# ESTABLISHMENT AND EFFICACY OF STEM BORER BIOLOGICAL CONTROL AGENTS RELEASED IN KENYA

**BY**

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## DECLARATION

I, **Esther Adhiambo Abonyo** (I80/92846/2013) declare that this thesis is my original work and to the best of my knowledge, has not been presented for the award of a degree in any other College or University.

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## DEDICATION

I dedicate this work to my sons and daughter. Fidel Jones, Tyrone Hans and Alexa Angel, my beautiful reasons for living.

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## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| ABC | Augmentative Biological Control |
| ANOVA | Analysis of Variance |
| BIOCLIM | Bioclimatic Prediction and Modelling System |
| CBC | Classical Biological Control |
| CIBC | Commonwealth Institute of Biological Control |
| CIMMYT | International Maize and Wheat Improvement Centre |
| FAO | Food and Agriculture Organization of the United Nations |
| Ft | Feet (unit of numerical measurement) |
| HSD | Honestly Significant Difference |
| *icipe* | International Centre of Insect Physiology and Ecology |
| IITA | International Institute of Tropical Agriculture |
| IPM | Integrated Pest Management |
| PCPB | Pest Control and Produce Board |
| KALRO | Kenya Agricultural and Livestock Research Organization |
| NSBB | Noctuid Stem Borer Biodiversity Project |
| SNK | Student-Newman-Keuls multiple range test |
| SSA | Sub Saharan Africa |

## ABSTRACT

Agriculture dominates the economy of most African countries with most farmers in Sub-Saharan Africa growing mainly maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* Moench.). However, production of these crops is greatly constrained by lepidopteran stem borers. In Kenya, the four important stem borer pests are *Chilo partellus* Swinhoe, *Chilo orichalcociliellus* Strand, *Busseola fusca* Fuller and *Sesamia calamistis* Hampson. Due to losses associated with these pests, the International Centre of Insect Physiology and Ecology's Biological Control programme spearheaded release of egg parasitoid *Telenomus isis* Polaszek in Eldoret/Kitale and Wundanyi in 2005. The larval parasitoid *Cotesia flavipes* Cameron was released in North and South Coast in 1993, Eastern region and Kisumu in 2002. The larval parasitoid *Cotesia sesamiae* Cameron was redistributed from Kitale to Taita Hills in 2006 and the pupal parasitoid *Xanthopimpla stemmator* Thunberg was released in Eastern region of Kenya in 2002. However, there is limited information on the establishment status, spread and impact of these agents. Due to this information gap, this study was carried out from March 2014 to September 2016 to determine the establishment status of the above biological control agents and assess their spread and impact on target stem borer pests. Surveys were carried out on maize and sorghum farms at sites where the biological control agents were previously released. In each field, 100 maize or sorghum plants were inspected for stem borer infestation. Ten infested plants were destructively sampled and recovered immature stem borers collected and taken to the laboratory for rearing. Percentage infestation and parasitism computed from this data was then compared to figures recorded before the parasitoids were released in respective localities. Results revealed that *T. isis* established in Eldoret/Kitale and Wundanyi with mean egg parasitism varying significantly between the two localities ($W$=57, $p$=6.8e$^{-10}$). In comparison to pre-release figures, egg parasitism in Eldoret showed a significant reduction ($V$=326, $p$=0.00072) while in Wundanyi, a significant increase was recorded ($V$=211, $p$=0.00035). Surveys in moist lowland, dry and moist mid altitude agro-ecological zones (AEZ) where *C. flavipes* had been released indicated that infestation was 28.9±3.1, 22.5±7.42 and 2.71±0.4% respectively, figures which were significantly lower in comparison to infestation levels documented before parasitoid release ($V$=3, $p$=4.26e$^{-10}$; $t$=41.63, $df$=54, $p$=2.2e$^{-16}$ and $V$=0, $p$=2.24e$^{-08}$ respectively). Current parasitism levels were 36.1±3.0, 25.3±3.3 and 5.5±2.5% in the moist lowland, dry and moist mid altitude AEZ respectively and were higher compared to parasitoid pre-release figures ($V$=10.96, $p$=5.22e$^{-15}$; $V$=1213, $p$=0.0002 and $V$=1950, $p$=0.0009 respectively). The larval parasitoid, *Cotesia flavipes* is steadily suppressing stem borer population in areas in which it was released. Following the highland *Cotesia sesamiae* redistribution to Taita hills, overall stem borer infestation was computed at 19.8±2.5% and this varied significantly among the three stem borer species, *B. fusca, C. partellus* and *S. calamistis* ($\chi^2$=16.86, $df$=2, $p$=0.00022). The highland *C. sesamiae* was not recovered from Taita hills and thus parasitism was generally low (10.8±4.3%) and did not show significant variation in comparison to pre-release records ($V$=3, $p$>0.05). *Xanthopimpla stemmator* was not recovered from the Eastern region of Kenya during this survey. This suggests a failure to establish though there is need to sample alternative and wild hosts before this is declared. An insecticide exclusion method was also used to evaluate the effect of parasitoids on maize yield in moist mid altitude and lowland AEZ. In both experiments, infestation varied among treatments ($F_{2,9}$=5.835; $p$<0.05 and $F_{2,87(0.05)}$=6.92; $p$<0.05 respectively). Mean weight of harvested maize (yield) did not vary among treatments indicating

that though there was a decrease in infestation coupled with increase in parasitism this did not translate into increased yield. It is recommended that for maize and sorghum yields to be increased, there is need to introduce *C. flavipes* egg parasitoids which can manage the pest before it reaches the most destructive larval stage.

# CHAPTER ONE

## 1.0 GENERAL INTRODUCTION

### 1.1 Background

Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* Moench.) are important cereal crops in in Sub-Saharan Africa (SSA) (Seshu Reddy, 1988; FAO, 1998; Kfir, 1998). Grown mainly by small scale farmers, maize and sorghum production rarely meets local demands of the ever growing human population. Production of these crops is constrained by several factors varying from field pest infestations, poor soil fertility and unreliable climate (Seshu Reddy, 1989; Brownbridge, 1991; Odindo, 1991; Saxena *et al*., 1991; Brownbridge and Onyango, 1992). Important among these constraints are field insect pests among which lepidopteran stem borers are most injurious (Brownbridge, 1991; Odindo, 1991; Overholt, 1998). Stem borer infestations account for yield losses ranging from 10 to 100% depending on composition of the pest community and infestation dynamics (Nye, 1960; Youdeowi, 1989; Seshu Reddy and Walker, 1990; Overholt *et al.*, 1997; Hassan *et al*. 1998; De Groote *et al.* 2003; Ong'amo *et al*., 2006a).

In Eastern and Southern Africa, important stem borer pests are the crambids; *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* Strand, the pyralid *Eldana saccharina* Walker, and the noctuids, *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Seshu Reddy, 1989; Polaszek and Khan, 1998; Overholt *et al.*, 2001). The afore-mentioned stem borer pests are indigenous to African continent except for *C. partellus* which was accidentally introduced in 1930's from Asia (Tams, 1932). In Kenya, the main pests include *B. fusca, S. calamistis, C. partellus* and *C. orichalcociliellus* (Seshu Reddy, 1998; Songa *et al*., 2001). Due to losses associated with stem borer pest infestations, management of these pests has been considered an important step towards increasing maize and sorghum production (Saxena, 1986). Biological control is one of the management strategies that has been initiated to reduce stem

borer pest populations. International Centre of Insect Physiology and Ecology's (*icipe*) Biological Control (BC) programme initiated the release of the egg parasitoid *Telenomus isis* Polaszek (Yaovi *et al.*, 2009b), larval parasitoids *Cotesia flavipes* Cameron (Overholt *et al.*, 1994a; Omwega *et al.* 1995) and *Cotesia sesamiae* Cameron and the pupal parasitoid *Xanthopimpla stemmator* Thunberg for the management of stem borer pests.

One of the criticisms of biological control is its lack of predictability in terms of agent establishment and success (Goolsby *et al.*, 2005). In the Kenyan context, there is limited information on establishment, success or failure of aforementioned biological control agents. It is against this background that this study was undertaken to determine the establishment, spread and impact of released biological control agents on target stem borer pest populations.

### 1.2 Problem statement

Cereal production in SSA is greatly constrained by field insect pests, the most injurious being lepidopteran stem borers (Brownbridge, 1991; Odindo, 1991; Overholt, 1998; Overholt et al., 2001). Due to losses associated with stem borer pest infestations, different management strategies have been initiated. However, conventional control strategies have not been without shortcomings. The use of pesticides is costly and less effective when applied after stem borers are already inside the stem (Guang and Oloo, 1990; Seshu Reddy and Sum, 1991; Bosque-pérez, 1995; Bonhof, 2000). Cultural and habitat management strategies are unpredictable ( Nwanze and Mueller 1989; Oloo, 1989). For these reasons, research has been focused on biological control as it provides an environmentally sustainable and cost effective solution to most insect pest problems (Waterhouse, 1998; Goolsby *et al*., 2005). Research has led to the importation and subsequent release of *T. isis, C. flavipes, C. sesamiae* and *X. stemmator* against stem borer pests in Kenya. However, save for *C. flavipes*, the

establishment status of the rest of the parasitoids is unknown. It is also mportant to assess whether these parasitoids have spread from the farms in which they were released into new areas. Further to this, documentation on the impact of these biological control agents on target stem borer population and if this has translated into yield increase is also lacking.

### 1.3 Justification

There is need to document quantitative data on the presence, spread and action of these biological control agents. Such documentation will provide a basis upon which informed decisions regarding the next course of action in management of stem borer pests will be made based on assessment of their effectiveness. Such records are also needed to back-up policies geared towards reducing resistance to biological control in sectors other than agriculture (including environmental and water resource management).

### 1.3 Project description

#### 1.3.1 Scientific question

Have biological control agents released for management of lepidopteran stem borer pests established and spread from their points of release? Have they reduced populations of target pests?

#### 1.3.2 Hypothesis

The biological control agents released for the management of stem borer pests established and have resulted in reduced pest population and cereal crop losses in Kenya.

### 1.3.3 Objectives of the study

#### *1.3.3.1 General objective*

To evaluate the establishment, spread and effectiveness of stem borer pest parasitoids (*T. isis, C. flavipes*, *C. sesamiae* and *X. stemmator*) in Kenya and determine environmental factors influencing their optimal action

#### *1.3.3.2 Specific objectives*

i) To assess the establishment and spread of *Telenomus isis* in areas in which it was released in 2005 (Kitale/Eldoret and Wundanyi)

ii) To assess the establishment, spread and impact of *Cotesia flavipes* in AEZs in which it was released between 1993 and 2002 and determine environmental factors favouring its optimum action

iii) To assess the establishment, spread and impact of highland *Cotesia sesamiae* in Taita Hills where it was released in 2006

iv) To assess the establishment, spread and impact of *Xanthopimpla stemmator* in dry mid-altitude AEZ where it was released in 2002

v) To evaluate the impact of parasitoids on maize yield (through insecticide exclusion method) in moist mid-altitude and moist lowland AEZ

### 1.3.4 Expected output

This study will compare the prevailing stem borer pest levels vis á vis rates that were documented before release of the biological control agents. Establishment status of each parasitoid will also be documented. Parasitism rates attributable to the biological control agents will indicate their effectiveness against target stem borers. Results on success or failure of establishment, spread and impact of biocontrol agents will be used to gauge the

next course of action towards stem borer pest management (need for augmentative or alternative biocontrol agent release) in Kenya.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 The importance of maize and sorghum in Sub-Saharan Africa

The most widely cultivated cereal crops in Kenya are maize (*Zea mays*) and sorghum (*Sorghum bicolor*) (FAO, 1991; CIMMYT, 1992). Maize is exotic to the African continent and was first introduced into East Africa in the 16$^{th}$ century (Seshu Reddy, 1998). Since then, acreage under maize cultivation has increased. This may be attributed to high productivity and availability of many varieties developed for diverse ecological conditions (Schulthess *et al.,* 1997). In Kenya, maize is the main staple food for majority of households with 125kg annual per capita consumption (Pingali, 2001) and contributes to about 12% of the rural households' income (Argwings-Kodeck *et al*., 1998). Maize stalks can be used for mulching, fuel, animal feed and as construction material. Maize is also used as a raw material for many industrial products and medicine (IITA, 2009). Despite its importance, maize grain yields in SSA are among the lowest in the world (FAO, 1998).

Sorghum, a native of African continent, is reported to have originated in the Sudan/ Ethiopia border region (De Wet, 1978). It is a traditional staple crop for millions of people and is widely cultivated due to it's resistance to drought (Haile and Hofsvang, 2001). It is also grown as feed for poultry and livestock in the form of grain, forage and fodder (Seshu Reddy, 1983). In addition, sorghum provides raw material for many industrial products. Sorghum grain yield in Eastern Africa is generally low despite the vast acreage under sorghum cultivation (FAO, 1984).

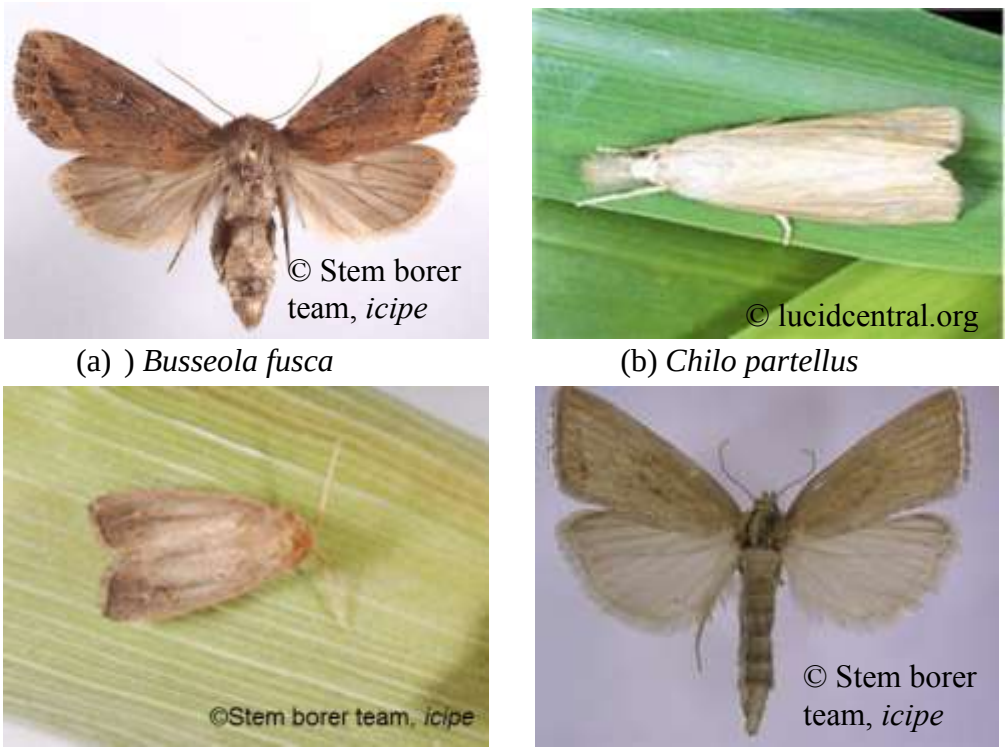
### 2.2 Constraints to maize and sorghum production in Sub-Saharan Africa

Production of maize and sorghum in the region is generally low. Notable factors limiting the production of these cereals in tropical Africa include field insect pests, poor climatic

conditions, low soil nutrients, weeds, soil fertility and plant diseases (Bowden, 1976). Among these constraints, the most important is crop damage caused by insect pests.

### 2.2.1 Important insect pests in maize and sorghum production in Kenya

In SSA, the most important insect pests of maize and sorghum are lepidopteran stem and ear borers belonging to the families Noctuidae, Crambidae and Pyralidae (Ngi-Song and Overholt, 1997a; Polaszek, 1998; Van den Berg *et al*, 1998). Importance of these pests varies greatly with the region, agro-ecological zone and season (Schulthess *et al*., 1997; Ongámo *et al*., 2006a). In East Africa, *B. fusca* and the invasive *C. partellus* are the major pests (Seshu Reddy, 1983; Khan *et al.*, 1997) whereas *S. calamistis* and *C. orichalcociliellus* (Fig. 2.1) are minor pest species (Bonhof *et al*., 1997; Songa *et al*., 2001; Zhou *et al*., 2001). In Kenya, the most dominant and economically important stem borer is the exotic species, *C. partellus* (Overholt *et al*., 1994a) which is dominant at low to mid elevations (Seshu Reddy, 1983).




(a) ) *Busseola fusca* (b) *Chilo partellus*

(c) *Sesamia calamistis* (d) *Chilo orichalcociliellus*

**Figure 2.1**: Economically important stem borers in Kenya

## 2.3 Damage and yield losses attributed to stem borer insect pests

All stem borer species characteristically go through four developmental stages: egg, larva, pupa and adult (Fig. 2.2). Life cycle begins with emergence of adults. Mating follows immediately after emergence by males finding females with the help of pheromones released by female moths (Overholt *et al.*, 2001). Gravid moths oviposit eggs on suitable young leaves that are later attacked by first instar larvae upon hatching (Omwega *et al*., 2006).

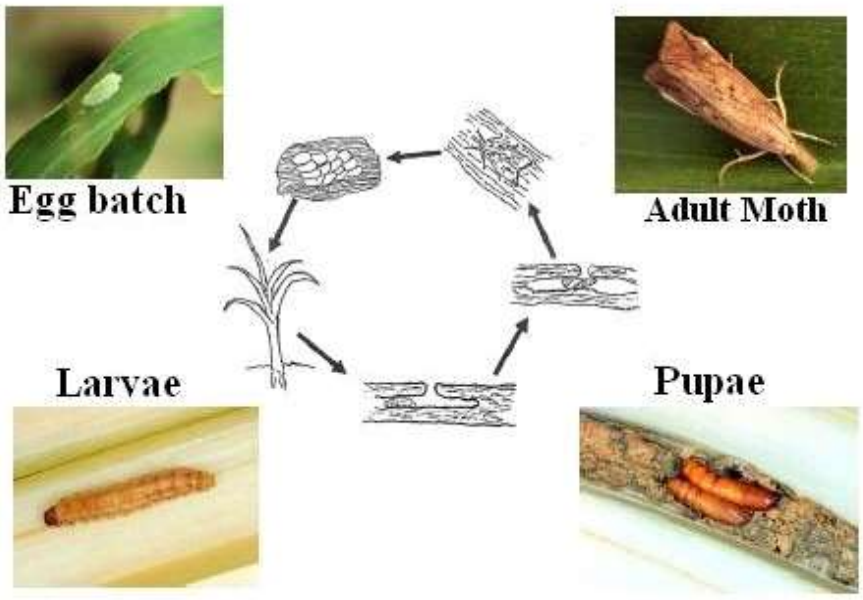



**Figure 2.2:** General stem borer life cycle ©Lucidcentral.org

Plant injury is caused by early instars of stem borers feeding in the plant's whorl and later burrowing into the stem through the growing point or directly through the stalk. Larvae cause foliar damage, dead heart, stem tunnelling, stem lodging and breakage (Fig. 2.3), all of which may contribute to the final loss in yield. Infested plants exhibit poor growth as translocation of nutrients is affected when the stem tissue is damaged. Infestation also results in reduced yield and plants that are more susceptible to lodging and secondary infections (Ngi-Song and Overholt, 1997b). Direct loss may be caused when larvae attack grains on the maize cob or sorghum head (Seshu Reddy, 1983; Ampofo, 1986; Unnithan, 1987).


(a) Window panes


(b) Dead heart


(c) Holes on maize stalk


(d) Frass-filled stem


(e) Shot holes

**Figure 2.3:** Damage symptoms caused by stem borer feeding on maize and sorghum plants

Together, stem borer pests can cause losses of between 18 and 88% on unprotected crop (Walker, 1983; Warui and Kuria, 1983; Ampofo, 1986; Seshu Reddy and Walker, 1990). *Chilo partellus* and *B. fusca* strongly limit yields of maize and sorghum crops from 20-80% depending on region, borer population and crop phenology during infestation (Seshu Reddy, 1983; Khan *et al.*, 1997). In maize, if infestation occurs in young plants, the growing point may be destroyed resulting in dead heart and no yield will be obtained (Bonhof *et al*., 1997). If plants are attacked at a more mature stage, the damage is less devastating with losses

estimated at 20-60% of the potential yield (Warui and Kuria, 1983; Seshu Reddy, 1988; Youdeowi, 1989; Bosque-Perez and Mareck, 1991). Reported yield losses in maize attributed to *C. partellus* in East Africa reach as high as 73-100% (Seshu Reddy and Walker, 1990). Estimated losses in sorghum yield by stem borer pests in Kenya ranged from 18-27% (Walker, 1967). On late planted sorghum, infestations by these insects can cause substantial grain yield losses (Seshu Reddy, 1983).

### 2.4 Control of lepidopteran cereal stem borers

Cereal losses incurred from stem borer infestation have necessitated the use of various pest management strategies. In Kenya, control methods such as chemical, biological, cultural as well as planting of resistant crop varieties have been used in the management of stem borer pests.

#### 2.4.1 Chemical control

Commercial insecticides have been used for stem borer control with varying degrees of success. In Kenya, Thunder® 145 O-TEQ and Bulldock® 262.5 EC are registered for use against stem borers (PCPB, 2008). However, the pressure and need to discontinue the use of synthetic insecticides as a pest management strategy cannot be overemphasized. Numerous drawbacks associated with their use are a major concern. Some of the drawbacks include: increasing development of resistance by target organisms, accumulation in soil, water, animals and agricultural products, disruption of ecological balance and the cost of insecticides (Overholt, 1992). The high cost is of particular importance in third world countries where valuable foreign exchange currency has to be used in the importation of such chemicals (Brownbridge and Onyango, 1992). Moreover, chemical control is ineffective once borers tunnel into the stem's meristematic tissue (Guang and Oloo, 1990; Seshu Reddy,1998). Other concerns regarding use of insecticides are the need for reapplication and effect on non-target organisms (Kumar, 1984).

### 2.4.2 Cultural control

Cultural control methods are the most relevant and economical approaches of stem borer control available to resource-poor farmers in Africa as they disrupt the build-up of pest populations (Khan *et al*., 1997). These techniques include destruction of crop residues, intercropping, crop rotation, manipulation of planting dates, rogueing of infested plants and tillage methods (Odindo, 1991). Many cultural practices are labour intensive but have little adverse effects on the environment. However, understanding stem borers' behaviour and relationships with their respective host crops are important for the development of efficient management strategies (Kfir *et al*., 2002a). Cultural control is severely strained by lack of management capabilities of farmers especially in areas where farming communities lack extension services (Harris, 1989). In addition to this, most African farmers have not adopted cultural control options (Nwanze and Mueller, 1989) since their effectiveness is not guaranteed (Van den Berg *et al.,* 1998).

### 2.4.3 Biological control

Biological control is an environmentally sound and effective means of reducing pests and their associated effects through the use of natural enemies. Classical biological control (CBC) is whereby natural enemies from a pest's native home are introduced to control exotic pests (Van Lenteren *et al.,* 2006). This strategy (CBC) has successfully been used to suppress populations of introduced lepidopteran pests in the tropics (Ngi-Song *et al*., 1996). Augmentative Biological Control (ABC) on the other hand is whereby exotic or native natural enemies are periodically released to manage a pest population (Van Lenteren *et al.,* 2006). A range of naturally occurring biological control agents such as parasitoids, predators and disease-causing pathogens have been reported for different growth stages of stem borers (Bonhof *et al*., 1997; Overholt, *et al*., 1997; Schulthess *et al.,* 1997). For small-scale farmers in developing countries where capital, scientific knowledge and pest management technology

are a major constraint in crop production, biological control is considered to offer the best prospects for crop protection against major pests (Greathead and Waage, 1983).

### 2.5 Biological control initiatives against stem borers in Kenya

In Eastern Africa, the role of natural enemies (parasitoids, predators and pathogens) as a cause of population fluctuations in stem borer pests of maize and sorghum has been investigated by several workers (Ingram, 1958; Schmutterrer, 1969; Mohyuddin and Greathead, 1970, Mathez, 1972 and Seshu Reddy, 1983, 1985). International Centre of Insect Physiology and Ecology's (*icipe*) Biological Control programme initiated the importation and release of *T. isis, C. flavipes, X. stemmator* and the redistribution of *C. sesamiae* within the country.

#### 2.5.1 Release of the egg parasitoid, *Telenomus isis* Polaszek

While *icipe* focused on CBC, the International Institute of Tropical Agriculture (IITA) focused on the 'redistribution'approach which aimed at exchanging natural enemy species and strains between African regions (Schulthess *et al.*, 1997). *Telenomus isis,* an egg parasitoid commonly obtained from noctuid stem borers in West Africa and Cameroon in Central Africa (Ndemah *et al.*, 2001; Schulthess *et al*., 2001; Chabi-Olaye *et al*., 2006b) was imported and released in South East Kenya in 2005 (Yaovi *et al*., 2009b) against *B. fusca*. Generally, egg parasitoids are an important source of mortality because the pest is killed before reaching the destructive stage (Temerak, 1981). In West Africa, egg parasitism by the scelionids *Telenomus busseolae* Gahan and *T. isis* has been reported to reach 95% (Schulthess *et al*., 2001; Ndemah *et al*., 2003).

