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STUDIES ON SPECIES DIVERSITY, HOST PREFERENCE, HOST
SUITABILITY, LONGEVITY AND FECUNDITY OF NATIVE EGG
PARASITOIDS OF STEMBORERS IN SEMI-ARID KENYA

BY

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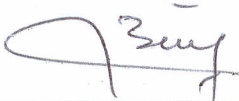


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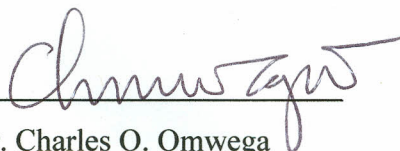


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DEDICATION

I dedicate this MSc. dissertation to my parents the late Mr. Francis Tom Okoth and the late Mrs. Judith Atieno Okoth for taking me to school.

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ABSTRACT

Infestation and damage by lepidopteran stemborers is one of the major causes of low maize yields in Kenya. Among the biological management options of stemborers, egg parasitoids are some of the potential agents that can be included in the integrated management strategy for the pests. Information on the species diversity and the role of egg parasitoids in stemborer control is lacking in the semi-arid eastern and central regions of Kenya. The aim of this study was to determine the egg parasitoid species diversity and to assess their contribution in enhancing lepidopteran stemborer eggs' mortality in semi-arid eastern and central regions of Kenya. This was first investigated through field surveys by collecting stemborer eggs from farmers' maize fields in Kitui and Thika districts. No egg batches were recovered from the plants in Kitui in eastern province and Thika in central province during the long rains, of 2003 unlike the short rains, 2003, where 9 egg batches of *Chilo partellus* and 19 batches of *Sesamia calamistis* were recovered from Thika district. Five out of the 19 egg batches of *S. calamistis* yielded *Telenomus busseolae* (Gahan). On-station studies were also conducted where maize plants were artificially infested with *Chilo partellus* and *Sesamia calamistis* eggs in order to determine egg parasitism in the eastern region. Egg parasitism ranged from 0.34 –12.54% in both Katumani and Kiboko. However, Kiboko had significantly higher parasitism levels than Katumani during both the long ($t=5.04$; $df=2209$; $P<0.0001$) and short rainy seasons of 2003 ($t=11.73$; $df=2021$; $P<0.0001$). The parasitoid species recovered from the stemborer' eggs in Kiboko were *T. busseolae*, *T. isis*, *Telenomus* spp., *Trichogramma* spp. and *Trichogrammatoidae* spp., while in Katumani, it was *Trichogramma* spp. The abundance of the parasitoids in descending order was *T. busseolae*, *T. isis* (Polaszek), *Trichogramma* spp., *Telenomus* spp., and *Trichogrammatoidae* (58.9, 17.81, 13.01, 9.59 and 0.68 % respectively) at Kiboko. If confirmed, this would be the first record of *T. isis* in East Africa. The suitability of different ages of stemborer eggs was determined using the dominant egg parasitoid, *T. busseolae*. Ages 1-3 days of both *B. fusca* and *S. calamistis* were found to be successfully parasitized and suitable for the development of *T. busseolae*. However, *T. busseolae* took a longer time to develop in older eggs of *S. calamistis*. A Y- tube experiment was used to compare the preference by *T. busseolae* for the pheromones produced by *B. fusca* and *S. calamistis* females, but no significant difference in choice for either of the stemborer species was observed ($\chi^2=1.667$; $df=1, 60$; $p=0.1967$). Similarly, in the field, *T. busseolae* females had non-preference for either *B. fusca* or *S. calamistis* ($\chi^2=0.72$; $df=1$; $P>0.05$) eggs for parasitization. In a study to determine the longevity and fecundity of *T. busseolae*, the females begun ovipositing on the first day of their emergence, and average daily oviposition rate decreased with the age of the female and was considerably higher on the first day than any subsequent day for both *B. fusca* and *S. calamistis*. The mean longevity and the mean fecundity of *T. busseolae* females on *B. fusca* and *S. calamistis* did not vary significantly. This study has generated baseline information on egg parasitoids, which can be used in the development of a biological management strategy for stemborers in the semi-arid Kenya.

CHAPTER 1: INTRODUCTION

Maize (*Zea mays*) is the main staple food in Kenya. However, on-farm yields have been generally low (ISAA, 2001). One of the major causes of these low yields is infestation and damage by lepidopteran stemborers (Seshu Reddy and Walker, 1990). Various methods have been used for the management of the stemborers and include host plant resistance (Seshu Reddy, 1998), chemicals (Bonhof *et al.*, 2001), cultural and biological control (Overholt, 1998).

Although chemical control methods have the advantage of being effective in controlling stemborers, there has been increasing concern about their impact on the environment, including the effect on non-target beneficial organisms, pollution of water and food poisoning (Bosque- Pérez and Schulthess, 1998). Use of insect resistant crop plants although favourable for use by farmers, has a disadvantage in that there is a possibility of evolution of resistant insect biotypes that would render the technology ineffective. Some cultural control practices such as destruction of crop residues have not been adopted because in most cases, farmers use the stalks for other purposes such as mulching and as fodder (Swaine, 1957).

Biological control is one of the favourable options for controlling stemborers, as it is safe to the environment, and once established, is self-sustaining. Biological control of lepidopteran stemborers in Kenya has mainly focused on the stemborer larvae through use of the exotic parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). *Cotesia flavipes* was introduced at the Kenyan Coast in 1993 and has since established (Omwega *et al.*, 1995). In spite of *C. flavipes* having a good searching ability, and being able to oviposit on *Busseola fusca* larvae (Fuller), it does not develop successfully on the larvae (Ngi-Song *et al.*, 1995) and it attacks the pest when damage has already started or occurred. On the other hand, parasitism of the

stemborer pupae has been too low to regulate and maintain the stemborer populations below economic damage levels (Songa *et al.*, 2002a). Egg parasitoids of cereal stemborers have also been reported in Kenya, although their contribution in the control of this pest has not been assessed.

Egg parasitoids are important because they kill the stemborers before they reach the larval stage, which is the damaging stage of these pests (Wajnberg and Hassan, 1994). Despite the fact that parasitism by indigenous stemborer parasitoids is low, they are preferred because they are adapted to the environment and host conditions in contrast to exotic species (Smith, 1996), and should therefore be integrated with other methods of control. Furthermore, the national regulatory agencies are reluctant to licence the importation of exotic organisms (Wajnberg, 1994).

Apart from being environmentally safe and effective, biological control through the use of egg parasitoids has not been extensively used in the management of stemborers in Kenya and East Africa as a whole. This is because information on the species diversity, host preference, host suitability, longevity and fecundity of the potential egg parasitoids is lacking. In order to design a multistage biological control programme in which egg parasitoids constitute an integral part of the control agents, it is important to generate the above stated information.

1.1 Problem statement

Human population growth in Kenya like the other countries in sub-Saharan Africa is estimated to rise by 3.0–3.5% per annum over the next twenty years (Mugo *et al.*, 2002). The projected population increase will be reflected on the increased demand for the already limited main food resource, maize. However, maize

production in Kenya has not been able to cope with the ever-increasing human demand. Damages and losses associated with stemborer infestations are among the causes of low maize production. Typically, these losses range between 11 and 45% of the potential maize yield (Seshu Reddy and Walker, 1990; CIMMYT, 2004).

In Kenya, attempts to use biological control agents to reduce stemborer damages are yet to provide sustainable control. Partly, this may be attributed to over reliance on larval parasitoids, which have a delayed effect as they attack the larvae already causing damage to the plants (Kfir and Bell, 1993). Because of this limitation, it is anticipated that increasing the egg stage mortality of maize stemborers (through the use of egg parasitoids) will have a great potential of reducing damages associated with stemborers. Consequently, information on longevity, progeny production and development time of the selected candidates, as well as host suitability are of great importance for such pest management intervention. In order to use egg parasitoids effectively, this study aimed at determining the diversity of indigenous parasitoids species, the suitability of indigenous stemborer species, the longevity and fecundity of the parasitoids.

1.2 Hypotheses of study

- i) The species diversity of egg parasitoids of maize stemborers in eastern and central Kenya is the same.
- ii) The species diversity, relative abundance and parasitism levels between upper midland zone 4 and lower midland zone 5 Agroecological zones (AEZ's) in semi-arid eastern Kenya is the same.
- iii) The age of stemborer eggs does not affect their suitability to their parasitoids.

- iv) The longevity and fecundity of egg parasitoids is not affected by stemborer host species.

1.3 OBJECTIVES OF THE STUDY

1.3.1 General Objective

To determine the species diversity of parasitoids of stemborer eggs, and assess their net contribution to egg mortality of lepidopteran stemborers of maize in semi-arid eastern and central regions of Kenya.

1.3.2 Specific Objectives

- 1 To assess the species diversity of native egg parasitoids of stemborers in maize, and their performance in semi-arid eastern and central Kenya.
- 2 To determine egg parasitoids species diversity, relative abundance and parasitism levels in two semi-arid Agroecological zones (AEZ's) in eastern Kenya.
- 3 To compare the host suitability and preference of two stemborer species to the dominant egg parasitoid.
- 4 To determine the longevity and fecundity of the dominant egg parasitoid females.

CHAPTER 2: LITERATURE REVIEW

2.1 Maize production and its economic importance

Maize originated in Central America and was introduced into Africa by the Portuguese in the 16th century. Maize is the most important cereal in sub-Saharan Africa, as its production and consumption exceeds that of other cereals such as wheat or sorghum (ISAA, 2001); and shortage of maize is synonymous to famine in the region (ISAA, 2001; CIMMYT, 2004). Per capita annual consumption levels are estimated at 79 kilograms in the eastern and southern African regions and 125 kilograms in Kenya (CIMMYT, 2004).

Despite its importance, maize production in Kenya is quite low, averaging 1,385 kg/ha, compared to a world average of 4,230 Kg/ha (FAO, 2000). This is mainly due to various factors including low and erratic rainfall (FAO, 2003), low soil fertility, pests and diseases (ISAA, 2001). Lepidopteran stemborers are the most important insect pests of maize in Kenya (Seshu Reddy and Walker, 1990; Songa *et al.*, 2001).

2.2 Lepidopteran stemborers of maize

In Kenya, the most important species of stemborers are *Chilo partellus* (Swinhoe) (Crambidae), found in the warm lowland areas of the coast (Warui and Kuria, 1983) and in the semi-arid eastern part of the country (Songa *et al.*, 2002a), while *Busseola fusca* Fuller (Noctuidae) is found in the cooler and higher altitude areas (Harris and Nwanze, 1992). *Sesamia calamistis* (Noctuidae), although considered less important, dominates elevations of up to 2600m (Tams and Bowden, 1953). While *Chilo orichalcociliellus* (Crambidae) is limited to the coastal lowland areas (Overholt *et al.*, 2001).

Ofomata *et al.* (1999a) postulated that *C. Partellus* has largely displaced *C. orichalcociliellus* in the Southern coast of Kenya. *Sesamia cretica* Ledere (*Noctuidae*) was reported in the extreme northern part of the country (Nye, 1960), while *Eldana saccharina* (Walker) (*Pyralidae*), a serious pest of sugar cane in southern Africa (Atkinson, 1980), is also found along the sugar cane belt in Kenya (Maes, 1998).

