CROSS-MATING AND OVIPOSITION BEHAVIOUR BETWEEN STRAINS OF *COTESIA SESAMIAE.* CAMERON (HYMENOPTERA: BRACONIDAE*)* FROM KITALE, MOMBASA AND MOUNT KENYA.

By

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree or any other award in any other University.

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DEDICATION

To my husband Peter, son Willy, daughter Anne, my mother and late father for constant love and support.

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ACRONYMS & ABBREVIATIONS

ARPPIS– African Regional Postgraduate Programme in Insect Science

ASAL – Arid and semi-arid lands

DAAD – German Academic Exchange Service

FAO – Food and Agricultural Organisation

ICIPE – International Centre for Insect Physiology and Ecology

WHO – World Health Organisation

SAS – Statistical Analysis Software

C – Cured

UC – Uncured

Cs – *Cotesia sesamiae*

Sc – *Sesamia calamistis*

Bf – *Busseola fusca*

Cp – *Chilo partellus*

C.s Msa C – *Cotesia sesamiae* Mombasa cured

C.s Kita UC – *Cotesia sesamiae* Kitale uncured

C.s Msa UC – *Cotesia sesamiae* Mombasa uncured

C.s Kita C – *Cotesia sesamiae* Kitale cured

C.s Mk1C – *Cotesia sesamiae* Mount Kenya 1 cured

C.s Mk1 UC – *Cotesia sesamiae* Mount Kenya 1 uncured

CI-Fm- (Known as female mortality type), is the death of fertilized eggs.

CI-MD- (Known as the male development type), one of the two possible outcomes assumed in an incompatible cross: eggs fertilized with a sperm from an infected male develop into males.

CI- Phenomenon induced by Wolbachia whereby crosses between a wolbachia infected male and a healthy female are incompatible.

K-Kitale

M-Mombasa

F-Mt Kenya

DEFINITIONS OF TERMS

Allopatric   Geographically isolated populations.

Avirulence   Inability to cause disease/ complete development of a progeny in an

                       organism.

Avirulent strain   A strain that does not develop in certain populations, in this case

                       Cotesia strain not developing in all populations of *B. fusca*

Biological control   A method of pest control using natural enemies such as predators/

                       parasitoids to regulate pest populations.

Biotype   A group of individuals that are genetically identical forming a

                       physiologically distinct race within a species.

Cured   Individuals disinfected with an antibiotic.

Encapsulation   An immune response by the host larvae that involves engulfing of

                       parasitoids eggs.

Endoparasitoids   Parasitoids that feed and develop within the body of their hosts.

Parasitoid   An insect whose larvae feed exclusively on the body of another

                       arthropod, its host, eventually killing it.

Primary parasitoid   A species attacking the free-living organisms (herbivore or

                       predator), it is attacked by a secondary or hyperparasitoid.

Resistant strain   Group of a population not allowing infection or succumbing to

                       death unlike all others in the same population.

Semiochemical   Any chemicals that carries information about the emitter to a

                       receiver.

Susceptible   Liable to infection or succumbing to death example *B. fusca* larvae that is liable to attack by *Cotesia* parasitoids.

Susceptible strain   Group in a population allowing infection or succumbing to death as opposed to other members of same population.

Sympatric   Populations of hosts and parasitoids in the same geographic regions.

Synomone   A semiochemical that is adaptively advantageous to both the sender and the receiving organism. It is typically a pheromone that induces a behavioural or physiological change in an individual.

Uncured   Individuals not disinfected with an antibiotic.

Variation   Genotypic and phenotypic differences between individuals in a population or between parents and their offspring.

Virulence   The collective properties of a parasitoid that renders it pathogenic to the host.

Virulent strain   *Cotesia* strains that successfully completes development in *B .fusca* larvae.

Wolbachia   An endocytoplasmic bacterium responsible for various reproductive modifications in Arthropods

ABSTRACT

Stemborers cause 50% yield losses in cereal crops including maize, sorghum and sugarcane. In Kenya, stemborers include *Busseola fusca*, B. phaia, *Sesamia calamistis*, *Eldana saccharina*, *Chilo orichalcociliellus* and *C. partellus*. Control options for the various stemborers include use of pesticides, early planting, intercropping with non-cereals and host plant resistance. In addition to being expensive, pesticides are environmentally unfriendly and are not fully effective due to the cryptic feeding behavior of the larvae. Thus the search for more efficient and convenient control methods for these pests is still ongoing. One of the other methods still being researched on is biological control where natural enemies, predators, parasitoids and pathogens, are used to lower the population of the pest to below economic injury level. Predators cannot keep the stemborers below economic injury levels, as pathogens and nematodes do not regulate the stemborer numbers. Parasitoids feed on immature stages of other insect host stages and kill them in the process. In Kenya *C. sesamiae* is one of the parasitoids that has been studied in regard to the control of stemborer pests. The relative importance of *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae), a gregarious larval parasitoid of lepidopteran stemborers, varies significantly between regions. Two biotypes of the parasitoid have been described in Kenya: the coastal biotype, which is unable to develop in the noctuid *B. fusca* (Fuller) and the western biotype, which develops successfully in this host. Thus several strains of *C. sesamiae* from Kenya are envisaged for introduction into regions where *B. fusca* is the main pest icluding certain parts of Kenya such as Wundanyi (Taita-Taveta District,), and Central, and western Africa. In this study the reproductive compatibility of three populations of *C. sesamiae* was investigated using the noctuid *S. calamistis* and *B. fusca* as hosts. The effect of cross mating on the mating and oviposition behavior of *C. sesamiae* strains as well as the influence of parental strain of *C. sesamiae* on the fitness and sex ratio of their hybrid progeny was investigated. Further, the effect of *Wolbachia* treatment on the reproductive compatibility of the three strains was assessed. The three strains of *C. sesamiae* from coastal regions, central (Mt Kenya) and western (Kitale) were cross-mated among themselves giving rise to homogenic and heterogenic crosses. The mated females (whether cured or not) oviposited in fourth instar larvae of *S. calamistis* and *B. fusca* as hosts. Duration of searching, courting, foraging, mating and ovipositing was recorded. Data on searching, mating time and cross mating parameters were analyzed using analysis of variance (ANOVA). Means were separated by the Student-Newman-Keuls (SNK) test. The results showed that homogamic crosses of Mombasa uncured and Mt Kenya cured took a long time to mate ($20.27\pm0.63a$ and 12.28 ± 0.27) respectively. The number of Progeny produced varied among the crosses with cured Mombasa male mated with cured Kitale female having the highest with ($33.0\pm0.1a$) and a sex ratio of 8% compared with uncured Mombasa male mated with uncured Kitale female having the lowest with ($9.3\pm0.1c$) and a sex ratio of $0.6\pm0.1abcd$, therefore curing affects mating behavior, progeny and sex ratio. The F1 individual were neither compatible among themselves nor with the parents. All parameters were highly significant apart from mean number of cocoons. The output of this work has helped to determine the effect of cross mating and oviposition behaviour of *C. sesamia* strains and the influence of crossing on sex ratio and progeny size and has helped to understand the possible consequences of the introduction and redistribution of *C sesamiae* for the sustainable control of stem borers in Africa.

1 INTRODUCTION

1.1 Background

Maize, *Zea mays* L. (Poaceae), is a staple food for majority of sub-saharan African countries (Ransom *et al.*, 1997; CIMMYT and EARO, 1999). However, yields are low partly because it is mostly grown by resource-poor, small-scale farmers. Other factors responsible for low production include unreliable rainfall, low soil fertility, insect pests and diseases (Kumwenda *et al.*, 1996). Lepidopteran stemborers are generally considered to be the most important group of insect pests that attack maize, sorghum and sugarcane in many areas of sub-saharan Africa (Polaszek, 1998; Overholt *et al.*, 2001; Kfir *et al.*, 2002). Yield losses in areas with high chronic infestations range from 10 to 70% (Cardwell *et al.*, 1997; De Groote *et al.*, 2003).

In East and Southern Africa (ESA), the noctuid *Busseola fusca* (Fuller) and the crambid *Chilo partellus* (Swinhoe) are the most economically important stemborers in the high- and lowland areas, respectively, while the noctuid *Sesamia calamistis* Hampson, the crambid *Chilo orichalcociliellus* Strand and the pyralid *Eldana saccharina* Walker are of minor importance (Bonhof *et al.*, 1997; Songa *et al.*, 2001; Le Rü *et al.*, 2006 a, b). Parasitism by the gregarious larval parasitoid *C. sesamiae* is usually below 7% (Jiang *et al.*, 2006) though it can reach 75% in some areas (Kfir, 1995; Gitau, 2007).