#### 2.5.2 Release of larval parasitoid, *Cotesia flavipes* Cameron

One of the natural enemies that has been used against stem borer pest, *C. partellus,* is the gregarious, koinobiont (does not kill host immediately after parasitization), larval

endoparasitoid *C. flavipes*. Two biological control attempts have been made in East Africa to increase the natural mortality of *C. partellus*. The first attempt was made by the Commonwealth Institute of Biological Control (CIBC) during which nine parasitoid species were imported from India and released in Kenya, Uganda and Tanzania from 1968 to 1972. Post release surveys showed that none of the parasitoids established (CIBC, 1968-1972). In the second attempt, *Cotesia flavipes*, was released in 1993 in Southern Coastal area of Kenya and post release surveys demonstrated that it established (Overholt *et al*., 1994a; 1997). Surveys have recorded reduced stem borer densities by 50% and increased maize yields by 10% as a result of this establishment (Overholt *et al*., 1997, Zhou and Overholt, 2001; Zhou *et al.,* 2001). Parasitism by *C. flavipes* has been rising steadily and may eventually become an important factor in management of stem borer population in some areas (Bonhof *et al.*, 1997).

### 2.5.3 Release of larval parasitoid, *Cotesia sesamiae* Cameron

A survey of the indigenous natural enemies of stem borers has been carried out in Kenya, and the number of species recovered was reported to be more than 40 (Bonhof *et al*., 1997). Among these is the braconid, *Cotesia sesamiae*, a native, gregarious larval endoparasitoid which attacks stem borer larvae throughout SSA (Mohyuddin and Greathead, 1970; Polaszek and Walker, 1991; Kfir, 1992; Bonhof *et al*., 1997). Two *C. sesamiae* biotypes with variation in developmental success in *B. fusca* exist in Kenya (Gitau, 2006; Mucheru *et al*., 2009). The virulent strain present in the highlands can successfully develop in *B. fusca* while the avirulent strain present at the coast is encapsulated by the pest's immune response (Ngi-Song *et al.,* 1995; 1998; Mochiah *et al*., 2002; Gitau *et al.,* 2006). This difference was exploited in a redistribution programme whereby the highland biotype was released in Taita hills in 2006 to manage *B. fusca* (*icipe*, unpublished data).

### 2.5.4 Release of the pupal parasitoid, *Xanthopimpla stemmator* Thunberg

Indigenous parasitoids of African stem borers have expanded their host ranges to include the exotic *C. partellus* but do not appear to effectively regulate its densities to acceptable levels (Oloo and Ogeda, 1990; Kfir, 1992). Among the most frequently reported indigenous pupal parasitoid in Eastern and Southern Africa is the solitary *Dentichasmias busseolae* (Heinrich: Ichneumonidae) which has formed a new association with *C. partellus*, (Zwart, 1998). However, pupal parasitism of *C. partellus* by indigenous species in Eastern Africa is low and usually less than 3% (Oloo and Ogeda, 1990). To complement the action of the larval parasitoid, an exotic, solitary, idiobiont (kills host immediately after parasitization), pupal parasitoid *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) was imported by *icipe* in 2000 for assessment as a biocontrol agent against *C. partellus* and released in 2002 in the Eastern region (*icipe* and KALRO, unpublished data). *Xanthopimpla stemmator* attacks pupae of *C. partellus* and several other stem borers in Asia (Vinson, 1942; Moutia and Courtois, 1952). The parasitoid had been introduced into several countries in Africa with varying degrees of success (Muli *et al*., 2006). *Xanthopimpla stemmator* was found to be competitively superior to *D. busseolae* suggesting that they could coexist as *X. stemmator* was not able to attack pupae in ears as was *D. busseolae*. Laboratoty studies showed that when *C. partellus* pupae were multiparasitized by *D. busseolae* and *X. stemmator,* either of the two parasitoids emerged, no pupa yielded both (Muli *et al*., 2006). Introduction of *X stemmator* is an attempt to re-establish an old association with *C. partellus* and to establish new associations with the native African borers (Gitau *et al*., 2005).

## 2.6 Importance of dispersal/ spread of biocontrol agents

Typically in CBC, a natural enemy is released at a few locations in a new environment and then relied upon to disperse through the environment and colonize other suitable habitats. Maximum suppression of pest densities is attained once the natural enemy population has

colonized all suitable habitats and population growth has stabilized to fluctuate above and below some equilibrium density (Overholt *et al.,* 1997). The rate of dispersal in a new habitat may be useful in predicting the amount of time before equilibrium is reached and may also help to determine the optimal spatial arrangement for future releases (Sallam *et al*., 2001).

The probability that a biological control agent will find a potential target host depends strongly on the dispersal ability of the agent. Dispersal is influenced by wind direction and host distribution. Good searching and dispersal abilities are considered valuable indicators of successful biological control agents (Sallam *et al*., 2001). Knowledge of dispersal characteristics is also essential for researchers to assess to what extent the agent could disperse into other habitats. The encounter probability between the agent and its host depends on the mechanism of dispersal, its life span, the local climate and habitat conditions in the area of release (Sallam *et al*., 2001).

**2.7 Post release surveys done in Kenya**

In Eastern and Southern Africa, egg parasitism is generally low (Yaovi *et al.*, 2006; Okoth *et al.,* 2006) and does not play an important role in population dynamics of stem borers. Following the release of *T. isis* in South East Kenya in late 2005, post release surveys done in 2006 indicated that egg parasitism was considerably low (Yaovi *et al.*, 2006). Subsequent surveys in the same region showed higher parasitoid diversity but egg parasitism remained low (37.9±1.71%) (Yaovi *et al*., 2009b). Further surveys to assess changes in parasitism have not been carried out.

Determining the actual impact of an introduced biological control agent on a target population is a difficult and often, a long term undertaking (Overholt, 1998). After *C. flavipes* release in 1993, the parasitoid was recovered during the season of release from *C. partellus* and two native stem borers, *C. orichalcociliellus* and *S. calamistis* (Overholt *et al*., 1997). A

single stem borer parasitized by *C. flavipes* was found in 1994 despite intensive sampling. A few recoveries were made in the subsequent years of 1995 and 1996 though parasitism remained low (Overholt *et al.*, 1997). In 1997, the number of recoveries increased dramatically and 6% parasitism was recorded (Zhou *et al.*, 2001). During the next two years an increase in parasitism was observed with average parasitism of about 13% being recorded in 1999 (Zhou *et al.*, 2001). The impact of *C. flavipes* on stem borer populations in Coastal Kenya was also investigated (Zhou *et al*., 2001). A ratio-dependent, host–parasitoid model was used to estimate the stem borer density with and without the parasitoid. A reduction of 1.1-1.6 stem borers per plant, equivalent to a 32-5% decrease in the stem borer density was shown. As there is not yet evidence that *C. flavipes* density has reached equilibrium, it may continue to increase and provide greater suppression of stem borers in future (Kfir *et al*., 2002a; Jiang *et al*., 2006). However, it is uncertain how long it will take before the population density of *C. flavipes* will peak and at what density it will level off (Overholt, 1998).

No post release survey has been carried out since the release of *C. flavipes* in dry mid altitude and moist mid attitude agro-ecological zones in Kenya. Similarly, no surveys have been carried out following the release of *C. sesamiae* and *X. stemmator*.

### 2.8 Effect of environmental factors on establishment of parasitoids

Establishment of biological control agents is determined by abiotic and biotic factors (Sanda and Sunusi, 2014). In CBC, preliminary studies are normally conducted to assess the similarity of climatic conditions between countries of collection and release (Van den Bosch and Messenger, 1973; Van Lenteren *et al*., 2006). Environmental factors operate together in an interactive manner in natural ecosystems (Getu and Overholt, 2004). Among the environmental factors, temperature and moisture are considered the most important in affecting life history processes of insects (Van Lenteren *et al*., 2006). Predictive modelling

studies have shown that with a change in climatic conditions, the stem borer pest incidence may also change (Khadioli *et al.,* 2014; Mwalusepo *et al*., 2015). Given that there is a functional response between stem borers and their parasitoids, it follows that with changes in climatic conditions, parasitism will also be affected. Temperature has a major influence on key processes that determine establishment and subsequent spread of introduced natural enemies (Van Lenteren *et al*., 2006). Studies have shown that the impact and rate of parasitism of *C. flavipes* in East Africa varied from one agro-ecological zone to another (Kfir *et al*., 2002a). The reasons for this is largely unknown but temperature is mentioned as one of the major factors responsible for variability in performance of the parasitoid (Jiang *et al*., 2004). Aspects of interest in this study (biocontrol agent establishment, spread and effectiveness) are dependent on prevailing environmental conditions which may either reinforce their success or failure.

# CHAPTER THREE

## 3.0 GENERAL MATERIALS AND METHODS

### 3.1 Study area

The biological control agents, *T. isis, C. flavipes, C. sesamiae* and *X. stemmator* were released for management of stem borer pests in Kenya between 1993 and 2006. This study was undertaken at bench-mark sites at which each of these parasitoids were released. *Telenomus isis* was released against *B. fusca* in South Eastern (Wundanyi) and Rift Valley (Kitale/Eldoret) Kenya in 2005 (Yaovi *et al*., 2009b). *Cotesia flavipes* releases against *C. partellus* were done at the Kenyan coast (Kilifi and Kwale) in 1993 (Overholt *et al.,* 1994a) and in the Eastern (Masii, Mulutu, Kitui) and Western regions (Kisumu) of Kenya in 2002. The virulent strain of *C. sesamiae* was redistributed from Kenyan highlands (Kitale) to the coastal Taita Hills against *B. fusca* in 2006. *Xanthopimpla stemmator* was released against *C. partellus* in Eastern region (Machakos and Kitui) of Kenya in 2002. Coordinates of the release sites are provided in table 3.1. These regions were selected for releases because of the annual occurrence of high densities of stem borers and the predominance of the exotic stem borer *C. partellus* (Zhou and Overholt, 2001). Data was collected between March 2014 and September 2016 to include both short and long rain seasons. The study sites were grouped in Agro-Ecological Zones (AEZ) (Fig. 3.1) as they represent regions with distinct production potentials (Hassan *et al.,* 1998).

**Table 3.1:** GPS coordinates of sites at which *Telenomus isis*, *Cotesia flavipes*, *Cotesia sesamiae* and *Xanthopimpla stemmator* were released between 1993 and 2006 by *icipe*

| Locality | Latitude | Longitude |
|---|---|---|
| *Cotesia flavipes* | | |
| Dry- mid-ltitude AEZ | | |
| | S01°26.415′ | E037°28.295′ |
| | S01°21.105′ | E037°57.171′ |
| Moist lowland AEZ | | |
| | S03°36.373′ | E039°50.535′ |
| | S04°33.447′ | E039°07.569′ |
| | S03°48.816′ | E039°36.950′ |
| Moist mid-altitude AEZ | | |
| | S00°10.082′ | E034°53.631′ |
| | S00°10.123′ | E034°55.241′ |
| | S00°09.862′ | E034°55.631′ |
| ***Telenomus isis*** | | |
| Kitale/Eldoret | | |
| | N00°36.628′ | E035°18.437′ |
| | N00°39.903′ | E035°18.208′ |
| | N00°46.336′ | E035°17.722′ |
| | N00°34.274′ | E035°18.639′ |
| | N00°36.298′ | E035°11.949′ |
| | N00°43.166′ | E035°09.545′ |
| | N00°51.251′ | E035°07.507′ |
| | N00°57.101′ | E035°03.890′ |
| | N01°01.719′ | E035°06.205′ |
| | N00°00.995′ | E035°12.379′ |
| | N00°55.588′ | E035°16.322′ |
| | N00°52.297′ | E035°15.001′ |
| | N00°46.792′ | E035°17.714 |
| Wundanyi | | |
| | S03°25.903′ | E038°21.366′ |
| | S03°24.275′ | E038°21.376′ |
| | S03°23.483′ | E038°20.338′ |
| ***Cotesia sesamiae*** | | |
| | S03°25.903′ | E038°21.366′ |
| | S03°24.275′ | E038°21.376′ |
| | S03°23.483′ | E038°20.338′ |
| ***Xanthopimpla stemmator*** | | |
| | S01°26.415′ | E037°28.295′ |
| | S01°21.105′ | E037°57.171′ |

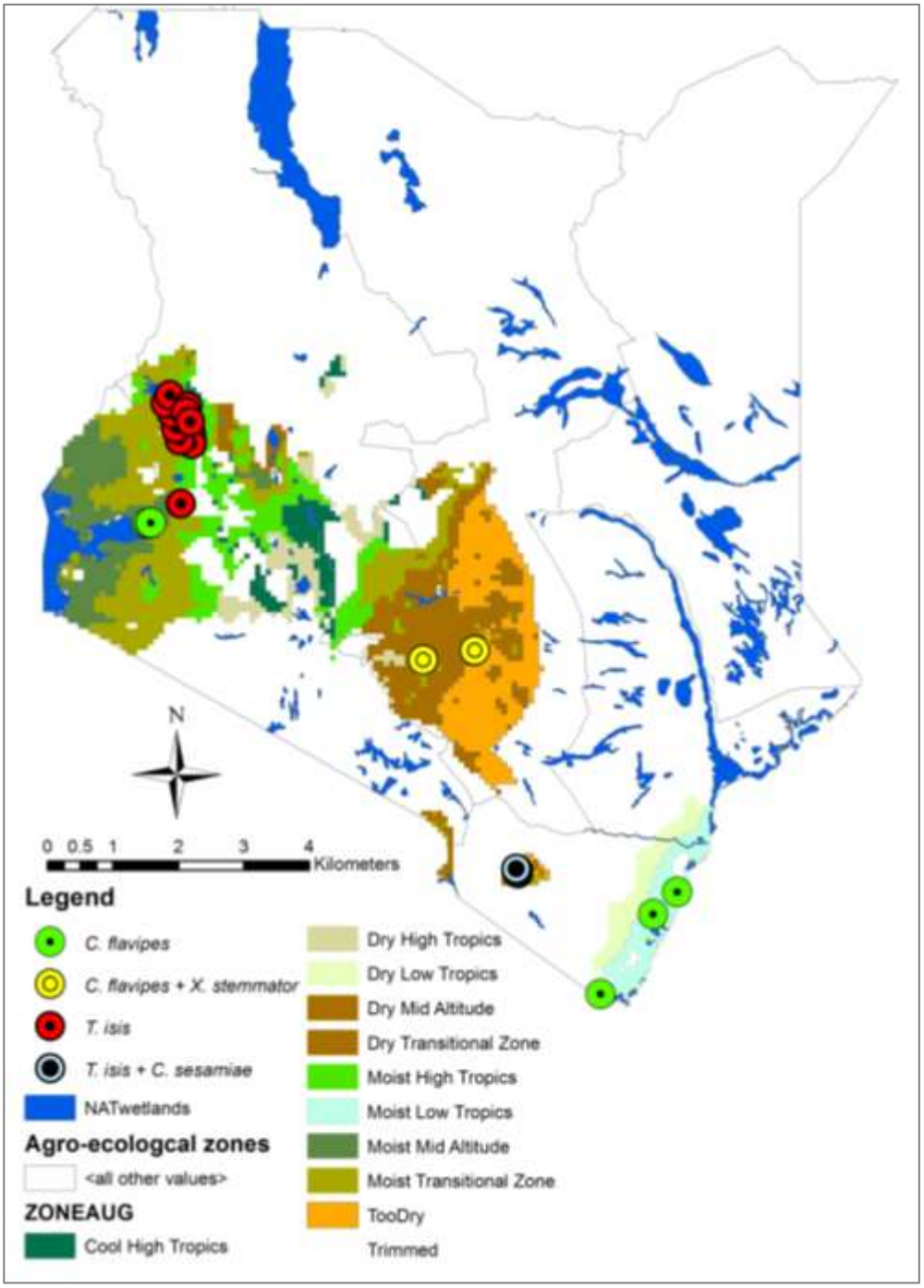



**Figure 3.1:** Map of Kenya showing various biological control release sites and Agro-ecological zones in which the sites are located

### 3.2 Sample size determination

#### 3.2.1 Number of plants per sampling field

In each field, 100 maize and / or sorghum plants were inspected for stem borer infestation as estimated from the equation below described by Zar (1999)

$$n = \frac{Z^2_{\alpha(2)}}{4Dd^2} \qquad \text{Equation 1}$$

Where $Z_{\alpha(2)}$ is the standard normal deviate (1.96), *d* permitted error (0.1) resulting in a uniform number of plants in all farms, *D* is the design effect (1). The number has been rounded to 100 to correct for errors associated with attrition.

$$n = \left\{ \frac{1.96^2}{4 \times 1 \times 0.1^2} = 96.04 \approx 100 \right\} \text{ maize/sorghum plants}$$

Uniform number of plants were inspected in each field. Information regarding infestation and density levels were computed from this data, with fields as replicates.

#### 3.2.2 Number of fields sampled

The number of maize fields surveyed in each locality was determined from the quotient of the total number of plants in each locality as estimated in equation 2 divided by 100 plants which were inspected in each field (Equation 1)

$$n = \left( \frac{Z_{\alpha/2}}{\delta} \right)^2 q/p \qquad \text{Equation 2}$$

Where *n* is the total number of maize plants required in each locality, δ the reliability level expressed as a fixed proportion of the mean (0.05), $Z_{\alpha/2}$ is the standard normal deviate at 95% (1.96). *p* is the proportion of land under maize and sorghum cultivation, while *q* = 1-*p*. For example, the total number of maize plants required in

regions where proportions under maize/sorghum cultivation is unknown will be estimated as:

$$\left\{ n = \left( \frac{1.96}{0.05} \right)^2 0.5/0.5 = 1536.64 = 1540 \right\} \text{ maize /sorghum plants.}$$

### 3.3 Sampling, rearing and identification of stem borers and parasitoids

In each farm, a total of 100 maize plants were inspected for stem borer infestation during which 10 infested maize stems were destructively sampled and dissected (Fig. 3.2a and b). Immature stem borer stages were collected, identified and categorized (as small {1$^{st}$ and 2$^{nd}$ instars}, medium, {3$^{rd}$ and 4$^{th}$ instars} and large {5$^{th}$ and 6$^{th}$ instars}). Identified larvae were placed individually in glass vials containing artificial diet (Onyango and Ochieng-Odero, 1994) (Fig. 3.3a) and transported to the laboratory at *icipe* where they were reared at ambient temperatures of 24-25$^{\circ}$C and a relative humidity of 55-65%, with a 12:12 light: dark photoperiod. Samples were inspected daily for parasitoid cocoons (Fig. 3.3b), pupal development, pupal parasitoid and adult moth emergence. Pupae were transferred into plastic jars lined with wet paper towels (Fig. 3.3c). Humidity in the jars was maintained by moistening the soft paper towels once every 2 days using a few drops of distilled water. Larval parasitoids and adult stem borer moths were identified and recorded.

Additional information collected during sampling included: region, county/district, number of fields sampled, plants sampled, stem borer stage (small, medium or large), plant stage (grain filling, silking, tasseling, harvest), borer location (tassel, stem, whorl, cob) and borer species.

**Figure 3.2:**(a) Inspection of maize plants for stem borer infestation

**Figure 3.2:** (b) Dissection of infested maize plants to recover immature stem borer stages

**Figure 3.3**(a): Stem borer larvae in glass vials containing artificial diet (b) Parasitoid cocoons from stem borer larva (c) Plastic jars lined with moist paper towel where stem borer pupae are reared

### 3.4 Collection of data on environmental factors favouring optimum action of released biological control agents

Climatic data for the sampled localities were extracted from BIOCLIM to deduce prevailing conditions in areas with the lowest pest abundance coupled with high biological control agent action or levels of parasitism. Emphasis was laid on parameters such as temperature, rainfall and humidity.

### 3.5 Statistical analyses

For egg parasitoids, the number of eggs and egg batches were subjected to Wilcoxon rank sum test to test for differences between the localities. Three kinds of parasitization parameters

were computed: (a) Mean egg parasitism (b) Discovery efficiency (c) Parasitism efficiency These constituted proportion data and were analysed using Wilcoxon rank sum test (*W*) to differentiate between localities and one sample Wilcoxon test (*V*) to separate between results obtained before and after parasitoid release.

For the larval and pupal parasitoids, percentage infestation was computed by expressing the number of infested plants as a percentage of the total plants inspected in respective farms. To estimate larval densities in respective farms, the total number of stem borer larvae collected from dissected plants was expressed as a proportion of the number of infested plants. Parasitoid cocoons that were spun from appropriate larval stages were expressed as a proportion of respective field densities in order to compute percentage parasitism. Percentage infestation, larval density and parasitism were subjected to Shapiro-Wilk test for normality upon which abnormal data was appropriately transformed. Normal data was analysed using One-Way ANOVA and significantly different means separated using Tukey's HSD test. Data which failed the normality test was subjected to Kruskal-Wallis rank sum test and significantly different means separated using Nemenyi post-hoc test ($p < 0.05$). One sample *t*-test and Wilcoxon rank sum test were used to compare mean infestation and parasitism levels obtained before and after the release of parasitoids. In order to determine which climatic parameters prevailing in sampled localities had the greatest effect on stem borer species infestation and parasitism, data was extracted from BIOCLIM and subjected to multiple regression analysis using the vegan package in R. To estimate the impact of parasitoids on cereal yield, number of exit holes, tunnelling length, plant height and stem diameter were analysed to test their respective effects on cob weight. Plant height, stem diameter, number of nodes and yield from non-infested and infested plants were compared using Mann-Whitney test. Mean yield between infested and non-infested plants were compared using student's *t*-test while Pearson's correlation test was used to determine the effect of tunnel length, exit

holes and cocoon cases on maize yield. GLM was used to determine the effect of plant height, stem diameter, mean larval density, infestation and parasitism on maize yield. Two-way ANOVA was used to assess the effect of interaction between maize stage and treatment on the maize yield. Generalized Linear Model (GLM) was used to find stages of infestation and parasitism that significantly affected yield. Stem borer parasitism was subjected to Friedman rank sum test to compare the means.

# CHAPTER FOUR

# 4.0 ESTABLISHMENT OF *Telenomus isis* POLASZEK, AN EXOTIC EGG PARASITOID OF NOCTUID STEM BORER PESTS IN KENYA

## 4.1 Introduction

Maize is one of the most important cereal crop in Sub-Saharan Africa (SSA) and an important staple food for more than 1.2 billion people in the region (Seshu Reddy, 1988; FAO, 1998). Introduced into Africa in the 1500s, maize recruited indigenous pests some of which currently constrain its production (Nye, 1960; Polaszek and Khan, 1998; Overholt *et al*., 2001). Important among the recruited indigenous pests are the phytophagous lepidopteran stem borers (Brownbridge, 1991; Odindo, 1991) which generally vary in their distribution among different regions in Africa (Kfir *et al*., 2002a; Ong'amo *et al*., 2006). In Eastern and Southern Africa, important stem borer pests are *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Family: Noctuidae), *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* Strand (Family: Crambidae) (Polaszek and Khan, 1998; Seshu Reddy, 1998; Overholt *et al.*, 2001). Among the aforementioned stem borer pests, *B. fusca* and *C. partellus* constitutes a major proportion of the stem borer pest community in Kenya (Seshu Reddy, 1983; Bonhof *et al.*, 1997; Songa *et al*., 2001; Zhou *et al*., 2001) and thus results in considerable maize yield losses.

Due to losses associated with stem borer pest infestations, different management approaches have been initiated to keep pest populations below economically damaging levels. Focus on the management of *B. fusca* in Kenya led to the importation of an egg parasitoid, *Telenomus isis* Polaszek (Hymenoptera: Scelionidae), from West Africa in 2005(Yaovi *et al*., 2009a). *Telenomus isis* (Hymenoptera: Scelionidae) is a solitary egg parasitoid of noctuid stem borers in West and Central Africa (Setamou and Schulthess, 1995; Moyal, 1998; Ndemah *et al*., 2001; Schulthess *et al.*, 2001; Chabi-Olaye *et al*., 2006a). In West and Central Africa, *T. isis*

co-exists with *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) in the *T. busseolae* complex where together, they suppress populations of *B. fusca* and *S. calamistis* (Setamou and Schulthess, 1995). However, of the species in the *T. busseolae* complex found in West Africa, only *T. busseolae* exists in East Africa. It is on this background that *T. isis* was released in Kenya in late 2005 to synergistically work with *T. busseolae* to suppress populations of the dominant *B. fusca* in midaltitude / highland zones (Schulthess *et al.,* 1997; Yaovi *et al*., 2009a).

Importation and release of *T. isis* was part of a redistribution approach initiated by IITA with an aim of exchanging natural enemies and strains between African regions. However, as with other biological control agents released in new environments, questions on the spread and establishment of *T. isis* in East Africa remain unanswered. A post-release survey done in 2006, reported overall egg parasitism rates of about 37.9±1.71% in Wundanyi (Yaovi *et al*., 2009a). However, the aforementioned post release survey was done barely one year after the release. This may in turn have resulted in erroneous conclusions and recommendations. No post release survey has been carried out in Eldoret, another *T. isis* release site. This study was thus undertaken to assess the establishment and dispersal of *T. isis*, and determine changes in egg parasitism in Wundanyi and Kitale/Eldoret.

## 4.2 Methodology

### 4.2.1 Description of the study area

Surveys were carried out in two localities, Kitale/Eldoret and Wundanyi; localities at which *T. isis* was released in 2005 (Fig. 4.1). Eldoret is a high altitude area (1500-2400 masl) while Wundanyi stretches across mid to high altitude areas (1110-2900 masl). In Eldoret, *T. isis* releases were done on 13 farms (Fig. 4.1). These farms were however close to one another and were collapsed during sampling to avoid overlaps among transects. In Wundanyi, *T. isis* was released at three sites: Josa, Wesu and at the Wundanyi prison's farm (Fig. 4.1). This

study was undertaken during the month of April 2014, the period during which stem borer eggs were considerably abundant in the field (Yaovi, *pers. com*).

