IMPACT ASSESSMENT OF NATURAL ENEMIES ON STEM BORER POPULATIONS AND MAIZE YIELD IN THREE AGROECOLOGICAL ZONES IN MOZAMBIQUE

BY
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DECLARATION BY THE CANDIDATE

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

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To my daughter Fainesse and my son Cayane, my mother and wife for their patience, love and support

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ABSTRACT

Lepidopteran stem borers are the major pests limiting the production of maize and grain sorghum under subsistence farming conditions of Southern Africa. Classical biological control has traditionally emphasized the control of introduced pests through the importation and/or introduction of coevolved natural enemies from the pest's native home. It is based on the assumption that coevolved natural enemies are best adapted to locating and successfully attacking the target host. On this basis, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) and *Xanthopimpla stemmator* (Thunberg) (Hymenoptera: Ichneumonidae) were introduced in Southern African countries for biological control of *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and *Chilo sacchariphagus* (Bojer) (Lepidoptera: Pyralidae). The current studies were conducted to evaluate the establishment and spread of *C. flavipes* and *X. stemmator* in the release locations, assess the impact of natural enemies on the stem borer populations and maize yield, assess the effect of temperature on the development of *X. stemmator* in three host species, and finally, to study population growth parameters of *X. stemmator* and *Dentichasmias busseolae* (Heinrich) (Hymenoptera: Ichneumonidae). Several parasitoids including indigenous and the exotic species were recovered from egg, larval and pupal stem borer development stages. Egg parasitism of more than 80% due to *Trichogramma bournieri* (Hymenoptera: Trichagrammatidae) was reported on *C. partellus* eggs in the Southern region of Mozambique. *C. flavipes* was recovered at all release and other sampling sites. The highest percent parasitism (33.8%) due to *C. flavipes* was reported at Maracuene, one of the 1996 release sites. This introducrd exotic larval parasitoid was reported to increasingly becoming the more abundant in relation to *C. sesamiae*. The exotic pupal parasitoid *X. stemmator* was recovered only from *C. partellus* pupae during the release season (2002/2003) and one year after its release, but it was not recovered in subsequent seasons. Results from field experiments indicated that damage levels due to stem borer attack varied from location to location. High damage levels were reported in the areas where *C. partellus* is dominant followed by the areas dominated by *Busseola fusca* Fuller (Lepidoptera: Noctuidae). However, stem borer density was higher at high elevation zones dominated by *B. fusca* compared to low and mid elevation zones. Yield losses varied from 28.8% to 34.5% across the regions. Yield losses were observed to increase (from 28.9 to 43.3, 34.5 to 40.8 and from 31.2 to 36.4% at low, mid and high elevation zones respectively) when natural enemies were excluded from the maize plots. The impact of natural enemies on maize yield increase was high at lowland zones (26.1%) and lowest at high elevations (7.6%). Laboratory experiment indicated that while *X. stemmator* successful developed in *C. partellus*, *B.fusca* and *S. calamistis* stem borer species, the more suitable host was *C. partellus*. The parasitoid developed faster at high temperatures and slowly at low temperatures. The lower temperature threshold for *X. stemmator* reared on *C. partellus* was 9.76°C and the maximum threshold of 35°C. These results indicate that this exotic parasitoid could survive and remain active at low to mid elevations and could not survive at high elevations where temperatures during winter are usually below 9°C. However, in the areas where minimum temperatures are above 10°C, *X. stemmator* could be an important agent of biological control against *C. partellus* in Mozambique.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Lepidopteran stemborer larvae are the most economically important pests of maize and sorghum in Mozambique. Among the stemborers attacking cereals, the spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and the maize stalk borer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae) are considered to be the most important species in Mozambique (Gonçalves, 1970; Segeren *et al.*, 1991). The pink stalk borer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) is of minor importance. Kfir (1992; 1998) speculated that the borer is kept under control by its natural enemies, which prevent serious outbreaks.

In Mozambique, *C. partellus* is the most abundant stemborer species at lower altitudes (0 - 200 m) and in warm zones, while *B. fusca* dominates at higher altitudes (1000 - 1500 m) and cooler areas (Sithole, 1988; Davies *et al.*, 1995; Segeren *et al.,* 1995). The infestation levels can be very high and fields with 100% of plants infested are frequently observed in southern Mozambique where *C. partellus* is the most abundant stemborer (Berger, 1981; Segeren *et al.*, 1991; Cugala *et al.*, 1999). Yield losses due to stemborer attacks are reported to range from 20 to 40% on research stations, and are more than 50% in the small-scale farmers' fields (Segeren *et al.,* 1991).

One of the most destructive stemborer, *Chilo partellus*, is thought to have been accidentally introduced into Africa in 1930 from Asian continent (Tams, 1932). Since its arrival, *C. partellus* has spread to eastern and southern Africa, often becoming the

most damaging stemborer of maize and sorghum particularly in the warmer lowland areas (Overholt, 1998).

Attempts at controlling stemborers in Mozambique have been made using cultural practices (Oever 1990) such as sanitation (burning the crop residues, cleaning and/or burning wild hosts around the fields), intercropping and management of sowing date. However, destroying crop residues and wild host plants by burning will suppress off-season survival of stemborers and parasitoids, and results in a loss of nitrogen. Sowing date is strongly dependent on rainfall, and cannot be greatly modified. Segeren *et al.* (1996) reported no differences on the stemborer infestation in a maize monoculture and in a maize/cowpea intercrop.

Insecticides have been used on large-scale farms (Ariyanayagan 1983; Jimenez and Mugabe 1990), but there is little or no use of insecticides by small scale farmers (Leeuwen and Zucula 1987) because of the high cost and hazardous effects on environment and human beings (Berger 1994; Kfir 1995; Overholt 1995)). Non-selective insecticides kill not only the target pests, but also natural enemies and other non-target organisms, and can induce the development of pesticide resistance (Gifford and Mann 1967). In addition, chemical controls must be carefully timed to coincide with the limited period when early instar stemborer larvae are found outside of plant tissues (Mohyuddin and Greathead 1970; Segeren *et al.* 1991).

Stemborer control by insecticides is difficult because the damaging larvae are protected inside the plant stems (Kfir, 1998). They are vulnerable to pesticides during the first instar stage, when they are feeding exposed on the leaves before penetrating inside stems (Davies *et al.*, 1995; Kfir, 1997a).

1.2 Problem statement and justification

In Mozambique, biological control is viewed as a potential strategy for the management of the exotic stemborer *C. partellus*. In general, indigenous natural enemies are not able to keep introduced stemborer species below economic injury levels (Overholt *et al.*, 1994; Seshu Reddy, 1998). Due to its status as an introduced species, *C. partellus* has been the target of classical biological control attempts in Africa. The Commonwealth Institute of Biological Control imported and released nine species of parasitoids from India against *C. partellus* in Uganda, Tanzania and Kenya from 1968 to 1972 (CIBC 1968-72). In South Africa, several exotic parasitoid species were released from 1977 to 1993 (Kfir, 1995). However, no establishments were reported in either of the programmes. A third attempt to introduce exotic parasitoids for control of *C. partellus* was started in Kenya by the International Centre of Insect Physiology and Ecology (ICIPE) which focused on the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) (Overholt, 1998).

The exotic larval parasitoid, *C. flavipes*, was released in Kenya in 1993 and became permanently established (Overholt, 1998). The parasitoid's impact has recently been evaluated, and a reduction of 37% of the total stemborer population and a reduction of 53% of *C. partellus* population has been observed in some areas (Zhou *et al.*, 2001).

Cotesia flavipes was introduced into southern and central Mozambique between 1996 and 2000 to increase the natural suppression of *C. partellus* population, and it became established at all release sites, and is spreading to other areas (Cugala, 2002; Cugala and Omwega, 2001; Cugala *et al.*, 1999; 2001). However, *C. flavipes* is

having little impact as a biological control agent of stem borers in the areas where *B. fusca* is the dominant species. Thus, due to its drill and sting attack strategy, the exotic pupal parasitoid *Xanthopimpla stemmator* (Thunberg) (Hymenoptera: Ichneumonidae) was considered a promising candidate for release to increase the natural suppression of stem borers in Mozambique because it will not compete with the exotic larval parasitoid, *C. flavipes*.

Considerable information is available on the geographical distribution and biology of *X. stemmator* (Moore and Kfir, 1986; Hailemichael *et al.*, 1994; Gitau *et al.*, 2005). However, data on its use as biological control agent against maize and sorghum stem borers and intrinsic rate of natural increase are lacking. Additionally, there is no information on the population dynamics of *C. flavipes* and its impact on the target stemborers and other lepidopteran larvae in Mozambique. In addition, many indigenous parasitoids have been reared from cereal stem borers species in Mozambique, but there has been no assessment of their importance as naturally occurring factors limiting stemborer populations.

1.3 Research questions

What role do parasitoids play in the stemborer population dynamics and maize yield?

i) Are they protecting the crop from stemborer's attack?

ii) Is *C. flavipes* spreading from the release sites to non-release areas?

iii) Is *C. flavipes's* population building up and then increasing stemborer population suppression?

iv) Can *Xanthopimpla stemmator* established at agro-ecologically different zones and contribute to stemborer population's suppression?

1.4 Hypothesis

i) Parasitoids play a significant role in the stemborer population dynamics and maize yield

ii) *C. flavipes* is permanently established and it is spreading from the release to non-release sites

iii) Levels of stemborer parasitism due to *C. flavipes* are increasing

iv) *X. stemmator* can establish at agro-ecologically different zones

1.5 Objectives

1.5.1 General objective

To investigate the impact of natural enemies on the cereal stemborers population and maize yield in Mozambique.

1.5.2 Specific objectives

a) To determine the establishment of *C. flavipes* at the release sites and its spread to new areas in Mozambique

b) To assess the incidence of egg parasitoids of cereal stemborer eggs in small scale farmers' fields

c) To evaluate the impact of *C. flavipes* and indigenous natural enemies on the cereal stemborers population dynamics

d) To assess yield losses due to stemborers attack at different agroecological zones relative to stemborer species composition

e) To investigate the establishment and spread of *Xanthopimpla stemmator* in three different agro-ecological locations in Mozambique

f) To assess the effect of temperature and host species on the development of the pupal parasitoids *Dentichasmias busseolae* and *Xanthopimpla stemmator*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of maize and sorghum

Maize (*Zea mays* L.) and to a lesser extent sorghum (*Sorghum bicolor* L.) are the major staple food sources for the majority of people throughout Southern Africa (Kfir, 1998). In Mozambique, maize and sorghum are grown for home consumption or cash income. Maize is the most widely grown crop occupying more than 30% of the land under cultivation (Ministério de Agricultura Moçambique, 1977), and more than 95% of the annual production is produced by small scale farmers. Crop production is limited due to losses caused by pests, often resulting in very low yields (Segeren *et al.*, 1995). Although pesticides may be effective, they are not affordable to small-scale farmers (Skoroszewski and van Hamburg, 1987). The larvae of stemborer moths that infest these crops are one of the main factors which contribute to significant reductions in yields.

2.2 Pest status of cereal stem borers in Mozambique

Of the twenty-one species of lepidopterous cereal stem borers found in Africa, five are of relevance to southern Africa and include as: *Busseola fusca* Fuller (Noctuidae), *Sesamia calamistis* Hampson (Noctuidae), *Chilo partellus* (Swinhoe) (Crambidae), *Chilo sacchariphagus* (Bojer) (Crambidae) and *Eldana saccharina* (Walker) (Pyralidae). Lepidopteran stemborer larvae are the most economically important pests of maize and grain sorghum in Mozambique. Among the stemborers attacking cereals crops, the spotted stalk borer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and the maize stalk borer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae) are considered to be the most important species (Gonçalves 1970; Segeren *et al.*,

1991). The pink stalk borer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) is of minor importance. Kfir (1992, 1998), and it is speculated that the borer is kept under control by its natural enemies, which prevent serious outbreaks.

2.3 Biology and life history

The description and biology of the three most common cereal stemborers in Mozambique are well documented (Ingram, 1958; 1983; Gonçalves, 1970; Harris, 1962; Harris, 1989a, 1989b; Segeren *et al.*, 1991; Berger, 1993; Overholt and Maes, 2000).

2.3.1 The spotted stalk borer, *Chilo partellus*

Adult *C. partellus* emerge from pupae in the afternoon and early evening and are active at night (Gonçalves, 1970; Harris, 1989a). Females lay eggs in batches of 10-80 overlapping eggs on the undersides of leaves. Young larvae ascend the plants to enter the leaf whorls, where they start to feed (Sithole, 1988; Segeren *et al.*, 1991). Later instar larvae tunnel into stem tissue and pupate after 2-3 weeks. The larvae are cream coloured with four longitudinal stripes of spots along the body and a brown head capsule. In cold and/or dry conditions, larvae enter to diapause in stems, stubbles and other crop residues, where they may spend up to 6 months before pupating when favourable conditions recur during the next growing season (Harris, 1989a; Kfir, 1992; Segeren *et al.*, 1995). Segeren *et al.* (1991), reported that there are at least six generations per year.