2.2.1 Biology of lepidopteran stemborers

The female adult moths mate soon after emergence from pupae after which they oviposit eggs on the leaf blades in case of *C. partellus* and *E. saccharrina*, sheaths in case of *B. fusca* and *S. calamistis*, or even next to the midribs of maize plants in the case of *C. partellus* (Cheng, 1994; Overholt *et al.*, 2001). Eggs hatch within 4 - 7 days and the newly emerged neonates enter the leaf whorls, bore through the whorl base, and enter the stems where they feed for a period ranging from two weeks to two months chewing an exit hole just before they pupate (Kuniata, 1994; Overholt *et al.*, 2001). In some species like *C. partellus*, *C. orichalcociliellus* and *B. fusca*, larvae may enter a resting stage known as diapause (Ofomata *et al.*, 1999b). Pupation lasts between 10-20 days, before the adults emerge. It is the stemborer larvae that cause damage to crop plants (Overholt *et al.*, 2001).

2.2.2 Damage caused by lepidopteran stemborers

Stemborers damage crops by initially feeding on the leaf tissues, and then proceed to feed and tunnel within the stem, and occasionally on the maize cobs (Swaine, 1957; Seshu Reddy, 1998). Pinholes and windowpanes are salient features of stemborer feeding on leaves (Overholt *et al.*, 2001). Sometimes, stemborers bore directly from the whorls into the stem resulting in death of the central shoot, a condition referred to as 'dead heart' According to Kfir (1998), stem tunnelling by the

larvae weakens the stem and interferes with the translocation of nutrients and metabolites in the plants resulting in malformation of grains, stem breakage and stunted growth. Prior to pupation, the late instar larvae chew an exit hole, offering entry points for bacteria and fungal pathogens (Overholt *et al.*, 2001). In the reproductive stage, feeding may be concentrated in the cobs and tassels (Seshu Reddy, 1998).

Every year, stemborers affect 87% of maize plantations in Kenya, leading to yield losses in the range of 11-45%. (CIMMYT, 2004). It has been reported that, maize grain yields can be reduced by about 30% of the potential yield by an average stemborer density of about 2.25 larvae per plant (Songa *et al.*, 2001). These losses are considerable given that maize must be imported to meet shortfall in domestic demand in Kenya and for individual farmer this destruction is significant since many people live on less than US \$1 per day (CIMMYT, 2004).

2.3 Management of stemborers

Various methods have been used to manage stemborers, with varying degrees of success (van den Berg and Ebenebe, 2001), and they include, host plant resistance, cultural control, chemical control and biological control.

2.3.1 Host plant resistance

In the broadest sense, plant resistance is defined as "the consequence of heritable plant qualities that result in a plant being relatively less damaged than a plant without the qualities" whereas in practical agricultural terms, an insect-resistant crop cultivar is one that yields more than a susceptible cultivar when confronted with insect pest invasion (Teetes, 1996). Resistance of plants is relative and is based on comparison with plants lacking the resistance characters, i.e., susceptible plants

(Eigenbrode and Trumble, 1994). Traits conferring host plant resistance (HPR) to insects and pathogens are among the most important for crop improvement and insect resistance has also been introduced into several hundreds of crop varieties during the last 20 years (Smith, 1989).

Host plant resistance is one of the most important and promising ways of reducing stemborer damage and subsequent yield losses in target crops, either through resistance to initial attack, antibiosis once attack has occurred or tolerance to attack (Seshu Reddy, 1998). Research at the International Center of Insect Physiology and Ecology (ICIPE) on the evaluation of maize and sorghum lines showed good levels of resistance/ tolerance to stemborer attack (Ampofo *et al.*, 1986). Seshu Reddy (1985) identified various varieties of sorghum as sources of sorghum resistance to the stemborer complex in East Africa. Currently there is on going work to breed maize resistant to stem borers by CIMMYT (International Maize and Wheat Improvement Centre) in collaboration with Kenya Agricultural Research Institute (KARI).

2.3.2 Cultural control

Cultural control is defined as the use of regular farm management practices, which are specifically designed to destroy or prevent pests from causing damage to crops (Omolo and Seshu Reddy, 1985). Most of these practices are labour intensive, and are mostly employed where labour surpluses exist. According to Kumar (1984), cultural control methods represent some of the oldest crop protection strategies in traditional agriculture. Their use seems to be unspectacular and generally little appreciated, but is nevertheless dependable and the partial control they offer, contributes reduction on the dependence on chemical pesticides.

In stemborer management, cultural practices are aimed at disrupting or slowing down the population build-up of the stemborers (Seshu Reddy, 1998). Some workers have recommended complete destruction of stalks by burning after harvesting (Ajayi, 1978). However, farmers do not commonly practice this method as the stalks are put into other uses such as mulching and building (Swaine, 1957). Early planting as a means of cultural control plays a significant role in lowering the number of borers per plant (Warui & Kuria, 1983). Soil tillage reduces stemborers populations through mechanical damage either by burying them deeply or by exposing them to harsh weather conditions (Seshu Reddy, 1998). Intercropping sorghum with a non-host plant has been reported to reduce damage by *C. partellus* in sorghum (Omolo and Seshu Reddy, 1985).

2.3.3 Chemical control

Chemical control is the reduction of insect populations or prevention of insect injury by the use of materials to poison them. The control of stemborers using chemicals is more difficult as they feed in sheltered areas (Leslie, 1993), and most of their life cycle is spent within plant tissues that cannot be reached by contact insecticides (Jotwani, 1983). However, chemicals reduce stemborers populations if applied at the correct time, before the larvae bore into the stems (Warui & Kuria, 1983). Chemicals that have been used to control stemborers in Kenya include Endosulfan (Thiodan), Coumaric acid (Dipterix), Permethrin (Ambush) and beta-Cyfluthrin (Bulldock) (Bonhof *et al.*, 2001). The majority of resource poor peasant farmers in Africa cannot afford to buy insecticides, and furthermore, these insecticides are seldom available on time and usually require application equipment and knowledge, which is inadequately available to the farmers (Bosque- Pérez and

Schulthess, 1998; Bonhof *et al.*, 2001). In addition, insecticides often have potential negative impacts on the environment, are a health hazard to humans and are not necessarily compatible with other control methods such as natural enemies (Bosque-Pérez and Schulthess, 1998).

2.3.4 Biological control

Biological control involves the importation, augmentation and conservation of beneficial organisms such as parasitoids, predators and pathogens for the regulation of population densities of other organisms (van Driesch and Bellows, 1996). Classical biological control involves the importation and establishment of an exotic natural enemy into a new environment for the management of its co-evolved host (pest) (which may have been accidentally introduced into the new environment) (Knutson, 1998). There have been various cases of the use of classical biological control for the management of introduced pests in Africa. For example, natural enemies have been imported and released in West Africa (Scheibelreiter, 1980), South Africa (Carnegie and Leslie, 1979) and East Africa (Overholt, 1998) for the management of various crop pests. Augmentation of natural enemies is the action taken to increase the populations or beneficial effects of natural enemies, while conservation of natural enemies refers to actions deliberately taken to protect and maintain populations of natural enemies (van Driesch and Bellows, 1996; Knutson 1998).

Natural enemies play an important role in regulating the populations of lepidopterous stemborers in Africa (Polaszek, 1992). Predators of stemborers such as earwigs, ladybird beetles, ants and several spider species have been reported to regulate stemborers populations in Kenya (Dwumfour, 1990; Bonhof *et al.*, 2001). Parasitoids of stemborers pupae, larvae and eggs have also been reported in various parts of Kenya (Oloo and Ogeda, 1990; Ogol *et al.*, 1998; Songa *et al.*, 2002a, 2002b).

2.3.4. 1 Species diversity of lepidopteran stemborer parasitoids

2.3.4.1.1 Pupal parasitoids

The indigenous pupal parasitoids reported in Kenya include *Pediobius furvus* (Gahan) (Hymenoptera: Eulophidae), *Dentichasmias busseolae* (Heinrich) (Hymenoptera: Ichneumonidae), *Psilochalsis soudanensis* (Steffan) (Hymenoptera: Chalcididae), *Dolichogenidia polazeki* Walker (Hymenoptera: Braconidae) and *Procerachasmias nigromaculatus* (Cameron) (Hymenoptera: Ichneumonidae) (Oloo and Ogeda, 1990; Bonhof *et al.*, 1997; Songa *et al.*, 2002a). However, according to Songa *et al.* (2002a) parasitism by these indigenous parasitoids has been generally low. An exotic parasitoid, *Xanthopimpla stemmator* (Thunberg) (Hymenoptera: Ichneumonidae) was imported into Kenya by ICIPE from Sri-Lanka via South Africa for laboratory trials and laboratory host range bioassays at ICIPE, Kenya. The findings of the studies indicated that *X. stemmator* can attack and complete development in all the major stemborer species in Eastern and Southern Africa (Gitau, 2002).

2.3.4.1.2 Larval parasitoids

Indigenous larval parasitoids in Kenya include *Cotesia sesamiae* Cameron and *Chelonus curvimaculatus* Cameron (Hymenoptera: Braconidae) (Songa *et al.*, 2002a). The exotic larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was also introduced in Kenya in the early 1990s, and has since established (Omwega *et al.*, 1995; Overholt, 1998). Although a wide range of larval parasitoids attack stemborer larvae, percentage parasitism is seldom higher than 10% (Oloo, 1989;

Skovgård and Päts, 1996; Songa *et al.*, 2002a) and is usually too low to keep the stemborer populations below economic damage levels (Oloo and Ogeda, 1990).

2.3.4.1.3 Egg parasitoids

Most of the stemborer egg parasitoids reported in Africa belong to the families of Trichogrammatidae and Scelionidae (Polaszek, 1998). According to Bonhof *et al.* (1997) Hymenopteran parasitoids of the genus *Trichogramma* (Trichogrammatidae) and *Telenomus* (Scelionidae) are the most abundant and widespread egg parasitoids of cereal stemborers in East Africa. Several species of the Trichogrammatidae have been reported attacking lepidopteran stemborers in Kenya, and these include *Trichogramma* sp. nr. *exiguum* Pinto and Planter on *C. Partellus* (Ochiel, 1989), *Trichogramma* sp. nr. *mwanzai* Schulten and Feijen on *C. partellus* (Ngi-Song, 1990) and *Trichogramma bournieri* on *C. partellus* (Abera, 2001).

Scelionidae is a large family with approximately 95 genera in the world, whose members are commonly encountered in a variety of habitats. All scelionids attack eggs, and the vast majority are solitary parasites. The family is broken down into three subfamilies: Scelioninae, Telenominae, and Teleasinae (Midwest Institute for Biological Control, 2000). The *Telenomus* species reported on lepidopteran stemborers in Kenya include *Telenomus bini* Polaszek & Kimani, *Telenomus busseolae* Gahan, *Telenomus nemesis* Polaszek & Kimani, *Telenomus thestor* Nixon and *Telenomus ullyetti* Nixon (Polaszek and Kimani, 1998).

2.4 *Telenomus* species

2.4.1 Distribution and host range of *Telenomus* species

Telenomus species belong to the sub-family Telenominae. Polaszek and Kimani (1998) identified and listed the host range and distribution of ten species of *Telenomus* as primary parasitoids of the eggs of African cereal stemborers (Table 1).