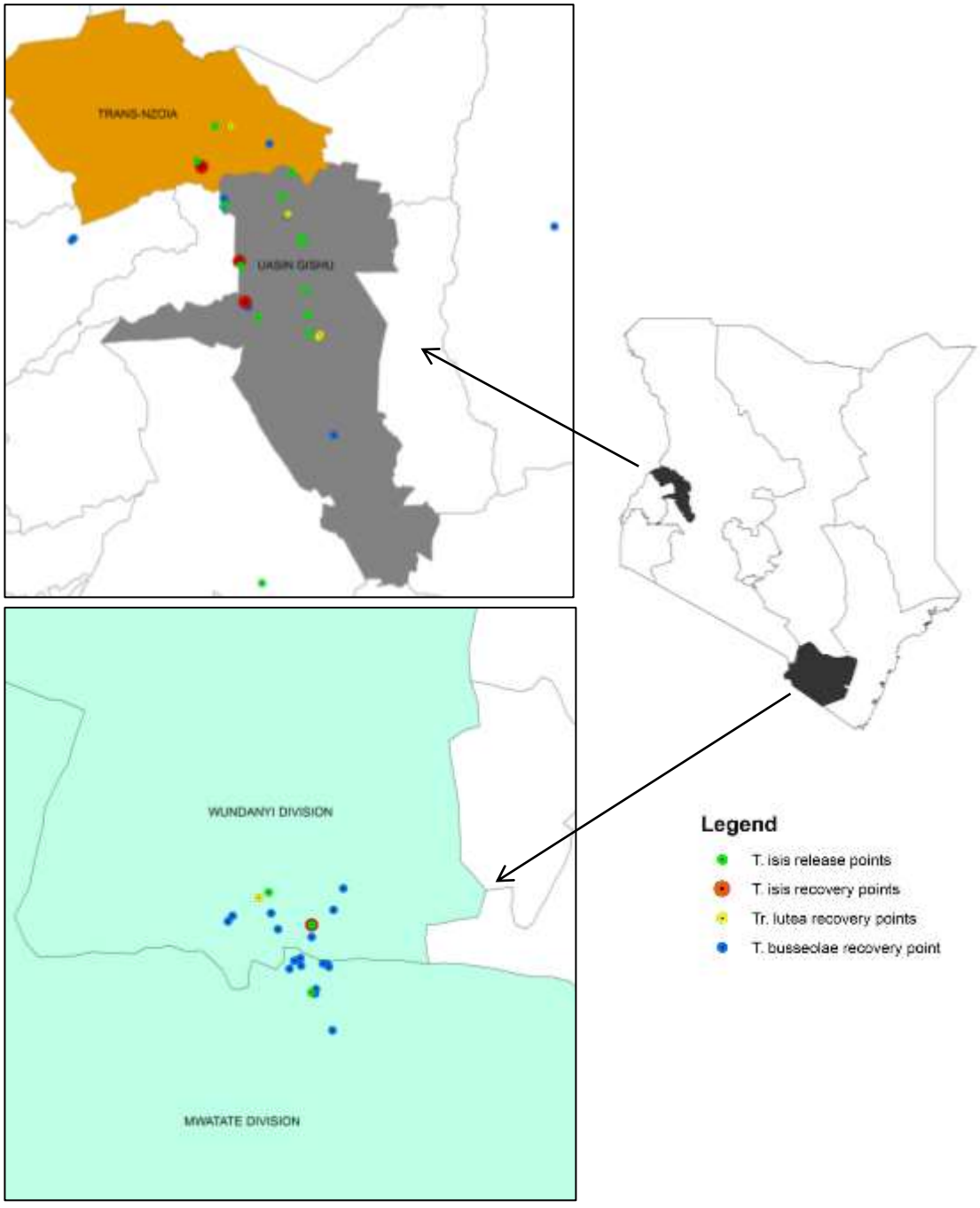



**Figure 4.1**: Sampled points in Uasin Gishu, Trans-Nzoia and Taita Taveta counties where egg parasitoids of noctuid stem borers were released (in 2006) and recovered in 2014

### 4.2.2 Sampling protocol

Maize farms with pre-tasseling plants were identified radially along the four cardinal compass directions from points of parasitoid release and marked for sampling. A total of 52 and 21 fields were sampled in Kitale/Eldoret and Wundanyi respectively. During sampling, marked fields were divided into five subplots; one at each corner and one at the centre of the field. All plants in each subplot were inspected for noctuid stem borer eggs (Fig 4.2a and b). The number of plants inspected, number of egg batches per plant and the number of eggs per batch were recorded. Egg batches were collected and individually placed in glass vials, plugged with cotton wool and labelled. Collected eggs were taken to the laboratory at *icipe* where they were incubated at 25±1°C and 60-70% relative humidity. Incubated eggs were checked and recorded each day for emergence of stem borer larvae or parasitoids. Recovered parasitoids were identified using keys developed by Polaszek (1998).

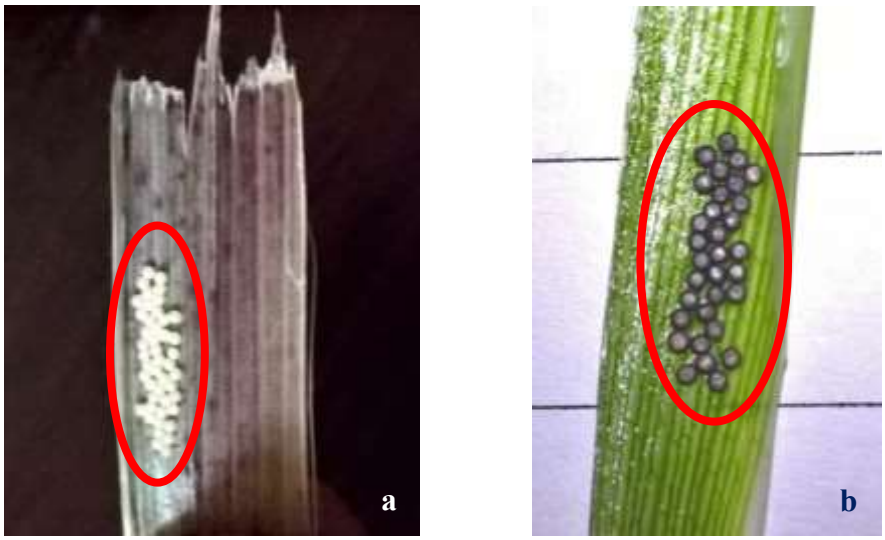



**Figure 4.2:** (a) Fresh noctuid stem borer eggs laid on maize blade (b) Parasitized noctuid stem borer eggs laid on maize blade

### 4.2.3 Statistical analyses

Number of eggs and egg batches were subjected to Wilcoxon rank sum test to test for differences between the localities. Three kinds of parasitization parameters were computed: (a) Mean egg parasitism per field was calculated as percentage of eggs parasitized within an individual egg batch averaged over all egg batches in the field. (b) Discovery efficiency was calculated as the percentage of egg batches with parasitoids per field (Bin and Vinson, 1991). (c) Parasitism efficiency was calculated as the percentage of eggs parasitized within discovered egg batches averaged over all egg batches per field (Bin and Vinson, 1991). Egg parasitism, discovery efficiency and parasitism efficiency constituted proportion data and were analysed using Wilcoxon rank sum test (*W*) to differentiate between localities and one sample Wilcoxon test (*V*) to separate between results obtained before and after parasitoid release.

## 4.3 Results

### 4.3.1 Egg parasitoid community and their relative contribution

Three egg parasitoid species, the indigenous *T. busseolae* and *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae) and the exotic *T. isis* were identified. Though these parasitoids were present in the two localities, there was only a single instance in Eldoret where there was multiple species parasitism in one egg batch. The observed multiple parasitism involved *T. busseolae* and *Tr. lutea*.

In terms of species importance, *T. busseolae* was recovered from 21 fields, *T. isis* from 3 fields and *Tr. lutea* from 6 fields in Eldoret (Fig. 4.1). In Wundanyi, *T. busseolae* was recovered from 20 fields, *T. isis* from 1 field and *Tr. lutea* from 2 fields (Fig. 4.1). Of the three parasitoid species, *T. busseolae* dominated the parasitoid community constituting 89.9 and 97.8% of the total parasitoids collected in Eldoret and Wundanyi respectively.

*Trichogrammatoidea lutea* constituted 6.8 and 0.2% in Eldoret and Wundanyi respectively while *T. isis* constituted 3.3 and 2.1% in Eldoret and Wundanyi respectively. *Telenomus isis* was only recovered from fields where releases had been done in 2005.

### 4.3.2 Stem borer egg batches and general parasitism

Collected number of egg batches varied among the sampled localities, Eldoret (1,168) and Wundanyi (683), with each locality giving a total of 51,944 and 15,529 eggs respectively. The mean number of egg batches per field varied significantly between the localities ($W$ = 292, $p$<0.05) with relatively higher mean observed in Wundanyi (32.5±5.2) compared to Eldoret (22.5±6.3) (Table 4.1). In Eldoret, mean number of egg batches recovered (22.5±6.3) was significantly higher than in pre-release period (2004) (2.1±0.2) ($V$=1147,$p$<0.05). This pattern was also depicted in Wundanyi where mean number of egg batches recovered during this study (32.5±5.2) was significantly higher than in 2004 (1.0±0.1) ($V$=228, $p$<0.05) (Table 4.1). Egg batch size varied between the two localities. In Eldoret, the sizes fluctuated between 0 to 196 eggs with a variance of 660.4 eggs while in Wundanyi, it fluctuated between 2 to 144 eggs with a variance of 176.23 eggs.

Differences were also observed in the mean number of eggs per batch (Table 4.1). Mean number of eggs per batch were significantly higher in Eldoret (30.4±3.0) compared to Wundanyi (21.3±0.7) ($W$=292, $p$<0.05). In Eldoret, mean number of eggs per batch (30.4±3.0) was significantly lower than pre-release period (34.2±1.4) ($V$=1198, $p$<0.05) while in Wundanyi, mean number of eggs per batch collected during this study was significantly lower ($V$=228, $p$<0.05) (Table 4.1).

**Table 4.1:** Mean number of eggs per batch and egg batches per field collected for detection of egg parasitoids in Eldoret and Wundanyi in April 2014.

| Locality | Period | Mean number of eggs/batch | Mean number of egg batches/field |
|---|---|---|---|
| **Eldoret** | 2004 | 34.2±1.4[a] | 2.1±0.2[a] |
| | 2015 | 30.4±3.0[b] | 22.5±6.3[b] |
| | *V*-value | 1198 | 1147 |
| | *p*-value | 3.41e $^{-06}$ | 2.93e $^{-05}$ |
| **Wundanyi** | 2004 | 25.7±1.4[a] | 1.0±0.1[a] |
| | 2015 | 21.3 ± 0.7[b] | 32.5±5.2[b] |
| | *V*-value | 228 | 228 |
| | *p*-value | 4.77e $^{-06}$ | 9.87e $^{-05}$ |
| **Eldoret** | 2015 | 30.4±3.0[a] | 22.5±6.3[a] |
| **Wundanyi** | 2015 | 21.3 ± 0.7[a] | 32.5±5.2[b] |
| | *W*-value | 416 | 292 |
| | *p*-value | 0.113 | 0.0019 |

Mean (±SE) within columns followed by the same lower case superscripts are not significantly different ($p>0.05$). Pre-release period is denoted by 2004 while post-release period is denoted by 2015

Generally, there was evidence of differences in egg parasitism between the localities. Of all egg batches collected in Wundanyi, 361 were parasitized compared to 83 found parasitized in Eldoret. This was consistent with mean egg parasitism observed in Eldoret (7.31±2.04%) and Wundanyi (52±5.79%) which were significantly different ($W=57$, $p<0.05$) (Table 4.2). These were however different from parasitism values observed in respective localities in 2004 before release of *T. isis*. In Eldoret, egg parasitism prior to release of *T. isis* (10.0±0.45%) was significantly higher than after the release (7.31±2.04%) ($V=326$, $p<0.05$) while in Wundanyi, it was significantly higher after *T. isis* release (52±5.79%) compared to pre-release (25.9±1.96%) ($V=211$, $p<0.05$) (Table 4.2).

The observed variations in egg parasitism were similar to variations in discovery efficiency (Table 4.2). The mean discovery efficiency observed in Wundanyi (55.4±6.11%) was

significantly higher compared to observations made in Eldoret (8.9±2.23%) ($W$=87, $p$<0.05). This, however, was different from observations made in respective localities in 2004. In Eldoret, mean discovery efficiency in 2004 was significantly higher (14.2±1.9%) compared to what was recorded during this study (8.9±2.2%) ($V$=299, $p$<0.05). In contrast, mean discovery efficiency in Wundanyi during this study was significantly higher (55.4±6.1%) compared to pre-release results (32.9±1.7%) ($V$=199, $p$<0.05).

Results of this study show that parasitism efficiency was relatively low with no significant difference between Eldoret (7.31±2.04%) and Wundanyi (2.41±0.51%) ($W$ = 397, $p$ > 0.05) (Table 4.2). However, observed parasitism efficiency in Eldoret (7.31±2.04) was lower compared to pre-release period (63.6±1.53%) ($V$=0, $p$<0.05). Similar difference was observed in Wundanyi where the observed parasitism efficiency (2.41±0.51%) was lower compared to the pre-release period (79.9±1.15%) ($V$=0, $p$<0.05).

**Table 4.2:** Egg parasitism, discovery and parasitism efficiency of egg parasitoids during long rains of 2014 in Eldoret and Wundanyi.

| Locality | Period | Egg parasitism | Discovery Efficiency | Parasitism efficiency |
|---|---|---|---|---|
| **Eldoret** | 2004 | $10.0\pm0.5^{a}$ | $14.2\pm1.9^{a}$ | $63.6\pm1.5^{a}$ |
| | 2015 | $7.3\pm2.04^{b}$ | $8.9\pm2.2^{b}$ | $7.3\pm2.04^{b}$ |
| | *V*-value | 326 | 299 | 0 |
| | *p*-value | 0.0007223 | 0.0002803 | $1.35e^{-10}$ |
| **Wundanyi** | 2004 | $25.9\pm2.0^{a}$ | $32.9\pm1.7^{a}$ | $79.9\pm1.2^{a}$ |
| | 2015 | $52\pm5.8^{b}$ | $55.4\pm6.1^{b}$ | $2.4\pm0.5^{b}$ |
| | *V*-value | 211 | 199 | 0 |
| | *p*-value | 0.0003538 | 0.003913 | $9.54e^{-07}$ |
| **Eldoret** | 2015 | $7.3\pm2.0^{a}$ | $8.9\pm2.2^{a}$ | $7.3\pm2.0^{a}$ |
| **Wundanyi** | 2015 | $52\pm5.8^{b}$ | $55.4\pm6.1^{b}$ | $2.4\pm0.5^{a}$ |
| | *W*-value | 57 | 87 | 397 |
| | *p*-value | $6.80e^{-10}$ | $6.08e^{-09}$ | 0.061 |

Mean (±SE) within columns followed by the same lower case superscripts are not significantly different ($p>0.05$). Pre-release period is denoted by 2004 while post-release period is denoted by 2015

### 4.4 Discussion

This study revealed the presence of three egg parasitoids, *T. busseolae*, *T. isis* and *Tr. lutea* in the noctuid stem borer eggs in Kenya. Presence of these parasitoids corroborates findings by Bruce *et al*. (2009) who in addition to these three species reported the presence of *Telenomus spp* and *Tr. bournieri* in Wundanyi. The existence of *T. isis* in the egg parasitoid community in Eldoret and Wundanyi confirms its establishment in localities in which it was released in 2005. Before the release of *T. isis* in Kenya, key factors such as climatic tolerance and host suitability were studied as both biotic and abiotic factors are involved in determining species establishment (Godfray and Waage, 1991; Goolsby *et al*., 2005; Pilkington and Hoddle, 2006; 2009; Chabi-Olaye *et al.,* 2001). Based on results of these studies, predictions

regarding favourable areas in East Africa were made. Predicted favourable areas included the mid and high altitude zones (Chabi-Olaye *et al*., 2001; Bruce *et al*., 2009). Presence of *T. isis* in the release areas as witnessed in this study confirms that predicted release areas were actually favourable for *T. isis*.

Parasitoids have been reported to experience complex interactions with other parasitoids and hyperparasitoids (Cusumano *et al*., 2012). Such interactions may in some cases result in multiple species parasitism. There were concerns before release of *T. isis* that it would face competition from indigenous egg parasitoids through such interactions that would in turn limit its establishment. These concerns emanated from results of detailed studies of extrinsic and intrinsic competition between egg parasitoids, especially between *T. busseolae* and *T. isis* (Agboka *et al*., 2002). Contrary to above fears, this study indicates that there is no multiple parasitism involving *T. isis*. The lack of multiple species parasitism cases by *T. isis* may be explained by its characteristic avoidance mechanism. Studies show that *T. isis* and its conspecific, *T. busseolae*, strongly avoid multiple species parasitism indicating interspecific host discrimination (Agboka *et al*., 2002). Of the three parasitoids collected in the two localities, Eldoret and Wundanyi, *T. busseolae* dominated the parasitoid community. Similar dominance of parasitoid community by *T. busseolae* was noted by Polaszek and Kimani-Njogu (1998), Schulthess *et al.* (2001) and Bruce *et al.* (2009) who attributed it's dominance to faster development and its competitive superiority in cases of superparasitism (Chabi-Olaye *et al*., 1997; 2001). Despite the dominance of *T. busseolae*, results of this study indicate that *T. isis* has established though it was only present in fields of previous release.

Despite *T. isis* establishment, this study showed persistence of stem borer pests in crop fields. This is evident in the observed number of stem borer egg batches on maize plants with majority of plants having single egg batches. Single egg batches observed on maize plants is not unique to this study as such a trend was reported in similar study (Bruce *et al*., 2009).

This observation is attributed to the female noctuid stem borer oviposition characteristic in which they are able to recognize and avoid plants with egg batches (Setamou and Schulthess, 1995; Ndemah *et al*., 2003; Chabi-Olaye *et al*., 2005). This behaviour is used to reduce competition for the food resource and increased mortality associated with emigration to other food sources.

The observed mean number of eggs per batch was higher in Eldoret compared to Wundanyi. This may be explained by the difference in farming practices in the two localities. In Eldoret, most fields are monocultures of maize grown for commercial purposes while in Wundanyi there is mixed cropping as most households farm for domestic consumption. The variation in egg batch sizes may be explained by the clutch size plasticity hypothesis. The theory hypothesizes that female lepidoptera preferentially oviposit larger egg clutches in habitats that ensure survival of F1 generation (Dethier, 1959). Larger maize monocultures in Eldoret provide more food compared to smaller farms in Wundanyi where mixed cropping is practised. In contrast, the mean number of egg batches per field was generally higher in Wundanyi compared to Eldoret. Variation in the number of egg batches between Wundanyi and Eldoret may be attributed to differential adjustment in female response to habitat quality. Studies on oviposition plasticity and number of batches show that female's decision to allocate her reproductive investment is based upon variation in quality of encountered plants (Fordyce, 2005). Habitat quality in Wundanyi is relatively poor compared to Eldoret and female moths must thus lay several egg batches to ensure survival of the progeny.

Contrary to expectations of the biological control programme, a decrease registered in Eldoret and the difference in activity of *T. isis* in the two localities may be attributed to prevailing cropping systems. In Eldoret, there is only one maize growing season that runs over seven months. The main host, *B. fusca* enters into diapause in the absence of the main crop host (maize) (Le Rü *et al.*, 2006) and this limits availability of eggs that could otherwise

host *T. isis.* This may explain the observed low parasitoid action. In contrast, Wundanyi is characterized by two maize growing seasons and this ensures availability of eggs to host *T. isis* for a longer period in the year. In addition to favourable season, Wundanyi is endowed with a network of rivers which are used by farmers for irrigation during the dry season. This ensures availability of maize plants (Bruce *et al*., 2009) and thus *T. isis* hosts, stem borer eggs. This explains the higher egg parasitism in Wundanyi and validates predictions by Chabi-Olaye *et al.* (2001) who projected that the availability of host eggs during off season would aid in *T. isis* establishment in mid-altitude areas. Besides the cropping systems, availability of other suitable hosts of *T. isis* resulted in increased parasitism. Non-diapausing *S. calamistis*, is found in mid-altitude Wundanyi where it is able to support the parasitoid population when the main host may not be available. This validates the suggestion that presence of significant populations of suitable, non-diapausing non-target species would increase the chance of establishment of *T. isis* in Kenya (Bruce *et al*., 2009). Studies in other regions have demonstrated that maintaining noctuid populations all year round can lead to *T. isis* outcompeting *T. busseolae* (Chabi-Olaye *et al*., 2006b). Witholding the conditions in Wundanyi (that necessitate for host availability all year round) and if *T. isis* reaches the spreading phase, this may be achieved.

The difference observed in parasitism parameters computed for the localities may have also arisen from average size of egg batches. Larger egg batches recorded in Eldoret resulted in lower parasitism as in such, central eggs are inaccessible for parasitization. In contrary, smaller egg batches recovered from Wundanyi resulted in higher parasitism as a larger number of the eggs were accessible to the parasitoid. Parasitization starts from peripheral eggs such that smaller egg batches are easily reached into by the parasitoid compared to large egg batches whose central rows may be left unparasitized (Alexandri and Tsitsipis, 1990).

Through this study, it is evident that the introduction of *T. isis* in Kenya was a success as the parasitoid has established. However, it is probable that further spread and impact of *T. isis* may have been achieved if releases had been done repeatedly over a period of time. This has been practiced in other classical biological control programmes such as *C. flavipes* Cameron which then resulted in extensive spread (Omwega *et al*., 1995; 1997; 2006; Overholt *et al*, 1997). Similar release practices were proposed by Lockwood *et al*. (2001) who asserted that species introduced in large and consistent quantities are more likely to survive unlike species introduced in small numbers with only a few release events. Like in other organisms, establishment of a new species is followed by spread whereby the organism becomes part of the new habitat's fauna before expanding its range to produce significant impact in composition, structure and ecosystem processes. This study thus projects that levels of *T. isis* will rise and spread to favourable areas as there is evidence of steady increase in egg parasitism. It is recommended that extensive releases be done in suitable areas in order to increase populations of *T. isis* and to realize impact on noctuid stem borer pest population in Kenya.

# CHAPTER FIVE

## 5.0 ESTABLISHMENT AND SPREAD OF *Cotesia flavipes* CAMERON AND ITS IMPLICATION ON *Chilo partellus* (SWINHOE) ACROSS THREE AGRO-ECOLOGICAL ZONES IN KENYA

### 5.1 Introduction

Lepidopteran stem borers constitute one of the most important constraints to maize and sorghum production in SSA (Brownbridge, 1991; Odindo, 1991; Schulthess *et al*., 1997). In Kenya and East Africa in general, the most important cereal stem borer pests are the crambids *Chilo partellus* (Swinhoe), *Chilo orichalcociliellus* Strand, the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Nye, 1960; Bonhof *et al*., 1997; Overholt *et al*., 2001). These stem borer pest**s** are indigenous in Africa except for *C. partellus* which originated from Asia (Tams, 1932; Nye, 1960; Bleszynski, 1970; Van Hamburg, 1979). *Chilo partellus* was accidentally introduced in Africa in the 1930's (Tams, 1932) and owing to its excellent colonizing abilities, it has since spread to various countries (Duerden, 1953; Nye, 1960; Ingram, 1983; Harris, 1990; Overholt *et al*., 1994a). In Kenya, *C. partellus* is the most damaging and important pest OF of maize and sorghum (Seshu Reddy, 1983; Overholt *et al*., 1997) since it was first reported in 1950s (Nye, 1960). Cereal yield losses associated with its infestation is estimated to reach as high as 73-100% in maize and 88-100% in sorghum (Seshu Reddy, 1983; Seshu Reddy and Walker, 1990).

Management of *C. partellus* populations is considered an important step towards increasing maize and sorghum production and has thus been the focus of various management initiatives (Overholt *et al*., 1997). Two classical biological control attempts targeting *C. partellus* population have been undertaken in Eastern Africa. The first one was undertaken by the Commonwealth Institute for Biological Control (now International Institute of Biological

Control) and it involved introduction of nine parasitoid species from Rawalpindi, Pakistan into Kenya, Uganda and Tanzania. These organisms failed to establish (CIBC, 1968-1972). The second attempt was initiated in Kenya by *icipe*'s Biocontrol Programme in 1991 (Overholt, 1993). Following a foreign exploration in the pest's native range during this attempt, *Cotesia flavipes* was selected from Sindh region of Pakistan and introduced in Kenya (Overholt *et al*., 1994a). The parasitoid was released along the Kenyan coast (Kilifi and Kwale) in July 1993 (Overholt *et al*., 1994a). Surveys to assess its establishment were carried out later and results showed that *C. flavipes* had established (Overholt *et al*., 1994b, Omwega *et al*., 1995; Overholt *et al*., 1997). Stem borer parasitism by *C. flavipes* progressively increased reaching levels of 0.03 to 7.1 in 1997 (Overholt *et al*., 1997). These figures were however relatively low and at the time, *C. flavipes* did not appear to be an important mortality factor for stem borers (Overholt *et al*., 1997). A subsequent assessment demonstrated an increase in parasitism to 13% in 1999 (Zhou *et al*., 2001). In dry mid altitude tropics, *C. flavipes* was released in experimental fields at three sites (Katumani, Ithookwe and Kiboko) in 1997 to 1998. Post release surveys carried out within the same season and year of release led to recovery of the parasitoid in release fields (Songa *et al*., 2001). However, the time between releases and post release surveys done in the region was too short to deduce establishment or measure spread. In moist mid altitude tropics, releases were done at three sites in Kisumu in 2000 (*icipe* and KALRO, unpublished data). Further to this, additional releases were done on farmer's fields in dry mid altitude tropics (Masii, Mulutu and Kitui) in 2002 (*icipe* and KALRO, unpublished data). No post release surveys have been carried out following the latter two releases.