2.3.2 The maize stalk borer, *Busseola fusca*

The female lays eggs in batches of 30-100, inserted between the leaf sheath and the stem (Harris, 1989a). After hatching, the larvae feed on the young blades of the leaf

whorl and then, suspended from silk strands, spread to neighbouring plants (van Hamburg, 1987). They penetrate into the stems by boring through the whorl base. Occasionally, they destroy the growing point and tunnel downward. After six to eight instars which take 30-45 days, they pupate in the tunnel. The larvae are cream to brown coloured with brown panancula (Sithole, 1988). According to the same author, depending on the agro-ecological conditions and the presence of suitable host plants, two or more generations per year may occur.

2.3.3 The pink stalk borer, *Sesamia calamistis*

The female lays up to 350 eggs (Ingram, 1958) in batches of 10-40, arranged in two to four rows and inserted between the lower leaf sheaths and stems. After hatching, the larvae leave the oviposition site and penetrate directly in the stems (Harris, 1962). Sithole (1988), reported that *S. calamitis* was a unique stemborer because its feeding habits were different from those of *B. fusca* and *C. partellus*. No feeding marks were found on the leaves of the host plant. During the larval stage, which lasts for 30-60 days, larvae may attack a number of young stems. The larvae are pink coloured with small brown pinnacula (Segeren *et al.*, 1991). Pupation takes place in the stem, or between the stem and the leaf sheath, and lasts for 10-12 days at 25°C. Two generations per year were observed in southern Africa (Sithole, 1988).

2.4 Stemborer species Distribution, composition and relative abundance

Lepidopteran stemborers occur regularly in maize and sorghum with different degrees of importance according to their abundance at each of the three agro-ecological zones in Mozambique. Sithole (1988), reported that the distribution and relative abundance of stemborers were significantly influenced by climatic factors. *C. partellus* was more abundant at low elevations where temperatures are high,

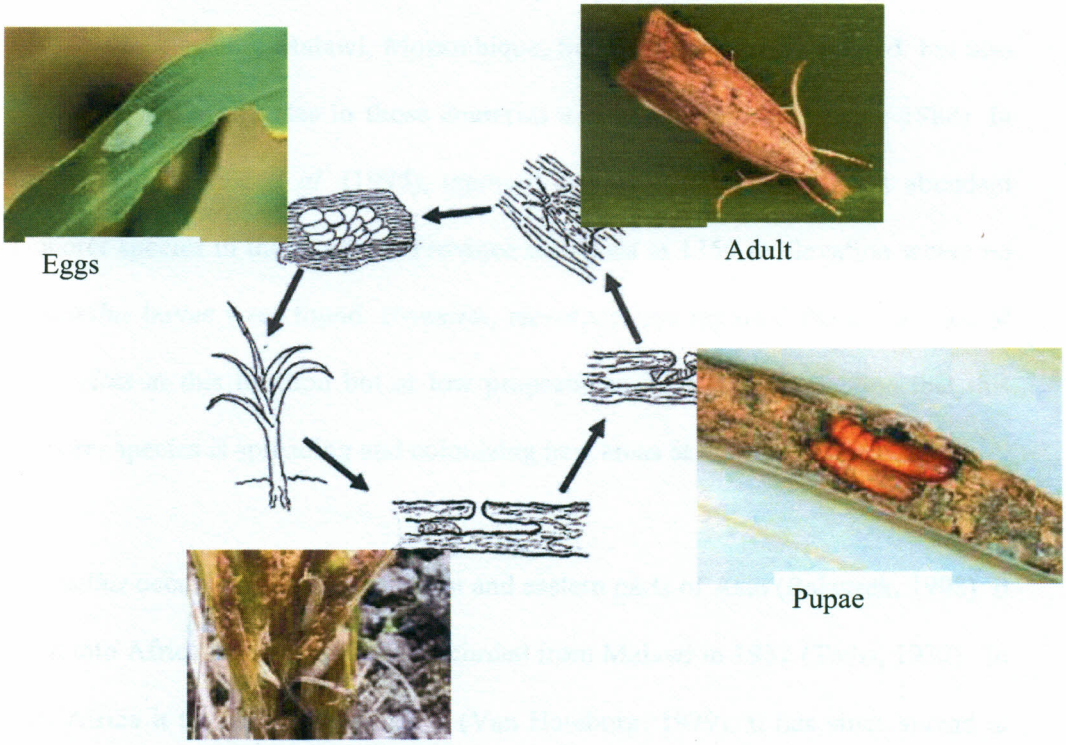

Plate 1 – General stemborer life cycle

whereas *B. fusca* was generally considered to be the most damaging stemborer at high elevation (>1500 m altitude) where temperatures are low. *S. calamistis* was most abundant at moderate elevations where temperatures are also moderate but rarely reaches damaging levels.

It was observed that *B. fusca* was the dominant stemborer at elevations above 900 m in Botswana, Lesotho, Malawi, Mozambique, South Africa and Swaziland, but also occurred at lower altitudes in those countries and in Zimbabwe (Sithole, 1988). In Mozambique, Davies *et al.* (1995), reported that *B. fusca* was the most abundant stemborer species in the Northern Province of Niassa at 1350 m elevation where no *C. partellus* larvae were found. However, recent surveys reported the occurrence of *C. partellus* at this location but at low proportions. This is an indication that this stemborer species is spreading and colonizing new areas at high elevation altitudes.

C. partellus occurs throughout southern and eastern parts of Asia (Polaszek, 1998). It spread into Africa where it was first recorded from Malawi in 1932 (Tams, 1932). In South Africa it first appeared in 1958 (Van Hamburg, 1979). It has since spread to most of the lowland areas (below 1500 m) within countries in eastern and southern Africa (CIE, 1989).

After its introduction, *C. partellus*, became the most destructive pest of maize and grain sorghum in the warm, low-altitude regions of southern Africa (Van Hamburg, 1979). In South Africa, it was first regarded to be mainly a pest of sorghum, but later became increasingly important in maize (Kfir, 1992).

In Mozambique, infestation levels of 100% have been reported in the areas where *C. partellus* is the most abundant species (Berger, 1981; Segeren *et al.*, 1991; Cugala *et al.*, 1999). *C. partellus* is the most abundant stemborer species at lower altitudes (0 - 200 m) and in warm zones, while *B. fusca* dominates at higher altitudes (1000 - 1500 m) and cooler areas (Sithole, 1988; Davies *et al.*, 1995; Segeren *et al.*, 1995). *S. calamistis* is of minor importance at all elevations (Segeren *et al.*, 1995) and cannot be considered an economic pest species in Mozambique. The three stemborer species were found occurring in the same area at medium to higher elevation zones (500-900 m altitude) (Cugala *et al.*, 1999; 2001; Cugala and Omwega, 2001).

2.5 Host plants of stemborers

The original host plants of all cereal stemborers were wild grasses. However, Harris (1989b), reported that maize and sorghum were the most important cereal crop hosts and to a lesser extent pearl millet and sugarcane.

The three stemborers species have different preferences for host plants. It has been reported that although *C. partellus* causes severe damage and losses in maize, it prefers sorghum as its host plant, while *B. fusca* prefers maize (Sithole, 1988; Kfir, 1998). *S. calamitis* was found to be associated with *C. partellus* in the same host plants (Cugala *et al.*, 1999; 2001; Cugala and Omwega, 2001).

In addition to the cultivated plants, several non-cultivated wild host plants were recorded as alternative hosts for the stemborers (Ingram, 1958; Gonçalves, 1970; Khan *et al.*, 1997). Some authors have argued that alternative hosts are detrimental because they serve as a stemborer reservoirs (Ingram, 1958; Harris, 1962), whereas others have pointed out that natural enemies can persist and increase their

populations during the non-growing season in the alternative stemborer host plants (Overholt, 1999). Additionally, Khan *et al.* (1997) suggested that certain wild host grown in proximity to maize fields would act as trap plants. Similarly Schulthess *et al.* (1997) found a negative relationship between the abundance of wild hosts and stemborer infestation in maize fields, suggesting that wild hosts may divert stemborers away from maize.

2.6 Damage and yield losses due to stemborers

2.6.1 Stemborers damage

Stemborers are of the greatest importance as pests of maize and sorghum in Africa. The three stemborer species attacking maize and grain sorghum in Mozambique produce similar symptoms of damage (Sithole, 1988; Segeren *et al.*, 1991). Damage is caused by larvae, which at first feed on the young leaves (*C. partellus* and *B. fusca*) (Berger, 1993), but soon tunnel into the stems (Seshu Reddy, 1998; Harris and Nwanze, 1992). *S. calamistis* bores into stems with little or no leaf feeding, which is different from the other stemborers which leave feeding marks in the leaf plants. During the early stages of crop growth, larvae may kill the growing points, resulting in the symptom called `deadheart' and consequent loss of crop stand. At later stages of crop growth, extensive tunnelling inside the stems occur (Harris and Nwanze, 1992).

2.6.2 Yield losses due to stemborers

Crop infestation and subsequent damage by lepidopteran stemborer larvae are a major constraint in the production of cereal crops. Crop loss is a reduction in both quantity and quality of the yield.

Plate 2 – Typical damage of stemborers in maize: a) dead heart symptom, b) moth exit hole, c) damage on inflorescence and d) damage on cob

Differences in maize and/or sorghum yields have been associated with the time of stemborer infestation, stage of crop development and geographical locations (Seshu Reddy, 1998). In Mozambique, the infestation levels can be very high and fields with 100% of plants infested are frequently observed in southern Mozambique where *C. partellus* is the most abundant stemborer (Berger, 1981; Cugala *et al.*, 2001). Yield losses due to stemborer attacks are reported to range from 20 to 40% on research stations, and more than 50% or a complete crop loss in the small scale farmers' fields (Segeren *et al.*, 1991).

In Nigeria, Usua (1968) reported that a stemborer density of 1 to 2 *B. fusca* larvae per plant reduced yield by about 25% while Harris (1962) observed grain losses of 26 to 28% due to *B. fusca* stem tunnelling. Ogwaro (1983) reported a complete yield loss when the infestation levels were 97% of plant infestation. In South Africa, yield losses of over 50% due to *C. partellus* infestations in maize and sorghum have been reported (Revington, 1986). In Kenya, Seshu Reddy (1985) obtained yield losses due to *C. partellus* ranging from 74.4 to 87.7%. Taneja and Nwanze (1989) observed grain yield reductions of 60% in sorghum when *C. partellus* infestations occurred between 15 and 30 days after crop emergence under conditions of no insecticide application.

Several methods have been used to assess yield losses caused by insect pests in the field. Several workers have used natural infestation in quantifying crop losses (Harris, 1962; Usua, 1968; van Rensburg and Hamburg, 1975; Seshu Reddy, 1985; Ampofo, 1988). The same author described yield losses (L) in relation to attack by insect (i) pests and expressed it as the reduction in potential maximum or pest free yield (Y_m) according to the following formula:

$$L = \frac{Y_m - Y_i}{Y_m} \times 100\%$$

L = percent yield loss
where Y_m = potential maximum yield
Y_i = yield under insect pest infestation

This method is often used to compare yields from insecticide protected or caged plots with unprotected or uncaged plots. Artificial infestation is also used and it involves mass rearing of the insect pest in the laboratory, and enables different levels of infestation by introducing known pest densities in the field at predetermined growth stage of the crop (Seshu Reddy, 1989; Ampofo, 1988; Taneja and Nwanze, 1989), and then its impact on yield is evaluated.

2.7 Control methods

The control of stemborers control by conventional means is difficult because the damaging larvae are protected inside the plant stems (ICIPE, 2000). They are vulnerable to pesticides and/or many natural enemies during the first instar stage, when they are feeding and exposed on the leaves before penetrating inside stems (Davies *et al.*, 1995).

Dent (1991), argued that instead of a single tactic, an emphasis should be given to the use of combined methods aimed to provide cheap but long-term reliability with the minimum of harmful side effects. Thus, Hahn and Caveness (1987), considered that the various methods of control must be viewed as complementary and not competitive or alternative approaches. The challenge is to find the best combination of methods for a given agricultural system.