2.4.2 Biology of *Telenomus* species

The parasitoids of the genus *Telenomus* are solitary with proovigenic egg production, having no measurable preoviposition period (Dass and Parshad, 1983). Marking of hosts by *Telenomus* has been reported in many species (Strand and Vinson, 1983); after oviposition, the females rub or scratch the host chorion with the ovipositor (Higuchi and Suzuki, 1996). The eggs of Telenominae hatch less than 20 hours after deposition, the first instar has well-developed mandibles and is very mobile (Navasero and Oatman, 1989). The larval period varies from 4 days at 30⁰C (Hernández and Díaz, 1996) to 7 days at 15.5⁰C (Gómez de Picho, 1987). Pupation, which occurs within the host egg, varies from 4.6 days at 30⁰C (Hernández and Díaz, 1996) to 15 days at 15.5⁰C (Gómez de Picho, 1987).

The total developmental time from egg deposition to adult emergence varies from 13.7 days at 23⁰C to 7 days at 34⁰C with ambient humidity having no influence on developmental rate. When the development of immature *Telenomus* is complete, the adult chews a small exit hole in the host chorion, through which it emerges. In general, males emerge 24 hours before females and remain on the egg mass from which they emerged or search for other egg masses with parasitoid hosts to await the emergence of females (Schwartz and Gerling, 1974). Sex ratios are usually female biased when larger egg masses are attacked by a single mated female of *T. busseolae*

(Bayram *et al.*, 2004). Chabi - Olaye *et al.* (1997) reported the longevity of *T. busseolae* to be 11.0 days and 21.7 days at 30°C and 20°C respectively. Copulation occurs immediately or soon after the female emerges from her host egg (Cave, 2000).

2.4.3 Population dynamics of *Telenomus* species

Ndemah *et al.* (2002) observed higher egg parasitism by *Telenomus* species on maize surrounded by *Pennisetum purpureum* L. (80%) compared to maize monocrop plots (58%) in Cameroon. Higher egg parasitism corresponding to higher egg numbers was also reported in the same study. According to Ndemah *et al.* (2003), egg parasitism by the *Telenomus* species is higher in the second than in the first rainy season of the year and was attributed to obligate diapausing in *B. fusca* and scarcity of alternative host plants, which may harbour non-diapausing insect hosts such as *S. calamistis* in Cameroon. Schulthess *et al.* (2001) observed in southern Benin, clear seasonal trends in egg parasitism by *T. busseolae* (quadratic) and *T. isis* (quadratic and cubic) trends and a steady decline in parasitism towards the dry season.

Table 1. Distribution and hosts of *Telenomus* species that parasitize eggs of African cereal stemborers (Polaszek and Kimani 1998).

Species	Host range	Distribution
<i>Telenomus applantus</i> Bin & Johnson	<i>E. saccharina</i>	Gabon, Ghana, Ivory Coast and Nigeria
<i>Telenomus bini</i> Polaszek & Kimani	Pyralidae & Crambidae	Ghana, Ivory Coast, Malawi, Senegal, Tanzania & Kenya
<i>Telenomus creusa</i> Polaszek & Kimani	Crambidae	Malawi
<i>Telenomus nemesis</i> Polaszek & Kimani	Crambidae	Ghana, Mozambique, Senegal & Kenya
<i>Telenomus nephele</i> Nixon	Crambidae	Cameroon, Ghana, Ivory Coast, Malawi, Mali & Senegal
<i>Telenomus soudanensis</i> (Risbec)	Crambidae & Pyralidae	Senegal & Mali
<i>Telenomus thestor</i> Nixon	Arctiidae & Crambidae	Ivory Coast, Senegal, DRC, Uganda & Kenya
<i>Telenomus versicolor</i> Bin & Johnson	Crambidae	Ghana, Ivory Coast, Malawi & Senegal
<i>Telenomus isis</i> Polaszek	Noctuidae	Benin, Togo, Cameroon
<i>Telenomus busseolae</i> Gahan	Noctuidae, Pyralidae	Ubiquitous in Africa

CHAPTER 3: MATERIAL AND METHODS

3.1 Selection of study sites

Due to population pressure, many households in Kenya have been forced to move to land with lower yield potential, most of which fall in the transitional and semi-arid zones that are less suitable for maize growing (FEWS, 1996). The semi-arid and arid areas represent over 80% of Kenya's land mass and 50% of the country's arable land (Jaetzold and Schimidt, 1983). Maize continues to be the dominant crop in these areas (FEWS, 1996). Three of the agroecological zones (AEZ's) in the semi-arid region of eastern Kenya, including the lower midland zone five (LM5), lower midland zone four (LM 4) and the upper midland zone four (UM 4), and which cover the areas of Kiboko, Kitui and Katumani respectively, constitute a predominant part of arable land in semi- arid eastern Kenya. These sites were therefore selected for the present field study. In addition, Thika district was chosen because it is also semi arid but is located at a higher altitude where some species of stemborers such as *B. fusca*, that do not occur in lower semi arid eastern Kenya is found.

3.2 Description of the study sites

Surveys to collect stemborer eggs from maize plants in farmers' fields were conducted in two districts: Thika (Central Province) and Kitui (Eastern Province). On-station trials to determine species diversity, relative abundance and parasitism levels in two semi arid Agroecological zones were conducted at the KARI-Katumani field station at; latitude S $01^{\circ} 34.940'$; longitude E $037^{\circ} 14.293'$ and altitude 1490 m. and Kiboko at; latitude S $02^{\circ} 13.095'$; longitude E $037^{\circ} 42.947'$ and altitude 830 m (Figure 1). Laboratory experiments on host preference, host suitability, longevity and fecundity were conducted at the KARI -Katumani and the ICIPE laboratories.

Katumani field station is in Machakos district within the Eastern province of Kenya and falls under the upper midland zone 4 (UM4) Agro-Climatic zone (Appendix 3) (Jaetzold and Schmidt, 1983). It has a bimodal rainfall pattern, with a short dry period dividing the cropping season into a long and short rainy season. The long rainy season starts in April and ends in August while the short rainy season starts in October and ends in January. The rainfall for Katumani was 246 mm and 221.2 mm, for the long and short seasons respectively (Appendix 3 and 4), and the mean temperature was 19.5 and 19.6 °C for the long and short seasons respectively (Appendix 3 and 4). The soil is a well-drained, dark reddish-brown sandy clay, classified as oxic paleustalf (chromic luvisol) (Barbar and Thomas, 1979). The annual cereal crops that are commonly grown in this station include maize, sorghum, and pearl millet, and are cultivated during both seasons. There are no annual crops in the field during the short dry spell separating the two seasons. Perennial crops grown include cassava, mangoes and sweet potatoes.

The Kiboko field station is in Makueni district of the Eastern province and falls under the lower midland zone 5 (LM5) Agro-Climatic zone (Appendix 3) (Jaetzold and Schmidt, 1983). The long rainy season starts in April and ends in August while the short rainy season starts in October and ends in January. Kiboko received of 90.5 mm and 403 mm of rainfall during long and short rains respectively in the course of the study period. The mean temperature was 25.5 and 24.6°C during the long and short rains (Appendix 3 and 4). Kiboko has irrigation facilities and hence allows continuous cropping of cereals and other crops. The annual crops that are commonly grown in this field station include maize, sorghum and pearl millet, while the perennial crops include pigeon peas and bananas.

3.3 Insect cultures

Colonies of *T. busseolae* were established using individuals collected from egg batches of *S. calamistis* in maize fields at Kiboko. The parasitoids were maintained on *S. calamistis* eggs in transparent glass vials of 2.3 cm diameter and 7.3 cm length and were provided with fresh 20 % honey droplets streaked on the inside surface daily (Agboka *et al.*, 2002). Parasitoid colonies were maintained both at the ICIPE and Katumani laboratories in rearing rooms under a photoperiod of L12; D12 and controlled conditions of $27\pm 1^{\circ}\text{C}$, 65-70% relative humidity (RH). The parasitoids were left for 12-24 hours to allow for mating before experiments were set.

The parasitoids were separated individually into small preservation bottles and then placed in an incubator at 15°C to slow down their movement, before observing them under the microscope at 40 magnification using medium light. The male and females of *T. busseolae* were easily distinguished based on sexual dimorphism shown by their antennal structures, which are filiform in males and clubbed in females (Bayram *et al.*, 2004). A few individuals from the colonies were reidentified at each generation using Polaszek and Kimani (1998) keys to make sure that there was no contamination of the colony. The stemborers eggs used in the studies were obtained from KARI Katumani and ICIPE Animal Rearing and Containment Unit. The maize variety used for the on-station and laboratory studies was the drought resistant Katumani composite B.

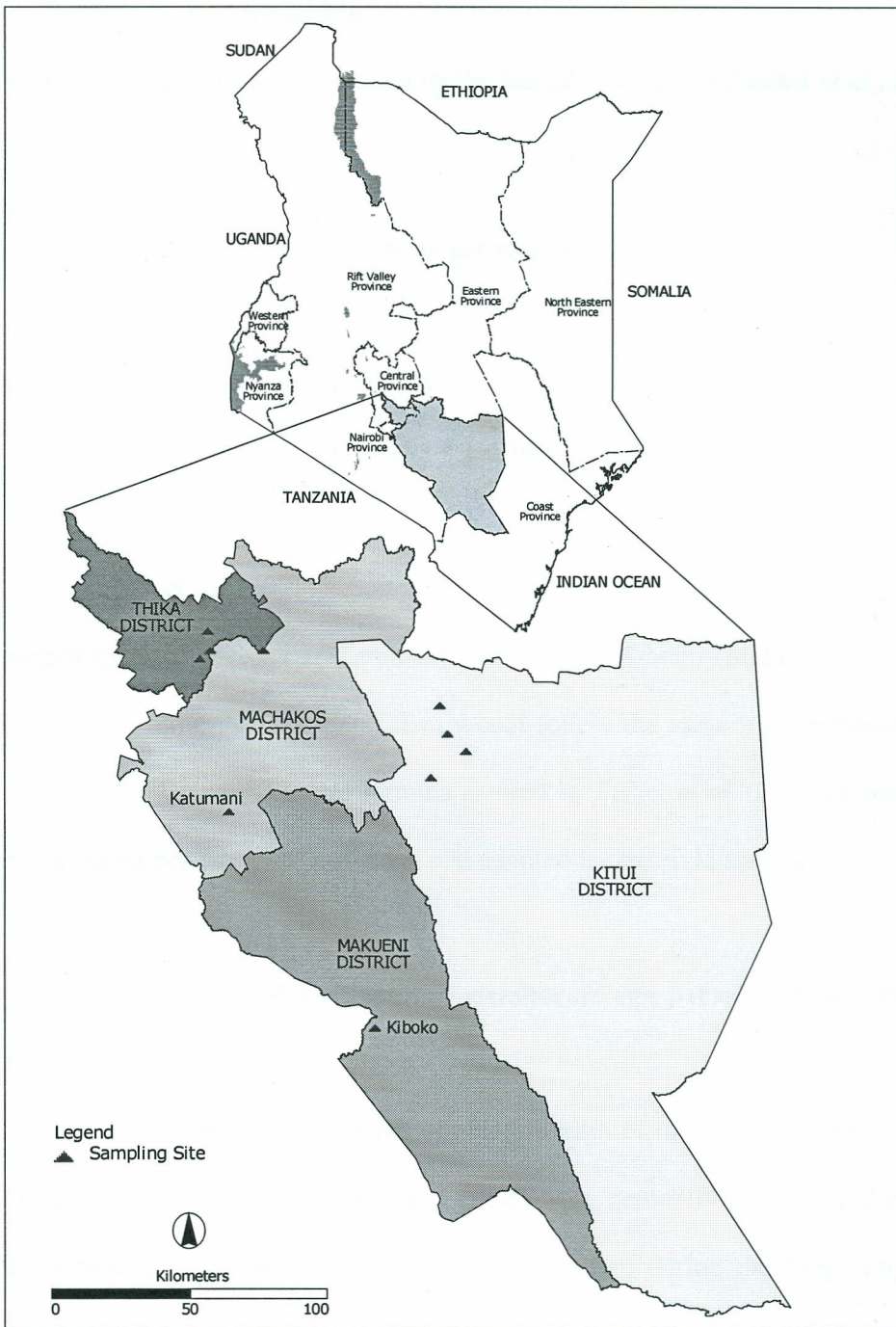


Figure 1. Map of Kenya Showing the Sampling Sites

3.4 Sample size determination

The sample size (n) was determined by the formula as used by Fischer *et al.*, (1998).