Maximum pest suppression can only be achieved once the parasitoid reaches host populations in all suitable habitats and thus spread is an important aspect in parasitoid impact. It was thus projected that a characteristic *C. flavipes* 'equilibrium' density needed to be reached before

its impact on stem borer pest population is measured (Overholt *et al*., 1997). However, no additional surveys have been carried out at the coast during the last seventeen years. In this study infestation levels in the three agro-ecological zones *vis a vis* rates of parasitism attained by *C. flavipes* were assessed. Such information is needed in order to fully assess and vouch for the potential of this biological control agent as a pest management measure.

## 5.2 Methodology

### 5.2.1 Description of study area

This study was carried out in three different Agro-Ecological Zones (AEZ) as classified by the Kenya Maize Database Project (Corbett, 1998). Surveys were carried out between March 2014 to September 2016. Transects along which surveys were done ran across moist lowland, dry mid-altitude and moist mid-altitude AEZs in Kenya (Figure 5.1). These areas were also previous *C. flavipes* release points (Kilifi and Kwale in moist lowland AEZ, Masii, Mulutu and Kitui in dry mid-altitude AEZ and Kisumu in moist mid-altitude AEZ).

#### *5.2.1.1 Moist lowland tropics*

Moist lowland tropics are characterized by temperatures ranging between 20 and 31°C, receives around 500-1,000 mm of rainfall annually, with most areas situated below 400masl (Corbett, 1998). The main maize growing season falls between March and August while the short rains come in October with a decrease being observed in January and February. The amount, reliability and duration of rainfall are the main factors limiting agricultural production in this zone. A variety of crops including pulses, industrial and root crops, fruits and cereals are cultivated with maize being the most important food crop (Rocheleau *et al*., 1995).

#### *5.2.1.2 Dry mid-altitude tropics*

In the dry mid-altitude zone, temperatures range from 14 to 33°C with annual precipitation varying between 300 to 550 mm. This amount is considered low and unreliable (Bernard *et al*., 1989). Long rains are received between March and May while the short rains are received between October and December (Moore, 1979). The region is predominantly semi-arid and lies at an altitude of 700-1,400 masl (Corbett, 1998). Production systems in the area have moved from agropastoral to mixed farming systems (Rocheleau *et al*., 1995). Bananas, sweet potatoes, green grams, cow peas, pigeon peas, pumpkins, beans and maize are the most common food crops grown in the area. Dry forest and savannah ecosystems have also been converted to agricultural lands besides being burnt to produce charcoal (Rocheleau *et al*., 1995).

#### *5.2.1.3 Moist mid-altitude tropics*

This zone is characterized by temperatures ranging from 13 to 30°C, receives more than 550 mm of rainfall annually with most areas situated between 1,110 and 1,500 masl (Corbett, 1998). There is commercial farming of sugarcane while maize, sorghum and pearl millet are mainly grown as food crops. This area has two maize growing seasons, March to June/July (long rainy season) and September to December (short rainy season) (Rocheleau *et al*., 1995).

### 5.2.2 Study design and stem borer sampling protocol

Bench mark sites were marked in farms at which *C. flavipes* had previously been released (Fig. 5.1). Survey farms were subsequently marked in the four cardinal compass points at every 15, 30 and 45 km (Fig. 5.2). Stem borers were sampled and reared using the procedure described under General Materials and Methods in Chapter three.

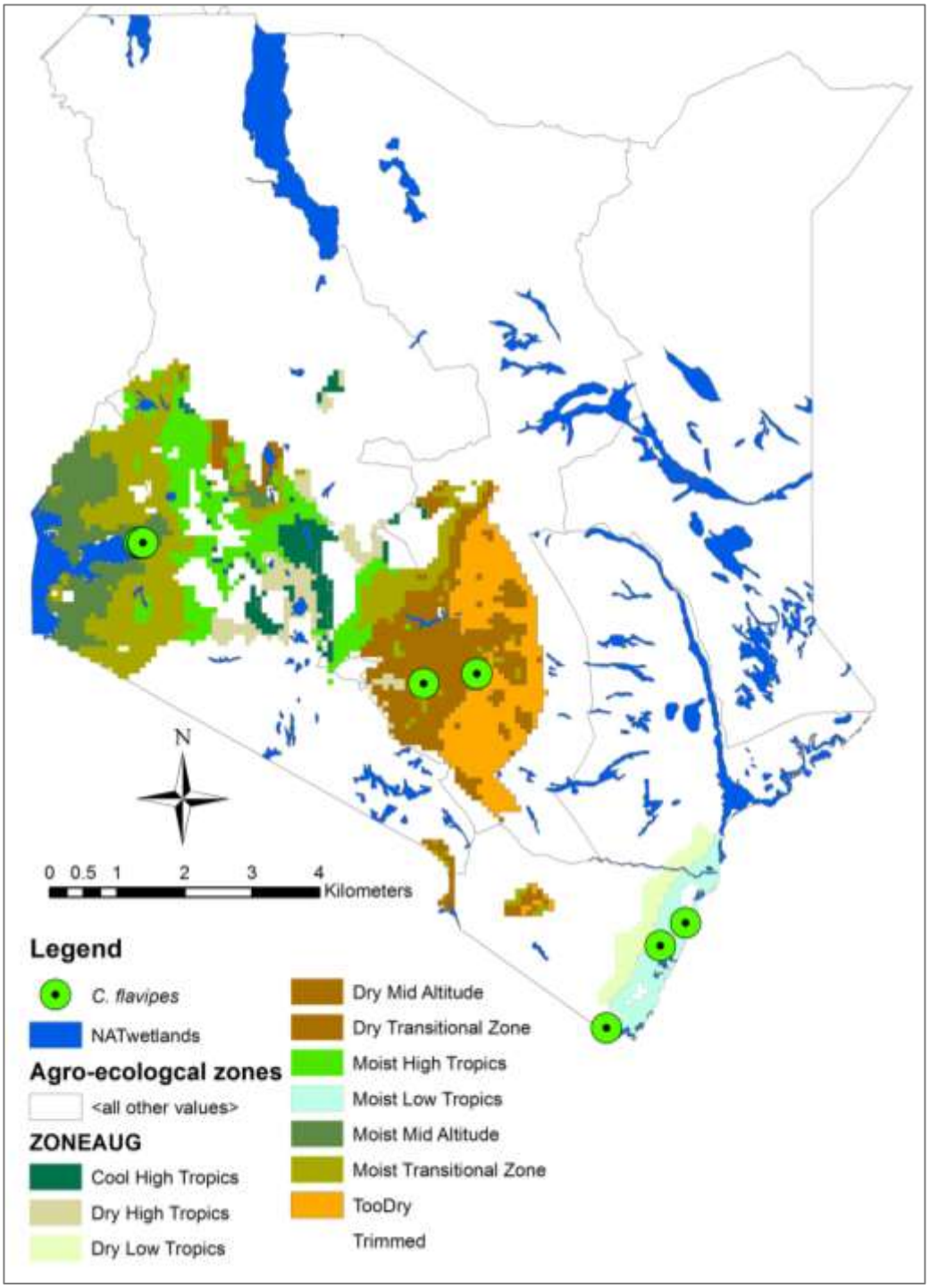



**Figure 5.1:** *Cotesia flavipes* release points in moist mid-altitude (Kisumu in 2002), dry mid-altitude (Masii, Mulutu and Kitui in 2002) and moist lowland (Kilifi and Kwale in 1993) AEZs in Kenya

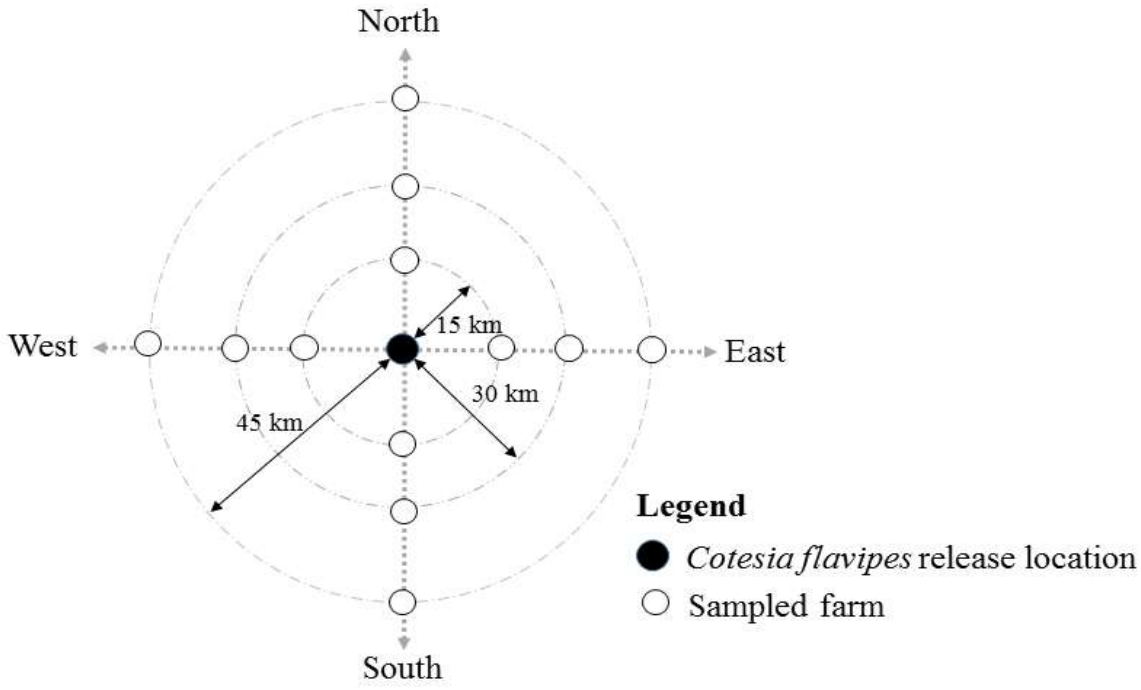



**Figure 5.2**: *Cotesia flavipes* release point and sampling transect radiating in four main compass point directions at 15, 30 and 45 km distances

### 5.2.3 Statistical analyses

Farms sampled at each point along different transects were treated as replicates and resulting data used to estimate means of infestation and parasitism for respective distance intervals. Percentage infestation was computed by expressing the number of infested plants as a percentage of the total plants inspected in respective farms. To estimate larval densities in respective farms, the total number of stem borer larvae collected from dissected plants was expressed as a proportion of the number of infested plants. Parasitoid cocoons that were spun from appropriate larval stages were expressed as a proportion of respective field densities in order to compute percentage parasitism. Percentage infestation, larval density and parasitism were subjected to Shapiro-Wilk test for normality upon which abnormal data was appropriately transformed. Normal data was analysed using One-Way ANOVA and

significantly different means separated using Tukey's HSD test. Data which failed the normality test was subjected to Kruskal-Wallis rank sum test and significantly different means separated using Nemenyi post-hoc test ($p < 0.05$). One sample *t*-test and Wilcoxon rank sum test were used to compare mean infestation and parasitism levels obtained before and after the release of parasitoids. In order to determine which climatic parameters prevailing in sampled localities had the greatest effect on stem borer species infestation and parasitism, data was extracted from BIOCLIM and subjected to multiple regression analysis using the vegan package in R.

### 5.3 Results

#### 5.3.1 Stem borer composition, diversity and larval density

In moist lowland AEZ, stem borer species recovered were *Chilo spp* and *S. calamistis*. *Chilo spp* were the most dominant and constituted 98.5% of total stem borers collected with the exotic *C. partellus* constituting 96.4% while the indigenous *C. orichalcociliellus* constituted 1.7%. *Sesamia calamistis* constituted 1.8% of total stem borers collected (Table 5.1). Mean larval densities significantly varied among the stem borer species ($\chi^2$=100.58, $p$<0.05; Table 5.1). *Chilo partellus* exhibited a significantly higher larval density (14.41±3.16) compared to both *C. orichalcociliellus* (0.23±0.07) and *S. calamistis* (0.15±0.05). Mean larval density by *C. orichalcociliellus* and *S. calamistis* were not significantly different.

In dry mid altitude AEZ, stem borer species recovered were *C. partellus, S. calamistis* and *B. fusca. Chilo partellus* predominated the stem borer pest community by constituting 71.2%. *Sesamia calamistis* constituted 26% while *B. fusca* constituted 2.8% of the stem borers collected (Table 5.1). Mean larval density showed significant variation among the three stem borer species ($\chi^2$=74.92, $p$<0.05). Highest larval density was exhibited by *C. partellus* (2.4±0.37) followed by *S. calamistis* (0.80±0.19) and *B. fusca* (0.06±0.03). *Sesamia*

*calamistis* and *B. fusca* did not show a significant difference in their mean larval densities (Table 5.1).

**Table 5.1:** Percentage composition and density of stem borer species recovered infesting maize and sorghum in the three agro-ecological zones between 2014 and 2016.

| Agro-Ecological Zone | Stem borer species | % composition | Larval density ( $\bar{x}$ ±SE) |
|---|---|---|---|
| **Moist lowland** | *Chilo partellus* | 96.4 | $14.4\pm3.2^{b}$ |
| | *Chilo orichalcociliellus* | 1.7 | $0.2\pm0.1^{a}$ |
| | *Sesamia calamistis* | 1.8 | $0.2\pm0.1^{a}$ |
| | $\chi^2$ value | | 100.58 |
| | *df* | | 2 |
| | *p* value | | 2.20E-16 |
| **Dry mid-altitude** | *Chilo partellus* | 71.2 | $2.4\pm0.4^{b}$ |
| | *Sesamia calamistis* | 26 | $0.8\pm0.2^{a}$ |
| | *Busseola fusca* | 2.8 | $0.1\pm0.0^{a}$ |
| | $\chi^2$ value | | 74.92 |
| | *df* | | 2 |
| | *p* value | | 2.20E-16 |
| **Moist mid-altitude** | *Chilo partellus* | 58.0 | $0.2\pm0.1^{b}$ |
| | *Sesamia calamistis* | 21.4 | $0.1\pm0.0^{ab}$ |
| | *Busseola fusca* | 19.1 | $0.1\pm0.0^{ab}$ |
| | *Eldana saccharina* | 1.5 | $0.005\pm0.005^{a}$ |
| | $\chi^2$ value | | 10.47 |
| | *df* | | 3 |
| | *p* value | | 0.015 |

Larval density $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different ($p$>0.05)

In moist mid altitude AEZ, four stem borer species were recovered; *C. partellus, S. calamistis, B. fusca* and *Eldana saccharina* Walker. *Chilo partellus* was the most dominant (58.02%), followed by *S. calamistis* (21.37%), *B. fusca* (19.08%) and *E. saccharina* (1.53%). Results showed a significant difference in mean larval densities among the four stem borer species ($\chi^2$=10.47; $p$<0.05) (Table 5.1). *Chilo partellus* exhibited significantly higher larval

density (0.21±0.08) followed by *S. calamistis* (0.07±0.02) and *B. fusca* (0.06±0.02). Mean larval densities of these three species were significantly higher in comparison to *E. saccharina* (0.005±0.005).

### 5.3.2 Stem borer parasitoid composition and diversity

In moist lowland AEZ, four larval and three pupal parasitoids were recovered (Table 5.2) which together had a Shannon diversity index of 0.7. The larval parasitoids included *C. flavipes*, *Cotesia sesamiae* (Cameron), *Chelonus curvimaculatus* (Cameron) and Tachnidae. Pupal parasitoids recovered were *Pediobius furvus* (Gahan), *Dentichasmias busseolae* Heinrich and *Xanthopimpla stemmator* Thunberg. The larval parasitoid community was dominated by the exotic *C. flavipes* (75.87%) followed by *C. sesamiae* (18.32%), *C. curvimaculatus* (0.33%) and Tortricidae (0.02). Pupal parasitoid community was dominated by *P. furvus* (0.7%) followed by *D. busseolae* (0.05%) and *X. stemmator* (0.05%). The hyperparasitoid *Aphanogmus fijiensis* (Ferriére) was also recovered (4.63%). Parasitoids were recovered from *C. partellus* only.

In dry mid altitude AEZ, four larval parasitoids, one pupal parasitoid and one hyperparasitoid were recovered (Table 5.2) and together they had Shannon diversity index of 0.7. The larval parasitoids were *C. flavipes* (76.47%), *C. sesamiae* (7.64%), *C. curvimaculatus* (0.41%), *Dolichogenidea polaszeki* Walker (0.14%) and *Sturmiopsis parasitica* (Curran) (0.03%). The pupal parasitoid was *D. busseolae* which constituted 0.17% of the total parasitoids collected while the hyperparasitoid, *A. fijiensis*, constituted 15.1%.

In moist mid altitude AEZ, *C. flavipes* was the only larval parasitoid collected and it constituted 69.57% of the parasitoids. Two pupal parasitoids, *Psilochalsis soudanensis* (Steffan) and *P. furvus*, were collected and they constituted 28.26% and 1.81% of the

parasitoid community respectively. The Shannon diversity index of parasitoids in this zone was 0.7. All these parasitoids emerged from *C. partellus* only. (Table 5.2).

**Table 5.2:** Percentage composition of parasitoids and stem borer species attacked in different agro-ecological zones between 2014 and 2016**.**

| Parasitoid species | Guild | Moist lowland AEZ | | Dry mid-altitude AEZ | | Moist mid-altitude AEZ | |
|---|---|---|---|---|---|---|---|
| | | % composition | Host | % composition | Host | % composition | Host |
| *Cotesia flavipes* | Larval | 75.9 | *Chilo partellus* | 76.5 | *C. partellus, S. calamistis, B. fusca* | 69.6 | *Chilo partellus* |
| *Cotesia sesamiae* | Larval | 18.3 | *Chilo partellus* | 7.4 | *C. partellus, S. calamistis, B. fusca* | - | - |
| *Chelonus curvimaculatus* | Larval | 0.3 | *Chilo partellus* | 0.4 | *C. partellus, B. fusca* | - | - |
| Tachnidae | Larval | 0.05 | *Chilo partellus* | - | - | - | - |
| *Dolichogenidea polaszeki* | Larval | - | - | 0.1 | *S. calamistis* | - | - |
| *Sturmiopsis parasitica* | Larval/pupal | - | - | 0.03 | *B. fusca* | - | - |
| *Pediobius furvus* | Pupal | 0.7 | *Chilo partellus* | - | - | 28.3 | *Chilo partellus* |
| *Xanthopimpla stemmator* | Pupal | 0.05 | *Chilo partellus* | - | - | - | - |
| *Dentichasmias busseolae* | Pupal | 0.05 | *Chilo partellus* | 0.2 | *C. partellus* | - | - |
| *Psilochalsis soudanensis* | Pupal | - | - | - | - | 0.4 | *Chilo partellus* |
| *Aphanogmus fijiensis* | Hyperparasitoid | 4.6 | *Chilo partellus* | 15.1 | *C. partellus, S. calamistis* | 1.8 | *Chilo partellus* |

### 5.3.3 Stem borer infestation across the three agro-ecological zones

Stem borer infestation in moist lowland AEZ was estimated at 28.94±3.11%. Mean infestation varied significantly across distances at which sampling was done ( $\chi_3^2$ =10.72; $p$<0.05). The least infestation levels were detected at parasitoid release points (11±3.46%) while the highest (35.58±9.8) were at farms sampled farthest from release points. Infestation progressively increased from release points to the furthest points sampled (Table 5.3). Infestation levels significantly reduced between 1983 (92%-pre-release period) and 2015 (current study) ($V$=3; $p$<0.05) (Table 5.4).

In dry mid altitude AEZ, stem borer infestation was computed at 22.47±7.42%. There was no significant difference in infestation levels across sampling distances ($F_{3,51}$=0.4; $p$>0.05). Infestation levels were 22.4±6.7%, 24.8±2.1%, 20.6±2.6% and 20.7±3.3% at 0, 15, 30 and 45 km respectively (Table 5.3). A comparison between infestation levels recorded before *C. flavipes* release (92% in 1997) revealed a significant decrease from pre-release figures ($t_{54}$=41.63; $p$<0.05) (Table 5.4).

Overall stem borer infestation in moist mid altitude AEZ was relatively low (2.71±0.44%). There was no significant difference in stem borer infestation levels across distances sampled ( $\chi_3^2$ =1.53; $p$>0.05). Higher infestation levels recorded at 0 km (3.0±3.0%) and 15 km (3.1±0.6%) did not vary significantly from lower infestation levels recorded at 30 km (2.6±0.9%) and 45 km (2.0±1.1%) (Table 5.3). Results obtained showed a significant decrease in stem borer infestation levels when compared to levels obtained in 2006 ($V$=0, $p$<0.05) (Table 5.4).

### 5.3.4 Stem borer parasitism across the three agro-ecological zones

Stem borer parasitism in moist lowland AEZ, was 36.1±3.0%. Parasitism levels varied significantly with sampling distance ($F_{3,48}$=7.44; $p$<0.05) (Table 5.4). The highest rate of parasitism was recorded at parasitoid release sites (52.0±10.9%) and this was not significantly different from parasitism at farms situated 15 km from release points (49.1±4.2%). Parasitism in farms located 30 km (24.1±3.1%) and 45 km (28.5±5.4%) away from release points did not differ significantly with each other though there was significant variation with farms at 0 and 15 km points (Table 5.3). There was a significant increase in parasitism levels compared to results recorded during the last post release survey (3% in 1994) ($t_{51}$=10.96; $p$<0.05) (Table 5.4).

In dry mid altitude AEZ, stem borer parasitism was estimated at 25.3±3.3%. There was no significant difference in parasitism levels across distances sampled ($\chi_3^2$ =4.34; $p$>0.05) (Table 5.4). Parasitism levels were 26.6±7.6, 31.9±6.0, 23±5.8 and 14.9±6.7% at 0, 15, 30 and 45 km respectively (Table 5.3). A significant variation was observed when parasitism results from this study were compared to pre-release figures (10% in 1998) ($V$ = 1213, $p$<0.05) (Table 5.4).

Parasitism levels in the moist mid altitude AEZ were low (5.5±2.5%) compared to results from moist lowland and dry mid-altitude AEZ. There was no significant difference in parasitism levels across distances sampled ($\chi_3^2$ =0.86; $p$>0.05). No parasitoids were recovered at release points (0.0±0.0%). Parasitism progressively increased with distance, that is, 3.2±2.4, 9.9±6.8 and 6.3±6.3% at 15, 30 and 45 km respectively (Table 5.3). There was significant variation in parasitism rates when results of this study were compared to pre-release rates obtained in 1999 (4%) ($V$=195, $p$<0.05) (Table 5.4).

**Table 5.3:** Mean stem borer infestation and parasitism ( $\bar{x} \pm SE$ ) in different AEZs in reference to initial *Cotesia flavipes* release sites

| | **SAMPLED AGRO-ECOLOGICAL ZONES (AEZ)** | | | | | |
|---|---|---|---|---|---|---|
| | **Moist lowland** | | **Dry mid-altitude** | | **Moist mid-altitude** | |
| Distance from release points | Infestation | Parasitism | Infestation | Parasitism | Infestation | Parasitism |
| 0 KM | 11.0±3.5[a] | 52.0±10.9[b] | 22.4±6.7[a] | 26.6±7.6[a] | 3.0±3.0[a] | 0.0±0.0[a] |
| 15 KM | 28.6±5.2[b] | 49.1±4.2[b] | 24.8±2.1[a] | 31.9±6.0[a] | 3.1±0.6[a] | 3.2±2.4[a] |
| 30 KM | 32.7±3.4[b] | 24.1±3.1[a] | 20.6±2.6[a] | 23.0±5.8[a] | 2.6±0.9[a] | 9.9±6.8[a] |
| 45 KM | 35.6±9.8[b] | 28.5±5.4[a] | 20.7±3.3[a] | 14.9±6.7[a] | 2.0±1.1[a] | 6.3±6.3[a] |
| *df* | 3, 48 | 3, 48 | 3, 51 | 3, 51 | 3, 37 | 3, 37 |
| *F* value | - | 7.44 | 0.4 | - | - | - |
| $\chi^2$ value | 10.72 | - | - | 4.34 | 1.53 | 0.86 |
| *p*-value | 0.013 | 0.00034 | 0.75 | 0.23 | 0.67 | 0.84 |

Percentage infestation and parasitism $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different ($p$>0.05)

**Table 5.4:** Stem borer infestation and parasitism levels before and after *C. flavipes* release in moist lowland, dry mid altitude and moist mid altitude tropics in Kenya.

| AEZ | Period | Infestation | Period | Parasitism |
|---|---|---|---|---|
| Moist lowland | Pre-release (1983) | 92[a] | Post-release (1994) | 3 |
| | 2014 | 28.9±3.1[b] | 2014 | 36.1±3.0[b] |
| | *V*-value | 3 | *t* value | 10.96 |
| | | | *df* | 51 |
| | *p*-value | 4.26E-10 | *p*-value | 5.22E-15 |
| Dry mid-altitude | Pre-release (1997) | 92[a] | Pre-release (2001) | 10[a] |
| | 2014 | 22.5±7.4[b] | 2014 | 25.3±3.3[b] |
| | *t* value | 41.63 | *V*-value | 1213 |
| | *df* | 54 | | |
| | *p*-value | 2.20E-16 | *p*-value | 0.0002 |
| Moist mid-altitude | Post-release (2006) | 37.4±3.8[a] | 1999 | 4[a] |
| | 2014 | 2.7±0.4[b] | 2014 | 5.5±2.5[b] |
| | *V*-value | 0 | *V*-value | 195 |
| | *p*-value | 2.24E-08 | *p*-value | 0.0009 |

Percentage infestation and parasitism $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different ($p$>0.05)

### 5.3.5 Climatic variables affecting infestation and parasitism

Climatic variables which exerted the greatest effect on infestation and parasitism are shown in table 5.5. Mean diurnal range, temperature seasonality and annual precipitation exerted the greatest effect on infestation ($p$<0.05). Annual mean temperature, mean diurnal range and isothermality exerted the greatest effect on parasitism ($p$<0.05).

