HOST RANGE OF *STURMIOPSIS PARASITICA* (CURRRAN) (DIPTERA: TACHINIDAE) AND INTERSPECIFIC COMPETITION WITH *COTESIA SESAMIAE* (CAMERON) (HYMENOPTERA: BRACONIDAE IN KENYA

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DECLARATION BY CANDIDATE

This thesis is my original work, and has not been presented for a degree in any other University or any other award.

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DEDICATION

To my family, my brothers and my sisters and to my friends

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ACRONYMS AND ABBREVIATIONS

ANOVA- Analysis of Variance.

ARQU- Animal Rearing and Quarantine Unit

CAN- Calcium Ammonium Nitrate.

DAM- Days After Mating.

ESA-Eastern and Southern Africa.

ICIPE- International Centre of Insect Physiology and Ecology.

IRMA- Insect Resistant Maize for Africa.

KARI- Kenya Agricultural Research Institute.

L: D- Light: Day.

NPK- Nitrogen, Phosphorous and Potassium.

RH- Relative Humidity.

SNK- Student- Newman- Keul´s

ABSTRACT

Cereal stemborers in Kenya include *Busseola fusca*, *B. phaia*, *Sesamia calamistis*, *S. botanephaga*, *Eldana saccharina*, *Chilo orichalcociliellus* and *Ch. partellus*. The stemborers cause 50% yield losses in cereal crops including maize, sorghum and sugarcane. Damage to the host plants results from the feeding and excavating of tunnels by the larvae. The different stemborer control options include pesticides, early planting, intercropping with non-cereals and host plant resistance. Pesticides are expensive and pose a health and environmental hazard. Moreover, they are not fully effective due to the cryptic feeding behavior of the pest larvae. Therefore there is need to search for more efficient and convenient control methods for these pests. Biological control is one such method where natural enemies are used. These include predators, parasitoids and pathogens. Predators do not keep the stemborers below economic injury levels. Pathogens and nematodes are not of great importance in regulating stemborer numbers. Parasitoids feed on immature stages of other insect host stages and kill them in the process. In Kenya, the parasitoids which have been studied in regard to the control of stemborer pests include *Telenomus* species, *Tricogramma*, *Cotesia sesamiae*, *Pediobius furvus* and *Dentichasmias busseolae*. However, the biocontrol status of *Sturmiopsis parasitica* in Kenya has not been fully investigated. The West African *S. parasitica* race parasitizes diapausing *B. fusca*, non diapausing *E. saccharina* and *S. calamistis*, while the Zimbabwe race parasitizes *B. fusca* but is encapsulated in *S. calamistis* and *E. saccharina*. The suitability of the West African race of *S. parasitica* in controlling the cereal stemborers has not been investigated in Kenya. The aim of this study was to determine the suitability of this biotype as an effective natural enemy through assessment of host range and interspecific competition with *Cotesia sesamiae* for its possible redistribution in Kenya. In this study, the suitability of *S. parasitica* as an efficient biocontrol agent was determined by inoculating planidia of *S. parasitica* in fourth instar larvae of different stemborer hosts. Host suitability and acceptability of four graminaceous pests namely, the noctuids *Sesamia calamistis*, *Busseola fusca*, the crambid *Chilo partellus* and the Pyralid *Eldana saccharina* were evaluated for their ability in supporting parasitoid development. The findings of the present study showed that, all the hosts were acceptable for larviposition but suitability varied greatly. *Sesamia calamistis* was the most suitable and preferred host while *B. fusca* and *E. saccharina* showed no significant difference. *Ch. partellus* was the least suitable host. However the parasitoid had the longest developmental period in *B. fusca* Interspecific competition was determined by exposing the most suitable stemborer host larvae to the parasitoids in the following sequence: *S. parasitica*, *Co. sesamiae* and then *Co. sesamiae*, *S. parasitica* at various time intervals. *Sturmiopsis parasitica* outcompeted *Co. sesamiae* irrespective of time interval between parasitism and whether it was the first or second species to parasitize. Moreover, *S. parasitica* had significantly higher rates of emergence in the presence of *Co. sesamiae*. Similarly *Co. sesamiae* took longer to develop in the presence of *S. parasitica* and had a high cocoon-to-adult mortality. The implication of this is that *Co sesamiae* may be extirpated in the highlands of East and Southern Africa. The present observations indicate the potential usefulness of *S. parasitica* as a biological control agent.

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background

Stemborers are of great economic importance in many African countries where they cause upto 50% yield loss (Bosque-Pérez and Schulthess, 1998). They are polyphagous and their host plants are found in three main families namely, Gramineae, Cyperaceae and Typhaceae. The lepidopteran stemborers belong to three main families namely, the Pyralidae, Noctuidae and Crambidae. About 12 stemborer species have been recorded in Africa and all are indigenous except *Chilo partellus* (Crambidae) which originated from the Indian sub-continent (Bleszynski, 1970). In Kenya, *Ch. partellus* (Swinhoe), *Busseola fusca* (Noctuidae) Fuller, *Sesamia calamistis* (Noctuidae) Hampson, *Chilo orichalcociliellus* (Crambidae) (Strand) and *Eldana saccharina* (Pyralidae) (Walker) cause yield loss of up to 18 % and 88% in maize and sorghum respectively (Warui and Kuria, 1983). The feeding activities of their larvae in the stem affects the translocation of nutrients leading to malformation of grains and results in yield loss in maize, sorghum and sugarcane. Such losses have great impact on food security and poverty alleviation since the affected cereals are an important part of traditional diets of millions of people in Kenya and other parts of Africa. Moreover, their by-products such as maize germ and bran are used as livestock feeds. These crops are usually grown by small, as well as large-scale farmers who produce about 75% and 25% respectively.

The damage to the crops results when the adult female moths oviposit on aerial parts of the host plants including the stems and leaves. The early instar larvae

feed superficially on the leaves, leaf sheaths and other succulent plant tissue. Older larvae excavate extensive feeding tunnels inside the stem. Mature larva pupates after forming a moth egress window and the adult emerges after 7 to 10 days (Harris, 1962).

Stemborer control methods include use of pesticides and cultural control methods such as early planting and intercropping with non cereals (Minja, 1990). Pesticides are expensive and pose a health and environmental hazard and hence the need to search for alternative methods. Various novel control options exists and focus mainly on three main avenues namely, biological control which involves the use of natural enemies. Other methods include habitat management and the use of natural pesticides such as botanicals.

Natural enemies, which include parasitoids, predators and pathogens, have been successfully used to control stemborers (Overholt *et al.*, 1994). They are not only relatively safe to the environment but are also compatible with cultural methods. Natural enemies in different ecological regions have different host ranges, suggesting diverse biotypes (Conlong, 1976). For example, in Kenya, there are two biotypes of the parasitoid *Cotesia sesamiae* (Cameron), namely the inland biotype that successfully parasitizes *B. fusca* and the coastal biotype that is encapsulated in *B. fusca* (Ngi-song *et al.*, 1998). The importance of parasitoids, their geographical and temporal distribution against lepidopterous borers in Africa has been studied with a view to their possible redistribution (Schulthess *et al.*, 1997). This approach will expand the geographical range of natural enemy species and strains in Africa. The West African race of

Sturmiopsis parasitica (Curran) is the most common larval parasitoid found there on *S. calamistis* and *E. saccharina* (Schulthess *et al.*, 1997). This race was introduced against *E. saccharina* on sugarcane in South Africa in 1999 but failed to establish (Conlong, 2000) and a stock culture has been maintained at the ICIPE since the year 2004 to determine its efficacy in the control of *B. fusca*.

1.2 Problem statement and justification

The stemborers namely, *B. fusca*, *S. calamistis*, *Ch. orichalcociliellus* *Ch. partellus* and *E. saccharina* are of great economic importance in several African countries including Kenya where they cause high yield losses (Appendix I) (Bosque Pérez and Schulthess 1998). For example, four seasons of on farm crop assessment in Kenya, showed an average maize crop loss of 13.5% or 0.4 million tons, valued at US $ 80 million (De Groote *et al.*, 2003). Their control by chemical methods is unsatisfactory due to their cryptic feeding behavior. Natural control by *Co. sesamiae*, an important indigenous larval parasitoid, has not been effective in maintaining the abundance of pests below economic threshold (Mohyuddin and Greathead, 1970). The ESA strain of *S. parasitica* has shown high levels of parasitism on *B. fusca* but is however, encapsulated in *S. calamistis* and *E. saccharina* and therefore does not complete its lifecycle. It is therefore not efficacious in the control of the stemborers pests and therefore *B. fusca* and other pests thrive and damage the crops in the absence of adequate density of this parasitoid. In West Africa, *S. parasitica* is one of the most common parasitoids on both *S. calamistis* and *E. saccharina*, which are the key pests in the region. *Busseola fusca* is not a major pest in West Africa it and has

been hypothesized that, the Western African biotype of *S. parasitica* is actively maintaining its abundance below economic threshold. This biotype thrives on *S. calamistis* and *E. saccharina* larvae during the off-season and maintains *B. fusca* below economic levels during the next growing season. These observations indicate the great potential of the West African *S. parasitica* as a biological control agent and it would therefore be desirable to release it against *B. fusca* in Kenya. The use of the West African biotype as an effective biological control agent will greatly alleviate losses to enhance achievement of the per capita consumption of 98 kilograms. But before introducing *S. parasitica*, there is a need to establish its suitability as a control agent against various pests and its ability to co-exist with the native larval parasitoid namely, *Co. sesamiae*. Though the borers in ESA are the same species as in West Africa, phylogenetic analyses has separated *B. fusca* and *E. saccharina* populations on maize in Africa into three mitochondrial clades which vary in host range (Sezonlin *et al.*, 2006, Assefa *et al.*, 2006). This study is aimed at elucidating the host range of *Sturmiopsis parasitica* and its interspecific competition with *C. sesamiae*.

1.3 Research questions

i. Will *Sturmiopsis parasitica* successfully parasitize and achieve equal percentage parasitism rates in the various hosts, namely *B. fusca*, *Ch. partellus*, *S. calamistis* and *E. saccharina*?

ii. Will *S. parasitica* successfully parasitize a host already parasitized by *Co. sesamiae*?

iii. Will *Co. sesamiae* successfully parasitize a host already parasitized by *S. parasitica*?

1.4 Null hypotheses

i. *Sturmiopsis parasitica* will not parasitize *B. fusca. Ch. partellus S. calamistis* and *E. saccharina* and achieve different percentage parasitism.

ii. *S. parasitica* will not parasitize a host already parasitized by *Co. sesamiae*.

iii. *Co. sesamiae* will not parasitize a host already parasitized by *S. parasitica.*

1.5 Objectives

1.5.1 General objective

The objective of the present study is to determine the host range of *Sturmiopsis parasitica* and its interspecific competition with *Cotesia sesamiae* with a view to assessing its suitability for use as biological control agent against stemborers in Kenya.

1.5.2 Specific objectives

i. To determine the acceptability and suitability of various stemborer hosts namely, *B. fusca*, *Ch. partellus*, *S. calamistis* and *E. saccharina* for the development of *S. parasitica*.

ii. To assess the success rate of *S. parasitica* in parasitizing a host that is already parasitized by *Co. sesamiae*.

iii. To evaluate the success rate of *Co. sesamiae* in parasitizing a host that is already parasitized by *S. parasitica*

CHAPTER 2

LITERATURE REVIEW

2.1 Economic importance and geographical distribution of the different cereal stem borers

In East Africa, the economically important stemborers include the noctuids *B. fusca* and *S. calamistis* and the crambids *Ch. orichalcociliellus*, *Ch. partellus* and the pyralid *E. saccharina* (Minja, 1990). *Busseola fusca* is the most damaging indigenous stem borer of maize and sorghum in sub-Saharan Africa where maize is grown at higher altitude (Kfir *et al.*, 2002). *Chilo partellus* is the dominant and most economically important exotic stem borer in many parts in the lowlands of East and South Africa (Overholt *et al.*, 1994). Plant damage by stemborers result from the feeding and stem tunneling by the borer larvae that leads to death of the growing point and the weakening of the stem. This interferes with the normal translocation of nutrients and metabolites in the affected plant and leads to malformation of grains, early leaf senescence, plant stunting and direct ear damage (Bosque-peréz and Schulthess, 1998).

Reports indicate high levels of *B. fusca* parasitism by the East African strain of larval parasitoid *Sturmiopsis parasitica* in Zimbabwe and parts of Tanzania but is encapsulated in *S. calamistis* and *E. saccharina* and therefore has no alternative hosts when *B. fusca* larvae diapauses during off-season (Mohyuddin and Greathead, 1970). The West Africa race of *S. parasitica* is the most common larval parasitoid found in *S. calamistis* and *E. saccharina* (Schulthes *et al.*, 1997).