Various control strategies, which include chemical, cultural, and, host plant resistance, have been used but none has provided a complete solution (Sétamou *et al.*, 1995; Schulthess and Adjala, 1999; Ndemah and Schulthess, 2002; Bruce *et al.*, 2004). More

recently, the International Centre for Insect Physiology and Ecology (ICIPE) put emphasis on biological control programes. ICIPE has focused on classical biological control of C. *partellus* using the Asian larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) and the ichneumonid pupal parasitoid *Xanthopimpla stemmator* Thunberg.

While habitat management techniques and classical biological control with C. *flavipes* have been shown to significantly reduce C. *partellus* populations, no satisfying solutions presently exist for the control of B. *fusca* (Gitau, 2007; Bruce, 2008). Schulthess *et al.*, (1997) proposed the exchange of natural enemy species and strains between African regions. Surveys in Africa have shown that several parasitoid species, common in some regions, are scarce or absent in other regions (Gounou *et al.*, 1994; Bosque-Pérez *et al.*, 1994; Bonhof *et al.*, 1997; Ndemah *et al.*, 2001; Conlong, 2001; Songa *et al.*, 2001; Gounou and Schulthess, 2006). A good example is C. *sesamiae*, which is the most common larval parasitoid of stemborers in East and Southern Africa but scarce in Western Africa (Schulthess *et al.*, 1997; Polaszek, 1998; Ndemah *et al.*, 2007).

1.2 Statement of the problem

In Kenya, C. *sesamiae* exists as two biotypes that differ in their ability to parasitize B. *fusca*. The first biotype is C. *sesamiae* from western Kenya which is able to complete development in B. *fusca* larvae, while the second is the coastal biotype whose eggs are encapsulated by haemocytes in B. *fusca* larvae (Ngi-Song *et al.*, 1998; Mochiah *et al.*, 2002; Gitau *et al.*, 2006).

Insects across several genera including *Cotesia* spp. are often contaminated by *Wolbachia* spp. a bacteria that infects arthropods and is assumed to play an important role in progeny production and parasitoid population dynamics and may affect establishment of a parasitoid in a new area (Blanca *et al.*, 2006). The Coastal strain of *C. sesamiae* is known to be infected with *Wolbachia* sp. whereas the Kitale strain is not (Ngi-Song, 1995; Mochiah *et al.*, 2002b). *Wolbachia* sp. infection is known to cause unidirectional incompatibility whereby crosses between males from an infected population from the Kenyan coast and females from an uninfected population from inland Kenya do not yield female offspring and this is referred to as female mortality type. It has been refuted that *Wolbalchia* sp. infection causes cytoplasmic incompatibility of the female mortality type in some of the *C. sesamiae* strains (Ngi-Song *et al.*, 1998; Mochiah *et al.*, 2002a). Furthermore the Mount Kenya strain of *C. sesamiae* (MK1), which is similar to the Coastal strain, is unable to develop on *B. fusca* (Blanca, personal communication) and it is the only strain, which is bi-infected by *Wolbachia* sp. The MK1 strain of *C. sesamiae* is genetically closer to the Kitale than the Coastal strain (Gitau, 2007).

1.3 Justification

For many smallholder farmers in sub-Saharan Africa, maize and sorghum production is seriously hampered by stemborers. Most farmers cannot afford insecticides for stem borer control. Also, chemical control using systemic insecticides provides no protection against the borers feeding in the cob (Sétamou *et al.*, 1995; Ndemah and Schulthess, 2002). In addition, the disruption of natural enemies due to extensive pesticide use has often led to

resurgence and pest population explosion besides the concern of the pesticide residue on the marketable produce (Ikin *et al.*, 1993). Therefore, in recent years, self-sustainable control methods such as biological control have been given increased emphasis by many research institutions including ICIPE. Biological control, utilizes natural enemies to reduce the damage caused by noxious organisms to tolerable levels (DeBach and Rosen, 1991). Over the past 60 years, there have been numerous attempts to introduce exotic parasitoids into Africa and the Indian ocean Islands for biological control of exotic and native stem borers, but only a few have established.

Experiments conducted by Gounou *et al.* (2008) which involved crossing the Nigerian *C. sesamiae* strains with the Kenyan ones, showed that reproductive compatibility did not explain regional differences in the relative importance of *C. sesamiae*. Their study assessed the uni-directional (one way) compatibility of different *C. sesamiae* populations. Their work helped to predict the chances of establishing different *C. sesamiae* strains and their hybrids in East and western Africa. During country-wide surveys in West Africa and Cameroon, Bosque-Pérez, *et al.* (1994), Gounou *et al.* (1994), Schulthess *et al.* (1997), Conlong (2001), Ndemah *et al.* (2001), and Ndemah *et al.,* (2007) found that several parasitoid species, common in other regions in Africa, were very scarce or absent in western Africa and the vice versa. For example, *C. sesamiae* is exceedingly rare in western Africa, but is the most common larval parasitoid recovered from *B. fusca* and *S. calamistis*. This opens opportunities for exchange of natural enemy species and strains between African regions to control indigenous cereal stemborers as proposed by Schulthess *et al.* (1997). The cross-mating between the three strains of *Cotesia sesamiae*

from coastal Kenya, Mt Kenya and Kitale were aimed at yielding compatible strains for future establishment in various African regions where the parasitoid is scarce or non-existing. This study attempted to cross-mate the three strains and come up with the most compatible strains with the least effect on *wolbalchia* sp. infection and introduces them in the areas where B. *fusca* is endemic.

1.4   Null Hypotheses

a) The mating and oviposition behaviour of Coastal, Mt Kenya and Kitale, strains of *Cotesia sesamiae* are not affected by cross mating.

b) The sex ratio of the hybrid progeny is not affected by the parental strains used in the crosses.

c) There is no difference in sex ratio, progeny size and mating and oviposition behaviour between hybrid and pure bred progeny of the three strains of *Cotesia sesamiae*.

d) *Wolbachia* infection has no effect on the mating behaviour and sex ratio of the progeny.

1.5   Objectives

1.5.1   General objective

To investigate the degree of reproductive compatibility of three *C. sesamiae* populations using *S. calamistis* and *B. fusca* as hosts.

1.5.2   Specific objectives

a.   To determine the effect of cross mating on the mating and oviposition behavior of three strains of *C. sesamiae* found in Kenya.

b.   To investigate the influence of parental strain of *C. sesamiae* on the sex ratio of their hybrid progeny

c.   To evaluate the sex ratio, progeny size and mating behaviour of F1 progeny and their crosses of the three strains of *C. sesamiae.*

d.   To assess the effect of *Wolbachia* infection on the sex ratio, progeny size and mating behaviour of the three strains of *C. sesamiae.*

2   LITERATURE REVIEW

2.1   Stemborer diversity and economic importance

The global demand for cereals in the year 2020 was in the past estimated at 2.1 billion metric tones and the tendency for a rise in maize demand had been shown (James, 2003). This maize demand was estimated at 582 million metric tones compared with 760 million metric tones for wheat and 503 million metric tones for rice. Globally the demand for maize, rice and wheat in 2020 was estimated to be 45%, 32 % and 30% respectively (James, 2003). This translates to 72% substantial growth for maize production in developing countries, and only 18% growth in developed countries. Different methods need to be sought and existing ones reinforced to manage this increase in demand of maize in tropical Africa (FAO, 2002).

In addition to low and unreliable rainfall and soil infertility as major factors responsible for low grain yields, infestation due to insect pests is also a major constraint (Grisley, 1997; Bonhof, 2000). Yield losses in areas with high populations of stem borers could be as high as 73% (De Groote *et al.*, 2003).   In areas with chronic pest problems, due to multiple stem borer species attack, yield losses vary between 10 and 75% (Cardwell *et al.*, 1997; Songa *et al.*, 2001, Songa *et al.*, 2007; Ndemah and Schulthess 2002; Buadu *et al.*, 2003).

In sub-Saharan Africa predictable lepidopteran stem- and cob-boring pests hinder maize production and consequently the yield. The stem borer menace mostly hits the small-

scale farmers who use resource-poor systems to grow the maize. The farmers lack farm inputs such as seeds and fertilizers.