**Table 5.5**: Effects of climatic parameters on infestation and parasitism

| **BIOCLIM variable** | ***df*** | ***F* value** | ***p* value** |
|---|---|---|---|
| **Infestation** | | | |
| Annual mean temp | 1 | 8.76 | 0.004 ** |
| Mean diurnal range | 1 | 48.53 | 1.607e-10 *** |
| Isothermality | 1 | 8.00 | 0.005 ** |
| Temperature seasonality | 1 | 20.03 | 1.683e-05 *** |
| Maximum temperature of warmest month | 1 | 7.62 | 0.007 ** |
| Annual precipitation | 1 | 15.91 | 0.0001 *** |
| Precipitation of coldest quarter | 1 | 3.63 | 0.059 . |
| **Parasitism** | | | |
| Annual mean temp | 1 | 20.56 | 1.326e-05 *** |
| Mean diurnal range | 1 | 12.47 | 0.00058 *** |
| Isothermality | 1 | 18.96 | 2.740e-05 *** |
| Temperature seasonality | 1 | 3.81 | 0.053 . |
| Precipitation of wettest quarter | 1 | 5.86 | 0.017 * |
| Precipitation of driest quarter | 1 | 7.65 | 0.007 ** |

### 5.4 Discussion

In classical biological control, natural enemies are released and relied upon to establish and spread beyond release points. *Cotesia flavipes* was released in moist lowland AEZ in 1993 (Overholt *et al*., 1994a). Releases were also done in moist mid-altitude AEZ in 2000 and central and eastern parts of dry mid-altitude in 2002 (*icipe* and KALRO, unpublished data). It is important to note that before 2002, release of *C. flavipes* had been carried out in experimental fields in southern part of dry mid-altitude AEZ (Songa *et al*., 1999). Following the afore-mentioned releases, establishment and spread of the parasitoid had only been documented for the moist lowland AEZ (Overholt *et al*, 1994, Omwega *et al.*, 1997; Overholt *et al*., 1997). This study is hereby confirming the establishment and spread of *C. flavipes* in dry mid-altitude and moist mid-altitude AEZ. It is difficult to attribute establishment in the

moist mid altitude AEZ to the official releases in 2000 or accidental escape from *icipe*, Mbita point field station where they had been quarantined for pre-release studies as the presence may be from both sources (Omwega *et al*., 1995).

The main objective of *icipe*'s Biological Control programme was to reduce stem borer pest populations in maize and sorghum crop by introducing *C. flavipes* in areas where *C. partellus* was the main pest (Overholt *et al*., 1994a). Across the three agro-ecological zones, *C. partellus* dominated the stem borer pest community corroborating reports from moist lowland (Nye, 1960; Van Hamburg, 1979, Overholt *et al*, 1994) and dry mid-altitude tropics (Songa *et al*., 1999, 2002a; Ong'amo *et al*., 2006a). However, this is contrary in moist mid-altitude tropics where previous reports indicated that *B. fusca* dominated (Zhou *et al*., 2001; Ong'amo *et al.*, 2006a). Formerly known to be a lowland tropical and dry mid-altitude species, the dominance demonstrated by *C. partellus* from these results validate findings that the pest's distribution has expanded into mid and high altitude areas. Further to this, in two of the zones, *C. partellus* was the only stem borer host from which parasitoids were recovered. This corroborates reports that the exotic stem borer has a larger number of parasitoids attacking it in comparison to native borers (Zhou *et al*., 2003).

This study was carried out to assess the current stem borer and parasitoid levels in order to discern whether the introduced parasitoid has succeeded or failed in suppressing target pest population. Observed stem borer infestation levels were significantly lower compared to figures recorded prior to release of parasitoid's in all the three agro-ecological zones. In the moist lowland tropics, stem borer infestation levels on maize farms were estimated at 92% (Seshu Reddy *et al*., 1983) before the parasitoid was released. Surveys carried out in 2005 yielded lower infestation levels (77.2%, Ong'amo *et al*., 2006a). Stem borer infestation levels recorded from this study are significantly lower. Together, these data shows that stem borer infestation rate has been decreasing steadily over the years. A similar trend was observed in

dry mid-altitude AEZ where infestation levels were higher (82-92%) before release of *C. flavipes* (Songa *et al.,* 2001) but reduced to 62% later after release and to the current level of 22.47%. In the moist mid-altitude AEZ, previous reports of infestation levels were estimated to vary between 8-100% (Seshu Reddy *et al*., 1983) and later to 33.72% (Ongámo *et al*., 2006a) and reduced further to the current level of 2.71%. Reduction in stem borer infestation levels is evident across all the three AEZs, an indication that *C. flavipes* has successfully suppressed the stem borer pest population as sites with lower infestation levels exhibited higher parasitism.

Parasitism by *C. flavipes* following release has been assessed through different studies. In moist lowland AEZ, post release parasitism rates were recorded at 0.05 to 3 (Overholt *et al*., 1997), 7.1% in 1997 (Overholt *et al*., 1997) and 13% in 1999 (Zhou *et al*., 2001). This study demonstrated a significant increase in parasitism levels. Similarly, in dry mid-altitude AEZ, a rise in parasitism level was observed. Parasitism levels recorded in the central and eastern parts of this zone were significantly higher than levels observed in the southern part (Songa *et al.*, 2001). In moist mid-altitude, parasitism rates were previously recorded at 3.5% (Khan *et al.,* 1997), 2.2% (Ogeda, 1999) and 4% in surveys carried out in 1999 (Zhou and Overholt, 2001). Current parasitism levels demonstrated a significant increase. This study thus confirms a rise in parasitism levels which in turn have suppressed pest populations in all the three agro-ecological zones.

Temperature and precipitation exerted the greatest effect on both infestation and parasitism levels, results that corroborate reports by Van Lenteren *et al*. (2006) and Jiang *et al*. (2004). These parameters define the synchrony between the life cycle of stemborers and *C. flavipes,* an interaction that may explain the impact of natural enemy on the pest population. This knowledge can be used in more informed choice of release localities both within and across Kenya's borders.

Generally, despite a rise in parasitism levels by *C. flavipes* across the surveyed regions, observed levels were still low compared to what is in the pest's native range. In India, 80% parasitism by *C. flavipes* is observed in maize (Singh *et al.*, 1975) with 0-43% being recorded in maize-sorghum intercrops (Subba Rao *et al*., 1969). Nonetheless, this scientific investigation has birthed some knowledge regarding the success of *C. flavipes* across various habitat types. Kenya has now joined the list of various other countries in which *C. flavipes* has successfully decreased *C. partellus* populations.

# CHAPTER SIX

# 6.0 CEREAL STEM BORER SPECIES COMPLEX AND ESTABLISHMENT STATUS OF HIGHLAND *Cotesia sesamiae* (CAMERON) IN COASTAL TAITA HILLS, KENYA

## 6.1 Introduction

*Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), is one of the important field pests of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L) Moench) in Sub-Saharan Africa (SSA) (Nye, 1960; Seshu Reddy, 1983). Before the introduction of maize from MesoAmerica in the 16$^{th}$ century (Chastanet, 1998) and the extensive cultivation of sorghum, *B. fusca* subsisted on non-cereal wild host plants (Nye, 1960). Following the introduction and extensive cultivation of these crops, *B. fusca* shifted to cultivated habitat (Sezonlin *et al.*, 2006) where yield losses associated with its infestation was estimated at 10-14% (Van den Berg *et al.*, 1991). In Kenya, these losses are mostly incurred in regions considered the 'bread basket' of the country (Hassan, 1998; De Groote, 2002) and characterized as high potential zones (highland tropics, moist transitional and moist mid-altitude) (Corbett, 1998). Distribution of *B. fusca* is favoured by low temperatures, an attribute found in the afore mentioned high potential zones (Harris and Nwanze, 1992; Kfir, 1995; 1998; Ngi-Song *et al*., 1995; Ndemah *et al.*, 2001; Le Rü *et al.*, 2006; Ong'amo *et al*., 2006a).

*Busseola fusca* infestations cause considerably higher yield losses among small scale farmers who cannot afford chemicals but rely mainly on naturally available enemies. Various natural enemies including forty parasitoids, five predators and eight pathogens have been reported to attack *B. fusca* in East Africa (Bonhof *et al.*, 1997). Of these, the most common is the braconid, larval endoparasitoid, *Cotesia sesamiae* (Cameron) (Mohyuddin and Greathead, 1970; Omwega *et al*., 1995). In addition to *B. fusca*, *C. sesamiae* can attack *Chilo partellus* (Swinhoe), *Chilo orichalcociliellus* (Strand) and *Sesamia calamistis* Hampson (Polaszek and

Walker, 1991). Several strains of *C. sesamiae* varying in their insect host ranges have been found in Africa (Mucheru *et al*., 2009). In Kenya, studies have shown that two *C. sesamiae* biotypes with variation in developmental success in *B. fusca* exist. The virulent strain (found in highlands) is able to develop successfully while the avirulent strain (found at the coast) is encapsulated by *B. fusca* (Ngi-Song *et al.,* 1995; 1998; Mochiah *et al*., 2002; Gitau *et al*., 2006). Evolution of biotypes were the result of trade-offs brought by local adaptation to host community structure (Dupas *et al*., 2008) and is of vital importance in pest management.

*Coteia sesamiae* distinct populations have been utilized in the biological control redistribution programme during which natural enemies were exchanged between African regions and different localities within a country to manage indigenous stem borer pests (Schulthess *et al*., 1997). In Kenya, the virulent highland *C. sesamiae* population was collected and released at three localities in Taita Hills in 2006 (*icipe*, unpublished data). However, no post release surveys have been undertaken to ascertain the establishment and spread of the parasitoid from release sites, a gap that was addressed through this study.

## 6.2 Methodology

### 6.2.1 Description of the study area

This study was undertaken in Taita Hills at sites where *C. sesamiae* had been released. Survey farms were marked around Josa, Wesu and Prison's farm (Fig. 6.1). Taita Hills is in the coastal region and stretches across mid to high elevations. The areas within the mid elevations lie between 1000-1800masl, with temperatures ranging within 17-32°C and receive between 500-1000mm of rainfall annually. These areas are generally moist. The areas within high elevations lie above 1800masl with temperatures ranging within 7-24C° and receive above 1000mm of rainfall annually. These areas are generally wet (Corbett, 1998). Taita hills has bimodal rainfall pattern with long rains received between March to May/June and short rains falling from September/ October to December.

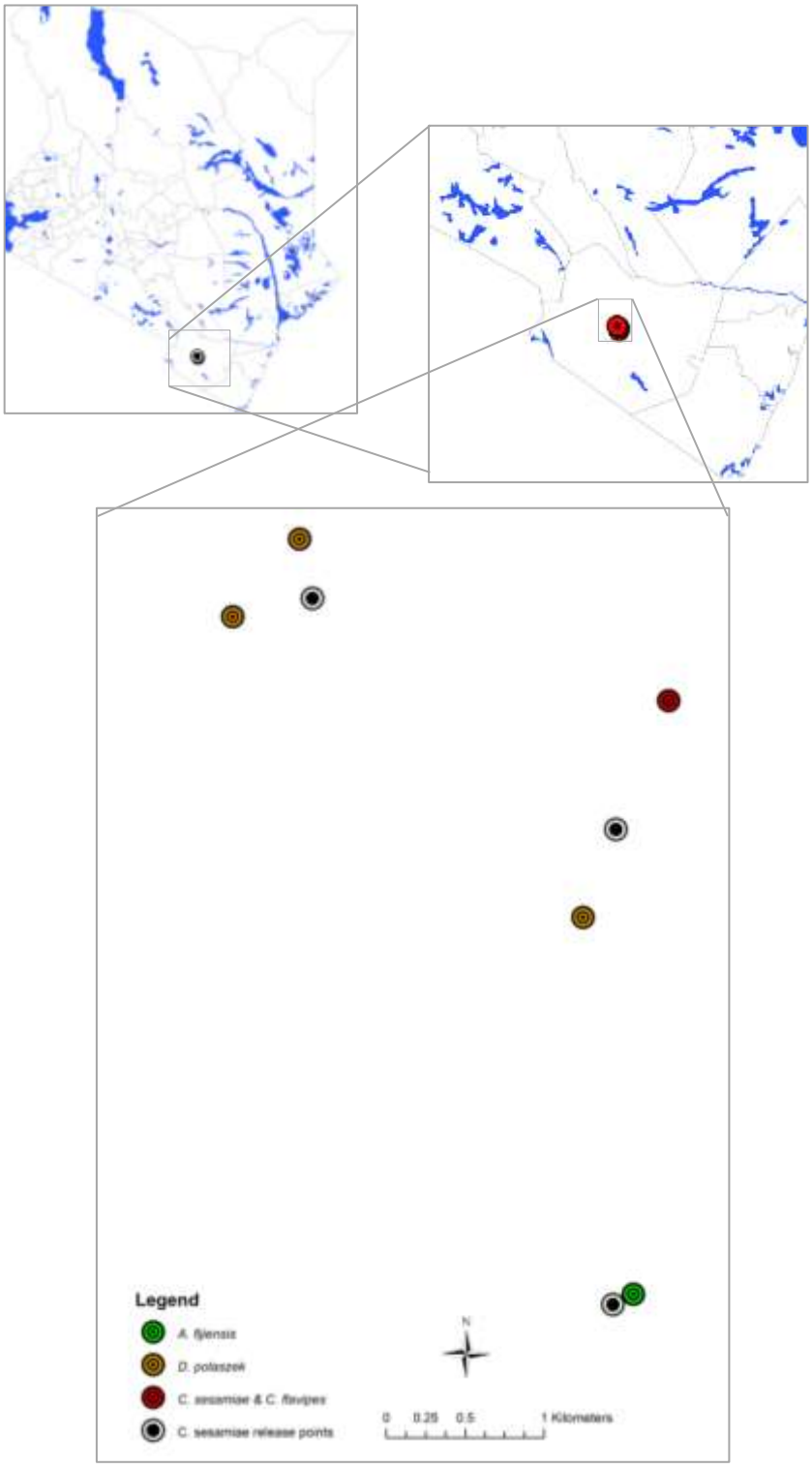



**Figure 6.1:** Points at which various parasitoids were recovered during the long rains of 2014 in Taita Hills.

### 6.2.2 Sampling protocol

A total of 12 farms were sampled during the long rains of 2014 and in each farm immature stem borer stages were sampled, reared and identified following the protocol detailed under General Materials and Methods in Chapter Three. Larvae which spun cocoons were placed in empty vials and emerging parasitoids were identified and counted. *Cotesia flavipes* and *C. sesamiae* were morphologically separated using the shape of male genitalia (Kimani-Njogu and Overholt, 1997).

### 6.2.3 Statistical analyses

Percentage infestation was computed by expressing the number of infested plants as a percentage of the total number of plants inspected. Larval densities were obtained by expressing the total number of stem borer larvae collected from dissected plants as a proportion of number of infested plants. To estimate percentage parasitism, suitable larval stages that were parasitized were expressed as a proportion of the total number of stem borer larvae collected. Percentage infestation and parasitism were tested for normality using Shapiro-Wilk normality test after which data that failed ($p<0.05$) were transformed. Data that failed to normalize after transformation were subjected to Kruskal-Wallis rank sum test and significantly different means separated using Nemenyi post-hoc test ($p<0.05$). Wilcoxon rank sum test was used to compare rates of infestation and parasitism computed during this study with parasitoid pre-release rates.

## 6.3 Results

### 6.3.1 Stem borer species composition and diversity

Three species of stem borers; *B. fusca*, *C. partellus* and *S. calamistis* were found occurring in Taita Hills. The most dominant was *B. fusca* which occurred at altitudes ranging within 1362-1736masl. *Busseola fusca* constituted 85.5% of the total stem borers collected. *Chilo partellus* which accounted for 9.7% of stem borers collected was found at 1099-1453masl. The least abundant stem borer species was S. *calamistis* which constituted 4.8% and was found at altitudes ranging within 1362-1712masl (Table 6.1). Mean larval density of the three stem borer species varied significantly ( $\chi_2^2$ =17.64; $p$<0.05) (Table 6.1). *Busseola fusca* exhibited a significantly higher larval density (2.5±0.5) in comparison to *C. partellus* (0.3±0.1) and *S. calamistis* (0.1±0.05). Mean larval density of *C. partellus* and *S. calamistis* did not show significant difference.

**Table 6.1:** Percentage composition, mean larval density and altitude at which stem borer species were recovered during the long rains of 2014 in Taita Hills

| Stem borer species | % composition | Larval density ( $\bar{x}$ ±SE) | Altitude (masl) |
|---|---|---|---|
| *Chilo partellus* | 9.7 | 0.3±0.1[a] | 1099-1453 |
| *Sesamia calamistis* | 4.8 | 0.1±0.1[a] | 1362-1712 |
| *Busseola fusca* | 85.5 | 2.5±0.5[b] | 1362-1736 |
| $\chi^2$ value | | 17.64 | |
| *df* | | 2 | |
| *p* value | | 0.00015 | |

Larval density $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different ($p$>0.05)

### 6.3.2 Parasitoid and hyperparasitoid species composition and diversity

Parasitoid species recovered from Taita Hills were *Dolichogenidea polaszeki* Walker, *C. sesamiae*, *C. flavipes* and the hyperparasitoid *Aphanogmus fijiensis* (Ferriére). The most dominant parasitoid was *C. sesamiae* which constituted 35.0% of the parasitoids collected and was only recovered from *C. partellus*. The exotic *C. flavipes* was recovered from *C. partellus*, while *Dolichogenidea polaszeki* was recovered from *B. fusca* larvae only. No parasitoid was recovered from *S. calamistis* despite the availability of the appropriate larval stages in the field (Table 6.2). Parasitism among the parasitoid species was not significantly different ($\chi^2_3$=2.56; $p$>0.05). The solitary larval parasitoid *D. polaszeki* parasitized the highest number of stem borers though the *C. sesamiae* and *C. flavipes* numbers were higher owing to their gregarious nature (Table 6.2).

**Table 6.2:** Parasitoid species recovered, their percentage composition, stem borer species and life stage attacked and their respective rates of parasitism during long rains of 2014 in Taita Hills

| Parasitoid species | % composition | Guild | Stem borer species parasitized | Parasitism ($\bar{x}$ ±SE) |
|---|---|---|---|---|
| *D. polaszeki* | 5.1 | Larval | *B. fusca* | 3.1±1.8[a] |
| *C. sesamiae* | 35.0 | Larval | *C. partellus* | 0.4±0.4[a] |
| *C. flavipes* | 29.1 | Larval | *C. partellus* | 0.8±0.8[a] |
| *A. fijiensis* | 30.8 | Hyperparasitoid | *C. partellus* | 0.7±0.7[a] |
| $\chi^2$ value | | | | 2.56 |
| *df* | | | | 3 |
| *p* value | | | | 0.47 |

Percentage parasitism by parasitoid species mean $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different ($p$>0.05)

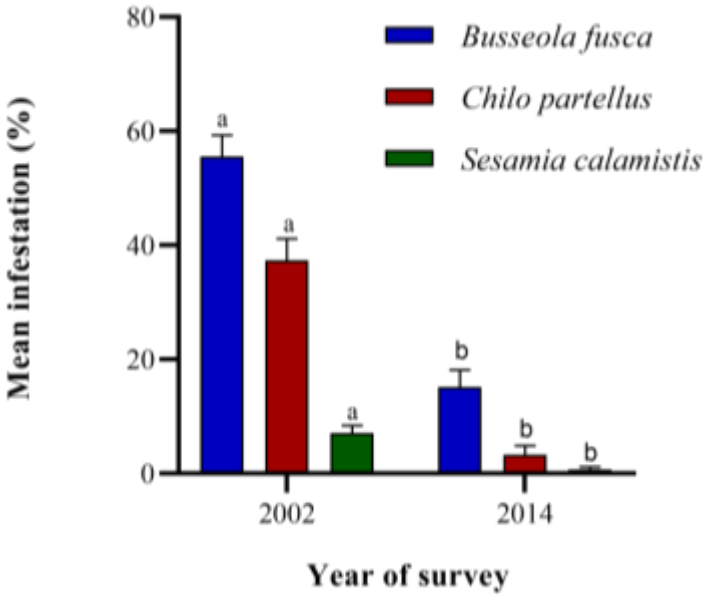
### 6.3.3 Stem borer infestation and parasitism levels

Mean stem borer infestation levels in Taita Hills were 19.2±2.5%. Infestation was significantly different among the three stem borer species ($\chi^2_2$=16.86; $p$<0.05). Mean infestation by *B. fusca* was 15.2±3.0% which was significantly higher than infestation by *C. partellus* (3.3±1.6%) and *S. calamistis* (0.7±0.4%). Mean infestation levels by *C. partellus* and *S. calamistis* were not significantly different (Table 6.3). The current record of *B. fusca* infestation was significantly lower than figures recorded during a *C. sesamiae* pre-release survey in 2002/2003 ($V$=0; $p$<0.05). Significantly lower percentage infestation by *C. partellus* and *S. calamistis* ($V$=0; $p$<0.05) was also observed in comparison to pre-release results (Fig. 6.2a).

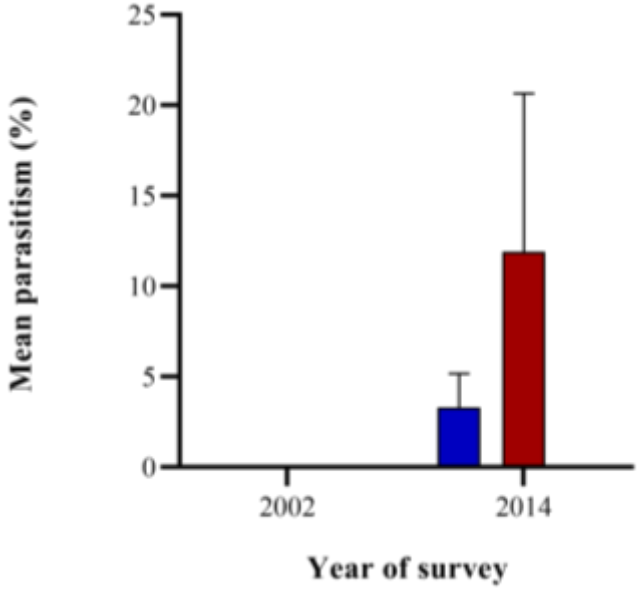
**Table 6.3**: Percentage infestation and parasitism of stem borer species recovered in Taita Hills during long rains of 2014.

| Stem borer species | % infestation | % parasitism |
|---|---|---|
| *Chilo partellus* | 3.31±1.6[a] | 11.9±8.8[a] |
| *Sesamia calamistis* | 0.70±0.4[a] | 0.0±0.0[a] |
| *Busseola fusca* | 15.2±3.0[b] | 3.3±1.9[a] |
| $\chi^2$ *value* | 16.86 | 2.95 |
| *df* | 2 | 2 |
| *p value* | 0.00022 | 0.23 |

Overall levels of parasitism were 10.8±4.3%. There was no significant difference in parasitism rates among the stem borer species ($\chi^2_2$=2.95; $p$>0.05). Though *C. partellus* exhibited a higher parasitism rate (11.9±8.8%), it was not significantly different from *B. fusca* (3.3±1.9%) and *S. calamistis* which did not yield any parasitoids (Table 6.3). There was no significant difference in *C. partellus* parasitism rate recorded during this study in comparison with pre-release records ($V$=3; $p$>0.05). The same trend was demonstrated by *B. fusca* ($V$=6; $p$>0.05) (Fig. 6.3b).