2.1.1 *Busseola fusca*

Busseola fusca is distinguishable from other *Busseola* species by the characteristics of the male and female genitalia. The male genitalia are strong slender, club like processes from the sacculus and are occasionally asymmetrical. The female genitalia has an asymmetric sclerotization of minutely spined lamella vaginalis (Nye, 1960). This noctuid maize stem borer is indigenous to tropical Africa where it is a major pest of maize and sorghum but also attacks pearl millet, sugarcane and some wild grasses at higher elevations of 600m above sea level in Eastern and Southern Africa (Harris, 1989, Kfir, 1995) and from sea level to over 2000m above sea level in W. Africa (Tams and Bowden, 1953, Cardwell *et al.*, 1997, Ndemah *et al.*, 2001). It is the most important pest in sub-Saharan Africa and in Kenya it accounts for 15% to 81% maize loss. It has 6-7 larval instars which take 30-45 day to develop. The second generation larvae in the final instar enter diapause during the dry season in mature maize and sorghum stubbles for upto six months. Pupation is initiated by the onset of the rains.

2.1.2 *Eldana saccharina*

The pyralid, *Eldana saccharina*, is easily recognizable by the characteristics of the fore wings. The longitudinal veins are brown with two distinct dark spots in the anterior half. It is a pest of graminaceous crops in West Africa and occurs in all suitable areas of sub-Saharan Africa from15°N to 30°S (Bosque Pérez and Schulthess, 1998). In ESA it is found on sugarcane but prefers the cyperus sedges where it feeds in the culms and the inflorescence (Atkinson, 1980). In Western Africa *E. saccharina* feeds mainly on crops such as maize, sugarcane,

sorghum and millet and is rarely found on wild sedges and grasses (Bosque Pérez and Schulthess., 1998).

2.1.3 *Sesamia calamistis*

Sesamiae calamistis, a noctuid, is identified by the characteristics of the fore wings, which have a lighter distal margin. The male antennae are bipectinate. In the male genitalia the juxta is flask shaped in the female genitalia the ductus bursae is unsclerotized. This pest of cultivated host crops such as maize, sorghum and millet as well as wild grasses such as *Pennisetum purpureum*, *Panicum maximum* and *Setaria splendida* is common in many African countries (Harris, 1962). In the moist transitional zone which lies above 1200m and rainfall above 550mm per year accounts for 2.7% loss.

2.1.4 *Chilo partellus*

The genus *Chilo* contains many species which are difficult to distinguish except through the head profile and male and female genitalia. In the male the aedigus has a bulbous basal projection and a ventral arm. The female genitalia has heavily sclerotized swollen ostial pouch. The crambid, *Ch. partellus*, is indigenous to the Indian subcontinent. It spread to Africa earlier last century and was first recorded in Malawi in 1932 (Jepson, 1954). *Ch. partellus* has since spread to several countries in the lowland areas of Eastern and Southern Africa (Bate *et al.*, 1991). Maize and sorghum are the major hosts of *Ch. partellus*, although it has been observed infesting and damaging rice, finger millet, wheat, pearl millet and sugarcane in farms. Its wild hosts include grasses such as *Andropogon* species, *Sorghum halepense*, *S. verticilliforum* and *Panicum*

maximum (Sithole, 1990). Maize yield losses attributed to *Ch. partellus* and *Ch. orichalcociliellus* in Kenya is about 15% (Hugo *et al.*, 2001).

2.2 Life history of stemborers

The adult moths of *B. fusca*, *S. calamistis*, *Ch. partellus* and *E. saccharina* oviposit on leaves and stem of the host plant. The eggs are commonly in clusters that vary from a few to several hundreds and hatch into larvae in 3 to 6 days at 25°C. The early instar larvae feed cryptically on succulent plant tissue in leaf sheath, whorls, tassels and cobs of maize, whereas the older larvae feed exclusively in tunnels inside the plant stem. The duration of the larval stage is 25 to 45 days with 6-8 instars. The pupal stage lasts for 7-10 days whereas the adult female lifespan ranges from a few days to two weeks with 1-3 days preoviposition period (Harris, 1962). The adults feed on nectar and water. The larvae enter diapause in cold and dry conditions in the stems, stubble and other plant residues where they spend up to 6 months before pupating when conditions are favorable during the subsequent growing season (Harris, 1962).

2.3 Current approaches to pest management

Integrated pest management is the current approach as no single management option is adequate. It is a pest control strategy that uses an array of complementary methods including mechanical devices, physical devices, genetic, biological, legal, cultural management, and chemical management

2.3.1 Cultural control methods

These practices include the manipulation of wild host plants, management of crops residues and planting dates, crop rotation, polyculture, plant density, physical removal of infected plants, soil solarizing and fertility management, trap crops, variety selection and water management. Each of these control options could affect a specific developmental stage of the stemborers life cycle. . It is the oldest and the first line of defence against pests and is the most relevant and economic method for the resource poor African farmer (Van den Berg *et al.*, 2001). However, for cultural methods to be effective, co-operation of farmers in a given region is essential because moths emerging from farms where the cultural control measures have not been effected can infest the crops which are under control (Kfir, 1992a). Moreover, lack of water for irrigation is major constraint and cultural control methods are not always practical as farmers often plant after first rains (Van den Berg *et al.*, 2001).

2.3.2 Resistant crops

Resistant crop cultivars are developed by identification and transfer of insect resistance genes across plant species through biotechnology. They provide an inherent control that involves no environmental problems and they are generally compatible with other control methods (Bosque-Pérez and Schulthess, 1998). Examples of the use of bioengineered crops in insect control include insect resistant maize for Africa (IRMA) a developed *Bt.* maize strain that has a gene for resistance against stemborers (Odame and Mbote, 2000). Although there is steady increase in bioengineered crops, there is still need to fully establish their long term environmental impact.

2.3.3 Chemical control

Synthetic pesticides used against stemborers include carbofuran, cabaryl, deltamethrin, endosulfan trichlorfon and pyrethroids (Sithole, 1990).They came into use after the second world war and their research was based on the Nazi nerve gas. Organochlorines include insecticides such as DDT, endosulfan and gamma-BHC. They are hazardous to the environment due to their persistence and their use is inappropriate (Dent, 1991). Another class of insecticides namely organophosphates are systemic in action and are less persistent. However, the timing of their application is critical to ensure a high level of efficiency (Dent, 1991). Pyrethroids are the most commonly used contact activity insecticides (Krause *et al.*, 1996). Carbamates are systemic insecticides applied in the soil and include carbofuran and carbaryl. Natural insecticides include botanicals such as the neem tree extracts *Azadirachta indica* and pyrethrins like Buhach, Chrysanthemum Cinerariaefolium, Ofirmotox, Insect Powder and Firmotox. Pesticidal control poses a health hazard and the pests always develop resistance after prolonged exposure and their utilization of limited food sources. Generally, pesticides are expensive and may also kill non target fauna such as the natural enemies since most farmers do not apply them properly and this has renewed the need for biological control (Howarth, 1991).

2.3.4 Biological control

This is the use of natural enemies such as parasitoids, predators, pathogens, antagonists or competitor populations to suppress a pest population, thus making it less abundant and damaging (Van Driesche and Bellows, 1996). Natural

enemies play an important role in the control of lepidopterous borers in Africa (Bosque-Pérez and Schulthess, 1998). Biological control options are safe to public health and environmentally friendly, thus giving a promising alternative to the use of pesticides in pest management (Pimentel and Andow, 1984). Among the parasitoids that have generated great interest in recent times and may have the possibility of redistribution for use as biological control agents are *S. parasitica* and *Co. sesamiae*. This is due to their existence as different biotype with varying stemborer host range (Schulthess *et al.*, 1997). The exchange or redistribution of natural enemies between regions of a continent as a solution to lepidopterous cereal stemborers problems has been proposed by several researchers (Rao, 1965., Mohyuddin and Greathead, 1970., Mohyuddin *et al.*, 1981, Mohyuddin *et al*, 1991).

2.3.4.1 Insect parasitoids

Parasitoids are insects whose immature stages feed on other insect host stages for their own self propagation and usually kill the hosts in the process, unlike true parasites which may not kill their hosts Godfray (1994). The adults stages are however, free living and feed on pollen, nectar and honeydew. Parasitoids use sensory cues to locate and evaluate the appropriate developmental stage of the host for its own offspring development (Baaren and Nenon, 1996). Parasitoids usually evaluate the acceptability of the insect host, the number of eggs to be oviposited and, in many instances, the sex of the eggs. There are more than 40 species of indigenous parasitoids in Kenya which include egg, larval and pupal parasitoids (Appendix 2). The egg parasitoids include *Telenomus* and *Trichogramma* species and are the most abundant and wide-spread in the

country. The larval parasitoids include *Co. sesamiae* and *S. parasitica* while the pupal parasitoids are *Pediobius furvus* (Gahan) (Hymenoptera: Eulophidae) and *Dentichasmias busseolae* (Heinrich) (Hymenoptera: Ichneumonidae) (Bonholf *et al.*, 1997). The braconid, *Co. sesamiae*, is the most common parasitoid of stem borer larvae at the coast of Kenya and in other areas of East and Southern Africa (Kfir, 1992c). *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) is an exotic larval parasitoid native to Indo-Australian region that was introduced by the International Centre of Insect Physiology and Ecology into Kenya to control the exotic stemborer *Ch. partellus* in1991 (Overholt *et al.*, 1994).

The mechanisms of larval competition and elimination which enable intrinsically superior parasitoid species to kill or eliminate the eggs or larvae of potential competitors have been identified (Mackauer 1973). Suppression can result from some action of the adult or a process between the immature stages (Fisher, 1961, 1971., Salt, 1961., Vinson and Iwantsch, 1980). For example aggression is common among the first instar larvae of many species of solitary parasitoids and most first instars have large and commonly sickle shaped mandible that can be used to bite and physically attack other larvae in the same host. In contrast to later stages, are often amandibulate or have reduced or non functional mandibles, (Salt 1961, Clausen, 1962). Although fighting takes place among larvae that are approximately of the same age, in some species the first instar will attack and kill older stages that are amandibulate and hence unable to defend themselves (Chow and Mackauer 1984, 1986).

2.3.4.1.1 *Sturmiopsis parasitica*

Sturmiopsis parasitica is a dipteran solitary larval parasitoid in the family Tachinidae and is indigenous to Africa. Morphological studies and DNA sequencing of cytocrome oxidase I suggest that there are at least two distinct populations of this parasitoid, one occurring in ESA and the other one in West Africa (Dittrich *et al.*, 2006, Barraclough, 2004). The adults are medium sized flies about 8mm long, basally black but with lighter grey to white pollinosity, especially dorsally on the thorax and in wide bands across the abdominal tergites. The females have whitish frons and two long downwardly directed fronto-orbital bristles on either side of the head. The frons in males is smoky grey and lacks the bristles. On mating, a female produces 500-900 maggots after a gestational period of 12-19 days. The larval period usually takes 12-14 days, whereas pre-pupal period is 12 hours and the emergence of imago from the puparium is after 12-19 days. The attack strategy is 'planidial ingress' where the female deposits first instar maggots (planidia) on moist frass at the stemborer tunnel entrance. The planidia are negatively phototactic and this guides them into the proximity of the host larvae feeding in the enclosed tunnel where they penetrate with their mouth hooks and develop. The fourth larval instar of the host is the preferred developmental stage.

2.3.4.1.2 *Cotesia sesamiae*

The Afrotropical parasitoid *Co. sesamiae* is polyphagous and attacks crambids, noctuids and pyralids. This species has black or dark brown abdomen and a forewing span of about 1.8mm long. The antenna in females is robust and shorter than the body length whereas in males they are slender and 1.4 times as

long as body. The basal half of antenna is usually completely yellowish brown. The legs and hind coxae are yellowish and dark basally. The yellowish hind coxae are dark basally. The cocoons of *Co. sesamiae* are loosely grouped and can be distinguished from those of *Co. flavipes*, which are closely packed. Egg to adult development is completed in about 18.5 days at 30°C and 21.4 days at 21-26.5°C (Ullyet, 1935 and Mohyuddin, 1971). Mating starts immediately after emergence, especially in bright light. The species is highly fecund with each parasitized larva capable of producing up to 100 progeny. The average larval period is 11 days, and the total pre-pupal and pupal periodl being 5.5 days at 28.5 ± 2°C.

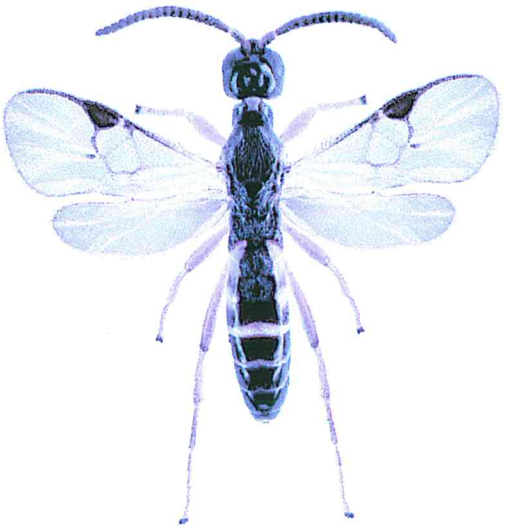


Plate 1 **Adult female *Cotesia sesamiae***

Plate 2 **Adult female *Sturmiopsis parasitica***

CHAPTER 3

3.0 GENERAL MATERIALS AND METHODS

3.1 Study site

The study was conducted at the International Centre of Insect Physiology and Ecology (ICIPE), Kasarani, Nairobi, Kenya It is located on 1° 13'- 11.41" S, 36° 53'- 40.76" E and about 13 kilometers from GPO to the east of the city of Nairobi. Within the ICIPE, the insectary, where the ARQU is located, occupies the north eastern part of the compound. The insects are reared in insectaries build in conformity to international safety control standards. The green houses occupy the north western part of the compound next to the plots where maize (*Zea mays*) for the experiments was planted.