2.7.1 Chemical control

The use of insecticides for the control of *C. partellus* in maize results in significant reduction of infestation, and an increase in yield, when applied to plants 3-5 weeks old (Berger, 1981; Segeren *et al.,* 1991). However, in the marginal rainfall areas of southern Africa, because of low profit margin, farmers cannot afford the cost of chemical control against stemborers (Kfir, 1998). Synthetic insecticides have been shown to be effective for the control of *C. partellus* when properly used and in repeated applications. The timing of insecticide application is crucial, as control measures are effective against young larvae only. Older larvae penetrate the stalks and are difficult to control with insecticides (Kfir, 1998).

In Mozambique, insecticides (Cypermethrin 20 EC) have been used on large scale farms (Ariyanayagan, 1983; Jimenez and Mugabe, 1990), but there is little or no use of insecticides by small scale farmers (Leeuwen and Zucula, 1987) because of the high cost and hazardous effects on environment and human beings (Berger, 1994; Kfir, 1995; Overholt, 1995). Non-selective insecticides kill not only the target pests, but also natural enemies and other non-target organisms, and can induce the development of pesticide resistance (Gifford and Mann, 1967).

In addition, chemical controls must be carefully timed to coincide with the limited period when early instar stemborer larvae are found outside of plant tissues (Segeren *et al.,* 1991). Chemical control should be applied if all other integrated pest management tactics sach as cultural control, host plant resistance, natural enemies, are unable to keep an insect pest population below an economic threshold, (DeBach and Rosen, 1991).

2.7.1.1 Economic threshold levels for stemborers infestation

The economic threshold levels (ETL) is defined as the level of damage at which control measures should be implemented to prevent an increase on pest population from reaching the economic injury level (Dent, 1991). Economic threshold models are dynamic and can be influenced by several factors such as yield potential of the crop, cost of insecticides application and market value of the yield (van den Berg *et al.*, 1997).

The concept of economic threshold levels (ETL) serves as an important tool in the decision making on the management of stemborers species. The importance of ETL in formulating any pest management programme has been highlighted by (Dent, 1991; van den Berg, 1998; Pedigo, 1999). Despite its great significance, it is often the weakest component of pest management programme (van den berg and Nur, 1998).

Seshu Reddy and Sum (1992) indicated large differences in the economic injury level of *C. partellus* on maize in Kenya, based on the price of various insecticides. They found the ETL for *C. partellus* on maize in Kenya to be when the mean infestation level per plant was 3.2 and 3.9 larvae per plant at 20 and 40 days after crop emergence respectively.

The ETL for stem borer can also be defined in terms of incidence of plants with visible damage, since this is a non-destructive method; it was considered a practical method of establishing infestation levels (van den Berg *et al.*, 1997). In South Africa, the ETL for the control of *B. fusca* in commercial maize farming systems is when 10% of plants show damage symptoms. Sithole (1994) reported a 16% of plant

infestation by *B. fusca* as ETL in Zimbabwe. This was considered the larval infestation levels that had the potential to cause economic yield loss if chemical control was not applied.

In South Africa, the ETL for *C. partellus* was estimated as 40% of plants showing symptoms of larval feeding in the whorls (van den Berg, 1998). The reasons for the differences between the ETL values for *B. fusca* and *C. partellus* are due to the lower comparative injuriousness of *C. partellus* on maize in South Africa (Bate and van Rensburg, 1992). van den Berg and Nur (1998) considered that these ETLs values are too low and often reached, but they provide for the timely application of control measures and to exclude control measures during the periods of low population levels.

In Mozambique, very few studies have been conducted on ETL especially on maize and sorghum stemborers. However, the ETL has been considered in some commercial seed production farms as an average of 10 out of 100 plants per hectare showing damage symptoms in the whorl. The practice has been to apply insecticides in a pre-determined schedule according to the crop stage starting at four weeks after crop emergence and repeated each two weeks, with a total of four applications during the crop development period (Segeren *et al.*, 1991). This may lead to unnecessary increase in costs of insecticide acquisition and application to farmers.

2.7.1.2 Economic threshold levels on small scale farmers' situation

The application of the ETL concepts to the small scale farmers is questionable, because the use of chemical control is not common. Infestation levels higher than

10% are common in maize or sorghum fields of small scale farmers throughout Africa and ETLs does not apply to these farmers (van den Berg and Nur, 1998).

2.7.2 Cultural control

There are many agricultural practices that make the environment less favourable to insect pest population. Cultural practices such as sanitation (burning the crop residues, cleaning and/or burning wild hosts around the fields), intercropping and management of sowing date have been recommended for stemborer control (Oever, 1990; Seshu Reddy, 1998). The destruction of crop residues after harvest may decrease the abundance of stemborers since these insects spend their entire immature life on or in plants. However, cultural practices often must be practised on a wide scale in order to be effective. The destruction of wild host plants in the proximity of fields has also been suggested as one means of suppressing the density of stemborers (Khan *et al.*, 1997; Kfir, 1998).

Harris and Nwanze (1992) and Kfir (1998), reported that crop residues are important for carrying over stemborer populations from one growing season to next. In rural areas where dry stems are used for fencing and building, it may not be possible to reduce carry-over populations. However, in commercial farms, it might be possible to reduce the pest status of borers by destroying their refuge sites, involving all farmers in a region, because moths emerging from untreated fields can infest adjacent crops (Kfir, 1992). However, destroying crop residues and wild host plants by burning will suppress off-season survival of stemborers and parasitoids, but will result in a loss of nitrogen.

Intercropping maize or sorghum with non-host plants has been recommended to manage stemborer populations. Intercropping is already practised in many low-input agricultural systems in Africa, but stemborers are still causing severe losses (Overholt *et al.*, 1994). For example, Segeren *et al.* (1995), reported no differences on the stemborer infestation in a maize monoculture and in a maize/cowpea intercrop.

Sowing date could be used to avoid severe borer infestations. Segeren *et al.* (1991; 1995), found that maize sown early in the season was less affected by *C. partellus* than maize sown later, because early in the season, the *C. partellus* populations is still low and the susceptible crop stage did not coincide with the peak of stemborer density. However, sowing date is strongly dependent on rainfall, and cannot be greatly modified. Khan *et al.* (1997), found that surrounding maize with Napier grass and intercropping with the legume *Desmodium* reduced stemborer infestation on maize in Kenya. The female moths are trapped on the susceptible trap plants surrounding the main crop and at the same time are repelled from the main crop by the repellent intercropped plants.

2.7.3 Host plant resistance

Dabrowiski and Nyangiri (1983), considered host plant resistance as a pest management tactic that is economical and demands little or no change in farmer practices. Several cultivars of maize and sorghum with some degree of resistance to *C. partellus* have been identified.

Host plant resistance offers a long-term solution to the management of stemborers and is the most compatible with other control methods in Integrated Pest Management (IPM) Programmes (Harris and Nwanze, 1992). Resistant cultivars of

maize and sorghum against cereal stemborers could play an important role in IPM programmes.

The resistance mechanism, which is based on antibiosis and antixenosis, apparently causes stress in stemborers larvae, making then more susceptible to the insecticides (van de Berg, 1994). Other mechanisms involved in *C. partellus* and *B. fusca* resistance in maize and sorghum include reduced feeding, reduced tunnelling, tolerance of plants to leaf damage, deadheart and stem tunnelling (Seshu Reddy, 1998). However, currently there are no available maize and sorghum varieties resistant to stemborer's infestation that are agronomically acceptable by farmers in Mozambique (van de Berg, 1997).

Efforts have been made in Africa to identify sources of stemborers resistance in cereal crops, but high levels of resistance have not been found. However, resistant lines or hybrids with good general combining ability have been identified and are uder field trials. For example, hybrids of sorghum bred in South Africa exbibited greatest tolerance to stem borer damage and therefore suffered low yield losses (Kfir *et al.*, 2002).

2.7.4 Biological control

DeBach and Rosen (1991), defined biological control as "the use of predators, parasitoids, nematodes, and pathogens to maintain the density of a species at a lower density than would occur in their absence" that is different from natural control that is "the collective action of environmental factors to maintain the members of a population within certain upper and lower limits over a period of time".

Natural control refers to both the biotic and abiotic agents, whereas biological control refers only to biotic agents. DeBach and Rosen (1991) argued that biological control should include other pest management tactics such as host plant resistance and the release of sterile insects. However, they stated that for the purpose of consistency and clarity, it was more useful to limit the concept of biological control to the use of natural enemies to regulate pests.

2.7.4.1 Indigenous natural enemies

A wide range of egg, larval and pupal parasitoids of stemborers has been recorded from the stemborer species in maize, sorghum and sugar cane in Africa (Kfir, 1992; 1995; 1997a; Polaszek, 1998). Several native parasitoid species attack three of the four stages in the life cycle of stemborers including species that attack eggs, larvae and pupae parasitoids (Gonçalves, 1970; Cugala and Omwega, 2001; Cugala *et al.*, 1999; 2001).

The most abundant and widespread parasitoids in Mozambique are the egg parasitoids *Trichogramma spp.*, the larval parasitoids *C. sesamiae*, *Stenobracon* (=*Euvipio*) *rufa*, *Chelonus curvimaculatus* and *Sturmiopsis sp.*, and the pupal parasitoids *Pediobius furvus* Gahan (Hymenoptera: Eulophidae), and *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae). Gonçalves (1970), Berger (1981), Segeren *et al.* (1991) and Davies *et al.* (1995), reported the gregarious larval parasitoid, *Cotesia sesamaie* Cameron (Hymenoptera: Braconidae) as an important mortality factor. Gonçalves (1970) recorded 60% egg parasitism in *C. partellus* and Davies *et al.* (1995) 20% larval parasitism in *B. fusca* in Mozambique. In South Africa, Kfir (1995) reported a peak parasitism of 70% on *B. fusca* larvae and 100% on pupae.

Despite the high parasitism levels by native parasitoids, they are considered not to be able to prevent economically significant damage (Kfir, 1992; Kfir and Bell, 1993; Overholt *et al.*, 1994). However, Kfir, (1992) speculated that native egg, larval and pupal parasitoids play an important role in curtailing stemborer populations and that without their activities, annual yield losses would be much higher. Mohyuddin and Greathead (1970) concluded that indigenous natural enemies have a potential as biological control agents against cereal stemborers.

In general, indigenous natural enemies are not able to keep stemborer populations below economic injury levels (Overholt *et al.*, 1994; Seshu Reddy, 1998). However, there are no detailed and published results available on their impact on the stemborer populations in Mozambique.

2.7.4.2 Classical biological control

The introduction of natural enemies strategy is often referred to as 'Classical Biological Control' (CBC) and rests on the premise that many organisms (insects and weeds) became pests because they have been introduced or spread to new areas, leaving behind their coevolved natural enemies (Overholt, 1993; Debach and Rosen, 1991).

The introduction approach refers to importing and introducing new species of natural enemies into the system. This approach is generally considered to be appropriate for the control of introduced pests, such as *C. partellus* (Overholt, 1993). It involves identification of the pest's natural enemies in the area of origin and the introduction of these into the pest's new home. If successful, the natural enemy will become

established and maintain the pest at a lower density than would occur in the absence of the natural enemy (Debach and Rosen, 1991).

Classical biological control is viewed as a potential strategy for stemborer population management, particularly against *C. partellus*, because of its status as an introduced pest. Because of the low impact of native natural enemies on the stemborer populations, a biological control program was initiated in 1968 in Africa. Many parasitoid species were imported and released in Kenya, Uganda and Tanzania, but none of the species established (CIBC, 1968-72) and stemborers continued to be important pests of maize and sorghum. The International Centre of Insect Physiology and Ecology (ICIPE) is leading a project on the introduction of *C. flavipes* into eastern and southern Africa. *C. flavipes* is a parasitic wasp that attacks *C. partellus* throughout Asia and is now established in Kenya (Omwega *et al.*, 1995). Results from Kenya show that the parasitoid population has increased each year since it was first released in 1993 (Zhou *et al.*, 2001).

After establishment, *C. flavipes* has spread from the release sites and has colonized new areas. The parasitoid's impact has recently been evaluated, and a reduction of 37% of the total stemborer population and a 53% of *C. partellus* population has been observed in some areas (Zhou *et al.*, 2001). *C. flavipes* has also been released in other African countries such as Uganda, Zanzibar, Somalia, Mozambique, Malawi, Zambia and Zimbabwe. *C. flavipes* establishment has been reported from Uganda, Mozambique and Malawi (Kfir *et al.*, 2002).

2.8 Biological control of cereal stemborers in Mozambique

Early studies of stemborers in Mozambique by Gonçalves (1970), Berger (1981) Segeren *et al.* (1991) and Davies *et al.* (1995), reported the gregarious larval parasitoid, *C. sesamaie* as an important mortality factor. However, all findings led to the similar conclusion that the native natural enemies were ineffective in controlling stemborer populations. In South Africa, Kfir (1992), recorded high parasitism levels of *B. fusca* pupae by the parasitoid *Procerochasmias nigromaculatus* (Cameron). In Mozambique, Gonçalves (1970) recorded 60% egg parasitism of *C. partellus* and Davies *et al.* (1995) 20% larval parasitism on *B. fusca*.

