephalothrips abdominalis*	Thrips	_	*	1.0	Thysanoptera	T & K
34	*Vuilletia houardi*	Thrips	_	*	0.9	Thysanoptera	T & K
35	*Ceratothripoides brunnens*	Tomato thrips	_	*	0.8	Thysanoptera	T & K
36	*Scirtothrips* sp.2	Thrips	_	*	0.8	Thysanoptera	T & K
37	*Ecacanthothrips tibialis*	Thrips	_	*	0.7	Thysanoptera	T
38	*Thrips revelatus*	Thrips	_	*	0.7	Thysanoptera	T
39	*Sericothrips* sp	Thrips	_	*	0.6	Thysanoptera	T
40	*Neosmerinthothrips sp*	Thrips	_	*	0.5	Thysanoptera	T
41	*Diarthrothrips* sp	Thrips	_	*	0.3	Thysanoptera	K

42	*Frankliniella williamsi*	Thrips	_	*	0.3	Thysanoptera	T
43	*Rhipiprothrips* sp	Thrips	_	*	0.3	Thysanoptera	T & K
44	*Stenchaetothrips*sp	Thrips	_	*	0.3	Thysanoptera	T
45	*Elaphrothrips* sp	Thrips	_	*	0	Thysanoptera	T
46	*Pselaphothrips pomeroyi*	Thrips	_	*	0	Thysanoptera	T
47	*Stephanothrips* sp	Thrips	_	*	0	Thysanoptera	T
48	*Urothripine* sp.	Thrips	_	*	0	Thysanoptera	T
49	*Craspedothrips* sp	Thrips	_	*	0	Thysanoptera	T
50	*Apis mellifera*	Honey bee	Pollinator	*	0	Hymenoptera	T & K
51	*Componotus maculatus*	Ant	_	*	0	Hymenoptera	T & K
52	*Oecophylla longinoda*	Weave ant	Predator	*	0	Hymenoptera	T & K
53	*Crematogaster* sp.	Ant	_	*	0	Hymenoptera	T & K

_ represents no description of the arthropod status; * shows less than 0.1 percent of the overall abundance. T=Taita Hills whereas K= Mount Kilimanjaro study area where the species were sampled.

Species accumulation curve revealed 51 species of arthropods for Taita Hills being steep towards asymptote (Figure 3.2a) whereas 42 species for Mount Kilimanjaro with the curve appearing less steep towards asymptote (Figure 3.2b).

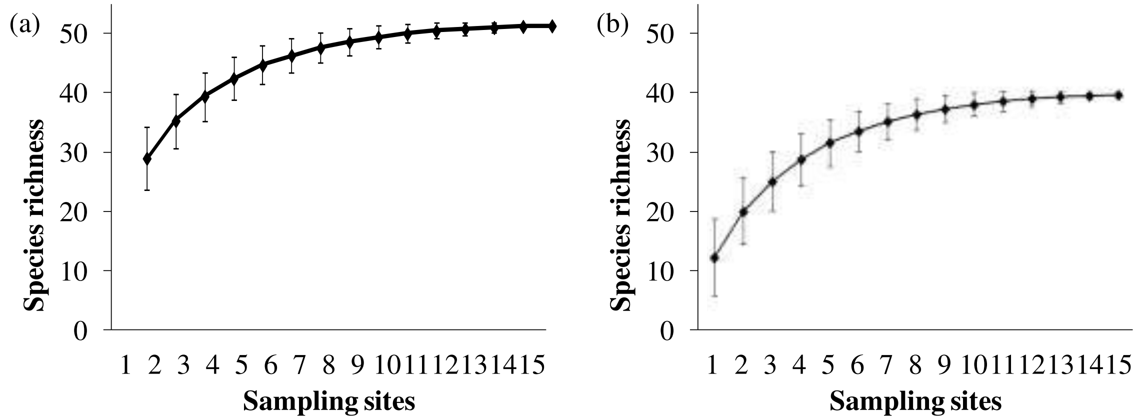


Figure 3.2a & b: Species accumulation curve of arthropod fauna recorded on avocado plants between August 2012 and July 2014 for both study areas (a) Taita Hills and (b) Mount Kilimanjaro. The bars are standard errors. Sampling sites are study blocks along altitudinal gradient with each one comprising of at least a hundred avocado trees.

Species richness estimators (Boot, Jacknife and Chao) predicted that a number of between 71 ± 13 and 54 ± 3 (mean ± se) species for Taita Hills, and between 51 ± 9 and 43 ± 1 for Mount Kilimanjaro (Table 3.3).

Table 3.3: Observed species richness and non-parametric species richness estimators for the combined study areas of Mount Kilimanjaro and Taita Hills transects.

Habitat	Observed species	Chao	Jacknife 1	Boot	Sampling blocks (n)
Taita Hills	51	71.51 ± 13.77	56.62 ± 4.23	54.43 ± 3.09	15
Mount Kilimanjaro	42	51.14 ± 9.81	50.20 ± 7.51	43.12 ± 1.36	15

3.3.2 Overall abundance of key avocado insect pests

The most abundant insect species in the two study areas were also the key pests of avocado fruits as explained in subsequent sections. The insect pests were; the Asian invasive fruit fly (*Bactrocera invadens*), false codling moth (*Thaumatotibia leucotreta*), greenhouse thrips (*Heliothrips haemorrhoidalis*) and common blossom thrips (*Frankliniella schultzei*) (Table 2).

3.3.2.1 *Bactrocera invadens* and False codling moth

Out of a total of 860,882 overall arthropod individuals recorded , there were 647,999 specimens of *Bactrocera invadens* collected along the study transects of Mount Kilimanjaro (453,599; 70%) and Taita Hills (194,400; 30%) in two consecutive avocado seasons between August 2012 and July 2014. During the same period, an overall abundance of 66,484 specimens of false codling moths (FCM) were sampled in Mount Kilimanjaro, Tanzania (50,219; 76%) and Taita Hills, Kenya (16,265; 24%). The mean abundance of *Bactrocera invadens* and false codling moth recorded in Mount Kilimanjaro and Taita Hills differed significantly (p<0.0001; Wilcoxon signed rank test).

3.3.2.2 *Heliothrips haemorrhoidalis* and *Frankliniella schultzei* (Thysanoptera: Thripidae)

Two species from genus Thripidae; *Heliothrips haemorrhoidalis* Bouche and *Frankliniella schultzei* Trybom, were the most abundant thrips on avocado crop within the two study areas (Table 3.2). *Heliothrips haemorrhoidalis* was mostly found on young fruits and leaves whereas *Frankliniella schultzei* was recorded on flowers. Of the total number of Thysanopteran individuals (4,446) collected in the two study transects for two avocado seasons, greenhouse thrips (*Heliothrips haemorrhoidalis*) represented 22.2% (872) of the population whereas common blossom thrips (*Frankliniella schultzei*) accounted for 34% (1670). The mean abundance of *Heliothrips haemorrhoidalis* collected in Taita Hills (mean±se; 2.58±0.2) was higher and statistically significant compared to mean population from Mount Kilimanjaro transect (mean±se: 0.86±0.1, Wilcoxon signed rank test: p<0.001, n=180). Similarly, the mean abundance of *Frankliniella schultzei* collected in Taita Hills (mean±se; 5.42±0.77) was higher and statistically significant compared to mean population from Mount Kilimanjaro transect (mean±se: 0.91±01, Wilcoxon signed rank test: p<0.001, n=180).

3.3.3 Avocado fruit damage by key avocado insect pests

Observation of mature avocado fruits during harvesting seasons revealed that percentage damage levels was highest due to attacks by Asian invasive fruit fly (*Bactrocera invadens*) followed by false codling moth (*Thaumatotibia leucotreta*) and greenhouse thrips (*Heliothrips haemorrhoidalis*). Laboratory observation of mature avocado fruits from both Taita Hills and Mount Kilimanjaro farmlands during two avocado harvesting seasons revealed a high of 29.3% ± 2.7 (mean ± se) incidence of proportional fruit infestation by Asian invasive fruit fly (*Bactrocera invadens*) in lowlands below 1200m a.s.l and 2.6 ± 0.5 in highlands above 1500m a.s.l. For false codling moth, incidence of proportional mature avocado fruits infestation in lowlands was lower with (mean ± se) 20.4 ± 1.9 and 9.1 ± 0.95 in highlands.

3.3.3.1 Proportional avocado fruit damage by the Asian invasive fruit fly (*Bactrocera invadens*)

There was a significant difference in incidence of percentage infestation of harvested avocado fruits by *Bactrocera invadens* in three agro-ecological zones (p<0.0001; Kruskal-Wallis chi-squared test). Avocado fruit infestation was higher in lowland areas followed by sub-montane zone while the ever cold uppermost montane region had least. Significant difference was also revealed in Tukey's HSD pair wise comparison of mean percentage infestation incidences of avocado fruits by *Bactrocera invadens* between agro-ecological zones ($p<0.001$; Figure 3.3a & b).

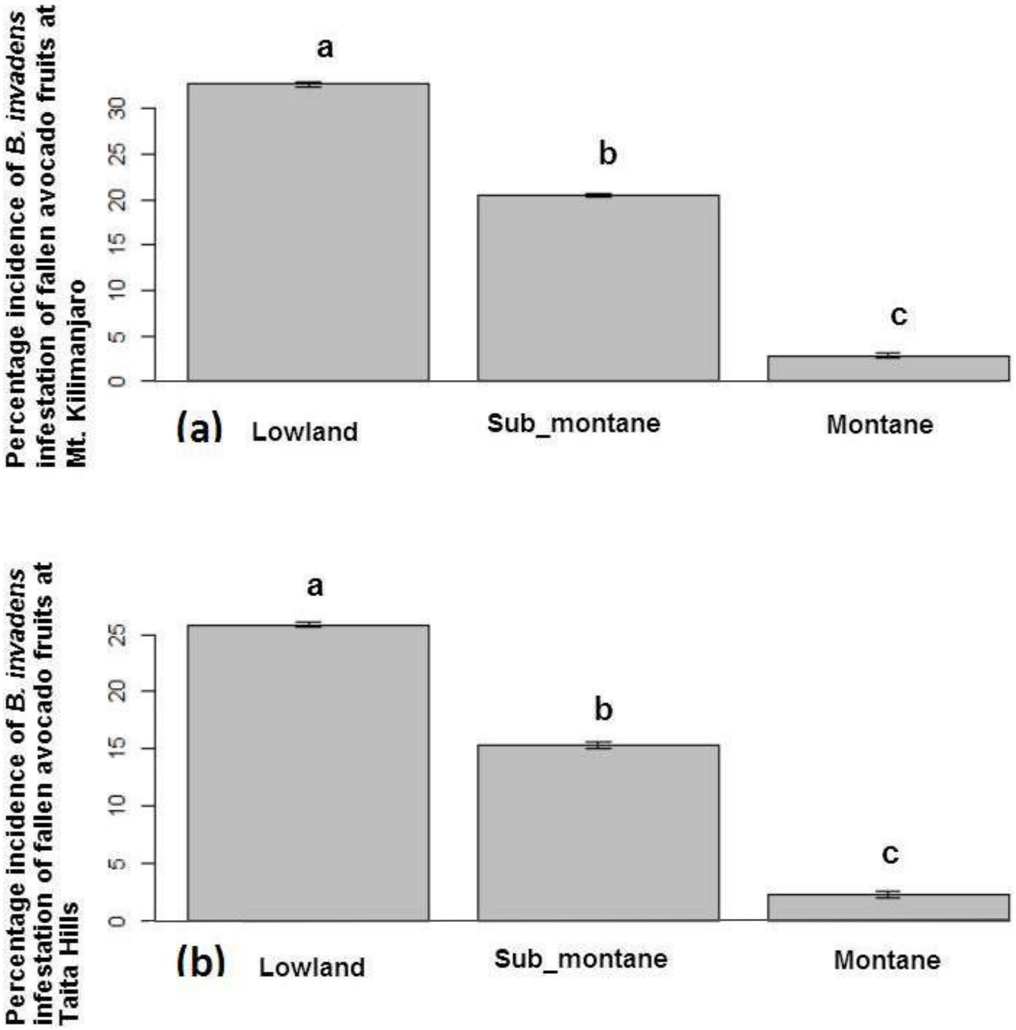


Figure 3.3a & b: Percentage incidence of *Bactrocera invadens* infestation of fallen avocado fruits across altitudinal zone in Taita Hills and Mount Kilimanjaro transects. The zones were categorized as Lowland (900-1199m a.s.l.), Sub-montane (1200-1499m a.s.l.) and Montane region (1500-1799m a.s.l). Different letters (a, b and c) denote significantly differing mean abundance between agro-ecological zones in each transect. The bars are standard errors.

Avocado fruit infestation at Taita Hills was higher in lowland areas followed by sub-montane zone while the ever cold uppermost montane region had the least. At Taita Hills, there was no significant difference between montane and highland Figure 3.4b). However, percentage infestation ± se of on tree avocado fruits by *Bactrocera invadens* was significant at the three agro-ecological zones in Mount Kilimanjaro ($p<0.0001$; Kruskal-Wallis chi-squared test; Figure 3.4a). Tukey's HSD pair-wise comparison of mean percentage infestation incidence of avocado fruits that were still on tree by the Asian invasive fruit fly (*Bactrocera invadens*) was significant in most of the paired agro-ecological zones within the two transects.

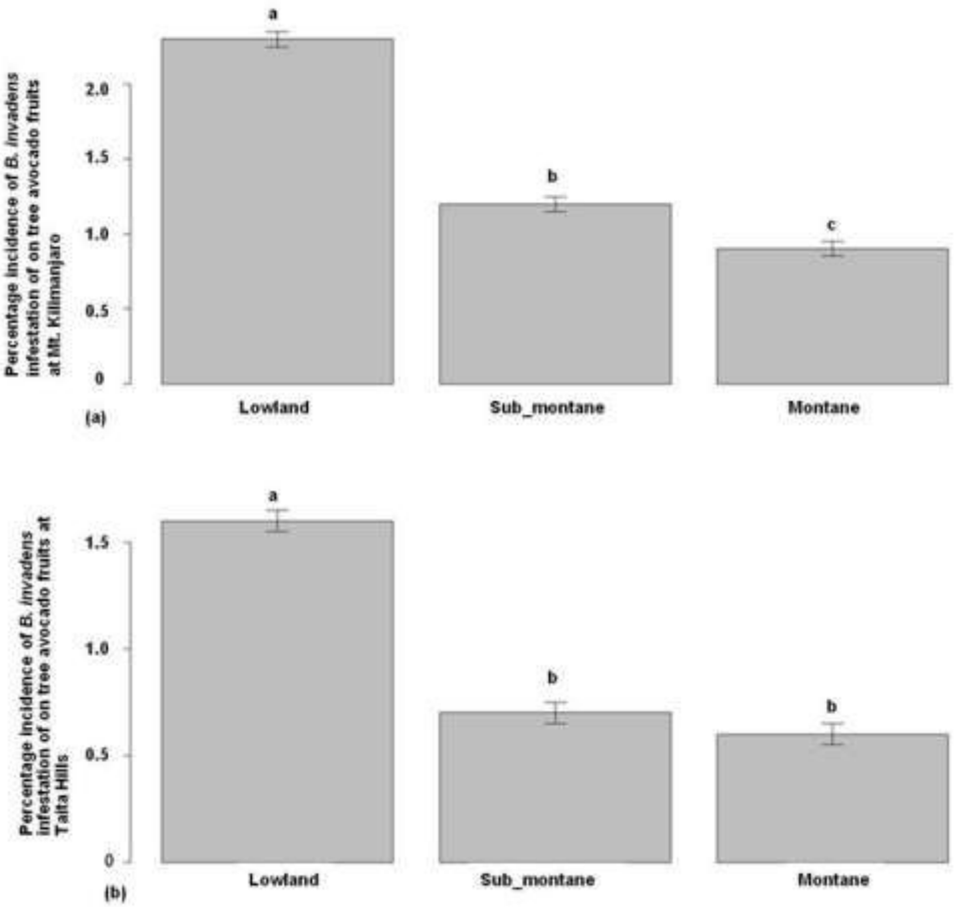


Figure 3.4a & b: Percentage incidence of *Bactrocera invadens* infestation of on tree avocado fruits across altitudinal zone in Taita Hills and Mount Kilimanjaro transects. The zones were categorized as lowland (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane region (1500-1799m a.s.l). Different letters (a, b and c) denote significantly differing mean abundance along agro-ecological zones of each transect. Same letter (b) denotes lack of significant difference in mean abundance in paired agro-ecological zones in Taita Hills.

3.3.3.2 Proportional avocado fruit damage by false codling moth (*Thaumatotibia leucotreta*)

Proportional incidence of fruit infestation by false codling moth (*Thaumatotibia leucotreta*) was significant across the three agro-ecological zones (P <0.001; Kruskal-Wallis chi-squared test; Figure 3.5a & b) in Taita Hills and Mount Kilimanjaro transects (Figure 3.5a & b). Tukey's HSD pair-wise comparison of mean percentage infestation incidence of avocado fruits that were still on tree by the false codling moth (*Thaumatotibia leucotreta*) was significant in both transects; Taita Hills and Mount Kilimanjaro (p<0.001; Figure 3.5a & b).

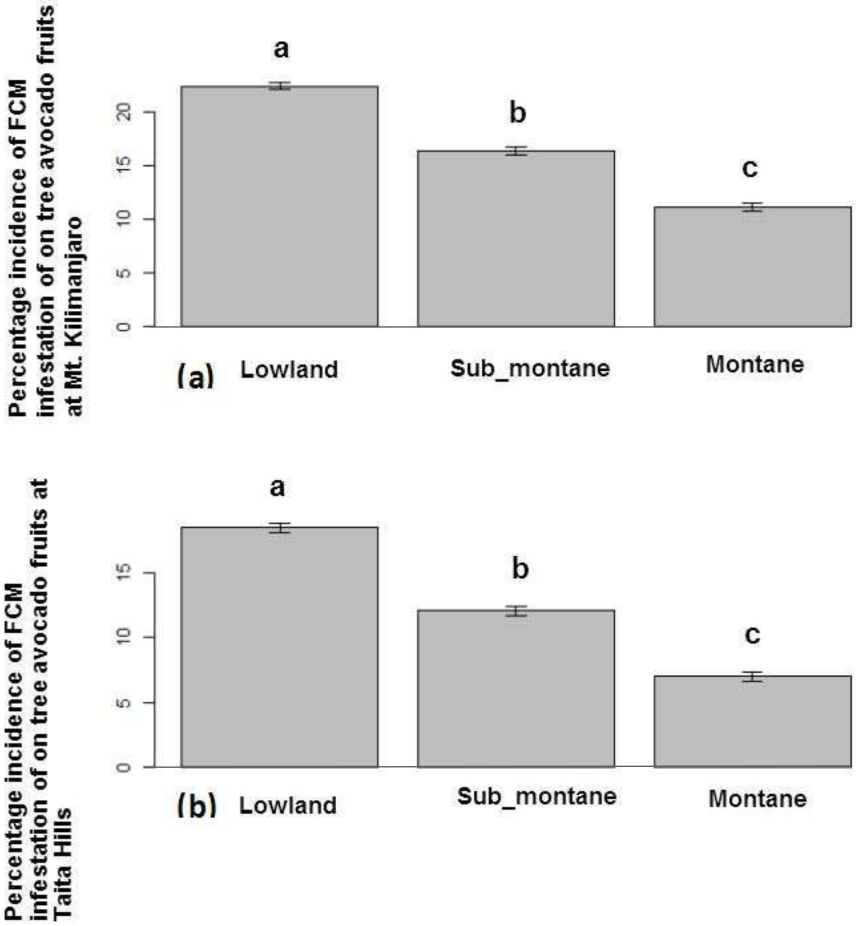


Figure 3.5a & b: Percentage incidence of false codling moth (*Thaumatotibia leucotreta*) infestation of on tree avocado fruits across altitudinal zones of Taita Hills and Mount Kilimanjaro transects. The zones were categorized as Lowland (900-1199m a.s.l.), Sub-montane (1200-1499m a.s.l.) and Montane region (1500-1799m a.s.l). Different letters (a, b and c) denote significantly differing mean abundance in paired agro-ecological zones of each transect.

3.3.3.3 Proportional avocado fruit damage by *Heliothrips haemorrhoidalis*

Mean percentage avocado fruit infestation rate by *Heliothrips haemorrhoidalis* was similar in both transects of Taita Hills, Kenya (15.8%) and Mount Kilimanjaro, Tanzania (15.5%). In Taita Hills, infestation was higher (21.0%) in Sub-montane zone (1200-1499m a.s.l.) followed by Lowland region (17.5%) (900-1199m a.s.l.) and the least was in Montane zone (1500-1799m a.s.l) at 9.0% (Figure 3.6a). Similarly in Mount Kilimanjaro transect, sub-montane zone (1200-1499m a.s.l.) led with (19.5%) followed by lowland region (16.0%) (900-1199m a.s.l.) with the least being witnessed in the Montane zone (1500-1799m a.s.l) at 11.0% (Figure 3.6b).

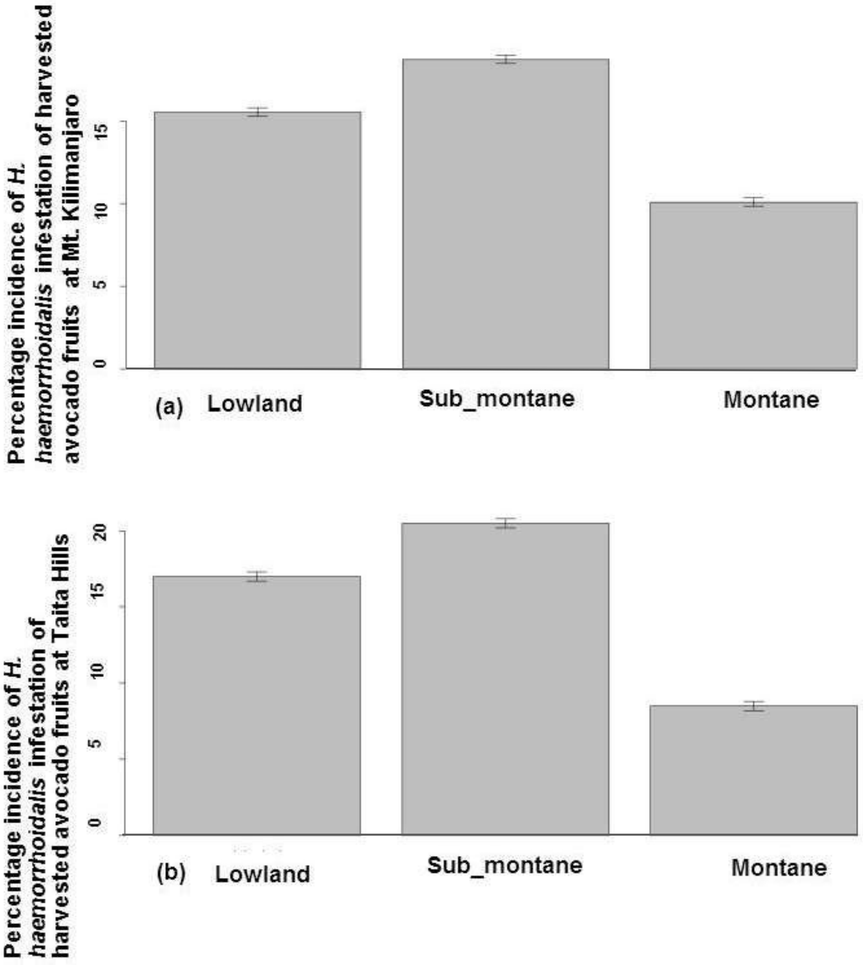


Figure 3.6a & b: Percentage incidence of *Heliothrips haemorrhoidalis* infestation of harvested avocado fruits along altitudinal zones of (a) Mount Kilimanjaro and (b) Taita Hills transects. The bars are standard errors.

3.3.4 Changes in mean abundance of key avocado insect pests along altitudinal zones

3.3.4.1 Asian invasive fruit fly (*Bactrocera invadens*)

Results from parapheromones lures revealed that *Bactrocera invadens* (Diptera: Tephritidae) was highly abundant in altitudes below 1500m but rare and seasonal in higher elevations. The highest mean abundance of *Bactrocera invadens* was recorded within lowland region (900-1299m a.s.l) of Mount Kilimanjaro (mean ± se: 24.45 ± 2.9) followed by Sub-montane zone (1200-1499m a.s.l) (16.40 ± 2.5) and least was montane section (1500-1799m a.s.l) with 1.86 ± 0.4; Figure 3.7i-a). In Taita Hills, highest mean abundance of *Bactrocera invadens* was collected in lowland region (mean ± se: 15.90 ± 1.5) followed by Sub-montane zone (4.06 ± 0.6) and least was montane (1500-1799m a.s.l) with 0.11 ± 0.03 (Figure 3.7i-b). Population density of *Bactrocera invadens* declined gradually with increasing elevation up to 1800m a.s.l where it reached zero: Taita Hills transect ($F_{1,\ 178}$ = 136.50, r = -0.65, slope = -0.033, n = 180, p<0.0001; linear regression) and Mount Kilimanjaro ($F_{1,\ 178}$ = 59.61, r = -0.50, slope = -0.09, n = 180, p<0.0001; linear regression). Comparison of mean abundance of *Bactrocera invadens* across all altitudinal zones of Taita Hills and Mount Kilimanjaro revealed a significant difference (P<0.001, df=2; Kruskal-Wallis chi-squared test). Tukey's HSD pair wise comparison of mean abundance of *Bactrocera invadens* between elevational zones in Taita Hills and Mount Kilimanjaro revealed a significant difference (p<0.001; Figure 3.7i-a & b).

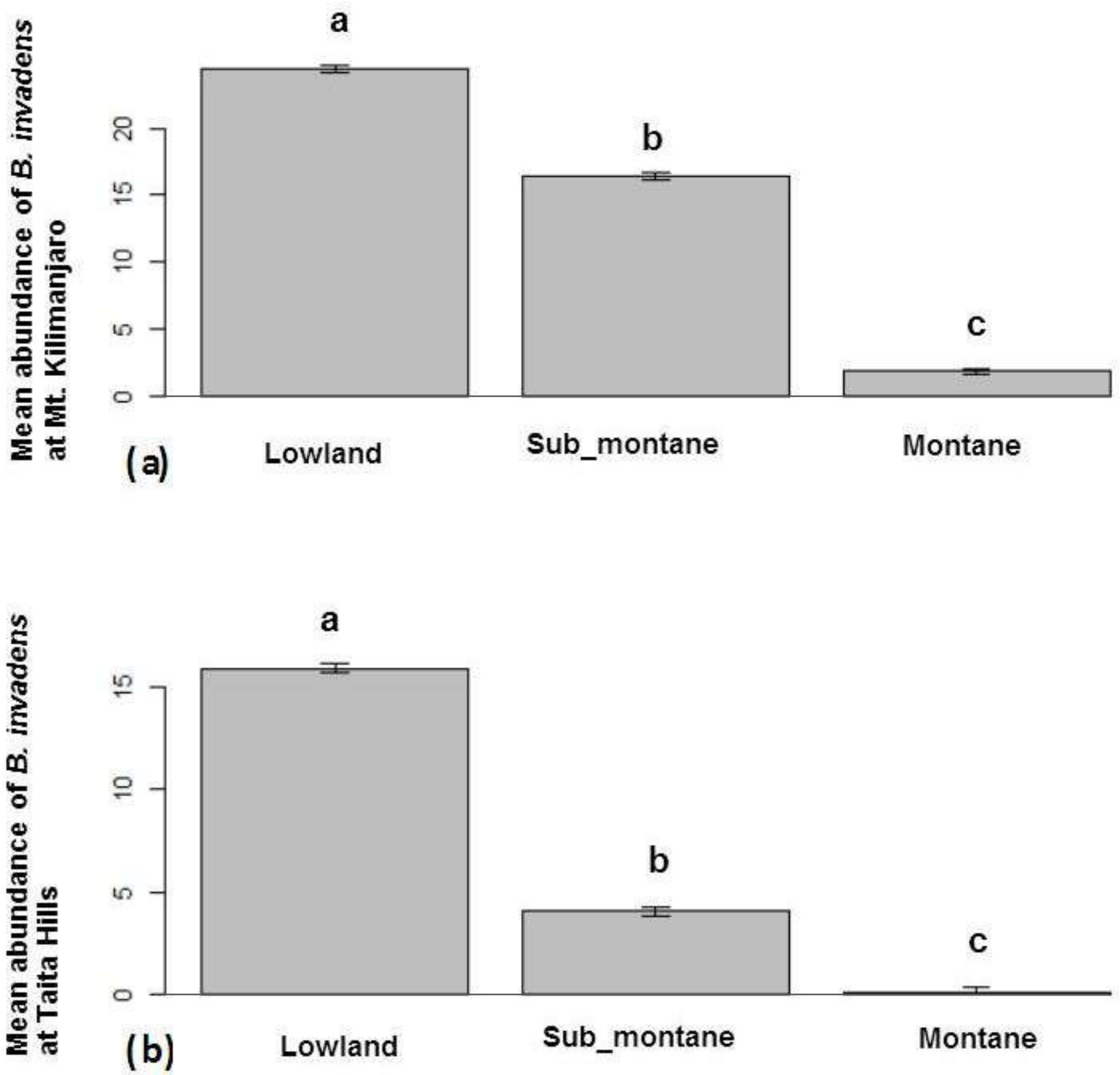


Figure 3.7i-a & b: Mean abundance of *Bactrocera invadens* within agro-ecological zones of (a) Mount Kilimanjaro and (b) Taita Hills. The altitudinal zones were categorized as lowland region (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane zone (1500-1799m a.s.l). Different letters (a, b and c) denote significantly differing mean abundance between agro-ecological zones in each transect.

3.3.4.1.1 Geographical distribution of the Asian fruit flies (*Bactrocera invadens*)

Spatial mapping of mean abundance of Asian fruit fly (*Bactrocera invadens*) revealed a geographical distribution preference to lowland and sub-montane altitudinal zones ranging from 800 up to 1500m a.s.l. (Figure 3.7ii-a & b).

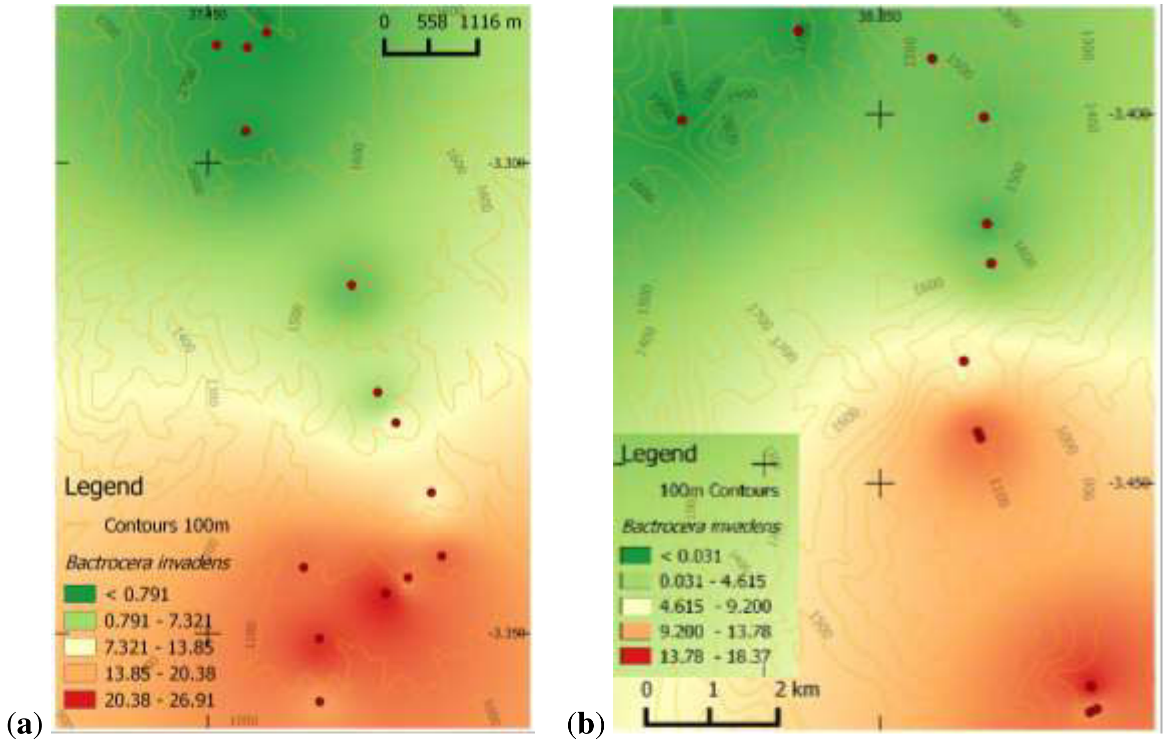


Figure 3.7ii-a & b: Geographical distribution of *Bactrocera invadens* mean abundance along altitudinal gradient of (a) Mount Kilimanjaro, Tanzania and in (b) Taita Hills, Kenya transects. Red shows highest whereas green reveals least mean abundance of *Bactrocera invadens*. The map was generated using kriging method by interpolating mean monthly abundance of *Bactrocera invadens* using QGIS version 1.8.0.