3.2 Maize plants growing

Hybrid maize variety 513, was used in these experiments. Two seeds were planted in each hole 5-7 cm deep at spacing of 30 cm and 60 cm within and between rows respectively behind the green houses at the ICIPE, Nairobi. With each hole,15gms of compound fertilizer containing nitrogen, phosphorus and potassium (NPK) was also applied and hole sealed. Top dressing using 15gms of Calcium Ammonium Nitrate (CAN) was done when the maize was 45 cm in height. About 1000 maize plants were established in phases of 100 plants per every week. During dry spells sprinkler irrigation was carried out. Eight week old maize stems were used in the experiments.

3.3 Parasitoid culture maintenance

Sturmiopsis parasitica cultures were raised from puparia imported from South Africa to the Animal Rearing and Quarantine Unit (ARQU), ICIPE in 2004. The parasitoids were maintained on *Sesamiae calamistis*. This race was originally introduced into South Africa from West Africa to control *Eldana saccharina* on sugarcane. The cultures were maintained on fourth instar larvae of *S. calamistis* collected from ARQU. On emergence the adult parasitoids were fed on 50% honey- water solution in perspex cages measuring 12×12×8 cm.

A freshly emerged female was put together with three, 4 days old males in a cage. To enhance mating, the cage was exposed to bright light. Twelve to fourteen days after mating (DAM), the females were dissected and the uteri ruptured into distilled water to release the planidia (Nargarkatti and Rao, 1975). A fine camel hair brush was used to transfer 8 active planidia to the ventral surface of a host larva which had been previously wiped clean with cotton wool soaked in distilled water. The inoculated larvae were then reared in an artificial diet in vials (2.5× 7.5 cm) at 26±1°C, 65-80% RH and a photoperiod of 12:12 (L:D). Larvae were checked daily until parasitoid emergence and the cycle was repeated to maintain the culture.

3.4 Stemborer culture maintenance

Busseola fusca, *S. calamistis*, *Ch. partellus* sand *E. saccharina* to be used in the host suitability experiments were reared on artificial diet at the International Centre of Insect Physiology and Ecology (ICIPE), Kenya. The artificial diet included sugar, maize leaf/sorghum leaf powder, ascorbic acid, brewers yeast, vitamin E and bean powder (Onyango & Ochieng- Odero, 1994). *Busseola fusca* was collected from maize fields in Kitale, Machakos, Western Kenya and the Mount Kenya region, and reared in the laboratory for eight generations before it was used in the experiments. *Eldana saccharina* was collected from sorghum at Mbita, Lake Victoria region, and had been reared for 23-26 generations. *Sesamia calamistis* was collected from maize and sorghum in Kitale and reared for 2-5 generations while *Ch. partellus* was obtained from maize in farms at the Kenyan coast and from sorghum in Mbita and was reared for 65-70 generations. New insects collected from the field were added at least three times each year to rejuvenate the colonies. All laboratory experiments were carried out at 26 ± 1°C, 12:12 (L: D) and 65 – 80% relative humidity (RH).

3.5 Host suitability experiments

Suitability is defined as the ability of a host to support development of *S. parasitica* from planidia inoculation to adult. The stemborers described in 3.4 were used to investigate suitability by inoculating the planidia of *S. parasitica* into the 210 larvae of each of the stemborers.

3.6 Host acceptability experiments

Acceptability is defined as the ability of the parasitoid to recognize and successfully parasitize a stemborer host larvae feeding inside a 6-8 week old maize stem. The larva of each of the stemborers were offered individually in a maize stem and observed. Larviposition signified acceptance.

3.7 Host preference experiments

Preference is defined as the choice for larviposition on either of the two species of stemborer larvae on offer. Two different stemborer larvae in different maize stems were placed 29 cm in a cage and a parasitoid released equidistance between the stems and observed. Larviposition on either stem indicated preference.

3.8 Determination of success rate of *S. parasitica* in parasitizing a host already parasitized by *Co. sesamiae*

This was the Cs-Sp parasitizing sequence whereby *Co. sesamiae* parasitized the stemborer larva first followed by *S. parasitica*. The larva was then reared individually in artificial diet in a vial. Parameters recorded were development time, sex ratio, percentage parasitism and potential growth index

3.9 Determination of success rate of *Co. sesamiae* in parasitizing a host already parasitized by *S. parasitica*.

The same protocol as in section 3.8 above was used except that *S. parasitica* parasitized first followed by *Co. sesamiae* (Sp- Cs).

3.10 Data analysis

The proportions of hosts from which parasitoids emerged and % preference were compared using Chi-square (Sokal and Rohlf, 1981). The data on parasitism, parasitism sequences, time interval between parasitism, development time, total adult progeny, mortality and female progeny produced per stemborer host larvae were compared between host species using Analysis of Variance (ANOVA) using the General Linear Protocol (PROC GLM, SAS Institute 2001). Ratio and percentage data was arcsine square root transformed before ANOVA was done. Student-Newman-Keul's (SNK) and Bonferroni mean separation procedures were used when ANOVA yielded significant differences. The significance level was set at $P \leq 0.05$.

CHAPTER 4

4.0 A COMPARATVE STUDY OF THE ACCEPTABILITY AND SUITABILITY OF VARIOUS STEMBORER HOSTS FOR *STURMIOPSIS PARASITICA* DEVELOPMENT

4.1 Introduction

In Africa, there are two known biotype of *S. parasitica* with varying stemborer host ranges (Schulthess *et al.*, 1997). The first is the East and Southern African *S. parasitica* biotype which successfully completes development in *B. fusca* but is, however encapsulated in *S. calamistis* and *B. fusca*. The second is the Western African biotype which successfully completes development on *B. fusca*, *S. calamistis* and *E. saccharina* (Barraclough, 2004). The ESA *S. parasitica* biotype has therefore no alternative host when *B. fusca* larvae diapauses during off season. It survives by synchronizing its larval development with that of the diapausing host larva. An active population of this parasitoid is therefore not available to maintain *B. fusca* below damaging levels in the next growing season. The W. African biotype maintains active population of the parasitoid in *S. calamistis* and *E. saccharina* during off season which prevents *B. fusca* from reaching damaging levels in the next growing season. The geographical variations in host range in *S. parasitica* has not been investigated in Kenya. The aim of the current study was to determine the suitability of various stemborers hosts in Kenya by the W. African biotype of *S. parasitica* with a view to assessing its potential as a biological control agent.

4.2 Materials and Methods

4.2.1 Host suitability

Host suitability is the ability of a parasitoid to successfully develop in the pest. To assess host suitability, thirty 4th instar larvae of each of the stemborer species namely, *Ch. partellus*, *B. fusca*, *S. calamistis* and *E. saccharina*, were inoculated with 8 planidia, which in pre-experiment had been shown to produce the highest parasitism. Planidia of *S. parasitica* females 12-14 DAM, (days after mating) were obtained as outlined in section 3.3 above. The larvae were then reared individually on an artificial diet until puparium formation, death of larvae or pupation. (Onyango and Ochieng- Odero, 1994). Parasitoid puparia were transferred into glass vials (2.5 × 7.5cm) until emergence of the parasitoid.

For each host, development period from inoculation of the host larva to puparia emergence and from puparia to adult parasitoid emergence, total progeny and number of females that emerged were recorded. The proportion of the host larvae that produced parasitoid puparia was estimated. For each stemborer host, 210 larvae were used. Seven sets of ten larvae were inoculated with planidia from the same *S. parasitica* female. The potential growth index (PGI) of *Co. sesamiae* was computed as the product of the percentage of stung larvae producing adult progeny, the mean number of adult progeny produced per host larva, and the sex ratio (proportion of females), divided by the immature period of the parasitoid, i.e., the maggot to adult emergence development time (Sétamou *et al.*, 1999).

Formula:

$$PGI = \frac{\%\ \text{larvae yielding adult progeny} \times \text{mean number of adult progeny} \times \text{sex ratio (female proportion)}}{\text{Development duration.}}$$

4.2.2 Host acceptability

Host acceptability is the ability of the parasitoid to recognize and successfully parasitize a suitable host. To assess host acceptability, fourth instar larvae of each of the stemborer species, namely *B. fusca*, *Ch. partellus*, *S. calamistis* and *E. saccharina* in maize stems were offered for parasitism to *S. parasitica* in a no choice. Three-cm-long larval feeding tunnels were made in 15-cm long maize stem by boring 3-mm-diametre tunnel using a cork borer. Each host larva was introduced head first into the tunnel using soft forceps. Parafilm was tied wrapped around the stem at the site of the tunnel opening to prevent larval escape. The infested stem was then supported vertically in a one liter clear plastic jar closed with a lid which had a hole covered by a net for ventilation. The stemborer larva was allowed to feed overnight. The Parafilm was unwrapped and the presence of frass at the tunnel opening confirmed prior to introduction of the parasitoid. Female, *S. parasitica* 12-14 DAM, was then released into the jar and then observed for foraging behavior, larvipositions, number of planidia larviposited, as well as superparasitism. After recording larvipositions, some of the larvae were dissected after 2, 4 and 8 days to confirm successful parasitization.

4.2.3 Host preference

Host preference was assessed in dual choice tests using all combinations of *B. fusca*, *Ch. partellus*, *E. saccharina* and *S. calamistis*. Two larvae of different stemborers species, in different maize stems offered as described above in Section 3.3.2. The stems were held vertically on a clay base 20 cm apart in a cage. A female *S. parasitica* that had mated 12-14 days earlier was released at equidistant between the maize stems and observed until the parasitoid larviposited. Those that failed to larviposit were dissected to establish fertility. For each pair of different stemborers, ten parasitoid females were used.

4.3 Data analysis

The proportions of hosts from which parasitoids emerged and % preference were compared using Chi-square (Sokal and Rohlf, 1981). The data on parasitism, duration of development, adult progeny, mortality and female progeny produced per stemborer host larvae were compared between host species using Analysis of Variance (ANOVA) using the General Linear Protocol (PROC GLM, SAS Institute 2001). Percentage and ratio data were arcsine square root transformed before ANOVA. Student-Newman-Keul's (SNK) test was applied when ANOVA yielded significant differences. The significance level was set at $P \leq 0.05$.

4.4 Results

4. 4.1 Host acceptability and suitability

Results showed that all the borer host species namely *Ch. partellus*, *S. calamistis*, *B. fusca* and *E. saccharina* were accepted for oviposition. The highest percentage moth emergence was recorded on *Ch. partellus* followed by *B. fusca* and *E. saccharina* which showed no significant while the lowest was recorded on *S. calamistis* ($\chi 2$ =111.653, P<0.0001). The highest puparia emergence was recorded in *S. calamistis* followed by *B. fusca* and *E. saccharina* which showed no significant difference while lowest was recorded on *Ch. partellus* ($\chi 2$=131.848, P=0.0001). Host death was not significantly different across all the species (Table I).