$$n = \frac{Z^2 pq D}{d^2}$$

Where: n = sample size
 p = Proportion of the target area (0.07)
 $q = 1-p$ (0.93)
 $Z = 1.96$
 $d : \alpha = 0.05$
 D = Design effect (=1)

$$n = \frac{1.96^2 * 0.07 * 0.93 * 1}{0.05^2} = 100$$

Songa *et al.* (2001) reported mean parasitism of 7 % on *C. partellus* by *C. flavipes* in three seasons at Katumani (Machakos), Kiboko (Makueni) and Ithokwe (Kitui). Assuming that egg parasitism would follow the same trend p becomes 0.07 and $q = 0.93$. Using the above formula as used by Fisher *et al.* (1998), a sample size of 100 plants per farm was arrived at and adopted for the field studies.

3.5 Species diversity of lepidopteran stemborers egg parasitoids in eastern and central Kenya

Fifteen farmers' fields were sampled in each of the districts; Thika and Kitui (Fig 1). A total of 100 plants per farm (field) were randomly sampled and examined for stemborer eggs once (at the 6th – 8th leaf stage) during the long rainy season (Appendix 1). However, during the short rains, the number of farmers was reduced to four in each district, and the sampling intensity increased. Four fields, each of approximately 0.25ha, with one-week old maize seedlings were selected in both Thika and Kitui (Appendix 2). Sampling started at one week after emergence (WAE) and was repeated once per week for four consecutive weeks. Each field was partitioned into four quadrats for sampling purposes. The crops cultivated by each farmer were

recorded in both seasons (Appendix 6-8). In each quadrat, the leaves and leaf sheaths of 50 randomly selected maize plants were carefully inspected for stemborer egg batches thus 200 maize plants were examined per farm. The egg batches were collected after cutting a piece of leaf or sheath containing the batch. The egg batches from each plant were placed in a glass vial, plugged with cotton wool, labeled and incubated in the insectary at $27\pm 1^{\circ}\text{C}$ and 65-70% RH until hatching of neonates or emergence of parasitoid. The hatched neonates were reared on artificial diet up to adult stage and taken to ICIPE for identification. Eggs that failed to develop were discarded after 3 weeks. Emerged parasitoids were preserved in 70% ethanol and taken to ICIPE, Nairobi for identification.

3.6 Determination of egg parasitoids species diversity, relative abundance and parasitism levels in two semi-arid Agroecological zones

Three plots measuring 20 m. x 10 m. each were established at the KARI field stations: Katumani and Kiboko, during the long and short rains of 2003. The distance between the plots was 2m. Maize was planted at a spacing of 90 cm between and 30 cm within the rows, at an interval such that the first plot was three weeks older than the second, and the second plot 3 weeks older than the third during the long rains, and 2 weeks during the short rainy season between the succeeding plots. All the recommended agronomic practices were adhered to, but no chemical insecticides were applied to the maize plants. Each plot was divided into four quadrats, and at the sixth leaf stage, one batch of 20-25 eggs of *C. partellus* and *S. calamistis* were attached to each of 25 randomly selected plants per quadrat respectively. Egg batches of *C. partellus* were stapled on the leaf blades (Plate 1) while those of *S. calamistis* were inserted in the leaf sheath (plate 2) (Overholt *et al.*, 2001). Plants infested with

eggs of *C. partellus* were identified with square pale yellowish tags, while those infested with *S. calamistis* eggs were identified with triangular pale yellowish tags attached on the leaves.

After two days, the egg batches were collected, counted and placed individually in a glass vial, plugged with cotton wool, labeled, and incubated separately in the insectary at $27\pm 1^{\circ}\text{C}$ and relative humidity (RH) of 65-70%, until borer or parasitoid emergence. After 3 weeks, the eggs that failed to develop were discarded. The plots were re-infested at the eighth leaf stage using the same procedure. A colony of the dominant egg parasitoid was established using the favourable host stemborer species, and these were used for the laboratory studies on longevity, fecundity, host suitability and host preference at ICIPE laboratories. Data on temperature and rainfall was recorded daily during the study period. *Telenomus busseolae* was found to be the dominant parasitoid species in Kiboko field site (Table 3). Since *T. busseolae*, is a parasitoid of the noctuids (Polaszek, 1998), it was therefore prudent to carry out further laboratory studies to determine the suitability, host preference, longevity and fecundity of *T. busseolae* females on two noctuid stemborers *B. fusca* and *S. calamistis*, which are major pests of maize in Kenya and specifically in the moist mid-cool and dry high tropics.

3.7 Host suitability and host preference studies

3.7.1 Host suitability

3.7.1.1 Determination of post-ovipositional exposure time

It was necessary to determine the length of time in which a parasitoid should



Plate 1. Infesting *Chilo partellus* on the leaf blade of maize

3.7.1.2 Effects of host age on parasitoid *Stenobothrus*

The suitability of 1-day, 2-day and 3-day old eggs of *S. furra* and *S. caloricus*



Plate 2. Infesting *Sesamia calamistis* in a leaf sheath of maize

3.7 Host suitability and Host preference studies

3.7.1 Host suitability

3.7.1.1 Determination of parasitoid exposure time

It was necessary to determine the length of time in which a parasitoid should be exposed to host eggs in order to produce the highest progeny. Hence, one female of the native *T. busseolae* recovered from Kiboko was released in a glass vial containing 100 ± 5 eggs of *S. calamistis* for six different randomly selected time periods: 1, 4, 8, 12, 16, and 24 hours respectively, and replicated 10 times. After each respective time period, the eggs were removed and placed in clean vials, and then incubated at $27 \pm 1^{\circ}\text{C}$ and RH of 65-70%, for parasitoid emergence. After emergence, the progeny produced was counted and averaged for each time regime and the parasitoids killed in absolute ethanol and discarded.

3.7.1.2 Effects of host age on parasitoid fitness parameters

The suitability of 1-day, 2-day and 3-day old eggs of *B. fusca* and *S. calamistis* to *T. busseolae* was determined by the effects of these host ages on the parasitoid's fitness parameters. The parasitoid fitness parameters used were; the progeny it produced, the time it takes to develop in a host and the proportion of females it produces on the host (Jeffrey *et al.*, 2004). This was achieved by releasing a single 12-24 hours old *T. busseolae* female, separately in glass vials containing 1-day, 2-day and 3-day old batch of 100 ± 5 eggs of *S. calamistis* and *B. fusca*, and then plugging them with cotton wool. The experiment was conducted at the Katumani laboratories and the treatments were replicated 30 times. After 24 hours the eggs were removed, placed in a clean vial, and incubated at $27 \pm 1^{\circ}\text{C}$ and RH of 65-70% until parasitoid emergence. The development time was taken to be the number of days the parasitoids

took to emerge from parasitized eggs; progeny production was the total number of parasitoids emerging from the parasitized eggs and female sex ratio was the proportion of females from the total progeny produced.

3.7.2 Host preference studies

Two experiments were conducted in order to determine host preference by the dominant parasitoid species. These were carried out in a Y-tube olfactometer (Plate 3) and in the field.

3.7.2.1 Olfactometer choice experiment

Maize plants were planted singly in pots in a screen house. At the 6th leaf stage, each plant was exposed in a cage for oviposition to either a single female *S. calamistis* or *B. fusca*. Ten plants were used per borer species. Before commencement of the study, the bias of location of an olfactometer arm was tested using empty chambers. For the preference test, the odour sources were a leaf sheath containing either a *S. calamistis* or *B. fusca* egg batch. The room was illuminated by a red light (Fiaboé *et al.* 2003). For each set, five *T. busseolae* females were tested. A -day old female, at a time, was introduced into the Y-tube olfactometer using a camel hairbrush. After each run, the olfactometer was cleaned with water and an odourless soap and the positions of the odor source switched. A parasitoid was considered to have made a choice when it crossed a 2 cm mark and stayed for 15 seconds in one of the arms of the olfactometer.

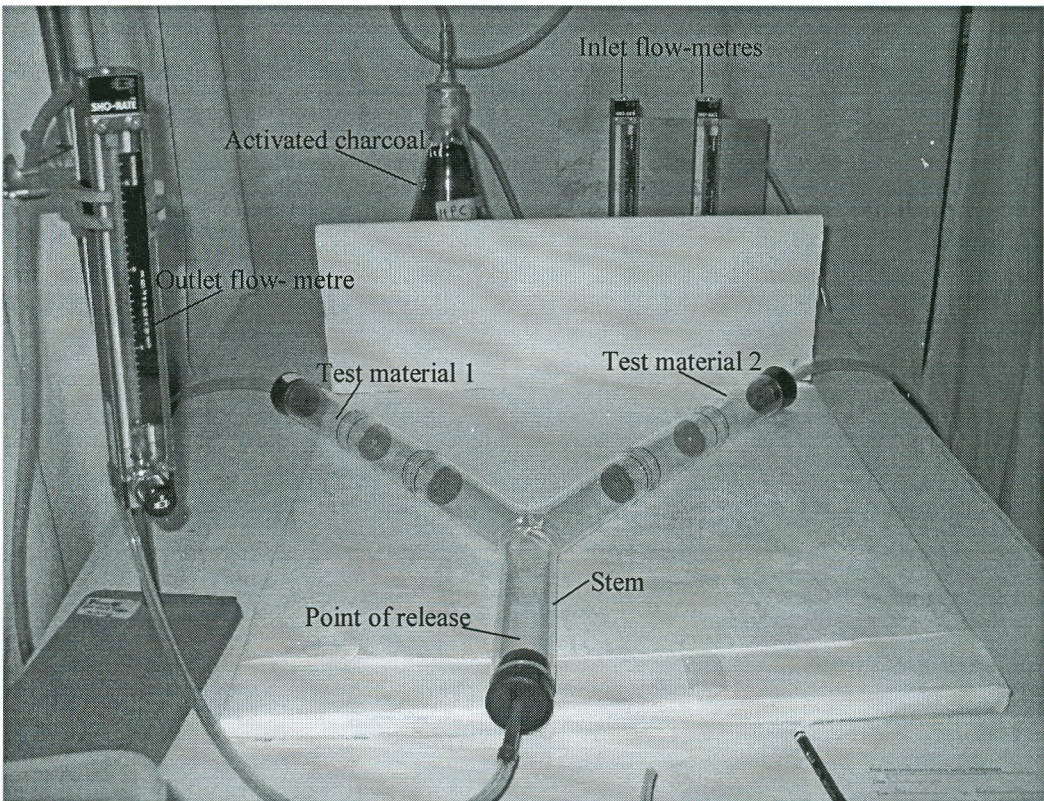


Plate 3. Y-tube olfactometer

3.7.2.2 Field experiment

Three Plots of maize each measuring 20 m x 10 m were established at the Kiboko field station during the short rainy season of 2003. The distance between the plots was 2m. Maize was planted at a spacing of 90 cm between and 30 cm within the rows. At the sixth- leaf stage, 100 plants were randomly infested with freshly laid egg batches (20 ± 5 egg/batch) of *S. calamistis* eggs or *B. fusca* eggs per plot. In both cases, the eggs were inserted in the leaf sheaths. The plants infested with *S. calamistis* eggs were tagged on the leaves with pale yellowish triangular tags, while the plants infested with *B. fusca* eggs were tagged with pale yellowish square tags. After two days, the eggs were collected from the field and incubated in clean glass vials at $27 \pm 1^{\circ}\text{C}$ and a RH of 65-70% for three weeks, until neonate or adult parasitoid

emergence. The egg batches of each stemborer species parasitized by *T. busseolae* was recorded.