There are 12 stem-borer pest species recorded in Africa, but the most important and widely distributed are *C. partellus, C. orichalcociliellus, B. fusca, S. calamistis, S. cretica* and *E. saccharina. Chilo partellus,* The latter was introduced from the Indian subcontinent and was first recorded in Africa from Malawi in 1930 (Tams, 1932) while the others are indigenous to Africa. In East Africa, the major stem borer pests include the exotic crambid *C. partellus*, the indigenous noctuid *B. fusca* while *S. calamistis C. orichalcociliellus* Strand, *S. cretica* are of minor importance (Tams, 1932; Jepson, 1954; Ingram, 1958; Nye, 1960; Mohyuddin and Greathead, 1971; Seshu Reddy, 1983; Minja, 1990; Kfir *et al.*, 2002; Le Ru *et al.*, 2006a, b). B*usseola. fusca* is the most damaging species in the highlands while, *C. partellus* is the predominant species in the lowlands (Ofomata *et al.,* 2000; Zhou *et al.,* 2001; Ong'amo *et al.,* 2006).

2.2   General biology of cereal stemborers

Most stem borers species have similar life cycles (Girling, 1978; Harris, 1990). Adult moths lay eggs in clusters inside the leaf sheath or on lobes, and eggs hatch into first instar larvae within 4-8 days.   Depending on temperature, the larval period lasts 25-45 days with 6-8 larval instars. Early instar larvae migrate to the whorl of the plants where they feed on succulent young tissue. The older larvae bore tunnels in the stem where they remain until pupation. Before pupation, the mature larva construct pupal chambers in one end of the feeding tunnel and chew an exit hole through the stem, leaving a thin layer of epidermal tissue for egress of adult moth. Pupal period lasts for 7-10 days and adult life

span is 4-14 days depending on the species and environmental conditions (Girling, 1978; Shanower *et al.,* 1993). During the adult stage, the moths mate and lay eggs (Smith *et al.,* 1993).

2.3 Stemborer damage and crop losses

Chilo partellus and *B. fusca* larvae feeding produce window paning and pinholes (Plate 2.1) while other stem borer larvae cause stem tunneling (Plate 2.2) on attacked maize and sorghum plants. Newly hatched larvae of *Chilo* spp and *Busseola fusca* feed initially by scraping in whorls of growing plants, producing characteristic window-paning and pinholes. Later, the larvae tunnel into the stems and may kill the central leaves and growing point, producing deadhearts (Plate 2.3). Plants affected have poor growth and reduced yield and are more susceptible to wind damage and secondary fungal infestations (Seshu Reddy, 1998) These damages eventually result in crop losses as a consequence of early leaf senescence, interference with translocation of metabolites and nutrients that result to malformation of grains, stem breakage, plant stunting, lodging and direct damage to the ears (Bosque-Pérez and Mareck, 1991). Larvae also bore into the maize cobs, destroy flowers, tassel and feed on the developing grains. Grain damage (Plate 2.4) by lepidopterous borers also predisposes maize to pre- and post-harvest infestations by storage beetles, infections by *Aspergillus flavus* Link and *Fusarium verticillioides* Sacc. (Nirenberg) and subsequent contamination with mycotoxins such as aflatoxin and fumonisin (Sétamou *et al.,* 1998; Cardwell *et al.,* 2000; Schulthess *et al.,* 2002; Ako *et al.,* 2003) The mycotoxins later on cause serious food poisoning (Hell *et al.,* 2000).

Plate 2.1 Leaf damage by Sesamiae calamistis on maize plant

Plate 2.2 Sesamiae calamistis feeding on maize cob grains

Plate 2.3 Maize plant showing the dead heart symptom during early attack by *Sesamiae calamistis*

Plate 2.4 *Sesamiae calamistis* feeding on maize cob grains

2.4   Stem borer management

Various control strategies have been tried, some with partial success, but all have limitations and none has provided a complete solution. However, habitat management, as a component of integrated pest management (IPM) of crop pests has received research attention during the past three decades (Zethner, 1995).

2.4.1. Chemical control

A number of insecticides have been tested in the field and can be recommended for use (Ajayi, 1978). Granules of the systemic insecticides carbofuran, applied into the planting hole at planting, followed by a side- dressing 6 weeks later is able to control *Sesamia* larvae (Ajayi, 1987; Uvah and Ajayi, 1991). Malathion 50 EC, basudin 10 G and basudin 60 EC are also effective (Sagnia, 1983). Earlier studies showed that neem products are as efficient as carbofuran against stem borers such as *S. calamistis* and *E. saccharina* (Bruce *et al.*, 2004). Despite being effective in controlling stem borers, pesticides are expensive and kill non-target fauna such as the natural enemies and most farmers do not know how to apply them properly (Howarth, 1991). Despite the limitations of chemical control, in some countries a number of large- scale farmers and government-controlled farms have reported applying insecticides for stem-borer control based mainly on calendar of operations (Seshu Reddy, 1985a). In Africa, control of maize and sorghum stem borers exclusively by insecticides by resource-limited small-scale farmers is uneconomical and not practical Due to the a forementioned limitations there is a renewed need for other control methods such as biological control because of its benefits to the environment and cost effectiveness.

2.4.2. Cultural control

Cultural control is the most relevant and economical method of stem borer control for resource poor farmers in Africa and includes destruction of crop residues, intercropping, crop rotation, manipulation of planting dates and tillage methods (Schulthess *et al.*, 2004; Chabi-Olaye *et al.*, 2005). Some cultural- control measures have become routine practices employed by farmers. Therefore, they can after minor changes towards being more effective, be profitably integrated into the farming system. In addition, socio-economic aspects may also influence the acceptability of management practices, since practical realities may have an effect on the adoption of otherwise effective cultural-control measures. Khan *et al.* (1997); Schulthess *et al.* (1997) and Ndemah *et al.* (2002) showed that some wild grass species attracts the female moth to oviposite more on them than on maize. However failure of co-operation among farmers where some control the moth as others fail to, causes moths emerging from untreated fields to infest adjacent crops. This is a major constraint on cultural control due to lack of management capabilities among farmers, especially where farmers lack the support of adequate extension services (Harris, 1989a). In subsistence farming systems in Africa where farmers normally intercrop cereals with other crops and lack of water is a major constraint, manipulation of sowing dates and management of plant densities is not always practical as farmers often plant after the first rains (Polaszek, 1998).

2.4.3. Host plant resistance

Resistant crop varieties provide an inherent borer control that creates no environmental problems and they are generally compatible with other insect control methods (Bosque-

Pérez *et al.*, 1998; Kfir *et al., 2002*). The process of identification and transfer of insect resistance genes to develop resistant crop cultivars across plant species through biotechnology is already in practice in developed countries. Efforts are underway in Africa to identify sources of stem borer resistance in cereal crops, but high levels of resistance have not been found (Ajala *et al.*, 2002; Kfir *et al., 2002*). Nevertheless, this method, which complements other control methods, may solve the borer problem.

2.4.4. Biological control

In Africa, natural enemies including parasitoids, predators and pathogens play an important role in the control of lepidopterous borers (Bosque-Pérez and Schulthess, 1998). Biological Control options are environmentally safe and an alternative to the use of pesticides in pest management (Pimentel and Andow, 1984) Classical biological control refers to an approach whereby natural enemies of a pest in its aboriginal home are introduced to the area the pest has invaded. When successful, classical biological control provides an environmentally and ecologically suitable means of pest control (Mochiah, 2002). This has resulted in mounting criticism of chemicals in pest control, including increasing public fear and the banning of pesticides. The development of resistance to pesticides has renewed enthusiasm for classical biological control introduction (Howarth, 1991). More recently, IITA and ICIPE put more emphasis on biological control methods. For example, the control of the maize stem borer, *C. partellus*, in Kenya and East and Southern Africa is attributed to a gregarious endoparasitoid, *Cotesia flavipes* Cameron, which was introduced from Asia by the ICIPE (Omwega *et al.*, 1995; Overholt *et al.*, 1997, 2003). The use of indigenous natural enemies to control indigenous stemborer pests

is gaining importance in African regions. The use of native natural enemies to control pests is preferable because the impact of native enemies on non-target insects is assumed to be less than that of exotic enemies (Stiling, 1993, Huffaker *et al., 1977 ;*).