4.4.2 Host preference.

In dual-choice tests, over 70% of the *S. parasitica* females chose *S. calamistis* over *B. fusca*, *C. partellus* or *E. saccharina* but there were no differences among the other species (Fig. 1). Initially, the parasitoid actively moved up and down the maize stem and locating the tunnel, then it proceeded to remove frass using the labellum and it then turned and curved the cerci towards the stem near the tunnel opening with the head and thorax assuming a parallel posture to the maize stem and larviposited between 7- 31 planidia. The parasitoid larviposited up to five times at different tunnel openings and more than once at some and occasionally superparasitism was observed (i.e. < 4%). There was a 3-5 minute interval between each larvipositions each lasting upto 36 seconds. After the initial larvipositions, the parasitoid became very active and moved up and down

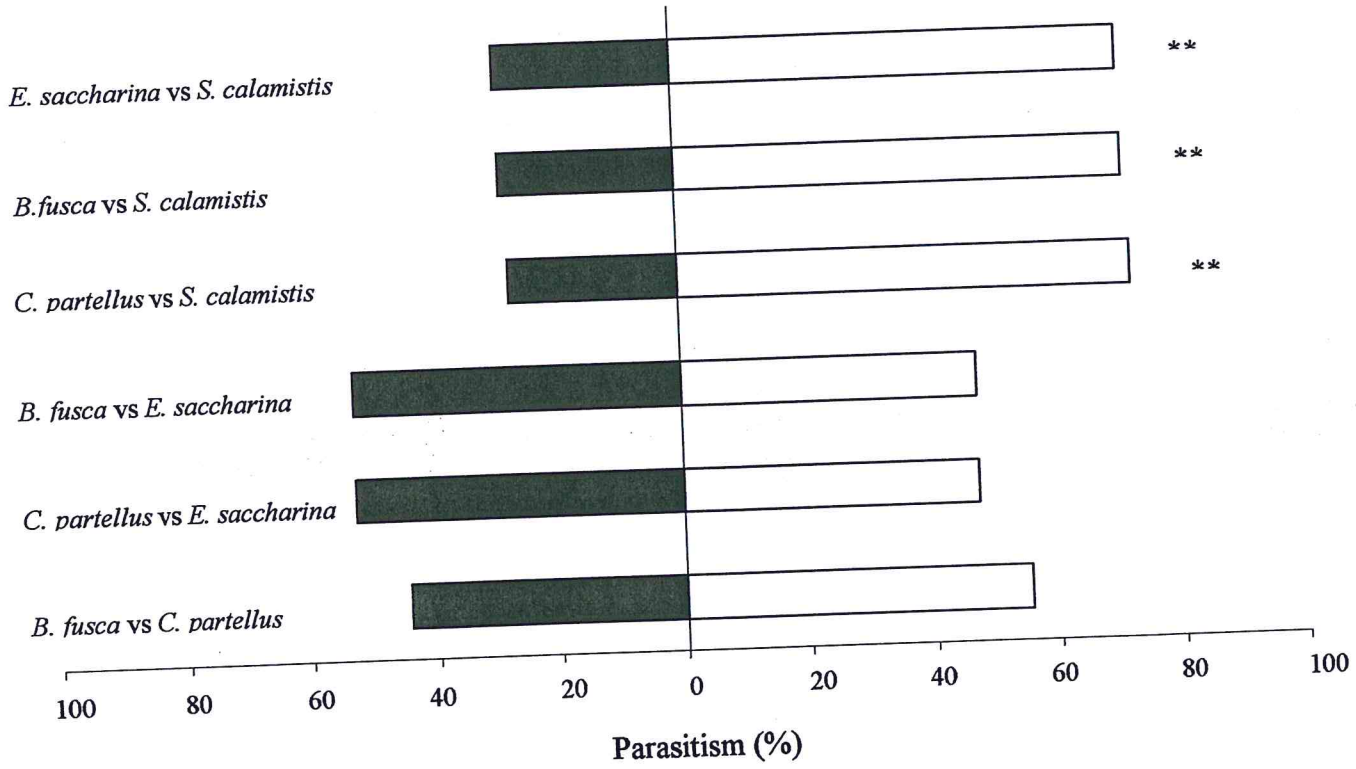
the maize stem rapidly searching for other tunnel opening. Super-parasitism was also observed in a no choice design. However, when unparasitized hosts were introduced into the experiment arena with the parasitized host still in there, the parasitoid was observed to avoid super-parasitism. When the larviposited planidia failed to find the tunnel opening, they were then observed to lift their anteriors and stand on their tail and move the head from side to side, thereafter, the planidia were observed to crawl towards the tunnel opening.

Table 1. Percentage of larvae of four stemborers species parasitized by *Sturmiopsis parasitica* yielding puparia, that died, or from which moths emerged

	B. fusca	*Ch. Partell*	*E. saccharina*	*S. calamistis*	χ^2	P
N	210	210	210	210		
Moth (%)	55.45B	68.10A	56.67B	19.05C	111.653	0.0001
Puparia (%)	37.91B	23.81C	34.76B	75.24A	131.848	0.0001
Death(%) due to Other causes	6.64A	8.09A	8.57A	5.71A	1.632	0.6521

Means followed by the letters in the same row are not significantly different at $P \leq 0.05$

The mean duration of development of females and males combined, females alone and males alone varied significantly across host species (F= 22.1; df= 3,356; P= 0.0001) (F= 11.9, df= 3,205, P= 0.0001) (F= 2.3, df= 3,149, P= 0.0491) The combined male and female parasitoid development periods from highest to the lowest were as follows *B. fusca*. *E. saccharina* S. *calamistis* and *Ch. partellus* respectively. The females showed the shortest development period *Ch. partellus* larvae while the development in the other hosts was longer but not significantly different (Table 2).



** Significant difference

Figure 1: Dual choice tests for preference of different stemborer species by the parasitoid *S. parasitica*

Table 2. The mean duration of development in days (± SE) of total progeny of *S. parasitica* that emerged from larvae of the four stemborer hosts.

Hosts	Development days		
	Male and female	Female	Male
B. fusca	31.3± 0.42 a	32.4 ± 0.64 a	29.0± 0.8 a
Ch. Partellus	26.3± 0.37 d	26.1 ±0.49 b	26.6± 0.6 b
S. calamistis	28.6± 0.13 c	29.9 ±0.34 a	27.6± 0.2 a
E. saccharina	29.9± 0.34 b	30.1±0.41 a	29.6± 0.6 a
F	22.01	11.95	2.31
Df	3, 356	3, 205	3, 152
P	0.0001	0.0001	0.0491

Means followed by the same lower case letters in the same column are not significantly different (Student-Newman- Keul´s multiple comparison test, P≤ 0.0001

The female to male ratio was not significantly different per host and between host species. Similarly, there was no significantly difference in development duration among hosts (Table 3). The highest potential growth index was calculated in the larvae of *S. calamistis* and the lowest in *Ch. partellus*

Table 3. Mean potential growth index, sex ratio (female) and duration of development in days (± SE) of *S. parasitica* emerging from the larvae of four stemborer hosts

Host	Female	Male	Sex ratio	PGI
B. fusca	31.8 ± 0.3a	29.0 ± 0.8a	0.64 ± 5.6	0.71 ± 0.008
Ch. Partellus	26.3 ± 0.4b	26.6 ± 0.6b	0.58 ± 7.0	0.52 ± 0.006
S. calamistis	29.9 ± 0.3a	27.6 ± 0.2a	0.59 ± 5.8	1.45 ± 0.009
E. saccharina	28.6 ± 0.1a	29.6 ± 0.6a	0.55 ± 4.0	0.68 ± 0.0069
F	11.95	2.31	0.51	
DF	3, 205	3, 149	3, 351	
P	< 0.0001	0.0491	0.67	

Means followed by the same letters in the same column are not significantly different at P≤ 0.005

4.5 Discussion

The larvae of the stemborers hosts namely, *S. calamistis*, *B. fusca*, *E. saccharina* and *Ch. partellus* were all acceptable for larviposition to West Africa *S. parasitica* biotype. This is a solitary koinobiont larval parasitoid. The parasitoid employs planidial ingress attack strategy where the female larviposits on moist frass at the tunnel opening on the maize stem. The results, showed a highly significant percentage parasitism among the four stemborer host species as indicated by the variable parasitoid retrievals. *Sesamia calamistis* and *Ch. partellus* were the most and least suitable host respectively. *Busseola fusca* and *E. saccharina* showed no significant but were better than *Ch. partellus*. *Chilo partellus* was the smallest host and the parasitoid had the shortest mean development duration probably because of its nutritional inadequacy. As an exotic pest, this is a new association which may interfere with the development of the parasitoid as they are not coevolved. *Sesamia calamistis* had the highest percentage parasitism probably because it is widely distributed in sub Saharan Africa (Bosque Pérez and Mareck 1990). This signifies that *S. parasitica* and *S. calamistis* have coevolved. *Eldana saccharina* is not as good as S. calamistis because it no as widely distributed. *B. fusca* diapause during off season and this may have affected the parasitoid performance.

The combined male and female development duration varied significantly among all the stemborer hosts. Female development duration was the same in *B. fusca*, *S. calamistis* and *E. saccharina* but varied significantly in *Ch. partellus* while there was no significant difference in the male development duration. This is probably because *S. parasitica* and these stemborers have coevolved for a

long period. Previous observations established that host suitability for parasitoid development inside a host was dependent on diverse factors, including, the ability to evade the host's immune defense systems, competition with other parasitoids, environmental influences, the presence of toxins detrimental to parasitoid's eggs or larvae and hosts nutritional adequacy (Vinson and Iwantsch,1980). However, all the four stemborer host larvae species were suitable for the development of this biotype of *S. parasitica*, this corroborates with work reported by David *et al.*, (1981), working with *Sturmiopsis inferens* Townsend on *Chilo infuscatellus* Snellen in India

The observations in the current study are not concordant with reports by Chinwada *et al*, (2004) that indicated no reported recoveries from *S. calamistis* due to planidial encapsulation inside the host. However, the percentage parasitism of the parasitoid in *Ch. partellus* in their work was similar to that observed in the present study. While up to eight puparia were obtained per host larva in the earlier work by Chinwada *et al*, (2004) a maximum of only two was obtained rarely in this study. In solitary species, normally only one larva completes its development to adult stage in each host with supernumerary larvae being eliminated by some form of physiological suppression or by physical combat among larvae Mackauer (1973). Puparial recoveries in the current study were dependent on the stemborer host species which corroborates studies by Chinwada *et al*, (2004). However, the inoculation method or the development stage of the host when egression of pupariating maggots occurred were not important factors in this study. Clearly, this brings into sharp focus the

differences in the ESA and W. African biotypes and the need for a very meticulous understanding of the biology of natural enemies and their hosts.

The current study also conforms to the observation by Chinwada *et al.* (2004) that the higher the number of planidia inoculated per host larva, the better the chances of successful parasitization. In deed preliminary observations showed inoculation of 8 or more planidia per host larva produced the highest successful parasitization. This conforms to what naturally happens when the female *S. parasitica* larviposits at the tunnel opening on host plant stem where upto 31 planidia are larviposited in a single larviposition. In a solitary parasitoid, where only one offspring can successfully develop in the host, it is of interest to note the high number of planidia larviposited per host larva. The high number of planidia larviposited per host could be a survival strategy to suppress the host immune defense systems and allow the fittest litter to develop. This may explain why inoculation of many planidia per host larva in the laboratory gave a higher rate of successful parasitization.

Parasitoids repress the immune systems of their host by injecting chemical substances such as polyDNA virus and venom with the eggs that interfere with the systems (Edson *et al.*, 1981, Stoltz and Guzo, 1986, and Fleming, 1992). *S. parasitica* larviposits at the tunnel opening on the plant stem, so this immune repression substances can only be in the larviposited planidia. The planidia must traverse the larvae feeding tunnel to locate the host feeding inside. However, not all the larviposited planidia will successfully locate the host. This may explain why upto 31 planidia are larviposited. Apparently, a good number

must locate the host, penetrate, and inject the substance to successfully compromise the host immune system for the fittest planidia to successfully develop. This was corroborated in inoculation experiment in the laboratory where the host inoculated using eight or more number of planidia gave the highest successful parasitization per host. In the no choice design, superparasitism was observed, probably as the parasitoids' way to enhance successful parasitism and due to its high fecundity of upto 900 fertile planidia.

It is clear that, although the planidia are larviposited at the same time, not all will locate the host feeding inside the plant stem concomitantly. Those planidia that will precede will be older and hence amandibulate and may be killed by the younger mandibulate ones which locate the host later. During late larval development, the competitor is eliminated by physiological repression through asphyxiation or starvation (Mackauer, 1973). The observation that the parasitoid larviposits upto five times with three- five minutes interval, corroborates earlier observation that a single female distributes her planidia over several borer tunnel entrances Chinwada *et al.*, (2004). The removal of frass using the labellum appears to be a search for cues for presence of the host larva feeding inside the stem. The frass removal is observed to take more time prior to the female parasitoid's first larviposition. The female takes much shorter time to evaluate subsequent tunnel opening apparently having already been experienced after the inaugural larvipositon. The larviposited planidia was observed to lift its anterior extremity and almost stand on the tail and swing the head from side to side especially if it is crawling away from the tunnel opening and immediately there after begins to crawl towards the tunnel. The intricate behavior exhibited by

some of the larviposited planidia prior to entering the tunnel may be interpreted as a search for cues to orient them towards the tunnel opening.

The planidial attack guild associated with this parasitoid allows high accessibility to cryptically feeding larvae than the ingress and sting strategy associated with *Co. sesamiae*. In the later guild, the parasitoid is attracted to the tunnel entrance by ordour from hosts or frass, and subsequently it enters the tunnel and attacks the host. As observed with *B. fusca* and *Ch. partellus* as hosts, parasitoid mortality inside maize stem, can be more than 50% (Potting *et al.*, 1993, Takasu and Overholt 1997). Moreover, *C. sesamiae* is exceedingly rare in Cameroon, it will be important to introduce *S. parasitica*, which has a high genetic plasticity in terms of host range and/or host plant preference (Ndemah *et al.*, 2001). *S. parasitica* has only been found once in Cameroon on *B. fusca* (Ndemah *et al.*, 2001, 2007).