**(a)**




**(b)**

**Figure 6.2:** (a) Comparison of *C. partellus*, *S. calamistis* and *B. fusca* infestation and (b) parasitism levels before and after *C. sesamiae* release in Taita Hills. Percentage infestation with same letters above the bars are not significantly different ($p$>0.05)

## 6.4 Discussion

Three stem borer species were found in Taita Hills; namely *B. fusca*, *C. partellus* and *S. calamistis*, results that corroborated findings by Seshu Reddy (1983). Among the above three pest species, *B. fusca* is reportedly the most important pest in highlands of East and South Africa (Nye, 1960; Kfir *et al.*, 2002a). This pest has been found occurring above 1000masl (Seshu Reddy, 1983; Harris and Nwanze, 1992, Zhou *et al.*, 2001; Mulaa, 1995; Le Rü *et al.*, 2006; Ong'amo *et al.*, 2006b; Calatayud *et al.*, 2014), areas considered high altitude. Considering that Taita Hills lies in mid to high altitudinal range, the stem borer community was dominated by *B. fusca*, upholding findings by previous researchers. Earlier reports from mid to high altitudinal zones documented *B. fusca* as the predominant species followed by *S. calamistis*. However, results obtained from this survey indicated that *C. partellus* was the second most dominant stem borer species. In addition to the growing proportions of *C. partellus*, it was found occurring at altitudes ranging between 1099 to 1453masl. Previously known to occur in lowland tropical and dry mid altitude zones (below 600masl) (Nye, 1960; Seshu Reddy, 1983), these results validate findings that the exotic *C. partellus* distribution is expanding into higher altitude areas (Ong'amo *et al*., 2006a). With it's superior competitive abilities, is it possible that it is displacing *S. calamistis* as an important pest in mid-altitude agro-ecological zones? This proposition is further supported by the observed average larval densities and rates of infestation of *C. partellus* which were also higher than *S. calamistis*. Predictive modelling studies done in Taita Hills forecast a future increase in proportion of *C. partellus* which may eventually become an economically important pest in the area (Mwalusepo *et al*., 2015).

The objective of the redistribution programme was to suppress *B. fusca* population in Taita Hills. However, parasitism rates by individual parasitoid species remained low. The same trend was demonstrated by parasitism figures obtained on individual stem borer species.

Various researchers also reported low parasitism by indigenous parasitoids (Oloo, 1989; Bahana, 1990, Overholt *et al*., 1994a). This study showed that parasitism level have not changed since the release of *C. sesamiae* in Taita Hills in 2006.

Contrary to the redistribution programme's expectation, *C. sesamiae* was not recovered from *B. fusca* which was the principal target stem borer species, neither was it recovered from *S. calamistis* which spatially overlaps in distribution with *B. fusca.* Interestingly, *C. sesamiae* was recovered from *C. partellus*. If *C. sesamiae* had been recovered from *B. fusca* during this survey, this could have warranted further tests (gel electrophoresis) to distinguish if it was the virulent highland or the avirulent coastal biotype. Since it was recovered from *C. partellus*, it was most probably the avirulent, coastal biotype as it was unable to parasitize *B. fusca* despite the host's availability as depicted by the stem borer composition results. Further to this, *C. sesamiae* recovery from *C. partellus* confirmed that indigenous parasitoids expanded their host range to include the exotic stem borer (Kfir, 1992; Zhou *et al.*, 2003). The other parasitoid, *C. flavipes,* was only recovered from its old association host, *C. partellus.*

In conclusion, the highland *C. sesamiae* did not establish in Taita Hills. This is explained by the low stem borer parasitism rates coupled with high *B. fusca* infestation rates. Parasitoid establishment and spread is dependent on suitable climatic parameters and host availability. Considering that climatic matching was done between the source of *C. sesamiae* and the recipient ecosystem, failure to establish cannot be attributed to difference in climatic parameters nor to non-availability of suitable hosts. Non-establishment in this area, may be attributed to the single release as CBC proponents agree that parasitoid establishment is enhanced by boosting natural enemy population through multiple releases (Sanda and Sunusi, 2014).

# CHAPTER SEVEN

# 7.0 PROSPECTING FOR FINE SCALE ESTABLISHMENT OF EXOTIC STEM BORER PUPAL PARASITOID *Xanthopimpla stemmator* THUNBERG IN KENYA

## 7.1 Introduction

Lepidopteran stem borers constitute important biotic factors that constrain maize and sorghum production in Sub Saharan Africa (SSA) (Brownbridge, 1991; Odindo, 1991; Schulthess *et al.*, 1997; 2007). However, losses associated with stem borer pest infestation varies among regions in SSA depending of stem borer community composition. In East Africa, the economically important lepidopteran stem borers are *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Family: Noctuidae) and *Chilo orichalcociliellus* Strand and *Chilo partellus* (Swinhoe) (Family: Crambidae) (Nye 1960, Bonhof *et al*. 1997, Overholt *et al*., 2001). All the aforementioned pest species are indigenous to African continent except for *C. partellus* (Nye 1960; Bleszynski, 1970; Van Hamburg, 1979) which was accidentally introduced from Asia in 1930s (Tams, 1932). Since its introduction, *C. partellus* has become one of the most economically important pests with losses associated with its infestation varying between 73 and 100% in maize, and 88 and 100% in sorghum (Seshu Reddy 1983; 1988; Ampofo, 1986; Seshu Reddy and Walker, 1990).

Due to economic importance of *C. partellus*, different management strategies including chemical, cultural, habitat management and host plant resistance have been utilized to reduce its populations (Seshu Reddy, 1985; Bonhof 2000; Kfir *et al*. 2002). Focus shifted towards biological control in order to find ecologically sound, technically and economically feasible techniques (De Bach, 1974; Sanda and Sunusi, 2014). A wide range of indigenous parasitoids including *Cotesia sesamiae* (Cameron), *Dolichogenidea polaszeki* Walker, *Chelonus curvimaculatus* (Cameron), (larval parasitoids), *Pediobius furvus* (Gahan), and *Dentichasmias buseolae* (Heinrich) (gregarious pupal parasitoids) and *Psilochalsis*

*soudanensis* (Steffan) (solitary pupal parasitoid) expanded their range to include this exotic species (Kfir 1992; Zhou *et al.*, 2003). However, the effect of this native parasitoid assemblage has been recorded at less than 5% and is considered negligible (Mohyuddin and Greathead, 1970; Oloo and Ogeda, 1990; Bonhof *et al.* 1997; Zhou *et al.*, 2003). International Centre of Insect Physiology and Ecology's (*icipe*) biological control programme thus spearheaded the importation and eventual release of the exotic, larval endoparasitoid *C. flavipes* Cameron from *C. partellus'* native range in 1993 (Overholt *et al*., 1994a).

To further suppress *C. partellus* population and build on stem borer natural enemy complex in Kenya, a solitary, idiobiont, pupal endoparasitoid, *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) was imported from South Africa in 2001. *Xanthopimpla stemmator* which is Asian in origin, is known to parasitize pupae of various lepidopteran stem borers. Prior to release in 2002, various pre-release studies were carried out regarding host suitability (Gitau *et al*., 2007), interspecific competition with native parasitoid species (Muli *et al*., 2006) and its performance in the field (Muturi *et al*., 2005). After these studies, releases were done in the Eastern region of Kenya, at two locations, Machakos and Kitui. Despite its potential, no post release assessments have been carried out to confirm its establishment. This study was thus undertaken to document fine scale establishment status and spread of *X. stemmator* since it's release in Kenya in 2002.

## 7.2 Methodology

### 7.2.1 Description of study area

This study was carried out in the Eastern region of Kenya where pupal parasitoid, *X. stemmator*, was released in 2002. *Xanthopimpla stemmator* was released on various farms in Machakos and Kitui counties (Fig. 7.1). The Eastern region is located in dry mid-altitude agro-ecological zone, characterized by temperatures ranging from 14 to 33°C. The area lies at

an altitude of 700-1,400 masl and receives annual rainfall varying between 300 and 550mm (Corbett, 1998).

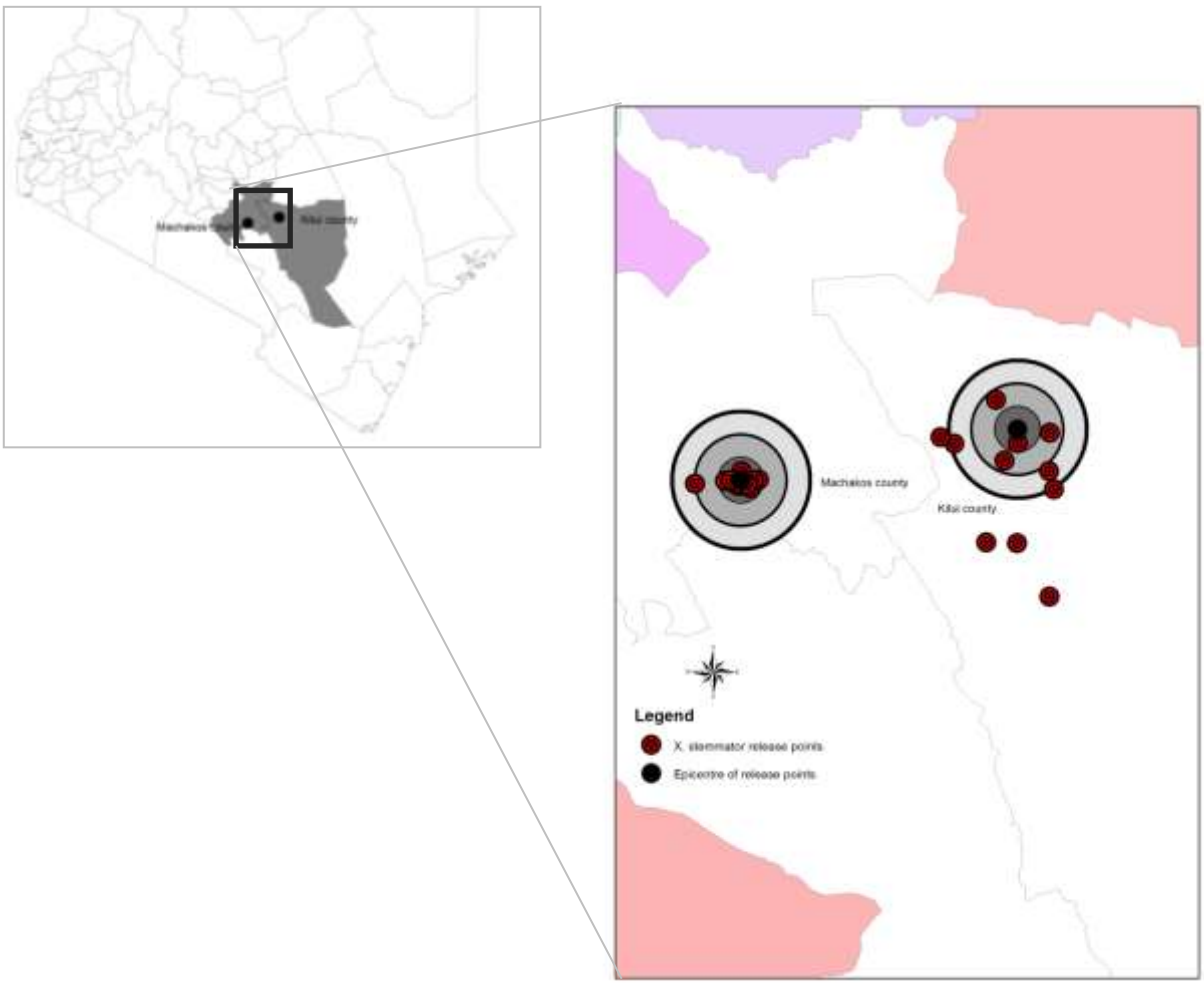



**Figure 7.1**: Release and sampled sites for *X. stemmator* in Machakos and Kitui counties, Eastern region of Kenya in June 2014

### 7.2.2 Sampling for stem borers

Maize farms around *X. stemmator* release sites were identified and marked for assessment of stem borer infestation and parasitism during the long rains of 2014. Marked farms radiated outwards along transects in the four cardinal compass directions as the terrain allowed. Stem borer infestation levels were assessed in farms at intervals of 15, 30 and 45 km along

transects laid in the cardinal directions from the release points (Fig. 7.1). Stem borer sampling, rearing and identification followed the protocol laid out in General Materials and Methods in Chapter three.

### 7.2.3 Statistical analyses

The number of infested plants was expressed as a percentage of the total plants inspected in respective fields and resulting data was used to compute percentage stem borer infestation. At each sampling distance, inspected farms were treated as replicates and the data pooled before analysis. Parasitoid cocoons that were spun from appropriate larval stages were expressed as a proportion of the respective field densities in order to compute percentage parasitism. Percentage infestation and parasitism were subjected to the normality test and data that failed the normality test were appropriately transformed before further analysis. Normally distributed data was analysed using One-Way ANOVA and significantly different means were separated using Tukey's HSD test. Data which failed the normality test was subjected to Kruskal-Wallis rank sum test and significantly different means were separated using Nemenyi post-hoc test ($p<0.05$). One sample *t*-test and Wilcoxon rank sum tests were used to compare mean infestation and parasitism levels obtained before and after parasitoid release.

## 7.3 Results

### 7.3.1 Stem borer species composition and diversity

A total of 5,500 maize plants were sampled from 55 farms surveyed during the study. Three stem borer species, *C. partellus*, *B. fusca* and *S. calamistis* were identified from the sampled larvae. *Chilo partellus* was the most dominant pest constituting 71.2% of the total stem borer population collected. The other two species, *S. calamistis* and *B. fusca,* were generally low constituting 26.0 and 2.8% of the total stem borer community respectively (Table 7.1).

**Table 7.1**: Stem borer species recovered, their percentage composition and pest density per infested plant in Eastern region of Kenya during the long rains of 2014

| Stem borer species | % composition | Larval density ( $\bar{x}$ ±SE) |
|---|---|---|
| *Chilo partellus* | 71.2 | $2.4\pm0.4^{b}$ |
| *Sesamia calamistis* | 26 | $0.8\pm0.2^{a}$ |
| *Busseola fusca* | 2.8 | $0.1\pm0.0^{a}$ |
| $\chi^2$ value | | 74.92 |
| *df* | | 2 |
| *p* value | | 2.20E-16 |

Larval density $(\bar{x} \pm SE)$ within column followed by the same lower case superscripts are not significantly different ($p$>0.05)

### 7.3.2 Parasitoid and hyperparasitoid species composition and diversity

Six parasitoid species were recovered during the survey with the ecological congeners, *C. flavipes* and *C. sesamiae* being the most abundant (Table 7.2). In addition to parasitoids, hyperparasitoid, *Aphagnomus fijiensis* (Ferriére) was also recovered from some larvae. Larval parasitoids dominated the stem borer parasitoid complex in the study area with only one pupal parasitoid, *Dentichasmias busseolae* Heinrich identified from the collection.

**Table 7.2:** Parasitoids recovered, their percentage composition, guild and host species in Eastern region of Kenya during the long rains of 2014

| Parasitoid species | Composition (%) | Guild | Stem borer species parasitized |
|---|---|---|---|
| *Cotesia flavipes* (Cameron) | 76.5 | Larval | *C. partellus, S. calamistis, B. fusca* |
| *Cotesia sesamiae* (Cameron) | 7.6 | Larval | *C. partellus, S. calamistis, B. fusca* |
| *Dolichogenidea polaszeki* Walker | 0.1 | Larval | *S. calamistis* |
| *Chelonus curvimaculatus* (Cameron) | 0.4 | Larval | *C. partellus, B. fusca* |
| *Dentichasmias busseolae* Heinrich | 0.2 | Pupal | *C. partellus* |
| *Sturmiopsis parasitica* (Curran) | 0.0 | Larval/pupal | *B. fusca* |
| *Aphanogmus fijiensis* (Ferriére) | 15.1 | Hyperparasitoid | *C. partellus, S. calamistis* |

### 7.3.3 Stem borer infestation and parasitism levels

During the survey, overall stem borer infestation was estimated to be 22.5±7.4% (Table 7.3). Further analysis revealed no difference in infestation across distances from parasitoid release points ($F_{3,51}$=0.4; $p$>0.05) (Table 7.3). Stem borer infestation levels significantly reduced after the release of *C. flavipes* and *X. stemmator* ($t_{54}$=41.6; $p$<0.05). Stem borer parasitism levels in this region were recorded at 25.3±3.3%. This was a significant increase from parasitism levels previously recorded following parasitoid release ($V$=1213; $p$<0.05). Pupal parasitism was estimated at 0.03±0.02% (Table 7.4).

**Table 7.3:** Stem borer infestation and parasitism levels ( $\bar{x} \pm SE$ ) across distances from release points of Kenya during the long rains of 2014

| Distance from release points | No. of farms | Infestation | Parasitism |
|---|---|---|---|
| 0 KM | 10 | 22.4±6.7[a] | 26.6±7.6[a] |
| 15 KM | 20 | 24.8±2.1[a] | 31.9±6.0[a] |
| 30 KM | 14 | 20.6±2.6[a] | 23.0±5.8[a] |
| 45 KM | 11 | 20.7±3.3[a] | 14.9±6.7[a] |
| *df* | | 3, 51 | 3, 51 |
| *F* value | | 0.4 | 4.34 |
| *p*-value | | 0.75 | 0.23 |

Percentage infestation and parasitism $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different ($p$>0.05)

**Table 7.4**: Overall stem borer infestation before and after parasitoid release in Eastern region of Kenya of Kenya during the long rains of 2014

| Period | Infestation (%) | Period | Parasitism (%) |
|---|---|---|---|
| Pre-release (1997) | 92[a] | Pre-release (2001) | 10[a] |
| 2014 | 22.5±7.4[b] | 2014 | 25.3±3.3[b] |
| *t* value | 41.63 | *V* value | 1213 |
| *df* | 54 | | |
| *p* value | 2.20E-16 | *p* value | 0.0002 |

Percentage infestation and parasitism $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different ($p$>0.05)

## 7.4 Discussion

Suppressing stem borer pest population is considered an important factor in enhancing maize production in tropical Africa. However, stem borer management interventions and their successful implementation varies among regions depending on the dominant/target pest species. During this study, *C. partellus* dominated the pest community in Eastern Kenya followed by *B. fusca* and *S. calamistis*, an observation that corroborated findings of Songa *et al*. (2002a, b; 2007). Except for *C. partellus*, all other stem borer species in the pest community are indigenous to the African continent. As an exotic species, *C. partellus* recruited several native natural enemies a characteristic that can explain the high number of natural enemies found associated with its larvae during the study. Similar observations were made in other studies during which native natural enemies reportedly expanded their host range to include the exotic *C. partellus* (Oloo and Ogeda, 1990; Kfir, 1992). Results obtained also uphold reports that *C. partellus* has a larger number of parasitoids attacking it in comparison to native stem borers (Zhou *et al*., 2003). This is attributable to it's dominance and thus availability for parasitization. Despite the high number of parasitoids associated with stem borers in this area, the list might not be exhaustive as the survey was limited to a certain distance and only on farms radiating from *X. stemmator* release points. Other researchers recovered much more parasitoids from the stem borer population in the same region (Songa *et al*., 2002a).

Growing dominance of *C. partellus* in the region due to limited success of indigenous natural enemies in suppressing its population informed the decision to introduce additional exotic natural enemies in the region. Larval parasitoid, *C. flavipes*, and pupal parasitoid, *X. stemmator*, were released in Eastern Kenya as part of classical biological control to augment population of indigenous natural enemies in the region. Collective action by the stem borer natural enemy assemblage within the Eastern region resulted in reduction of overall stem

borer infestation in comparison to levels observed before release of *C. flavipes* and *X. stemmator*. The observed reduction was consistent across all different sampled radii and similar patterns were observed with respect to parasitism levels. Generally, there was a considerable increase in parasitism compared to lower levels (0.1-5.69%) recorded before release of *X. stemmator* (Songa *et al.*, 2002a). Observations in the present study are consistent with findings of other studies that have shown an existence of positive relationship between the diversity of parasitoids and parasitism (Hawkins and Gagne, 1989; Hawkins and Gross, 1993). Despite the high diversity of parasitoids recovered during this survey, *D. busseolae* was the only pupal parasitoid recovered. It was however present in low numbers with significantly low resultant parasitism, results that are consistent with findings of previous studies (Mohyuddin and Greathead, 1970; Oloo and Ogeda, 1990). This study was undertaken 15 years after the release of *X. stemmator* in the region and contrary to research expectation, the study did not yield any *X. stemmator* specimen.

Pre-release host suitability studies revealed that *X. stemmator* has a broad host range and could successfully parasitize and develop in *C. partellus*, *S. calamistis* and *B. fusca* (Gitau *et al*., 2005). A range of reasons (excluding host suitability) could be advanced in an attempt to explain the lack of recovery within the surveyed fine scale. First, the releases were done on ten farms during short rains of 2002 in Kitui. However, no repeat releases were carried out. In Machakos, *X. stemmator* releases were done in the short rains of 2002 and 2003 and during the long rains of 2003 on an average of seven farms each time. CBC proponents agree that multiple releases boost the natural enemy population after the initial introduction in a new environment (Sanda and Sunusi, 2014). This is because the establishment process is marred by both biotic and abiotic factors whose effects can be abated by pumping in more and fresher individuals (Sanda and Sunusi, 2014). Biological control agent releases may need to be repeated sometimes over years to increase chances of establishment. Non-recovery of *X.*

*stemmator* is not unique to this study. In Mozambique, *X. stemmator* was only recovered during the release season and one year after its release but not in subsequent years (Cugala, 2007).

Secondly, the biological control programme's main objective that necessitated *X. stemmator*'s release was to suppress *C. partellus* population. *Chilo partellus* occurrence in wild habitat has been reported by various researchers (Songa *et al.,* 2002b, Ong'amo *et al*. 2006b; Otieno *et al*. 2006; Mohamed *et al*. 2007). Country-wide surveys on wild host plants in Kenya revealed that more than 95% of *B. fusca* and *C. partellus* were found on wild sorghum species providing a suitable refugia for *X. stemmator*. The wild habitat was however not sampled during this survey and this study cannot confirm the presence of *X. stemmator* in the wild. However, this gap needs to be explored before further decisions regarding the use of *X. stemmator* in management of *C. partellus* is made as wild host plants play an important role in the stem borer pest and parasitoid perennation (Muturi *et al.,* 2005; Mailafiya *et al.,* 2010).

Thirdly, an aspect of competition within the parasitoid community whose differentiation was demonstrated by the attack method used, was shown to be an important criterion in parasitoid selection (Muli *et al*., 2006). *Xanthopimpla stemmator* uses the "drill and sting" attack strategy (Smith *et al*., 1993) whereby the parasitoid pierces the stem to gain access to pupa in pupal chamber. While comparing the different attack strategies employed by pupal parasitoids, the "ingress and sting" attack strategy whereby the parasitoid seeks and attacks the stem borer host within the tunnel was thought to be superior to the "drill and sting" strategy (Muli *et al.,* 2006). *Xanthopimpla stemmator's* ovipositor length is about 0.52cm (Muturi *et al.,* 2005) and thus stem borer pupae in thin stemmed plants such as sorghum, millet and rice would be much readily available than those in large stemmed plants such as maize and sugarcane (Hailemichael *et al*., 1994). This emphasizes further, the importance of

sampling alternative hosts in order make a clear decision on whether *X. stemmator* established in the region or not.

Biological control success and failure reports from various countries inform decision making processes. Though reports of failed establishment of *X. stemmator* have also been made in South Africa where releases were done on maize and sorghum fields (Moore and Kfir, 1996, Kfir, 1997), its non-recovery during this study cannot be regarded as a non-establishment until wild and/or alternative hosts are sampled. This is because it successfully managed *Eldana saccharina* and *Chilo sacchariphagus* in sugarcane in South Africa (Conlong, 1994), Mozambique, Mauritius and Reunion (Moutia and Courtois, 1952; Moore and Kfir 1996; Conlong and Goebel 2002). Though *X. stemmator* was not recovered in maize fields during the study, the hope to use it in management of *C. partellus* in the area is ignited by the recovery of two specimen in maize fields in Lunga Lunga along Kenya/Tanzania border in a separate study (Abonyo, unpublished data). This result corroborated findings by Bonhof *et al*. (1997) who reported the parasitoid along the Kenyan Coast. Presence of *X. stemmator* along the Kenyan coast is thought to have come from influx of parasitoid populations from Tanzania, Uganda, Ethiopia, Zanzibar and Eritrea (Mailafiya, 2009) where releases had been done. These possible influxes of *X. stemmator* from neighbouring countries indicate that populations may have established in the respective countries. This study is therefore recommending repeated release of *X. stemmator* in selected multiple sites using populations from neighbouring countries.

# CHAPTER EIGHT

# 8.0 IMPACT OF PARASITOIDS ON STEM BORER PEST INFESTATION AND MAIZE YIELD

## 8.1 Introduction

Parasitoids have been used in different agro-ecosystems as biological control agents for many years (Rodriguez and Hawkins, 2000; Gullan and Cranston, 2005; Sanda and Sunusi, 2014). In classical biological control (CBC), parasitoids are released in areas where previously harmless insects have become pests after accidental introduction outside their native range (Greathead, 1990; DeBach and Rosen, 1991; Mohyuddin, 1991). *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is one of the exotic insects that became an important pest in Eastern and Southern Africa (ESA). Accidentally introduced in 1930's from Asia (Tams, 1932), *C. partellus* infestation has resulted in yield losses estimated at 73% in maize and 80-100% in sorghum (Seshu Reddy, 1983; Reddy and Walker, 1990; Kfir, 1994). As a result of these losses, *C. partellus* has been a target of various management strategies.