2.4.4.1 Parasitoids of stemborers

Wide ranges of egg, larval and pupal parasitoids of stem borers have been identified. The most abundant and widespread parasitoids in the East African region are the egg parasitoids *Telenomus* spp., and *Trichogramma* spp., the larval parasitoids *C. sesamiae* and *Sturmiopsis parasitica* (Curran) (Diptera: Tachinidae), and the pupal parasitoids *Pediobus furvus* (Gahan) (Hymenoptera: Eulophidae) and *Dentichasmias busseolae* Heinrich (Hymenoptera: Eulophidae) (Oloo and Ogeda, 1990; Bonhof *et al.*, 1997). The Braconidae is one of the largest families of Hymenoptera, and all except a few rare species are primary parasitoids. Together with the Ichneumonids, they account for over half the total number of parasitoid species associated with stem borers in Africa (Van Achterberg and Polaszek 1996). *Cotesia sesamiae,* is the most common indigenous parasitoid of stem borer larvae at the Kenya coast and in other areas of East and southern Africa (Mohyuddin and Greathead, 1970; Kfir, 1992).

2.4.4.1.1   Control of stem borers using *Cotesia sesamiae.*

The female parasitoid stings the hosts stem borer larvae and lay 40-60 eggs inside (Mochiah *et al.,* 2001). Developed larva from the egg after 10-12 days and spin white cocoons, which are loosely held together by strands of fine silk. The average egg to adult developmental is 18 days at 25^0C (Ngi-Song *et al.,* 1995; Mochiah *et al.,* 2001) The larval period on average is 11 days and the pre pupal and pupal period takes about   5.5

days at 28.5 ± 2^0C. Several factors could be responsible for the success of the *C. sesamiae* and *C. flavipes.* In their area of endemism, they attack several species of crambid, pyralid and noctuid stem borers (Mohyuddin, 1971; Nagarkatti and Nair, 1973; Beg and Inayatullah, 1980; Mohyuddin *et al.,* 1981; Goraya *et al.,* 1982; Shami and Mohyuddin, 1987). The relatively wide taxonomic range of suitable hosts, coupled with narrow habitat specificity, may favour their establishment. Stem borers often occur as species complexes, and a parasitoid that can exploit more than one host may be better adapted to colonise a new area than a monophagous parasitoid, due to a more constant availability of hosts. The parasitoids have a shorter generation time of 16-18 days in comparison to their hosts with 30-50 days and a high fecundity of 30-40 progeny per oviposition with female biased sex ratio. A high host-searching ability may also be involved. Wiedenmann and Smith (1997) demonstrated that, even at low densities, *C. flavipes* is able to successfully locate stem- borer hosts.

2.4.4.1.2 *Cotesia sesamiae* parasitoid

Cotesia sesamiae is an indigenous, gregarious, larval endoparasitoid that attacks mid– to late– instar stem borer larvae (Plate 2.5). Adults start mating immediately after emergence. Females locate stem borer larvae by volatiles emanating from the damaged gramineous host plants (Ngi-Song *et al.*, 1995). The females sting the host larvae and lay eggs inside . A female's clutch size is 40-60 eggs (Mochiah *et al.*, 2001). Fully developed parasitoid larvae emerge from the host larvae after 10 to 12 days and spin white cocoons loosely held together by strands of fine silk. The larval period on average is 11 days, and the pre-pupal and pupal in total being 5.5 days at $28.5\pm2°$C. The average egg to adult

developmental period is 18 days at 25°C (Ngi-Song *et al.*, 1995; Mochiah *et al.*, 2001).

Males emerge first and wait near the cocoons for the females to emerge (Ullyett, 1935).

Cotesia sesamiae is one of the most important native larval parasitoids in many countries

in sub-Saharan Africa (Ingram, 1958; Mohyuddin, 1971; Sheilbelreiter, 1980; Polaszek

and Walker, 1991; Walker, 1994; Kfir, 1995, 1998). *Cotesia sesamiae* has been used in

classical biological control programmes against *S. calamitis* in Mauritius, Reunion, and

Madagascar (Greathead, 1971). After its introduction into Mauritius, *C. sesamiae* became

the most abundant parasitoid of *S. calamistis* (Anon, 1954). Hailemichael *et al.,* (1997)

evaluated the on host acceptability and suitability of six gramineous borers occurring in

western Africa for *C. sesamiae*. These studies showed successful paratisization of *S.*

calamistis and *S. poephaga* Tams and Bowdens by *C. sesamiae*. Studies conducted in

South Africa identified *C. sesamiae,* as the most important mortality factor of *B. fusca*

(Mally, 1920; Du Plessis and Lea, 1943; Van Rensburg *et al. 1988;* Kfir and Bell, 1993).

Kfir (1990, 1992b) also recorded *C. sesamiae* as the most abundant parasitoid of *C.*

partellus. According to Kfir (1995), *C. sesamiae* was active throughout the season and

was by far the most abundant parasitoid of *B. fusca*. It was reared from about 90% of

parasitized larvae.

Cotesia sesamiae and the exotic *C. flavipes* overlap in their niches and they are closely

related (Polaszek and Walker, 1991). But in East and southern Africa *C. sesamiae* is

mostly recovered from borers attacking sorghum or maize (Matama *et al.*, 2006; Gitau,

2007; Wale *et al.*, 2007). The use of native natural enemies to control pests is preferable

because the impact of native enemies to non-target insects is assumed to be less than that of exotic enemies (Huffaker *et al.,* 1977; Stiling, 1993). In western Kenya particularly in the Kitale area, parasitism of *B. fusca* by *C. sesamiae* can be as high as 70% (Gitau, 2007). Similarly in South Africa, parasitism of *B. fusca* by *Cotesia sesamiae* on sorghum was as high as 75% and the parasitation is thought to keep *S. calamistis* under control (Kfir, 1995). In Kenya, *C. sesamiae* exists as two biotypes that differ in their ability to parasitise *B. fusca. Cotesia sesamiae* from western Kenya completes development in *B. fusca* larvae, but the eggs of the coastal biotype the eggs are encapsulated inside the *B. fusca* larvae (Gitau *et al.,* 2006). In recent surveys in Cameroon it was only obtained from the noctuids *Poeonoma serrata* Hampson and *Busseola quadrata* Bowden feeding on *Pennisetum purpureum* (Moench) and *Setaria megaphylla* (Steud) Dur. and Schinz, respectively, and never from *B. fusca* on maize (Ndemah *et al.,* 2007).

Plate 2.5.   Adult Male *Cotesia sesamiae*

Table 2.1. Host range and distribution of *Cotesia sesamiae* in Africa

Notuidae	Distribution	Association (old/new)	References
Busseola fusca Fuller	East Africa S. Africa W. Africa	Old	Mohyuddin & Greathead, 1970 Ullyett, 1935 Walker, 1994
Sesamia calamistis Hampson	It is found in all Sub-Saharan Africa	Old/ New	(Le Ru, Personal communication)
Spodoptera litura (F)	S.Africa	Old	Polaszek &Walker, 1991
Sesamia *spp*	All sub-Saharan Africa Africa	Old	(Le Ru, personal communication)
Pyralidae			
Chilo partellus (Swinhoe)	Mozambique East Africa, Sudan, Malawi, South Africa	New	Polaszek & Walker, 1991 Mohyuddin & Greathead, 1970 Mohyuddin & Greathead, 1970
Chilo Orichalcociliellus (Strand)	Mauritius Kenya Mozambique Tanzania	Old	Polaszek & Walker, 1991 Polaszek & Walker, 1991 Polaszek & Walker, 1991

3   MATERIALS AND METHODS

3.1   Insect for use

3.1.1   Stemborers and parasitoids in the experiments

Two stemborer species, *S. calamistis* and *B. fusca* were used in this study. Larvae

of the two species were obtained directly from the Animal Rearing and

Containment Unit (ARCU) of ICIPE Nairobi Kenya. Three times in a year, new

insects are collected from the field and added into the colony in order to ensure

constant supply of insects. Stem borer colonies were reared and maintained on

artificial diet in the laboratory as described by Onyango and Ochieng-Odero

(1994). Fourth instar larvae, the most suitable host stage for development and

reproduction of *C. sesamiae were* used (Ngi-Song *et al.*, 1995). The stem borer

larvae were removed from the artificial diet and introduced into glass jars (16 x 9

cm) containing pieces of maize stems. This was to make the larvae more attractive

to the parasitoids owing to the frass from maize: infested host plants release a

synomone that is attractive to parasitoids (Potting *et al.*, 1995). The set up was left

for 24 hours for borer to feed and produce frass prior to exposure to a mated

female *C. sesamiae.*

Three geographically located populations of *C. sesamiae* from western (Kitale),

central (Mount Kenya) and coastal regions of Kenya were used in this study. The

parasitoid colonies were initiated in 2006-2007 with materials collected from

maize and sorghum in the respective regions. At the coast and Mount Kenya, *C.*

Sesamiae was recovered from S. *calamistis* whereas in Kitale the parasitoid was recovered from *B. fusca*.