A parasitoid species specific to *B. fusca* would have little chance of establishing itself, since cues needed for host finding such as frass or synomones emitted by damaged plant are not produced during off season when the borer diapauses in larval stage (Kajita & Drake, 1996; Mohyuddin, 1971., van Leerdam *et al.*, 1985; Potting *et al.*, 1995). From the observations in the current study, it is conclude that, *S. parasitica* has the potential to reduce pest damage to crops due to *S. calamistis*, *B. fusca*, *E. saccharina* and *Ch. partellus* by attacking and maintaining the pest population below the economic threshold and hence contribute significantly to other pest management strategies in IPM. However, release in East African region should be cautious as both the East and West

African biotypes are known to mate and produce fertile progeny which will make it difficulty to judge the performance of the new entrant (Dittrich *et al*, 2006).

CHAPTER 5

5.0 THE SUCCESS RATES OF DEVELOPMENT OF *STURMIOPSIS PARASITICA* AND *COTESIA SESAMIAE* IN AN ALREADY PARASITIZED

5.1 Introduction

The essence of interspecific competition is that individuals of one species suffer a reduction in fecundity, survivorship or growth as a result of exploitation of resources or interference by individuals of another species. With exploitive competition, the more successful competitor is the one that more effectively exploits the shared resources. *Cotesia sesamiae* (Cameron) Hymenoptera: Braconidae), a gregarious larval endoparasitoid, is one of the most important native larval parasitoid in many countries of sub-Saharan Africa (Ingram, 1958; Mohyuddin, 1971; Scheilbelreiter, 1980; Polaszek and Walker, 1994; Kfir, 1995). Competition between species of insect parasitoids or predators can influence the size and structure as well as the stability of insect communities that include several trophic levels (Lawton, 1986., Price, 1986). In biological control, competition between species of introduced natural enemies or between introduced and native natural enemies of a pest have been invoked to explain why some species fail either to become established or to control the pest successfully (Turnbull, 1967, Ehler and Hall, 1982; Jalali *et al.*, 1988).

Interspecific competition between individuals belonging to different species is of great interest due to its symmetry, it has been an important evolutionary force

that has led to niche separation, specialization and diversification. Godfray (1994) reviewed the importance of competition as a factor influencing community structure. Thus, understanding the competitive interactions between biological control agents enhances the ability to understand and predict the interactions of natural enemy populations with their host. According to Mack and D'Antonio, (1998), species removed from or added to an environment which strongly interact with native species frequently produce ecosystem structure alteration. These alterations occur in both disrupted and intact systems resulting in profound changes in ecosystem processes that ultimately control plant and animal activities and direct species replacement (Knops *et al.*, 1999). *S. parasitica*, a solitary tachinid and *Co. sesamiae* a gregarious braconid are both endoparasitic larval parasitoids of various cereal stemborer pests in Sub Saharan Africa. In Kenya, *Co. sesamiae* is the most important larval parasitoids of some stemborers including *B. fusca* and *S. calamistis*. *B. fusca* is a major pest in Kenya where maize is grown at elevations greater than 600m (Nye, 1960). However, *Co. sesamiae* is more common in the wetter parts of Africa and levels of parasitism in Kenya and Uganda is upto 20% (Skovgård and päts, 1996) which does not maintain the pest at acceptable levels. S. parasitica has been proposed for release in ESA (Schulthess et al., 1997). However, before release, information on its target and non target species as well as on other indigenous natural enemies is necessary.

5.2 Materials and methods

5.2.1 Determination of success rate of *S. parasitica* in parasitizing a host already parasitized by *Co. sesamiae*

To determine the success rate of *S. parasitica* in parasitizing a host already parasitized by *Co sesamiae*, 30 fourth instar *S. calamistis* larvae were each exposed individually to a mated 24h old *Co. sesamiae* female for oviposition using the hand-stinging method whereby the stemborer host larva was held with soft forceps and offered to the parasitoid for oviposition (Overholt *et al.*, 1994) and thereafter inoculated with 8 planidia of *S. parasitica* at 0, 24 and 48 hours. Another 30 larvae parasitized by *S. parasitica* alone were used as control. In the results, experiment treatments are referred to in the results as *Sp-Cs* and controls as *Sp*. The larvae either died when the parasitoid emerged or due to undetermined causes or a moth emerged.

5.2.2 Determination of success rate of *Co. sesamiae* in parasitizing a host already parasitized by *S. parasitica*.

To determine the success rate of *Co. sesamiae* in parasitizing a host already parasitized by *S. parasitica*, the experiment was carried out as above in section 3.3.4 except that the *S. calamistis* larvae were first inoculated with 8 planidia of *S. parasitica* and thereafter exposed to *Co. sesamiae* at 0, 24 and 48h. 30 larvae exposed to *Co. sesamiae* alone were used as control. In the results, experiment treatments are referred to as *Cs-Sp* and the controls as *Cs*. After parasitization the larvae were reared individually on artificial diet according to the method of

Onyango and Ochieng-Odero (1994). They were then maintained in an incubator set at 26 ± 1°C, RH 70 ± 5%, and 12:12 (L: D) h photoperiod. Host larvae were inspected daily. The proportion of larvae that produced puparia or cocoons, development duration to puparia or cocoons and to adult emergence were recorded for *S. parasitica* and for *Co. sesamiae* respectively. Associated immature mortality, numbers of puparia or cocoons per larvae and the proportion of larvae that yielded both parasitoids and sex ratio as the proportion of female offspring were also recorded.

5.3 Data analysis

The data on parasitism, parasitism sequences, time interval between parasitism, duration of development, total adult progeny, mortality and female progeny produced per stemborer host larvae were compared using Analysis of Variance (ANOVA) using the General Linear Protocol (PROC GLM, SAS Institute 2001). Student-Newman-Keul's (SNK) and Bonferroni mean separation procedures were used when ANOVA yielded significant differences. Percentage and ratio data were arcsine square root transformed before ANOVA. The significance level was set at $P \leq 0.05$.

5.4 Results

5.4.1 Determination of success rate of *S. parasitica* in parasitizing a host already parasitized by *Co. sesamiae* and vice versa

When planidia of *S. parasitica* were inoculated onto the larvae of *S. calamistis* already infested with *Co. sesamiae*, both parasitoids emerged from the same host larvae in either parasitism sequence and in all time intervals between

parasitism. Multiparasitism reduced the PGI by 34% for *S. parasitica* and 96% for *C. sesamiae*, when compared with the controls. Time after parasitization and parasitizing sequence showed no significant difference (Table 4).

The percentage parasitism of *S. parasitica* in the controls and in multiparasitized hosts showed significant difference across all time intervals 0hrs ($\chi^2 = 10.9$, p= 0.0042), 24hrs ($\chi^2 = 16.3$, p= 0.0003) and 48hrs ($\chi^2 = 20.8$, p= 0.0001). It was lowest in the controls (Fig 2A).

The percentage parasitism of *Co. sesamiae* in the controls and in multiparasitized hosts showed significant difference across all time intervals, 0hrs ($\chi^2 = 10.5$, p= 0.0052) 24hrs ($\chi^2 = 8.0$, p= 0.0182) and 48hrs ($\chi^2 = 7.0$, p= 0.0302). I t was highest in the controls (Fig 2B).

Puparia-to-adult mortality was highest in multiparasitized hosts and lowest in the controls ($F = 0.3- 5.4$, $P = 0.005 - 0.38$). In the 0 hr interval there was no mortality in the controls. The mortality in controls was similar when S. parasitica parasitized first in the 24 and 48 hr intervals (Fig. 3A).

Cocoon-to-adult mortality was always lowest in the controls and similar in the two parasitizing sequences ($F = 12.8$-14.8, $P < 0.0001$; Fig. 3B).

In the control 100% of the *S. calamistis* larvae produced only one *S. parasitica* puparium, while 9.5-14.5% in the Sp-Cs parasitizing sequence produced two puparia The percentage larvae yielding both *S. parasitica* puparia and *Co.*

sesamiae cocoons varied between 7.8 and 12.2% and time intervals between parasitism was not significant. However, the mean number of puparia per larvae did not vary between the control and the sequences for any of the time intervals. Parasitizing sequence showed no significant difference (Table 5).

The number of *Co. sesamiae* cocoons which were produced per larvae were about three to four times higher in the control than in the multiparasitized larvae. However, parasitizing sequence and time after parasitization did not show any significant difference (Table 5).

The planidia-to-puparia duration of developmental varied in the 0h F=36.5, P =0.0001 interval only and it was shortest in the Sp-Cs sequence and longest in the control. Parasitism sequence showed significant difference when *S. parasitica* parasitized first F=23.6, P= 0.0001. In *Cotesia sesamiae* egg-to-cocoon developmental time was significantly different in all the time intervals between parasitism (0h F=6.52, P=0.0029, 24h F= 35.8, P= 0.0001, 48h F= 25.7, P= 0.0001). Parasitizing sequence showed significant difference Sp-Cs (F= 5.96, df= 2,56, P= 0.0046) Cs-Sp (4.09, df= 2,47, P= 0.0233) (Table 6).

Planidia-to-adult development duration varied significantly in all the time interval after parasitization 0h F= 6.8, P= 0.0001, 24h F= 93.6, P= 0.0001, 48h F= 88.7, P= 0.0001. There was a significant difference when *S. parasitica* parasitized first but there was no significant difference when *Co. sesamiae* parasitized first F= 3.6, P= 0.0279. Egg-to-adult development duration also varied significantly in all the time interval after parasitization 0h F= 7.8, P=

0.0001, 24h F= 41.2. P= 0.0001, 48h F= 20.5, P=0.0001 and when *S. parasitica* parasitized first F= 9.45, P= 0.0004. There was no significant difference when Co. sesamiae parasitized first (Table 7)

Planidia-puparia development period varied significantly at 0h interval F= 36.5, P= 0.0001 only and when *S. parasitica* parasitized first F= 23.6 P= 0.0001. Egg-cocoon development duration varied significantly different in all time intervals 0h F= 36.5, P= 0.0029, 24h F= 35.8, P= 0.0001, 48h F= 25.3, P= 0.0001 and in both parasitization sequences Sp-Cs F= 5.9. P=0.0046, Cs-Sp F= 4.1, P=0.0233. Puparia-to-adult development duration showed significant difference in all time intervals after parasitization 0h F= 24.4, P= 0.0001, 24h F= 44.8, P= 0.0001, 48h F= 71.1, P= 0.0001 and in the parasitization sequences Sp-Cs F= 4.95, P= 0.0081, Cs-Sp F= 4.94, P= 0.0082. Time interval showed no significant difference in cocoon-to-adult development duration but there was a significant difference when *S. parasitica* parasitized first Sp- Cs F= 3.9, P=0.0262 (Table 8).

Table 4. Mean potential growth index (± SE) in the controls (Sp or Cs) and in multiparasitized host in different parasitism sequences (Sp-Cs) where *S. parasitica* parasitized first and vice versa (Cs-Sp) at various time intervals 0, 24 and 48 hours.

	PGI		
Sequence	0hrs	24hrs	48hrs
S. parasitica			
Sp	1.87 ± 0.025	1.67 ± 0.012	1.89 ± 0.015
Cs-Sp	0.88 ± 0.015	1.30 ± 0.021	1.28 ± 0.016
Sp-Cs	1.00 ± 0.016	1.37 ± 0.019	1.10 ± 0.021
Co. sesamia			
Cs	1.16 ± 0.015	1.21 ± 0.013	1.16 ± 0.010
Cs-Sp	0.04 ± 0.001	0.06 ± 0.001	0.06 ± 0.001
Sp-Cs	0.05 ± 0.001	0.04 ± 0.001	0.07 ± 0.001