Despite the high levels of parasitism levels, paraistoids did not prevent economically significant damage on maize and sorghum (Kfir, 1992). The following parasitoids have been recorded in Mozambique, the egg parasitoids *Trichogramma sp.*, the solitary ovolarval endoparasitoid *Chelonus curvimaculatus*, the larval parasitoid *Stenobracon* (=*Euvipio*) *rufa* and the pupal parasitoids *Pediobius furvus*, *Dentichasmias busseolae* (Cugala *et al.*, 1999). All parasitoids listed above are indigenous to Africa, and their association with the exotic stemborer *C. partellus* is relatively new, they attack *C. partellus* as a new alternative host (Kfir, 1992).

During country-wide surveys in Mozambique parasitism was generally low, but several parasitoids were reared from the stemborers collected.. *Cotesia sesamiae* (Hymenoptera: Braconidae) was the most common larval parasitoid at most locations, but typically accounted for less than 5% parasitism (Cugala *et al.*, 1999).

Based on the finding that *C. partellus* was the dominant stemborer in many areas of Mozambique, and that parasitism by native parasitoids was generally very low, it was

decided to make releases of *C. flavipes*. The first releases were made at two sites in southern Mozambique in early November 1996 (Cugala *et al.*, 1999). Additional releases were made in the following years of 1998 to 2002 in several locations in the southern and central regions Mozambique.

During post release surveys, the parasitoid was recovered from all sites sampled 1 to 3 years after its first introduction, indicating that this exotic parasitoid had established in the southern and central regions of Mozambique and was spreading to new areas where it had not released.

2.9 Parasitoids of cereal stemborers

2.9.1 *Cotesia spp.* parasitoids

Cotesia species are among the most important natural enemies of cereal stemborers (Kimani-Njogu and Overholt, 1997). Three species are considered to be the most important, and these are *C. flavipes* from the Indo-Australian region, *C. sesamiae* from Africa and *Cotesia chilonis* (Matsumura), which has only been recorded from Japan and China (Kimani-Njogu and Overholt, 1997). The three species have been grouped together in the *C. flavipes* complex (Polaszek and Walker, 1991), although there is still some confusion regarding their taxonomic status (Kimani-Njogu and Overholt, 1997). All the three species are morphologically similar.

Cotesia flavipes is a gregarious endoparasitoid of pyralid and other stemborer larvae. Females lay eggs in the host's body cavity. About 40 eggs are laid in each host (Potting *et al.*, 1997). First instar parasitoid larvae hatch after 3 days and begin feeding internally. *C. flavipes* develops through three larval instars in the host body, and then emerges from the host by chewing through the integument. The egg-larval

period lasts about 14 days at 25°C. After emergence from the host, the last instar larvae spin cocoons and pupate. In the field, the cocoons can be found inside host feeding tunnels in graminaceous plants. The pupation takes about 6 days at 25°C, after which adults emerge.

The egg to adult development time is about 20 days (Ngi-Song *et al.*, 1995) and the sex ratio is usually female biased (60% - 70%). The adults are small wasps of about 3-4 mm in length. The length of antennae differentiates males from females. The antennae of males are approximately twice the length of the female antennae. The adult life span is short, approximately 34 hours at 25°C (if adults are not fed) and/or 51 hours (if adults are fed on 20% honey/water solution). Because of the short life span, *C. flavipes* must quickly mate after emergence and begin searching for hosts (ICIPE, 2000). Fertilised eggs (diploid number of chromosomes) become female and male, and unfertilised eggs (haploid number of chromosomes) become males.

2.9.1.1 *Cotesia spp.* host finding

Lews *et al.* (1990), highlighted the ability of parasitoids to locate and attack their hosts as a key factor which determines how a parasitoid population performs. Once a female *Cotesia spp.* has located a stemborer infested plant, it has to locate the host (stemborer larva) inside the plant stem. Host frass is an important cue in host finding. Laboratory work showed that hosts from natural diet (maize or sorghum) were much attractive to parasitoids than hosts from artificial diet (Ngi-Song *et al.*, 1999). Other studies demonstrated that larval frass, caterpillar regurgitations and holes in the stem were used in host location by *Cotesia* parasitoids.

Takasu and Overholt (1997), reported that after locating the exit hole of the stemborer tunnel, where larval frass has accumulated, the parasitoid female tries to enter the stemborer tunnel. This can take a long time because the tunnel is often blocked by larval frass and the female some time has to squeeze through small holes. Walker (1994), considered the dorso-ventrally flattened shape *of Cotesia flavipes* complex as an adaptation to facilitate the entrance in the tunnel.

A female parasitoid has a high probability of being bitten to death when it approaches the host towards the head (Takasu and Overholt, 1997). However, the majority of the females are able to successfully parasite the host before being killed. A female *C. flavipes* needs only a few seconds to inject the eggs into its host (Takasu and Overholt, 1997). Each female *C. flavipes* has around 150 eggs available for oviposition and can parasiteze 3 to 4 hosts.

2.9.1.2 *Cotesia spp.* Host range

A parasitoid can have preference for particular host or plant species. Parasitoids may distinguish between the odours of two different host species feeding on the same plant. However, Kimani-Njogu and Overholt (1997) did not find differences in *C. flavipes* searching time on plants infested with *C. partellus* and *B. fusca*. Similarly, *C. flavipes* did not make distinction between stemborer species feeding on the same plant species. They concluded that *C. flavipes* was not specific with regard to the host species, but was host habitat specific. The volatile and contact stimuli released by maize plants being fed on by different species of stemborers are very similar, and the parasitoid will attack all stemborer species found in the stem.

A recent survey in Mozambique revealed that stemborers often occur sympatrically in species complexes. Thus, it is likely that any introduced natural enemy will encounter not only the target species, but also other stemborers. *C. flavipes* does not appear to discriminate between different species of stemborers and will readily oviposit in not only *C. partellus*, but also in several native African stemborers including *Chilo orichalcociliellus*, *S. calamistis*, *B. fusca* and *Eldana saccharina* (Ngi-Song, *et al.*, 1995; Overholt *et al.*, 1997). The exotic, *C. flavipes* has been recovered from native stemborer species *B. fusca* and *S. calamistis* in Mozambique (Cugala *et al.*, 1999; 2001), Uganda (Matama-Kauma, 2000) and Ethiopia (Emana, 2001).

Thus, the ability of *C. flavipes* to establish in a new area will largely depend on their ability to develop in the stemborers present. A species complex, which includes the target species (*C. partellus*), and non-target species (*B. fusca* and *S. calamistis*), may preclude establishment, as many of the parasitoid progeny will be encapsulated inside the non-suitable host. Field investigations where *C. flavipes* is released in areas where *C. partellus, B. fusca*, *S. calamistis* and other lepidopteran larvae occur may provide greater insight into the effect of *C. flavipes* on the target and non-target stemborer species under field conditions in Mozambique.

2.9.2 Pupal parasitoids: *Dentichasmias busseolae* and *Xanthopimpla stemmator*

2.9.2.1 *Dentichasmias busseolae*

The pupal parasitoids are widespread in southern Africa. The most common are *D. busseolae* and *Pediobius furvus*. *D. busseolae* is a solitary pupal endoparasitoid that attacks cereal stemborer pupae in Africa. The *C. partellus* is considered as its main host. Bahana (1990) recovered *D. busseolae* only from *C. partellus* pupae and not from *Eldana saccharina* or *B. fusca* which occurred together with *C. partellus*, thus

it was thought that the parasitoid was monophagous to *Chilo* species under field conditions. The adult parasitoid emerges from the host pupa. The first record of this species as a parasitoid of stemborers was made by Ingram (1958) from *C. zonellus* (=*partellus*) in Uganda where it performs well in dry areas.

Bahana (1990) reported that the rate of development of *D. busseolae* was shortest at high temperature (30°C) and lowest at low temperature (15°C). However, it could complete its development at 10°C and that there was high mortality at 35°C. Thus, the author concluded that the parasitoid could develop within the temperature limits where *C. partellus*, its main host, exhibited optimum development and survival.

In Western Kenya, it was reported that *D. busseolae* and *P. furvus* were the most common pupal parasitoids found and that the lack of host availability during the dry season was thought to limit their incidence in the area (Bahana, 1990). Thus, it was concluded that indigenous natural enemies were unlikely to have an impact on the pest population in the area.

2.9.2.2 *Xanthopimpla stemmator*

Xanthopimpla stemmator is a solitary pupal endoparasitoid of stemborers. It has wide geographical distribution and occurs in warm low altitude areas. The adult parasitoid locates the pupal chamber in the stem and drills through the plant wall. Eggs are laid in the pupal chamber but only one develops into the next larval stage and then to the adult wasp. Pupation takes place in the host pupal chamber and the adult parasitoid emerges from the pupal case.

Xanthopimpla stemmator was introduced into Mauritius from Sri-Lanka in 1939 and became established against sugar cane borer, *Chilo sacchariphagus* and *S. calamistis* (Moutia and Courtois, 1952). It was also introduced into Reunion from Mauritius in 1962 where it successfully established on *C. sacchariphagus* in sugar cane.

Attempts have been made to introduce it from Mauritius into South Africa against *E. saccharina* in sugarcane (Conlong, 1994) and against *C. partellus* in maize and sorghum (Kfir, 1992). The species was recovered a few times after releases, but it did not permanently establish. Recently, *X. stemmator* was introduced into Mozambique from South Africa previously imported from Mauritius against *Chilo sacchariphagus* in sugar cane and its establishment was reported at the release location (Conlong and Goebel, 2002).

Xanthopimpla stemmator is a pupal parasitoid that readily accepts and attack pupae enclosed in a stem or wrapped tissue but rarely attack pupae that are naked (Smith *et al.*, 1993). However, no studies have so far been conducted on its ecological impact on native pupal parasitoids, such as *D. busseolae* in Sourthen Africa.

2.9.3 Life table statistics

According to Maia *et al.* (2000), life tables are appropriate to study the dynamic of animal population, especially arthropods, as a process for estimating parameters related to the population growth potential. The methods for construction, description and analysis of life tables for animal population have been well studied and described. The population growth potential of insects can be used as an indicator in studies that aim to assess environmental effects of agricultural technologies and practices such as the assessment of potential biological control agents. The

parameters usually estimated from life tables includes net reproductive rate (Ro), the intrinsic rate of natural increase (r_m), the mean generation time (T), the doubling time (Dt) and the finite rate of increase (λ).

Net reproductive rate (Ro) is the mean net contribution per female to the next generation, expressed in total of offspring females per female during the entire oviposition period. It represents the expected number of young per individual entering the population and gives an indication of potential population growth. Population must grow if Ro>1 and Ro<1 is an indication of a declining population. The mean generation time (T) is the mean time span between the birth of individuals of a generation and that of the next generation. Doubling time (Dt) is the time span necessary for doubling the initial population. The finite rate of increase (λ) is a multiplication factor of original population at each time period. It represents the number of individuals added to the population per female/day

These parameters provide data on the parasitoids, which may be useful in planning in their utilization as biological control agents against cereal stem borers in biological control programmes.

CHAPTER THREE

3.0 INCIDENCE OF CEREAL STEM BORER EGG PARASITOIDS AND THEIR RELATIVE IMPORTANCE IN SMALL SCALE FARMER'S FIELDS

3.1 Introduction

The importance and advantages of biological control methods in pest management strategies for crop protection are well recognized. Egg parasitoids are among the most important biological control agents of a number of major insect pests including stemborers of maize and sorghum (Temerak, 1981).

In Mozambique, little attention has been given to indigenous natural enemy species and especially egg parasitoids. Egg parasitism has been considered to be an important naturally occurring mortality factor affecting stem borer populations as the pests are killed before they damage the crops (Temerak, 1981). In West Africa Setamou and Schulthess (1995) indicated that egg parasitism due to *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) was among the major factors that contributed to the low *S. calamistis* densities found. In Mozambique, *C. partellus* egg parasitism of 60-80% by *Trichogramma sp.* were reported, but the data were rather scanty (Gonçalves, 1970, Berger, 1981). Thus, the present study aimed at evaluating the incidence and relative abundance of egg parasitoids in maize fields in three agro-ecological zones in Mozambique.

3.2 Materials and Methods

3.2.1 Site description

Surveys were conducted during 2003/04 growing season, in small-scale farmers' fields at several locations in the southern region lowland area at Guija, Nhassune and Nhamatanda (<200 m asl), with mostly *C. partellus* and some *S. calamistis*; in the central region mid to high elevation area at Chimoio and Manica (500 to 900 m altitude asl), where *C. partellus* and *B. fusca* occur with proportions of 61% and 32%, respectively, and few *S. calamistis* and in the northern region high elevation area (>1300 m asl) at Lichinga dominated by *B. fusca* (>90% of the stemborer population) and some *S. calamistis* and *C. partellus* .