3.3.4.2 False codling moth (*Thaumatotibia leucotreta*)

Thaumatotibia leucotreta (Lepidotera:Tortricidae) were fairly distributed across all elevation ranges sampled from 800 to 1800m a.s.l (Figure 3.8i-a & b). The difference in mean abundance of *Thaumatotibia leucotreta* between the three elevational zones in Taita Hills and in Mount Kilimanjaro transects was significant (P<0.05, df=2; Kruskal-Wallis chi-squared test). In Taita Hills, the mean abundance of *Thaumatotibia leucotreta* was almost even in all agro-ecological zones: lowland region (mean ± se: 0.65 ± 0.03), Sub-montane zone (0.63 ± 0.03) and montane (1500-1799m a.s.l) with 0.54 ± 0.04) (Figure 3.8i-a). In Mount Kilimanjaro, the highest mean abundance of false codling moth (*Thaumatotibia leucotreta*) was recorded in Sub-montane zone (1200-1499m a.s.l) (2.02 ± 0.2) followed by lowland region (900-1299m a.s.l) (mean ± se: 1.98

± 0.15) and least was montane section (1500-1799m a.s.l) with 1.58± 0.1; Figure 8i-b). There was no significant difference in Tukey's HSD pair wise comparison of mean abundance of *Thaumatotibia leucotreta* (false codling moth) between agro-ecological zones in Taita Hills and Mount Kilimanjaro (p>0.06; Figure 3.8i-a & b).

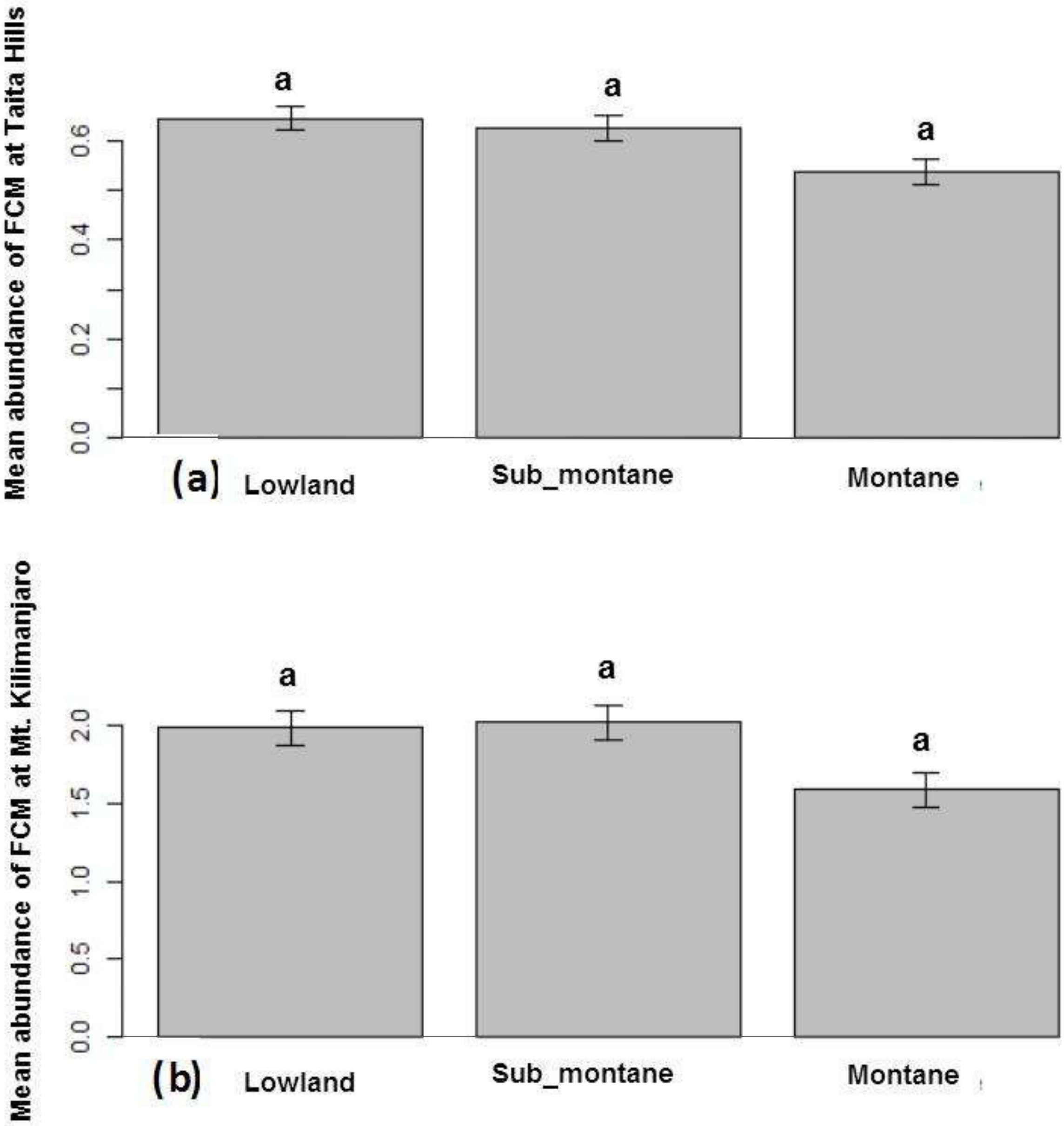


Figure 3.8i-a & b: Mean abundance of false codling moth (*Thaumatotibia leucotreta*) within agro-ecological zones of (a) Taita Hills and (b) Mount Kilimanjaro. The altitudinal zones were categorized as lowland region (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane zone (1500-1799m a.s.l). Same letter (a) denote lack of significant difference in mean abundance in paired agro-ecological zones in each transect.

3.3.4.2.1 Geographical distribution of false codling moth

Geographical distribution of false codling moth (*Thaumatotibia leucotreta*) mean abundance sampled was evenly spread along the altitudinal gradient of Taita Hills and Mount Kilimanjaro (Figure 3.8ii-a & b).

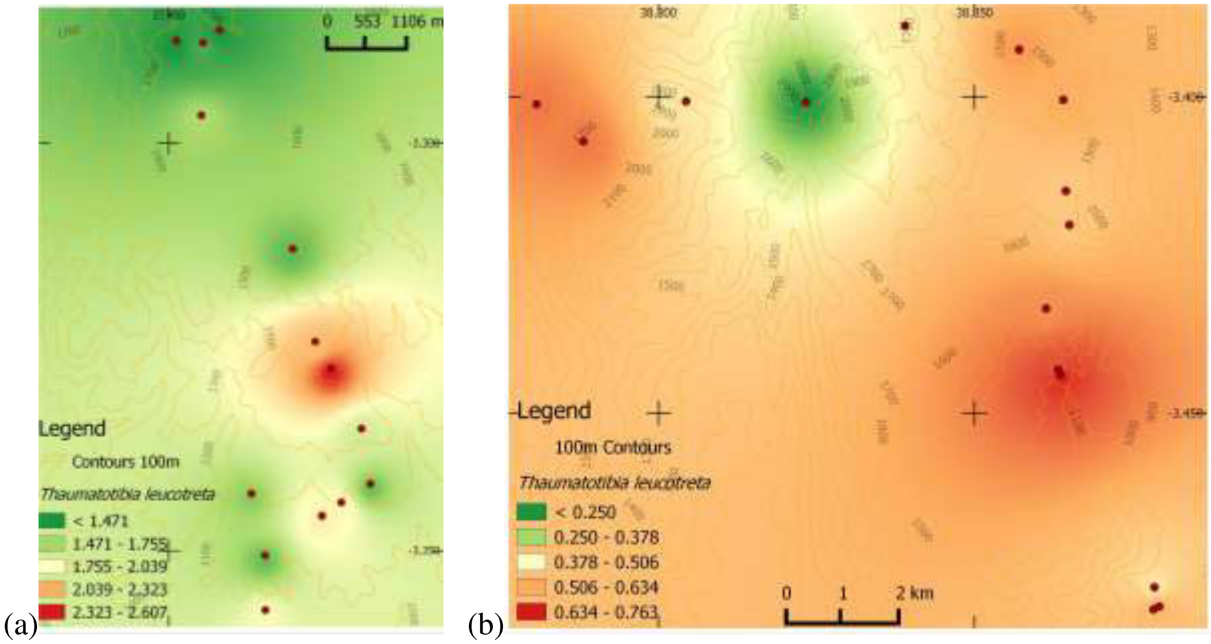


Figure 3.8ii-a & b: Geographical distribution of false codling moth (*Thaumatotibia leucotreta*) mean abundance along altitudinal gradient of (a) Mount Kilimanjaro, Tanzania and in (b) Taita Hills, Kenya transects. Red shows highest whereas green reveals least mean abundance of false codling moth.

3.3.4.3 *Heliothrips haemorrhoidalis* (Greenhouse thrips)

Heliothrips haemorrhoidalis was abundant in mid-altitudinal range (1200-1499m a.s.l.) in Taita Hills however its population was higher in montane zone (1500-1799m a.s.l.) of Mount Kilimanjaro (Figure 3.9i-a & b). Spatial distribution scenario was more less the same for *Heliothrips haemorrhoidalis* (Figure 3.9ii-a & b). Comparison of mean abundance of *Heliothrips haemorrhoidalis* across all altitudinal zones of Taita Hills and Mount Kilimanjaro revealed a significant difference. Tukey's HSD pair wise comparison of mean abundance of *Heliothrips haemorrhoidalis* across all elevational zones in Taita Hills revealed a significant difference. For

Mount Kilimanjaro transect, *Heliothrips haemorrhoidalis* revealed a significant difference in only two pairs (lowland and montane; sub-montane and montane) except between lowland and sub-montane.

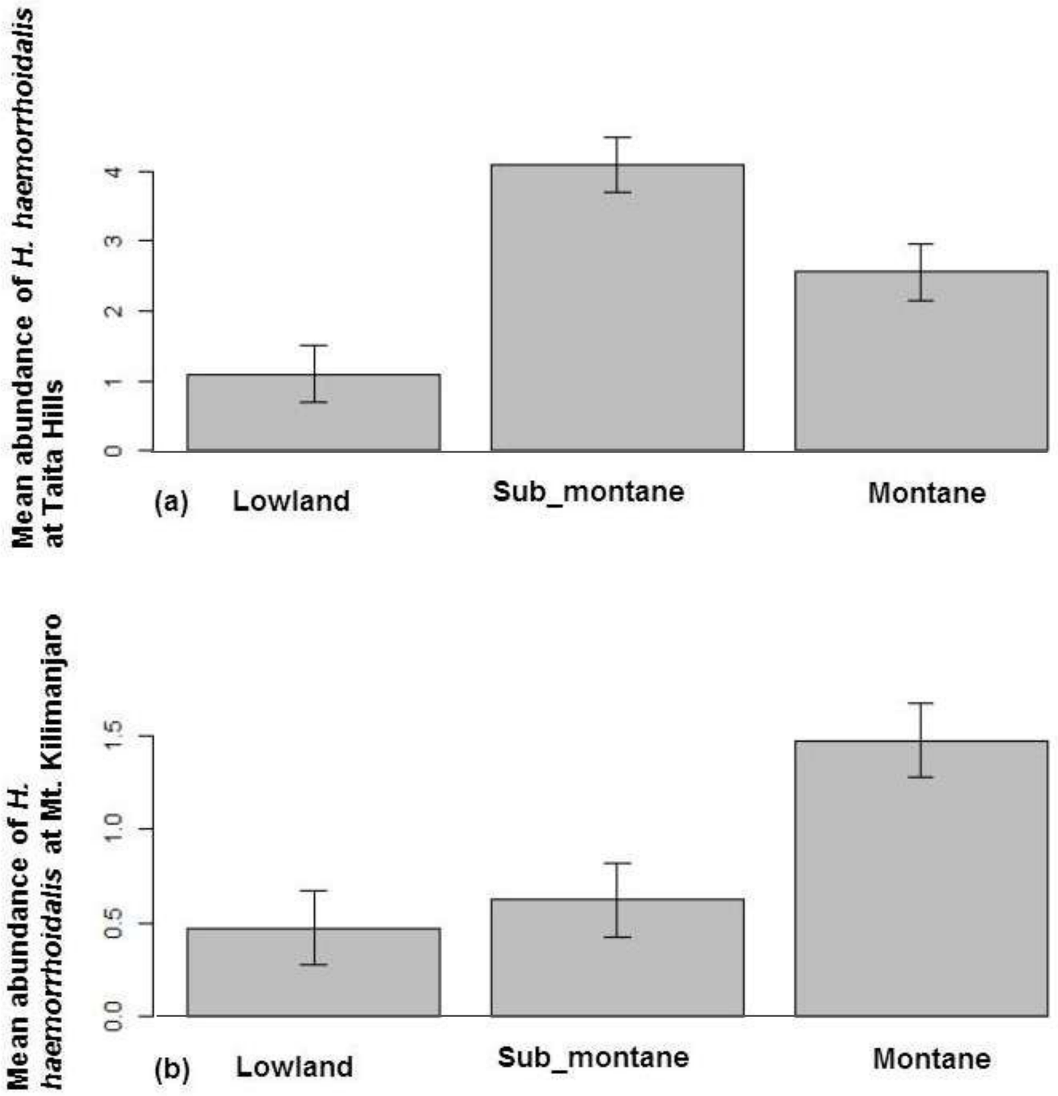
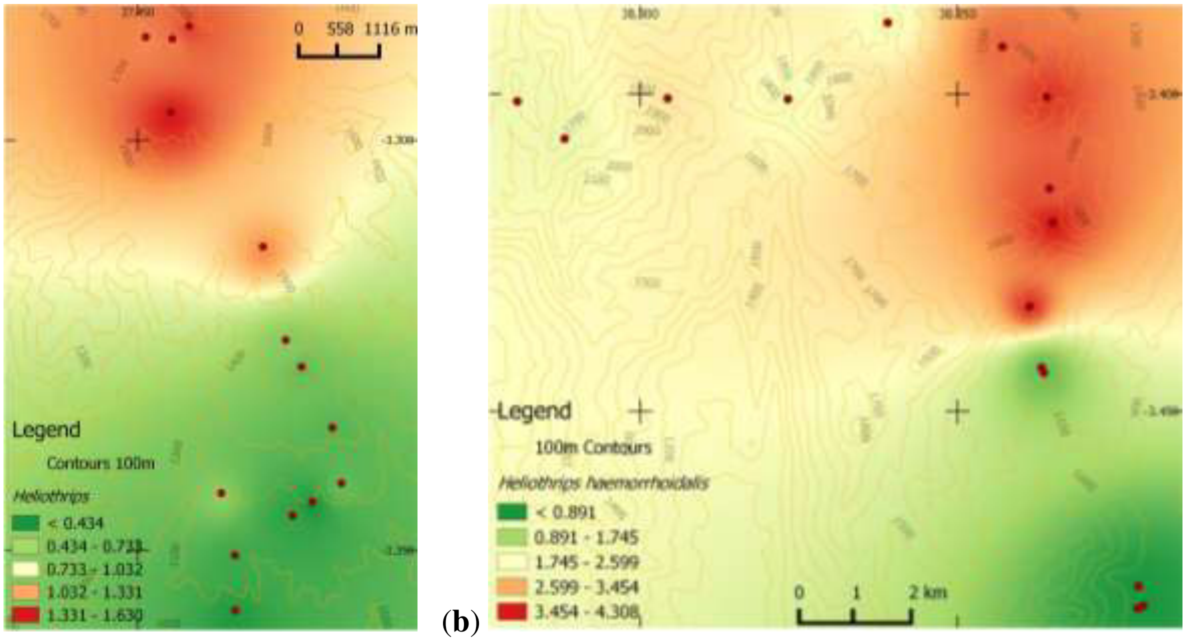


Figure 3.9i-a & b: Distribution of *Heliothrips haemorrhoidalis* within agro-ecological zones of Taita Hills (a) and Mount Kilimanjaro (b). The altitudinal zones were the lowland region (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane (1500-1799m a.s.l).



(b)

Figure 3.9ii-a & b: Geographical distribution of *Heliothrips haemorrhoidalis* mean abundance along altitudinal gradient of (a) Mount Kilimanjaro, Tanzania and in (b) Taita Hills, Kenya transects. Red shows highest whereas green reveals least mean abundance of *Heliothrips haemorrhoidalis*.

3.3.4.4 *Frankliniella schultzei* (Common blossom thrips)

Frankliniella schultzei was abundant in mid-altitudinal range (1200-1499m a.s.l.) in Taita Hills however its population was higher in montane zone (1500-1799m a.s.l.) of Mount Kilimanjaro (Figure 3.10a & b). The spatial distribution scenario was more less the same for *Frankliniella schultzei* (Figure 3.11a & b). The means of *Frankliniella schultzei* revealed a significant difference only in Taita Hills. Tukey's HSD pair wise comparison of mean abundance of *Frankliniella schultzei* between agro-ecological zones in Taita Hills revealed a significant difference in only two pairs except between montane and lowland. For Mount Kilimanjaro transect, the mean abundance of *Frankliniella schultzei* between agro-ecological zones revealed a significant difference in only two pairs (lowland and montane; sub-montane and montane) except between lowland and sub-montane.

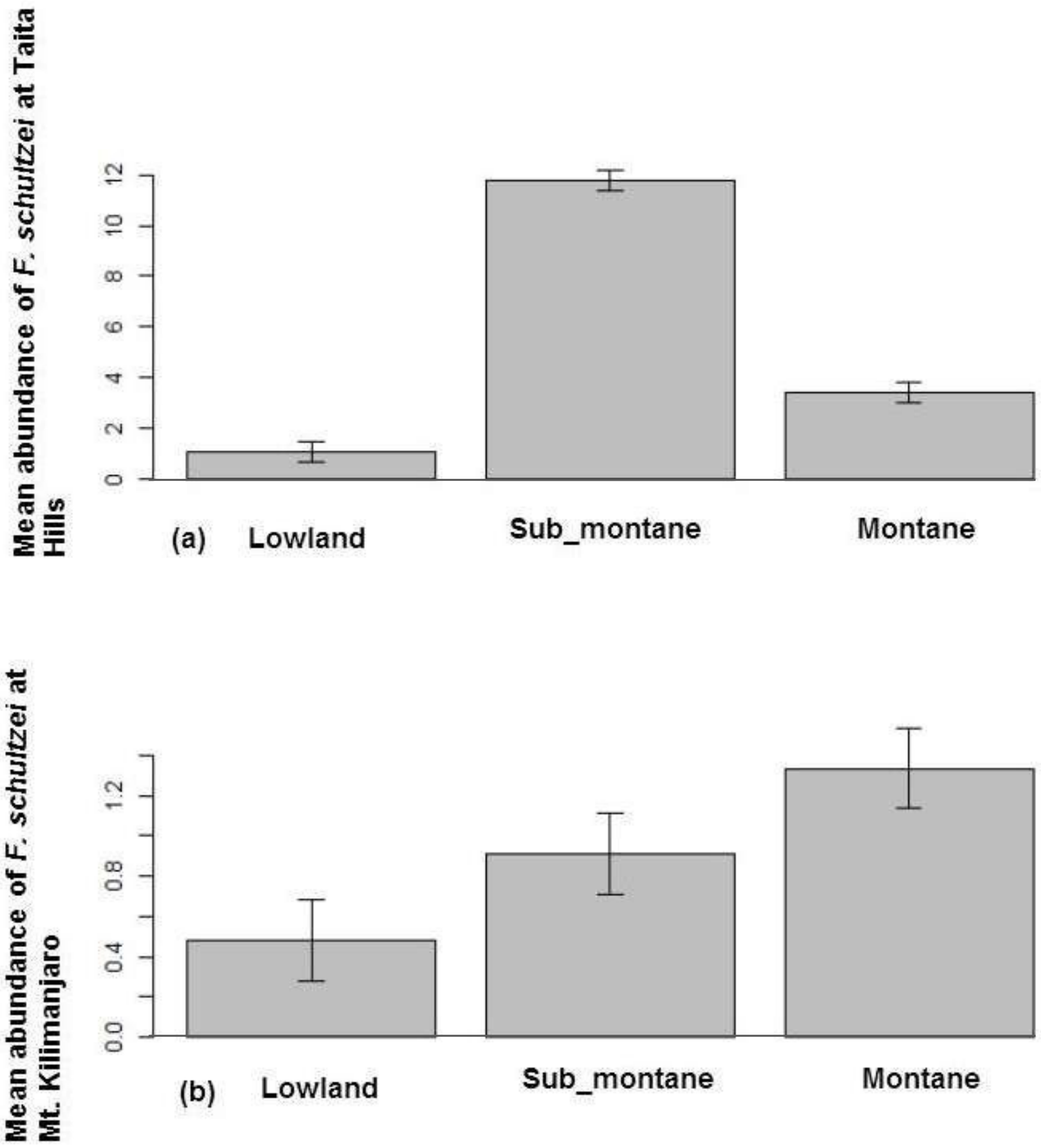
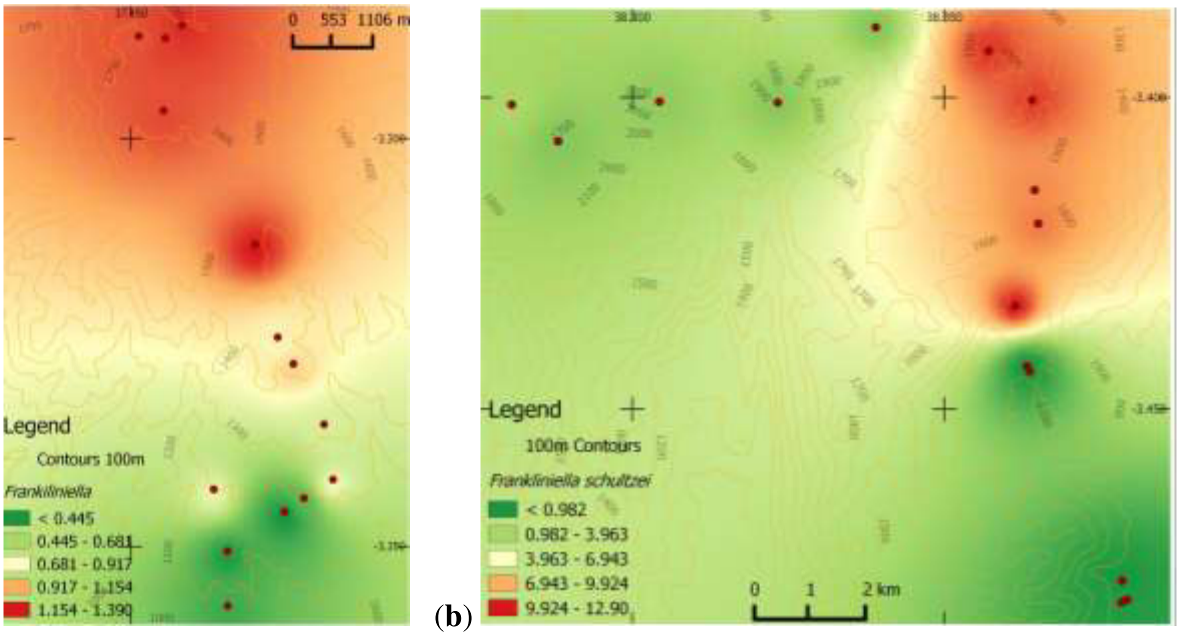


Figure 3.10a & b: Distribution of *Frankliniella schultzei* within agro-ecological zones of (a) Taita Hills and (b) Mount Kilimanjaro. The altitudinal zones were the lowland region (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane (1500-1799m a.s.l).



(b)

Figure 3.11a & b: Geographical distribution of *Frankliniella schultzei* mean abundance along altitudinal gradient of (a) Mount Kilimanjaro, Tanzania and in (b) Taita Hills, Kenya transects. Red shows highest whereas green reveals least mean abundance of *Frankliniella schultzei*.

3.3.5 Seasonal changes in population density of avocado key pest species

Weather and avocado plant seasonality (Appendix 5a & b; 6a & b) were defined based on mean rainfall of the two study areas which differed significantly (P<0.0001) between most paired seasons for both Taita Hills and Mount Kilimanjaro. Comparison of change in abundance of the key avocado insect pests (*Bactocera invadens*, *Thaumatotibia leucotreta*, *Heliothrips haemorrhoidalis* and *Frankliniella schultzei*) between different seasons in the two study areas is described below.

3.3.5.1 Seasonal changes in mean abundance of *Bactrocera invadens* and false codling moth

A significant difference was observed in mean abundance of the Asian invasive fruit fly (*Bactrocera invadens*) within all weather seasonality in Taita Hills (P>0.002, df=3, chi-squared=15.24; Kruskal-Wallis test; Figure 3.12a) and Mount Kilimanjaro (P<0.0001, df=3, chi-squared=53.01; Kruskal-Wallis test; Figure 3.12b). Significant difference in Tukey's HSD pair wise comparison of mean abundance of Asian invasive fruit fly (*Bactrocera invadens*) was observed between Cold season against Dry season, and also between Dry season against Short rain season both in Taita Hills and Mount Kilimanjaro (Figure 3.12a & b).

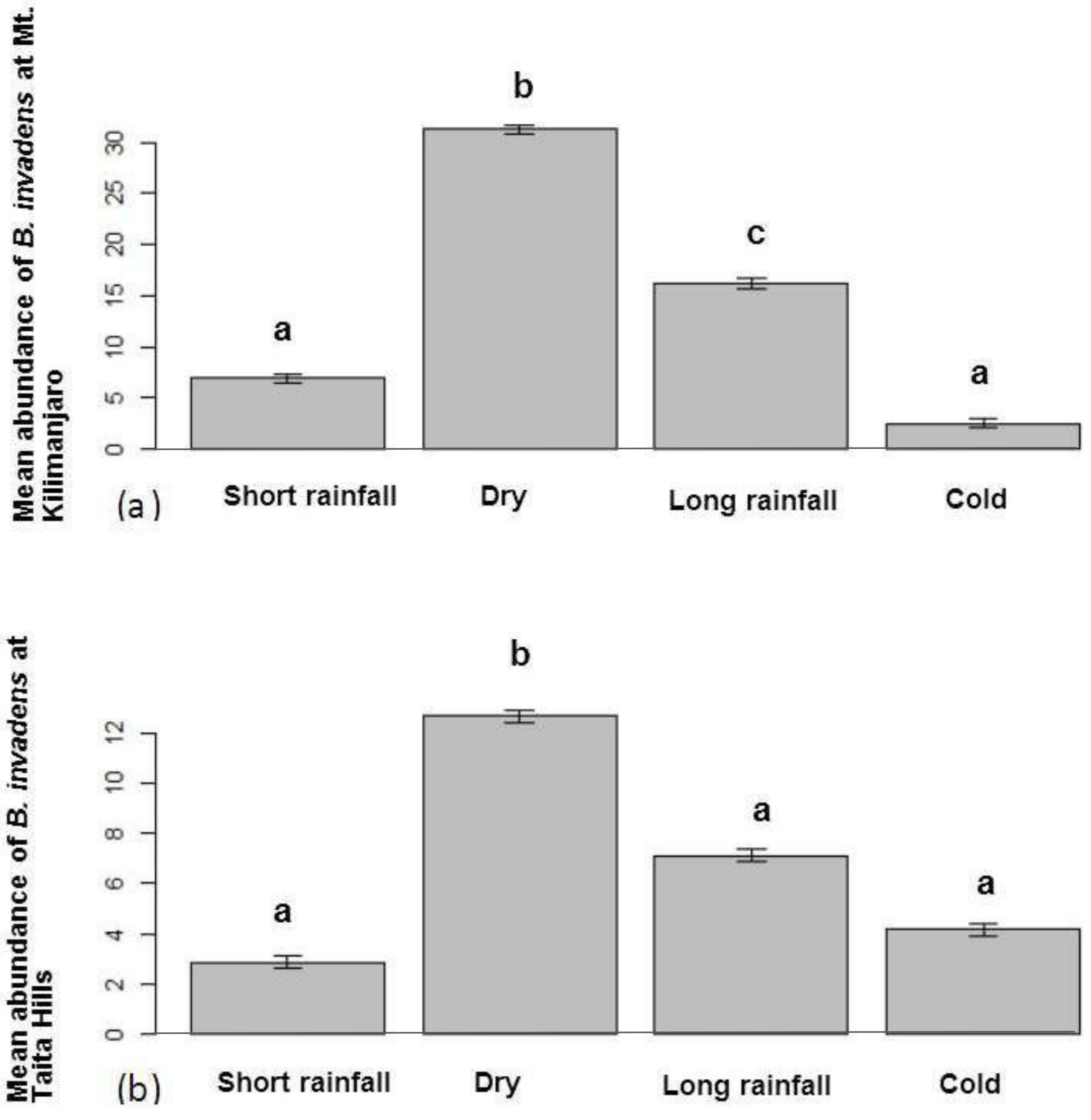
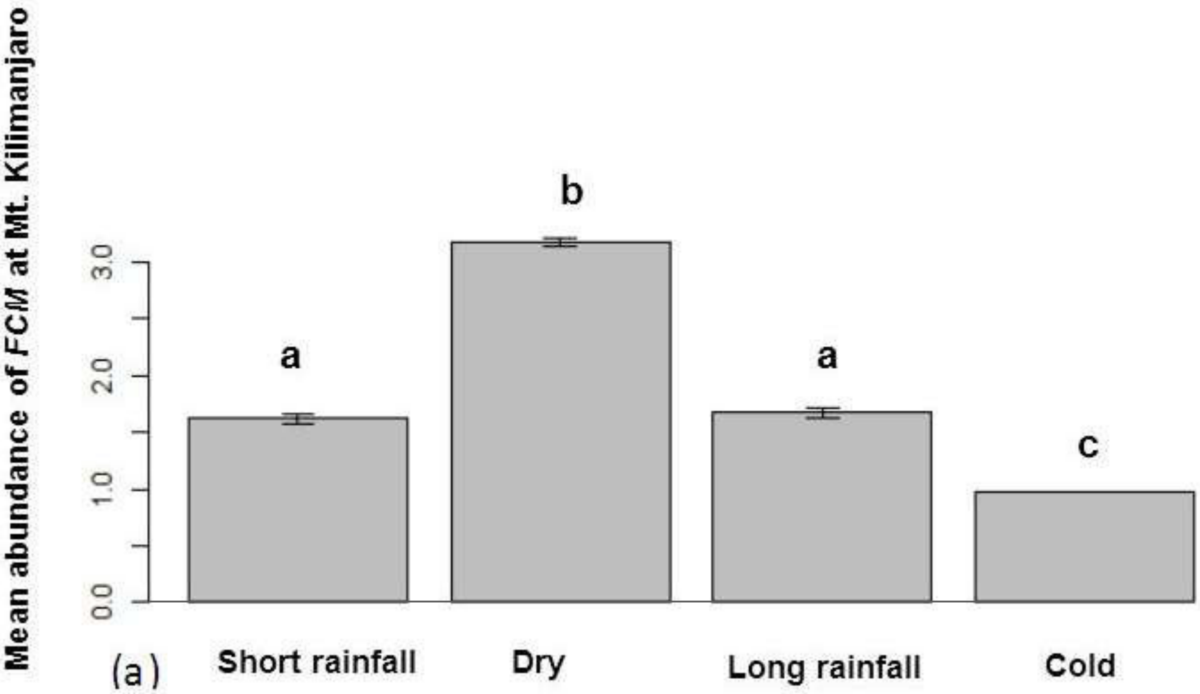


Figure 3.12a & b: Seasonal abundance of *Bactrocera invadens* in (a) Mount Kilimanjaro study area and (b) Taita Hills transect within a single year (12 months). Short rain (September, October & November), Dry (December, January & February), Long rain (March, April & May) and Cold season (June, July & August). Different letters (a, b and c) denote significantly differing mean abundance along annual seasonality. Same letter (a) denotes lack of significant difference in mean abundance in paired seasonal abundance in Taita Hills and Mount Kilimanjaro study areas.