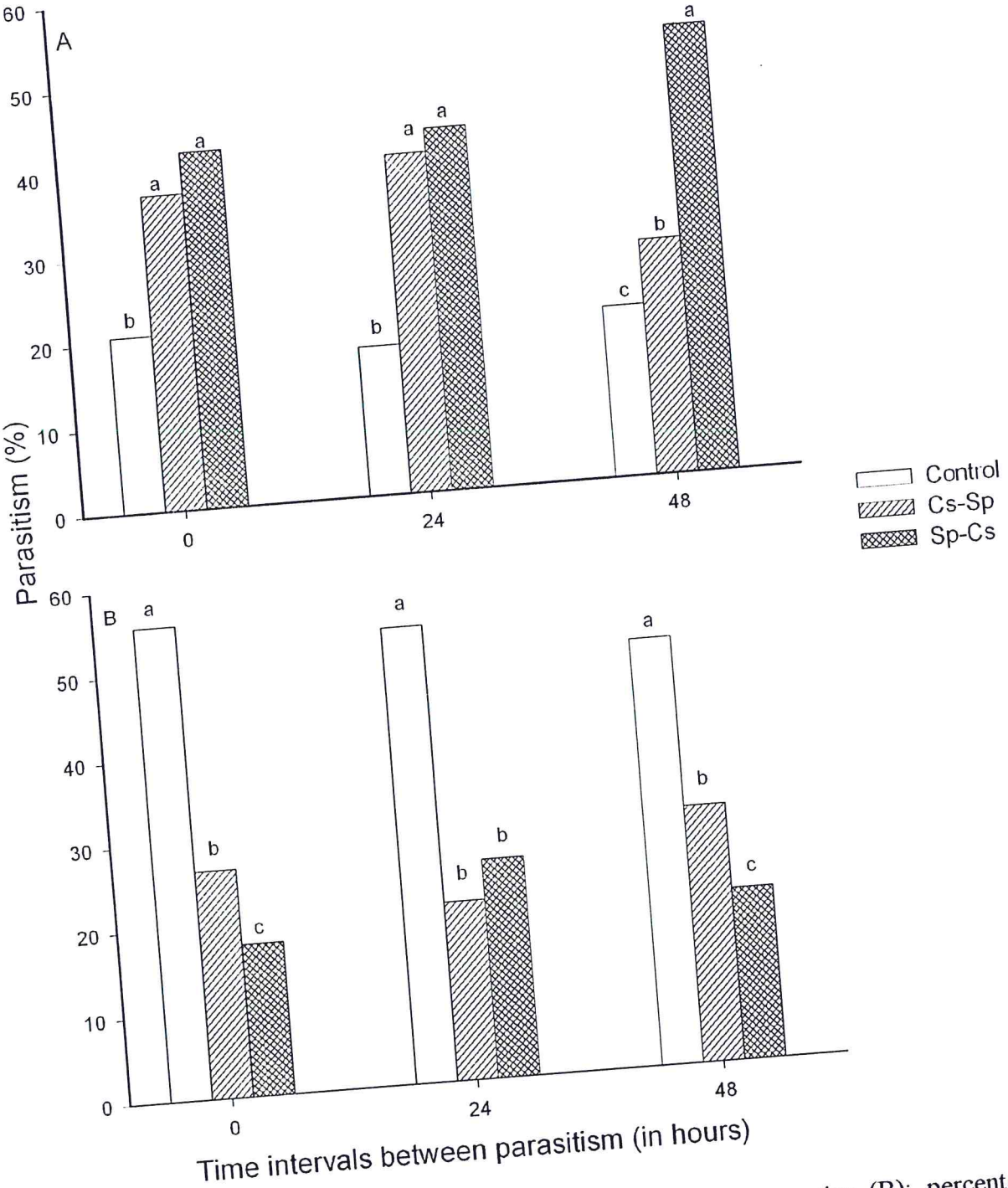


Figure 2: Comparison of *S. parasitica* (A) or *Co. sesamiae* (B); percentage parasitism in controls Sp or Cs) and multiparasitized host larvae in different parasitism sequence Sp-Cs where *S. parasitica* parasitized first followed by *Co. sesamiae* and vice versa at various time intervals (0, 24 and 48 hrs.) between parasitism

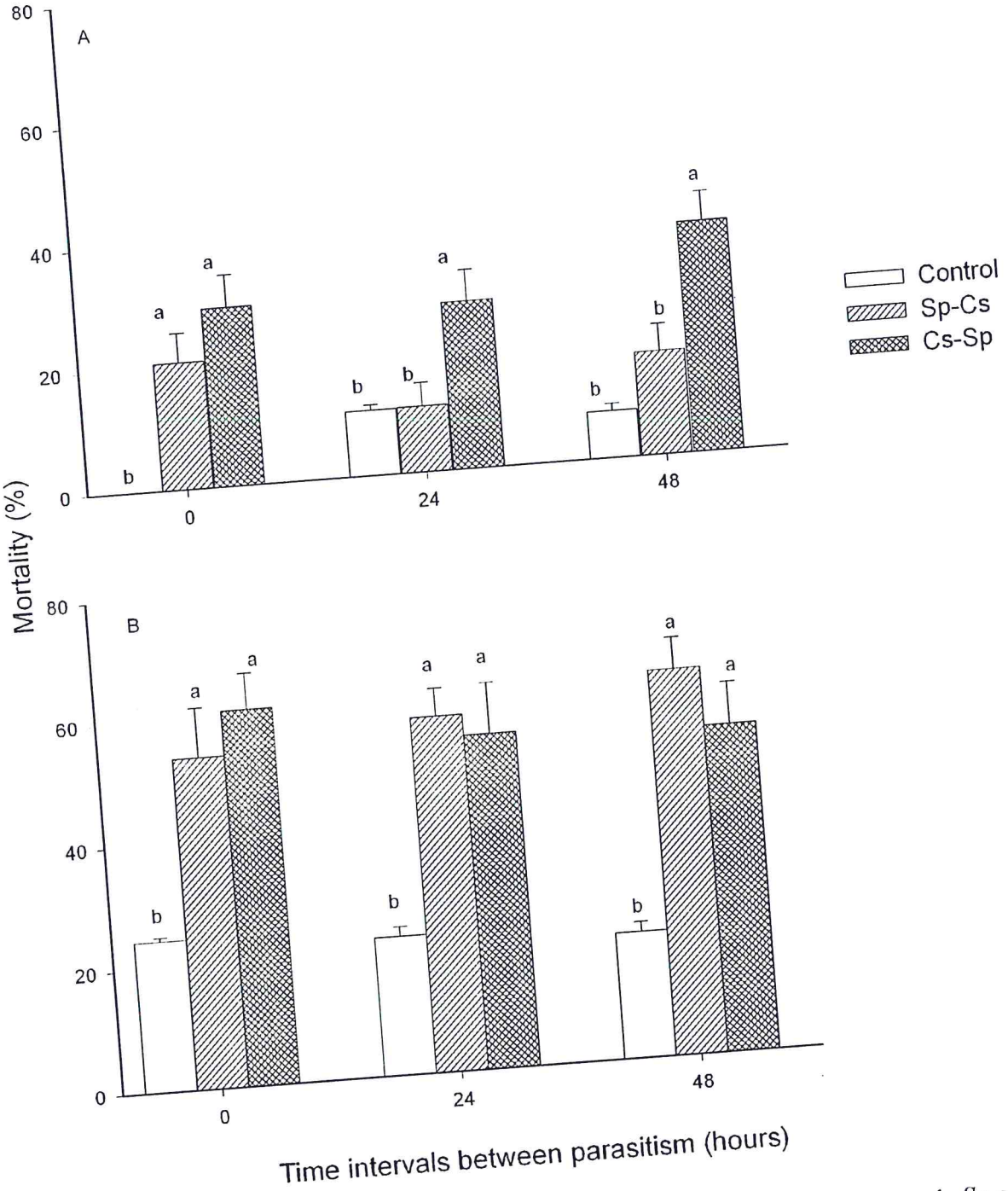


Figure 3. Mortality (%) of puparia (A) and cocoons (B) (± SE); in controls *Sp* or *Cs* and in multiparasitized host in different parasitism sequence (*Sp-Cs* where *S. parasitica* parasitizes fist followed by *Co. sesamiae* and vice versa Cs-Sp) at various time intervals between parasitism (0, 24 and 48 hrs).

Table 5. The mean number of puparia or cocoons (± SE) in controls (Sp, Cs) and multi-parasitized host (Sp- Cs where *S. parasitica* parasitized first and vice versa Cs- Sp) at various time intervals (0, 24, or 48 h) between parasitism

Sequence	0hr	24hr	48hr	F	DF	P
		Number of puparia/larvae				
Sp	1.00 ± 0.0	1.00 ± 0.0	1.00 ± 0.0	0	2, 82	0
SP-Cs	1.09 ± 0.03	1.13 ± 0.04	1.20 ± 0.04	0.27	2, 211	0.7628
Cs-Sp	1.04 ± 0.02	1.03 ± 0.02	1.03 ± 0.02	0.15	2, 207	0.8574
F	2.01	4.78	3.47			
DF	2, 171	2, 170	2, 159			
P	0.1377	0.0496	0.0436			
		Number of cocoons /larvae				
Cs	70.1 ± 4.5a	59.2 ± 4.0a	63.0 ± 3.1a	0.79	2, 72	0.4576
Sp-Cs	22.6 ± 3.9b	17.8 ± 3.1c	22.6 ± 2.8b	1.43	2, 56	0.2487
Cs-Sp	23.2 ± 3.4b	29.4 ± 4.5b	28.1 ± 4.3b	0.77	2, 47	0.4689
F	20.89	25.92	26.39			
DF	2, 55	2, 57	2, 63			
P	0.0001	0.0001	0.0001			

Means followed by the same lowercase letters in the same column and means followed by the same uppercase letters in the same row are not significantly different at P≤0.05 Student-Newman-Keul's (SNK test).

Table 6. The mean duration of development in days (±SE) of puparia and cocoon in controls (Sp or Cs) and in multiparasitized host in different parasitism sequence (Sp-Cs where *S. parasitica* parasitized first and vice versa Cs- Sp) at various time intervals (0, 24, or 48 h) between parasitism

Sequence	0hr	24hr	48hr	F	DF	P
		Puparia developmental time (days)				
Sp	15.5 ± 0.4a	14.5 ± 0.3	14.5 ± 0.3a	2.87	2,82	0.0626
Sp-Cs	12.9 ± 0.1cC	14.6 ± 0.2B	14.9 ± 0.2bA	23.61	2,211	<0.0001
Cs-Sp	14.2 ± 0.1b	13.6 ± 0.28	14.3 ± 0.2	23.61	2,206	0.0544
F	36.55	2.39	1.59			
DF	2, 168	2, 170	2, 161			
P	<0.0001	0.0945	0.2076			
		Cocoons developmental time (days)				
Cs	11.5 ± 0.1b	11.9 ± 0.1b	11.7 ± 0.1b	9.08	2,71	0.0503
Sp-Cs	12.3 ± 0.3bB	13.9 ± 0.2aA	13.9 ± 0.4aA	5.96	2,56	0.0046
Cs-Sp	13.4 ± 0.2a	14.2 ± 0.2a	14.8 ± 0.4a	4.09	2,47	0.0233
F	6.52	35.80	25.27			
DF	2,55	2, 57	2, 62			
P	0.0029	<0.0001	<0.0001			

Means followed by the same uppercase letters in the same column and means followed by the same lower case letters in the same row are not significantly different at P≤0.05 Student-Newman-Keul's (SNK test).

Table 7. The mean duration of development in days(± SE) of *S. parasitica* and *C. sesamiae* in the controls (Sp or Cs) and in multiparasitized host in different parasitism sequences (*Sp-Cs* where *S. parasitica* parasitized first followed by *C. sesamiae* and vice versa *Cs-Sp*) at various time intervals (0, 24, 48hrs) between parasitism

Parasitoid/ Sequence	0 hr	24hr	48hr	F	DF	P
S. parasitica (days)						
Sp	26.0 ± 0.2b	25.4 ± 0.3b	25.6 ± 0.3c	0.93	2, 78	0.3986
Cs-Sp	29.8 ± 0.2a	29.8 ± 0.2a	30.3 ± 0.2a	2.27	2, 176	0.1062
Sp-Cs	29.8 ± 0.2aB	30.4 ± 0.2aA	29.6 ± 0.2bB	3.65	2, 186	0.0279
F	61.77	93.63	88.68			
DF	2, 137	2, 159	2, 144			
P	0.0001	0.0001	0.0001			
Co. sesamiae (days)						
Cs	17.4 ± 0.2b	17.3 ± 0.2c	17.5 ± 0.3b	6.45	2, 72	0.0527
Cs-Sp	19.9 ± 0.8a	18.8 ± 0.8b	20.6 ± 0.5a	1.54	2, 39	0.2279
Sp-Cs	20.5 ± 0.3Ab	22.1 ± 0.3aA	20.3 ± 0.4aB	9.45	2, 46	0.0004
F	7.79	41.18	20.55			
DF	2, 58	2, 46	2, 53			
P	0.0010	0.0001	0.0001			

Means followed by the same lower case letters in the same column and means followed by the same upper case letters in the same row are not significantly different at P≤ 0.05 (Student-Newman-Keul's SNK test).