3.2.2 Sampling procedures

At each location, 20 to 30 maize fields in the vegetative stage (i.e., a total of 134 fields) were selected at approximately 500 m intervals. From each field, 50 plants were randomly selected and inspected for stem borer egg batches. Destructive sampling was done in areas where *B. fusca* is dominant, as the egg batches are hidden within the leaf sheath. Egg batches collected were placed individually in glass vials and after counting, the eggs were taken to the laboratory and reared until stemborer larvae borer or adult parasitoid emerged (Setamou and Schulthess, 1995). Specimens of parasitoids obtained were sent to South African Sugar Research Institute (SASRI), South Africa and INRA (Entomology and Biological Control Unit), Antibes, France, for species identification. Parasitism was calculated as means of percent egg batches parasitized; percentage of eggs parasitized per field and percentage of eggs parasitized within parasitized egg batch.

3.2.3 Data analysis

To compare the various sampling sites, data were subjected to analysis of variance (ANOVA) (PROC GLM, SAS Institute, 1999) and means separated by Student-Newman-Keuls (SNK) multiple range test when ANOVAs were significant ($P < 0.05$). In order to normalize the data, percent parasitism and the number of insects were subjected to square root and logarithmic transformation before analysis. The relationships between the number of egg per plant, percent of egg parasitism, percent of plant infestation and number os stem borer larvae per plant were performed using correlation matrix analysis (PROC CORR and REG, SAS)

Egg batch distribution patterns within sampling sites were assessed by Taylor's Power Law (Taylor, 1961). This law postulates a consistent relationship between variance S^2 and mean m:

$$S^2 = am^b \quad \ln S^2 = \ln a + b \ln m$$

where b is a measure of dispersion of the species, with $b > 1$ indicating an aggregated distribution, $b = 1$ randomness, and $b < 1$ regular distribution, while a is considered a mere scalar factor without biological meaning. These coefficients were computed by regressing the natural logarithm of the plant variance ($\ln S^2$) against the natural logarithm of mean density ($\ln m$), for each field or sampling occasion.

3.3 Results

3.3.1 Stemborer egg distribution and abundance

C. partellus was found to be the most abundant stem borer species damaging young maize plants at the most of locations in Mozambique. *C. partellus* eggs were collected from all fields at the southern lowland areas while *B. fusca* eggs were found only at the northern high altitude site of Lichinga. No parasitoids were recovered

from *B. fusca* egg collected at the later site. Thus *B. fusca* data were excluded from the analyses. Significantly higher (P<0.05) *C. partellus* egg batch density was found in the lowland site of Nhassune, followed by Chimoio, Nhamatanda and Guija, while the lowest was found in the mid-altitude site of Manica (Table 3.1).

Egg batch size was smallest at Manica and it was significantly low compared to the other sites, while the largest was reported at Chimoio. The percent of plant with egg and the number of eggs per plant were significantly higher ($P<0.05$) at the southern lowland site of Nhassune compared to Guija, Nhamatanda and Manica. There were no significant differences among the sampling sites on the egg batch per plant (Table 3.1).

Table 3.1 – Mean *C. partellus* egg incidence and density on small scale farmers' field in Mozambique (±SE)

Location	Egg batch/ field	Egg batch size	Percent plant with eggs	Egg batch/ plant	Number eggs/plant
Guija	6.3±0.47b	25.1±0.55a	11.5±0.53b	0.13±0.12a	3.2±0.94b
Nhassune	10.9±0.52a	23.8±0.16a	25.1±0.46a	0.22±0.30a	8.3±1.07a
Nhamatanda	5.1±0.59b	34.4±0.69a	10.1±0.74b	0.10±0.21a	3.5±0.97b
Chimoio	7.1±0.62b	40.0±0.27a	14.2±0.71b	0.14±0.02a	4.9±1.99ab
Manica	0.3±0.21c	9.5±0.31b	0.6±0.35c	0.00±0.0a	0.2±0.00c

Mean values with the same letter within a column are not statistically different ($P=0.05$)

3.3.2 Distribution and abundance of egg parasitoids

Egg parasitoids were collected from four out of five sampling sites. They were identified as *Trichogramma bournieri* Pintureau and Babault (Hymenoptera: Trichogrammatidae). The percent egg batch parasitism varied from 0.0% at Manica to 95.9% at Nhassune (Table 3.2). A high egg batch parasitism indicates a good host finding capacity of the parasitoid. Egg parasitism varied significantly between

locations and was between 2 and 69.7% (P<0.05). Likewise, numbers of parasitoids emerging from parasitized eggs varied significantly between 1 and 2.3 (P<0.05).

Table 3.2 – Mean of *C. partellus* egg and egg batch parasitism (±SE)

Location	Egg batch with parasitoids	Egg batch parasitism	Egg parasitism	Adult parasitoid/ egg batch	Adult parasitoid/ egg
Guija	4.1 ± 3.3a	71.1 ± 2.8a	33.4±0.74b	8.8 ± 0.58b	1.2±2.10b
Nhassune	9.6 ± 3.9a	95.9 ± 5.6a	69.7±0.73a	29.8±0.69a	2.3±0.10a
Nhamatanda	0.5 ± 0.3b	7.8 ± 1.1b	2.0±0.63d	0.6 ± 0.39d	1.0±0.18c
Chimoio	3.3 ± 0.0	69.8±4.2a	18.3±1.47c	4.6 ± 0.98c	1.0±0.00c
Manica	0.0c	0.0c	0.0d	0.0d	0.0c

Values in the same column with the same letter are not statistically different (*P*=0.05)

With exception of Manica where b value was less than 1, the present study revealed b values greater than 1 at all the other sampling sites (Table 3.3), which indicated that the *C. partellus* egg batches had aggregated distribution within these sites.

Table 3.3 – Coefficients of Taylor's Power Law and *C. partellus* egg batches distribution patterns at different locations and host species

Location	n	Intercept (loga)	Slop (b)	r^2	P>F	Dispersion patterns
Chimoio	71	0.159	1.94	0.9762	0.0001	Aggregation
Guija	190	0.165	1.47	0.9174	0.0001	Aggregation
Manica	60	0.004	0.93	0.8848	0.1589	Uniformity
Nhamatanda	101	0.110	1.73	0.9051	0.0001	Aggregation
Nhassune	261	0.283	1.59	0.9132	0.0001	Aggregation

3.3.3 Correlation coefficients between egg abundance and plant infestation by stem borer larvae

The relationships between percentage of plant infestation by stem borer larvae and egg batch size, number eggs/plant and percentage of egg parasitism are presented in Table 3.4.

Table 3.4 – Correlation coefficients between various egg batch and egg parasitism parameters

	Egg batches	Ebs	Pp egg	eggbp	Pepara	Nept	P.inf. L
Egg batch size (Ebs)	0.2926 0.0233	1.000					
Percent plant with eggs (Pp. egg)	0.7894 0.0001	0.3401 0.0078	1.000				
Egg batch parasitism (eggbp)	0.6543 0.0002	-0.0450 0.7328	0.0497 0.7061	1.000			
Percent egg parasitism (Pepara)	0.4493 0.0003	-0.0287 0.8272	0.1608 0.2197	0.7527 0.0001	1.000		
Number eggs per plant (Nept)	0.7226 0.0001	0.3969 0.0017	0.9694 0.0001	-0.0166 0.8995	0.0893 0.4974	1.000	
Plant infestation by larvae (P inf. L)	0.3304 0.0099	0.1909 0.1439	0.3042 0.0181	0.4125 0.0011	0.3184 0.0131	0.2323 0.0740	1.000
Stem borer larvae per plant	0.0118 0.9287	-0.0547 0.6780	0.1365 0.2982	-0.0825 0.5307	-0.1444 0.2709	0.1376 0.2941	0.3395 0.0079

The first line within a row represents the coefficient of correlation (pairwise correlations) and the second line the significance probability (P=0.05)

The plant infestation by larvae was significantly positively correlated with percent plant with eggs (r^2=0.3042, P=0.0181), egg batch parasitism (r^2=0.4125, P=0.0011), percent egg parasitism (r^2=0.3184, P=0.0131) and plant infestation by larvae (r^2=0.3304, P=0.0099). However, plant infestation by larvae was positively correlated but non-significantly with egg batch size (r^2=0.1909, P=0.1439) and with number of eggs per plant (r^2=0.2323, P=0.0740).

Negative non-significant relationship occurred between stem borer larvae per plant and egg batch size (r^2=-0.0547, P=0.6780), percentage of egg parasitism (r^2=-0.1444, P=0.2709) and egg batch parasitism (r^2=-0.0825, P=0.5307) and positively with number of eggs/plant (r^2=0.1376, P=0.2941), but it was significantly positive with plant infestation by larvae (r^2=0.3395, P=0.0079) (Table 3.4).

3.4 Discussion

High plant infestation by eggs recorded at Nhassune a lowland area in the southern region of Mozambique where only *C. partellus* eggs were collected. Previous studies conducted by Gonçalves (1970) and Berger (1981) reported similar trends for the low-land areas (<500 m asl) and mainly in the southern region. Cugala (2002), stated that the high infestation levels observed in the southern region Mozambique may have been due to the continuous cultivation of maize that makes host available throughout the year.

In Mozambique, *C. partellus* egg parasitism of 60-80% due to *Trichogramma sp.* have been reported by Gonçalves (1970) and Berger (1981). *Trichogramma bournieri* was also reported parasitizing *Chilo sacchariphagus* eggs on sugar cane (Conlong and Goebel, 2002). In the present study, the high levels of parasitism on *C. partellus* eggs due to *T. bournieri* indicated that this parasitoid is the most abundant in the country and may constitute an important factor of stemborer natural mortality and control.

The aggregated distribution of *C. partellus* eggs found at most sampling sites was expected. Similarly, Overholt *et al.* (1994) reported that the distribution of *C. partellus* larvae using Taylor's Power Law was aggregated with b values ranging from 1.47 to 1.20, which are within the range of b values found in the present study.

Taylor (1961), argued that populations with high density and variability would produce b values higher than 1. Setamou and Schulthess (1995) working in Benin also argued that *S. calamistis* female adults recognize and avoid ovipositing on plants

already infested with eggs because overcrowding would either kill the plant or force the young larvae to disperse, which would lead to high mortality.

Ndemah *et al.* (2001) and Chabi-Olaye *et al.* (2005) reported that stemborer natural dispersion changed from regular at low batch density to random and aggregated at medium to high batch density. In the present study, data presented in Table 3.3 indicated that the highest b value was reported in Chimoio (b=1.94) where stem borer density was 2.7 larvae/plant and the lowest at Manica (b=0.93) with stem borer density of 2.0 larvae/plant. The b values greater than 1 suggest that stem borer egg batches were of high densities. The b value less than 1 (b<0.93) may have been due to the low density of *C. partellus* egg batches collected at Manica.

In the present study the collected egg parasitoids were identified as those of *Trichogramma bournieri*. Conlong and Goebel (2002) reared and identified the same species from *Chilo sacchariphagus* Bojer eggs collected on sugar cane at the Mafambisse and Marromeu Sugar States in Mozambique. *T. bournieri* was also reared from *C. partellus* eggs collected from maize in the Comoros (Bournier, 1993) and in Kenya (Bonhof, 2000; Haile *et al.*, 2002).

The results in the present study were very preliminary and did not allow for an assessment of the impact of *T. bournieri* on the populations of *C. partellus*. First larval stages of *C. partellus*, like *B. fusca*, migrate from the oviposition site on leaf blades or from within leaf sheaths, respectively, to the whorl, where they cause the typical 'window' damage. Thereafter they either penetrate into the stem or disperse to other plants. Since it can be expected that dispersal is density dependent, egg

parasitism would lead to decreased dispersal and fewer plants infested with stem borers.

Berger (1989) stated that egg batch size and the number of eggs/plant are likely to be of importance for the ballooning behaviour of newly hatched larvae, migration to non-infested plants and subsequent increase in the levels of plant infestation. However, *T. bournieri* is not very host specific and was found parasitizing both noctuid and pyralid species on various crops (Pintuerau and Babault, 1988), and even formed a new association with *C. partellus*. Thus, its impact on *C. partellus* is probably limited and the question arises if exotic species from the area of origin of the pest such as *Trichogramma chilonis* Ishii should be considered in a biocontrol program to complement the larval parasitoid *C. flavipes*.