Similarly, a significant difference was observed in mean abundance of false codling moth (*Thaumatotibia leucotreta*) within all weather seasons in Taita Hills (P<0.01, df=3, chi-squared=34.99; Kruskal-Wallis test; Figure 3.13a) and Mount Kilimanjaro (P<0.0001, df=3, chi-squared=100.10; Kruskal-Wallis test; Figure 3.13b). There was significant difference (p<0.04) in Tukey's HSD pair wise comparison of mean abundance of *Thaumatotibia leucotreta* (false codling moth) between Cold season against Dry season, Long rainfall season against Dry season, and also between Dry season against Short rain season both in Taita Hills and Mount Kilimanjaro (Figure 3.13a & b).



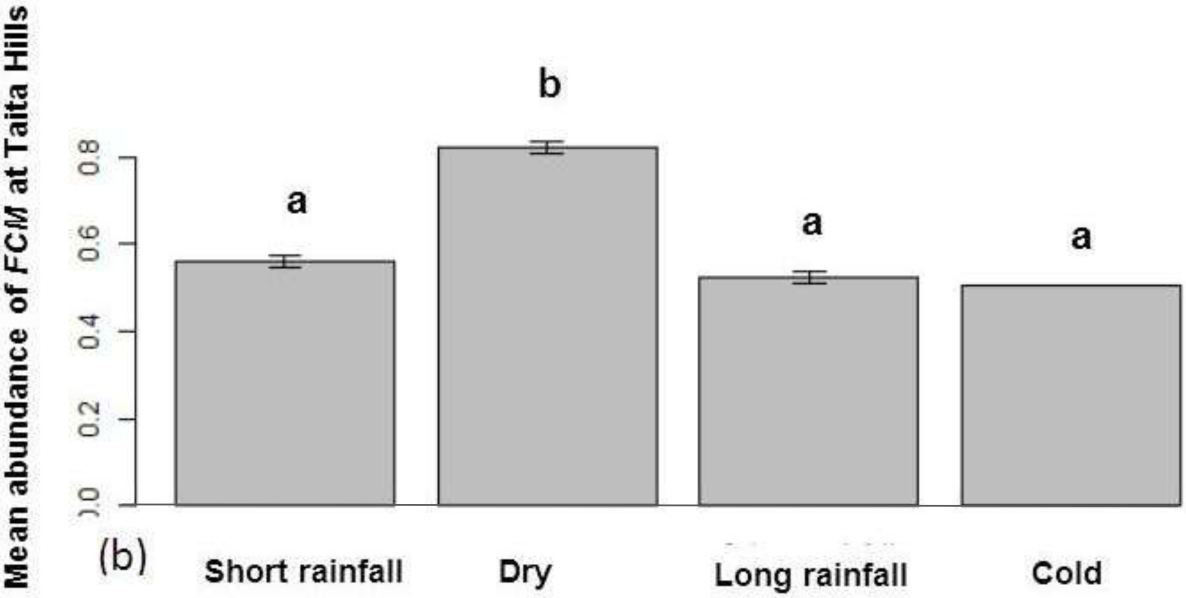


Figure 3.13a & b: Seasonal abundance of false codling moth in (a) Mount Kilimanjaro study area and (b) Taita Hills transect within a single year (12 months). Short rain (September, October & November), Dry (December, January & February), Long rain (March, April & May) and Cold season (June, July & August). Different letters (a, b and c) denote significantly differing mean abundance along annual seasonality. Same letter (a) denotes lack of significant difference in mean abundance in paired seasonal abundance in Taita Hills and Mount Kilimanjaro study areas.

3.3.5.2 Seasonal changes in mean abundance of *Heliothrips haemorrhoidalis* and *Frankliniella schultzei*

A significant difference was observed in mean abundance of greenhouse thrips (*Heliothrips haemorrhoidalis*) and common blossom thrips (*Frankliniella schultzei*) within all avocado phenological seasons in Taita Hills and Mount Kilimanjaro transects (P>0.0001, df=3, chi-squared=15.24; Kruskal-Wallis test; Figure 3.14a & b; Figure 3.15a & b).

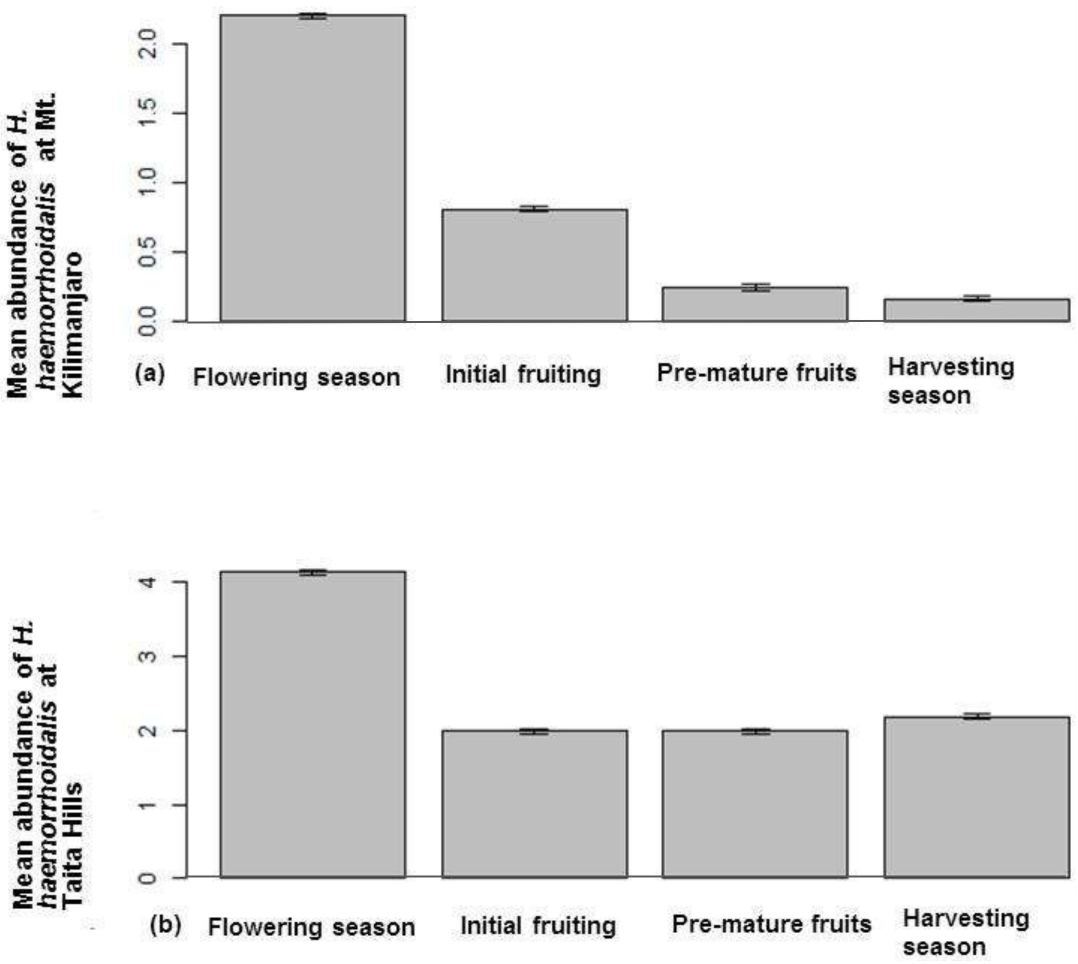


Figure 3.14a & b: Seasonal abundance of greenhouse thrips (*Heliothrips haemorrhoidalis*) in (a) Mount Kilimanjaro study area and (b) Taita Hills transect within a single year (12 months). Flowering season (August, September & October), Initial avocado fruiting (November, December & January), Pre-mature season (February, March & April) and Harvesting season (May, June & July).

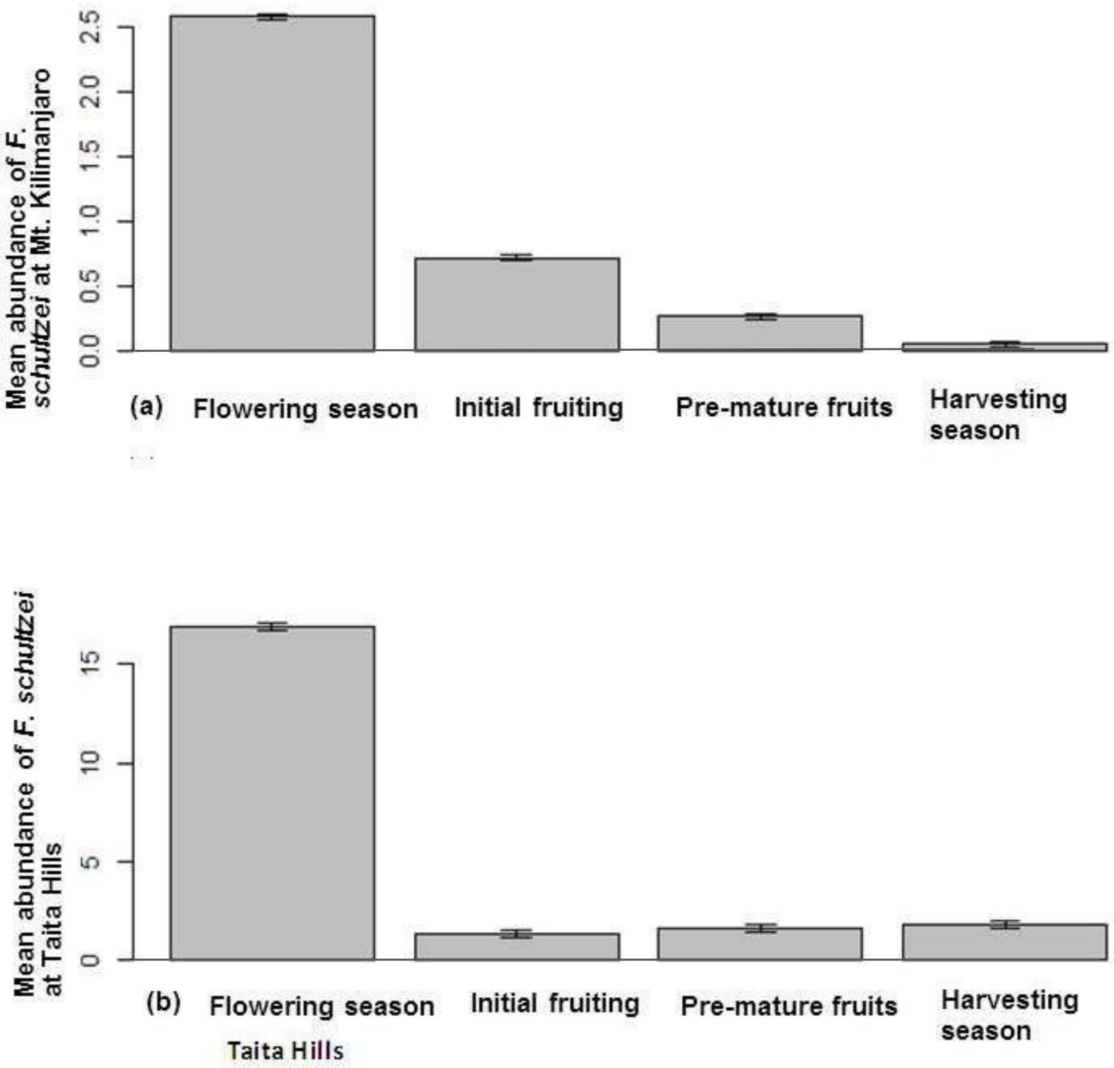


Figure 3.15a & b: Seasonal abundance of common blossom thrips (*Frankliniella schultzei*) in (a) Mount Kilimanjaro study area and (b) Taita Hills transect within a single year (12 months). Flowering season (August, September & October), Initial avocado fruiting (November, December & January), Pre-mature season (February, March & April) and Harvesting season (May, June & July).

3.3.6 Annual population trend of the key avocado pest species

3.3.6.1 Mean monthly population density trend of *Bactrocera invadens* in each transect

Annual overall population fluctuation of the Asian invasive fruit fly (*Bactrocera invadens*) revealed similar patterns in Taita Hills and Mount Kilimanjaro. A peak period was recorded between November and March of the following year and then it reduced drastically up to May.

The population of *Bactrocera invadens* then increased substantially in June during avocado harvesting season. August and September had the least population density. Mean monthly abundance of *Bactrocera invadens* was significantly different. A significant difference was observed in mean abundance of *Bactrocera invadens* within all calendar months recorded in Taita Hills ($P>0.03$, df=11; chi-squared = 21.03; Kruskal-Wallis test) and Mount Kilimanjaro during the same period ($P<0.0001$, df=11; chi-squared = 50.86; Kruskal-Wallis test).

Annual population trend of the Asian invasive fruit fly (*Bactrocera invadens*) varied greatly depending on the elevational zones of Mount Kilimanjaro and Taita Hills (Figure 3.16a & b). Annual population density variations was more pronounced in lowland region (900-1199m a.s.l.), sub-montane zone (1200-1499m a.s.l.) but there was very minimal changes in upper montane (1500-1799m a.s.l) section of transect (Figure 3.16a & b). Generally, peak mean population density in each agro-ecological zone was between November and March of the following year. The population then reduced drastically in April and May but witnessed a significant increase in June (Figure 3.16a & b) except in the upper elevated montane zone (1500-1799m a.s.l). There was no significant difference in monthly mean abundance of *Bactrocera invadens* in montane zone (1500-1799m a.s.l of Taita Hills ($P = 0.16$, df=11; Kruskal-Wallis chi-squared test).

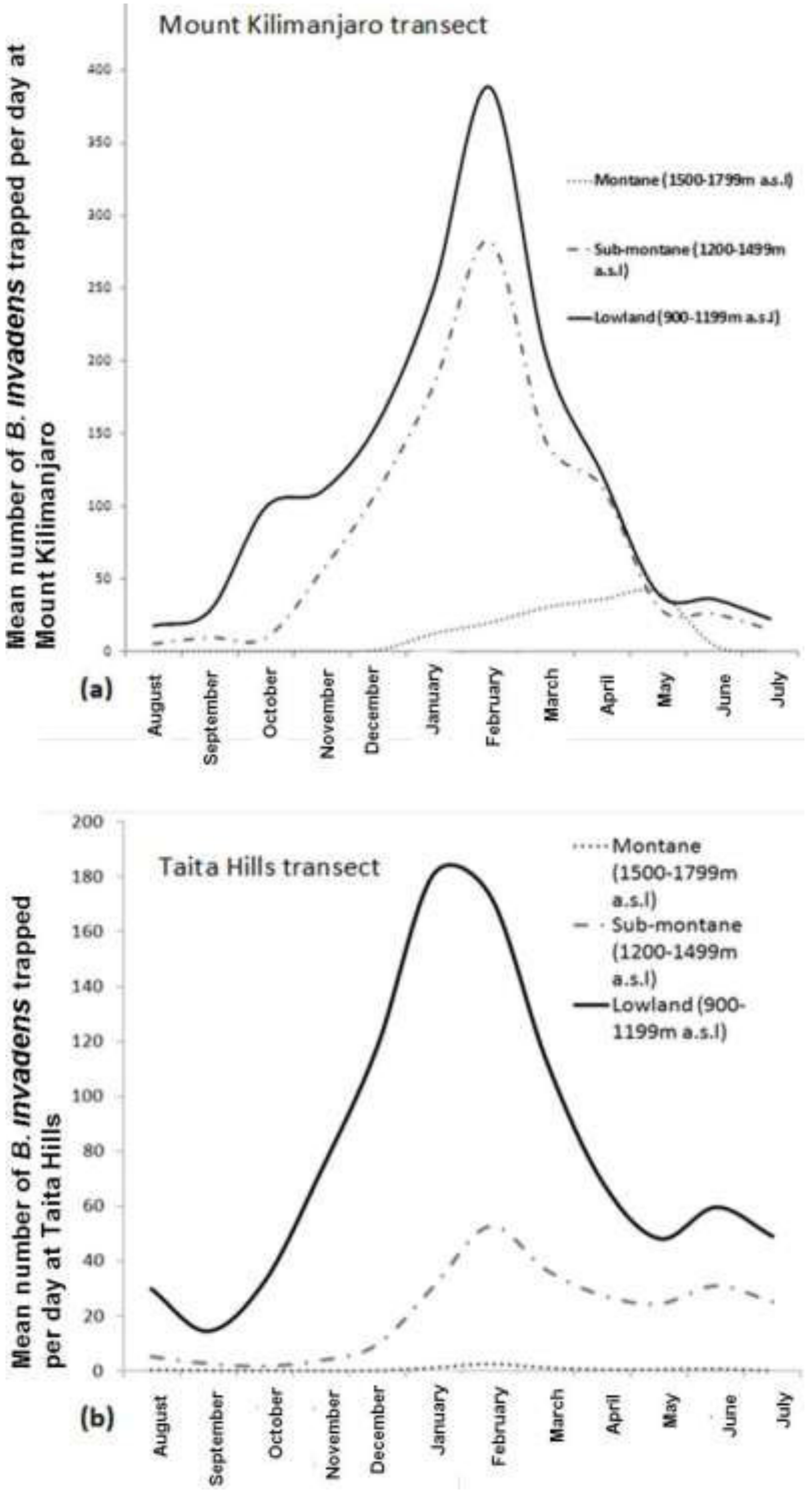


Figure 3.16a & b: Mean abundance trend of *Bactrocera invadens* describing a twelve month variation in population from August to July the following year in each agro-ecological zone along (a) Mount Kilimanjaro and (b) Taita Hills transects. The zones were categorized as lowland (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane region (1500-1799m a.s.l).

3.3.6.2 Mean monthly population density trend of false codling moth in each transect

Annual overall population density trend of false codling moth (*Thaumatotibia leucotreta*) had a similar pattern both in Mount Kilimanjaro, Tanzania and Taita Hills, Kenya. Peak months with highest mean abundance were December, January, February and March. A significant difference was observed in mean abundance of *Thaumatotibia leucotreta* within all calendar months recorded in Taita Hills ($P>0.03$, df=11; Kruskal-Wallis chi-squared test) and Mount Kilimanjaro ($P<0.0001$, df=11; Kruskal-Wallis chi-squared test).

Annual population density trend of false codling moth (*Thaumatotibia leucotreta*) were similar in each of the three agro-ecological zones of Mount Kilimanjaro and Taita Hills (Figure 3.17a & b). Peak mean population density was in December but density reduced drastically between February and May and witnessed a small increase in June within lowland and sub-montane zones (Figure 3.17a & b). There was significant difference in monthly mean abundance of false codling moth in all zones ($P<0.05$, df=11; Kruskal-Wallis chi-squared test).

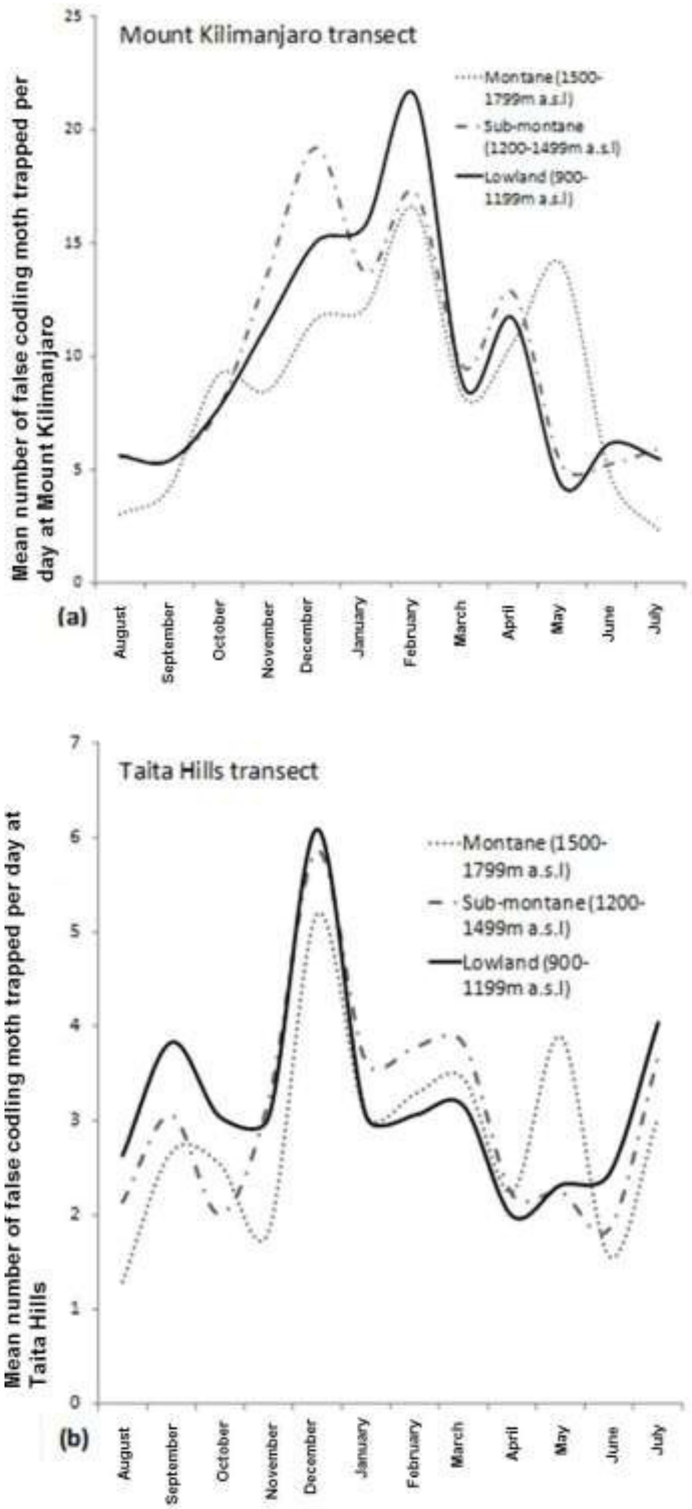


Figure 3.17a & b: Abundance trend of *Thaumatotibia leucotreta* (false codling moth) describing a twelve month changes in population from August to July the following year in each agro-ecological zone along (a) Mount Kilimanjaro and (b) Taita Hills transects. The zones were categorized as lowland (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane region (1500-1799m a.s.l).

3.3.6.3 Mean monthly population density trend of greenhouse thrips and common blossom thrips

Highest mean monthly abundance peak for greenhouse thrips (*Heliothrips haemorrhoidalis*) and common blossom thrips (*Frankliniella schultzei*) was observed from August to October in each of the two study areas. The population of the two thrip species declined rapidly in the following year during the months of March, April and May but increased from late July. There was significant difference in monthly mean abundance of *Frankliniella schultzei* and *Heliothrips haemorrhoidalis* within the avocado season/ year ($P<0.005$, df=11; Kruskal-Wallis chi-squared test). Tukey's HSD pair wise test revealed a significant difference in mean abundance of *Heliothrips haemorrhoidalis* and *Frankliniella schultzei* between months when avocado plant is at peak flowering stage and fruit harvesting (September VS May and October VS June).

Annual population density trend of greenhouse thrips (*Heliothrips haemorrhoidalis*) and common blossom thrips (*Frankliniella schultzei*) were more or less similar in each of the three agro-ecological zones of Mount Kilimanjaro and Taita Hills (Figure 3.18a & b; 3.19a & b).

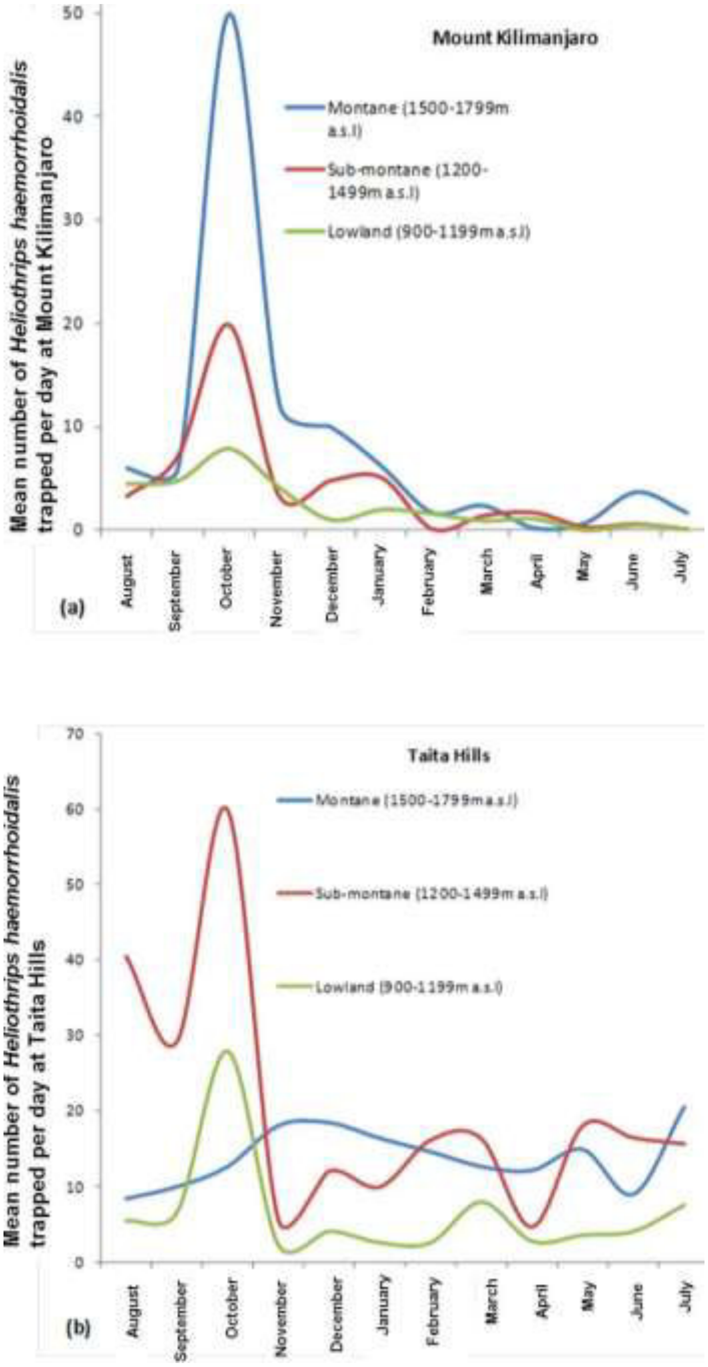


Figure 318a & b: Abundance trend of *Heliothrips haemorrhoidalis* describing a twelve month changes in population from August to July the following year in each agro-ecological zone along (a) Mount Kilimanjaro and (b) Taita Hills transects. The zones were categorized as lowland (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane region (1500-1799m a.s.l).

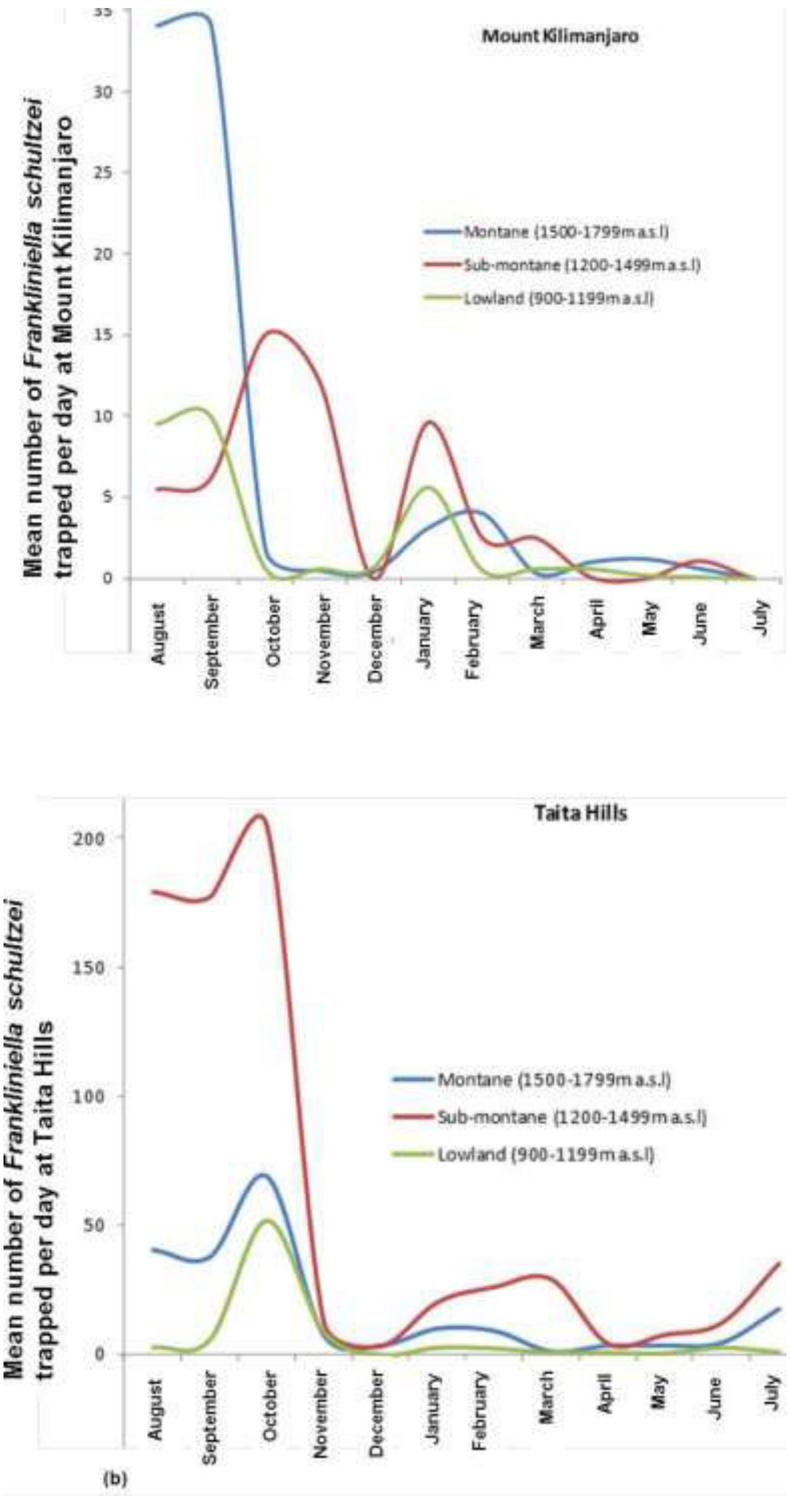


Figure 3.19a & b: Abundance trend of *Frankliniella schultzei* describing a twelve month changes in population from August to July the following year in each agro-ecological zone along (a) Mount Kilimanjaro and (b) Taita Hills transects. The zones were categorized as lowland (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane region (1500-1799m a.s.l).

3.3.7 Characterizations of abundance of key avocado insect pests using environmental variables

Overall abundance of *Bactrocera invadens* recorded in Taita Hills and Mount Kilimanjaro had a strong positive correlation with temperature (r = 0.81; Table 4a) but negatively correlated with elevation and humidity (Table 3.4a). For false codling moth (FCM), an overall positive correlation (r = 0.46; Table 3.4b) was recorded with temperature and negative correlation with humidity and elevation but the majority of variables weakly correlated with abundance of FCM, *Thaumatotibia leucotreta* (Table 3.4b). The mean abundance of greenhouse thrips (*Heliothrips haemorrhoidalis*) and common blossom thrips (*Frankliniella schultzei*) correlated weakly with most of environmental variables (Table 3.4c & d).

Table 3.4: Spearman's rank correlation coefficient of abundance of avocado insect pests with different environmental variables.