The exotic larval parasitoid, *Cotesia flavipes* Cameron (Hymenoptera, Braconidae), is among biological control agents used in management *C. partellus*. Two *C. flavipes* releases have been made in East and Southern Africa. In the first release, *C. flavipes* from Rawalpindi, Pakistan, was introduced in Uganda, Kenya and Tanzania by the Commonwealth Institute of Biological Control (CIBC) (now International Institute of Biological Control) between 1968 and 1972. Post release studies showed that the parasitoid did not establish (CIBC, 1968-1972). In the second release, *C. flavipes* from Sindh region in Pakistan was introduced at three sites in Southern Coastal area of Kenya in 1993 (Overholt *et al*., 1994a). Following the second attempt, surveys revealed that *C. flavipes* had established (Overholt *et al*., 1994a). Subsequent surveys showed that parasitism by *C. flavipes* was rising steadily. This was

reflected in initial recoveries made in 1995 and 1996 during which parasitism was estimated at 0.05-3% (Overholt *et al*., 1997), 7.1% in 1997 and 13% in 1999 (Zhou *et al*., 2001). Though there was a general increase in parasitism, there are doubts on whether parasitoids can effectively reduce stem borer pest populations below economically damaging levels (Kfir, 1997; Jiang *et al*., 2006; Kipkoech *et al*., 2009). These doubts arise due to the fact that there is limited quantitative data on impact of parasitoids on stem borer pest population and the resulting cereal yields (Kfir *et al*., 2002b; Cugala *et al*., 2006).

The limited quantitative data available on the impact of parasitoids on lepidopteran pest populations is attributed to methodological challenges associated with low densities, mobility of the lepidopteran pests and the endophagous behaviour of the larvae (Kfir, 2004). Due to these challenges, very few studies using insecticidal check method have been undertaken (Kfir, 2002b; Cugala *et al.*, 2006). Insecticidal check method, first described by DeBach (1946), is considered as a good experimental technique for evaluating the efficacy of natural enemies (Jones, 1982, Kenmore *et al*., 1984; DeBach and Rosen, 1991; Luck *et al*., 1999). This method has been used to estimate the impact of parasitoids on stem borer pest population in South Africa (Kfir *et al*., 2002b) and Mozambique (Cugala *et al*., 2006). Studies were done in regions characterized by stem borer community dominated by *Busseola fusca* (Fuller) and *C. partellus* respectively. They both observed significantly higher infestation in plots sprayed with a selective insecticide, dimethoate and higher parasitism in unsprayed plots, results they both attributed to partial elimination of parasitoids. These studies were however done on small plots measuring 333m$^2$ in South Africa and 100m$^2$ in Mozambique. Results from such small plots may not be effectively used to determine the effect of natural enemies on pest population and the yields in large farmers' fields because results are likely to be blurred by movement of natural enemies (van Driesche and Bellows, 1996).

Despite limitations associated with aforementioned exclusion experiments (Kfir, 2002b; Cugala *et al.,* 2006), no such study has been undertaken in East Africa, a region where *C. flavipes* was released more than 20 years ago. Extrapolation of results from the above exclusion experiments in the East African context may not be feasible due to ecological differences. It is on this background that this study was initiated in the moist mid-altitude agro-ecological zone (AEZ) in Kenya, a region dominated by *C. partellus* with an aim of assessing the impact of *C. flavipes* on stem borer pest population and maize yield.

## 8.2 Methodology

### 8.2.1 Description of the study area

The study was carried out in twelve farmers' fields during the short rain-growing season between September 2015 and February, 2016 in Oluch village, Homa-Bay County (Table 8.1). Oluch village is located in moist mid-altitude AEZ, at an elevation of 1000-1800masl between 0°27.949′ to 0°28.001′ S and 34°33.192′ to 34° 33.919′E. The area is characterised by temperatures ranging from 17 to 32°C with bimodal rainfall ranging between 500 and 1000mm annually. Bimodal rainfall received in the area allows cultivation of maize in both long and short rainy season.

### 8.2.2 Study design and insecticide treatment

The twelve fields were purposively selected within a radius of one kilometre so as to limit differences that may arise with respect to ecological characteristics. Selected fields were assigned three different insecticide treatments (A, B and C), and replicated four times. Four maize fields designated for Treatment A (Fig. 8.1) were sprayed with stem borer pesticide, Thunder® 145 O-TEQ, to exclude stem borers, while fields designated for Treatment B were sprayed with Dimethoate 40 EC to exclude parasitoids. Maize plants in fields designated as Treatment C were not treated with any insecticide and were thus used as controls.

**Table 8.1**: Estimated number of maize plants in different experimental fields in Homabay county during the short rains of September, 2015 to February, 2016

| Treatment | Replication | Farm size (Acres) | No. of plants | Estimated no. of plants/acre |
|---|---|---|---|---|
| A (Stem borer excluded) | I | 0.503 | 6760 | 13439 |
| | II | 0.178 | 3440 | 19326 |
| | III | 0.600 | 10472 | 17453 |
| | IV | 0.611 | 2808 | 4596 |
| B (Parasitoid excluded) | I | 0.842 | 10360 | 12304 |
| | II | 0.687 | 6800 | 9898 |
| | III | 0.438 | 5016 | 11452 |
| | IV | 0.306 | 2200 | 7190 |
| C (Control) | I | 1.191 | 8580 | 7204 |
| | II | 1.058 | 12470 | 11786 |
| | III | 0.531 | 6630 | 12485 |
| | IV | 0.574 | 2890 | 5034 |


**Figure 8.1**: Experimental field designated treatment A for exclusion experiment in Oluch Village, Homabay County in October 2015

Two millilitres of the stem borer pesticide, Thunder® 145 O-TEQ, was mixed with one litre of water and sprayed onto pre-selected fields designated as A to exclude stem borers from maize plants. The two active ingredients in Thunder, Imidacloprid and Beta-Cyfluthrin, act through quick knock-down effect by blocking acetylcholine receptors in insect nerve cells and by keeping sodium channels in membranes of nerve cells open respectively (Bayer Crop Science, 2013). To exclude stem borer parasitoids, 2mm of the parasitoid insecticide, Dimethoate 40 EC, was mixed with one litre of water and sprayed on maize plants in pre-selected fields in Treatment B. Dimethoate 40 EC, is a selective organophosphate compound with both systemic and contact action. Dimethoate inhibits the enzyme acetylcholinesterase resulting in nerve damage and death (Fukuto, 1990).

The first stem borer pesticide spraying was done three weeks after germination of maize plants (Fig. 8.2) and three subsequent applications done after every two weeks. The first dimethoate application was done four weeks after germination and two subsequent treatments were done after every two weeks.

**Figure 8.2**: Spraying of maize plants using the stem borer excluding insecticide (Bulldock) in Oluch Village, Homabay County in October 2015

### 8.2.3 Sampling protocol

Sampling for stem borer infestation, densities and parasitism was done once during the 5$^{th}$ week after germination. During each sampling session, all plants in respective treatment fields were inspected for stem borer infestation. The observed number of infested plants were expressed as a percentage of total plants inspected in respective fields. The total number of stem borer larvae collected was used to estimate larval densities in respective fields. Stem borer sampling, rearing and identification was done following the protocol detailed in Chapter Three under General Materials and Methods. At the end of the study, dry maize cobs were harvested and their respective weights recorded (Fig. 8.3).

### 8.2.4 Estimating effect of infestation and parasitism on maize yield

The effect of infestation and parasitism on maize yield was estimated using a hypothetical approach. In this approach, ten infested and ten non-infested maize plants were identified and tagged in all the farmers' experimental fields. The following parameters were recorded from the tagged plants during harvest: height and diameter, tunnelling length, number of exit holes, number of cocoon cases and maize cob weights with husks. These parameters were statistically compared to determine their respective effects on observed yields.

### 8.2.5 Statistical analyses

Data on the percentage infestation, larval density, stem borer parasitism and cob weight (with husk) from individual fields, were pooled in respective treatments and used as replicates during analysis. Before analysis, the aforementioned parameters were tested for normality using Shapiro-Wilk's test. Percentage infestation, larval density and yield were normally distributed and One-way ANOVA was thus used to compare variations among treatments. Tukey's post-hoc test was used to separate means where treatments were found to be

significantly different ($p$<0.05). Percentage parasitism was log transformed $(Log_{10}x+1)$ before subjection to One-way ANOVA and means separated using Tukey's post-hoc test. Number of exit holes, tunnelling length, plant height and stem diameter were analysed to test their respective effects on cob weight. Plant height, stem diameter, number of nodes and yield from non-infested and infested plants were compared using Mann-Whitney test. Yield data was standardized to yield per acre in order to correct for differences in farm sizes. Mean yield between infested and non-infested plants were compared using student's *t*-test while Pearson's correlation test was used to determine the effect of tunnel length, exit holes and cocoon cases on maize yield. GLM was used to determine the effect of plant height, stem diameter, mean larval density, infestation and parasitism on maize yield.

**Figure 8.3**: Weighing of maize harvested from respective treatment fields at the end of exclusion experiment in February 2016 in Oluch Village, Homabay County

## 8.3 Results

### 8.3.1 Stem borer diversity, infestation and mean larval density

*Chilo partellus* was the only stem borer species found occurring in the fields during this study. Stem borer infestation varied among treatments (A, B and C) ($F_{2,9}$=5.835; $p$<0.05; Table 8.2). Infestation in treatment A (0.5 ± 0.3%) was significantly lower compared to treatment B (2.0±0.2%) and C (1.5± 0.4%). Like infestation levels, there was evidence of variation in mean larval density among treatments ($F_{2,9}$=8.887; $p$<0.05; Table 9.2). Larval density was significantly lower in Treatment A (0.5±0.4) compared to Treatment B and C where densities were 2.4±0.4 and 2.7±0.4 respectively (Table 8.2).

### 8.3.2 Parasitoid species composition and pest parasitism

A total of 118 cocoon masses were recovered from parasitized stem borers during the study. *Cotesia flavipes* was the only parasitoid species recovered and thus constituted 100% of parasitoid community. However, there was evidence of variation in level of parasitism among treatments ($F_{2,9}$ =91.97; $p$<0.0001). Parasitism was significantly high in treatment C (23.5±6.8%) compared to Treatment A (00.00±0.00) and B (0.3±0.1) where populations of stem borers and parasitoids were manipulated respectively (Table 8.2).

**Table 8.2**: Mean $(\bar{x} \pm SE)$ stem borer infestation, larval density, parasitism and maize yield among different insecticide treatments

| Treatment | Infestation (%) | Larval density | Parasitism (%) | Yield (kg/acre) |
|---|---|---|---|---|
| A | 0.5±0.3[a] | 0.5±0.4[a] | 0.0±0.0[a] | 915.3±294.2[a] |
| B | 2.0±0.2[b] | 2.4±0.4[b] | 0.3±0.1[a] | 841.3±223.9[a] |
| C | 1.5±0.4[ab] | 2.7±0.4[b] | 23.5±6.8[b] | 587.0±047.7[a] |
| $F_{2,9}$ | 5.835 | 8.887 | 91.97 | 0.641 |
| $p$-value | <0.05 | <0.01 | <0.0001 | >0.05 |

Mean $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different ($p$>0.05) (Tukey's post hoc test).

### 8.3.3 Maize yield

After correcting for difference in farm sizes, Treatment A produced relatively higher yield (915.3±294.2kg/acre) followed by Treatment B (841.3±223.9kg/acre) and Treatment C (587.0±47.7kg/acre). However, statistical comparison did not reveal any differences in mean yield among the treatments ($F_{2,9}$=0.64; $p$>0.05) (Table 8.3).

#### *8.3.3.1 Effects of plant diameter, plant height and number of nodes on maize yield*

*i) Comparison of infested and non-infested maize plants*

On average, infested maize plants were significantly shorter (171.6±4.3cm) compared to non-infested plants (202.6±3.5cm) ($W$=4033; $p$<0.05) (Table 8.3). Significant variations were also observed in the number of nodes between infested (10.4±0.2) and non-infested plants (11.3±0.2) ($W$=4814.5; $p$<0.05) (Table 8.3). However, plant diameter did not vary between infested (2.1±0.0) and non-infested plants (2.1±0.0) ($W$=6003; $p$>0.05) (Table 8.3). Statistical comparison of yield weights revealed significant variation between infested (100.9±8.1) and non-infested plants (199.1±6.4) ($W$=2579.5; $p$<0.05).

**Table 8.3**: Mean ($\bar{x} \pm SE$) plant height, number of nodes, diameter and yield weight of infested and non-infested plants.

| Infestation status | Plant height(cm) | Number of nodes | Plant diameter (cm) | Yield weight(Kg) |
|---|---|---|---|---|
| Infested plants | 171.6±4.3[a] | 10.4±0.2[a] | 2.1±0.1[a] | 100.849±8.1[a] |
| Non-infested plants | 202.6±3.5[b] | 11.3±0.2[b] | 2.1±0.0[a] | 199.056±6.4[b] |
| *W* value | 4033 | 4814 | 6003 | 2579.5 |
| *p*-value | <0.001 | < 0.001 | > 0.05 | < 0.001 |

Mean (± *SE*) within columns followed by the same lower case superscripts are not significantly different ($p$>0.05)

*ii) Effect of infestation and parasitism on cob weights*

Regardless of the status of infestation, GLM analysis revealed that performance of maize plant is positively affected by maize plant height ($b$=0.51; $t$=4.20; $p$<0.0001) and diameter ($b$=48.15; $t$=4.01; $p$<0.0001). Further GLM analysis of stem borer infestation parameters (tunnelling length and exit holes) revealed their respective negative effect on cob weights (Table 8.4). Tunnelling length caused during stem borer feeding negatively affected cob weights ($b$=1.65; $t$=-3,524.01; $p$<0.05). The number of exit holes indicating number of stem borers that completed their life cycle within the maize stems, also negatively affected cob weight though insignificantly ($b$=-7.08; $t$=-1.86; $p$>0.05) (Table 8.4). The number of cocoon cases did not have a significant effect on cob weight ($b$=-4.34; $t$= -0.49; $p$>0.05).

**Table 8.4**: Estimated effect of different growth, infestation and parasitism parameters on cob weights

| Term | b | SE | T | *p*(T) |
|---|---|---|---|---|
| (Intercept) | -47.209 | 24.558 | -1.92 | 0.05582 |
| Height of maize plant (cm) | 0.510 | 0.121 | 4.20 | 0.00004 |
| Maize plant diameter (cm) | 48.154 | 11.997 | 4.01 | 0.00008 |
| Tunnelling length (cm) | -1.650 | 0.469 | -3.52 | 0.00052 |
| Number of exit holes | -7.083 | 3.800 | -1.86 | 0.06362 |
| Number of cocoon cases | -4.338 | 8.936 | -0.49 | 0.62783 |

## 8.4 Discussion

*Chilo partellus* was the only stem borer pest collected during the study contrary to results from previous surveys in which *B. fusca* reportedly dominated the stem borer community in this region (Seshu Reddy, 1983; Ongámo *et al*., 2006a, b). The predominance of *C. partellus* had previously been reported in low altitude areas (Nye, 1960; van Hamburg, 1979; Seshu

Reddy, 1983), an observation attributable to its competitive advantage over indigenous stem borer species. However, with climate change, predictive models showed that *C. partellus* was likely to expand it's distribution range beyond low altitude areas (Khadioli *et al.,* 2014; Mwalusepo *et al*., 2015). Recovery of *C. partellus* from mid-altitude zone during this study validated the predictive models. Other researchers have reported similar range expansion by *C. partellus* into mid and high –altitude areas (Kfir, 1997a,b; Kfir *et al*., 2002a; Songa, 1999; Overholt *et al.,* 2000).

Found associated with *C. partellus*, was its old association parasitoid, *C. flavipes* whose establishment is hereby confirmed in moist mid-altitude zones despite there being no official release (Omwega *et al*., 1995). Its presence is attributed to a population of parasitoids which escaped from a laboratory at *icipe*, Mbita point field station where they had been quarantined for pre-release studies (Omwega *et al*., 1995). *Cotesia flavipes* was the only parasitoid recovered contrary to earlier surveys during which the native, gregarious larval endoparasitoid *Cotesia sesamiae* Cameron was also recovered (Zhou and Overholt, 2001).

The direct effect of stem borer infestation on maize production and contribution of biological control agents have rarely been quantified with two documented studies having been done on small plots (Kfir *et al.,* 2002b; Cugala *et al*., 2006). The high cost involved in replicating experiments using larger plots was cited as a constraint and thus plots measuring 333m$^2$ in South Africa (Kfir *et al.,* 2002b) and 100m$^2$ in Mozambique (Cugala *et al*., 2006) were used, 10 years after the initial release of *C. flavipes* in the latter. However, small plots have been found to be ineffective in determining the effect of natural enemies on pests because results are blurred by movement of natural enemies across plots (van Driesche and Bellows, 1996). Larger plots measuring between 720 to 4820m$^2$ were used in this study after 23 years since the initial release of *C. flavipes* in Kenya in order to overcome this challenge.

Following the study, generated results provided evidence on the impact of C. *partellus* infestation on maize yield and contribution of *C. flavipes* in limiting losses associated with infestations. Low infestation observed in Thunder treated fields were attributed to mortality induced by the insecticide which killed majority of first instar larvae with only a few that had penetrated into stems surviving. The higher percentage infestation recorded in treatment B was as a result of partial removal of natural enemies by dimethoate application, an observation that corroborated findings from South Africa and Mozambique (Kfir, 2002b; Cugala *et al*., 2006). In South Africa, direct effects of natural enemies on *B. fusca* and *C. partellus* populations were assessed and significantly higher infestation was recorded in plots sprayed with dimethoate with higher parasitism being recorded in unsprayed plots (Kfir, 2002b). In Mozambique, a similar experiment was carried out on *C. partellus*, *B. fusca* and *S. calamistis* and similar results were obtained following natural enemy exclusion (Cugala *et al.*, 2006).

In treatment B, parasitoid action was considerably reduced by dimethoate application. However, 100% elimination of natural enemies is often not achieved by insecticide application (Kidd and Jervies, 2005) and this explained the low parasitism rate in treatment B in comparison to treatment C. This is in agreement with results obtained from a similar study carried out by Cugala *et al*. (2006) where parasitism in control plots were higher than in stem borer and parasitoid-excluded fields. This study revealed a general trend in increase of parasitism in the region. In 1997, Khan *et al.* (1997) recorded about 3.5% parasitism while Ogeda recorded about 6.1% in 1999 (Ogeda, 1999). Results of this study demonstrate an increase in parasitism (23%) in control fields. Contrary to variations in stem borer pest infestation and parasitism among treatments, there was no evidence of variation in maize yield among treatments. This contradicted earlier reports that natural enemies (including *C. flavipes*) played a considerable role in stem borer population suppression resulting in

increased maize yield (Cugala *et al.*, 2006). It is worth noting that maize yield is affected by both biotic and abiotic factors. Manipulated stem borer and parasitoid populations are only part of the biotic factors that may affect yield. However, comparison of yields from infested and non-infested plants showed that infestation contributes to yield loss depending on the health of the plant.

Plant height and diameter played an important role towards yield as they together showed positive influence. In contrast, infestation parameters particularly tunnel length and number of exit holes negatively affected the yield. Tunnel length is considered a good indicator of stem borer damage as it results in destruction of the stem tissue (Polaszek, 1998; Cherry *et al*., 1999; Midega, 2013) leading to stem weakening and lodging (Santiago *et al*., 2003). The observed low yield in infested plants was thus attributed to stem tunnelling which interfered with translocation of water and nutrients to actively photosynthesizing parts of a plant resulting in reduced plant growth, seed setting and grain sizes (Kalule *et al*., 1997; Polaszek 1998; Malvar *et al*, 2008) thus reduced yield (Ajala and Saxena, 1994; Songa *et al*., 2001; Midega, 2013). The other infestation parameter, number of exit holes, showed a strong positive correlation to tunnelling length indicating that with an increase in the number of stem borers that completed their life cycle within the maize stem, there was an associated increase in crop damage.

Cocoon cases are an indication of parasitoid presence and action on the target pest. Parasitism being a numerical response, an increase in number of exit holes and tunnelling lengths was observed to be accompanied by an increase in cocoon cases. However, larval parasitoids attack 3$^{rd}$, 4$^{th}$ or 5$^{th}$ instar larvae of the pest after damage had been done on maize. Results obtained in this study upheld reports that the number of stem borers, exit holes and tunnelling length are the most important factors affecting yield in maize crop (Songa *et al*., 2001).

According to the same author, these were then followed by plant height and plant diameter in that order of importance. Generally, this study revealed a reduction in stem borer infestation levels in the area (1.45% in control fields) compared to earlier studies in which stem borer infestation levels were estimated at 8-100% (Seshu Reddy *et al.*, 1983) and 33.72% (Ongámo *et al*., 2006a). Reduced infestation levels were attributed to increase in parasitism by *C. flavipes*. However, given the *modus operandi* of the parasitoid, decreased infestation coupled with increased parasitism did not translate into improved maize yield. Optimum yield may be realised when biological control involving both egg and larval parasitoids are used. Further research to identify egg parasitoids of *C. partellus* in the pest's native range or that have expanded their host range to include *C. partellus* in Kenya needs to be done.

# CHAPTER NINE

# 9.0 IMPACT OF PARASITOIDS ON LEPIDOPTERAN STEM BORER INFESTATION LEVELS AND MAIZE YIELD AT THE KENYAN COAST

## 9.1 Introduction

In Sub-Saharan Africa, farmers mainly grow maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* Moench.) for both domestic consumption and income (Seshu Reddy, 1989; Odindo, 1991; Overholt, 1992; Chamberlain *et al.*, 2006). Production of these crops is however constrained by several factors key among them being field pest infestations (Seshu Reddy, 1983; Saxena *et al*., 1991; Brownbridge and Onyango, 1992; Bosque-Perez and Schulthess, 1998; Overholt, 1998). In Eastern and Southern Africa (ESA), there are five important stem borer pests of maize and sorghum. These are *Busseola fusca* Fuller and *Sesamia calamistis* Hampson (Family: Noctuidae), *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* Strand (Family: Crambidae) and *Eldana saccharina* Walker (Family: Pyralidae) (Polaszek and Khan, 1998; Seshu Reddy, 1998; Overholt *et al.*, 2001). *Busseola fusca* and *C. partellus* are major pests in the region, while *S. calamistis*, C. *orichalcociliellus* and *E. saccharina* are minor pests (Bonhof *et al*., 1997; Songa *et al*., 2001; Zhou *et al*., 2001). In Kenya, *C. partellus* and *B. fusca* constitute the major proportion of stem borer pest community (Seshu Reddy, 1983; Khan *et al.*, 1997). In Kenya, a country where production of sufficient food for a rapidly increasing population is a challenge, 73% yield losses caused by *C. partellus* in small farmers'fields is of great concern (Seshu Reddy and Walker, 1990). Due to such high losses, various management strategies have been initiated to reduce *C. partellus* population in cereal crops.

The koinobiont larval parasitoid, *Cotesia flavipes* Cameron, from Sindh region of Pakistan was imported and released in Kenya to suppress *C. partellus* population (Overholt *et al*., 1994a). Since the release in 1993, various post release surveys have been carried out and

these showed a steady increase in parasitism levels (Overholt *et al*., 1994a, 1997; Omwega *et al*., 1997; Zhou *et al*., 2001). However, information regarding impact of the parasitoid on stem borer pest population and associated maize yield is lacking. Various methods for evaluation of parasitoid impact on pest populations have been developed. These include introduction and augmentation, cages and other barriers, removal of natural enemies, prey enrichment, direct observations and evidence of feeding (Luck *et al*., 1988). Removal of natural enemies with insecticides, first described as the insecticidal check method by DeBach (1946) is considered a good experimental technique for evaluating efficacy of natural enemies (Jones, 1982; Kenmore *et al*., 1984; DeBach and Rosen, 1991; Luck *et al*., 1999). Using insecticidal check technique, this study was undertaken to provide quantitative data on the impact of parasitoids on stem borer pest population and the associated maize yield in moist lowland agro-ecological zone (AEZ) of Kenya.

## 9.2 Materials and methods

### 9.2.1 Description of the study area

The study was undertaken at Kenya Agricultural and Livestock Research Organisation (KALRO), Mtwapa station (S 03°55.897′; E 039°43.918′; Elevation 17m), located in moist lowland tropics. Moist lowland AEZ is characterized by temperatures ranging from 22-32°C and an average precipitation of 500-1000mm/year (Corbett, 1998). During this study, Hybrid 4 (PH4) maize variety was planted in an experimental plot measuring 127 by 37.25m during the long rains of 2014 (May-September).

### 9.2.2 Experimental layout and insecticide treatment

Randomized Complete Block Design (RCBD) approach was adopted for this study. The experimental plot was divided into 30 subplots each measuring 35.25 by 3m with buffer zones left between each subplot. Each subplot had five rows with 48 hills in each row and

two plants per hill giving a total of 480 plants per subplot. Buffer zones had two rows with 48 hills in each row and two plants per hill giving a total of 192 plants per buffer zone. Maize grown subplots were subjected to three different treatments (A, B and C). Treatments A and B were treated with Bulldock® 262.5 EC (*Beta cyfluthrin*) and Dimethoate 40 EC respectively. Treatment C was not treated with any insecticide and served as a control. This layout design took care of differences associated with both experimental and replication errors.

The stem borer pesticide, Bulldock® 262.5 EC, was mixed with water at a ratio of 2ml/litre and sprayed onto pre-selected subplots to exclude stem borers from maize plants (Fig. 9.1). Bulldock® is a synthetic pyrethroid, acting through contact and as stomach poison. Dimethoate 40 EC, was mixed with water at a ratio of 2ml/litre and applied to exclude parasitoids in pre-selected subplots. Dimethoate is a selective organophosphate compound with both systemic and contact action. The first Bulldock spraying was done three weeks after germination and two subsequent applications were done after every three weeks. The first dimethoate application was done five weeks after germination and two subsequent applications done after every three weeks (Fig. 9.2).