Results on the relationship between egg abundance and plant infestation corroborate with results reported by Setamou and Schulthess (1995) who observed positive relationship between dead hearts and percentage of plant infested by eggs, and between percentage parasitism and number of dead hearts. They suggested that this may be due to a higher attraction of fields with high borer populations to the parasitoid. They also reported poor correlation between percentage of egg parasitism and number of *S. calamistis* larvae and concluded that this have been due to the fact that many stem borers had completed their immature development or had been killed by their natural enemies.

The current study is part of a regional program on biological control of cereal stem borer populations aimed at evaluating the role of indigenous egg parasitoids on the management of stem borer populations. High levels of parasitism have been

previously reported on *C. partellus* egg batches in Mozambique (Gonçalves, 1970; Berger, 1981). However, *C. partellus* continues to be the most destructive pest of maize in the country. It is not clear why the exotic stem borer is the most important pest of maize in the low and mid altitude zones of Mozambique despite the higher levels of egg parasitism due to *T. bournier*.

CHAPTER FOUR

4.0 ESTABLISHMENT AND SPREAD OF *Cotesia flavipes* CAMERON (HYMENOPTERA: BRACONIDAE) IN MOZAMBIQUE

4.1 Introduction

The exotic larval endoparasitoid, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), has been released in Eastern and Southern Africa as a biological control agent against cereal stem borers. In Mozambique, *C. flavipes* was released for the first time in 1996 for biological control of *C. partellus*. Other releases were made in the subsequent years in several locations in the southern and central provinces in the areas where *C. partellus* is the most abundant stem borer species.

Post release survey have revealed that *C. flavipes* has become established at all release sites with levels of parasitism being less than 10% and that the parasitoid is spreading to new areas (Cugala *et al.*, 1999; 2001; Cugala and Omwega, 2001). *C. flavipes* was recovered from all the three stem borer species (*C. partellus*, *B. fusca* and *S. calamistis*) found on maize in Mozambique.

There is no information on the distribution and abundance of *C. flavipes* and its possible impact on the target stem borers and other lepidopteran borer species in Mozambique which was the main aim of the present study.

4.2 Materials and methods

Surveys on stemborers and associated parasitoids were conducted during the 2002/2003 and 2003/2004 growing seasons in Chibuto, Guijá, Ilha Josina Machel, Magude and Maracuene in the Southern Mozambique. All these locations are

lowland (<500 m asl) warm area, where previous work had shown that *C. partellus* was the dominant stemborer followed by *S. calamistis* (Segeren *et al.* 1991; Cugala *et al,* 2001) while *B. fusca* has not been recorded from this area.

At each location, 20 small-scale farmers' fields were selected within a ca 50 km radius. From each field, 20 maize plants were randomly sampled and inspected for borer damage symptoms. Plant growth and damage variables recorded included plant height, stem diameter, total number of internodes, number of internodes bored, number of borer holes, tunnel length, and percent ear damage and cob weight.

The plants were dissected in the field to assess the number of stemborers, then they were separated according to their respective species. Stemborer larvae and pupae collected were placed individually in vials. Fresh maize stem pieces were provided to larvae as food. The vials were plugged with cotton wool and taken to the laboratory and kept until adult moth or parasitoid emergence. All parasitoids that emerged were recorded according to the host from which they were reared and identified to species. *Cotesia spp.* were identified by the shape of the male genitalia (Polaszek and Walker, 1991).

4.2.1 Data analysis

Data from different locations were subjected to analysis of variance (ANOVA) (PROC ANOVA, SAS Institute, 1999) and means separated by Student-Newman-Keuls (SNK) multiple range test when ANOVAs were significant ($P<0.05$). The relationships between the plant growth parameters and damage parameters, stemborers and parasitoids abundance were determined using correlation matrix and regression analysis (PROC CORR and REG, SAS). The data on number of insects

and proportions were square root and arcsine of square root transformed respectively to normalize data before being subjected to analysis. Paired comparisons on *C. sesamiae* and *C. flavipes* abundance were performed using t-test (PROC TTEST SAS Institute, 1999)

4.3 Results

4.3.1 Stemborer damage and plant growth variables

Plant growth variables such as height, stem diameter and number internodes and damage variables including exit holes, stem tunnelling and cob damage are shown in Table 4.1. Significant differences ($P<0.05$) between locations were found for plant height, stem diameter, numbers of internodes, number of exit holes and stem tunnelling. Maracuene tended to have the highest values for plant growth variables while Guijá had the lowest (Table 4.1). Guijá also had the highest number of borer holes while I. J. Machel had the lowest.

Number of internodes was higher in Maracuene and Guija and the lowest in I.J.Machel and Magude. The highest number exit holes was recorded in Guija and the lowest in I.J.Machel (Table 4.1). Maracuene had the lowest stem tunnelling and together with Guijá the lowest percent cob damage. Stem tunnelling was significantly higher at Guija and I.J. Machel. The lowest stem tunnelling was observed at Maracuene, while the percentage of cob damage was significantly high at most of the 2002 release sites when compared with the 1996 release site of Maracuene (Table 4.1).

4.3.2 Stemborer density, species composition and percent parasitism in southern Mozambique

Table 4.2 shows the stemborer species composition and density in the different locations. *C. partellus* was the most abundant stemborer species at all locations, accounting for 94.8%, while the remainder was *S. calamistis*. Because the survey was conducted when the plants were at tasseling stage, 88.9% of the stemborers collected were larvae. *C. partellus* densities varied significantly within locations. Highest densities were observed in Chibuto and Guijá followed by I.J. Machel and Magude, and lowest in Maracuene.

At all sites, both *C. flavipes* and *C. sesamiae* were reared from *C. partellus*. From *S. calamistis*, only one *C. flavipes* cocoon mass was obtained at Maracuene. The levels of parasitism due to *C. flavipes* on *C. partellus* was significantly highest ($P<0.05$) in Maracuene, the 1996 release site, followed by I. J. Machel and Magude and the lowest in Guija and Chibuto. There were no differences ($P<0.05$) in clutch size while sex ratio varied significantly different between Guijá and Maracuene with a lower value for the latter location (Table 4.2). *Cotesia sesamiae* parasitism did not vary between locations. *Cotesia flavipes* tended was the most abundant larval parasitoid at all sampling locations. Paired comparisons showed that *C. flavipes* was the most abundant at all sampling locations than its African congener *C. sesamiae* (Table 4.3).

Table 4.1 - Plant growth and damage variables (± SE) at the different release sites in the southern regions of Mozambique during the 2002/2003 growing season

Location	N	Plant height	Stem diameter	Number of internodes	Number of borer holes	Stem tunnelling	Cob damage (%)
Chibuto	400	96.0 ± 1.3b	1.8 ± 0.4b	8.9 ± 0.4b	8.0 ± 1.1b	12.3 ± 1.3ab	8.8 ± 1.9a
Guijá	400	32.9 ± 1.6c	1.4 ± 0.6c	9.3 ± 0.4ab	9.9 ± 0.8a	14.1± 1.1a	5.5 ± 1.3b
I.J.Machel	400	105.9 ± 1.7ab	1.7 ± 0.4b	8.4 ± 0.6c	5.1 ± 1.1c	13.6 ± 2.0a	12.2 ± 1.9a
Magude	400	33.5 ± 1.5c	1.9 ± 0.4a	8.2 ± 0.6c	7.0 ± 0.8b	10.1 ± 1.1bc	9.8 ± 1.9a
Maracuene	400	123.0 ± 3.9a	1.9 ± 1.4a	9.6 ± 0.6a	8.0 ± 1.3b	8.4 ± 1.5c	1.7 ± 0.6c
Cv (%)		26.5	16.1	17.0	38.7	44.8	42.1

Means followed by the same letter in the same column are statistically different (SNK, P=0.05)

Table 4.2 – Stemborer density, species composition, percent parasitism and clutch size in southern Mozambique (± SE)

	C. partellus/ plant	*S. calamistis*/ plant	*C. flavipes*				*C. sesamiae*	
			n	Parasitism %	Clutch size	Sex ratio	n	Parasitism %
Chibuto	3.3 ± 0.63a	0.1 ± 0.15a	142	10.8 ± 0.4c	31.5 ± 1.1a	0.72 ± 0.3ab	86	6.7 ± 0.3a
Guijá	3.2 ± 0.57a	0.2 ± 0.25a	97	7.6± 0.4c	34.3 ± 1.4a	0.85 ± 0.2a	60	4.7 ± 0.3a
I.J.Machel	2.1 ± 0.49b	0.2 ± 0.26a	146	17.8 ± 0.4b	28.9 ± 1.7a	0.82 ± 0.2ab	43	5.2 ± 0.3a
Magude	2.4 ± 0.44b	0.1 ± 0.21a	95	9.9 ± 0.4b	32.2 ± 1.8a	0.79 ± 0.3ab	45	4.7 ± 0.3a
Maracuene	1.6 ± 0.37c	0.3 ± 0.27a	212	33.5 ± 0.5a	38.6 ± 1.3a	0.67 ± 0.3b	46	7.3 ± 0.3a
Cv (%)	18.9	22.1		104.1	26.2	25.6		144.5

Means followed by the same letter in the same column are not statistically different (SNK, P=0.05)

Table 4.3 – Abundance of *C. flavipes* and *C. sesamiae* at different sampling sites (± SE)

Locations	Abundance n (%)		P>t	$P>r^2$
	C. flavipes	*C. sesamiae*		
Chibuto	142(62.3)±0.03	86(37.7) ±0.02	0.0001	0.0705
Guija	97(61.8)±0.24	60(38.2) ±0.12	0.0013	0.2876
I.J. Machel	146(77.2)±0.05	43(22.8)±0.01	0.0001	0.0488
Magude	95(67.9)±0.24	45(32.1)±0.32	0.0001	0.0678
Maracuene	212(82.2)±0.43	46(17.8) ±0.21	0.0001	0.0389

4.3.3 Correlation coefficients between plant growth parameters, stemborer damage and parasitoids abundance

Relationships between plant growth parameters, stemborer damage and parasitoid abundance are presented in Table 4.4. Plant height and diameter were negatively correlated with proportion of internodes damaged and number of stemborers collected. The proportion of internodes bored was significantly positively correlated with stemborer abundance while it was negatively correlated with *C. flavipes* abundance (Table 4.4).

There were significant positive correlations between damage variables (proportion internodes infested, stemborers holes, tunnel length and cob damage) and stemborer abundance ($P<0.0001$ to $P=0.0002$). The relationship between number of holes in the stalks and the mean tunnel length was positively correlated. *C. flavipes* parasitism was negatively correlated with proportion of internodes bored and stemborer density and positively with cob damage (Table 4.3). There was a weak positive non significant correlation between *C. flavipes* and *C. sesamiae* ($r^2=0.0717$, $P=0.0537$).

Table 4.4 – Correlation coefficients between plant growth parameters, stemborer damage and abundance and parasitoids

	Pth	Std	Nintd	Pintnb	Meh	Tleng	Cobd	Stbd	Cf
Plant height (Pth)									
Stem diameter (std)	0.0312 0.1629	1.0000							
Number internodes (Nintd)	0.0252 0.2603	0.0752 0.0008	1.0000						
Proportion internodes bored (Pintnb)	-0.06645 0.0039	-0.0502 0.0247	-0.2873 0.0001	1.0000					
Moth exit holes (Meh)	-0.0352 0.1153	0.0316 0.1573	0.2557 0.0001	0.1215 0.0001	1.0000				
Tunnel length (tleng)	-0.0012 0.9612	0.0693 0.0019	0.0979 0.0001	0.0409 0.0672	0.3067 0.0001	1.0000			
Cob damage (cobd)	0.0148 0.5056	-0.0406 0.0695	0.0615 0.0060	0.0517 0.0208	0.1642 0.0001	0.0703 0.0016	1.0000		
Stem borer density (stbd)	-0.0320 0.1521	-0.0125 0.5764	0.0462 0.0388	0.0948 0.0001	0.2278 0.0001	0.0837 0.0002	0.1229 0.0001	1.0000	
C. flavipes (Cf)	0.0554 0.0034	-0.0037 0.8666	0.0103 0.6439	-0.0709 0.0015	0.0258 0.2495	0.0304 0.1741	0.0383 0.0867	-0.0419 0.0608	1.0000
C. sesamiae (Cs)	0.0099 0.6588	-0.0345 0.1225	0.0246 0.2706	0.0039 0.8584	-0.0144 0.5198	-0.0147 0.5087	0.0053 0.8126	0.0144 0.5188	0.0717 0.0537

4.4 Discussion

C. partellus and *S. calamistis* were found at all five locations where the survey was conducted. Previous studies by Cugala *et al.* (1999; 2001), Cugala and Omwega (2001), Segeren *et al.* (1995) and Gonçalves, (1970) reported similar trends for the low-land areas. Cugala (2002) suggested that the high infestation levels observed in the southern region Mozambique may have been due to the continuous maize cultivation contributing for food availability during the year and consequently to the increase of stemborer populations (Segeren *et al.*, 1991).