3.8 Fecundity and Longevity of female adults parasitoids

Thirty females of *T. busseolae* were selected 12-24 hours after emergence, placed in a clean glass vials individually and provided with 100 ± 5 freshly laid eggs of either *S. calamistis* or *B. fusca* after every 24 hours until the parasitoid died. For each female parasitoid, the host eggs exposed on each day were placed separately in clean vials on the following day and then incubated at $27 \pm 1^{\circ}\text{C}$ and a RH of 65-70%. The mortality of the parasitoids was recorded daily until they all died. Progeny production was estimated as the number of parasitoids emerging from the parasitized eggs. Emerging parasitoids were counted and sexed. The experiment was conducted under a photoperiod of 12L: 12D at $27 \pm 1^{\circ}\text{C}$ and RH of 65-70%.

3.9 Data analysis

Data on percentage parasitism and female sex ratio were arc-sine transformed while absolute insect counts were log-transformed. Parasitism data for the three plots at each site were pooled. Mean parasitism between and within the two sites (Kiboko and Katumani) in the two seasons was compared using paired t-tests. The relative abundance of the parasitoid species recovered in the two sites was calculated as a percentage. Data on the determination of parasitoid exposure time was log-transformed and the mean progeny produced between the six time regimes was compared using Analysis of Variance (ANOVA). ANOVA was also used to compare mean progeny produced, sex ratio and development time amongst 1-day, 2-day and 3-day old eggs of *B. fusca* and *S. calamistis*, and the Least Significant Difference test (LSD) was used to separate the means (SAS –Institute, 2003).

In the Y-tube experiment, Chi-square test was used to compare the frequency of the parasitoid's choice between *B. fusca* and *S. calamistis* eggs. In the field experiment, the percentage of egg batches of each stemborer species parasitized by *T. busseolae* was calculated and Chi-square (equal proportions) test used to test for the variation in the parasitism between *B. fusca* and *S. calamistis*.

The mean longevity and fecundity of *T. busseolae* females on *B. fusca* and *S. calamistis*, the mean daily progeny produced and the daily proportions of females produced were calculated and Chi-square (equal proportions) test used to test for the variation in the longevity and fecundity between *B. fusca* and *S. calamistis*.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Results

4.1.1 Species diversity of egg parasitoids in Kitui and Thika districts during the long and short rainy seasons of 2003

During the long rainy season of 2003, no egg batches were recovered from both Kitui and Thika districts, while in the short rainy season, 9 batches of *C. partellus* and 19 batches of *S. calamistis* were recovered from Thika district with none of the *C. partellus* batches producing parasitoids and 5 egg batches of *S. calamistis* yielding *T. busseolae*. No egg batches were recovered from Kitui during the short rainy season. The low egg batch recovery from the farmers' fields necessitated more focus on on-station studies, which were carried out in Katumani and Kiboko where plants were artificially infested with stemborer egg batches.

4.1.2 Determination of species diversity and relative abundance of stemborers' egg parasitoids in two semi - arid Agroecological zones of eastern Kenya

The parasitoid species obtained from stemborer eggs in Kiboko were *T. busseolae*, *T. isis*, *Telenomus* spp., *Trichogramma* spp. and *Trichogrammatoidae* spp., while in Katumani, it was *Trichogramma* spp. The abundance of the parasitoids in Kiboko in descending order was *T. busseolae*, *T. isis*, *Trichogramma* spp., *Telenomus* spp. and *Trichogrammatoidae* (Table 2).

Table 2. Parasitoid species recovered from maize plants at Katumani and Kiboko during the long and short rainy season of 2003

Site	Parasitoid	Host eggs	Percent abundance
Kiboko	<i>T. busseolae</i>	<i>S. calamistis</i>	62.32 (n= 86)
	<i>T. isis</i>	<i>S. calamistis</i>	18.84 (n= 26)
	<i>Telenomus</i> spp.	<i>S. calamistis</i>	10.14 (n = 14)
	<i>Trichogramma</i> spp.	<i>S. calamistis</i>	7.97 (n = 11)
		<i>C. partellus</i>	n=107
	<i>Trichogrammatoidae</i>	<i>S. calamistis</i>	n=1
<i>C. partellus</i>		-	
Katumani	<i>Trichogramma</i> spp.	<i>C. partellus</i> ,	(n = 16)
		<i>S. calamistis</i>	(n = 8)

n= total number of egg batches of *C. partellus* and *S. calamistis* parasitized.

Data analysis was on arc-sine transformed but the percentages presented in this table are non-transformed.

4.1.3 Parasitism levels at Katumani and Kiboko during the long and short rainy seasons of 2003

During both the long and short rainy seasons of the year 2003, Kiboko had a significantly higher mean percentage parasitism than Katumani ($t = 5.04$; $df = 2209$; $P < 0.0001$ and $t = 11.73$; $df = 2022$; $P < 0.0001$ respectively). However, the short rainy season had a significantly higher mean parasitism than the long rainy season at both Katumani ($t = 2.81$; $df = 2070$; $P < 0.0001$) and Kiboko, ($t = 10.58$; $df = 2160$; $P < 0.0037$), (Table 3).

Table 3. Percentage parasitism Mean (\pm SE) between Katumani and Kiboko during the long and short rainy seasons of 2003

Site/Season	LR	SR	t	df	P
Katumani	0.34 \pm 0.15 (n=1139)	1.39 \pm 0.36 (n=1028)	2.81	2160	<0.0051
Kiboko	2.45 \pm 0.40 (n=1077)	12.54 \pm 0.89 (996)	10.58	2071	<0.0001
t	5.04	11.73			
df	2209	2022			
P	<0.0001	<0.0001			

All egg parasitoid species

Data analysis was on arc-sine transformed but these figures presented in this table are non-transformed percentages.

LR- Long rains

SR- Short rains

4.1.4 Host suitability and Host preference studies

4.1.4.1 Host suitability

4.1.4.1.1 Determination of parasitoid exposure time

There was a significant difference in the number of progeny produced by *T. busseolae*, among the different exposure times ($F=10.43$; $df=5,54$; $P=0.0001$). The progeny production increased with the exposure time and mean progeny was highest at 24 hours. It was lowest in 1 hour. 24 hours was therefore selected as the time for exposure of *T. busseolae* females in the subsequent host suitability experiments (Fig. 2).

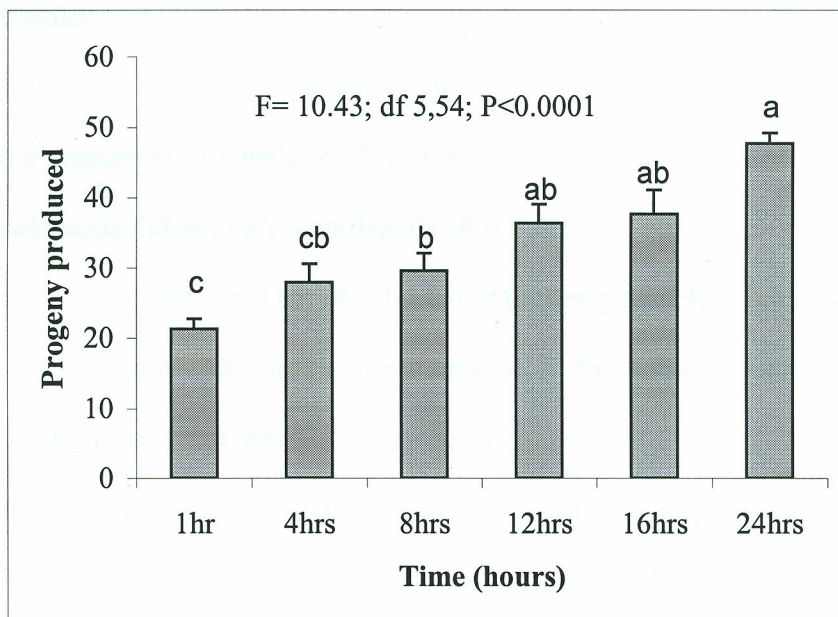


Figure. 2. Progeny produced by *T. busseolae* mean (\pm S.E) at different time regimes $n=10$; Number of replications (female parasitoids used) per treatment.

Data was log-transformed before analysis, but the means presented in this figure are non-transformed. Means followed by the same letter on the bars are not significantly different at $P<0.05$ (SNK test).

4.1.4.1.2 Effects of host age on fitness parameters of *T. busseolae* on *B. fusca* and *S. calamistis*

There was no significant variation, in the progeny produced by *T. busseolae* amongst the three ages of *B. fusca* eggs and *S. calamistis* ($F=1.79$; $df = 2, 87$; $P = 0.17$ and $F = 2.97$; $df = 2, 87$; $P = 0.06$ respectively). Likewise, there was no significant difference in the progeny produced by *T. busseolae* between ages 1 and 3 on *B. fusca* and *S. calamistis* eggs ($F= 0.24$; $df= 1, 58$; $P= 0.06$ and $F= 2.03$; $df= 1, 58$; $P= 0.16$ respectively), while Progeny production on age 2 on *B. fusca* was significantly higher than that of *S. calamistis* ($F= 12.56$; $df= 1, 58$; $P= 0.0008$) (Table 4).

Similarly, there was neither a significant difference in the proportion of females of *T. busseolae* amongst the three ages of *B. fusca* nor *S. calamistis* eggs ($F = 1.78$; $df = 2, 87$; $P = 0.18$ and $F = 0.22$; $df = 2, 87$; $P = 0.80$ respectively). Likewise the proportion of females of *T. busseolae* between ages 1, 2 and 3 of *B. fusca* and *S. calamistis* did not vary significantly ($F = 0.00$; $df = 1, 58$; $P = 0.96$; $F = 2.16$; $df = 1, 58$; $P = 0.15$ and $F = 0.01$; $df = 1, 58$; $P = 0.94$ respectively) (Table 5).