To avoid mixing of the parasitoid populations, rearing and cross mating experiments were conducted in separate rooms at $25 \pm 2°C$, 50-80% relative humidity and 12:12h (L: D) photoperiod (Overholt *et al.*, 1994).

3.2   Cross-mating experiments

The various populations of *C. sesamiae* were allowed to emerge singly by separating individual dark cocoons from the cocoon masses. Immediately after emergence, the naïve male and female parasitoids were paired and allowed to mate in transparent glass vials measuring 5 x 2 cm, (height x diameter). Soon after mating, the pairs were fed on 20% honey/water solution (Overholt *et al.*, 1994) dipped in cotton wool which was placed on a plastic cup. *Wolbachia.* is a bacteria that infects arthropods affecting their reproductive compatibility. Parasitoids were cured of *Wolbachia* by incorporating an antibiotic, 2% rifadine (Rifampicin), into the diet where parasitized larva are reared until cocoon formation. During mating, both cured and uncured strains of heterogenic and homogenic crosses were mated. Heterogenic refers to those of different strains, for example kitale strain crossed with Mombasa strain. Homogenic are those of same strain, for example Mombasa male mated with Mombasa female as indicated in table 3.1.The set ups were left undisturbed for at least 3 hours before being exposed to the *Wolbachia* cured host

larvae for oviposition experiments. Only mated females of *C. sesamiae* were used

for the oviposition experiment in vials measuring (7.5 by 2.5). Sex ratio, progeny,

number not forming cocoons, number dead in cocoons and mating behaviour, were

assessed for both cured and uncured couples. The searching, foraging and

oviposition period of the parasitoid on the stem borer larvae were assessed during

crossings through out the experiments.

Table 3.1. Heterogenic and homogenic crosses.

MALE		Kitale	FEMALE Mombasa	Mt Kenya
Treatment	Strain			
			Cured	
	Kitale	X	X	X
	Mombasa	X	X	X
Cured	Mt Kenya	X	X	X
			Uncured	
	Kitale	X	X	X
	Mombasa	X	X	X
	Mt Kenya	X	X	X
			Cured	
	Kitale	X	X	X
	Mombasa	X	X	X
Uncured	Mt Kenya	X	X	X
			Uncured	
	Kitale	X	X	X
	Mombasa	X	X	X
	Mt Kenya	X	X	X

Searching time (T1), time taken to locate hosts (T2), and time taken for oviposition

(T3) were recorded. Each parasitized host larva was transferred and reared singly

in vials measuring (7.5 by 2.5cm) containing artificial diet.

For each geographical location, 15 females were tested for cross mating. Thereafter, the host larvae were reared on artificial diet and monitored daily for cocoon formation or pupation or host mortality for 12-14 days. The percentage of hosts from which parasitoid cocoons emerged, brood size (i.e. number of cocoons), total progeny, sex ratio as the proportion of female offspring, mortality of mature parasitoids expressed as the percentage of adults emerging over number of cocoons, and developmental time were recorded. The experiments were conducted in the laboratory with conditions maintained as follows: temperature 25 ± 2^0C, relative humidity (RH) of 50-80% and a photoperiod of 12:12h (L: D)

3.3   Data analysis

Observations on searching and mating time and percent females that mated successfully in the cross mating experiment were carried out as a time series (in three or four sets). For the assessment of the reproductive potential of the different crosses and all observations in all experiments, only one set of time series was used. Differences in percentage females were analyzed using (ANOVA) PROC GLM (SAS Institute 2003). Tukey's test was used as a post ANOVA procedure to separate the means. The significance level was set at $P \leq 0.05$. Insect counts were log transformed and percentages and proportions were arcsine transformed before being subjected to ANOVA (Sokal and Rohlf, 1981). Back transformed means are presented in the tables of results and the graphs.

4 RESULTS

4.1 The effect of cross mating on mating and oviposition behaviour of *C. sesamiae* strains

The behavioural steps or courtship leading to copulation such as wing fanning by males was usually in form of continuous and sustained movement. Wings were spread out almost perpendicular to the long axis of the body, with less flapping than when flying. This observation was noted once males were introduced into the vials with females. The longest mating period was observed between crosses of uncured Mombasa males with uncured Mombasa females followed by cured Mt Kenya males with cured Mt Kenya females which were significantly different from all the other crosses but not significantly different from each other (Table 4.1). Crosses of cured Kitale males mated with cured Kitale females had the second longest period and were also significantly different from cured Kitale males mated with uncured Mombasa females, uncured Kitale males mated with uncured Kitale females, uncured Kitale males mated with cured Mt Kenya females, cured Mt Kenya males mated with cured Kitale females, cured Mt Kenya males mated with uncured Mt Kenya female and uncured Mt Kenya male mated with cured Kitale female, which had the shortest mating period. All the other crosses were not significantly different from each other (Table 4.1). The longest courting period was in two crosses, cured Kitale male mated with uncured Mombasa female and cured Kitale male mated with cured Mt Kenya female which were not significantly different from each other but were significantly different

from all other crosses and notably the cross cured Kitale male mated with cured Kitale female with one of the shortest courting period. The courting period among crosses in descending order was cured Kitale male mated with cured Mombasa female, uncured Mombasa male mated with uncured Kitale female, uncured Kitale male mated with cured Mt Kenya female and uncured Mt Kenya male mated with cured Mt Kenya female with the shortest period resulting from cured Kitale male mated with cured Kitale female which was significantly different from all the other crosses (Table 4.1).

The oviposition period was significantly different in only one crossing, cured Mombasa male mated with cured Kitale female with the shortest period while all others were not significantly different from each other. The longest periods in searching and foraging are in the same cross cured Mombasa male mated with cured Kitale female and the same cross gave the shortest period of Oviposition.

Table 4.1. Courting and mating periods for males and females from three populations of *C. sesamiae* from coastal and inland Kenya.

Cross			Mean ± SE time in seconds		
Male	Female	N	Courting(in seconds)	N	Mating(in seconds)
kc	kc	13	240.0±10.9f	13	17.46±0.9b
kc	ku	52	842.3±14.4bcedf	52	16.0±0.16bc
kc	mc	35	1721.7±35.8b	35	15.6±0.9bc
kc	mu	40	2835.0±36.1a	40	13.3±0.6c
kc	fc	55	2663.3±31.8ab	55	14.8±0.0bc
ku	kc	114	540.0±7.3fde	114	13.4±0.3bc
ku	ku	11	569.2±16.3fde	11	12.7±0.3c
ku	mu	24	673.1±23.1fde	24	14.3±0.6bc
ku	fc	26	423.5±10.2e	26	12.7±0.4c
ku	fu	65	554.8±8.2cedf	65	13.9±0.5bc
mc	kc	25	888.0±24.6cedf	24	14.0±0.7bc
mc	mc	21	948.6±16.0bcd	21	14.7±0.2bc
mu	ku	35	1290.3±36.7c	35	16.2±0.8bc
mu	mu	7	1414.3±30.7abc	7	17.9±1.0ab
fc	kc	52	693.5±16.2cedf	52	12.8±0.3c
fc	fc	11	272.7±8.4fde	11	20.3±0.6a
fc	fu	80	399.8±9.1fde	80	12.3±0.3c
fu	kc	94	930.0±11.6bcd	92	12.6±0.4c
fu	ku	86	471.4±7.0fde	86	14.5±0.6bc
fu	fc	58	486.2±7.4e	57	15.0±0.8bc
fu	fu	37	1094.9±21.8bcdef	37	13.8±0.6bc
DF			20, 936		20, 936
F-value			4.00		4.00
P-value			<.0001		<.0001
Means followed by same letter(s) are not significantly different (p<0.05), Tukey's test					

key:-

k-Kitale

m-Mombasa

f-Mt Kenya

c-Cured

u-Uncured

The four crosses with the longest foraging time were cured Kitale male mated with cured Mombasa female, uncured Kitale male mated with uncured Mt Kenya female, cured Mombasa male mated with cured Kitale female and uncured Mt Kenya male mated with uncured Mt Kenya female which were not significantly different from each other, but were significantly different from the others.