(a) Response variable: Asian invasive fruit fly (*Bactrocera invadens*)

	r-value	
Explanatory variables	**Taita Hills**	**Mount Kilimanjaro**
Temperature	0.82	0.80
Humidity	-0.51	-0.43
Rainfall	-0.32	0.01
Altitude	-0.85	-0.68
Distance	0.83	0.68
Latitude	-0.68	-0.68

(b) Response variable: False codling moth (*Thaumatotibia leucotreta*)

	r-value	
Explanatory variables	**Taita Hills**	**Mount Kilimanjaro**
Temperature	0.31	0.61
Humidity	-0.15	-0.53
Rainfall	-0.05	0.08
Altitude	-0.24	-0.15
Distance	0.10	0.15
Latitude	-0.15	-0.45

(c) Response variable: Greenhouse thrips (*Heliothrips haemorrhoidalis*)

	r-value	
Explanatory variables	**Taita Hills**	**Mount Kilimanjaro**
Temperature	-0.50	0.11
Humidity	0.35	-0.31
Rainfall	0.04	-0.07
Altitude	0.45	0.16
Distance	-0.45	-0.17
Latitude	0.50	0.16

(d) Response variable: Common blossom thrips (*Frankliniella schultzei*)

	r-value	
Explanatory variables	**Taita Hills**	**Mount Kilimanjaro**
Temperature	-0.44	0.12
Humidity	0.05	-0.40
Rainfall	-0.19	0.10
Altitude	0.35	-0.21
Distance	-0.37	-0.10
Latitude	0.42	0.10

Distance refers to the linear space in kilometres from the highest montane form to every sampling point along each study transect; Taita Hills (Vuria peak) and Mount Kilimanjaro (Uhuru summit).

Linear Mixed Effect model analysis revealed that temperature explained best change in abundance of the key avocado pest; Asian invasive fruit fly (*Bactrocera invadens*, Diptera: Tephritidae). In Mount Kilimanjaro, the change in mean abundance of *Bactrocera invadens* was best explained by a model with lowest Akaike Information Criterion (AIC) value that had both mean temperature and habitat (agro-ecological zones) as explaining variables (AIC= 1455.23, $R^2 = 0.53$, $F_{5,174} = 41.97$, $p < 0.0001$; linear mixed effect model) (Table 3.5). The best model (one) had the interaction term: agro-ecological zone by temperature. Based on evaluations of AIC values, other models could not best explain the data but had a significant influence on change in abundance of *Bactocera invadens* along the study transect (Table 3.5).

Table 3.5: Linear mixed effect models explaining change in abundance of *Bactocera invadens* along agro-ecological zones of Mount Kilimanjaro, Tanzania.

Model	**AIC**	**ΔAIC**	**R^2**	**p - value**	**Rank**
1. *Bactocera invadens* mean density vs interaction term (mean temperature by agro-ecological zones) **was the best model) --------->**	**1455.23**	0	**0.5337**	**<0.0001**	**1**
2. *Bactocera invadens* mean density vs mean temperature & habitats **without** interaction term (mean temperature by agro-ecological zones)	1485.83	30.6	0.4413	**<0.0001**	2
3. *Bactocera invadens* mean density vs mean temperature, mean humidity & agro-ecological zones	1487.83	32.6	0.4381	**<0.0001**	3
4. *Bactocera invadens* mean density vs mean temperature, mean humidity, rainfall & agro-ecological zones	1488.13	32.9	0.4401	**<0.0001**	4
5. *Bactocera invadens* mean density vs mean temperature, mean humidity, rainfall, elevation, distance & agro-ecological zones	1490.04	34.81	0.4388	**<0.0001**	5
6. *Bactocera invadens* mean density vs mean humidity & agro-ecological zones	1518.98	63.75	0.3283	**<0.0001**	6

Model one in Table 3.5 above was the best with all the p-values for all explanatory variables including interaction term were statistically significant (Table 3.6).

Table 3.6: Parameters estimates of the model (one) that explained best the change in abundance of *Bactocera invadens* (response variable) along agro-ecological zones of Mount Kilimanjaro, Tanzania.

Explanatory variables	**Parameter estimate**	**Std. error**	**t value**	**p-value**
(Intercept)	-170.057	22.03	-8	***
Mean temperature	8.902	1.01	9	***
Sub-montane zone (1200 - 1499m a.s.l)	63.979	28.87	2.22	*
Montane zone (1500 - 1799m a.s.l)	160.286	27.95	5.74	***
Mean temperature: Sub-montane zone	-2.975	1.35	-2.21	*
Mean temperature: Montane zone	-8.253	1.39	-5.95	***

The t-values reveal the strength and direction of main effects and also combined effects of parameters involved in the interaction between temperature and habitats. *** = highly significant and * = fairly significant. The model rank one had *Bactocera invadens* as the response variable (AIC= 1455.23, $R^2 = 0.53$, $F_{5, 174} = 41.97$, $p < 0.0001$; linear mixed effect model).

In Mount Kilimanjaro, the best model showed that abundance of *Bactrocera invadens* linearly increased with increase in mean temperature and the gradient was higher at lowland zone (0.456) followed by sub-montane (0.396) and the least change was in montane agro-ecological zone (0.189; Figure 3.20). Interaction of temperature and habitat on mean abundance of *Bactrocera invadens* was more pronounced in agro-ecological zones of Mount Kilimanjaro, Tanzania.

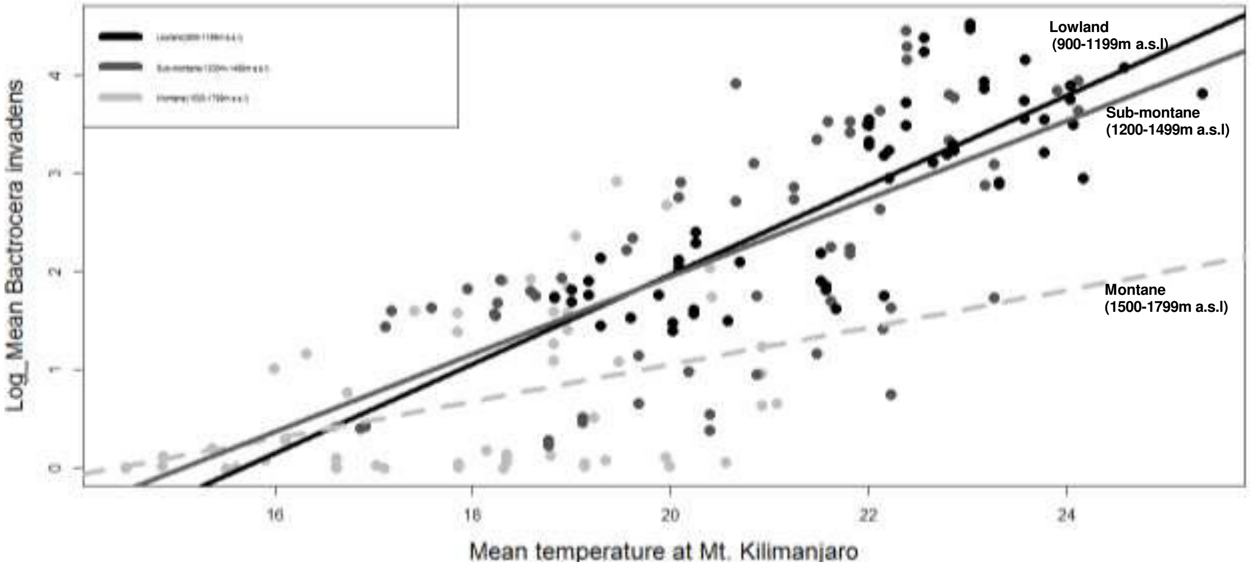


Figure 3.20: Relation between mean abundance of *Bactrocera invadens* and average temperature in agro-ecological zones of Mount Kilimanjaro, Tanzania. The black line = Lowland zone ($R^2 = 0.64$), dark grey = Sub-montane ($R^2 = 0.41$) whereas doted-grey = Montane ($R^2 = 0.17$).

Similarly in Taita Hills, the change in mean abundance of *Bactrocera invadens* was best explained by model one that had both habitat (agro-ecological zones) and mean temperature as explaining variables (Table 3.7). The best model had an interaction term: agro-ecological zone by temperature with the lowest Akaike Information Criterion (AIC= 1176.41) and highest adjusted R² = 0.5876 (Table 3.7). All other models had a significant influence on change in abundance of *Bactocera invadens* along agro-ecological zones of Taita Hills but did not best explain change in the data due to higher AIC values (Table 3.7).

Table 3.7: Linear mixed effect models explaining change in abundance of *Bactocera invadens* along agro-ecological zones of Taita Hills, Kenya.

Model	**AIC**	**ΔAIC**	**Adjusted R²**	**p - value**	**Rank**
1. *Bactocera invadens* mean density vs interaction term (mean temperature by agro-ecological zones) **was the best model) --------->**	**1176.41**	0	**0.5876**	**<0.0001**	**1**
2. *Bactocera invadens* mean density vs mean temperature , mean humidity, rainfall & agro-ecological zones	1185.27	8.86	0.5783	**<0.0001**	2
3. *Bactocera invadens* mean density vs mean temperature, mean humidity, rainfall, elevation, distance & agro-ecological zones	1188.4	11.99	0.5754	**<0.0001**	3
4. *Bactocera invadens* mean density vs mean temperature, mean humidity & agro-ecological zones	1190.78	14.37	0.5628	**<0.0001**	4
5. *Bactocera invadens* mean density vs mean temperature & habitats **without** interaction term (mean temperature by agro-ecological zones)	1200.81	24.4	0.5353	**<0.0001**	5
6. *Bactocera invadens* mean density vs mean humidity & agro-ecological zones	1222.95	46.54	0.4745	**<0.0001**	6

Model one in Table 3.7 above was the best with all the p-values for all explanatory variables including that of interaction term (temperature: habitats) were statistically significant as shown in Table 3.8.

Table 3.8: Parameters estimates of the model (one) that best explained change in abundance of *Bactocera invadens* (response variable) along agro-ecological zones of Taita Hills, Kenya (Table 2b-i above).

Explanatory variables	Parameter estimate	Std. Error	t value	p-value
(Intercept)	-67.01	12.398	-5.41	***
Mean temperature	3.7157	0.5543	6.704	***
Sub-montane zone (1200 - 1499m a.s.l)	40.543	16.46	2.463	*
Montane zone (1500 - 1799m a.s.l)	66.286	15.08	4.396	***
Mean temperature: Sub-montane zone	-2.12	0.7909	-2.68	**
Mean temperature: Montane zone	-3.667	0.744	-4.93	***

The p-values for all explanatory variables including that of interaction term (temperature: habitats) were statistically significant. The t-values reveal the strength and direction of main effects and also combined effects of parameters involved in the interaction between temperature and habitats. *** = highly significant, ** = significant and * = fairly significant. The model rank one had *Bactocera invadens* as the response variable (AIC= 1176.41, $R^2 = 0.59$, $F_{5, 174} = 52.0$, $p < 0.0001$; linear mixed effect model).

The best model in Taita Hills, revealed that abundance of *Bactrocera invadens* linearly increased with increase in mean temperature and the gradient was higher at sub-montane (0.278) followed by lowland zone (0.223) and the least change was in montane agro-ecological zone (0.036; Figure 3.21). Interaction of temperature and habitat on mean abundance of *Bactrocera invadens* was less pronounced in agro-ecological zones of Taita Hills, Kenya.

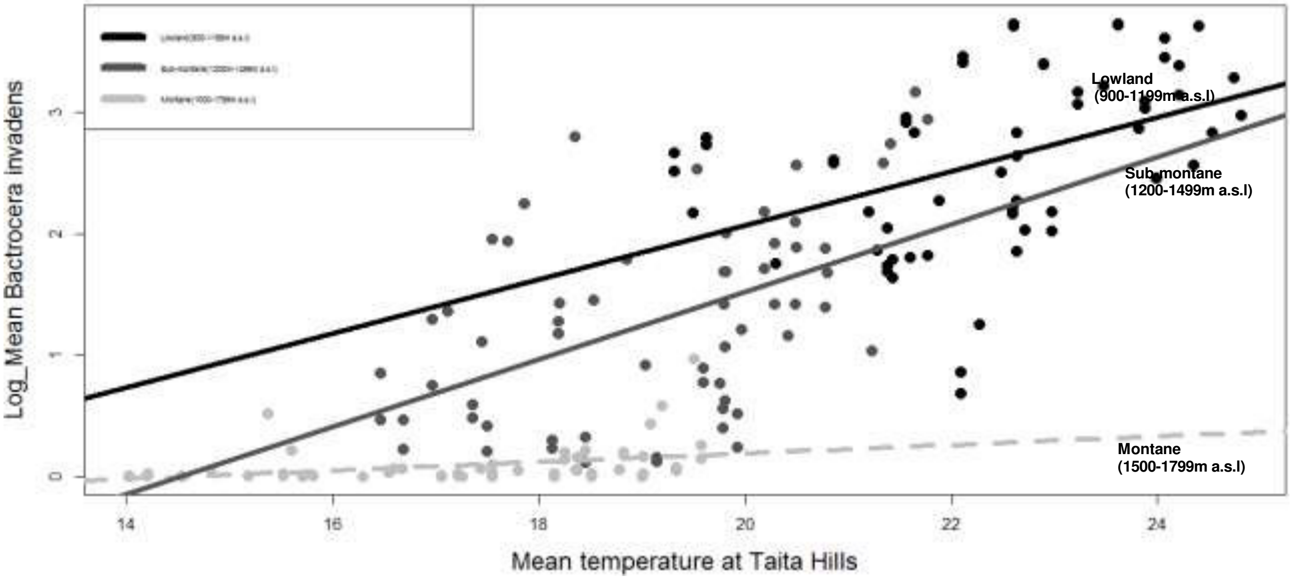


Figure 3.21: Relation between mean abundance of *Bactrocera invadens* and mean temperature in agro-ecological zones of Taita Hills, Kenya. The black line = Lowland zone ($R^2 = 0.28$), dark grey = Sub-montane ($R^2 = 0.24$) whereas doted-grey = Montane ($R^2 = 0.11$).

3.4 Discussion

3.4.1 Arthropod species accumulation curves and richness

Failure of the species accumulation curves to reach saturation point indicated that more species were likely to be found if additional sampling effort continued in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. Probably, if surveys are conducted over multiple years, the species accumulation curves for arthropods are expected to reach an asymptote due to a proportion of rare species which are often recorded in single encounters (Novotny & Basset, 2000). The singletons usually constitute a sizeable percentage of the species. This is a reported finding in several extensive long-term biodiversity studies of arthropods (Freund, 2005; Novotny & Basset, 2000). In this present study, beneficial arthropods including pollinators were not surveyed. However, Luvanga (2015) reports that friendly insect species including bees, beetles, ants and wasps were recorded on avocado flowers in Taita Hills. This study focused mainly on the key avocado insect pests.

3.4.2 Overall abundance of key avocado insect pests in Taita Hills and Mount Kilimanjaro

Study results confirmed that key avocado insect pests are Asian invasive fruit fly (*Bactrocera invadens*) and false codling moth (*Thaumatotibia leucotreta*) in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. Mount Kilimanjaro study area recorded high abundance of the key avocado insect pests than Taita Hills. This is because study area along southern slopes of Mount Kilimanjaro was a homogenous farmland with avocado trees being dominant as a fruit crop. Secondly, *Bactrocera invadens* and false codling moth could have adapted to avocado fruits as host due to its abundant availability along study transects. Consequently, the high number of avocado fruits and in particular those that fell on ground provided continuous reservoir for *Bactrocera invadens* and false codling moth along the Mount Kilimanjaro transect.

In the study area along Taita Hills, the avocado orchards were intertwined in between indigenous forest that probably provided refuge for natural competition of the key avocado insect pests and cold weather hence reduced population. Duyck *et al* (2007, 2006b, 2006a & 2004) reports that availability of indigenous habitats enhances numbers of pests' competitors, predators and parasitoids that enables natural control of pest species. Similarly, the overall population of *Bactrocera invadens* was very high in avocado orchards of Taita Hills compared to that of the false codling moth. The high abundance of the Asian invasive fruit fly (*Bactrocera invadens*) recorded over the sampling period is a feature that has been previously observed and described in other regions (Ekesi, *et al.*, 2009; Mwatawala *et al.*, 2009; Rwomushana *et al.*, 2008; Mohamed *et al.*, 2008). Ekesi *et al* (2009) and Rwomushana *et al* (2008) reports that the Asian invasive fruit fly infests multiple alternate hosts thereby increasing their chances of survivability in an agro-ecosystem.

The study concurs with published reports (Mound, 2010; Palmer, 1990) that Thripidae accounts for up to 60% of the total population of thrip species collected. The family Thripidae comprises of most pest species of avocado and the two most dominant species; *Heliothrips haemorrhoidalis* and *Frankliniella schultzei* are in family Thripidae. However, the red-banded thrips, *Selenothrips rubrocinctus* was not recorded in farmlands of Taita Hills and Mount Kilimanjaro as reported in avocado orchards of South Africa. The general results concur with findings by Mound (2010) and Palmer (1990). There was little variation in the number of species richness within the study areas but the population of thrips changed significantly depending on micro-environments. Namu (2005) reports that the number of species in an ecosystem remains the more-less the same but their ability to reproduce effectively and increase the population is heavily influenced by many spatiotemporal factors. The spatiotemporal factors include weather seasons, elevation gradient, number of productive host plants, temperature, relative humidity, predators and competitors.

3.4.3 Fruit infestation levels of avocado fruits by key insect pests within agro-ecological zones

3.4.3.1 Fruit damage levels by *Bactrocera invadens* and false codling moth

Fruit observation for infestation incidence by *Bactrocera invadens* revealed that the highest infestation was in avocado fruits that had fallen on the ground during harvesting and it was lower in fruits that were still on trees. Damage on unharvested fruits by *Bactrocera invadens* was mostly recorded on fruits with prior pericarp injury possibly due to feeding by birds or those with triangle mark from infestation by the false codling moth. These findings proves that the Asian invasive fruit fly (*Bactrocera invadens*) prefers ripening fruits (Mwatawala *et al*., 2009) and further confirm that it is an important post-harvest insect pest of avocado fruits. Infestation incidence of mature avocado fruits during peak harvesting season (June – July) by the Asian invasive fruit fly (*Bactrocera invadens*) insinuates that lowland (900–1199m a.s.l) and sub-montane zone (1200–1499m a.s.l) had a higher proportional damage than the montane (1500–1799m a.s.l). Lowland agro-ecological zone with relatively warmer temperatures witnessed higher infestation than the cold upper montane zones. Proportional avocado fruit infestation incidence correlated positively with the population density along study transects. This implies that interaction between temperature and agro-ecological zones strongly influenced infestation incidences in the two transects of Taita Hills, Kenya and Mount Kilimanjaro, Tanzania.

In contrast, fruits observations for infestation incidence by false codling moth (Thaumatotibia leucotreta) revealed that a significant infestation level of avocado fruits that were still on trees. This is because the false codling moth prefers to attack young avocado fruits that are still on tree (Prinsloo & Uys, 2015). Incidences were reported of re-infestation of fruits that had fallen on the ground. Our study, therefore, confirms that the false codling moth (*Thaumatotibia leucotreta*) is a pre-harvest fruit pest that prefers to attack immature avocado fruits that are still on tree across all agro-ecological zones of Taita Hills and Mount Kilimanjaro. (Prinsloo & Uys, 2015).

3.4.3.2 Fruit damage levels by *Heliothrips haemorrhoidalis*

Average avocado fruit infestation by *Heliothrips haemorrhoidalis* was higher in elevations below 1499m a.s.l (lowland and sub-montane). Infestation rate concurs with spatial distribution trends of *Heliothrips haemorrhoidalis* mean abundance in Taita hills and Mount Kilimanjaro. Areas below 1499m a.s.l. had ambient temperatures that enabled successful reproduction of *Heliothrips haemorrhoidalis* and consequently enhanced infestation. Constant cooler temperatures and very higher relative humidity could be used to explain the reason why proportional browning of the fruit skin was minimal in higher altitudinal zones. Proportional level of scarring on skin of mature avocado fruits was used to infer damage caused by the green house thrip. The results obtained concur with published reports that low temperature reduces developmental rate of insects (Khadioli *et al*., 2014a & b).

3.4.4 Geographical distribution of the key avocado insect pests along elevational gradients

3.4.4.1 *Bactrocera invadens* (Asian invasive fruit fly)

Spatial distribution patterns of key avocado insect pests along the two study areas of Taita Hills, Kenya and Mount Kilimanjaro, Tanzania were influenced by environmental characteristics of each agro-ecological zone. The findings from this study indicated that average abundance of *Bactrocera invadens* in the two study areas was greatly influenced by temperature and altitudinal gradient. This study revealed that abundance increased with increasing mean temperature and there was minimal presence of *Bactrocera invadens* or none below 15ºC or above 1800m a.s.l where very low temperatures were lethal for the fruit fly survival. Lowland region (900-1199m a.s.l) recorded the highest mean abundance of *Bactrocera invadens* followed by Sub-montane (1200-1499m a.s.l) and only singletons were recorded in the ever cold Montane zone (1500-1799m a.s.l). Agro-ecological zones in low altitudinal areas had warmer temperature and ambient humidity throughout the year that allowed efficient survival of *Bactrocera invadens*. In addition to avocadoes, there were a few clusters of productive mango crops between 900-1000 m a.s.l that enhanced dominance of *Bactrocera invadens* in lowland zones. Spatial distribution in this study suggested that the Asian invasive fruit fly (*Bactrocera invadens*) has over the years colonized extensively over warmer lowland areas along the study transects and established its efficient reproductive prowess leading to increased avocado fruit damage. This concurs with reports in some studies in Kenya that lowland inhabiting mango fruits are preferred hosts of Asian invasive fruit fly (*Bactrocera invadens*) and also were alternate hosts (Ekesi, *et al*., 2009; Rwomushana *et al*., 2008; Mohamed *et al*., 2008).

Statistical assessment using linear mixed effect models to establish which environmental variables explained best change in abundance confirmed that variation in mean abundance of *Bactrocera invadens* was best explained by a model with interaction term: temperature by agro-ecological zone or micro-habitats along each study area (Mount Kilimanjaro and Taita Hills). Impact of interaction of temperature and agro-ecological on mean abundance of *Bactrocera invadens* was more pronounced along Mount Kilimanjaro compared to Taita Hills. The effect of interaction term (temperature by agro-ecological zones) revealed that *Bactrocera invadens* population density is dependent on temperature and agro-ecological zones (Rwomushana *et al*., 2008) and both acted collectively. This means that the effect of temperature on population density of *Bactrocera invadens* was modified by altitudinal gradient or was specific to each elevational zone. Population trend of *Bactrocera invadens* in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania revealed that abundance increased with rising mean temperature but variation differed slightly depending on each agro-ecological zone within a transect. Habitat homogeneity along Mount Kilimanjaro study area was revealed with a strong overlap of temperature regimes compared to Taita Hills transect.

In Taita Hills, the interaction of temperature and habitat on mean abundance of *Bactrocera invadens* was less pronounced in agro-ecological zones due to ecosystem heterogeneity with relics of native forest intertwined in between avocado orchards. The lowland areas of Taita Hills transect had temperature regime that was independent of the upper zones. The higher elevated zones (Sub-montane and Montane) showed a strong overlap of temperature regimes in Taita Hills. Distribution pattern of montane zone could more often overlap that of sub-montane region but not with that of lowland areas. Consequently, the minimum adequate linear mixed effect model for Taita Hills revealed that abundance of *Bactrocera invadens* linearly increased with raising mean temperature and the gradient was higher at Sub-montane followed by lowland zone while the least change was in Montane uppermost agro-ecological zone. Higher changes in gradient in middle-placed Sub-montane zone (1200-1499m a.s.l) of Taita Hills suggest that this transitional zone has been experiencing extreme increase in temperature over years due to global warming and anthropogenic activities. The current data, therefore, supports the notion that upper elevated zones of Taita Hills and Mount Kilimanjaro are warming (Pellika *et al.,* 2009; Hemp 2005) and may enhance range expansion of the Asian invasive fruit fly (*Bactrocera invadens*) towards higher altitudes.

3.4.4.2 False codling moth (*Thaumatotibia leucotreta*)

False codling moth (*Thaumatotibia leucotreta*) was fairly distributed across all elevation zones along the study transect of Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. The spatial distribution of false codling moth was more or less similar and this suggested that false codling moth can reproduce and survive well in both lowland and highland zones of each transect in Taita Hills and Mount Kilimanjaro. The findings of this study concur with Newton (1998) who reported that false codling moth can thrive well in both lowlands and highlands of South Africa. This may be due to fact that false codling moths are nocturnal insects whose adults are active at night when temperatures are usually low (Newton, 1998). Females lay eggs at night but the immature stages requires warmer day temperatures to develop hence false codling moth can successfully thrive in both colder and warmer regions. Due to fair distribution of mean density along each transect, the change in abundance of false codling moth was influenced only by a number of environmental variables. The study, therefore, suggested that the agro-ecological zones or elevational gradient in the two study areas did not have any significant influence on change on abundance of false codling moth. This was confirmed by almost even distribution of false codling moth abundance along elevational gradient compared to the decreasing distribution of the Asian invasive fruit fly (*Bactrocera invadens*) along rising altitudinal gradient.

3.4.4.3 *Heliothrips haemorrhoidalis* and *Frankliniella schultzei*

The two most dominant pest species: *Heliothrips haemorrhoidalis* and *Frankliniella schultzei*, revealed a spatial trend that is similar to the one which is availed by mean richness of all the thrips sampled. Greenhouse thrips **(***Heliothrips haemorrhoidalis*) and common blossom thrips (*Frankliniella schultzei*) were most abundant in altitudes above 1200m a.s.l because host avocado plant were plentiful with favourable floral resources. Availability of host plants provided enough food resources for the two serous pests of avocado crop; *Heliothrips haemorrhoidalis* and *Frankliniella schultzei* (Palmer *et al*., 1992).

3.4.5 Temporal changes in abundance of the key insect pests

3.4.5.1 Annual population changes

The key avocado insect pests; Asian invasive fruit fly (*Bactrocera invadens*), false codling moth (*Thaumatotibia leucotreta*), greenhouse thrips (*Heliothrips haemorrhoidalis*) and common blossom thrips (*Frankliniella schultzei*) were present throughout the year in avocado orchards of Taita Hills and Mount Kilimanjaro but temporal population fluctuation was strongly influenced by either the time of the year or weather seasonality. The results, therefore, suggests that continued reservoirs for survival of immature stages of the key avocado insect pests throughout an avocado season was provided by avocado fruits (Copeland *et al*., 2002; Ekesi *et al*., 2006).

3.4.5.1.1 Annual population changes of *Heliothrips haemorrhoidalis* and *Frankliniella schultzei*

The two most dominant pest species: *Heliothrips haemorrhoidalis* and *Frankliniella schultzei*, revealed almost similar temporal pattern. Their relative abundance revealed greater temporal fluctuation within calendar months over a single avocado season that commences in August and ends in July of following year. The highest population peak was recorded in months of August, September and October with the later showing maximum amount of temporal variation. This is because during the three mentioned-months, it is also the time when avocado starts flowering and hence abundance of flowers and new leaves. Avocado plant gradually drops mature leaves during onset of flowering and develops new ones before the onset of fruiting; from late October of the same year. *Frankliniella schultzei* recorded highest abundance in months of August and October during the peak of flowering. Abundant florescence at the time provided enough floral resource for the avocado flower pest; *Frankliniella schultzei* hence high population. *Heliothrips haemorrhoidalis* (greenhouse thrip) recorded highest peak in month of October at initial fruiting stage and immergence of new avocado leaves. *Heliothrips haemorrhoidalis* sucks chlorophyll from young leaves and fruitlets leading to brownish scarring of mature fruits and leaves. Highest infestation usually takes place when pests are most abundant and for *Heliothrips haemorrhoidalis* it is October. The population density of the two pest; *Heliothrips haemorrhoidalis* and *Frankliniella schultzei*, reduced drastically in December up to May of the following year but started to incline from July. June and July period is peak avocado harvesting time which proceeds flowering. It is the time when economic damage of avocado fruits by *Heliothrips haemorrhoidalis* is witnessed. Some avocado plants do flower in late June and July hence an overall increase in abundance especially in lowland elevational zone that is slightly warmer than uppermost highlands. Minor temporal peaks are witnessed in months of January and February due to isolated flowering after short rains of November and December although the change is insignificant. Some avocado plants that do not flower in August and September do blossom in January and February of the following year.

3.4.5.1.2 Annual population changes of False codling moth (*Thaumatotibia leucotreta*)

Temporal population of false codling moth was almost evenly distributed throughout the year and in all agro-ecological zones of Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. Small peaks were witnessed between October through December to March and between June and July during the avocado harvesting season. The high population density of false codling moth between short rains and dry season (October - March) was due to the availablity of young host avocado fruits at the time hence the increase in abundance. Furthermore, temperatures were ambient for insect and avocado fruit development during the period between the short rains and dry season. A false codling moth (*Thaumatotibia leucotreta*) female mostly oviposits on young and occasionally on immature avocado fruits but their larvae do not mature from the fruit (Newton, 1998). Larvae emerge from the infested fruit and carefully drop onto the ground leaf litter for pupation. Mean population density of false codling moth reduced marginally during long rains (March - May) and cold season (June - August). Heavy rains and mist wash off majority of immature stages resulting in their death hence low population density. Furthermore, low temperatures during long rains and cold season could have reduced developmental rate of false codling moth (*Thaumatotibia leucotreta*).

3.4.5.1.3 Annual population changes of Asian invasive fruit fly (*Bactrocera invadens*)

The overall population of *Bactrocera invadens* peaked between November and March then reduced drastically in May but the abundance increased marginally in June during avocado harvesting season. The dry season peak abundance in lowland zone (900–1199m a.s.l) coincided with the ripening of mango fruit between November and January. Furthermore, high temperatures and ambient humidity in dry season enhances development of immature stages of fruit flies in infested fruits. Present study, therefore, confirms that *Bactrocera invadens* is a polyphagous insect pest that infests other fruit crops along the study transects. Mwatawala *et al* (2009) and Hanna *et al* (2008) reported that *Bactrocera invadens* reinvades with high population density during dry season (December, January & February) that comes immediately after the short rains of September, October & November. The population of *Bactrocera invadens* then reduced marginally on the onset of long rains in March and reduced drastically during the peak of heavy rains (April & May). Heavy rains are normally wet and washe off majority of immature stages resulting in low density. However, the small temporal peak of *Bactrocera invadens* during cold season (June- July) implied that availability of avocado fruits during harvesting season in June and July provided reservoir during the coldest season of the year.

Bactrocera invadens was recorded throughout the year in area below 1500m a.s.l. Ekesi (2006) and Copeland *et al* (2002) reports that the warmer lowland areas have different varieties of fruit crops in addition to avocado that provided alternate hosts of *Bactrocera invadens* than the subsequent higher zones. Temporal patterns of *Bactrocera invadens* in lowland region (900–1199m a.s.l) were more consistent with that of sub-montane zone (1200–1499m a.s.l) although the later habitat had few productive mangoes. Generally, sub-montane region was a transitional zone between lowland and upper most montane region (1500–1799m a.s.l) along the study transects. In Montane region (1500–1799m a.s.l) of the study area, the mean monthly populations were almost zero throughout the year deducing that *Bactrocera invadens* occurrence in highest elevated regions is seasonal. The upper elevated montane zone was not conducive for survival of *Bactrocera invadens* due to ever cold environment characterized by extremely misty condition throughout the year. In addition to low temperatures, the montane zone (1500–1799m a.s.l) had high rainfall, relative humidity and ever cloudy skies. This could have reduced developmental process of *Bactrocera invadens* as a result of inadequate ultra-violet heat required by insects for movement, reproduction and growth. Most immature stages remain in diapause for long in upper elevated areas hence very low temporal population in montane zones.