After its introduction into the African continent, *C. partellus* has become the most destructive and economically important pest of maize and grain sorghum in low-altitude regions of Southern Africa (van Hamburg, 1979). There is also evidence that *C. partellus* is displacing indigenous stemborer species in highvelds in Africa (Overholt, 1998). In Mozambique, *C. partellus* was recovered for the first time during the surveys conducted during the 2001/02 growing season at high elevation in the Northern Province of Niassa (Dinis, 2003). This is a clear evidence that, this exotic pest is spreading and colonizing the high altitude areas from where it was not reported previously (Davies *et al.*, 1995).

In Transvaal highveld area of South Africa, initially *B. fusca* was the only stem species found, but each year the population of *C. partellus* has increased to the point where it has become the dominant stem borer, while at the same time the population of *B. fusca* was found to be declining (Kfir, 1997b; Overholt, 1998). Thus, it has been postulated that *C. partellus* is displacing the indigenous stem borer *B. fusca*. If this is true in Mozambique, it is expected that *C. partellus* population will increase in the near future at the high elevations areas so far dominated by *B. fusca*. The number

of *C. partellus* larvae per plant reported at the 1996 release site was relatively low when compared with the numbers reported by Gonçalves (1970), Segeren *et al.* (1991) and Cugala *et al.* (2001), who, respectively, reported 10, 7 and 5 larvae per plant in the southern region. These results suggest that, the establishment of *C. flavipes* is having an impact on *C. partellus* population

The highest plant height and lowest stem tunnelling were observed at the 1996 release site. This may due to the fact that the low tunnel length did not significantly affect the plant functions. Kfir (1998), stated that stem tunnelling by larvae weakens the stem and interferes with translocation of nutrients and metabolites in the plant. These observations agree with the present results, which found, in general, that stem diameter, number internodes were low in the areas where stem tunnelling was high. However, Songa *et al.* (2001), working in Kenya reported that stemborer tunnel length had little effect on maize stem diameter.

The positive correlation between *C. flavipes* and plant growth parameters and the negative correlation of *C. flavipes* with plant damage at the 1996 release site and the significantly higher levels of parasitism due to *C. flavipes* may indicate the potential of the exotic parasitoid in reducing stemborer populations and consequently their damage to maize crop.

In Kenya, levels of parasitism of more than 60% with a concomitant 30%-50% reduction in stemborer populations were reported four years after the first releases of *C. flavipes* (Zhou *et al.*, 2001). Thus the present findings suggest that the exotic parasitoid populations is building up and increasing stemborer population's natural mortality in southern and central Mozambique.

Songa *et al.* (2001) reported that the numbers of stemborers, holes, stem tunnelling were the key factors affecting maize grain yield. The significantly positive correlation between damage parameters and stemborer density were similar to the findings of Kumar (1997). The negative correlation of number of the internodes infested and stemborer abundance with *C. flavipes* levels of parasitism indicate a positive effect of biological control on the plant via reduction of the pest.

Four years after *C. flavipes* was first released in southern Mozambique at Maracuene and Moamba, Cugala and Omwega (2001) reported low levels of parasitism at those locations. At that time, *C. sesamiae* was the most abundant larval parasitoid. However, recent studies reported a significant increase in the levels of parasitism due to *C. flavipes* at either location, i.e., 40,8% at Moamba (Cugala *et al.*, 2003) and 33,4% (in the present study), and at some release sites such as Xai-Xai, Manhiça and Machipanda, *C. flavipes* has become more abundant than *C. sesamiae* (Cugala *et al.* 2003).

These observations agree with the findings reported by Debach and Rosen (1991), Overholt (1998), Cugala *et al.* (2001) and Cugala and Omwega (2001) who stated that introduced exotic parasitoids may need time to adapt to the local environment for population build-up and spread from the release sites to other locations, and to produce significant impact on the stemborer populations and crop yield.

CHAPTER FIVE

5.0 IMPACT OF NATURAL ENEMIES ON STEMBORER INFESTATIONS AND YIELD LOSS IN MAIZE USING SELECTED INSECTICIDES

5.1 Introduction

The invasive stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and the indigenous species, *Busseola fusca* Fuller (Lepidoptera: Noctuidae) constitute a serious threat to maize and sorghum production in Mozambique. The pink stemborer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), which is of minor importance (Gonçalves, 1970; Segeren *et al.*, 1991), is kept under control by its natural enemies (Kfir, 1998).

A wide range of egg, larval and pupal parasitoids of stemborers had been recovered from stemborer species attacking maize and sorghum in Mozambique (Gonçalves, 1970; Segeren *et al.*, 1991; Davies *et al.*, 1995; Cugala *et al.*, 1999; 2001; Cugala and Omwega, 2001). Among them, the indigenous parasitoid *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) is the most important mortality factor of stemborer larvae.

Gonçalves (1970) recorded 60% parasitism of *C. partellus* eggs due to *Trichogramma sp.* while Davies *et al.* (1995) reported 20% parasitism of *B. fusca* larvae due to *Cotesia sesamiae*. The exotic larval parasitoid, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), was released in the Southern and Central regions of Mozambique in years of 1996, 1998, 1999 and 2000. The parasitoid has become established at the majority of release sites and is spreading to new areas where it was not released (Cugala *et al.*, 1999;

2001; Cugala and Omwega, 2001). Levels of parasitism of about 40% due to C. *flavipes* have been reported on *C. partellus* at one of the release sites (Cugala *et al.* 2003).

In South Africa, Kfir (2002) using a method of partial elimination of parasitoids by applying pesticides reported high infestation levels by stemborers on sorghum due to the removal of natural enemies by applying pesticides. Many indigenous parasitoids have been reared from cereal stemborers species in Mozambique, but there has been no assessment of their impact on stemborer populations. This study attempted to assess the extent to which the natural enemies prevented yield losses due to stemborers by excluding them with insecticides.

5.2 Materials and methods

5.2.1 Study sites

Trials were conducted in three agro-ecologically different zones. One location was Chokwe village (24°29.970'S; 32°57.942' E, elevation 80 m), a warm, lowland area in the southern province of Gaza where previous work had shown that *C. partellus* was the dominant stemborer (>90%), followed by *S. calamistis* (<10%) (Segeren *et al.* 1991; Cugala *et al.*, 1999; 2001). *B. fusca* has not recorded from this area. The second area was Machipanda (18°52'16"S; 32°47'96"E, elevation 800 m), a medium to high elevation zone in the Central Province of Manica. In Machipanda, both *B. fusca* and *C. partellus* occur with frequencies of 32% and 61% of the population for *B. fusca* and *C. partellus*, respectively (Cugala *et al.*, 2001). The third and high altitude location was Lichinga (>1000 m asl), where *B. fusca* is the dominant species (>90% of the stemborer population), followed by *S. calamistis*.

5.2.2 Experimental design

A plot of 50x50 m^2 was prepared at each study site. The plot was divided in 12 subplots of 10x10 m^2 for each separated each other by empty spaces of 2 m and 5m within and between replications respectively. A randomised complete block design (RCBD) with three treatments replicated four times was used. The treatments were 1) a control without insecticide application, 2) application of Dimethoate at a rate of 0.5 ml/l of water, which is 1/4 of the recommended dose of 2 ml/l of water, to exclude natural enemies as reported by Kfir (2002) and 3) a Cypermethrin treatment at 20 ml of insecticide/20 l of water to suppress both natural enemies and stemborers. These treatments will be referred to as unprotected, excluded and fully protected respectively.

To ensure stemborer infestation, maize was planted early in January 2003 and 2004 at each site to coincide with the peak of stemborer infestation (February-March). Maize was planted at inter-row spacing of 90 cm and inter-plant spacing of 45 cm. The chemical fertilizer NPK (12:24:12) at 200 Kg/ha was applied as basic fertilizer at planting date and ammonium nitrate (46% N) at 100 Kg/ha as top-dressing about four weeks after emergence. Chemical insecticides were applied according to a predetermined schedule at 15, 30, 45 and 60 days after crop emergence (DAE) as recommended by Segeren *et al.* (1991), at the doses of 0.5 ml/l of water (1/4 of the recommend dose of 2 ml/l of water) for Dimethoate and at 20 ml of insecticide/20 ml of water for Cypermethrin. According to Kfir (2002), the dose of Dimethoate applied suppresses natural enemies in maize.

5.2.3 Assessing stemborer abundance and parasitism

All plots were monitored at 4 weeks after crop emergence for assessment of egg batch densities and egg parasitism, at tesseling to evaluate numbers of larvae and pupae as well as associated parasitoids, and at harvest to determine yield losses due to stemborers and the impact of suppressing natural enemies. From each plot, 50 plants were randomly selected and inspected for egg batch infestation. After counting the number of eggs, the batches were placed individually in vials and reared until larva or parasitoid emergence. Parasitoids were counted and sent to South African Sugar Research Institute (SASRI), South Africa for species identification.

To assess stemborer larval and pupal abundance, samples of 10 plants were randomly selected from each plot and dissected. All larvae and pupae were placed individually in vials and taken to the laboratory and reared until adult or parasitoid emergence. The parasitoids were counted and recorded according to species. At tesseling, plant growth variables including plant height, stem diameter and number of internodes, damage indicators such as number of internodes bored, tunnel length, number of holes and grain damage were recorded from each plot and at each study site.

5.2.4 Yield and yield losses

The hand harvested grain was sun-dried until the moisture content was between 13 and 14% for grain yield determination after hand threshing. The grain was then weighted and the mean grain yield of each treatment calculated. Yield losses due to stemborer attack were estimated as differences between expected yield from the fully protected plots and yield from unprotected and exclusion plots according to the method described by Ampofo

(1988) and expressed as a percentage of potential yield harvested from fully protected plots. The following formula was used:

$$Yp = (Yfp - Yu)/Yfp*100$$

where Yp = yield loss in the presence of parasitoids, Yfp = yield of fully protected plots, Yu = yield of unprotected control plots.

The difference between the yield from fully protected plots and exclusion plots indicated the yield losses in absence of natural enemies:

$$Ya = (Yfp - Yex)/Yfp*100$$

where Ya – yield loss in the absence of parasitoids, Yex – yield of exclusion plots.

The impact of natural enemies (INE %) was estimated as the difference between the yield from unprotected and exclusion plots:

$$INE\ (\%) = (Yu - Yex)/Yu*100$$

5.2.5 Economic threshold levels determination

Economic threshold levels (ETL) was determined according to the method proposed by Cheshire *et al.* (1989) and Seshu Reddy and Sum (1992) considering 1) costs of insecticide application, 2) the market price of maize at different regions, 3) relationships between pest infestation and crop yield.

The following arithmetic expression was used:

$$\text{Loss Kg/ha} = \frac{\text{Cost of treatment/ha}}{\text{Price of crop/Kg}}$$

$$\text{EIL} = \frac{\text{Loss in Kg/ha}}{b}$$

where b is the regression coefficient of linear regression of yield and pest population densities. The ETL thus gives the density of stemborer larvae per plant estimated to cause loss equivalent to the cost of control. The ETL was estimated in relation to the cost of insecticide application at 40 days after crop emergence (DAE) at each location.

5.3 Data analysis

Data for each location were subjected to analysis of variance (ANOVA) (Proc GLM, SAS Institute, 1999). Means were separated using the Student Newman Keuls multiple range test when ANOVA was significant ($P < 0.05$). The relationships between the plant growth and damage variables, stemborers and parasitism levels were performed using correlation matrix and regression analysis (PROC CORR and REG, SAS). The data on the number of insects and proportions were square root and arcsine transformed, respectively, to normalize data before analysis.

5.4 Results

5.4.1 Stem borers and parasitism levels

C. partellus was almost the only stemborer species collected in the lowland area at Chokwe. At Machipanda, *C. partellus* and *B. fusca* accounted for 68.3% and 31.7%, respectively, of the total stemborer population, while at Lichinga, it was 93.3% *B. fusca*

and 6.7% *C. partellus*. Overall, numbers of *S. calamistis* were very low at all study sites and, thus, were excluded from the data analyses. In all three sites, stemborer population levels in the exclusion plots were significantly ($P<0.05$) higher when compared to unprotected control plots (Table 5.1).

In Chokwe, a mean of 5.8±3.2 stemborers per plant were significantly ($P<0.05$) higher in the exclusion plots compared to 2.9±2.3 stemborers per plant collected from the unprotected plots. In Machipanda a mean 6.8±3.2 stemborer/plant were significantly ($P<0.05$) higher from the exclusion plots compared to 3.4±2.2 stemborers/plant in unprotected plots. At Lichinga a mean density of 7.8±3.1 stemborers/plant was significantly higher in the exclusion plots compared to 4.8±2.4 borers/plant in the unprotected plots (Table 5.1).