Table 8. The mean duration of development in days ± SE of different life cycle stages of *S. parasitica* and *Co. sesamiae* in the controls (Sp or Cs) and in multiparasitized host in different parasitism sequence (Sp-Cs where *S. parasitica* parasitized first and vice versa Cs-Sp) at various time interval (0, 24 and 48 hrs) between parasitism

Sequence	0hr	24hr	48hr	F	DF	P
S. parasitica planidia - puparia						
Sp	15.5 ± 0.4a	14.5 ± 0.3	14.5 ± 0.3	2.87	2,82	0.0626
Sp-Cs	12.9 ± 0.1cC	14.6 ± 0.2B	14.9 ± 0.2A	23.61	2,211	<0.0001
Cs-Sp	14.2 ± 0.1b	13.6 ± 0.28	14.3 ± 0.2	23.61	2,206	0.0544
F	36.55	2.39	1.59			
DF	2, 168	2, 170	2, 161			
P	<0.0001	0.0945	0.2076			
		C. sesamiae egg – cocoon				
Cs	11.5 ± 0.1b	11.9 ± 0.1b	11.7 ± 0.1b	9.08	2,71	0.0503
Sp-Cs	12.3 ± 0.3aB	13.9 ± 0.2aA	13.9 ± 0.4aA	5.96	2,56	0.0046
Cs-Sp	13.4 ± 0.2a	14.2 ± 0.2a	14.8 ± 0.4a	4.09	2,47	0.0233
F	6.52	35.80	25.27			
DF	2,55	2, 57	2, 62			
P	0.0029	<0.0001	<0.0001			
		S. parasitica puparia - adult				
Sp	10.4 ± 0.5b	10.8 ± 0.5b	11.1 ± 0.5b	0.49	2,78	0.6160
Sp-Cs	14.8 ± 0.4aB	15.8 ± 0.5aA	15.9 ± 0.2aA	4.95	2,185	0.0081
Cs-Sp	15.1 ± 0.5aB	15.3 ± 0.3aB	16.4 ± 0.2aA	4.94	2,176	0.0082
F	24.38	44.79	71.16			
DF	2,146	2,152	2,141			
P	<0.0001	<0.0001	<0.0001			
		C. sesamiae cocoons – adult				
Cs	5.9 ± 0.2	5.3 ± 0.3	6.3 ± 0.5	1.67	2,72	0.1951
Sp-Cs	8.8 ± 0.4A	7.1 ± 0.3AB	6.8 ± 0.7B	3.95	2,47	0.0262
Cs-Sp	7.9 ± 1.5	8.6 ± 0.6	6.3 ± 0.4	1.09	2,39	0.3460
F	3.28	2.58	1.74			
DF	2,50	2,52	2,56			
P	0.0461	0.0862	0.1849			

Means followed by the same lower case letters in the same column and means followed by the same upper case letters in the same row are not significantly different at P≤ 0.05 (Student-Newman-Keul's SNK test.

Sex ratios varied between 0.43 – 0.63 for *S. parasitica* and 0.44 – 0.56 for *C. sesamiae* and there were no significant differences between parasitism sequences and the control or time intervals (Table 9).

Table 9. Sex ratio, (female) (± SE) of *S. parasitica* and *Co. sesamiae* in the controls (Sp or Cs) and in multiparasitized host in different parasitism sequences (*Sp-Cs)* where *S. parasitica* parasitized first and vice versa (*Cs-Sp)* at various time intervals (0, 24, 48hrs) between parasitism

Parasitoid/ Sequence	0hr	24hr	48hr	F	DF	P
S. parasitica						
Sp	0.59± 0.09	0.52± 0.10	0.44± 0.01	0.56	2,78	0.5728
Cs-Sp	0.54 ± 0.07	0.43± 0.06	0.51 ± 0.08	0.08	2,148	0.4508
Sp-Cs	0.63 ± 0.06	0.48 ± 0.06	0.45 ± 0.06	2.59	2, 184	0.0775
F	0.45	0.29	0.22			
Df	2, 144	2, 144	2, 121			
P	0.6389	0.7460	0.8010			
C. sesamiae						
Cs	0.51 ± 0.01	0.55± 0.09	0.45 ± 0.05	0.10	2, 18	0.9037
Cs-Sp	0.56 ± 0.09	0.45 ± 0.09	0.50 ± 0.05	0.66	2, 37	0.5209
Sp-Cs	0.48 ± 0.01	0.44 ± 0.09	0.51 ± 0.08	0.15	2, 48	0.8602
F	0.19	0.28	0.21			
DF	2, 31	2, 35	1, 37			
P	0.8264	0.7549	0.8148			

Means followed by the same lower case letters in the same column and means followed by the same upper case letters in the same row are not significantly different at P≤ 0.05 (Student-Newman-Keul's SNK test).

5.5 Discussion

In the current study, interspecific competition with *Co. sesamiae* reduced intraspecific competition in *S. parasitica* enhancing its progeny per larva with 9.5-14.5% producing two puparia each and 7.8-12.8% yielding both *S. parasitica* puparia and *Co. sesamiae* cocoons. However, multiparasitism reduced the PGI of both parasitoids. Each larva, parasitized by *S. parasitica* alone (control) produced only one puparia. In solitary parasitoids, only one individual can develop in a host whereas supernumerary individuals are eliminated by some form of physiological suppression or by physical combat among the larvae (Hubbard *et al.*, 1987, Mackauer, 1990). When multiparasitism does occur, the outcome of intrinsic competition is affected by differences in parasitoid development rates, egg numbers, the developmental stage of the host, the order in which oviposition occur and time interval between

first and second oviposition(van Strien-van Liempt, 1983, Tillman and Powell, 1992). The current study contradicts this work because *S. parasitica* did not show any significant difference due to parasitism sequence or time interval after parasitizing. This could be due to the developmental stage of *S. parasitica* which larviposits first instar planidia while *Co. sesamiae* lays eggs. However, *Co. sesamiae* corroborates the earlier work. Both parasitoids emerged from the same host and it is suggested that the host could be a realized niche allowing the two parasitoids to co-exist. However, multiparasitism reduced performance both parasitoids through cocoon-to adult and puparia-to adult mortality. Time interval between oviposition /larvipositions and the parasitization sequence had no significance. Development time was longer in the multiparasitized hosts but, Jiang *et al* (2004), showed that, developmental time depended on the quality and size of the host.

Bakker *et al*. (1985) suggested that for sympatric parasitoid species exploiting the same resources, multiparasitism is the best strategy when hosts are scarce, parasitoid females are not egg limited and the second egg laid into the host has some probability of survival. The ability to avoid multiparasitism or to use it as a means to compete with other foragers is an important aspect of parasitoid reproductive behavior that influences without any doubt parasitoid population stability (van Alpen and Visser, 1990). The current study corroborates the earlier work because both parasitoids emerge from the same host; however *S. parasitica* is competitively intrinsically superior to *Co. sesamiae* as its percentage parasitism and progeny production increased in interspecific competition across all time intervals and in parasitizing sequences. Parasitizing

sequence or time interval after parasitization had no impact on development duration or sex ratio of *S. parasitica* in the larvae of *S. Calamistis* whereas there was a significantly shorter development period when *S. parasitica* parasitized *B. fusca* larvae first at 0hrs and at 24hrs.

Mixing cultures makes it possible to study different parameters as a means of evaluating the performance of one species when in the presence of another (Gonzales-Candelas *et al.*, 1990). Development time and viability are among the parameters used. In the current study there was no significant difference in development duration of *S. parasitica* due to multiparasitism in the larvae of *S. calamistis*. When measuring the impact of interspecific competition on population growth we must take into account differences between competing species in amount of resource used and overlap in the set of resources used. *S. parasitica* and *Co. sesamiae* have size variation which influence the way the food resources are utilized. Interspecific competition is usually weaker than intraspecific competition because two species never utilize same resources with exactly the same efficiency.

There was no significant difference in sex ratio of both *S. parasitica* and *Co. sesamiae* due to parasitizing sequence or time interval after parasitization in the larvae of *S. calamistis*. The production of fertilized eggs depends on a wide variety of physiological and ecological mechanisms, and the operations of these mechanisms influence the sex ratio (Clausen 1939). Large variations in the sex ratio of hymenopterous parasitoids has been observed in the field and, since this group of insects is so frequently used as natural enemies in biological control

programmes, it is clearly important that the influence of a dynamic sex ratio should be considered in host parasitoid systems Wilson *et., al* (1982). In the current study, *Co. sesamiae* sex ratio was not affected by multiparasitism suggesting that they may co-exist.

CHAPTER 6

6.0 GENERAL DISCUSSION CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

The stem borers continue to cause great yield loss despite many attempts to control them. The methods that have been used to control the pests so far include cultural, chemical and biological approaches. In the current study, the host range of *S. parasitica* and the interspecific competition between *S. parasitica* and *Co. sesamiae* was investigated to assess suitability and acceptability of various stemborers occurring in Kenya and other parts of Africa to determine its potential as a biological control agent. The study showed that, the West African *S. parasitica* strain had 2-3 times higher rate of parasitism in *S. calamistis* than with any other host. In contrast, the Zimbabwe strain did not develop in this host species as a result of maggot encapsulation (Chinwada *et al.*, 2004). The most suitable host for the latter was *B. fusca*, which yielded 83.3% parasitism, which is comparable to the 75.2% with *S. calamistis* in the present study. The present observations that *Ch. partellus* was the least suitable, corroborates with the results by Chinwada *et al.*, (2004). *Chilo partellus* is increasingly invading the cooler areas in eastern Africa and in some areas in South Africa it has started to displace *B. fusca* (Kfir, 1997; Wale *et al.*, 2007). The present findings support the results from phylogenetic and morphological studies by Dittrich *et al.*, (2006) and Barraclough (2004), reported that, there are several populations of *S. parasitica* in Africa, which vary in their host range. Furthermore, all host species used in this study were suitable for development of the West African *S. parasitica* strain indicating that the geographic race of the

borer hosts played no or only a minor role (Sezonlin *et al.*, 2006; Assefa *et al.*, 2006).

In the present study, *S. par*asitica was able to distinguish between a suitable and less suitable host. It has been reported that kairomones in the frass and synomones produced by plants damaged by larvae might trigger larviposition activity by the tachinids (Clement *et al.*, 1985., Dicke and Sabelis, 1988., Stireman 2002). The West African strain of *S. parasitica* had a greater preference for *S. calamistis* compared to the other species Thus the cues produced by *S. calamistis* are host specific, probably consisting of saliva or haemolymph. It is not known if the Zimbabwe strain of *S. parasitica* attacks *S. calamistis* in the field, but if it does *S. calamistis* would form a reproductive sink. However, since both species are ubiquitous in sub-Saharan Africa and occur sympatrically in Zimbabwe (Chinwada *et al.*, 2008) it is suggested that *S. parasitica* learned to distinguish between a suitable and unsuitable host. This would suggest that the Zimbabwe strain is more specialized than the West African strain.

According to Stireman *et al.* (2004) relatively specialized tachinids tended to be associated with monophagous or narrowly oligophagous hosts. In the area in West Africa, where *S. parasitica* was collected, the dominant noctuid borer species is *S. calamistis* and *B. fusca* is rare (Schulthess *et al.*, 1997), while in Zimbabwe it is the reverse (Chinwada 2003). As shown by Gounou and Schulthess (2004), Le Rü *et al.*, (2006) and Ndemah *et al.*, (2007), *B. fusca* is oligophagous limited mostly to cultivated and wild sorghum species, while *S.*

calamistis attacks a wide range of grass and sedge species. This is probably a result of their strategy to survive periods when the crop is not present in the field: While *B. fusca* diapauses in the larval stage, *S. calamistis* is feeding on wild host plants (Gounou and Schulthess, 2004). *Ch. partellus*, on the other hand, has a similar narrow host plant range as *B. fusca* (Le Rü *et al.*, 2006) and also diapauses during the off-season. Thus, in West Africa, *S. parasitica* remains active during the entire dry season indicating that non-diapausing borers on wild host plants form an important reservoir for perennation of the parasitoid (Gounou *et al.*, submitted). In contrast, in Zimbabwe *S. parasitica* is carried over from one cropping season to the next by synchronizing its larval development with that of the diapausing *B. fusca* larvae (Chinwada and Overholt, 2001). Thus, the active population crashes to zero during the off-season.

In contrast to the Zimbabwe strain which yielded up to 6 puparia per host the West African strain never produced more than one except when multiparasitized, but even then it never exceeded more than 2 per host (Chinwada *et al.*, 2004). It appears that the West African strain tends to be solitary while the Zimbabwe strain is gregarious. In solitary species, normally only one larva completes its development to adult stage in each host with supernumerary larvae being eliminated by some form of physiological suppression or by physical combat among larvae (Clausen, 1962; Mackauer, 1973; Quicke, 1997). Several mechanisms for the elimination of supernumerary larvae such as physical combat among first instars of *Marquartia chalconota* Meigen (Mellini & Baronio 1971), anoxia for elimination of older

supernumerary maggots of *Lixophaga diatraeae* (Townsend) (King *et al.*, 1976) and probably physiological suppression during the second stadium of supernumerary maggots (Reitz, 1995), have been reported for tachinids. It is not clear, however, how *S. parasitica* eliminates competitors, but the competition appears to be greater in the West African biotype than in the Zimbabwe one.

Sétamou *et al.* (2005) and Jiang *et al.* (2004) showed that development time of *Co. flavipes* depended on the quality of its host *Ch. partellus* and it was longer in smaller hosts or hosts feeding on wild grasses which affected size of the host. They contributed it to a lower rate of development due to lack of adequate food supply. Puparia of *S. parasitica* are large compared to *Co. sesamiae* immatures. Thus, it is suggested that the extended development time and the low number of *Co. sesamiae* offspring in multiparasitized larvae was a result of scramble competition for host resources. In addition, Sétamou *et al.* (2005) also found that host quality can affect adult *Co. flavipes* emergence, which could explain the high cocoon-to-adult mortality of *Co. sesamiae* from multiparasitized larvae. Consequently, it can be expected that with the Zimbabwe strain of *S. parasitica*, which tends to be gregarious, interspecific competition should lead to the extirpation of *Co. sesamiae*.