CHAPTER FOUR

INFLUENCE OF TEMPERATURE ON PHENOLOGICAL DEVELOPMENT OF KEY AVOCADO INSECT PEST; THE ASIAN INVASIVE FRUIT FLY (*Bactrocera invadens*), AND ITS EGG PARASITOID (*Fopius arisanus*)

Abstract

Experiments were carried out in a secured African Fruit Fly Laboratory at ICIPE for two years to establish phenological development of the key avocado insect pest; *Bactrocera invadens*, and its egg parasitoid, *Fopius arisanus*. Main objective of this study was to generate development, reproduction, longevity and mortality rates of the pest and parasitoid at six constant temperatures (15, 20, 25, 30, 32.5 and 35°C), with a relative humidity of 75% ±5 and a L12:D12 photoperiod. The insects were fed daily on artificial food diet throughout the experiment. Phenological data collected was used in Insect Life Cycle Modeling software (ILCYM) to generate several constant temperature-dependent phenology models. The models clarified that both *Bactrocera invadens* and its egg parasitoid survives well in warmer temperatures between 25 and 30ºC but the two insects could not complete their development at extreme low or high temperatures. Findings of this study suggest that *Bactrocera invadens* and its egg parasitoid, *Fopius arisanus*, can thrive well in warmer lowland areas. The study further revealed that infestation of *Bactrocera invadens* eggs by its specific parasitoid, *Fopius arisanus*, was high in temperatures between 25 and 30ºC. These findings, therefore, suggest that *Fopius arisanus* can be utilized as one of the bio-control remedies aimed at reducing infestation of avocado fruits by the Asian invasive fruit fly (*Bactrocera invadens*) in warmer areas.

4.1 Introduction

Temperature is the single most important abiotic factor that influences development, survival, behavior, mortality and geographical distribution of most insect pests and related parasitoids (Chapter Three, Liu & Ye, 2009; Rwomushana *et al.*, 2008; Vayssières *et al.*, 2008; Duyck & Quilici, 2002; Brévault & Quilici, 2000; Vargas *et al.*, 1997; Fletcher 1987). Insects are cold-blooded organisms whose body temperatures are approximately the same as that of their environment (Yang & Rudolf, 2010; Logan *et al.*, 2006; Visser & Both, 2005; Schwartz, 1998). For this reason, development and survival of an ectotherm is intimately linked to a given range of atmospheric temperature. A significant increase or reduction in surrounding mean temperatures can severely affect koinobiont endoparasitoids such as *Fopius arisanus* (Hymenoptera: Braconidae) because their insect hosts must remain alive throughout in order for them to fully develop. *Fopius arisanus* is an egg parasitoid of Asian invasive fruit fly (*Bactrocera invadens*) (Ekesi & Billah, 2006). Successful parasitism of the Asian invasive fruit fly (*Bactrocera invadens*) eggs by *Fopius arisanus* depends on capacity of a fertile adult female parasitoid to locate a suitable host, overcome the host immune response and adapt to a constantly changing host environment. Therefore, understanding temperature-dependent life histories of *Bactrocera invadens* (Diptera: Tephritidae) and its egg parasitoid can assist in generation of models to analyze their development and survival rates in an ecosystem. Such information can assist in developing efficient integrated pest management (IPM) strategies that are aimed at reducing infestation of avocado fruits by insect pests (Rwomushana *et al.*, 2008).

The Asian invasive fruit fly (*Bactrocera invadens*) is a key insect pest that infests ripened avocado fruits in Taita Hills and Mount Kilimanjaro within East Africa (Chapter 3). The larvae of *Bactrocera invadens* Drew Tsuruta and White is a devastating pest of fruit varieties that lead to loss of livelihood (FAO, 2006). The pest is well established in lowland zones in at least twenty four countries in Africa where temperatures are relatively warmer thorough the year (Mwatawala, 2006). The highly aggressive Asian invasive fruit fly readily displaces less damaging indigenous fruit fly species in ecosystems it populates (Duyck, *et al*., 2007; Duyck, *et al*., 2006b; Duyck, *et al*., 2006a; Ekesi & Billah, 2006; Ekesi, *et al*., 2006; Duyck, *et al*., 2004). This reduces the number of indigenous natural enemies of fruit flies many of whom are dependent on competitors that are displaced by the Asian invasive fruit fly. Consequently, an exotic specific enemy of *Bactrocera invadens*; *Fopius arisanus* (Hymenoptera: Braconidae), was introduced in few lowland regions in Kenya and Tanzania as part of bio-control strategies in managing pest populations in mid- 2000s. *Fopius arisanus* (Hymenoptera: Braconidae) was introduced because it specifically attacks *Bactrocera invadens* eggs on or near fruit surface and the parasitoid present no risk to beneficial insect species (Rwomushana *et al*., 2008; Ekesi & Billah, 2006). Female *Fopius arisanus* specializes in ovipositing into eggs of the Asian invasive fruit fly (*Bactrocera invadens*) found mostly on ripened fruits (Chapter Three) and may be used in bio control of the pest in the avocado orchards at Taita Hills and Mount Kilimanjaro.

In order to fully understand interaction between the Asian invasive fruit fly and its egg parasitoid (*Fopius arisanus*) in envisaged fluctuating temperature scenarios, process-based mathematical models describing adult survival rate, oviposition, longevity, stage-specific development rates and mortalities are required. However, there is limited phenological data on the key avocado insect pest (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*). This study was, therefore, initiated to generate development, reproduction, longevity and mortality rates of

Bactrocera invadens and *Fopius arisanus* at six constant temperatures (15, 20, 25, 30, 32.5 and 35°C) using data generated from the laboratory-based life-cycle experiments. Insect Life Cycle Modeling software (ILCYM) was used to process phenology models or simplified mathematical models, which describe the basic physiological principles of the insect growth; development, survival and reproduction. Generally, non-linear models are implemented in ILCYM software because development of an insect stage is not linear at lower and upper developmental threshold temperatures (Kroschel *et al*., 2013; Tonnang *et al*., 2013; Sporleder *et al*., 2009; Sporleder *et al*., 2007; Nietschke, *et al*., 2007; Schoolfield *et al*., 1981; Sharpe & DeMichele 1977; Logan *et al*., 1976; Stinner *et al*., 1974). Generated phenological models can be used in simulating future geographical distribution patterns of the Asian invasive fruit fly (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*) as described in next chapter of this thesis.

4.2 Materials and methods

Temperature - dependent phenology experiments were performed between August 2013 and August 2015 at different constant temperatures (15ºC, 20ºC, 25ºC, 30ºC, 32.5ºC and 35ºC) on cohorts of individual life stages of *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*) following methods described by Sporleder *et al* (2007) and Nietschke *et al* (2007). The experiments were executed in controlled incubators model: Sanyo - MLR-350H553 (Appendix 4) with relative humidity of 75% ± 5 and a photoperiod of L12:D12 hours at the African Fruit Fly Laboratories within International Centre of Insect Physiology and Ecology (ICIPE), Nairobi. The *Bactrocera invadens* colonies used in the experiments were established from initial population that immerged from fruits collected during preliminary field sampling along study transects of the Taita Hills and Mount Kilimanjaro. To reduce inbreeding, several new

Bactrocera invadens individuals from study areas were incorporated into the reared colony every three months. Colonies of egg parasitoid (*Fopius arisanus*) were established from exposing infested avocado fruits to a reared colony at ICIPE. *Fopius arisanus* was recorded in singletons along the study transects and hence the use of ICIPE reared population for experiment. *Fopius arisanus* is an exotic egg parasitoid species to Africa that has been legally introduced in Kenya as a biological control of *Bactrocera invadens*. Both colonies of the pest and its parasitoid were maintained at room temperature (25°C ± 0.46) within at the African Fruit Fly Laboratories. Only one day old eggs, larvae, pupae and adults were used as cohorts of individual life stages during the experiments. The life cycle or phenology experiments were carried out to generate development time, development rate and mortality rate of immature stages (eggs, larvae and pupae), adult longevity and female fecundity (reproduction rate) on the key avocado insect pest (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*).

4.2.1 General experiment layout

For a full life cycle of *Bactrocera invadens*, a total of 3000 eggs, 3000 larvae, 3000 pupae, 1500 males, 1500 females and 450 fertile couples were observed at six different constant temperatures for development time, development rate, oviposition and mortality. The life cycle of *Fopius arisanus* which is a specialized egg parasitoid of *Bactrocera invadens* (the key avocado pest) was established in the laboratory environment. A total of 2500 *Bactrocera invadens* eggs exposed to parasitism by *Fopius arisanus* fertile females, 3000 males, 3000 females and 450 fertile couples were observed at six different constant temperatures. Also 3000 larvae and 3000 pupae were observed for parasitism by *Fopius arisanus* fertile females. Development time, development rate, female oviposition and mortality rate of *Fopius arisanus* was recorded daily from day one to death of last insect life cycle stage in each cohort. Only one day old life cycle stages of *Fopius arisanus* were used. A random experimental design was used and replicated five times.

4.2.1.1 Egg stage

4.2.1.1.1 *Bactrocera invadens* egg

Following protocols described by Rwomushana *et al* (2008) and Ekesi *et al* (2007), one day old eggs from the reared colony were obtained by exposing pierced avocado fruit domes to mature fertile *Bactrocera invadens* females. The avocado fruit domes were pierced several times with sterilized standard entomological pins. Gentle piercing of ripened fruit skin to make fine holes and removal of inner flesh facilitated easy oviposition by the *Bactrocera invadens* females. To avoid injury and stress, a very fine camel brush was used to collect newly oviposited eggs. A hundred newly laid eggs, one to four hours old, were placed on a 9cm diameter petri dish with a liquid diet in six different controlled incubators (model: Sanyo - MLR-350H553) with constant temperatures (15ºC, 20ºC, 25ºC, 30, 32.5ºC and 35ºC), relative humidity of 75% ± 5 and a photoperiod of L12: D12 hours. Each petri dish was maintained in a 30cm x 30cm x 30cm well aerated perspex cage. Both hatchability into larvae and mortality of the eggs was observed daily with experiments being replicated five times.

4.2.1.1.2 *Fopius arisanus* eggs

Five hundred *Bactrocera invadens* eggs, one hour old, were carefully placed onto ripened avocado fruit dome and exposed to fifty fertile couples of *Fopius arisanus* that were ten days old for parasitism. Fertile females of *Fopius arisanus* usually commence laying eggs from tenth day after immergence. The eggs exposed for parasitism were removed after four hours and placed in a growth media at different sets of incubators with constant temperatures of 15, 20, 25, 30, 32.5 and 35ºC. The *Bactrocera invadens* eggs were observed daily for emergence of both parasitoid (*Fopius arisanus*) and host larvae or only *Bactrocera invadens* larvae. All *Bactrocera invadens* (host) eggs were carefully dissected after 24 hours to observe presence of the parasitoid (*Fopius arisanus*) eggs. Each egg was placed in onto a 9cm petri dish with normal saline liquid and dissected under a stereo microscope. Normal saline (a sterile mixture of salt and water) was used to reduce surface density during dissecting. Parasitoid eggs were only found in eggs of *Bactrocera invadens* (host) that were infested. The experiments were replicated five times at each constant temperature.

4.2.1.2 Larval stage

4.2.1.2.1 *Bactrocera invadens* larvae

A hundred newly emerged larvae of *Bactrocera invadens*, one day old, were transferred onto transparent 7cm x 5cm x 3cm plastic rearing containers with a liquid diet in six different controlled incubators (model: Sanyo - MLR-350H553) with constant temperatures (15ºC, 20ºC, 25ºC, 30, 32.5ºC and 35ºC), relative humidity of 75% ± 5 and a photoperiod of L12: D12 hours. Each plastic rearing container was maintained in 30cm x 30cm x 30cm a well aerated perspex cage. Both pupal emergence and larval mortality was observed and recorded daily with experiments being replicated five times.

4.2.1.2.2 *Fopius arisanus* larvae

Five hundred *Bactrocera invadens* (host) eggs, one hour old, were placed onto one ripened avocado fruit dome using a fine camel brush and exposed to fifty fertile couples of *Fopius arisanus* that were ten days old for infestation. Of all larvae that emerged in a single day, a batch of one hundred larvae was randomly selected for observation at each of the six different constant temperatures (15ºC, 20ºC, 25ºC, 30, 32.5ºC and 35ºC) for development time, development rate and mortality. Larvae were observed daily for development of pupae of both pest and parasitoid. Every emerging pupa was dissected to observe presence of the parasitoid (*Fopius arisanus*) pupae. The lifecycle of parasitoid is dependent on host development. Larvae that died were also recorded**.**

4.2.1.3 Pupal stage

4.2.1.3.1 *Bactrocera invadens* pupae

For pupal development, a hundred one day old, were transferred onto a 9cm diameter petri dish in each of the six different controlled incubators (model: Sanyo - MLR-350H553) with constant temperatures (15ºC, 20ºC, 25ºC, 30, 32.5ºC and 35ºC), relative humidity of 75% ± 5 and a photoperiod of L12: D12 hours. Each petri dish was maintained in 30cm x 30cm x 30cm a well aerated perspex cage with moist but sterile sand at the bottom for pupation. Larvae in the late third instar usually hop onto the sand to pupate. Both emergence of new *Bactrocera invadens* adults and mortality of each pupa were observed daily and recorded with experiments being replicated five times. Pupal mortality was indexed by recording daily the number of enclosed puparia with sign of death (very dark colour and poorly formed). Puparia with signs of live are usually healthy and are pale.

4.2.1.3.2 *Fopius arisanus* Pupae

One hundred *Bactrocera invadens* (host) pupae were selected randomly from a population that emerged in single day and observed daily at six different constant temperatures for emergence of adult parasitoids (*Fopius arisanus*) or hosts (*Bactrocera invadens*). Development time and mortality was recorded daily. The selected hundred newly emerged pupae, one day old, were transferred onto a 9cm diameter petri dish in six different controlled incubators (model: Sanyo - MLR-350H553) with constant temperatures (15ºC, 20ºC, 25ºC, 30, 32.5ºC and 35ºC), relative humidity of 75% ± 5 and a photoperiod of L12: D12 hours. Each petri dish was maintained in 30cm x 30cm x 30cm a well aerated perspex cage with moist but sterile sand at the bottom for pupation because larvae in the last instar usually hop onto the sand to pupate. Both emergence of new *Fopius arisanus* adults and mortality of each pupa were observed daily and recorded with experiments being replicated five times. Pupal mortality was indexed by recording daily the number of enclosed puparia with sign of death; very dark colour and poorly formed.

4.2.1.4 Adult longevity

4.2.1.4.1 *Bactrocera invadens* (pest)

A total of 1500 males and 1500 *Bactrocera invadens* females who were one day old were observed for longevity and mortality at six different constant temperatures. The insects were fed on a diet composed of sugar and enzymatic yeast hydrolysate at a ratio of 2:1 as described by Rwomushana *et al* (2008) and Ekesi *et al* (2007). Sets of fifty newly (one day old) emerged *Bactrocera invadens* males only were transferred into a 30cm x 30cm x 30cm well aerated perspex cage in six different controlled incubators (model: Sanyo - MLR-350H553) with constant temperatures (15ºC, 20ºC, 25ºC, 30, 32.5ºC and 35ºC), relative humidity of 75% ± 5 and a photoperiod of L12: D12 hours. Similar but independent experiment was also carried out only on sets of fifty newly (one day old) emerged females of *Bactrocera invadens*. Mortality and survival of sets of fifty the males and females respectively was recorded daily at specific time of the day until the last *Bactrocera invadens* individual died in each cohort. The experiments were replicated five times.

4.2.1.4.2 *Fopius arisanus* (parasitoid)

A total of one day old 3000 males and 3000 *Fopius arisanus* females were observed for longevity and mortality at six different constant temperatures. The insects were fed on a honey. Sets of one hundred newly (one day old) immerged *Fopius arisanus* males only were transferred into a 30cm x 30cm x 30cm well aerated perspex cage in six different controlled incubators (model: Sanyo - MLR-350H553) with constant temperatures (15ºC, 20ºC, 25ºC, 30, 32.5ºC and 35ºC), relative humidity of 75% ± 5 and a photoperiod of L12: D12 hours. Similar but independent experiment was also carried out only on sets of one hundred newly emerged (one day old) females of *Fopius arisanus*. The experiments were replicated five times. Mortality and survival of sets of the one hundred males and females respectively were recorded daily at specific time of the day until the last *Fopius arisanus* individual died in each cohort.

4.2.1.5 Female oviposition rate or fecundity

4.2.1.5.1 Fecundity of *Bactrocera invadens* females

A total of 450 fertile couples of *Bactrocera invadens* were observed for oviposition rate (fecundity) at six different constant temperatures. The couples were fed on a diet composed of sugar and yeast as described by Rwomushana *et al* (2008) and Ekesi *et al* (2007). A healthy one day old male and a female were coupled and then transferred into a 12cm x 12cm x 12cm well aerated perspex cage with a 2cm ripened avocado fruit skin in six different controlled incubators (model: Sanyo - MLR-350H553) with constant temperatures (15ºC, 20ºC, 25ºC, 30, 32.5ºC and 35ºC), relative humidity of 75% ± 5 and a photoperiod of L12: D12 hours. The number of eggs oviposited by each female on the 2cm ripened avocado fruit skin was recorded daily at specific time of the day until the female died. In contrast, a dead male was replaced immediately by a newly emerged one from the reared colony stock in order to maintain the female fertility rate. The experiments were replicated five times.

4.2.1.5.2 Fecundity of *Fopius arisanus* females

A total of 450 fertile couples of *Fopius arisanus* were observed for oviposition rate (fecundity) at six different constant temperatures. Healthy one day old male and a female were coupled and then transferred into a 12cm x 12cm x 5cm well aerated perspex cage with a 2cm ripened avocado fruit skin with ten eggs of *Bactrocera invadens* in six different controlled incubators (model: Sanyo - MLR-350H553) with constant temperatures (15ºC, 20ºC, 25ºC, 30, 32.5ºC and 35ºC), relative humidity of 75% ± 5 and a photoperiod of L12: D12 hours. The couples were fed on drops of honey placed at roof top of the cages and moistened cotton wool at the bottom. All exposed *Bactrocera invadens* (host) eggs on 2cm ripened avocado fruit skin were carefully dissected after 24 hours to observe presence of the parasitoid eggs (*Fopius arisanus*). Each egg was placed onto a 9cm petri dish with normal saline and dissected under a stereo microscope. Normal saline was used to reduce surface density during dissecting. Parasitoid eggs were found only in eggs of *Bactrocera invadens* (host) that were infested. The experiments were replicated five times at each constant temperature. The number of eggs oviposited by each *Fopius arisanus* female into eggs of *Bactrocera invadens* on the 2cm ripened avocado fruit skin was observed daily at specific time of the day until the female died. In contrast, a dead male was replaced immediately by a newly emerged one from the reared colony stock in order to maintain the female fertility rate.

4.2.2 Data analysis and modelling

The phenology data of *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*) was analyzed using the Insect Life Cycle Modeling Software (ILCYM) linked to R-statistical programme interface as described by Mwalusepo *et al* (2015) and Khadioli *et al* (2014a & b). Data on the development time of different life stages and adult longevity were compared across constant temperatures using analysis of variance (ANOVA). Development times were log-transformed before analysis in order to normalize the data. This is because frequency distributions of insect development time are usually skewed towards the longer times and it is assumed that the distribution of development time of an insect at different temperatures is similar (Mwalusepo *et al*., 2015; Sharpe & DeMichele, 1977).

4.2.2.1 Temperature-dependent phenological models

4.2.2.1.1 Development rate model

Relationship between temperature and development rate of an insect species at a particular insect life stage was described by a non-linear model called Logan (Logan, 1988; Logan *et al*., 1976). The Logan model which is described below estimates optimum temperature where most favourable development occurs and also pinpoints maximum threshold temperature where no development took place.

$$r(T) = Y(\exp(p \cdot T)-\exp(p \cdot Tmax-(Tmax-T)/v))$$

Where r(T) = rate of development at temperature T; Y,p,v = constants values which are given and Tmax = Upper developmental threshold temperature in ºC (Logan, 1988; Logan *et al.*, 1976).

Temperature-dependent development of an insect stage does not follow a linear relationship hence the non-linear Logan model was fitted onto a simple linear regression to generate a phenology model that estimated optimum and maximum developmental temperatures (Sporleder *et al.*, 2007). The simple linear regression model was only used to estimate the lower development threshold.

4.2.2.1.2 Mortality rate model

Relationship between temperature and mortality rate of immature stages of the insect species at a particular insect life stage was described by a polynomial model (Mwalusepo *et al.*, 2015; Khadioli *et al.*, 2014a & b; Harcourt and Yee, 1982; Tanigoshi & Browne, 1978). The model is described below;

$$m(T) = \exp(b1+b2.T+b3.T^2)$$

Where M(T) is the rate of mortality at temperature T; and b_iT are parameters to be estimated.

4.2.2.1.3 Fecundity or oviposition rate model

Relationship between temperature and mortality rate of immature stages of the insect species at a particular insect life stage was described by a polynomial model (Mwalusepo *et al.*, 2015; Khadioli *et al.*, 2014a & b; Harcourt & Yee, 1982; Tanigoshi & Browne, 1978). The model is described below;

$$f(T) = \exp(b1+b2.T+b3.T^2)$$

Where f(T) is the fecundity (oviposition) rate at temperature T; and b_iT are parameters to be estimated.

4.2.2.1.4 Longevity rate model

Relationship between adult longevity of both females and males and temperature was explained by Stinner model (Mwalusepo *et al.*, 2015; Khadioli *et al.*, 2014a & b; Stinner *et al.*, 1974). The model is described below;

$$S(T) = c_i / (1+e^{k_1+k_2.T})$$

Where S(T) is longevity and c_i and k_i were parameters estimated at temperature T.

4.3 Results

4.3.1 Developmental time and adult longevity of *Bactrocera invadens*

The Asian invasive fruit fly (*Bactrocera invadens*) was able to develop in a wide range of temperatures. Mean developmental time of all life stages generally decreased with increase in temperature and hence temperature significantly influenced duration of each developmental stage. Mean development of egg varied from a high of 6 ± 0.09 days (mean ± se) at 15ºC to a low of 1 ± 0.02 at 35ºC ($F1,8 = 36.67$; $P < 0.0003$) (Table 4.1). Mean larval development changed from 32 ± 0.07 days at 15ºC (mean ± se) to a reduced 8 ± 0.09 at 35ºC, however, mean pupal development varied from 37 ± 0.05 (mean ± se) at 15ºC to 11 ± 0.03 at 30ºC (Table 4.1). At 35ºC the pupae did not develop into the next stage or failed to hatch into an adult *Bactrocera invadens*. The developmental time decreased as temperature increased up to optimum threshold.

The longevity of adult *Bactrocera invadens* decreased with increasing temperatures for both females and males although females lived longer than the males (Table 4.1).

Table 4.1: Mean ± se development time (days) or longevity of *Bactrocera invadens* life stages at five different constant temperatures.

Constant temperature (°C)	**Egg**	**Larvae**	**Pupae**	**Female**	**Male**
15	6 ± 0.09	32 ± 0.07	37 ± 0.05	62 ± 5.877	65 ± 3.093
20	4± 0.02	16 ± 0.04	19 ± 0.02	59 ± 5.028	56 ± 4.045
25	2± 0.03	11 ± 0.03	12 ± 0.01	39 ± 2.266	35 ± 3.164
30	1 ± 0.03	9 ± 0.04	11 ± 0.03	22 ± 0.963	22 ± 1.034
35	1 ± 0.02	8 ± 0.09	N/A	16 ± 0.302	15 ± 0.097

The images describing development time of *Bactrocera invadens* at most favorable constant temperature (30ºC) during the life-table experiments are described in Figure 4.1.

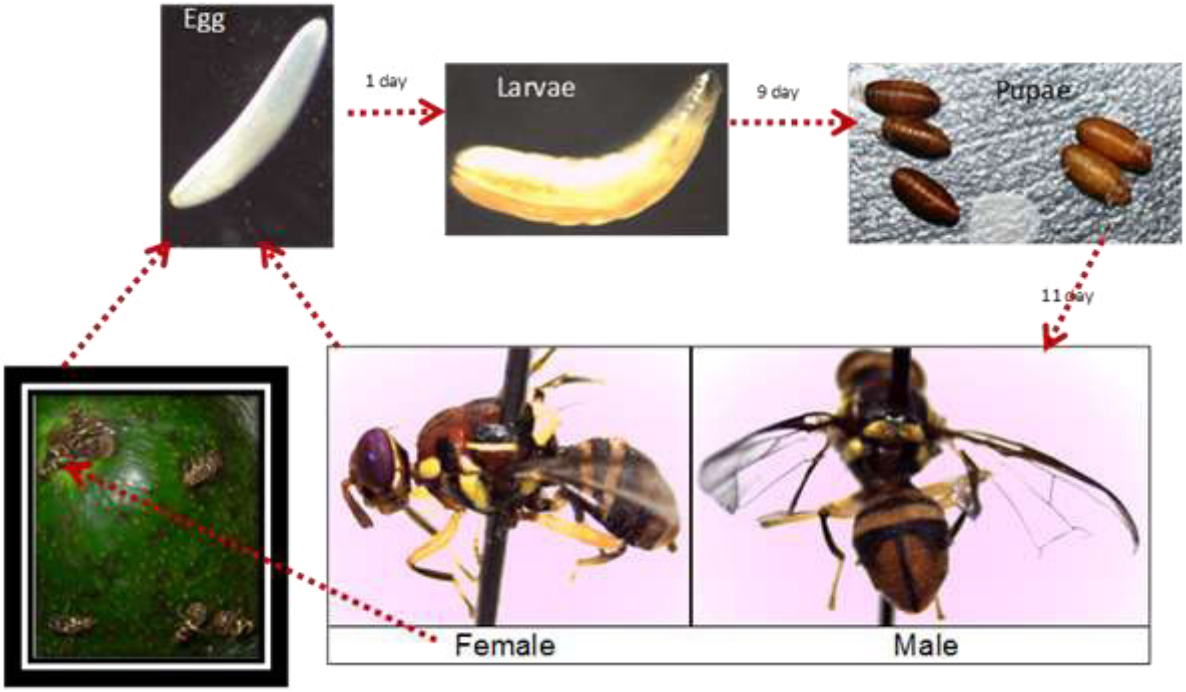


Figure 4.1: Images of life stages of the Asian invasive fruit fly (*Bactrocera invadens*, Diptera: Tephritidae) which is a key pest of avocado fruits in the Mount Kilimanjaro region. Pictures are of the representative specimens from a colony used during the life table experiments at the African Fruit Fly Laboratories in Nairobi and were captured using a Leica EZ4-D stereo-microscope with integral digital camera implemented on a Windows PC. The average length of *B. invadens* stages was as follows; male (1.01mm), female (1.05mm), egg (0.61mm), larva (1.13mm) and pupa (0.78mm).

4.3.2 Developmental rate of the Asian invasive fruit fly (*Bactrocera invadens*)

Developmental rate increased with temperature but the optimum and maximum levels varied among different immature stages. The developmental rate for egg and larvae could not go beyond 39ºC; (egg; Tmax = 38.82ºC) and (larvae; Tmax = 38.86ºC) (Figure 4.2a & b). Pupal stage did not develop beyond the maximum temperature (Tmax) of 33.84ºC (Figure 4.2c). 32.5ºC was the optimum temperature where development of *Bactrocera invadens* was most favourable for eggs and larvae however it was lower for pupae (Figure 4.2a, b & c).

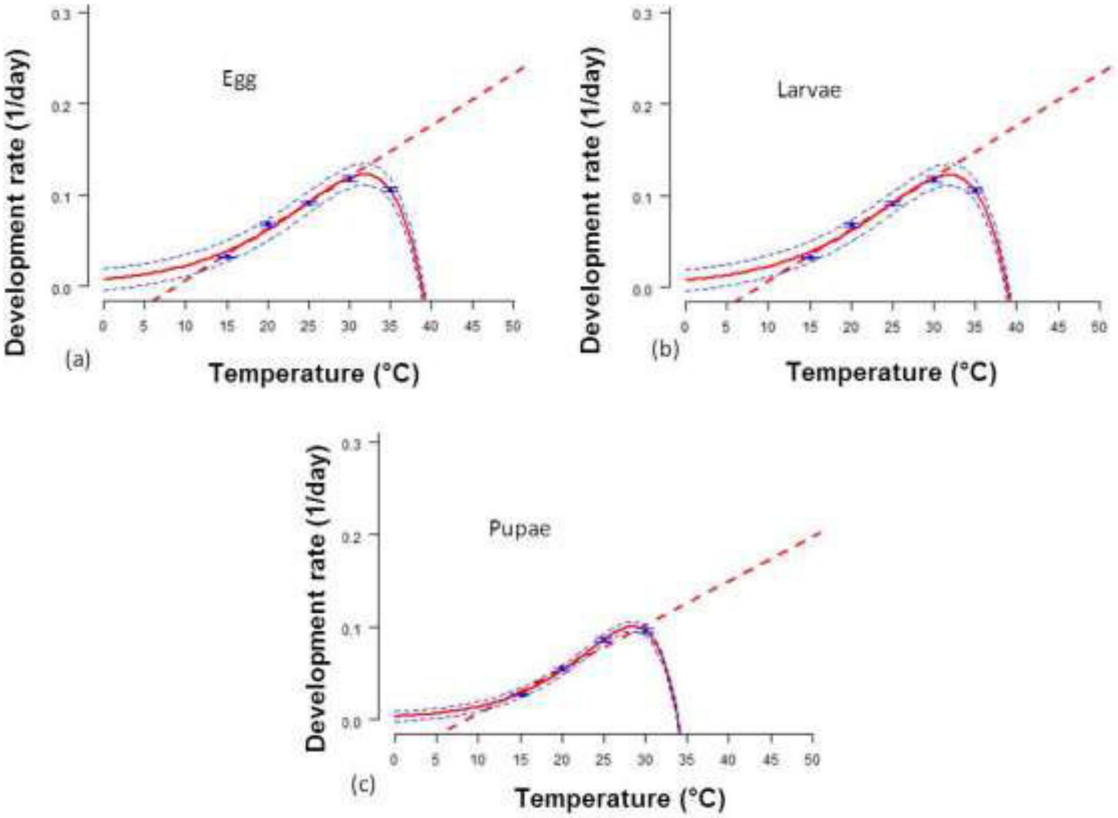


Figure 4.2: Temperature dependent development rate (1/developmental time) of (a) eggs, (b) larvae and (c) pupae for *Bactrocera invadens*. Non-linear Logan model (curved red line) was fitted onto a simple linear regression (straight red line) to generate the phenology model that estimated optimum and maximum developmental temperatures. Linear regression estimated the lower development threshold temperature. The blue lines represent 90% confidence interval. Blue points represent constant temperature ranges at which phenology experiments were carried out.

4.3.3 Mortality of *Bactrocera invadens* immature stages

Mortality of *Bactrocera invadens* eggs was high in extreme temperatures ranging from 31% at 15ºC to 79% at 35ºC (Figure 4.3).