Whereas the development time of *T. busseolae* did not differ significantly amongst the three ages of *B. fusca* ($F = 2.47$; $df = 2, 87$; $P = 0.09$), it varied significantly amongst the three ages of *S. calamistis*, with age 3 having the longest development time ($F = 8.44$; $df = 2, 87$; $P = 0.0004$) (Table 6). The development time of *T. busseolae* between age's 1, 2 and 3 of *B. fusca* and *S. calamistis* eggs did not vary significantly. ($F = 3.59$; $df = 1, 58$; $P = 0.06$; $F = 1.26$; $df = 1, 58$; $P = 0.27$ and $F = 0.01$; $df = 1, 58$; $P = 0.94$ respectively) (Table 6).

Table 4. Progeny production of *T. busseolae* on *B. fusca* and *S. calamistis* eggs of different ages Mean (\pm SE)

Host age (days)	<i>B. fusca</i>	<i>S. calamistis</i>	F	df	P
1	37.17 \pm 2.42	38.30 \pm 2.04	0.24	1,58	<0.62
2	42.03 \pm 0.17	31.87 \pm 1.64	12.56	1,58	<0.0008
3	36.53 \pm 1.69	32.17 \pm 1.85	2.03	1,58	<0.16
F	1.79	2.97			
df	2, 87	2, 87			
P	<0.17	<0.06			

Analysis was on log-transformed data, but the means presented in this table are non-transformed data. Means followed by the same letter in a row are not significantly different at $P < 0.05$ Least Significant Difference (LSD) test.

Table 5 Female sex ratio of *T. busseolae* parasitizing different egg ages of *B. fusca* and *S. calamistis* Mean (\pm SE)

Host age (days)	<i>B. fusca</i>	<i>S. calamistis</i>	F	df	P
1	0.73 \pm 0.13	0.73 \pm 0.01	0.00	1, 58	<0.96
2	0.68 \pm 0.20	0.72 \pm 0.02	2.16	1, 58	<0.15
3	0.71 \pm 0.02	0.72 \pm 0.01	0.01	1, 58	<0.94
F	1.78	0.22			
df	2, 87	2, 87			
P	<0.18	<0.80			

Analysis of female sex ratio was on arc-sine transformed data, but the ratios presented in this table are non-transformed data.

Table 6. Development time (days) of *T. busseolae* on *B. fusca* and *S. calamistis* eggs of different ages Mean (\pm SE)

Host age (days)	<i>B. fusca</i>	<i>S. calamistis</i>	F	df	P
1	17.17 \pm 0.11	16.87 \pm 0.11b	3.59	1,58	<0.06
2	17.00 \pm 0.17	17.20 \pm 0.09 ab	1.26	1,58	<0.27
3	17.40 \pm 0.10	17.57 \pm 0.15a	0.77	1,58	<0.38
F	2.47	8.44			
df	2, 87	2, 87			
P	<0.09	<0.0004			

Means followed by the same letter in a column are not significantly different at $P < 0.05$ (LSD test).

4.1.4.2 Host preference studies

There was no significant difference between choice of *B. fusca* and *S. calamistis* by *T. busseolae* in the Y-tube experiment ($\chi^2 = 1.667$; df 1, 60; $P = 0.1967$).

Similarly in the field experiment no significant difference in parasitism by *T. busseolae* was observed between *B. fusca* and *S. calamistis* ($\chi^2 = 0.72$; df =1; $P > 0.05$)

(Table 7).

Table 7. Preference for either *B. fusca* or *S. calamistis* eggs

Host	Percentage choice in Y-tube	Percentage parasitism in field
<i>B. fusca</i>	58.33	54.2
<i>S. calamistis</i>	41.67	45.8
χ^2	1.667	0.72
df	1	1
P	<0.1967	>0.05

4.1.5 Fecundity and longevity of *T. busseolae* females

Telenomus busseolae females began ovipositing on the first day of emergence. Average daily oviposition was considerably higher on the first day than on any subsequent day, and it decreased with the age of the female for both *B. fusca* and *S. calamistis* (Figs. 3 and 4). The *T. busseolae* females produced 67.3 and 67.2% of their total progeny on *B. fusca* and *S. calamistis* respectively within the first five days. There was neither a significant difference in the longevity ($\chi^2=0.03$; $df=1$; $P>0.05$) nor fecundity ($\chi^2=0.55$; $df=1$; $P>0.05$) of *T. busseolae* on *B. fusca* or *S. calamistis* eggs. The mean longevity of *T. busseolae* females on *B. fusca* was 26.1 days, and its mean progeny was 119.2, while the mean longevity on *S. calamistis* was 25.5 days and the mean progeny produced was 108.0 (Table 8). In both treatments (stemborer species), the proportion of females produced decreased with the age of ovipositing females (Figures 5 and 6).

Table 8. Mean longevity and fecundity of *T. busseolae* on *B. fusca* and *S. calamistis* eggs

Parameter	<i>B. fusca</i>	<i>S. calamistis</i>	χ^2	df	P
Longevity	26.13	25.47	0.03	1	>0.05
Fecundity	119.17	108.04	0.55	1	>0.05

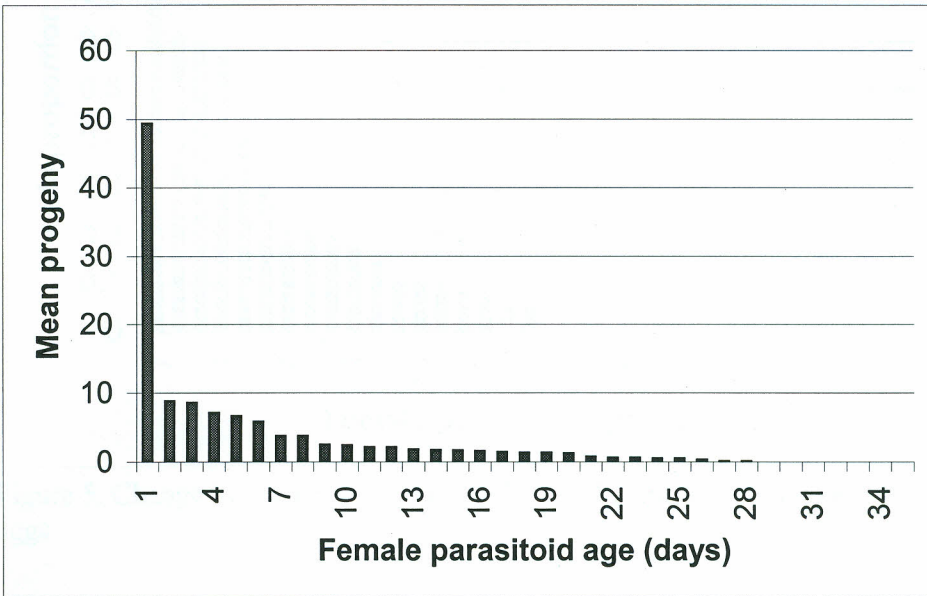


Figure 3. Mean daily progeny produced by *T. busseolae* on *B. fusca* eggs

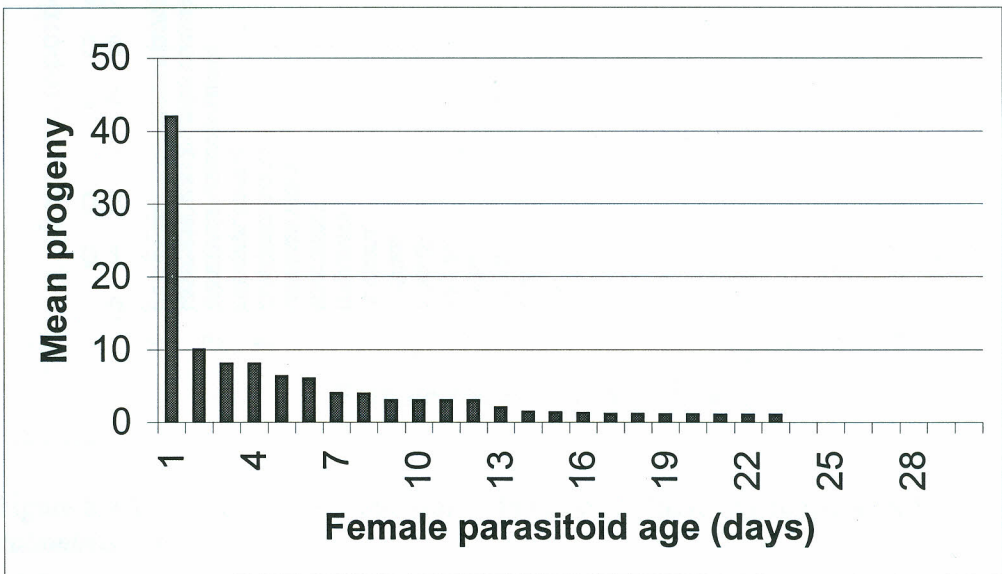


Figure 4. Mean daily progeny produced by *T. busseolae* on *S. calamistis* eggs

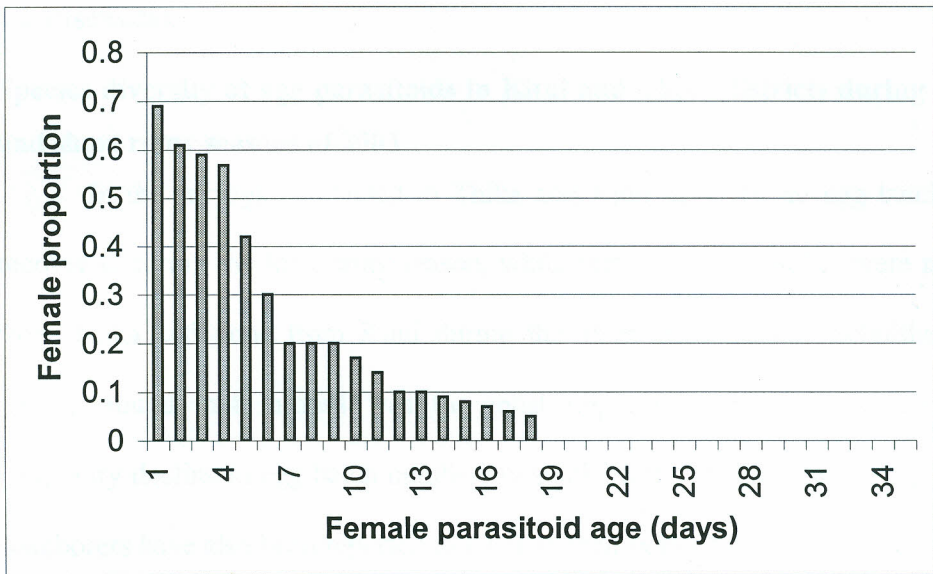


Figure 5. Change in female proportion with age of *T. busseolae* females on *B. fusca* eggs