In the Zimbabwe strain, parasitism and the numbers of larvae yielding more than one puparia increased with the number of maggots used for inoculation (Chinwada *et al.*, 2004). The same trend for parasitism was observed with the West African strain but the final number of puparia never exceeded one, except when multiparasitized. This suggests that the maggots had to overcome the

immune systems of the host and became increasingly successful as the number of maggots per larvae increased. Of the multi-parasitized larvae 7-14.5 % yielded 2 puparia. Thus, in the *Sp - Cs* sequence, although multiparasitism led to a decrease in parasitized larvae, the numbers of offspring produced was similar among treatments. The occurrence of two puparia per host might have been the combination of two factors. Firstly, *Cotesia sesamiae* injects polyDNA viruses together with the eggs to induce immune suppression in the host in order to prevent encapsulation of the eggs; this might have benefited both parasitoid species (Gitau *et al.*, 2006). Secondly, in the multi-parasitized larvae, inter-specific competition caused a drastic reduction in the number of *Co. sesamiae* successfully developing to pupae but concomitantly it might have allowed the survival of supernumerary *S. parasitica* larvae as a result of reduced intraspecific competition. Similarly, Iwao and Ohsaki (1996) studying the Intra- and inter- specific larval interactions between a solitary and gregarious tachinid and the gregarious *Cotesia glomerata* (L.) attacking the pierid *Pieris brassica* (L) showed that the gregarious tachinid could more often survive in the presence of *Co. glomerata*.

In the present observations multiparasitism negatively affected the performance of both parasitoids via reduced successful parasitism, cocoon-to adult mortality and brood size for *Co. sesamiae*, and increased puparia to adult mortality and extended life cycle. In most cases, however, time interval between ovipositions and oviposition sequence had no significant effect. In other parasitoid couplings in the stemborer-parasitoid system, the parasitism sequence and the time interval between ovipositions have been shown to influence the outcome of the

competition (Agboka *et al.*, 2002; Muli *et al.*, 2006; Muturi *et al.*, 2006). Competition may be reduced through niche separation where the two competitors attack different age classes of the host, but both *S. parasitica* and *Co. sesamiae* occupy the same niche. Probably, the lack of the effect of parasitism sequence and time interval between ovipositions very likely was the result of *S. parasitica*, which larviposits, having a developmental head start over *Co. sesamiae*, whose eggs require several days before they eclose.

Multiparasitism drastically reduced the performance of *Co. sesamiae*, expressed as the PGI. Thus, in this parasitoid coupling, the solitary *S. parasitica* outcompeted the gregarious *Co. sesamiae*. The two parasitoids occupy similar niches in terms of host species and life stages attacked, and probably use the same cues to find the host (Ngi-Song and Overholt, 1997) but *S. parasitica* has a considerably greater fecundity and adult longevity, and it is a much stronger flier than *Co. sesamiae*. A female *S. parasitica* produces up to 1700 eggs, half of which yield active maggots (Chinwada *et al.*, 2004), versus 160 eggs produced by a *Co. sesamiae* (Gitau, 2006) female, of which 25-60% yield cocoons. *S. parasitica* adults live up to 29 days versus 3 days for *Co. sesamiae* (Chinwada *et al.*, 2004; Mbapila and Overholt 2001). The tachinid larviposits up to 30 planidial larvae per site, of which only one usually makes it to pupa; consequently it could theoretically, parasitize at least 30 larvae versus 2-3 for *Co. sesamiae*. Furthermore, it has been shown that, *Cotesia* larvae display an aggressive behavior towards the parasitoids by spitting or biting them, and up to 40% are killed in the attempt to parasitize the larvae (Takasu and Overholt 1997, Potting *et al.*, 1999). Thus, for both parasitoids, even suitable hosts can form a

reproductive sink. On the other hand, the tachinid might have more problems in reaching borer larvae deeply imbedded in maize stems than the braconid. During its short life span of 1-2 hours a planidial larvae only moves a few centimeters while tunnel length might average between 10-20 cm and more ((Allen *et al.*, 1999, Chabi-Olaye *et al.*, 2005). Ndemah *et al*, (2002) found that the level of parasitism of *S. Calamistis* by S. *parasitica* was higher on wild grasses than maize and concluded that this was due to the considerably thinner stems that got easily damaged, which increased the accessibility of the host. However, wild grasses are qualitatively inferior to maize in terms of survival and weight of the host larvae (Shanower *et al.*, 1993); thus, for *S. parasitica* there is a trade-off between parasitism and host plant suitability.

6.2 Conclusions

1. All the stemborer hosts in this study were acceptable and suitable for development of *S. parasitica*.
2. *Sturmiopsis parasitica* successfully parasitized a host already parasitized *by Co. sesamiae* and vice versa respectively.
3. The West African *S. parasitica* can establish in the mid altitudes of ESA which has mixed populations of stem borers that have varying degrees of suitability without affecting populations of *Co. sesamiae* or *Co. flavipes*
4. In the ESA highlands where *B. fusca* dominates, the West African biotype may over-exploit the alternative non diapausing hosts which are scarce, during the off season and therefore will very likely not establish. Interspecific competition might cause local extinction of *Co. sesamiae*

6.3 Recommendations

1. Hybrid of crosses between ESA and the West African *S. parasitica* should probably be investigated for suitability and taxonomic differences before any field releases are done.
2. Since the present study showed that *S. parasitica* was superior to *Co. sesamiae* as a parasitoid, it recommended that the extrinsic interspecific competition between them should also be determined.
3. Determination of the effect of different temperature regimes and humidity is essential and semi field trials in screened houses as they play key roles in biological control.
4. The role of wild stem borers needs to be elucidated because they may be alternative reservoirs during off-season.

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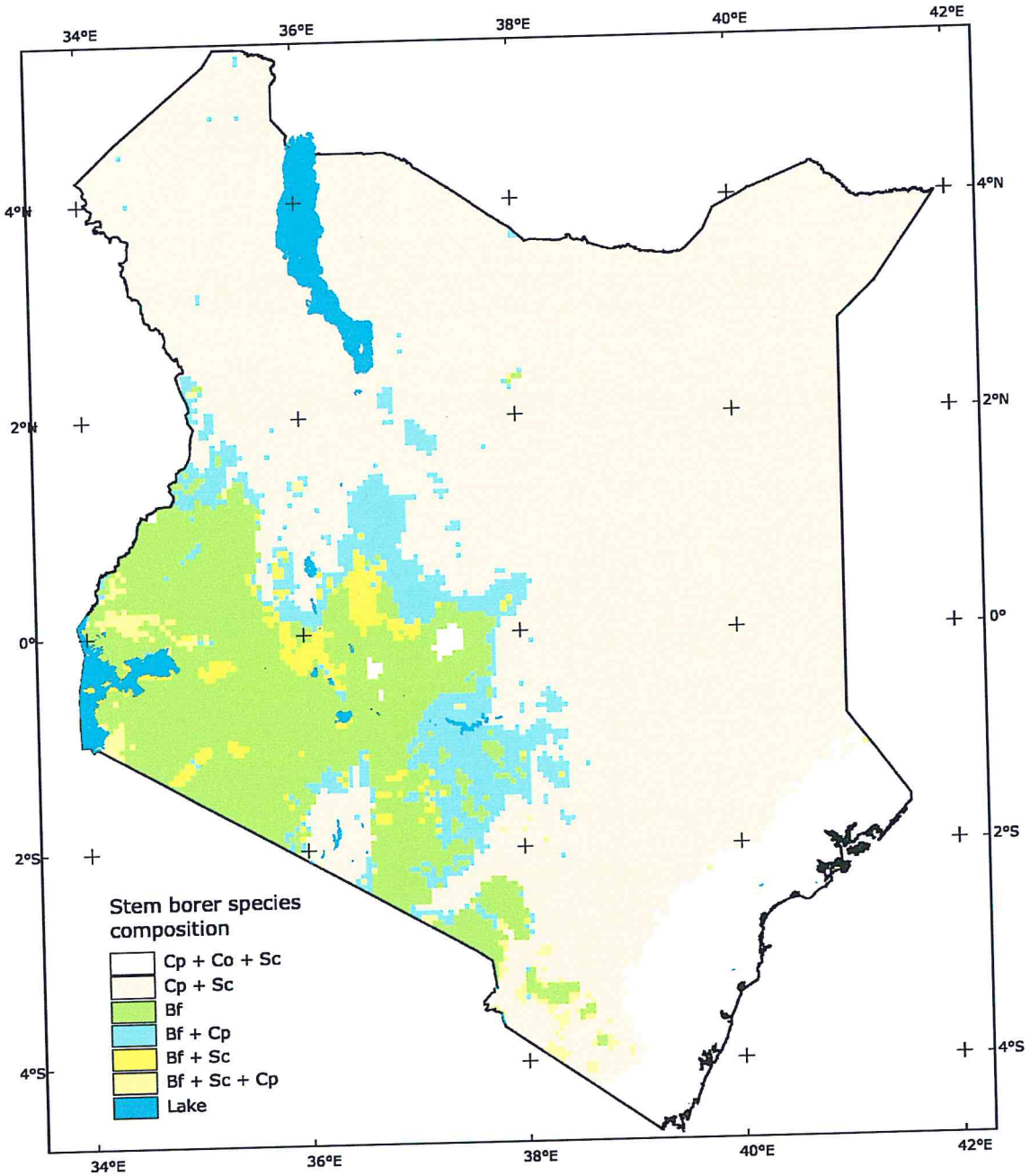
Appendix 1

Map of Kenya showing the distribution of various stemborers



Appendix 2

Primary parasitoids of common cereal stem borers in Kenya

Parasitoid	Host species	Host stage
Hymenoptera		
Bethylidae		
*Goniozus sp	C. sp	L
Goniozus indicus Ashmead	Co, Cp	L
Odontepyris transvaalensis De Buyson	?	
Braconidae		
Amyoson nyanzaense Quicke and Wharton	Bf, Cp	L
*Apanteles sp (ater group)	Cp	L
*Apanteles sp. Nr laevigatus (Ratzeburg)	Co	L
Bassus sublevis (Granger)	Cp, Es	L
*Bracon (Grabrobracon) sp.	Co, Cp	L
Bracon chinensis Szeplige´ti (Myosoma chinensis)	Cp	L
Bracon sesamiae Cameron	Bf, Cp, C. sp	
Chelous sp.	?	
Chelonus curvimaculutus Cameron	Co, Cp	E/L
Cotesia flavipes Cameron (Apanteles flavipes)	Cp	L
Cotesia ruficrus Haliday	?	L
Cotesia seamiae Cameron (Apanteles sesamia)	Co,Cp	L
*Dolichogenidae fuscivora Walker	Bf, Cp	L
Dolichogenidae polaszeki Walker	Bf, Cp	L

Euvipio rufa Szepligéti	Cp	L
Glytapanteles africanus Cameron (Apanteles africanus)	Cp	L
Glytapanteles maculitarsis Cameron (Apanteles maculitarsis)	Bf	?
Glyptomorpha spp	Co, Cp	L
Macrocentrus sesamivorus van Achterberg	Cp, Sc	L
*Meteorus sp.	Bf	L
Myosoma nyanzaensis Quicke and Wharton	Cp	L
Phanerotoma leucobasis Kriechbaumer	Bf	E/L
Rhaconotus sp.	Cp	L
Rhaconotus scirpophagae Wilkinson	Cp	L
Stenobracon rufus Szepligeti	Bf, Co, Cp, C. sp., Es, Sc, S. Sp	L
Chalcididae		
Anthrocephalus mitys Walker	Cp	P
*Brachimeria spp.	Co, C. sp, Bf, Cp Es	P
Psilochalcis soudanensis Steffan	Bf, Cp S. Es	P
Eulophidae		
Pediobius furvus Gahan	Bf, Cp. S. spp	P
*Tetrastichus sp. A	C. sp	?
Eurytomidae		
Eurytoma oryzivora? Delvare	Cp	P
Inchneumonidae		
Denticasmias busseolae Heinrich	Cp, Co, B.sp.	P
Pristomerus sp.	C.sp	?

Procerochasmias nigrimaculatus Cameron	Bf, Sc	P
Syzeutctus ruberrimus Benot	Co. Cp	L
Xanthopimpla sp.	Cp	P
Pteromalidae		
Norbanus sp.	Cp	L
Scelionidae		
*Telenomus busseolae Gahan	Bf	E
Telenomus sp.	Cp	E
Telenomus applanatus Bin and Johnson	Es	E
T. Nemesis Polaszek and Kimani	Co, C.Sp.	E
*Trichogramma sp.	Co, Cp, Sc	E
DIPTERA		
Chrolopidae		
*Polyodaspis sp.? robusta Lamp	Co, Cp	L
Muscidae		
*Antherigona sp.	C.sp	
Tachinidae		
Sturmiopsis parasitica Curran	Co	L

Bf= Busseola fusca

B.sp. = Busseola species

Co= Chilo orichalcociliellus

Cp= Chilo partellus

C. Sp= Chilo species

Es= Eldana saccharina

Sc= Sesamia calamistis

E= Egg

L= Larva

P= Pupa

?= Unkwon

*= incidental parasitoids or species of doubtful status

S.Sp. = Sesamia species