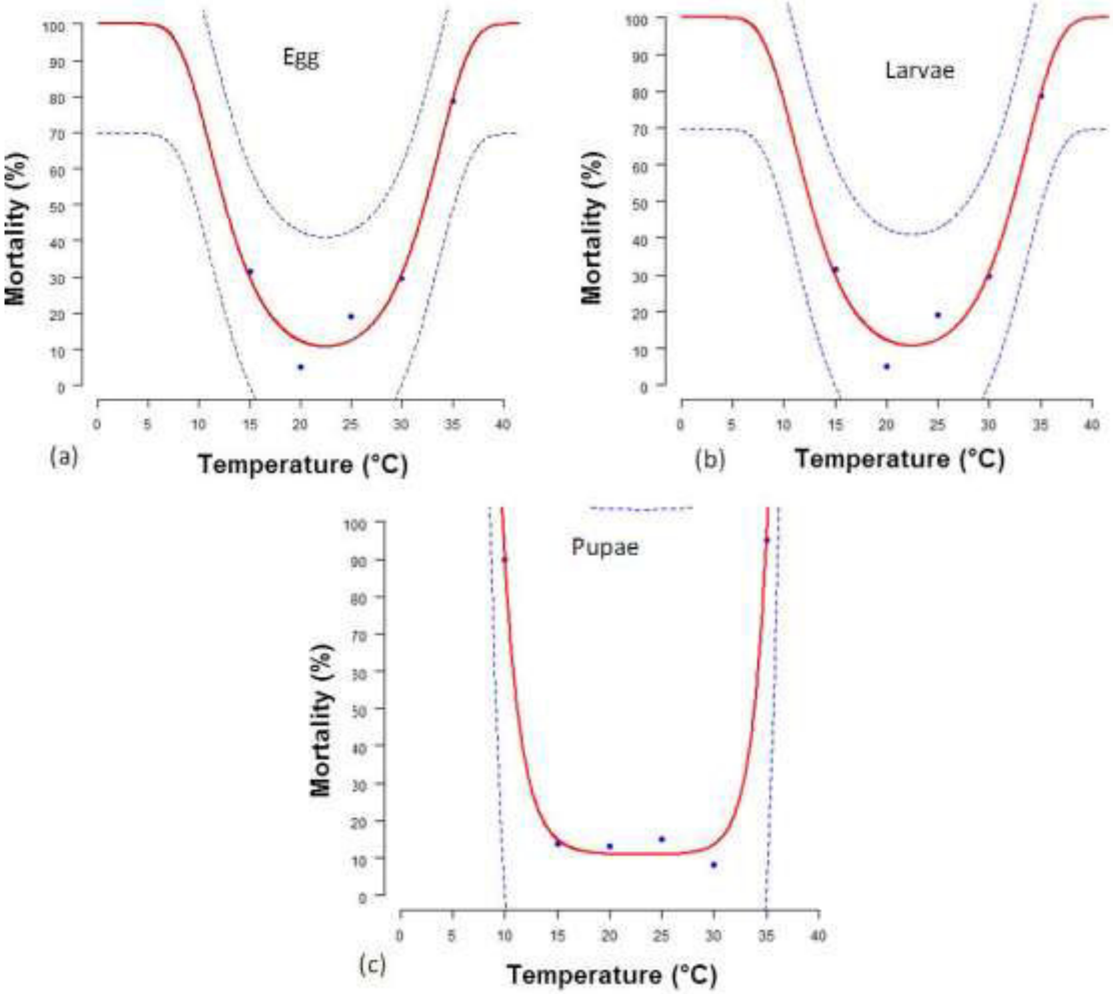


Figure 4.3: Temperature-dependent mortality rate of (a) eggs, (b) larvae and (c) pupae for *Bactrocera invadens* (red line). The blue lines represent 90% confidence interval. Blue points represent constant temperature ranges at which phenology experiments were carried out.

4.3.4 Fecundity (oviposition rate) of *Bactrocera invadens* female

The mean number of eggs laid per female increased with increasing temperature from 111 eggs at 15ºC to 343 eggs at 30ºC. At 25ºC the *Bactrocera invadens* female laid a mean of 232 eggs but the number reduced drastically to 65 eggs at 35ºC. The polynomial model estimated that the female pest could not lay eggs below 5ºC and above 36ºC (Figure 4.4).

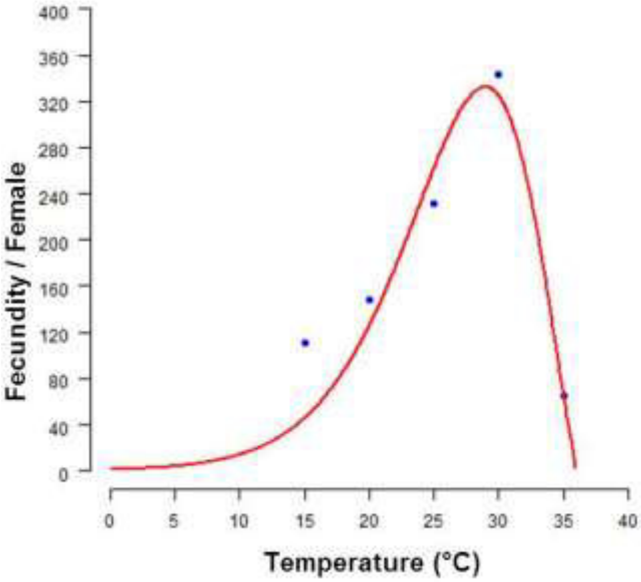


Figure 4.4: Temperature-dependent total egg production curves of *Bactrocera invadens* (red line). Blue points represent constant temperature ranges at which phenology experiments were carried out.

4.3.5 Phenology of *Fopius arisanus* (the egg parasitoid of *Bactrocera invadens*)

4.3.5.1 Developmental time and adult longevity of *Fopius arisanus*

Temperature significantly influenced duration (in days) of each developmental stage of *Fopius arisanus*. Mean developmental time of all life stages of *Fopius arisanus* generally decreased with increase temperature. Analysis of the life table data of *Fopius arisanus* (Hymenoptera: Braconidae) was combined at the larval-papal stages because it is a koinobiont endoparasitoid whose host (*Bactrocera invadens*) must remain alive throughout in order for them to fully develop. Constant temperatures had significant effects on the development time of different life stages of the *Fopius arisanus* (Table 4.2). The developmental time of the egg was 5 days to 1 day (F1, 8 = 45.56; P < 0.0001) at temperatures from 15 to 32°C. The developmental time decreased as temperature increased, whereas at the higher temperatures the developmental time was faster.

The longevity of adult *Fopius arisanus* decreased with increasing temperatures for both females and males although females lived longer than the males (Table 4.2).

Table 4.2: Mean ± se development time (days) or longevity of *Fopius arisanus* life stages at five different constant temperatures.

Constant temperature (°C)	**Egg**	**Larvae- Pupae**	**Female**	**Male**
15	5 ± 0.15	61 ± 0.48	29 ± 2.25	25 ± 3.01
20	4± 0.09	30 ± 0.67	26 ± 0.95	19 ± 1.60
25	1± 0.05	19 ± 0.18	19 ± 0.93	17 ± 1.93
30	1 ± 0.02	17 ± 0.11	13 ± 0.56	13 ± 0.77
32.5	1 ± 0.03	11 ± 0.08	12 ± 0.54	9 ± 0.50

The images describing development of *Fopius arisanus* during the life-table experiments are described in Figure 4.5.

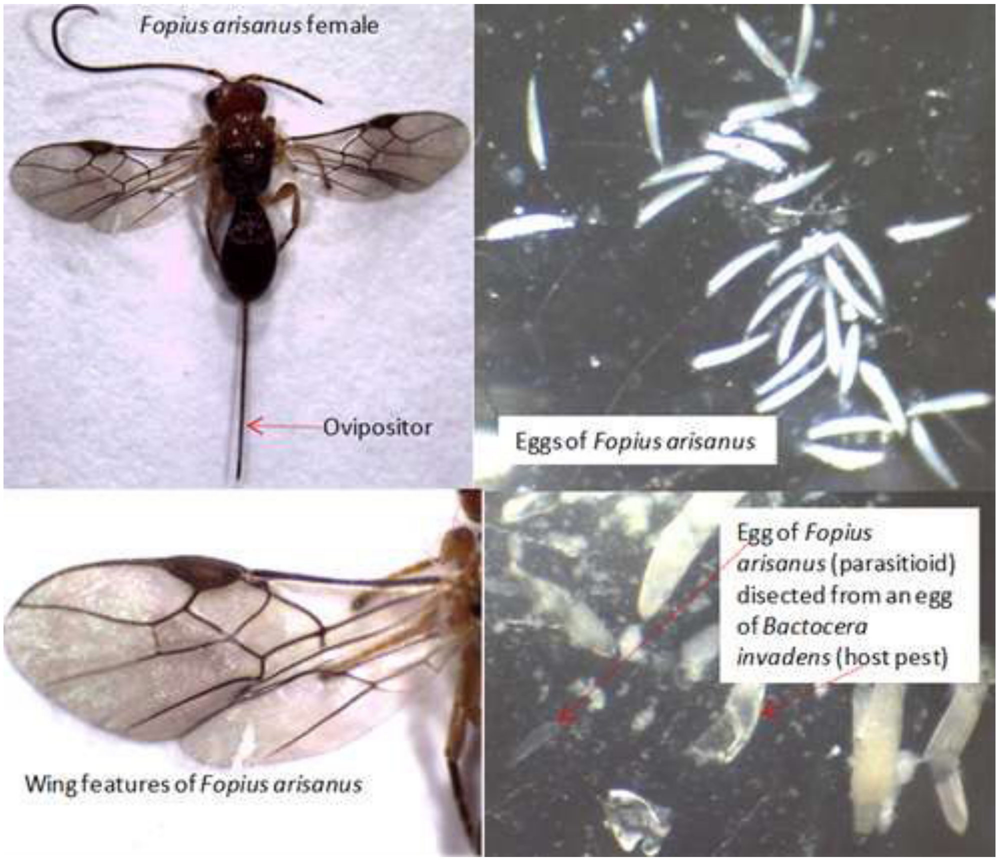


Figure 4.5: Images of some life stages of *Fopius arisanus* (the egg parasitoid of *Bactrocera invadens*). Pictures are of the representative specimens from a colony used during the life table experiments at the African Fruit Fly Laboratories in Nairobi and were captured using a Leica EZ4-D stereo-microscope with integral digital camera implemented on a Windows PC. The average length of *F. arisanus* male was 0.55mm whereas the female had 1.36mm with her ovipositor accounting for 0.78mm of the total length.

4.3.5.2 Developmental rate of *Fopius arisanus*

Developmental rate increased with temperature but the optimum and maximum varied among different immature stages. The developmental rate for all stages of *Fopius arisanus* could not go beyond 32ºC (Figure 4.6).

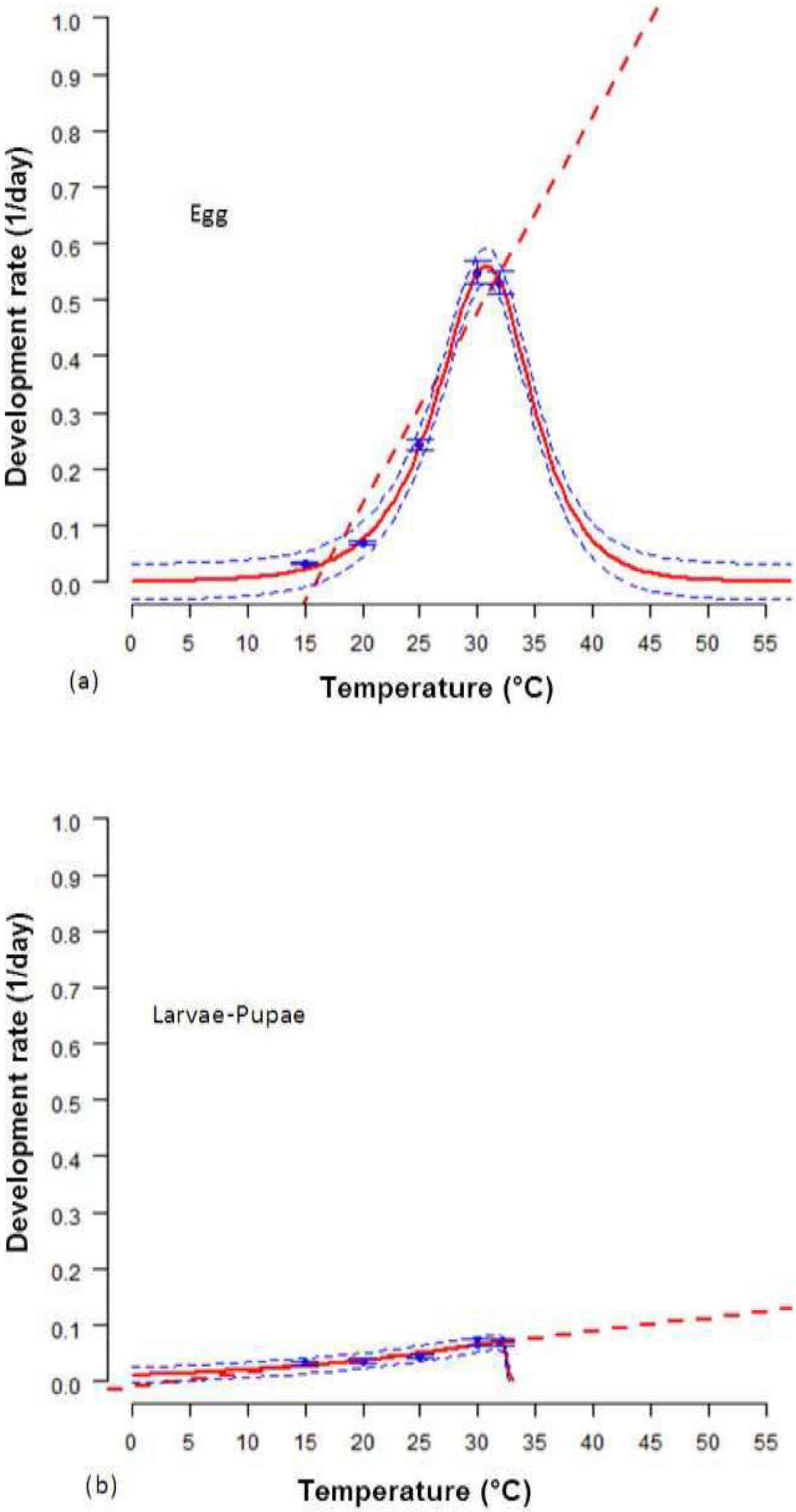


Figure 4.6: Temperature dependent development rate (1/developmental time) of (a) eggs, (b) larvae and (c) pupae for *Fopius arisanus* (red line). The blue lines represent 90% confidence interval. Blue points represent constant temperature ranges at which phenology experiments were carried out.

4.3.5.3 Mortality of *Fopius arisanus* immature stages

Mortality was high in extreme temperatures during larval-pupal stage with a mean average of 75% but comparably lower during the egg stage (Figure 4.7).

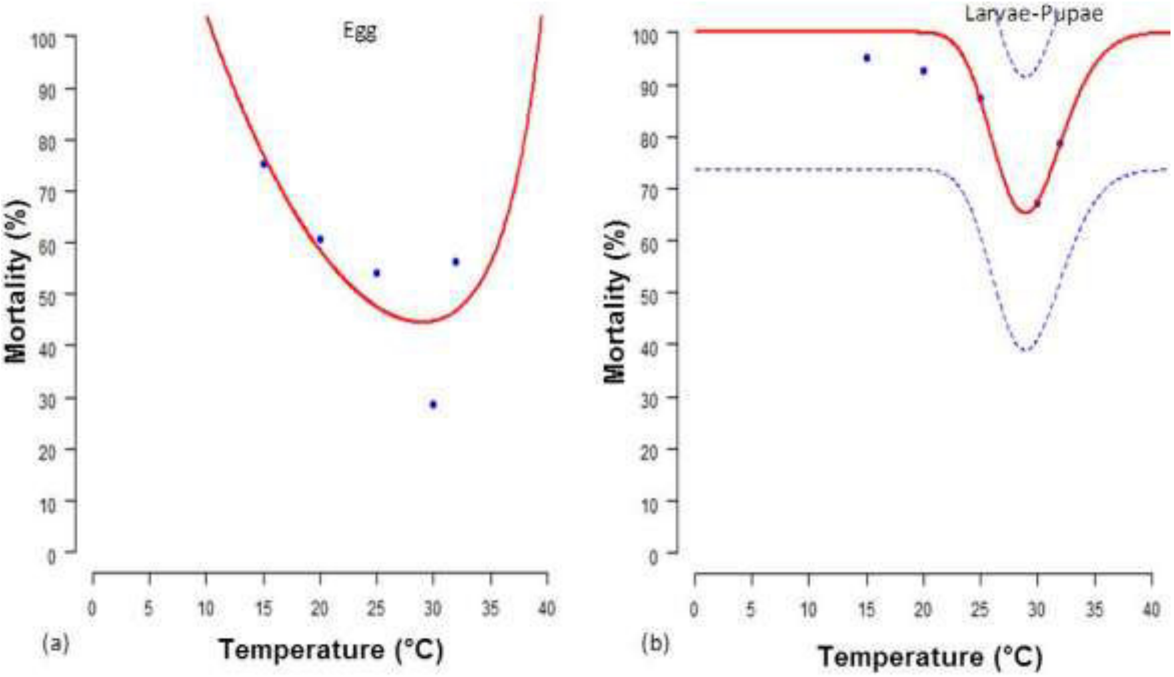


Figure 4.7: Temperature-dependent mortality rate of (a) eggs, (b) larvae and (c) pupae for *Fopius arisanus*(red line). The blue lines represent 90% confidence interval. Blue points represent constant temperature ranges at which phenology experiments were carried out.

4.3.5.4 Fecundity (oviposition rate) by a female *Fopius arisanus*

The mean number of eggs laid per female increased with increasing temperature from 2 eggs at 15ºC to 31 eggs at 30ºC. At 25ºC the *Fopius arisanus* female laid a mean of 28 eggs but the number reduced to 19 eggs at 32ºC. The model estimated that the female pest could not lay eggs below 15ºC and above 36ºC (Figure 4.8).

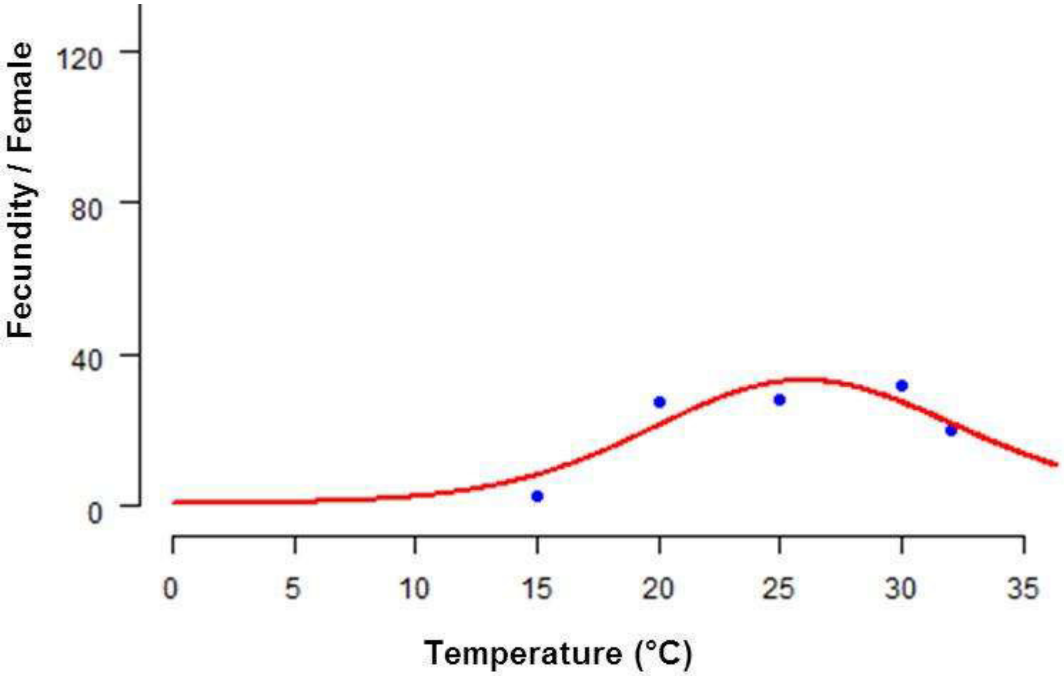


Figure 4.8: Temperature-dependent total number of egg oviposited (fecundity) by *Fopius arisanus*; red line). Blue points represent constant temperature ranges at which phenology experiments were carried out.

4.4. Discussion

4.4.1 Developmental time and longevity of *Bactrocera invadens* and *Fopius arisanus*

The study results confirmed that the developmental time (days) decreased with increasing temperature ranges. Development time for both *Bactrocera invadens* and *Fopius arisanus* was shorter in warmer temperatures between 25°C and 30°C than cooler ranges. Shorter developmental time of *Bactrocera invadens* suggests low risk of death hence high population influx and infestation by the pest species. The study revealed that temperature gradients <15°C and >36°C are unfavorable to the development of *Bactrocera invadens* and *Fopius arisanus* which concurs with findings by Keena (2006), Briere *et al* (1999) and Logan *et al* (1976). The results suggest that temperature has a vital role in the development of insect pest and its egg parasitoid. Females generally lived longer than males possibly due active feeding during their reproductive life. Furthermore, the egg parasitoid; *Fopius arisanus* (Hymenoptera:

Braconidae), of the Asian invasive fruit fly (*Bactrocera invadens*, Diptera: Tephritidae) was able to develop in a wide range of temperatures with 25°C being the most favorable for their survival.

4.4.2 Developmental and mortality rate of *Bactrocera invadens* and *Fopius arisanus*

Developmental rate of the Asian invasive fruit fly (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*) was faster between 25°C and 32°C but slower in temperatures below 15°C. This suggests the pest and its egg parasitoid do thrive well in warmer temperatures which are found in lowland regions. This concurs with results from previous chapter and Rwomushana *et al* (2008) on occurrence of *Bactrocera invadens* along elevation gradient. Results of chapter three in this thesis revealed that *Bactrocera invadens* was more abundant in warmer lowland areas than the colder highlands suggesting that mortality was high in temperatures below 15°C. The constant temperature of 15°C is equivalent to the colder highlands at Taita Hills and Mount Kilimanjaro. The study findings also revealed that mortality of *Bactrocera invadens* and *Fopius arisanus* was high in temperatures that were above 35°C implying that extreme hotter regions are unfavorable to survival of the insects. Lack of development of *Bactrocera invadens* pupae at 35°C and beyond suggested that it was the most vulnerable stage for the Asian invasive fruit fly.

4.4.3 Oviposition rate (Fecundity) by females of *Bactrocera invadens* and parasitization level by *Fopius arisanus*

The non-linear polynomial model predicted that temperatures between 25 °C and 30 °C were the better settings for oviposition by females of the Asian invasive fruit fly (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*). Optimum oviposition by the parasitoid; *Fopius arisanus*

(Hymenoptera: Braconidae), into eggs of Asian invasive fruit fly (*Bactrocera invadens*, Diptera: Tephritidae) was highest at warmer temperature range of 25°C. This study indicates that the female *Fopius arisanus* can successfully oviposit into eggs of *Bactrocera invadens* found on host ripening avocado fruits as described by Rwomushana *et al* (2008), Ekesi *et al* (2006) and Ekesi & Billah (2006). *Fopius arisanus* can, therefore, be used as a bio control of the Asian invasive fruit fly in avocado orchards in warmer lowland to midland regions of Taita Hills, Kenya and Mount Kilimanjaro, Tanzania.

CHAPTER FIVE

PREDICTING FUTURE GEOGRAPHICAL DISTRIBUTION OF *Bactrocera invadens* AND ITS PARASITOID IN TAITA HILLS AND MOUNT KILMANJARO UNDER CLIMATE-WARMING SCENARIOS USING TEMPERATURE-DRIVEN PHENOLOGY MODELS

Abstract

Phenological models generated in the previous chapter were compiled for stochastic simulation resulting in life table parameters that were used to simulate current year (2014) and future (2055) distribution maps of the Asian invasive fruit fly (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*) in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. The climate-driven distribution maps of the *Bactrocera invadens* and its egg parasitoid for Taita Hills and Mount Kilimanjaro were generated using a GIS environment implemented in the Insect Life Cycle Modeling software (ILCYM) and R statistical programme. Present-day spatial simulation was based on current daily minimum and maximum temperatures recorded from 1st January to 31st December 2014 along study transects of Taita Hills and Mount Kilimanjaro. Potential future (year 2055) minimum and maximum temperatures were generated using downscaled climate information from the WorldClim data base. Spatially referenced pest indices displaying risk establishment and number of generations per year were computed using the shape files of the study transects of Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. The study findings revealed that *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*) is established in warmer lowland regions below 1200m a.s.l but with continued global warming the insect species can expand its distribution range into higher altitudinal zones above 1700m a.s.l by the year 2055. The current simulated spatial patterns concur with geographical distribution of the Asian invasive fruit fly (*Bactrocera invadens*) based on its field mean abundance as described in Chapter Three. Establishment and generation indices of *Bactrocera invadens* by the year 2055 were high enough indicating that it will remain a serious insect pest of avocado fruits at the two study areas. Species distribution maps generated based on the establishment and generation indices can be used in designing effective bio-control strategies of *Bactrocera invadens* by pin-pointing exact location in Taita Hills and Mount Kilimanjaro for release of the egg parasitoid (*Fopius arisanus*) of Asian invasive fruit fly since their preferred current and future projected habitat conditions are almost similar.

5.1 Introduction

The United Nation's Inter-governmental Panel on Climate Change (IPCC) predicted a mean increase in global temperature of between 1.1ºC and 5.4ºC by year 2100 (IPCC, 2001b). IPCC (2007) further reported that average surface temperature increased by 0.6ºC for the past 100 years. The anticipated increase in temperature due global warming will potentially affect development of insect species in the next century (UNEP, 2009; Parmesan, 2007; Parmesan *et al.*, 1999). Generally, global warming is impacting negatively on world agriculture and more so horticulture sector in tropical countries in Africa (FAO, 2013 & 2003). Agro-ecosystems adjacent to montane tropical areas in Eastern Africa have been most affected due to drastic evolution of altitudinal gradients over decades into different micro-climate regimes as result of wanton deforestation and agricultural intensification. Climate-warming in agro-ecosystems adjacent to East African montane forests can enhance infestation of fruit crops by invasive insect pests such as *Bactrocera invadens* (Diptera: Tephritidae). This is due to reduction in diversity of wild host fruits. Chapter three of this thesis describes avocado is the most dominant fruit crop that is infested by *Bactrocera invadens* (Diptera: Tephritidae) leading to loss of livelihood in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. Chapter three of this thesis expounds on how *Bactrocera invadens* is adapted to avocado fruit host-ability in low-elevated slopes of the study areas. The highly aggressive Asian invasive fruit fly (*Bactrocera invadens*) readily displaces less damaging indigenous fruit fly species in ecosystems it populates (Ekesi *et al.*, 2009; Mohamed *et al.*, 2008; Rwomushana *et al.*, 2008; FAO, 2006; Mohamed, 2003). Therefore, ability to map current and future geographical distribution of the Asian invasive fruit fly (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*) using temperature-dependent phenology models is important in designing effective bio-control strategies of the invasive pest in the Mount Kilimanjaro region. However, detailed information on eco-physiological responses of *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*) to global warming in avocado agro-ecosystems adjacent to montane forests within East Africa is not available. Such metadata is required for simulating distribution patterns of avocado insect pests based on current and future climate change.

Knowledge of phenology and population dynamics of *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*) is required to develop Integrated Pest Management (IPM) strategies aimed at combating infestation of avocado fruits in Eastern Africa. Khadioli *et al*, (2014a & b) reports that understanding development, fecundity and mortality of a pest and its parasitoid has led to generation of phenological models. The models can be used to estimate physiological development and behavior of an insect pest and its parasitoid in response to projected increase in temperature. Temperature is emphasized because it is the most fundamental environmental variable influencing development, fecundity, behavior, mortality and distribution of insects (Chapter Three, Chapter Four, Liu & Ye, 2009; Rwomushana *et al.*, 2008; Vayssières *et al.*, 2008; Brévault & Quilici, 2000; Vargas *et al.*, 1997; Fletcher 1987). Temperature-dependent phenology models play a significant role in detecting habitat suitability and mapping spatial distribution of the pests and parasitoids using Insect Life Cycle Modeling software (Tonnang *et al.*, 2013; Sporleder *et al.*, 2009; Nietschke *et al.*, 2007). The Insect Life Cycle Modeling software (ILCYM) is important in assessment of impact of climate change on insect species (Kroschel *et al.*, 2013; Tonnang *et al.*, 2013; Sporleder *et al.*, 2009). This chapter, therefore, hypothesized that projected increase in temperature may accelerate development of insect pests such *Bactrocera invadens* resulting in more generations per year and consequently expand its distribution range leading to enhanced infestation of avocado fruits.

Models generated using temperature-dependent ILCYM software can be used to simulate geographical phytosanitary risk assessments aimed at developing IPM protocols for control of avocado insect pests. These include identification of potential release sites for parasitoids, simulating field performance of other classical bio-control methods and application frequencies. ILCYM is a process based on phenology models which describe the basic physiological principles of the insect growth; development, survival and reproduction (Sporleder, *et al*, 2009). ILCYM is based on detailed laboratory assessments that produce life-table parameters and allow the simulation of populations according to real or interpolated temperature data for a given region and time (Kroschel *et al*., 2013; Sporleder *et al*., 2007; Nietschke *et al*., 2007). The ILCYM software was, therefore, used in this study to generate phenological models and further simulate risk maps of the key avocado insect pest (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*) using data from the life-cycle experiments. *Fopius arisanus* was selected because it specifically attacks *Bactrocera invadens* eggs on or near avocado fruit surface and the parasitoid presents no risk to beneficial insect species. Species distribution models provide the best way to overcome sparseness typical of distributional data by relating them to a set of geographic and environmental predictors. Present and potential future knowledge on the geographical distribution of the insect pest and its parasitoid is crucial for spatial planning of avocado crop production in East Africa. However, comprehensive data on distribution of avocado pests based on their phenology is not available for Taita Hills and Mount Kilimanjaro. Collecting such data is very costly, labour intensive and highly technical. This study, therefore, adhered to the following objective: to generate risk maps of the major avocado insect pest and its associated parasitoid using species distribution models based on present and projected climate change scenarios in Mount Kilimanjaro, Tanzania and Taita Hills, Kenya

5.2 Materials and methods

5.2.1 Study areas

Potential spatial distribution patterns of key insect pest (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*) were simulated in avocado farmlands within Taita Hills, South-eastern Kenya and along southern slopes of Mount Kilimanjaro, Tanzania as described in Chapter Three.

5.2.2 Current (year 2014) and future projected (year 2055) temperature data

Current minimum and maximum daily temperatures for a calendar year were averaged from data collected along altitudinal gradient of Taita Hills and Mount Kilimanjaro transects for twenty four months between 1st January 2014 and 31st December 2014. Temperature (ºC) and relative humidity (%) were automatically recorded every 30 minutes at every 100m a.s.l along the study areas for the two sampling years using iButton™ data loggers from Maxim Integrated Products, Incorporation, USA (https://www.maximintegrated.com). The average daily minimum and maximum temperatures were computed from information recorded from the data loggers. Nine data loggers were set at every 100m a.s.l along altitudinal gradient ranging from 1000m a.s.l to 1800m a.s.l at each study site. The data loggers were hang on the lower canopy of avocado tree at a height of 1.5m above ground level with an allowance of free movement of sunlight and air. The geographical coordinates and elevation of each sub-block was recorded using a hand-held

Germin GPS model eTrex 30 (Table 5.1). Projected future (year 2055) daily minimum and maximum temperatures were generated using downscaled climate information from the WorldClim data-base.

Table 5.1: Elevation (m a.s.l) of major sites along study transects of (a) Mount Kilimanjaro, Tanzania and (b) Taita Hills, Kenya.

Locality		**Locality**	
(a) Mount Kilimanjaro	Elevation (m a.s.l)	(b) Taita Hills	Elevation (m a.s.l)
Marua	1780	Mgange	1800
Nduoni	1680	Mwanda	1700
Kongo	1600	Wundanyi	1570
Mahakama	1500	Josa	1300
Sumi	1300	Dembwa	1200
Uparo	1080	Kipusi	1100

5.2.3 Generation of life table parameters

All temperature-dependent phenological models generated in Chapter Four were combined for stochastic simulation resulting in life table parameters of the Asian invasive fruit fly (*Bactrocera invadens*) as described by Curry *et al* (1978) and Kroschel *et al* (2013). The life table parameters estimated using stochastic simulation tool in ILCYM were; intrinsic rate of natural increase (*rm*), net reproduction rate (Ro), gross reproductive rate (GRR), mean generation time (T) in days, finite rate of increase (λ) and doubling time (*Dt*) in days (Maia *et al.*, 2000; Carey, 1993). Resultant life table parameters were used to simulate current (year 2014) and future (year 2055) distribution maps of *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*) as described in Mwalusepo *et al* (2015) and Khadioli *et al* (2014a & b).