The two crosses with the shortest period in foraging were cured Kitale male mated with cured Kitale female and cured Mt Kenya male mated with cured Mt Kenya female and were not significantly different from each other. The longest searching period was in cured Mombasa male mated with cured Kitale female cross which was significantly different from all others while the shortest was in uncured Mt Kenya male mated with uncured Mt Kenya female cross which was also significantly different from all others (Table 4.2).

4.2 The influence of parental strain of C. *sesamiae* on the sex ratio of their hybrid progeny.

The sex ratio was expressed as a proportion of females and it was male biased. It indicates the highest numbers of females are arising from cured Kitale male mated with cured Kitale female crossing which is significantly different from all others. The lowest was from cured Mombasa male mated with cured Kitale female which was significantly different from all other crosses but gave one of the highest progeny sizes (Table 4.3).

Table 4.2. The mean time (± S.E) searching, foraging (males) and Oviposition (females) period of parasitoids   from three populations of C. sesamiae in coastal and inland Kenya.

Cross								
			Mean ± SE time in seconds				
Male	Female	N	Searching	N	Foraging	N	Oviposition
kc	kc	13	63.4±2.2bcde	13	2.4±0.4c	13	12.1±0.1a
kc	ku	48	50.3±3.8bcde	48	9.2±0.8abc	48	14.6±0.2a
kc	mc	22	129.7±6.3b	24	30.8±1.7a	24	15.5±0.9a
kc	mu	38	62.2±8.9bcde	38	11.6±0.5abc	38	12.0±0.9a
kc	fc	41	41.4±6.8ed	41	19.8±0.9ab	41	11.2±0.5a
ku	kc	91	139.7±7.3b	87	23.8±0.7ab	85	13.2±0.7a
ku	ku	11	49.7±3.7bcde	11	3.8±0.9bc	11	11.5±0.8a
ku	mu	24	67.5±5.5bcde	24	14.1±0.5abc	24	13.7±0.3a
ku	fc	25	58.3±6.8bcde	25	5.4±0.3bc	25	13.5±0.7a
ku	fu	63	123.2±2.9b	63	28.1±0.6a	63	12.8±0.6a
mc	kc	14	242.8±4.6a	13	27.9±1.9a	13	8.6±1.0b
mc	mc	20	43.1±9.9cde	20	6.4±0.3bc	20	14.5±1.0a
mu	ku	29	89.5±2.6bc	28	21.9±1.1ab	28	12.3±0.0a
mu	mu	7	56.9±3.3bcde	7	13.0±0.7abc	7	15.9±0.2a
fc	kc	52	70.7±6.1bcd	52	9.5±0.2bc	52	13.8±0.8a
fc	fc	10	58.8±2.4bcde	10	3.0±0.5c	10	13.7±0.0a
fc	fu	74	66.2±4.2d	74	6.9±0.3bc	74	15.3±0.0a
fu	kc	62	99.4±5.0cb	58	5.1±0.7bc	58	13.9±0.7a
fu	ku	74	92.5±9.8cb	74	10.3±0.3abc	74	11.7±0.6a
fu	fc	52	72.7±1.2bcd	52	20.1±1.7abc	52	13.3±0.6a
fu	fu	37	40.2±5.5e	37	24.6±0.2a	37	12.2±0.6a
DF			20, 806		20, 798		20, 796
F-value			7.50		2.81		2.70
P-value			<.0001		<.0001		<.0001

Means followed by same letter(s) are not significantly different (p<0.05), Tukey's test

Key:-

k-Kitale,   m-Mombasa,   f-Mt Kenya,   c-Cured,   u-Uncured

Table 4.3. Progeny, sex ratio, cocoons and dead cocoons mean numbers of crosses between the three *C sesamiae* in coastal and inland Kenya.

| Cross | | | | | Mean ± SE | | | | |
Male	Female	N	progeny	N	Sex ratio	N	No of cocoons	N	dead cocoons
kc	kc	10	14.2±0.9abc	10	1.0±0.0a	11	2.2±0.4a	11	9.1±0.3abcde
kc	ku	26	19.0±0.1abc	26	0.4±0.1abcd	16	3.4±0.7a	26	5.9±0.6cde
kc	mc	7	26.4±0.2abc	6	0.4±0.1abcd	8	7.5±0.5a	6	23.3±0.4a
kc	mu	14	13.6±0.4abc	13	0.5±0.0abcd	9	4.2±0.2a	14	14.3±0.4abc
kc	fc	23	29.0±0.3ab	23	0.2±0.1bcd	13	4.2±0.7a	26	16.1±0.1ab
ku	kc	41	18.2±0.6abc	39	0.4±0.1abcd	27	4.5±0.6a	37	6.0±0.5bcde
ku	ku	11	26.9±0.7abc	11	0.7±0.1ab	3	3.0±0.6a	11	10.3±0.7abcde
ku	mu	15	21.0±0.2abc	15	0.7±0.1abc	6	7.8±0.8a	11	9.7±0.4abcde
ku	fc	12	27.8±0.3abc	12	0.4±0.0abcd	12	3.2±0.3a	13	4.9±0.8de
ku	fu	35	25.1±0.7abc	35	0.3±0.1abcd	27	5.9±0.8a	23	5.7±0.6cde
mc	kc	2	33.0±0.1a	2	0.1±0.0c	8	6.5±0.7a	6	11.5±0.7abcd
mc	mc	7	29.0±0.4ab	7	0.5±0.0abcd	-	2.0±-a	8	8.5±0.1abcde
mu	ku	15	9.3±0.1c	14	0.6±0.1abcd	9	4.1±0.5a	11	14.8±0.4abc
mu	mu	6	30.7±0.9ab	6	0.2±0.0dc	4	1.5±0.3a	6	7.2±1.0abcde
fc	kc	37	35.0±0.9a	37	0.5±0.1abcd	20	2.1±0.5a	32	5.8±0.2de
fc	fc	9	10.3±0.5bc	9	0.4±0.0abcd	9	3.6±0.7a	9	10.6±0.2abcde
fc	fu	46	30.7±0.2ab	46	0.4±0.1abcd	32	3.9±0.6a	44	6.7±0.5abcde
fu	kc	48	32.7±0.6ab	44	0.2±0.1dc	30	3.1±0.8a	42	6.6±0.6bcde
fu	ku	36	11.6±0.6abc	36	0.4±0.1abcd	30	3.4±0.4a	36	5.9±0.7de
fu	fc	26	30.7±0.1ab	26	0.4±0.1abcd	15	5.8±0.3a	10	4.4±0.3e
fu	fu	9	28.8±0.8ab	9	0.3±0.0bcd	-	8.0±-a	11	7.4±0.4abcde
DF			20, 434		20, 425		20, 290		20, 392
F-value			4.35		4.04		1.26		5.58
P-value			<.0001		<.0001		0.2058		<.0001

Means followed by same letter(s) are not significantly different (p<0.05), Tukey's test

Key:- k-Kitale,   m-Mombasa,   f-Mt Kenya,   c-Cured,   u-Uncured

Two crosses gave the highest mean progeny number, cured Mombasa male mated with cured Kitale female and cured Mt Kenya male mated with cured Kitale female. They were not significantly different from each other but were significantly different from all others. The crosses between uncured Mombasa male mated with uncured Kitale female gave the lowest progeny size and was significantly different from all others. The highest mean number of dead cocoons was obtained from cured Kitale male mated with cured Mombasa female crosses which were significantly different from all other crosses. The lowest number of dead cocoons was from uncured Mt Kenya male mated with cured Mt Kenya female crosses which were significantly different from all other crosses (Table 4.3). The mean developmental time from egg to cocoon was highest in uncured Mombasa male mated with uncured kitale female crosses which was significantly different from all other crosses while the lowest was from uncured Mt Kenya male mated with uncured Kitale female crosses which was significantly different from all other crosses (Table 4.4).

The two crosses giving the longest (cocoon to adult) emergence time are cured Mombasa male mated with cured Kitale female and uncured Mt Kenya male mated with cured Mt Kenya female which were not significantly different from each other but were significantly different from all other crosses while the two giving the lowest are cured Kitale male mated with cured Mombasa female and cured Kitale male mated with uncured Mombasa female which were not significantly different from each other but were significantly different from all others. The cross between uncured Mt Kenya male mated with cured Mt Kenya female had the highest number of days for the total duration and was significantly different from all other crosses. The same crosses were one of the two with the longest cocoon-to-adult duration in days and were significantly different from all others (Table 4.4).