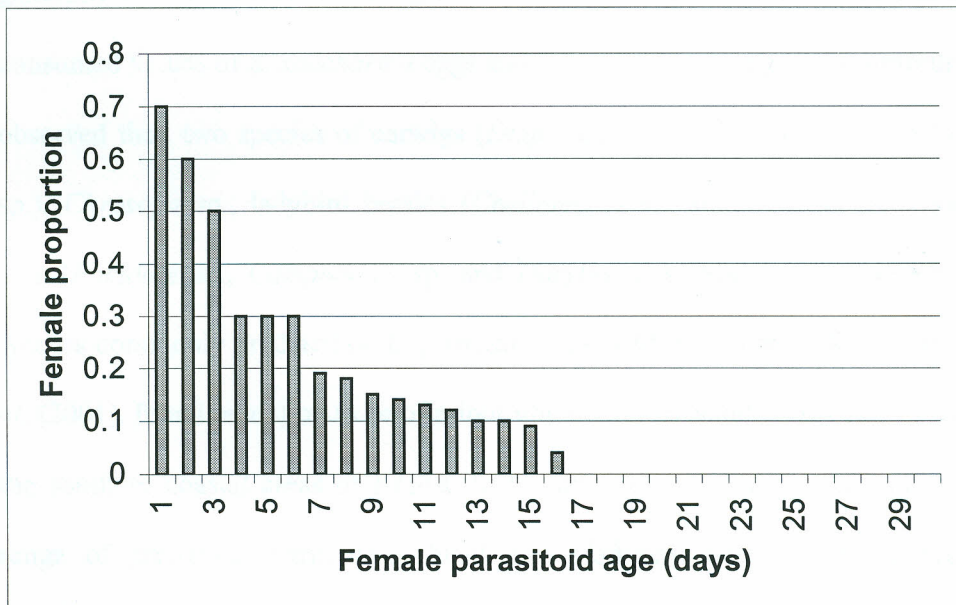


Figure 6. Change in female proportion with age of *T. busseolae* females on *S. calamistis* eggs

4.2 Discussion

Species diversity of egg parasitoids in Kitui and Thika districts during the long and short rainy seasons of 2003

In the surveys conducted in Thika and Kitui districts, no egg batches were recovered during the long rainy season, while very few egg batches were recovered from Thika and none from Kitui during the short rainy season. Schulthess *et al.* (2001), found that rainfall was the most important abiotic factor that caused temporary decline in egg batch numbers in southern Benin. Predators of lepidopteran stemborers have also been reported to regulate their populations in the field (Polaszek, 1998). Leslie (1988) showed that several species of ants and spiders predated on *E. saccharina* eggs and larvae, although their impact on *E. saccharina* populations was generally low. In contrast, Girling (1978) estimated that predacious ants in Uganda consumed 91.6% of *E. saccharina* eggs and 93% of neonate larvae. Dwumfour (1990) observed that, two species of earwigs (*Diaperasticus erythrocephalus* and *Forficula* sp.), *Chrysopa* sp., ladybird beetles (*Cheilomenes* sp. and *Gonocephalus simplex*), ants (*Pheidole* sp., *Camponotus* sp. and *Dorylus* sp.) *Orius* sp. and several spider species commonly predated on *C. partellus* eggs at Mbita Point, in Kenya. Bonhof *et al.* (2001), found predation to be of minor importance in stemborers eggs' mortality in the southern coastal areas of Kenya. In the present study, high numbers of a wide range of predators (particularly black ants, ladybird beetles and spiders) were observed on the plants during the surveys, with higher numbers being observed in Kitui than in Thika. The predators may have been responsible for the low stemborer egg numbers recovered from maize plants in the two study sites.

Determination of species diversity and relative abundance of stemborers' egg parasitoids in two semi - arid Agroecological zones of eastern Kenya

Trichogramma spp. egg parasitoids were recovered from both Katumani and Kiboko, while *T. busseolae*, *T. isis* and *Telenomus* spp. were only recovered from Kiboko. This is the first record of *T. isis* in East Africa. *Telenomus isis* has never been reported in East Africa (Polaszek, 1998), and the question has to be, whether it was accidentally introduced or the previous samplings were not exhaustive enough. Kiboko, which is, classified as low midland zone 5 (LM5) seems to experience similar climatic conditions as lowland humid tropics where the parasitoids of the genus *Telenomus* have been earlier reported in west Africa (Ndemah *et al.*, 2002). This probably explains the reason why there were no *Telenomus* species recovered from Katumani (upper midland zone 4 agro ecological zone) (UM4), which is at a much higher elevation and less humid than Kiboko. The present results suggest that parasitoids of the genus *Trichogramma* was widespread in both UM4 and LM5 agro ecological zones while parasitoids of the genus *Telenomus* and Trichogrammatoidae were restricted to the LM5 agro ecological zone. However, this is highly speculative and more field surveys to determine the species of egg parasitoids occurring in other sites within these two zones (UM4 and LM5) need to be done and for more seasons in order to obtain more conclusive results.

The predominant egg parasitoid species in Kiboko was *T. busseolae* followed by *T. isis*, *Trichogramma* spp., *Telenomus* spp. and *Trichogrammatidae* respectively. This finding is similar to the findings of Schulthess *et al.* (2001) who found *T. busseolae* to be the predominant species in southern Benin where both *T. busseolae* and *T. isis* occurred. *T. isis* has a considerably longer oviposition time than *T. busseolae* (Agboka *et al.*, 2002), lower fecundity, and a higher egg resorption than *T. busseolae* (Chabi- Olaye *et al.*, 2001a). According to Bell and Bohm (1975),

resorption in insects is an adaptive strategy allowing females to conserve their metabolic resources instead of laying eggs under unfavourable conditions. Agboka *et al.* (2002) reported parasitism of *T. isis* to be lower than that of *T. busseolae* by 16%, though the fecundity of the former could allow for complete exploitation of a batch of 20-25 eggs. They noted that, it appeared that *T. busseolae* exploited batches further and superparasitized sooner than *T. isis*. Superparasitism can be an adaptive reproductive strategy for parasitoids when hosts are scarce and the likelihood of finding unparasitized host is low (van Alphen and Visser, 1990). Mackauer (1990) noted that in cases of super- and multiparasitism among the solitary endoparasitoids, the older larva has the advantage over the younger one. Multiparasitism has been reported to be low between *T. busseolae* and *T. isis*, but when it occurs less than 24 hours after the first species to parasitize, *T. busseolae* wins over *T. isis* irrespective of the sequence of parasitism (Agboka *et al.*, 2002). This is because *T. busseolae* develops faster than *T. isis* (Chabi-Olaye *et al.*, 1997,2001a). These competitive advantages of *T. busseolae* over *T. isis* probably explain the dominance of *T. busseolae* over *T. isis* in this study with the assumption that they have co-existed. The dominance of *T. busseolae* over *Trichogramma* and *Trichogrammatoidae* is not well understood. Whereas *Trichogramma* and *Trichogrammatoidae* successfully parasitized both *C. partellus* and *S. calamistis* eggs, *T. busseolae* parasitized only *S. calamistis* in the present study, hence host competition may have been of non-significance in the dominance.

Discussion Kiboko had higher parasitism than Katumani during both long and short rainy seasons. This may be due to the different habitats and microclimatic conditions in the two localities. According to Ndemah *et al.* (2001), egg parasitism is extremely low in spring as a result of host scarcity during the off-season. Kiboko has irrigation

facilities, which allow continuous cropping of cereals and other crops throughout the year. Perhaps, this allows a higher build up of parasitoid population in the off-season at Kiboko in contrast to Katumani where there is no cropping in the off-season. High temperatures are known to favour rapid insect growth and development up to the maximum temperature limit characteristic of each insect species. This also explains why, Kiboko that has a higher temperature, hence rapid parasitoid development than Katumani, had a higher parasitism than Katumani.

Parasitism was higher in the short than the long rainy season at both Katumani and Kiboko. This is similar to the parasitism trends reported by Schulthess *et al.* (2001) and Ndemah *et al.* (2003). Egg batch densities increases in the course of cropping season and reach a peak in the early second season (Shulthess *et al.*, 2001 and Ndemah *et al.*, 2003). During the second season, the percentage of parasitized egg batches increases because of increased availability of hosts hence a reduced host searching time (Ndemah *et al.*, 2003). In the present study, although, equal numbers of egg batches were infested during both the long and short rainy seasons, these only acted as traps for the parasitoids and the parasitism realized was a reflection of parasitoid populations that already existed in the field.

In this study, low percentage parasitism was recorded at both Katumani and Kiboko, ranging from 0.34% to 12.34%. This is much lower than what has been reported in other studies elsewhere. For instance, Schulthess *et al.* (2001) reported parasitism means of up to 77% by *T. busseolae*, *T. isis* and *Lathromeris ovicidia* in Benin. Moyal (1998) reported 72% parasitism by *T. busseolae* on *B. fusca* in Côte d'Ivoire, while Ndemah *et al.* (2003) reported mean parasitism of 40.5 and 59.3 % in two regions of Cameroon by the parasitoids *T. busseolae* and *T. isis*.

At the Kenyan Coast, Mathez (1972) reported up to 90% parasitism of *C. partellus* eggs by *Trichogramma* sp., Skovgård and Päts (1996) reported 19-71% and 31-76% by *Trichogramma* sp. on *Chilo* spp. and *S. calamistis* in maize monocrop and maize intercropped with cowpea respectively, and Bonhof *et al.* (2001), reported egg mortality of 18-78% as a result of parasitism by *Trichogramma* spp.

Sex ratios

There was no significant difference in female sex ratio of *T. busseolae* between the three ages of *B. fusca* and *S. calamistis*. Similarly, there was no significant difference in female sex ratio between age's 1, 2 and 3 of *B. fusca* and *S. calamistis*. The female sex ratio was in the range of 0.68 and 0.73. This is close to the range of 0.63 and 0.72 reported for *T. busseolae* (Chabi-Olaye *et al.*, 1997). Sex allocation in arrhenotokous (haplodiploid) Hymenoptera and its evolutionary bases have been studied by several workers (Godfray, 1994). Unfertilized haploid eggs develop into males and fertilized (diploid) eggs develop into females in such parasitoids. In many parasitoid species, mating often occurs between siblings, brothers compete with each other for mates. This is normally associated with a female-biased sex ratio (Godfray, 1994). This explains the female biased sex ratio observed in the present study.

Progeny production and development time

The results of progeny production showed that *T. busseolae* could successfully parasitize and develop in 1-3 day old eggs of *S. calamistis* and *B. fusca*. This confirms the results of Chabi-Olaye *et al.* (1997) who found *T. busseolae* was capable of developing on the host embryo during the later stages of host-egg incubation. On the

other hand Chabi-Olaye *et al.* (1997) reported a significant difference in progeny production by *T. busseolae* on 1, 2, 3 and 5 day old eggs of *S. calamistis*, which was not observed in the present study. As reported by Chabi-Olaye *et al.* (2001a), the ability of a parasitoid to accept and develop in a range of host stages is an advantage during times of host scarcity, given that in the field a parasitoid may not find the most suitable host stage i.e. freshly laid eggs. In the present study, the development time varied significantly between the three ages of *S. calamistis* eggs and not for *B. fusca* eggs. The decreasing suitability of older eggs is common in egg parasitoids (Chabi-Olaye *et al.*, 1997). According to Pak *et al.* (1990), the non-suitability of older eggs can be attributed to desiccation, and as noted by Chabi-Olaye *et al.* (2004), the amount of food available for parasitoid progeny declines with host age. The latter explains the longer development time of parasitoids in older eggs of *S. calamistis*. However, in *B. fusca* eggs, there was no difference in development time of *T. busseolae* with the host ages. This is because the *B. fusca* has a longer egg duration than *S. calamistis* (F. Schulthess, unpublished data) and probably at the third day they have not significantly depleted the food resources.