Intrinsic rate of natural increase (*rm*) is the ratio of the total number of individuals in a closed population that has been subject to range of constant temperatures or constant age-specific schedules of fertility and mortality for a long period of time and has converged to be a stable population (Maia *et al*., 2000; Carey, 1993). Net reproduction rate (Ro) is defined as the average number of female offspring that would be born to a cohort of females during their lifetime if they experienced a fixed pattern of age-specific birth and death rates (Maia *et al*., 2000; Carey, 1993). Gross reproductive rate (GRR) describes the average numbers of eggs produced by each female in a cohort (Maia *et al*., 2000; Carey, 1993). Mean generation time (T) is the period in days required for a population to increase by a factor equal to the net reproductive rate; one generation to the next. In other words, the time (days) required for a newborn female to replace herself with the new one. Finite rate of increase (λ) is a multiplication factor of the original population at each time period (Maia *et al*., 2000; Carey, 1993). Doubling time (*Dt*) is the number the time span (days) necessary for doubling the initial population. The above-mentioned models have previously been used by Mwalusepo *et al* (2015), Khadioli *et al* (2014a & b), Fand *et al* (2014), Kroschel *et al* (2013) and Tonnang *et al* (2013) to predict distribution of insect pests and related parasitoids by generating spatial indices such as risk establishment (ERI) and number of generations (GI) (Mwalusepo *et al*., 2015).

5.2.4 Spatial analysis

5.2.4.1 Development of current (2014) and future (2055) risk maps of *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*) using phenology models

Geographical information system environment implemented in Insect Life Cycle Modeling software (ILCYM) was employed in generating risk maps using temperature-driven climate data following methods described by Kroschel *et al* (2013). Development of current (year 2014) and future (year 2055) potential risk maps of key avocado insect pest; *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*) was simulated using a complete process-based phenology model from objective three that describes the basic physiological principals of insect growth; development, survival and reproduction. Spatially referenced pest indices displaying risk establishment (ERI) and number of generations (GI) per year was computed using shape files of the study transects at Taita Hills and Mount Kilimanjaro (Sporleder *et al.*, 2009). Establishment index (ERI) ranges between 0 and 1 whereas generation index (GI) assumes any value that is calculated based on appropriate data input as explained by Sporleder *et al* (2009). Establishment index (ERI) identifies areas along the study region where the pest or its parasitoid may survive and become established whereas generation index (GI) estimated mean number of generations that may be produced by the insect species in a year.

The spatial simulation was based on current and future daily minimum and maximum temperatures as inputs as described in Nietschke, *et al.*, 2007. Current temperatures were collected along altitudinal gradient of Taita Hills and Mount Kilimanjaro transects for one year starting from 1st January 2014 to 31st December 2014. Projected future (year 2055) daily minimum and maximum temperatures were generated using downscaled climate information from WorldClim data-base and the information can be freely accessed at www.york.ac.uk/environment/research/kite/resources/ (Platts *et al.*, 2014). AIB climate scenario was selected as detailed in Khadioli *et al* (2014a & b). Calculation was done using an index interpolator, a sub-module of Insect Life Cycle Modeling software (ILCYM) as described in Mwalusepo *et al* (2015). Climate layers of the Taita Hills and Mount Kilimanjaro study areas were generated through interpolation of mean daily minimum and maximum temperature data on a 30 arc-second resolution grid often referred to as 1 km^2 resolution (Hijmans *et al.*, 2005). Other variables included in analysis were longitude, latitude and elevation. The year 2055 was selected to be used for a potential climate change scenario following protocol that an average of a thirty years continuous weather data is the minimum change in climate (IPCC, 2007, 2001 & 1996).

5.3 Results

5.3.1 Current and future predicted (year 2055) temperatures

The current (year 2014) mean annual temperature of Taita Hills study area was 19.56ºC±0.19 whereas the present average annual temperature of Mount Kilimanjaro transect was 20.14ºC± 0.66. Future (year 2055) temperatures of each study transect in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania were predicted to increase by average of 1.45°C (between 1°C and 1.9°C). A large margin in increment of temperature between current (year 2014) and predicted (2055) future was witnessed in mid elevated highlands (1300 – 1600m a.s.l) compared to lowland plains below 1200m a.s.l of the two study areas. Current and future projected temperatures of low-elevated zones of the study transect along Mount Kilimanjaro, Tanzania had a higher average maximum temperature than that of Taita Hills study area in Kenya (Table 5.2).

Table 5.2: Current (year 2014) and future predicted mean annual temperatures in different altitudinal zones at Taita Hills and Mount Kilimanjaro. *Future predicted temperatures for year 2055 was downscaled from the WorldClim data-base

	Mean annual temperature	
Taita Hills transect	**Current_2014**	**Predicted future_2055**
Lowland (1000m - 1200m a.s.l)	22.32±0.19	23.420
Sub-montane (1200m - 1500m a.s.l)	19.14±0.19	21.040
Montane (1500m - 1800m a.s.l)	17.22±0.21	19.120

	Mean annual temperature	
Mount Kilimanjaro transect	**Current_2014**	**Predicted future_2055**
Lowland (1000m - 1200m a.s.l)	21.85±0.22	22.950
Sub-montane (1200m - 1500m a.s.l)	20.66±0.25	22.560
Montane (1500m - 1800m a.s.l)	17.92±0.24	19.820

5.3.2 Life table parameters

The life table parameters which were estimated revealed that the avocado pest; Asian invasive fruit fly (*Bactrocera invadens*) can survive in different range of temperatures from 15°C to around 35°C (Table 5.3). The optimum population development was witnessed between 25°C to 32.5°C with 30°C having the highest intrinsic rate of natural increase (*rm*; Table 5.3; Figure 5.1). The cohorts at extreme temperatures had smallest intrinsic rate of natural increase (*rm*), finite rate of increase (λ) and net reproduction rate (Ro) values (Table 5.3; Figure 5.1). Descriptions of life table parameters are detailed in Khadioli *et al* (2014a & b), Fand *et al* (2014) and Kroschel *et al* (2013).

Table 5.3: Life table parameters of *Bactrocera invadens* at different constant temperature ranges. Where *rm* = intrinsic rate of natural increase, Ro = net reproduction rate, GRR = gross reproductive rate, T = mean generation time in days, λ = finite rate of increase, *Dt* = doubling time in days, *** = highly significant, ** = significant and * = least significant.

Constant temperature range	rm	Ro	GRR	T	λ (Lambda)	Dt
15	0.04	28.25	84.20	84.57	1.04	17.8
20	0.07	79.88	218.30	59.23	1.08	9.40
25	0.10	99.50	300.40	44.24	1.11	6.68
30	0.12	64.17	269.87	35.87	1.12	6.06
32.5	0.09	25.29	198.65	35.47	1.09	7.74
35	0.04	4.61	112.29	37.50	1.03	13.66
p-value	0.002**	0.02*	0.006**	0.002**	<0.0001***	0.003**

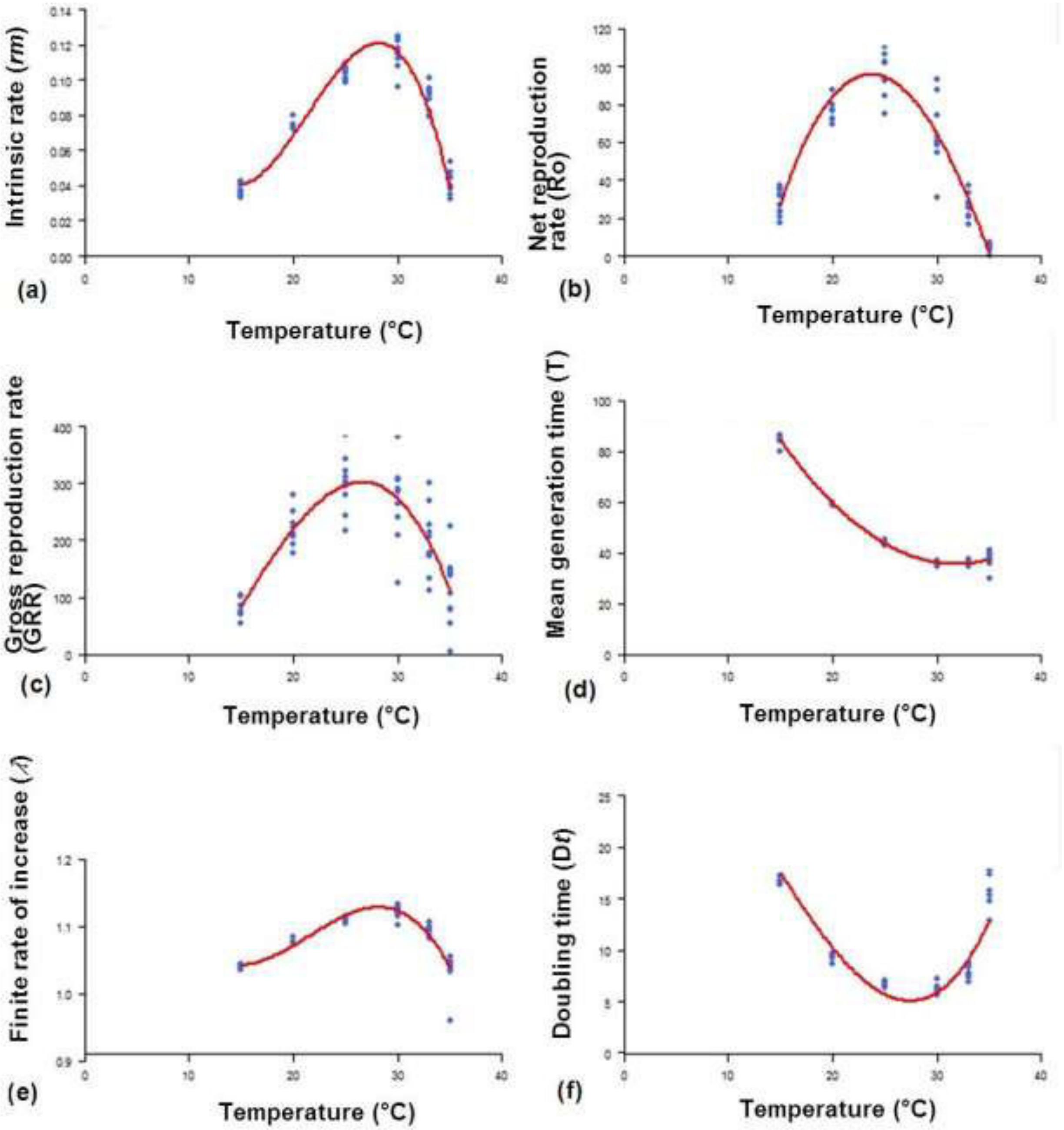


Figure 5.1: Life table parameters of the Asian invasive fruit fly (*Bactrocera invadens*) at constant temperature ranges**;** (a) intrinsic rate of natural increase, *rm*, (b) net reproduction rate, Ro, (c) gross reproductive rate, GRR, (d) mean generation time, T, (e) finite rate of increase, *λ* and (f) doubling time, *Dt*.

5.3.3 Current and future (year 2055) spatial distribution of *Bactrocera invadens* and its parasitoid (*Fopius arisanus*)

5.3.3.1 Establishment risk index (ERI)

5.3.3.1.1 Mapping establishment risk index of the Asian invasive fruit fly (*Bactrocera invadens*) in Taita Hills

In Taita Hills, current (year 2014) predictions of a mean establishment risk index (ERI) revealed a range between 0.74 in highlands above 1600m a.s.l (Wundanyi - Mgange) to high of 0.85 in lowlands below 1100m a.s.l or Kipusi (Table 5.4; Figure 5.2a). Potential future prediction for year 2055 showed that Asian invasive fruit fly (*Bactrocera invadens*) will have a higher establishment risk index (ERI) ranging from 0.76 to 0.89 (Table 5.4; Figure 5.2b). The study findings revealed that the whole of Taita Hills transect will experience increase in changes in distribution of the Asian invasive fruit fly (*Bactrocera invadens*) by end of 41 years (year 2055; Figure 5.2b; Table 5.4).

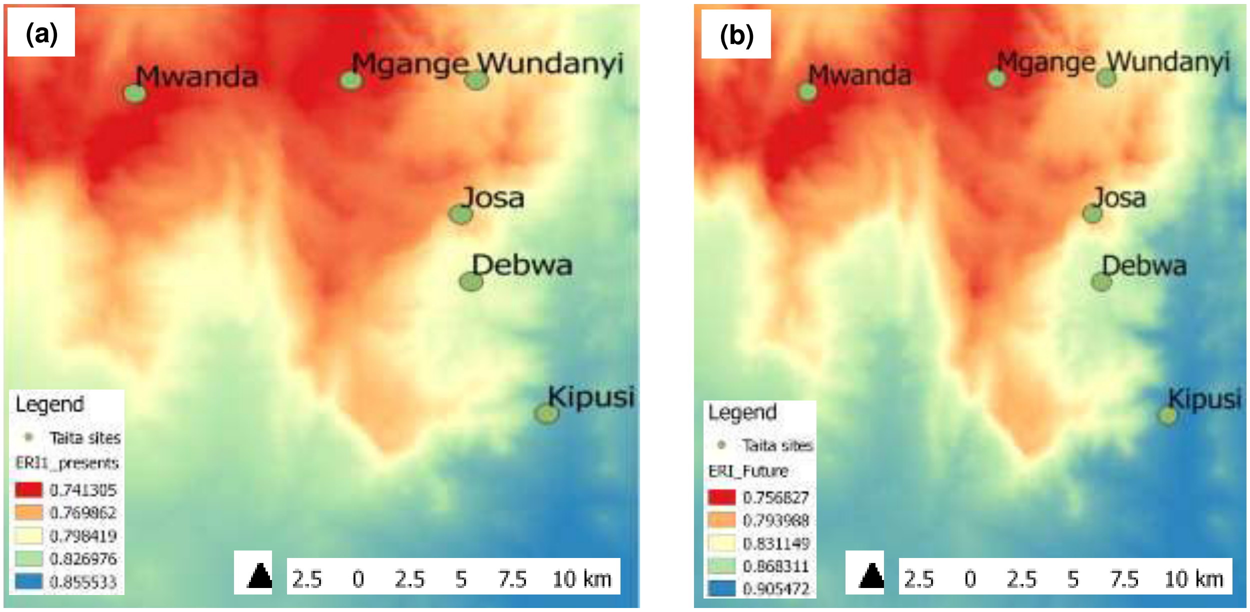


Figure 5.2: Establishment risk index (ERI)/ risk map of *Bactrocera invadens* at Taita Hills, Kenya (a) Current (year 2014) and (b) future (year 2055). Focal study points along increasing elevation were: Kipusi (1100m a.s.l), Dembwa (1200m a.s.l), Wundanyi (1570m a.s.l) and Mgange (1800m a.s.l).

Table 5.4: Current and future predicted mean Establishment risk index (ERI) of *Bactrocera invadens* in different altitudinal zones at Taita Hills

	Bactrocera invadens	
Taita Hills transect	**ERI_2014**	**ERI_2055**
Lowland (1000m - 1200m a.s.l)	0.846	0.893
Sub-montane (1200m - 1500m a.s.l)	0.784	0.813
Montane (1500m - 1800m a.s.l)	0.741	0.757

5.3.3.1.2 Mapping establishment risk index of the egg parasitoid; *Fopius arisanus*, of Asian invasive fruit fly in Taita Hills

Current (year 2014) predictions of establishment risk index (ERI) revealed a range between 0.50 in highlands above 1600m a.s.l (Wundanyi - Mgange) to high of 0.99 in lowlands below 1100m a.s.l or Kipusi locality (Figure 5.3a). Potential future prediction for year 2055 showed that the egg parasitoid (*Fopius arisanus*) of Asian invasive fruit fly will have a mean increment in establishment risk index (ERI) of 0.785 within highlands located above 1300m a.s.l (Josa) but a reduction in ERI will be witnessed in lowlands (Table 5.5; Figure 5.3b). The study findings revealed that middle zones including Dembwa (1200m a.s.l) and Josa (1300m a.s.l) will experience increase in changes in species distribution by end of 41 years (year 2055; Figure 5.3b; Table 5.5).

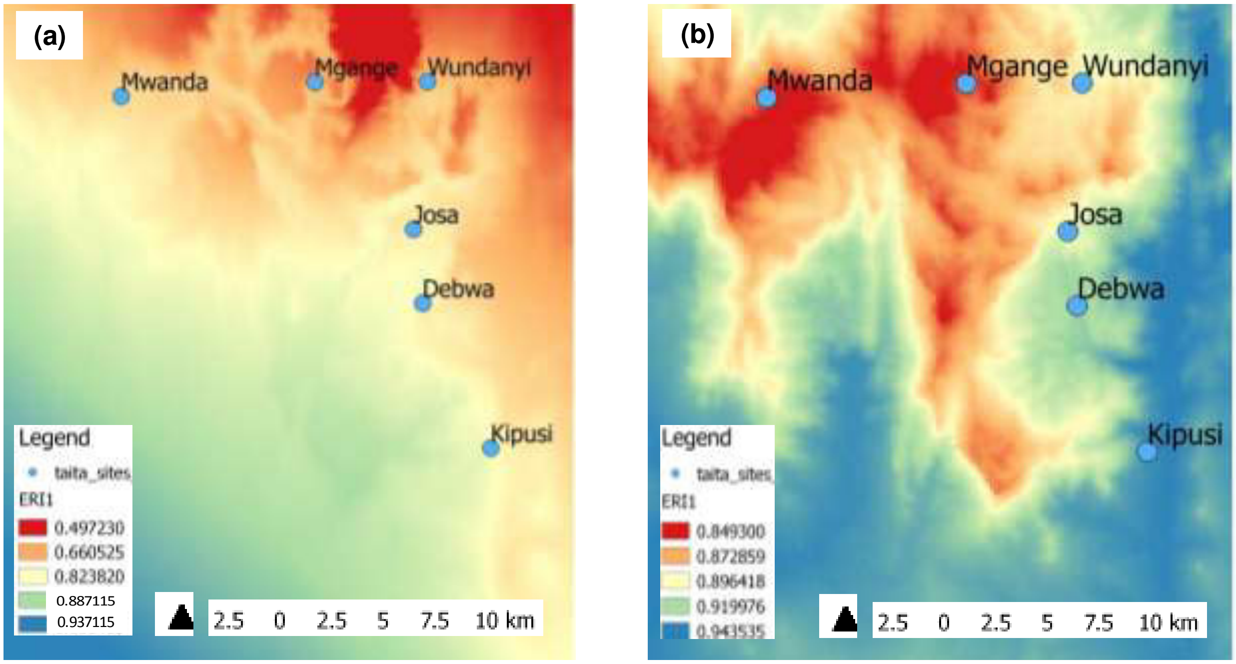


Figure 5.3: (a) Current (year 2014) and (b) future (year 2055) establishment risk index (ERI) of the egg parasitoid (*Fopius arisanus*) of Asian invasive fruit fly at Taita Hills, Kenya. Focal study points along increasing elevation were: Kipusi (1100m a.s.l), Dembwa (1200m a.s.l), Wundanyi (1570m a.s.l) and Mgange (1800m a.s.l).

Table 5.5: Current and future predicted mean Establishment risk index (ERI) of egg parasitoid (*Fopius arisanus*) in different altitudinal zones at Taita Hills

	Fopius arisanus	
Taita Hills transect	**ERI_2014**	**ERI_2055**
Lowland (1000m - 1200m a.s.l)	0.856	0.931
Sub-montane (1200m - 1500m a.s.l)	0.740	0.885
Montane (1500m - 1800m a.s.l)	0.582	0.849

5.3.3.1.3 Mapping establishment risk index of the Asian invasive fruit fly (*Bactrocera invadens*) in Mount Kilimanjaro, Tanzania

In Mount Kilimanjaro, current (year 2014) predictions of mean establishment risk index (ERI) revealed a range between 0.75 in highlands above 1600m a.s.l (Kongo – Marua) to high of 0.89 in lowlands <1100m a.s.l or below Uparo study area (Table 5.6; Figure 5.4a). Potential future prediction for year 2055 showed that Asian invasive fruit fly (*Bactrocera invadens*) will have an increment rate in establishment risk index (ERI) of 0.013 within highlands located above 1300m a.s.l (Sumi) but insignificant change in ERI will be witnessed in lowlands below Uparo (1100m a.s.l) (Table 5.6; Figure 5.4b). The study findings revealed that the highlands located above 1300m a.s.l (Sumi) will experience increase in changes in species distribution by end of 41 years (year 2055; Figure 5.4b; Table 5.6).

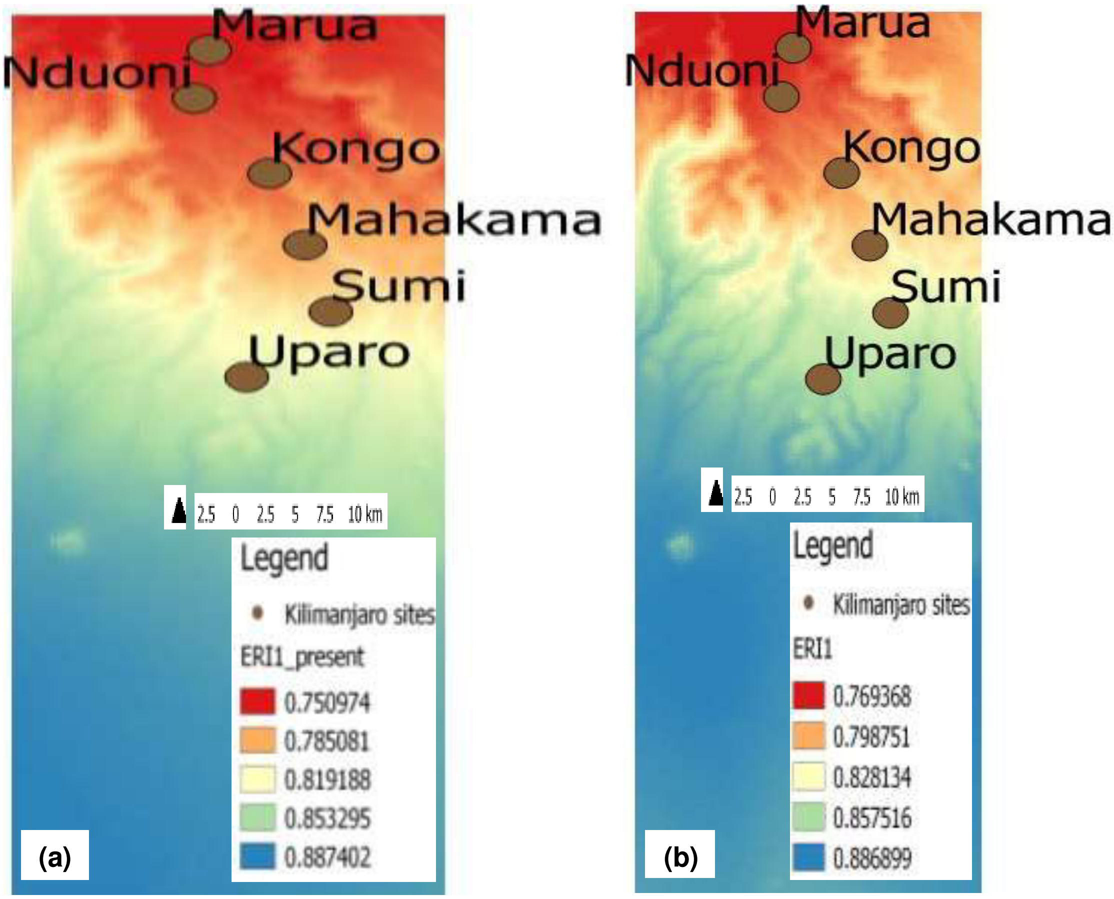


Figure 5.4: (a) Current (year 2014) and (b) future (year 2055) establishment risk index (ERI)/ risk map of *Bactrocera invadens* at Mount Kilimanjaro, Tanzania. Focal study points along increasing elevation were: Uparo (1080m a.s.l), Sumi (1300m a.s.l), Mahakama (1500m a.s.l) and Marua (1780m a.s.l).

Table 5.6: Current and future predicted mean Establishment risk index (ERI) of *Bactrocera invadens* in different altitudinal zones at Mount Kilimanjaro

	Bactrocera invadens	
Mount Kilimanjaro transect	**ERI_2014**	**ERI_2055**
Lowland (1000m - 1200m a.s.l)	0.853	0.868
Sub-montane (1200m - 1500m a.s.l)	0.785	0.814
Montane (1500m - 1800m a.s.l)	0.751	0.769

5.3.3.1.4 Mapping establishment risk index of the egg parasitoid; *Fopius arisanus*, of Asian invasive fruit fly in Mount Kilimanjaro, Tanzania

Current (year 2014) predictions of establishment risk index (ERI) revealed a range between 0.75 in highlands above 1600m a.s.l (Kongo – Marua) to high of 0.85 in lowlands <1100m a.s.l or below Uparo study area (Figure 5.5a; Table 5.7). Potential future prediction for year 2055 showed that the egg parasitoid (*Fopius arisanus*) of Asian invasive fruit fly will have an increment rate in establishment risk index (ERI) of 0.092 (Figure 5.5b). The study findings revealed that the whole of Mount Kilimanjaro transect will experience increase in changes in distribution of *Fopius arisanus* by end of 41 years (year 2055; Figure 5.5b; Table 5.7).

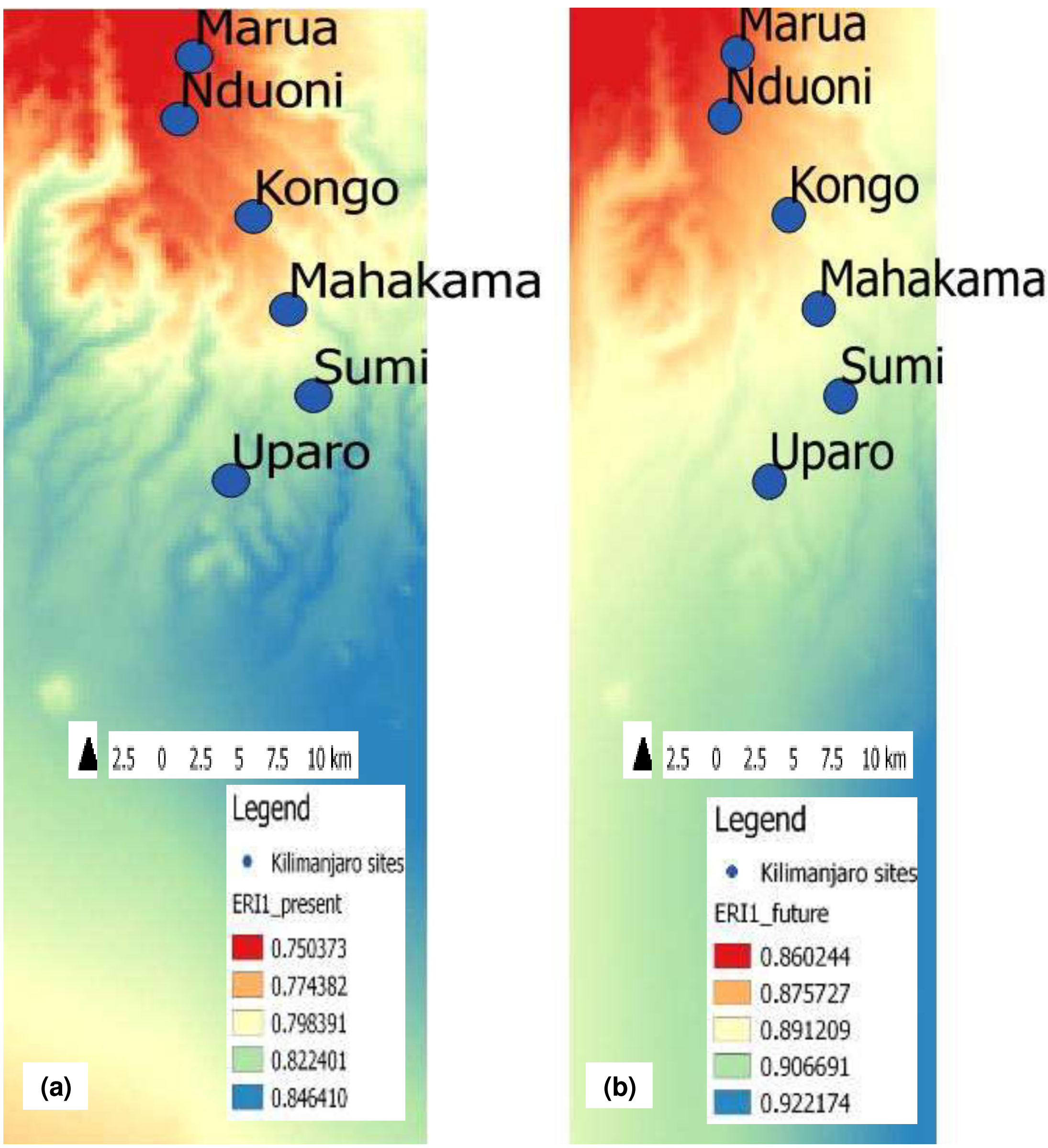


Figure 5.5: (a) Current (year 2014) and (b) future (year 2055) establishment risk index (ERI) of the egg parasitoid (*Fopius arisanus*) of Asian invasive fruit fly at Mount Kilimanjaro, Tanzania. Focal study points along increasing elevation were: Uparo (1080m a.s.l), Sumi (1300m a.s.l), Mahakama (1500m a.s.l) and Marua (1780m a.s.l).

Table 5.7: Current and future predicted mean Establishment risk index (ERI) of egg parasitoid (*Fopius arisanus*) in different altitudinal zones at Mount Kilimanjaro

	Fopius arisanus	
Mount Kilimanjaro transect	**ERI_2014**	**ERI_2055**
Lowland (1000m - 1200m a.s.l)	0.838	0.912
Sub-montane (1200m - 1500m a.s.l)	0.786	0.884
Montane (1500m - 1800m a.s.l)	0.750	0.868

5.3.3.2 Generation index (GI)

5.3.3.2.1 Mapping generation index of the Asian invasive fruit fly (*Bactrocera invadens*) in Taita Hills

In Taita Hills, predicted mean number of generations (generation index; GI) that can be produced by the Asian invasive fruit fly (*Bactrocera invadens*) in the current year (year 2014) ranged between 8 and 9 in highlands (1300 - 1800m a.s.l; Josa - Mgange) to 11 in lowlands below 1100m a.s.l or Kipusi (Figure 5.6a; Table 5.8). Number of generations of a population per year is reported as a whole number since it represents life. Potential future prediction for year 2055 showed that Asian invasive fruit fly (*Bactrocera invadens*) will have a slightly higher generation index (GI) ranging from 9 in highlands to 11 in lowlands (Figure 5.6b; Table 5.8). The study findings revealed that the whole of Taita Hills transect will experience increase in mean number of generations of the Asian invasive fruit fly (*Bactrocera invadens*) by end of 41 years (year 2055; Figure 5.6b; ; Table 5.8) compared to the current scenario (year 2014; Figure 35a). The increment will be by one generation of the Asian invasive fruit fly (*Bactrocera invadens*) at every 100m a.s.l in Taita Hills at end of 41 years (year 2055; Figure 5.6b).

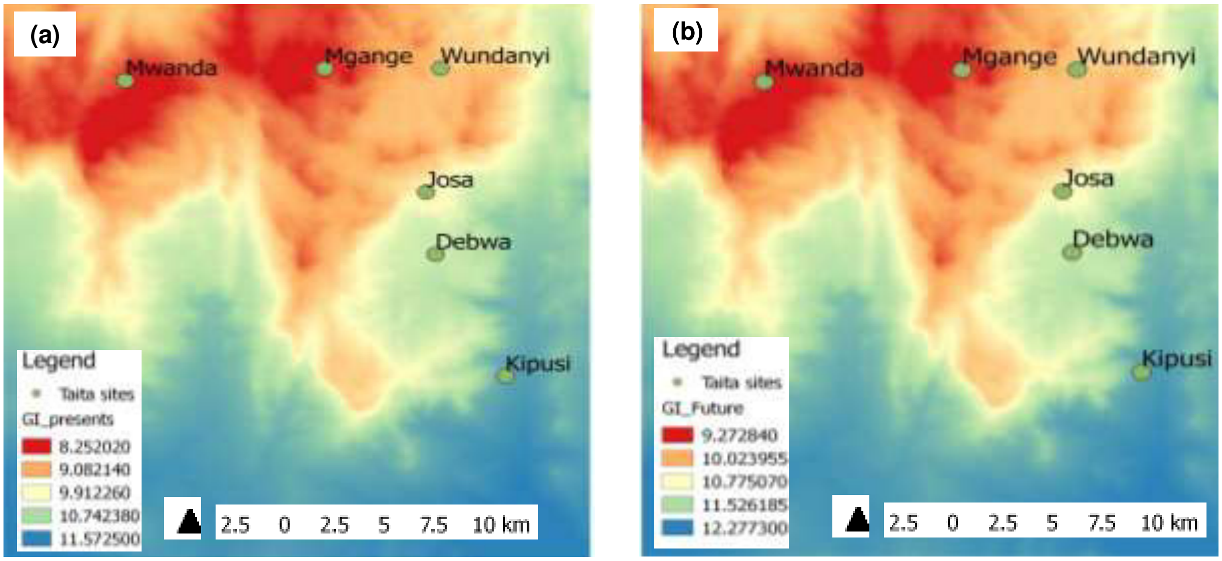


Figure 5.6: (a) Current (year 2014) and (b) future (year 2055) generation index (GI)/ risk map of *Bactrocera invadens* at Taita Hills, Kenya. Focal study points along increasing elevation were: Kipusi (1100m a.s.l), Dembwa (1200m a.s.l), Wundanyi (1570m a.s.l) and Mgange (1800m a.s.l).