Table 4.4. The mean developmental time ± S.E in (days): egg to cocoon, cocoon to adult and total duration.

| Cross | | | (Mean± SE) Duration in days | | | | |
Male	Female	N	Egg to cocoon	N	Cocoon to adult	N	Total duration
kc	kc	11	11.3±0.3efg	9	4.7±0.3ab	9	16.0±0.0b
kc	ku	30	12.1±0.1bcdef	26	4.2±0.2ab	26	16.3±0.1b
kc	mc	15	12.1±0.3bcdef	1	3.0±0.0b	1	15.0±0.0b
kc	mu	17	13.5±0.4ab	2	3.0±0.0b	2	17.5±0.5b
kc	fc	26	12.0±0.2cdef	4	3.8±0.3ab	4	16.5±0.3b
ku	kc	44	11.9±0.1cdef	38	4.1±0.1ab	38	15.9±0.1b
ku	ku	11	11.1±0.1fg	11	3.9±0.1ab	11	15.0±0.0b
ku	mu	18	12.9±0.2bc	15	3.6±0.2ab	15	16.4±0.3b
ku	fc	14	11.5±0.1fed	12	4.0±01ab	12	15.5±0.2b
ku	fu	44	11.9±0.2cdef	29	4.2±0.2ab	29	15.7±0.2b
mc	kc	11	11.4±0.3efg	8	5.3±0.3a	8	16.5±0.3b
mc	mc	8	13.0±0.0bc	6	4.0±0.0ab	6	17.0±0.0b
mu	ku	16	14.3±0.5a	12	3.6±0.4ab	12	17.1±0.3b
mu	mu	6	12.0±0.0cdef	6	4.0±0.0ab	6	16.0±0.0b
fc	kc	40	11.3±0.3efg	38	4.0±0.1ab	38	15.2±0.4b
fc	fc	9	12.3±0.4bcdef	9	4.0±0.2ab	9	16.3±0.6b
fc	fu	51	12.7±0.4bcde	42	4.5±0.1ab	42	17.2±0.4b
fu	kc	48	11.8±0.1cdef	44	4.1±0.1ab	44	15.9±0.1b
fu	ku	46	10.4±0.1g	34	4.6±0.1ab	34	15.1±0.2b
fu	fc	36	11.8±0.1fg	26	5.1±0.4a	26	19.5±0.4a
fu	fu	12	12.2±0.3cdef	9	4.0±0.0ab	9	16.1±0.4
DF			20, 512		20, 380		20, 380
F-value			11.58		3.32		2.47
P-value			<.0001		<.0001		0.0005

Means followed by same letter(s) are not significantly different (p<0.05), Tukey´s

Key:-

k-Kitale

m-Mombasa

f-Mt Kenya

c-Cured

u-Uncured

The total mortality was significantly different among the different crosses (DF 20, 940 F-value 4.05 and P-value <.0001). The two crosses with the highest total mortality were uncured Kitale male mated with uncured Kitale female and uncured Mombasa male mated with uncured Mombasa female and were not significantly different from each other but were significantly different from all other crosses. The two crosses with the lowest total mortality were uncured Mt Kenya male mated with cured Mt Kenya female and uncured Mt Kenya male mated with uncured Mt Kenya female which were not significantly different from each other but were from the rest of the crosses. (Figure 4.1)

The larval mortality was significantly different among crosses (df 20, 943, F-value 4.83 P-value <.0001). Three crosses, uncured Kitale male mated with uncured Kitale female, uncured Kitale male mated with uncured Mombasa female and uncured Mombasa male mated with uncured Mombasa female had the highest larval mortality and all were not significantly different from each other but are significantly different from all other crosses, some four crosses had no mortality these were, cured Kitale male mated with cured Kitale female, cured Kitale male mated with cured Mombasa female, cured Mombasa male mated with cured Kitale female and cured Mt Kenya male mated with cured Mt Kenya female and were not significantly different from each other but were significantly different from all other crosses (Figure 4.2).

Figure 4.1. Mean generation mortality in days (±SE) of three populations of *C. sesamiae* in coastal and inland Kenya
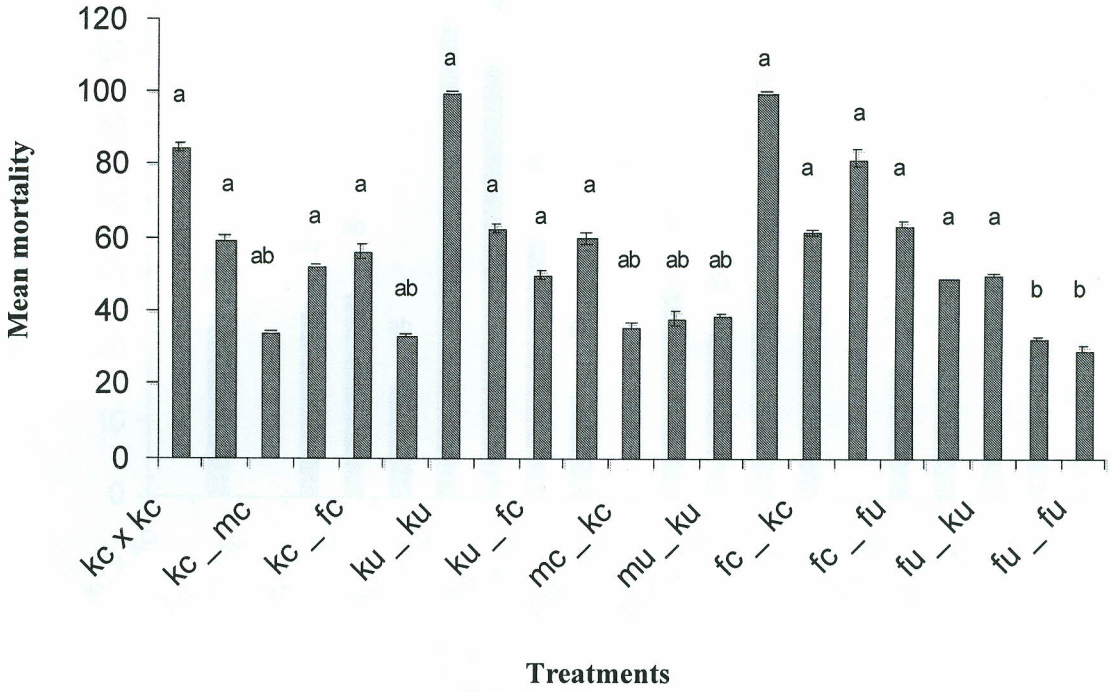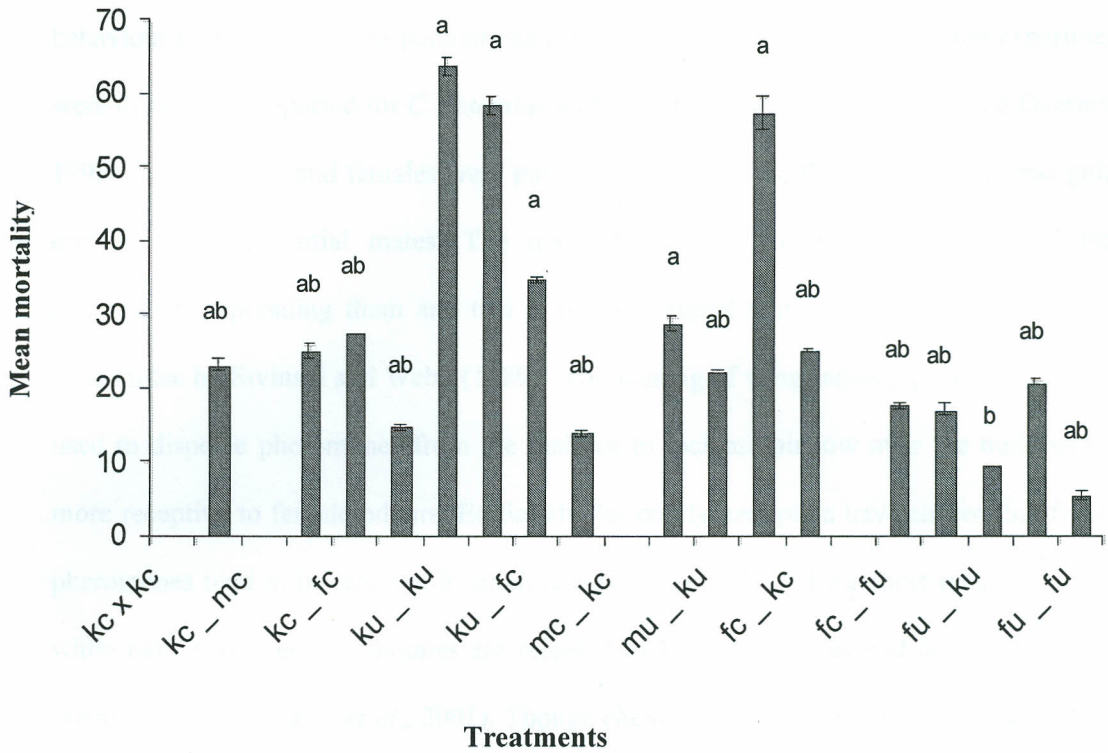