**Figure 9.1**: Spraying of the stem borer excluding pesticide, Bulldock, at KALRO Mtwapa in June 2014

**Figure 9.2:** Spraying of the parasitoid excluding pesticide, Dimethoate at KALRO Mtwapa in July 2014

### 9.2.4 Sampling protocol

Estimation of stem borer infestation, densities and parasitism was done at three different maize growth stages. Stem borer larvae were also collected from maize cobs during harvest. During each sampling session, all plants in each treatment subplots were inspected for stem borer infestation. The number of infested plants were expressed as a percentage of the total plants inspected in respective subplots to compute percentage infestation. In each treatment subplot, five infested plants were dissected and all immature stem borer stages collected and identified. Sampled stem borer larvae were reared and identified following the protocol detailed under General Materials and Methods in Chapter Three. At the end of the experiment (Fig. 9.3), maize was harvested, cobs dried for one week, shelled and weighed (kg).

**Figure 9.3:** Maize plants on experimental fields in KALRO, Mtwapa, ready for harvesting in September 2014

### 9.2.5 Statistical analyses

Data on percentage infestation, stem borer density, stem borer parasitism and yield (kg) from individual subplots were pooled in respective treatments and used as replicates during analysis. Percentage infestation, stem borer density and stem borer parasitism were also estimated at three maize growth stages; vegetative, early maturity and mature stages. Before analysis, aforementioned parameters were tested for normality using Shapiro-Wilk's test. Percentage infestation and yield data were normal. Percentage stem borer parasitism was arcsine transformed while larval density was square root transformed. Two-way Analysis of Variance (ANOVA) was used to compare variations in stem borer pest infestations and maize yield among the three maize growth stages in the three insecticide treatments. Tukey's pairwise comparison test was performed to separate means where treatments were found to be significantly different ($p<0.05$). Two-way ANOVA was used to assess the effect of interaction between maize stage and treatment on the maize yield. Generalized Linear Model (GLM) was used to find stages of infestation and parasitism that significantly affected yield. Stem borer parasitism was subjected to Friedman rank sum test to compare the means. Wilcoxon test was used to compare means between *Chilo spp* and *S. calamistis* parasitism.

## 9.3 Results

### 9.3.1 Stem borer species composition and abundance

A total of 632 immature stem borers (larvae (622) and pupae (10) were collected during the experiment. Majority of immature stem borers (71.5%) were collected during the vegetative stage, followed by early maturity (23.7%) and mature stage (4.7%). However, sizes of sampled stem borers varied among maize growth stages (Table 9.1). At vegetative stage, 34, 50 and 16% of collected immatures could be grouped as small, medium and large respectively. Similar variations were observed at early maturity in which 20, 19 and 61% of

collected immatures were categorised as small, medium and large respectively, while at mature stage, 18, 9 and 73% of total collection constituted small, medium and large stages respectively (Table 9.1). Immature stem borers were found on different maize plant parts during sampling. At vegetative stage, 74 and 26% of total collection were found on stems and tassels respectively while at early maturity 84% of the total collection were found on stems and 16% on the cobs. At maturity, immature stem borers were found on cobs (54%) and stems (46%) (Table 9.1).

On rearing, three stem borer species, *C. partellus*, *C. orichalcociliellus* and *S. calamistis* were identified from collected immature stages. *Chilo partellus* constituted 43, 52 and 25% while *C. orichalcociliellus* constituted 30, 35 and 8% of the total collections during the vegetative, early maturity and mature maize growth stages respectively. *Sesamia calamistis* constituted 27, 14 and 67% of all stem borers collected at vegetative, early maturity and mature maize stages respectively (Table 9.1).

**Table 9.1:** Percentage (%) stem borer stages, plant parts and community composition at different maize growth stages during long rains of 2014.

| | **Vegetative** | **Early maturity** | **Mature** |
|---|---|---|---|
| | | Stem borer size (%) | |
| Small | 33.9 | 20.0 | 18.2 |
| Medium | 50.7 | 19.2 | 9.1 |
| Large | 15.5 | 60.8 | 72.7 |
| | | Plant part (%) | |
| Tassel | 25.9 | 0.0 | 0.0 |
| Stem | 74.1 | 84.1 | 46.2 |
| Cob | 0.0 | 15.9 | 53.8 |
| | | Community composition (%) | |
| *C. partellus* | 43.1 | 51.7 | 25.0 |
| *C. orichalcociliellus* | 29.7 | 34.5 | 8.3 |
| *S. calamistis* | 27.2 | 13.8 | 66.7 |

### 9.3.2 Stem borer infestation levels

Stem borer infestation levels varied among treatments (A, B and C) during the study ($F_{2,87}$ = 6.92; $p$<0.05 (Table 9.2). Infestation was significantly low in treatment A (1.4±0.2%) compared to treatments B (2.4±0.2%) and C (2.2±0.2%). In addition to treatment differences, there was evidence that infestation levels varied with maize growth stages ($F_{2,87}$= 11.07; $p$<0.001). Infestation was significantly higher at vegetative stage (2.7±0.5%) compared to early maturity (1.9±0.3%) and mature maize stages (1.5±0.3%) (Table 9.2).

**Table 9.2:** Overall stem borer infestation across treatments and maize growth stages during long rains of 2014.

| **Overall infestation ( $\bar{x} \pm SE$ )** | | | |
|---|---|---|---|
| **Treatment** | | **Crop stage** | |
| A | 1.4±0.2[b] | Vegetative | 2.7±0.5[a] |
| B | 2.4±0.2[a] | Early maturity | 1.9±0.3[b] |
| C | 2.2±0.2[a] | Mature | 1.5±0.3[b] |
| $F_{2,87}$ | 6.92 | $F_{2,87}$ | 11.07 |
| $p$ value | 0.00162** | $p$ value | 5.208e$^{-05***}$ |

Mean (± *SE*) within columns followed by the same lower case superscripts respectively are not significantly different ($p$>0.05)

Interaction between maize stage sampled and treatment had a significant effect on infestation ($F_{2,81}$ = 3.383; $p$ = 0.013). At the vegetative stage, mean stem borer infestation levels varied among treatments ($F_{2,27}$=7.04; $p$<0.05). Infestation was relatively high in both treatment B (3.1±1.5%) and C (3.3 0.6%) which were not significantly different, but significantly higher than in treatment A (1.6±0.9%) (Table 9.3). Mean infestation levels by the pest during early maturity also varied among treatments ($F_{2,27}$=5.4; $p$<0.05). Infestation was significantly higher in treatment B (2.6±1.0%) compared to treatments A (1.2±1.1%) and C (1.8±0.7%),

which were not significantly different. Mean stem borer infestation levels on maize at mature stage did not vary among treatments ($F_{2,27}$=0.2; $p$>0.05).

Mean stem borer infestation in treatment A did not vary across maize growth stages ($F_{2,27}$ = 0.403; $p$>0.05). Contrary to this, in treatment B and C, stem borer infestation varied significantly ($F_{2,27}$=5.962; $p$<0.05 and $F_{2,27}$=28.21; $p$<0.05 respectively) (Table 9.3).

**Table 9.3:** Mean stem borer infestation among treatments at different maize growth stages during long rains of 2014.

| | **Mean infestation ( $\bar{x} \pm SE$ )** | | | **Statistics** | |
|---|---|---|---|---|---|
| | Vegetative | Early maturity | Mature | *F* value | *p* value |
| A | $1.6 \pm 0.3^{bA}$ | $1.24 \pm 0.3^{bA}$ | $1.4 \pm 0.2^{aA}$ | 0.403 | 0.672 |
| B | $3.1 \pm 0.5^{aB}$ | $2.6 \pm 0.3^{aAB}$ | $1.4 \pm 0.2^{aA}$ | 5.962 | 0.007** |
| C | $3.3 \pm 0.2^{aB}$ | $1.8 \pm 0.2^{bA}$ | $1.6 \pm 0.1^{aA}$ | 28.21 | $2.436e^{-07***}$ |
| $F_{2,87}$ | 7.04 | 5.4 | 0.21 | | |
| *p* value | 0.003** | 0.011* | 0.82 | | |

Mean (± *SE*) within columns and rows followed by the same lower case and upper case superscripts respectively are not significantly different ($p$>0.05).

### 9.3.3 Parasitoid species composition and abundance

A total of 98 cocoon masses were recovered from parasitized stem borers during the experiment. Emerging parasitoids were identified as *C. flavipes* and *C. sesamiae*. *Cotesia flavipes* was the most abundant parasitoid species constituting 90.43 and 94.89% of parasitoids collected during the vegetative and early maturity plant growth stages respectively (Table 9.4). No parasitoids were recovered from stem borer larvae sampled on mature maize plants despite the presence of parasitized larvae and parasitoid cocoons, as depicted in mean parasitism. Parasitoids were recovered from all three stem borer species.

**Table 9.4**: Abundance and percentage composition of parasitoid community at different maize growth stages during long rains of 2014.

| Parasitoid species | % Composition (*n*) | | |
|---|---|---|---|
| | Vegetative | Early maturity | Mature |
| *Cotesia flavipes* | 90.4 (1342) | 94.9 (446) | 0 |
| *Cotesia sesamiae* | 9.6 (142) | 5.1 (24) | 0 |

### 9.3.4 Stem borer pest parasitism

Mean stem borer pest parasitism in treatment A, B and C were 16.2±7.4, 20.9±4.9 and 20.7±6.9% respectively (Table 9.5). Pest parasitism levels did not vary among treatments ( $c_2^2$=3.558; $p$>0.05). However, there was evidence of variation in pest parasitism levels among different maize growth stages ( $c_2^2$=21.6; $p$<0.05). Significantly higher parasitism was recorded in both vegetative (22.4±5.2%) and early maturity stage (34.6±8.9%) compared to mature maize (0.8±0.8%) (Table 9.5).

**Table 9.5:** Mean stem borer parasitism $(\bar{x} \pm SE)$ among treatments in different maize growth stages during long rains of 2014.

| Treatment | Parasitism (%) | Maize stage | Parasitism (%) |
|---|---|---|---|
| A | 16.2±7.4[a] | Vegetative | 22.4±5.2[a] |
| B | 20.9±4.9[a] | Early maturity | 34.6±8.9[a] |
| C | 20.7±6.9[a] | Mature | 00.8±0.8[b] |
| $c_2^2$ value | 3.558 | $c_2^2$ value | 21.6 |
| *df* | 2 | *df* | 2 |
| *p* value | 0.169 | *p* value | 2.05E-05 |

Mean (± *SE*) within columns and rows followed by the same lower case superscripts are not significantly different ($p$>0.05).

At the vegetative stage, mean parasitism did not vary among treatments ( $c_2^2$=3.84; $p$>0.05). Parasitism was relatively high in treatment C (35.5±13.7%) compared to A (13.2±4.4%) and B (18.5±4.8%) (Table 9.6). Mean parasitism during early maturity stage varied among treatments ( $c_2^2$=6.22; $p$<0.05). Parasitism was significantly higher in treatment B (41.5±11.25%) compared to treatment A (35.5 ± 20.8%) and C (26.67±13.9%). Mean parasitism at mature stage did not vary among treatments ( $c_2^2$= 2; $p$>0.05) (Table 9.6).

**Table 9.6:** Mean $(\bar{x} \pm SE)$ stem borer parasitism in different insecticide treatments at various maize growth stages.

| | Mean parasitism (%) | | | Statistics | |
|---|---|---|---|---|---|
| Treatment | Vegetative | Early maturity | Mature | *F* | *p* value |
| A | 13.2±4.4[a] | 35.5±20.8[b] | 0.0 ± 0.0[a] | 7.69 | 0.021 |
| B | 18.5±4.8[a] | 41.5±11.3[a] | 7.9±2.5[a] | 10.764 | 0.0046** |
| C | 35.5±13.7[a] | 26.7±13.9[b] | 0.0 ± 0.0[a] | 10.137 | 0.0063** |
| Friedman $\chi^2$ | 3.84 | 6.22 | 2 | | |
| *p* value | 0.147 | 0.045* | 0.368 | | |

Mean (± *SE*) within columns and rows followed by the same lower case superscripts are not significantly different ($p$>0.05) (Tukey's/Friedman's rank sum test).

### 9.3.5 Maize yield

A total of 513.7kg of shelled dry maize was harvested from the experimental plot. On average, 20.8±2.1, 24.1±2.0 and 20.5±1.6kg were harvested from treatments A, B and C respectively. Statistical comparison did not reveal any significant difference in mean yield among treatments ($F_{2,27}$=1.148; $p$>0.05). Further analysis showed that stem borer density at early maturity stage had the greatest negative impact on maize yield ($b$=1.131; $t$=2.639; $p$>0.05). However, there was no evidence of impact of larval density at vegetative ($b$=0.062; $t$ = 0.368; $p$>0.05) and mature stage ($b$=0.086; $t$=1.201; $p$>0.05) on yield (Table 9.7). Like

larval density, stem borer infestation level at early maturity had the greatest impact on maize yield ($b$=2.185; $t$=2.363; $p$<0.05). However, this was not consistent at vegetative ($b$=-0.624; $t$ =-0.849; $p$>0.05) and mature stage ($b$=1.358; $t$=1.017; $p$>0.3186). Stem borer parasitism at early maturity had the greatest effect on maize yield ($b$=0.093; $t$=3.207; $p$<0.05). This however was not consistent for vegetative ($b$=0.059; $t$=0.702; $p$>0.05) and mature stages ($b$=0.037; $t$=1.357; $p$>0.05).

**Table 9.7:** Effect of stem borer larval density, infestation and parasitism on maize yield.

| Maize stage | Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|---|
| **Stem borer larval density** | | | | |
| Intercept | 17.81432 | 1.71219 | 10.404 | 9.23e-11 *** |
| Vegetative | 0.06221 | 0.16905 | 0.368 | 0.7158 |
| Early maturity | 1.13143 | 0.42875 | 2.639 | 0.0139* |
| Mature | 0.86365 | 0.71904 | 1.201 | 0.2405 |
| **% infestation** | | | | |
| Intercept | 15.7426 | 3.6103 | 4.361 | 0.000182*** |
| Vegetative | -0.9853 | 0.9835 | -1.002 | 0.325699 |
| Early maturity | 2.9848 | 1.2371 | 2.413 | 0.02318* |
| Mature | 2.1402 | 1.7872 | 1.198 | 0.241907 |
| **% parasitism** | | | | |
| Intercept | 18.0495 | 1.56297 | 11.548 | 9.77e-12 *** |
| Vegetative | 0.08436 | 0.11212 | 0.752 | 0.45859 |
| Early maturity | 0.12226 | 0.03891 | 3.142 | 0.00415** |
| Mature | 0.0565 | 0.0362 | 1.561 | 0.13069 |

## 9.4 Discussion

This study confirmed the presence of three stem borer pest species (*C. partellus*, *C. orichalcociliellus* and *S. calamistis*) in moist lowland AEZ corroborating findings by Mathez (1972), Seshu Reddy (1983), Overholt *et al*. (1994), Bonhof (2000) and Rwomushana *et al*.

(2005). Among the three species, *C. partellus* (43.2%) was the most abundant followed by *C. orichalcociliellus* (29.2%) and *S. calamistis* (27.6%). Even though this study was conducted under controlled field conditions, similar patterns of community composition were reported by Bonhof (2000) and Midega *et al*. (2004).

Generally, infestation levels recorded were lower than levels reported in the mid-1990's. Low infestation was however not consistent among the treatments. Relatively low infestation was observed in Bulldock treated subplots suggesting that the insecticide killed majority of first instar larvae. Initial Bulldock spraying was done three weeks after germination of maize plants to coincide with oviposition period. Being a contact and stomach poison, first instar larvae were killed by insecticide action upon emergence thereby successfully suppressing the 1$^{st}$ generation of stem borer population.

The first application of dimethoate five weeks after germination coincided with the presence of stem borer larval stages that are suitable for parasitization. Treatment with dimethoate was anticipated to suppress parasitoid action, while leaving stem borer infestation level unchanged. This explained why there was a very subtle difference in stem borer infestation levels observed in treatments B and C, where natural stem borer infestations and parasitoid action were not manipulated. Treatment B plots exhibited higher infestation level compared to treatments A and C, this was a direct effect of increased oviposition by stem borers. Similar results were documented by Kinzer *et al*. (1977) who showed an increase in lepidoptera that oviposited on maize crop as a result of spraying with dimethoate. In listing various possible limitations of insecticidal exclusion method DeBach (1946) stated that "Residues nontoxic to the host but toxic to its natural enemies may, entirely aside from the elimination of the natural enemies make conditions more favourable for host population increase". The above observed stem borer infestations was not consistent across maize

growth stages in all treatments. High infestation levels observed during the vegetative stage was attributed to diaspore population that formed the first generation in the season. This initial population was assumed to have had limited natural enemies that suppressed their numbers unlike subsequent maize growth stages where parasitoids affected pest populations.

Natural enemies identified in this maize ecosystem included the larval parasitoids, *C. flavipes* and *C. sesamiae*. *Cotesia flavipes* dominated the parasitoid community, an observation attributed to the dominance of pest community by its old association host, *C. partellus*. *Cotesia flavipes* has been introduced into more than 40 countries in the tropics and subtropics for biological control of *Chilo sp*. (Polaszek and Walker, 1991). However, host suitability studies have indicated that *C. flavipes* has expanded its host range to include indigenous species (Ngi-Song *et al.*, 1995; Overholt, *et al*., 1997; Zhou *et al*, 2003) findings that are upheld in this study. *Cotesia flavipes* was recovered from *C. partellus* and the indigenous *C. orichalcociliellus* and *S. calamistis.* The ability of *C. flavipes* to parasitize three stem borer species is essential for success of the biological control programme. Use of a highly specific parasitoid that attacked only *C. partellus* would have probably had no effect on overall stem borer population. Suppression of *C. partellus* could have resulted in an ecological void that could have been filled by indigenous species (Overholt *et al*., 1994a). Similar to *C. flavipes*, *C. sesamiae*, a native, gregarious larval endoparasitoid which fills an ecologically similar niche (Polaszek and Walker, 1991) was also recovered from *C. partellus*, *C. orichalcociliellus* and *S. calamistis* during the experiment. However, it was recovered at lower proportions and thus it would not be an important mortality factor. This finding emphasized on reports that *C. sesamiae* is an inferior competitor to *C. flavipes* on *C. partellus* (Mbapila and Overholt, 2001; Ngi-Song *et al*., 2001; Sallam *et al.*, 2002).

Parasitism is a numerical response of parasitoids to host populations. Percentage parasitism is estimated from the number of parasitized hosts expressed as a percentage of the total hosts (of suitable developmental stage) collected in respective treatments. In addition to the presence of suitable hosts, parasitoids select only the suitable host stages (Russell, 1987). Though infestation was relatively high at vegetative stage across all treatments, majority of the larvae were in the $1^{st}$ and $2^{nd}$ instar and were unsuitable for parasitization. To the contrary, majority of larvae collected during early maturity were in the $3^{rd}$, $4^{th}$ and $5^{th}$ instars of their development thus highest parasitism was exhibited at this stage of maize growth. Observed differences in larval instars among maize stages explained the observed variations in parasitism levels. Low percentage parasitism observed in treatment A was as a result of suppression of the pest (and thus host) population. Higher parasitism in dimethoate-treated subplots resulted from numerical response to increased stem borer population.

Levels of infestation and parasitism at early maturity stage of maize growth had the greatest impact on maize yield as they induced compensation and reduced stem borer population respectively. Stem borer attack on mature maize has been known to result in less devastating damage (Seshu Reddy, 1988; Youdeowi, 1989; Bosque-Perez and Mareck, 1991) and grain yield loss inspite of the larval density (Reddy and Sum, 1991). Contrary to variations in infestation and parasitism, there was no difference in mean maize yield among treatments. Levels of parasitism by *C. flavipes* on stem borers at the Kenyan coast have exhibited a steady rise but are still low compared to what is observed in the pest's native range. The low parasitoid action has not translated into changes in maize yield.

# CHAPTER TEN

## 10.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

### 10.1 General discussion

Maize and sorghum are the most important cereal crops grown in SSA (Seshu Reddy, 1988; FAO, 1998; Kfir, 1998). Acreage under maize and sorghum production has increased tremendously over time (Pingali, 2001; FAO, 2003), though cereals produced rarely meet demand of the ever increasing human population. Lepidopteran stem borer pest infestation is one of the most important constraints to maize and sorghum production in SSA. In Kenya, the most important cereal stem borer pests are the crambids *Chilo partellus* and *Chilo orichalcociliellus*, the noctuids *Busseola fusca* and *Sesamia calamistis*. The pyralid, *Eldana saccharina* is considered a minor pest (Nye, 1960; Bonhof *et al*., 1997; Polaszek and Khan, 1998; Seshu Reddy, 1998; Overholt *et al*., 2001). All of these pests are indigenous in Africa except for *C. partellus* which was introduced from Asia (Tams, 1932; Bleszynski, 1970). In order to increase maize and sorghum production, management of stem borers has been initiated by various institutions including *icipe*'s Biological Control programme. This led to the importation and release of the egg parasitoid *Telenomus isis*, the larval parasitoids *Cotesia flavipes* and *Cotesia sesamiae* and the pupal parasitoid *Xanthopimpla stemmator* along different timelines.

A solitary scelionid West African egg parasitoid, *Telenomus isis* was imported and released in Wundanyi and Eldoret in 2005 (Bruce *et al*., 2009). Since the post release survey done in Wundanyi in 2006, no other post release survey has been undertaken to confirm *T. isis* establishment. This study confirmed the establishment of *T. isis* in both localities. Besides *T. isis*, *Telenomus busseolae* and *Trichogrammatoidea lutea* were also recovered. However, *T. isis* was only recovered from fields where releases were previously done. There was an increase in egg parasitismas a result of increased egg parasitoid natural enemy assemblage.

*Cotesia flavipes* was released in mid lowland, dry mid-altitude and moist mid-altitude AEZ of Kenya. This survey confirmed the establishment of *C. flavipes* in the three zones. This has translated to reduction in stem borer infestation levels and an increase in parasitism rates. The virulent *Cotesia sesamiae* biotype was released in 2006 in Taita Hills to suppress B. fusca population. Since the release, no survey had been undertaken to assess establishment. In this survey, *C. sesamiae* was only recovered from *C. partellus* strongly indicating that it was the avirulent coastal biotype. This suggests that the virulent highland *C. sesamiae* did not establish in Taita hills. This was further confirmed by parasitism rates recorded which did not show significant variation from pre-release figures.

The solitary pupal endoparasitoid, *X. stemmator* was released in the eastern region of Kenya in 2002 to complement action of *C. flavipes.* During this survey undertaken to assess it's establishment status, the only pupal parasitoid recovered was the indigenous *Dentichasmias busseolae*. *Xanthopimpla stemmator*, was not recovered. However, the wild habitat which plays a big role in host parasitoid perennation (Mailafiya *et al.,* 2010) was not within the scope of this survey and was thus not sampled. It is important to carry out survey so as to assess if *X. stemmator* is present in the wild habitat before further decisions are made regarding the use of this parasitoid in the country.

Insecticidal check method was used to evaluate efficacy of *C. flavipes* in moist mid altitude and moist lowland AEZ of Kenya. Results revealed that stem borer infestation varied among treatments with plots where stem borers had been excluded portraying significantly lower infestation levels. The highest infestation levels were obtained from treatments where parasitoids had been eliminated. Stem borers infesting these treatment plots did not have parasitoids as a biotic factor limiting their population. Statistical comparison did not reveal any differences in mean yield among treatments suggesting that observed increase in parasitism did not translate into a change in cereal crop yield.

## 10.2 Conclusions

i. *Chilo partellus* is the most predominant stem borer pest species in moist lowland and mid altitude agro-ecological zones. It's old association parasitoid, *C. flavipes* established in the release areas and is dominating the parasitoid community within the above mentioned zones.

ii. There is a general decrease in infestation levels in the moist lowland, dry mid-altitude and moist mid-altitude agro-ecological zones since the parasitoid was released. This decrease is accompanied by an increase in stem borer parasitism.

iii. The rates of parasitism recorded during this study have not translated into increased cereal production.

iv. Larval parasioids play an important role in stem borer pest management in Kenya while pupal parasitoid action remained low.

v. *Telenomus isis* which was imported from West Africa has established in Kenya, though spread has not been achieved.

vi. The virulent highland *C. sesamiae* which was released in Taita hills did not establish.

vii. The exotic pupal parasitoid, *X. stemmator* did not establish in dry mid-altitude tropics but was recovered in moist-lowland AEZ. Its presence in this zone is attributable to possible influx from neighbouring countries where it had established.

## 10.3 Recommendations

i. In order to increase chances of parasitoid establishment, multiple releases should be done over various maize growing seasons and possibly years so as to boost the natural enemy population in new environment.

ii. To achieve maximum pest suppression, biological control agents targeting the pest's various developmental stages should be used. Emphasis should be laid on egg parasitoids which arrest pest development before they reach the most destructive larval stage. If such is done, increase in cereal yields may be realized.

iii. Foreign exploration of *C. partellus* egg parasitoids from its native range should be carried out.

iv. Augmentative releases of *T. isis* in Wundanyi and Eldoret should be carried out to boost the parasitoid population and increase chances of spread.

v. Before further decisions are made regarding the use of *X. stemmator* for management of *C. partellus*, sampling should be done in the wild habitat to confirm the parasitoid's absence.

vi. Further *X. stemmator* releases should be done using populations available in the neighbouring countries

vii. Host-parasitoid interaction models should be used to further assess the impact of *C. flavipes* on stem borer populations.

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