Oviposition preference

In the olfactometer experiment, there was no difference in choice by *T. busseolae* for *B. fusca* or *S. calamistis*, similarly; in the field experiment, there was no difference in parasitism between *B. fusca* and *S. calamistis* eggs. Female parasitoids respond to a hierarchy of physical and/ or chemical stimuli, which lead them to their potential hosts (van Alphen and Vet, 1985). In the olfactometer experiment, the odours that are emitted by the eggs, which aid in host finding were tested and the parasitoid showed no preference for either *B. fusca* or *S. calamistis*. This suggests that

in the field *B. fusca* and *S. calamistis* have equal chances of being discovered by the parasitoid. In the field experiment, where the parasitoid came into contact with the host eggs, the preference of actual factors that would lead to oviposition or lack of it on a host once the parasitoid had encountered the host, were examined. One such factor is the contact kairomones. Once parasitoids have found a microhabitat, the sequence of responses is often continued with a reaction to chemical stimuli deposited by the host or caused by the host's presence. The use of such contact kairomones is a common phenomenon in host finding (Weseloh, 1981). The responses to Kairomones are likely to differ between different species, depending on the distribution of hosts and kairomones (van Alphen and Vet, 1985). Likewise there was no significant difference in the parasitization of *B. fusca* and *S. calamistis* in the field. This suggests that the kairomones of *B. fusca* and those of *S. calamistis* are equally attractive to *T. busseolae*. Chabi- Olaye *et al.* (2001b) reported that *Telenomus* spp. were attracted to Kairomones of their hosts.

Adult female longevity and fecundity

The longevity of *T. busseolae* females was 26.13 days on *B. fusca* eggs and 25.47 days on *S. calamistis* eggs. Chabi –Olaye *et al.* (1997) reported the mean longevity of ovipositing *T. busseolae* females to range from 21.7 days at 20⁰ C to 11.0 days at 30⁰C. Cave and Gaylor (1989) reported mean longevity of *Telenomus reynoldsi* (Hymenoptera: Scelionidae) at 20, 25, 28 and 32⁰C to be 34.6, 23.1, 20.3 and 17.2 days respectively. Similarly, Chabi Olaye *et al* (2001a) found the longevity of *T. isis* to range from 11 days at 30⁰C to 24 days at 21⁰C. The mean fecundity of *T. busseolae* females was 119.17 and 108.04 on *B. fusca* and *S. calamistis* respectively. This is lower than the fecundity of 146.2 reported by Chabi-Olaye *et al.* (1997) on *S.*

calamistis. However it is higher than the fecundity of *T. isis*, which has been reported to be 82.1 (Chabi-Olaye *et al.*, 2001a). The non-significant variation in the longevity and fecundity of *T. busseolae* between *B. fusca* and *S. calamistis* eggs suggests that stemborer host species did not have an effect on the longevity and fecundity of *T. busseolae* in the present study. The difference in longevity and fecundity between the East African and West African populations of *T. busseolae* may suggest that they are of different strains. The decreased proportion of females with increased age of *T. busseolae* suggests the females ran out of male gametes as reported by Godfray (1994).

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

- 1) The species diversity of lepidopteran egg parasitoids is wider than earlier reported. The recovery of *T. isis* from Kiboko shows that there are some species in Kenya that have not yet been reported. However the determination as to whether *T. isis* is a different strain from the West African strain should be determined through genetic distancing studies.
- 2) Both *B. fusca* and *S. calamistis* are suitable hosts of *T. busseolae*. This was confirmed by the ability of *T. busseolae* females to successfully parasitize, develop and produce progeny on 1-day, 2-day and 3-day old eggs of both *B. fusca* and *S. calamistis*.
- 3) There was no difference in sex ratio and progeny production between 1-day, 2-day and 3-day old eggs of both *B. fusca* and *S. calamistis*. It can then be concluded that age of host (*B. fusca* and *S. calamistis*) does not affect the sex ratio and progeny production of *T. busseolae* females.
- 4) There was a significant difference in development time of *T. busseolae* between 1-day, 2-day and 3-day old eggs of *S. calamistis*. While in *B. fusca* there was no difference in development time between the ages. Thus, the age of *S. calamistis* eggs affected the development time of *T. busseolae* while the age of *B. fusca* eggs had no effect on the development time of *T. busseolae*. This makes *B. fusca* a more suitable host.
- 5) *Telenomus busseolae* females produce the highest progeny on the first day of their emergence.

5.2 Recommendations

- 1) There is need to conduct intensive and extensive field surveys in order to come up with a more comprehensive list of the species of egg parasitoids attacking lepidopteran stemborers in Kenya.
- 2) Pest and habitat management strategies that do not promote pest infestation or act as pest reservoirs and at the same time help to conserve key parasitoids in the field should be developed and farmers trained on these management strategies.
- 3) Understanding the interaction between the *Trichogramma*, *Trichogrammatoidae* and *Telenomus* parasitoids, will be of importance as they were recovered from the same locality and happen to share some hosts.

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Appendices

Appendix 1. Location of the field surveys conducted in Kitui during the long rains, of 2003

Region	Location	Farmer	Altitude (m)	Longitude	Latitude
Eastern	Masii	1	1225	E 37 ⁰ 49.944'	S 01 ⁰ 22.374''
Eastern	Masii	2	1225	E 37 ⁰ 27.640'	S 01 ⁰ 26.620''
Eastern	Masii	3	1280	E37 ⁰ 27.544'	S 01 ⁰ 26.603'
Eastern	Masii	4	1225	E 37 ⁰ 23.312'	S 01 ⁰ 20.488''
Eastern	Masii	5	1200	E 37 ⁰ 49.344'	S 01 ⁰ 20.074''
Eastern	Kwa vonza	6	1020	E037 ⁰ 48.853'	S 01 ⁰ 23.567'
Eastern	Kwa vonza	7	1080	E037 ⁰ 49.864	S 01 ⁰ 23.367'
Eastern	Kwa vonza	8	1000	E 37 ⁰ 57.097'	S 01 ⁰ 21.722''
Eastern	Mulutu	9	1075	E 37 ⁰ 57.186'	S 01 ⁰ 21.096'
Eastern	Mulutu	10	1100	E037 ⁰ 57.648'	S01 ⁰ 21.005'
Eastern	Kabati	11	1100	E 37 ⁰ 56.522'	S 01 ⁰ 16.266'
Eastern	Kabati	12	1100	E 37 ⁰ 21.918'	S 01 ⁰ 15.185'
Eastern	Kabati	13	1100	E 37 ⁰ 55.787'	S 01 ⁰ 16.503'
Eastern	Wikililye	14	1025	E 38 ⁰ 00.965'	S 01 ⁰ 24.205'
Eastern	Kyanika	15	1080	E38 ⁰ 00 .545'	S 01 ⁰ 22.918'
Central	Gatuanyaga	1	1380	E37 ⁰ 11.009'	S 01 ⁰ 04.455'
Central	Gatuanyaga	2	1375	E 37 ⁰ 11.116'	S 01 ⁰ 04.537'
Central	Gatuanyaga	3	1400	E 37 ⁰ 11.433'	S 01 ⁰ 04.565'
Central	Gatuanyaga	4	1250	E 37 ⁰ 19.651'	S 01 ⁰ 05.906'
Central	Gatuanyaga	5	1250	E 37 ⁰ 19.679'	S 01 ⁰ 05.911'
Central	Gatuanyaga	6	1275	E 37 ⁰ 21.012'	S 01 ⁰ 6.280'
Central	Gatuanyaga	7	1225	E 37 ⁰ 21.129'	S 01 ⁰ 6.366'
Central	Gatuanyaga	8	1250	E 37 ⁰ 20.284'	S 01 ⁰ 6.118'
Central	Gatuanyaga	9	1350	E 37 ⁰ 11.331'	S 01 ⁰ 4.486'
Central	Gatuanyaga	10	1300	E 37 ⁰ 11.841'	S 01 ⁰ 5.426'
Central	Munyu	11	1375	E 37 ⁰ 10.722'	S 01 ⁰ 6.399'
Central	Munyu	12	1375	E 37 ⁰ 10.699'	S 01 ⁰ 6.320'
Central	Munyu	13	1375	E 37 ⁰ 10.262'	S 01 ⁰ 6.676'
Central	Munyu	14	1350	E 37 ⁰ 8.953'	S 01 ⁰ 6.285'
Central	Komo	15	1350	E 37 ⁰ 8.640'	S 01 ⁰ 7.822'

Appendix 2. Locations of field surveys conducted in Kitui during the short rains of 2003

Region	Location	Farmer	Altitude (m)	Longitude	Latitude
Eastern	Kwa-vonza	1	1020	E 37 ⁰ 48.853'	S 01 ⁰ 23.567'
Eastern	Mulutu	2	1100	E 37 ⁰ 51.179'	S 01 ⁰ 21.116'
Eastern	Wikiliye	3	1025	E 38 ⁰ 00.965'	S 01 ⁰ 24.205'
Eastern	Kabati	4	1100	E 37 ⁰ 55.787'	S 01 ⁰ 16.503'
Central	Ngoliba	1	1380	E 37 ⁰ 11.482'	S 01 ⁰ 06.804'
Central	Gatwanyaga	2	1400	E 37 ⁰ 11.433'	S 01 ⁰ 4.565'
Central	Munyu	3	1375	E 37 ⁰ 10.722'	S 01 ⁰ 6.399'
Central	Komo	4	1350	E 37 ⁰ 8.640'	S 01 ⁰ 7.822'

Appendix 3. Study sites, their agro ecological zones and environmental characteristics in eastern Kenya.

Site	AEZ*
Katamani	UM4
Kiboko	LM5
Kitui	LM4

AEZ*- Agro ecological zone: UM4- Upper midland zone 4, LM4- Lower midland zone 4, and LM5- Lower midland zone 5 (Jaetzold & Schmidt, 1983).

Appendix 4. Long rains mean temperature and rainfall

Site	Temperature (°C)	Rainfall (mm)
Katamani	19.5	246
Kiboko	25.5	90.5

Courtesy of ICRISAT Kiboko and meteorological department in Katamani

Appendix 5. Short rains mean temperature and rainfall

Site	Temperature (°C)	Rainfall (mm)
Katamani	19.6	221.6
Kiboko	24.6	403

Courtesy of ICRISAT Kiboko and meteorological department in Katamani

Appendix 6. . Crops grown in each of the study farms in Kitui during the long rains of 2003

District	Location	Farmer	Crops			
			Maize	Beans	Cowpeas	Pigeon peas
Kitui	Masii	1	1	1	1	1
Kitui	Masii	2	1	0	1	1
Kitui	Masii	3	1	1	1	0
Kitui	Masii	4	1	1	0	0
Kitui	Masii	5	1	0	1	0
Kitui	Kwa vonza	6	1	1	0	1
Kitui	Kwa vonza	7	1	0	0	0
Kitui	Kwa vonza	8	1	0	0	0
Kitui	Mulutu	9	1	0	1	1
Kitui	Mulutu	10	1	1	0	0
Kitui	Kabati	11	1	0	0	0
Kitui	Kabati	12	1	1	0	0
Kitui	Kabati	13	1	1	1	0
Kitui	Wikililye	14	1	0	1	1
Kitui	Kyanika	15	1	0	1	0

1 – Means crop present

0 – Crop absent