Key for figure 4.1:

k-Kitale

m-Mombasa

f-Mt Kenya

c-Cured

u-Uncured

Figure 4.2. Mean larval mortality in days (±SE) of three populations of *C. sesamiae* in coastal and inland Kenya

Key for figure 4.2:

k-Kitale

m-Mombasa

f-Mt Kenya

c-Cured

u-Uncured

5   DISCUSSION

Cross mating had an effect on mating and oviposition behaviour of *C sesamiae* strains. The results of the current study showed that the sequence of events observed in the mating behaviour of all *C. sesamiae* populations tested in the reciprocal and backcross experiment were the same as reported for *C. sesamiae* and other *Cotesia* species (Kimani and Overholt, 1995). When males and females were put in the mating vials, they were able to recognize each other as potential mates. The male started by fanning their wings and then consequently spreading them and this is acoustic signal which has been documented in Braconidae by Sivinski and Webb (1989). The fanning of wings among parasitoids may be used to disperse pheromones from the male or to increase airflow over the male and be more receptive to female odours. Earlier studies on Hymenoptera have shown that female pheromones tend to be released in small quantities and are used for short-range attraction, while male-produced pheromones are released in larger quantities and are used for long range attraction (Ayasse *et al.*, 2001). Though chemical, tactile as well as visual stimuli are involved in the release and continuation of courtship in parasitic Hymenoptera (van den Assem and Jachmann, 1982), the behaviour of *C. sesamiae* males seemed to suggest that more emphasis is placed on visual stimuli, which elicit an aggressive behaviour of physical pursuit of females. Irrespective of the strain used, *C. sesamiae* male parasitoids started fanning as soon as females were offered to them. The females especially those from Mt Kenya and kitale exhibited behaviours suggesting eagerness to mate without waiting for males to go through all the features of courtship particularily when being crossed with Mt Kenya strain. This is an indication of the existence of biological and behavioral differences between inland and coastal strains. In the present study, mating time was on average longest for cured Mt Kenya male mated with cured Mt Kenya female, uncured Mombasa

male mated with uncured Mombasa female and cured Kitale male mated with cured Kitale female which are all homogamic and the strains are both either cured or uncured. These were three out of the total of twenty one crosses. This further confirms the biological and behavioral differences between the cured and uncured populations. The shortest mating time on average was from six crosses of which five were heterogamic with one strain either cured or uncured while the sixth one was homogamic of Kitale uncured. The crossings between homogamic and heterogamic strains seem to suggest that longer mating periods are achieved among homogamic strains when they are both cured or uncured while the shortest periods are among heterogamic where the two strains are a mixture of cured and uncured. Thus heterogamic crosses are more successful in mating due to their short periods. Earlier works by (Gounou *et al.*, 2008) showed that the longer the couple took to copulate the lower the percentage of successful mating and this has a negative effect on the larva producing the cocoons. It seems possible to generalize those males and females from the same strain and of similar physiological conditions spend more time mating. The results of the research are confirming clearly that crossing cured and uncured strains is increasing the compatibility especially in heterogamic crosses. The study reflected that the shortest courtship was followed by the longest mating among Kitale and Mt. Kenya homogamic crosses which were all cured. The shortest period of oviposition is also the longest time in searching and foraging according to this study and it has a negative effect on the percent larvae that produces cocoons similarly reported by (Gounou *et al.,* 2008) in their work. Most of the searching periods were short which meant more cocoons forming on the larvae and this results to successful parasitism.

The highest numbers of females were obtained in crosses where the males were from Kitale either cured or uncured when crossed with females from Kitale or Mombasa while cured or uncured. The Kitale homogamic cured cross had the highest female number indicating a high compatibility to support the works earlier done by (Mochiah *et al* 2002b), where crosses carried out using progeny reared on an antibiotic-supplemented medium indicated that antibiotic treatment restored compatibility. Apart from the highest with cured Kitale strains the other two with high female numbers were crosses involving uncured Kitale males and uncured females from Kitale and Mombasa. The results are supported by the works of Ngi-Song *et al.,* (1998), Ngi-Song and Mochiah (2001) and Mochiah *et al.* (2002a).The outcome of these crossings suggest that there are no differences between males and females in relation to reproductive behaviour when males or females are cured since we got more female progeny in both cured and uncured crosses between Kitale and Mombasa but these were when Mombasa was the uncured female and Mt. Kenya strain is exempted. This too brings out clearly that the strain type is important in the reproductive compatibility as the levels of *Wolbachia* infection vary. On average the sex ratio among crosses is male biased which is a result of incompatibility in reproduction between the strains and has caused reduced progeny a condition in haplodiploid insect species (Diel and bush, 1984; Pinto *et al.,* 1991). Therefore, it can be concluded that the three strains are compatible in reproduction since half of the crosses in the two parameters are not significantly different. The cocoon mean numbers are all the same but the numbers that died are highly significant with majority being in the same range of 10-4 (fig. 4.3). The study shows that when cured Mombasa male is crossed with cured Kitale female the progeny is high but with the lowest female numbers. The cocoons formed gave rise to more males than females indicating the females are the ones that might have died as dead

cocoons, supporting the finding of unidirectional compatibility by Mochiah *et al.,* (2002a). The inland crosses between uncured Mt. Kenya male and cured Mt. Kenya female had the lowest dead cocoons showing they are highly compatible unlike the cured Kitale male and Mombasa female that had high numbers of dead cocoons. The crosses where the highest mortality occurred were the homogamic of both kitale and Mombasa when uncured and this is attributed to high *wolbachia sp.* infection.

The longest developmental period was observed from crosses between inland and coast uncured and the lowest from inland crosses uncured. This could be attributed to *wolbachia sp.* infection bringing differences in mating compatibility as suggested by Ngi-song and Mochiah (2001). The observed influence of *Wolbachia sp.* infestation in the reproductive and survival behavior of the parasitoids included the variation in size of the F1 population progeny from case to case and the sex-ratios varying significantly in the twenty one crosses. The mean differences of various crosses in egg to cocoon, cocoon to adult and total duration in days were minor implying once mating occurs these parameters have small variations.

In the crosses cured kitale male mated with cured Kitale female, cured kitale male mated with cured mombasa female, cured mombasa male mated with cured Kitale female and cured Mt Kenya male mated with cured Mt Kenya female larval mortality was zero but in the cured Kitale male mated with cured Kitale female and cured Mt Kenya male mated with cured Mt Kenya female total mortality was over 80%,while cured Kitale male mated with cured Mombasa female and cured Mombasa male mated with cured Kitale female was

below 40% due to unknown reasons, however, varying temperatures influenced the cross

mating and oviposition behaviour.

6   CONCLUSIONS AND RECOMMENDATIONS

6.1   Conclusions

1. Cross-mating effect both prolonged and shortened searching, mating and oviposition time of the three *C. sesamiae* strains.

2. The parental strain compatibility had an effect on sex ratio and increased and decreased progeny production.

3. The F1 progeny did not mate successfully to allow assessment of the sex ratio, mating behaviour and progeny size.

4. The *Wolbachia* infection lowered the compatibility of the various strains which affected the sex ratio, mating behaviour and progeny size.

5. Majority of the crosses had low compatibility and six out of twenty one can be used for establishment of *Cotesia sesamiae.*

6. The curing improves on sex ratio, progeny size and the reproductive parameters.

7. Strains of Kitale should be more used as females and not with Mombasa males.

8. Exchange of *cotesia sesamiae* could commence in African regions.

6.2 Recommendations

1. The current study has established that cross mating affects searching, mating and oviposition but the role of temperature and light intensity in mating and oviposition should be investigated.

2. Should determine the *wolbachia* infection levels that have no effect on mating compatibility.

3. Investigate why only a few F1 progeny successfully mated

4. Evaluate the success of the cross bred progeny in the stem borer control in ESA.

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APPENDIX

Appendix 1: Map of Kenya showing stem borer distribution