Table 5.8: Current and future predicted mean generation index (GI) of *Bactrocera invadens* in different altitudinal zones at Taita Hills

	Bactrocera invadens	
Taita Hills transect	**GI_2014**	**GI_2055**
Lowland (1000m - 1200m a.s.l)	11.296	12.027
Sub-montane (1200m - 1500m a.s.l)	9.497	10.400
Montane (1500m - 1800m a.s.l)	8.252	9.273

5.3.3.2.2 Mapping generation index of the egg parasitoid; *Fopius arisanus*, of Asian invasive fruit fly in Taita Hills

In Taita Hills, predicted mean number of generations (generation index; GI) in the current scenario (year 2014) produced by *Fopius arisanus* ranged between 2 in highlands (1300 - 1800m a.s.l) to 3 in lowlands below 1100m a.s.l (Table 5.9). Potential future prediction for year 2055 showed that *Fopius arisanus* will have a higher index (GI) of up to 4 generations in lowlands <1100m a.s.l (Table 5.9).

Table 5.9: Current and future predicted mean generation index (GI) of egg parasitoid (*Fopius arisanus*) in different altitudinal zones at Taita Hills

	Fopius arisanus	
Taita Hills transect	**GI_2014**	**GI_2055**
Lowland (1000m - 1200m a.s.l)	3.122	3.785
Sub-montane (1200m - 1500m a.s.l)	2.933	3.159
Montane (1500m - 1800m a.s.l)	2.839	2.988

5.3.3.2.3 Mapping generation index of the Asian invasive fruit fly (*Bactrocera invadens*) in Mount Kilimanjaro, Tanzania

In Mount Kilimanjaro transect, predicted mean number of generations (generation index; GI) that can be produced by the Asian invasive fruit fly (*Bactrocera invadens*) in the current year (year 2014) ranged between 8 - 9 in highlands (1300 - 1800m a.s.l; Josa - Mgange) and 10 - 12 in lowlands below 1100m a.s.l or Kipusi (Figure 5.7a; Table 5.10). Potential future prediction for year 2055 showed that Asian invasive fruit fly (*Bactrocera invadens*) will have a slightly higher generation index (GI) ranging from 9 in highlands to 11 in lowlands (Figure 5.7b). The study findings revealed that the whole of Mount Kilimanjaro transect will experience increase in mean number of generations of the Asian invasive fruit fly (*Bactrocera invadens*) by end of 41 years (year 2055; Figure 5.7b; Table 5.10) compared to the current scenario (year 2014; Figure 5.7a). The increment will be by average of one generation at every 100m a.s.l in Mount Kilimanjaro study area at end of 41 years (year 2055; Figure 5.7b)

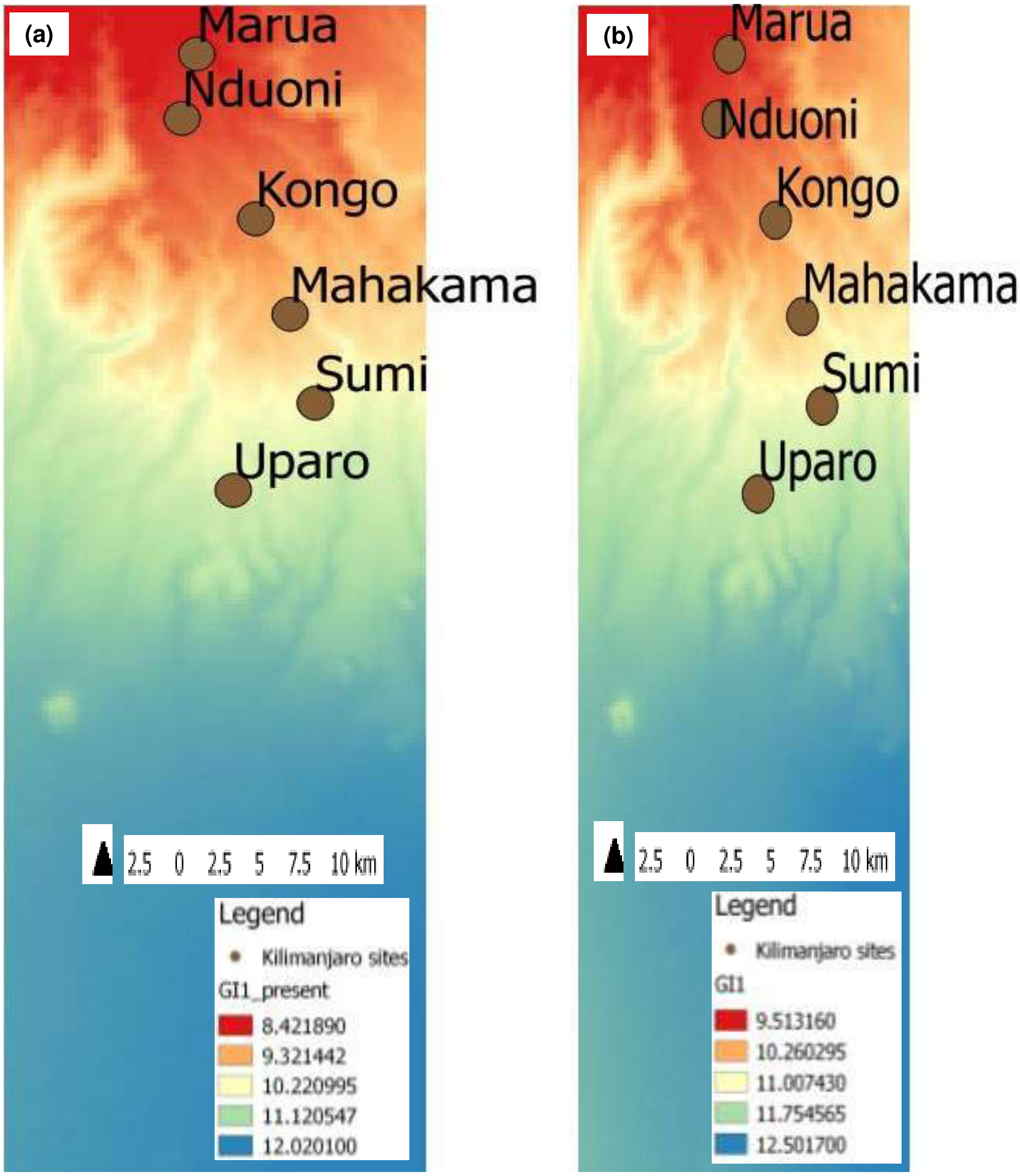


Figure 5.7: (a) Current (year 2014) and (b) future (year 2055) generation index (GI)/ risk map of *Bactrocera invadens* at Mount Kilimanjaro, Tanzania. Focal study points along increasing elevation were: Uparo (1080m a.s.l), Sumi (1300m a.s.l), Mahakama (1500m a.s.l) and Marua (1780m a.s.l).

Table 5.10: Current and future predicted generation index (GI) of *Bactrocera invadens* in different altitudinal zones at Mount Kilimanjaro

	Bactrocera invadens	
Mount Kilimanjaro transect	**GI_2014**	**GI_2055**
Lowland (1000m - 1200m a.s.l)	11.121	11.755
Sub-montane (1200m - 1500m a.s.l)	9.321	10.260
Montane (1500m - 1800m a.s.l)	8.422	9.513

5.3.3.2.4 Mapping generation index of the egg parasitoid; *Fopius arisanus*, of Asian invasive fruit fly in Mount Kilimanjaro, Tanzania

In Mount Kilimanjaro study area, predicted mean number of generations (generation index; GI) in the current scenario (year 2014) produced by *Fopius arisanus* ranged between 2 in highlands (1300 - 1800m a.s.l) to 3 in lowlands below 1000m a.s.l (Table 5.11). Potential future prediction for year 2055 showed that *Fopius arisanus* will witness stagnation of generation index (GI) in lowlands <1100m a.s.l (Table 5.11) and an increase in mean number of 3 generations in midlands (1300 - 1500m a.s.l; Table 5.11).

Table 5.11: Current and future predicted generation index (GI) of egg parasitoid (*Fopius arisanus* in different altitudinal zones at Mount Kilimanjaro

	Fopius arisanus	
Mount Kilimanjaro transect	**GI_2014**	**GI_2055**
Lowland (1000m - 1200m a.s.l)	3.411	3.934
Sub-montane (1200m - 1500m a.s.l)	2.243	3.483
Montane (1500m - 1800m a.s.l)	1.990	2.290

5.4 Discussion

Predictions revealed that *Fopius arisanus* (Hymenoptera: Braconidae) has almost similar geographical distribution range as its host *Bactrocera invadens* (Diptera: Tephritidae) along slopes of Mount Kilimanjaro and Taita hills but the generation and establishment indices of the egg parasitoid (*F. arisanus*) were very low. High geo-spatial indices means that the Asian invasive fruit fly (*Bactrocera invadens*) was more established in Mount Kilimanjaro and Taita hills than its egg parasitoid. The results of this study further shows that the Asian invasive fruit fly was more established in the lowland zones of Mount Kilimanjaro (Uparo area; <1200m a.s.l) and Taita hills (Kipusi area; <1180m a.s.l). The range of habitat preferences for *Fopius arisanus* was also the lowland zones (<1200m a.s.l) in the two study areas but the parasitoid had a lower generation and establishment indices than *Bactrocera invadens*. This is an expected findings for koinobiont endoparasitoids such as *Fopius arisanus* (Hymenoptera: Braconidae) because their development depends on survivability of their host (*Bactrocera invadens*). The population of the egg parasitoid (*Fopius arisanus*) is usually low as a result of dependence on hosts. Furthermore, *Fopius arisanus* is a non-invasive exotic insect species that is still adapting to regions of East Africa since its introduction in late 2000s. This study has, therefore, revealed that global warming could lead to expansion of invasive species such as the Asian invasive fruit fly (*Bactrocera invadens*) into new ecological niches. The upland expansion scenario is also reported by Kroschel *et al*., (2013).

The results of this study has confirmed that an increase of at least 1°C can alter distribution and abundance of ectotherm insects such as *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*) in both Mount Kilimanjaro and Taita hills study areas. The projected mean increase of temperature by 1.45°C in present findings concurs with publications by IPCC (2001b). Future projected increase in temperature (by years 2055) will, therefore, potentially affect insect survival, development, population size and geographical distribution of *Bactrocera invadens* and its egg parasitoid (*Fopius arisanus*). Overall increase in generation index (GI) from low level in year 2014 to higher projected values in 2055 confirms that an uphill shift in distribution of *Bactrocera invadens* and *Fopius arisanus* is influenced by rising temperatures in highlands. The midland zones (1300 – 1600m a.s.l) of the two study areas witnessed highest change margin in generation index (GI) and establishment index from year 2014 to 2055. Present findings predicted that the Asian invasive fruit fly (*Bactrocera invadens*) and its egg parasitoid (*Fopius* *arisanus*) will respond to temperature-driven climate change by range expansion towards higher elevation.

The margin of increase from year 2014 to 2055 was high along Mount Kilimanjaro transect compared to Taita Hills study area. The current data, therefore, suggests that the upper elevated zone of Mount Kilimanjaro transect is warming faster than that of Taita Hills probably due to enhanced human activities. The study area along the Mount Kilimanjaro is a homogeneous farmland with avocado forming major crop in the agro-ecosystem. Habitat homogeneity one of the main reason contributing to high temperatures at Mount Kilimanjaro study area was compared to Taita Hills transect whose ecosystem heterogeneity with relics of native forest intertwined in between avocado orchards reduced rate of regional warming. However, the range expansion of the Asian invasive fruit fly (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*) towards higher altitudes is expected to be higher in Taita hills than Mount Kilimanjaro. The envisaged shrink or depletion of relics of indigenous forests will enhance intense global warming in Taita Hills.

Understanding species distribution scenarios and knowledge of the temperature-dependent population growth potential is crucial for evaluating insect population dynamics and design advance pest control strategies for different avocado agro-ecological zones. The novel spatial information generated on the key avocado insect pest species (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*) along altitudinal gradients of Taita Hills and Mount Kilimanjaro can be utilized by concerned government agencies to develop efficient policies aimed at controlling *Bactrocera invadens* in avocado farming areas. Female *Fopius arisanus* specializes in ovipositing its eggs into the eggs of *Bactrocera invadens* found on ripening fruits (Ekesi, *et al*., 2009; Mwatawala *et al*., 2009; Rwomushana *et al*., 2008; Mohamed *et al*., 2008). *Fopius arisanus* can, therefore, contribute significantly to classical biological control of *Bactrocera invadens* in the Mount Kilimanjaro region but in combination with other environmental friendly methods.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

Reviewed literature on *Persea americana* Miller in conjunction with findings of this study confirmed that avocado is an important horticultural crop in Mount Kilimanjaro region. However, avocado plant is threatened by several insect pests hence the need to understand how climate change impacts on its farming. The survey of orchards revealed that major avocado insect pests of economic importance in Taita Hills, Kenya and Mount Kilimanjaro, Tanzania are the Asian invasive fruit fly (*Bactrocera invadens*), false codling moth (*Thaumatotibia leucotreta*), greenhouse thrips (*Heliothrips haemorrhoidalis*) and common blossom thrips (*Frankliniella schultzei*). Sugar ants (*Pheidole megacephala*) were common where scale insects were abundant whereas the spiralling whitefly (*Aleyrodicus dispersus*) was recorded only in warmer lower regions in Taita Hills and Mount Kilimanjaro feeding on sap of avocado plants.

Most of the arthropods were lowly populated non-pests which constituted majority of species that were recorded inhabiting avocado plants however few insects were advantageous. Beneficial insects included ladybird beetles (*Cheilomenes sulphurea* and *Cheilomenes lunata*) which are predator of aphids. Aphids were spotted on vegetables which are inter-cropped in avocado orchards at both Taita Hills and Mount Kilimanjaro. Predatory ant-mimicking thrips (*Franklinothrips megalops*) that feeds on other thrips including the sampled pest species was also recorded. Other beneficial arthropods sampled from avocado plants were weaver ant (*Oecophylla longinoda*) which is a predator of different insect larvae in warmer coastal regions of Africa and honey bees (*Apis mellifera*) were found pollinating avocado flowers. *Componotus* *maculatus* (Hymenoptera; Formicidae) is also a possible pollinator of avocado flowers since it feeds on sugary secretions from nectar. Similar findings on beneficial insects and also pests are also reported by Moritz *et al* (2013), Woin *et al* (2006), Moritz *et al* (2001), Palmer *et al* (1992) and Palmer (1990).

Field-based fruit observation for infestation incidence by key pests revealed that the invasive fruit fly (*Bactrocera invadens*) caused the highest damage levels to avocado fruits. *Bactrocera invadens* preferred infesting ripened fruits than immature ones and hence the insect was confirmed to be a post-harvest pest of avocadoes in altitudinal zones below 1200m a.s.l. Furthermore, temperature explained best the change in mean population density of *Bactrocera invadens* recorded along the study transects of Taita Hills and Mount Kilimanjaro. These findings on *Bactrocera invadens* concurs with published reports by Ekesi *et al* (2006) and Copeland *et al* (2002).

Chapter four described findings on how temperature influences phenological development of the key pest of avocado fruits (the Asian invasive fruit fly; *Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*). The generated phenology models clarified that both *Bactrocera invadens* and its egg parasitoid survive well in warmer temperatures between 25 and 30ºC but the two insects could not complete their development at extreme low or high temperatures. The study further revealed that infestation of *Bactrocera invadens* eggs by its specific parasitoid *Fopius arisanus* was high in temperatures between 25 and 30ºC. Findings of this study, therefore, suggest that *Bactrocera invadens* and its egg parasitoid, *Fopius arisanus*, can thrive well in warmer lower elevated areas. The results concur with published reports by Mohamed *et* *al* (2008), Ekesi *et al* (2007) and Ekesi & Billah (2006). The phenological models generated in this study describing oviposition, longevity, stage-specific development rates and mortalities can assist scientists to gain in-depth knowledge of life cycle of a pest species and hence its control. Phenological models complied for stochastic simulation resulted in life table parameters that were used to simulate current (year 2014) and future (year 2055) distribution maps of the Asian invasive fruit fly (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*) at Taita Hills in Kenya and Mount Kilimanjaro in Tanzania. Climate-driven distribution maps were generated using a GIS environment implemented in the Insect Life Cycle Modeling software (ILCYM) and R statistical programme (Sporleder *et al*., 2009; Sporleder, *et al*., 2007). Spatially referenced pest indices displaying risk establishment and number of generations per year were computed using the shape files of the two study transects; Taita Hills and Mount Kilimanjaro. The study findings simulated that *Bactrocera invadens* is established in warmer lowland regions below 1200m a.s.l but with continued global warming the insect species can expand its distribution range into higher altitudinal zones above 1700m a.s.l by the year 2055. The study findings infer that *Bactrocera invadens* will remain a serious pest of avocado fruits in the two study areas. Spatial results that the egg parasitoid (*Fopius arisanus*) can be well established in lowland regions of Taita Hills and Mount Kilimanjaro is important findings in bio-control of the Asian invasive fruit fly (*Bactrocera invadens*) in East Africa. This is because efficiency of bio-control strategies is built on extensive knowledge on how weather and climate influences biology and ecology of a pest and its specific parasitoid (Rwomushana *et al*., 2008; Griffin, 2000; Lux, 1999).

6.2 Conclusion

High species richness of arthropod species recorded on avocado crop can be explained by dominance of *Persea americana* trees in the agro-ecosystems that were initially native forests. Taita Hills had a high number of arthropod species than Mount Kilimanjaro because avocado orchards in the former transect is surrounded by numerous relics of indigenous vegetation which provides a wide range of hosts for the invertebrates. However, Mount Kilimanjaro transect which is a homogeneous farmland had a high population of key avocado insect pests than Taita Hills. The three key avocado insect pests were established by observation of mature avocado fruits at both transects during harvesting seasons which revealed that percentage damage levels was highest due to attacks by Asian invasive fruit fly (*Bactrocera invadens*) followed by false codling moth (*Thaumatotibia leucotreta*) and the least was by greenhouse thrips (*Heliothrips haemorrhoidalis*). Geographical distribution patterns of major avocado insect pests along altitudinal gradient of the two study areas were influenced by environmental characteristics of each agro-ecological zone. These findings can assist policy makers in designing best IPM strategies aimed at controling the key avocado insect pests and hence improve livelihood of farmers in Taita Hills and Mount Kilimanjaro. This may be achieved through bio-control methods including release of parasitoids or natural enemies of pests in affected agro-ecological zones. Control measures for *Bactrocera invadens* should be focused in lowland and midlands of the two study areas whereas false codling moth should be dealt with in all zones.

Phenology models revealed that an increase in temperature resulted in higher growth rates and shorter developmental times of the Asian invasive fruit fly (*Bactrocera invadens*) and its egg parasitoid (*Fopius arisanus*). These findings, therefore, suggest that *Fopius arisanus* can be utilized as one of the classical bio-control remedies aimed at reducing infestation of avocado fruits by the Asian invasive fruit fly (*Bactrocera invadens*) in warmer areas. Species distribution maps generated based on the establishment and generation indices can be used in designing effective bio-control strategies of *Bactrocera invadens* and further pin-pointing exact location in Taita Hills and Mount Kilimanjaro for release of the egg parasitoid (*Fopius arisanus*) of Asian invasive fruit fly. This is because their preferred current and future habitat conditions are almost similar.

Greenhouse thrips (*Heliothrips haemorrhoidalis*) and common blossom thrips (*Frankliniella schultzei*) were the most abundant thrips at the two study areas of Taita Hills in Kenya and Mount Kilimanjaro in Tanzania. Temporal or monthly variation of population density of thrips was more prominent compared to spatial changes. Peak population density of thrips coincided with main avocado plant flowering season and during formation of fruit-lets due to presence of adequate food resources. Montane and sub-montane zones with elevation above 1200m a.s.l. had a high mean population density compared to lowland areas which was explained by availability of favourable environment for survival of host avocado plants and thrip species. Thrips thrives well in zones with mild temperatures which were available in sub-montane region (1200m – 1600m a.s.l) along the study transects. The findings of this study can be used by entomologists to identify other Thysanopteran pest species of economic importance at Taita Hills and Mount Kilimanjaro.

6.3 Recommendations

- Comprehensive research is recommended to advance current knowledge on how global warming may influence future distribution of other key avocado insect pests.
- This study recommends that sufficient control of *Bactrocera invadens* in avocado farmlands

should be solved by use other control approaches such as bio-pesticides and orchard sanitation in addition to the parasitoids.

- In order to provide superior data for climate modeling, long-term research on biology, ecology and systematic of the Asian invasive fruit fly (*Bactrocera invadens*) and indigenous Tephritidae fruit flies in other avocado growing areas in East Africa is highly recommended.
- This study calls for a research on effectiveness of using natural enemies such as *Trichogrammatoidea cryptophlebiae* as an alternate bio-control method of *T. leucotreta* in East African fruit orchards.
- Control measures for thrip pests should commence months before the main population build-up in August (at onset of avocado flowering) in all altitudinal zones of both study sites.
- Additional research is required to develop a universal protocol for indexing infestation levels by major pests of avocado in East Africa.
- Research is also required to establish if weaver ants (*Oecophylla longinoda*) could also be used as another bio-control of avocado insect pests especially in warmer lowlands.
- Research on pest status and beneficial of ants (Hymenoptera; Formicidae) in relation to avocado crop and associated key insect pests is highly recommended.
- There is urgent need to establish local farmers-led stakeholder awareness committees to disseminate sustainable management strategies for controlling avocado insect pests in Kenya and Tanzania.
- Finally, further research should also be conducted to assess socio-economic status of avocado crop to the local farmers at Taita Hills in Kenya and Mount Kilimanjaro in Tanzania.

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APPENDICES

Apendix 1a: Asian invasive Fruit fly (*Bactrocera invadens*)

(a) *Thaumatotibia leucotreta*, female

(b) *Thaumatotibia leucotreta*, male

Apendix 1b: False codling moth (*Thaumatotibia leucotreta*)

Apendix 1c: Greenhouse thrips (*Heliothrips haemorrhoidalis*)

Apendix 1d: Common blossom thrips (*Frankliniella schultzei*)

Appendix 1a, b, c & d: Images of key avocado insect pests collected from avocado plants along the study transects of Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. 1a. *Bactrocera invadens*, 1b. false codling moth, 1c. Greenhouse thrips (*Heliothrips haemorrhoidalis*) and 1d. Common blossom thrips (*Frankliniella schultzei*).

Appendix 2a: False codling moths (*Thaumatotibia leucotreta*) damage on avocado fruits

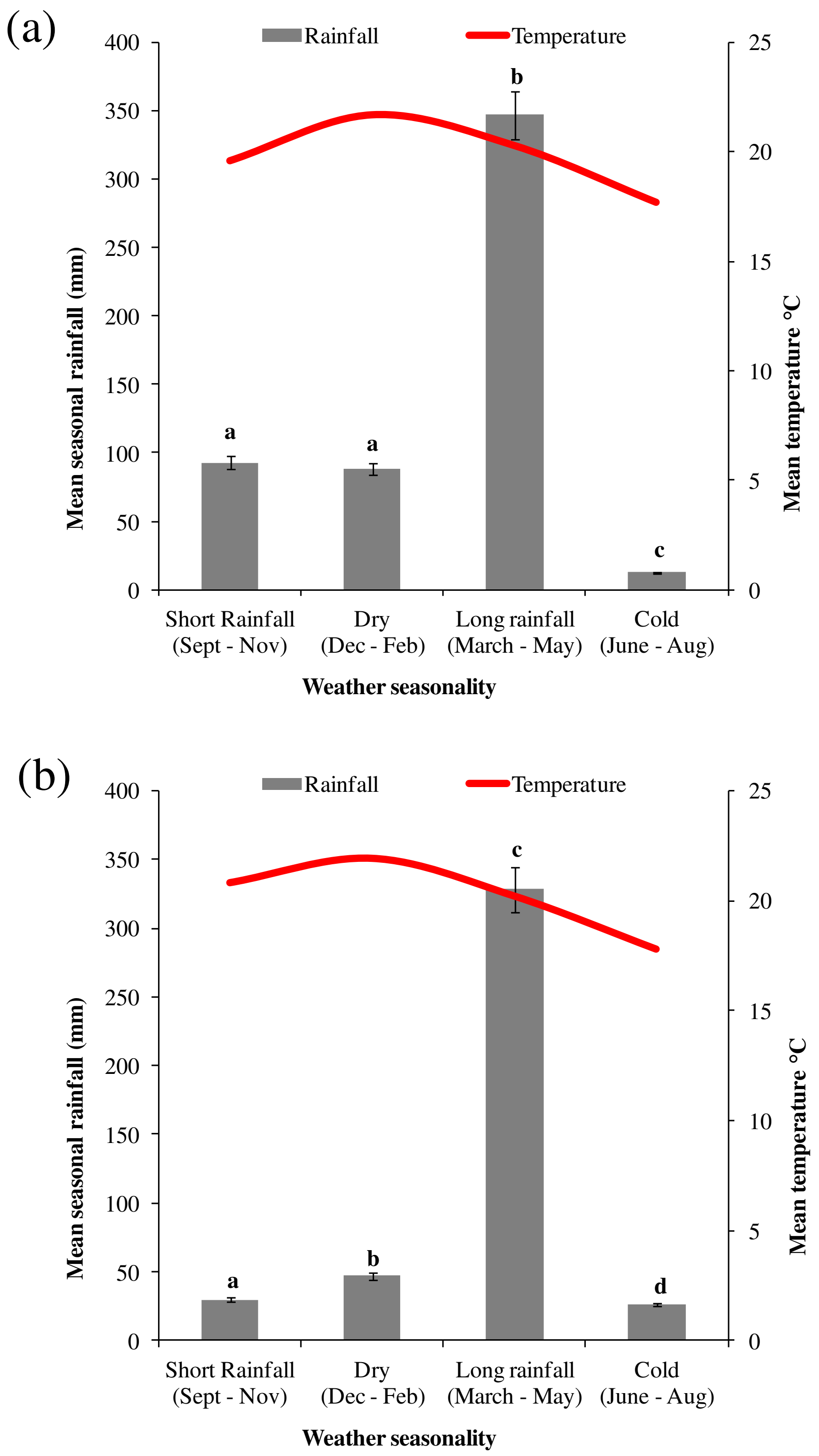
Appendix 2b: Infestation of avocado fruits by female Asian invasive Fruit flies (*Bactrocera invadens*). Both females and males of the Asian invasive Fruit fly (*Bactrocera invadens*) also feed on ripe avocado

Appendix 2c: Avocado leaves with pale stippled, bleaching or black excrement (above) and fruits with brown scars due to infestation by the greenhouse thrips (*Heliothrips haemorrhoidalis*)

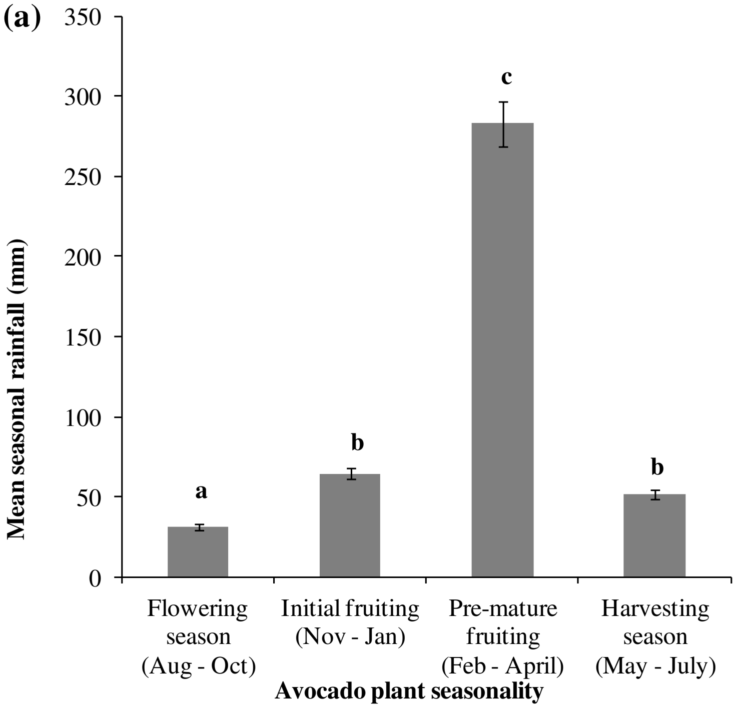
Appendix 2a, b & c: Infestation of avocado fruits by the key insect pests along study transects of Taita Hills, Kenya and Mount Kilimanjaro, Tanzania. 2a. False codling moth, 2b. *Bactrocera invadens* (inset) and 2c. Greenhouse thrips (*Heliothrips haemorrhoidalis*).

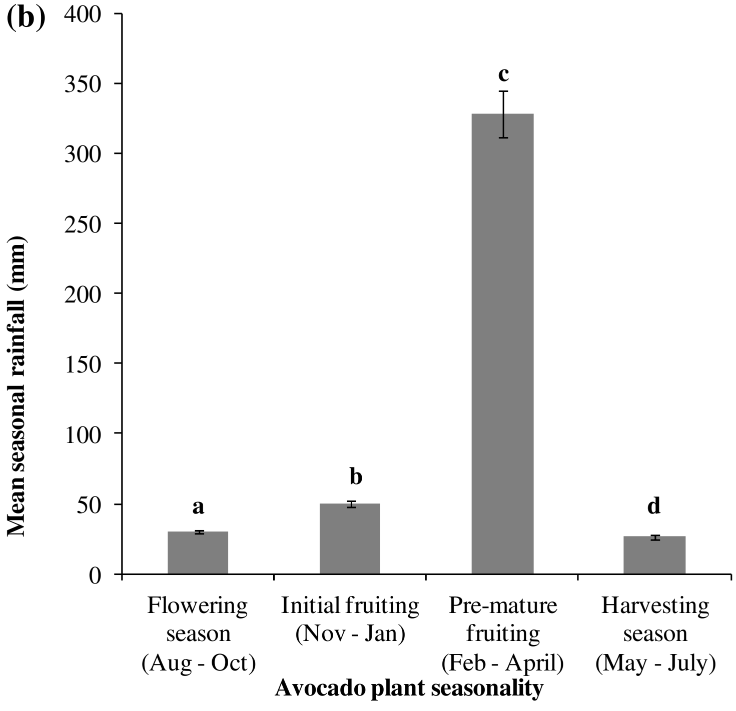
Appendix 3: Images of some of collected avocado fruits which were observed for emergency of the Asian invasive fruit fly (*Bactrocera invadens*). The avocado fruit infestation observation experiments were carried out in temporary laboratories along the study areas in warmer lowland plains (700m a.s.l) at Mwatate (Taita Hills, Kenya) and Uparo (Mount Kilimanjaro, Tanzania), during avocado harvesting months between May and July.

Appendix 4: Observation of the life-table (phenology) experiments that was carried out in an incubator with constant temperature at the African Fruit Fly Laboratories within International Center of Insect Physiology and Ecology (ICIPE), Nairobi.



Appendix 5a & b: Mean rainfall and temperature trend during different weather seasons at (a) Taita Hills and (b) Mount Kilimanjaro. Different letters denote significant difference whereas same letters denote lack of significance.





Appendix 6a & b: Mean rainfall during different avocado plant phenology at (a) Taita Hills and (b) Mount Kilimanjaro. Different letters denote significant difference whereas same letters denote lack of significance.