The parasitoids collected at the study sites included the larval parasitoids *C. flavipes*, *C. sesamiae* and *Sturmiopsis parasitica* (Curran) (Diptera: Tachinidae), and the pupal parasitoids *Dentichasmias busseolae* Heinrich, *Procerochasmias nigromaculatus* Heinrich (Hymenoptera: Ichneumonidae) and *Pediobius furvus* Gahan (Hymenoptera: Eulophidae) (Table 5.1). The highest level of parasitism due to larval parasitoids was observed in Machipanda due to *C. sesamiae* (20.4%) and 48.6% due to pupal parasitoids, *P. nigromaculatus* in Lichinga. At Chokwe, *C. sesamiae* was also the most abundant with 13.2% and 36.7% for the pupal parasitoid *D. busseolae* on *C. partellus*.

C. sesamiae was by far the most abundant larval parasitoids of *C. partellus* in Chokwe and Machipanda, while in Lichinga the pupal parasitoid *P. nigromaculatus* (48.6±10.4)

was the most abundant followed by the larval parasitoid *S. parasitica* (4.5±1.32). In general, the recovered parasitoids and levels of parasitism were more abundant on unprotected plots than in exclusion and fully protected plots at all study sites. The exotic parasitoid C. *flavipes* was recovered at Chokwe and Machipanda where it was released in 1999 but not in Lichinga. This was the first time *C. flavipes* was recovered at Chokwe (Table 5.1).

5.4.2 Plant growth and damage variables

In general, plant height, stem diameter and number of internodes were highest in fully protected and lowest in exclusion plots. In Chokwe, plant height and number of internodes were significantly higher on fully protected plots ($P<0.05$) than on unprotected and exclusion plots. However, the same parameters did not differed between fully protected and unprotected plots at Machipanda, but were significantly higher when compared to exclusion plots ($P<0.05$). At northern region of Lichinga, both stem diameter and number of internodes from fully protected plots were statistically similar to those from unprotected plots (Table 5.2).

Plant damage due to stemborer infestation especially proportion of internodes bored, stemborer holes, tunnel length/proportion of plant tunnelled and percent cob damage at maturity were significantly low on fully protected plots compared to exclusion and unprotected plots (Table 5.2).

Table 5.1 – Effect of Dimethoate (to exclude natural enemies) and Cypermethrin (to protect against stemborer infestation) treatments on stem borer density and parasitism at Chokwe (low altitude), Machipanda (medium altitude) and Lichinga (high altitude) study sites (±SE)

Location/Treatment	Stem borer[1] Infestation (%)	Stem borer Per plant	*Cotesia flavipes*	*Cotesia sesamiae*	*Sturmiopsis parasitica*	*Dentichasmias busseolae*	*Procerochasmias nigromaculatus*
a) *Chokwe*							
Unprotected	75±0.1b	2.9±2.3b	5.8±2.1a	13.2±4.2a	-	36.7±5.2a	-
Exclusion	90±1.2a	5.8±3.2a	0.0±0.0b	0.9±1.4b	-	10.3±2.4b	-
Fully protected	15±0.2c	0.9±0.8c	0.0±0.0b	0.0±0.0c	-	0.0±0.0c	-
Df	2, 119	2, 119	2, 119	2, 119		2, 119	
F	45.5	22.0	3.3	34.6		21.8	
P-values	<0.0001	0.0003	0.0474	<0.0001		<0.0001	
b) Machipanda							
Unprotected	45±0.4b	3.4±2.2b	15.4±3.1a	20.4±4.4a	7.2±3.9a	20.9±2.4a	0.0±0.0
Exclusion	60±0.8a	6.8±3.2a	2.2±1.3b	5.1±2.1b	1.5±0.4b	4.0±3.2b	0.0±0.0
Fully protected	5±0.0c	0.5±0.8c	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.0±0.0c	0.0±0.0
Df	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	
F	47.2	27.9	16.9	30.3	8.7	10.5	
P-values	<0.0001	<0.0001	<0.0001	<0.0001	0.0078	<0.0001	
c) *Lichinga*							
Unprotected	80±1.2a	4.8±2.4b	0.0±0.0	3.6±1.5a	4.5±1.3a	0.0±0.0	48.6±10.4a
Exclusion	95.5±1.5a	7.8±3.1a	0.0±0.0	0.0±0.0a	1.2±0.9a	0.0±0.0	2.6±1.2b
Fully protected	10±0.1b	1.2±1.6c	0.0±0.0	0.0±0.0a	0.0±0.0b	0.0±0.0	0.0±0.0c
Df	2, 119	2, 119		2, 119	2, 119		2, 119
F	56.0	78.8		5.1	8.3		27.0
P-values	<0.0001	<0.0001		0.0479	0.0004		0.0002

Means followed by same lowercase letter within column are not significantly different at $P<0.05$ (SNK)

[1] *C. partellus* and *S. calamistis* (at Chokwe); *C. partellus*, *B. fusca* and *S. calamistis* (at Machipanda) and *B. fusca*, *S. calamistis* and *C. partellus* (Lichinga)

The proportion of internodes bored by stemborer was significantly higher on exclusion than on the control (unprotected) and fully protected plots in Chokwe and Machipanda ($P<0.05$), while at Lichinga there were no significant differences in the number of internodes bored between the exclusion and unprotected plots, but both variables were significantly higher on protected plots compared to unprotected and exclusion plots ($P<0.05$) (Table5.2).

Significantly more tunnel length and/or proportion of plant tunnelled were observed from the exclusion plots than unprotected and fully protected ones at all sampling sites ($P<0.05$). Tunnel length (at Lichinga) and proportion of plant tunnelled (at Machipanda) were statistically similar in exclusion and unprotected plots. In Chokwe, both parameters were significantly higher on exclusion than on unprotected plots ($P<0.05$) (Table 5.2).

The highest proportion of cob damage at maturity was recorded at Chokwe from exclusion plots (54.8±29.8) and the lowest at Machipanda from fully protected plots (0.0). Percent cob damage was significantly higher on exclusion plots at Chokwe and Lichinga ($P<0.05$) than on unprotected plots. While at Machipanda, there were no significant differences between unprotected and exclusion plots for the same parameters, but both variables differed significantly from the fully protected plots ($P<0.05$) (Table 5.2).

5.4.3 Yields and yield losses

There were no significant differences in cob weight between unprotected and exclusion plots at the three study sites (Table 5.3). However, significantly higher cob weight was recorded in fully protected plots compared to the other treatments ($P<0.05$). The highest

Table 5.2 – Effect of suppressing natural enemies (exclusion plots) and stem borer (full protected plots) on plant growth and damage variables at the study sites (±SE)

Location/Treatment	Plant height (m)	Stem diameter (cm)	Number internodes	Proportion internodes bored	Number stemborer holes	Tunnel length (cm)	Proportion tunnelled plants	Percent cob damage
a) *Chokwe*								
Unprotected	1.95±0.5b	2.4±0.2a	11.9±1.9b	0.57±0.2b	8.8±5.5a	50.6±23.5b	0.28±0.2b	14.3±22.7b
Exclusion	1.69±0.4c	2.1±0.6b	11.6±2.3b	0.78±0.1a	9.9±4.4a	69.8±39.3a	0.45±0.3a	54.8±29.8a
Fully protected	2.47±0.3a	2.5±0.3a	13.6±1.8a	0.05±0.1c	0.8±1.3b	3.9±6.8c	0.05±0.0c	5.1±12.8b
Df	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119
F	35.9	7.8	10.4	200.9	56.8	64.1	46.5	51.3
P-values	<0.0001	0.0007	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
b) Machipanda								
Unprotected	2.82±0.4a	1.95±0.3b	10.8±1.4a	0.45±0.27b	4.6±2.8a	20.8±11.2b	0.11±2.3a	25.6±2.8a
Exclusion	1.58±0.5b	1.84±0.3b	9.8±1.6b	0.65±0.2a	3.9±2.1b	31.5±19.1a	0.23±0.1a	33.3±28.1a
Fully protected	3.10±0.3a	2.30±0.2a	11.4±1.8a	0.04±0.1c	0.25±0.5c	2.15±3.3c	0.01±0.0b	0.0±0.0b
Df	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119
F	18.3	22.9	9.4	156.6	51.9	49.5	1.2	27.2
P-values	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.2972	<0.0001
c) *Lichinga*								
Unprotected	2.22±0.5b	1.83±0.2a	12.1±1.9a	0.53±0.2a	8.1±5.6a	28.7±20.7a	0.14±0.1b	22.7±25.6ab
Exclusion	1.54±0.2c	1.57±0.3b	9.4±1.8b	0.57±0.2a	9.0±5.6a	32.1±18.7a	0.21±0.1a	31.1±34.5a
Fully protected	2.8±0.4a	1.92±0.2a	12.5±1.9a	0.06±0.1b	0.8±1.4b	2.6±3.6b	0.01±0.0c	10.6±22.7b
Df	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119	2, 119
F	98.5	18.4	31.4	113.4	41.5	39.5	41.6	5.1
P-values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0079

Means followed by same lowercase letter within column are not significantly different at P<0.05 (SNK)

grain weight was recorded from fully protected plots and it varied from 21.1±2.1 at Lichinga to 22.3±1.0 Kg/plot at Chokwe. Significantly higher grain weight was recorded for fully protected plots compared to unprotected and exclusion plots at all study sites ($P<0.05$) (Table5. 3). When comparing grain weight from fully protected plots with that of unprotected plots, the grain weight loss (yield loss) was estimated at 28.9%, 34.5% and 31.2% respectively in Chokwe, Machipanda and Lichinga (Table 5.3).

Table 5.3 – Effect of treatments on cob and grain weight and yield losses in the three treatments at the three study sites (±SE)

Location/Treatment	Cob weight	Grain weight kg/plot	Yield losses (%) from Unprotected plots*	Yield losses (%) from Exclusion plots**
a) *Chokwe*				
Unprotected	18.6±2.8b	17.1±1.5b	-	26.1
Exclusion	17.5±3.3b	12.6±1.48c	-	-
Fully protected	26.1±3.5a	22.3±1.0a	28.9	43.3
Df	2, 12	2, 12		
F	8.2	52.9		
P-values	0.0093	<0.0001		
b) Machipanda				
Unprotected	16.6±2.2b	14.1±2.6b	-	11.2
Exclusion	16.2±5.3b	12.9±5.5b	-	-
Fully protected	28.1±1.4a	21.4±3.4a	34.5	40.8
Df	2, 12	2, 12		
F	15.6	5.4		
P-values	0.0012	0.0290		
c) *Lichinga*				
Unprotected	18.2±1.70b	14.5±2.19b		7.6
Exclusion	17.6±0.91b	13.4±1.00b		
Fully protected	25.3±2.32a	21.1±2.01a	31.2	36.4
Df	2, 12	2, 12		
F	24.1	21.1		
P-values	0.0002	0.0004		

Means followed by same lowercase letter within column are not significantly different at $P<0.05$ (SNK). * = Yield losses in the presence of natural enemies (comparing fully protected and unprotected plots), ** = yield losses in the absence of natural enemies (comparing unprotected and exclusion plots and fully protected and exclusion plots for first and second values respectively)

In Chokwe, grain weight from unprotected plots was 26.1% higher compared to exclusion plots. At Machipanda and Lichinga, the impact of natural enemies was estimated as 11.2% and 7.6% respectively. The highest impact of natural enemies was recorded in Chokwe (where *C. partellus* was abundant), followed by Machipanda (where both *C. partellus* and *B. fusca* occurred) and the lowest in Lichinga (where *B. fusca* was the most abundant followed by *C. partellus*) (Table 5.3).

5.4.4 Correlation coefficients between plant growth, stemborers damage and grain weight

Across ecological zones, the correlation analysis showed that there were significantly positive correlations between grain weight and plant growth variables including plant height, stem diameter and number of internodes ($P<0.01$). However, in general, grain weight had significant negative correlation with all damage indicators including proportion of internodes bored, number of stem borer holes, tunnel length, cob damage and number of stem borer per infested plant ($P<0.01$) (Table 5.4). The correlation between plant growth variables, damage and grain weight varied from location to location (table 5.4).

Table 5.4 suggests that in general, stem borer abundance had positive significant correlation with damage variables such as proportion of internodes bored, number of stem borer holes, tunnel length and cob damage ($P<0.01$). Parasitism levels were negatively but not significantly correlated with plant height and number of internodes, but they had a positively significant correlation with grain weight, proportion of internodes bored and number of holes ($P<0.05$). There were positive but non-significant correlation between levels of parasitism and tunnel length, cob damage and stem borer

density. The multiple regression analysis indicated a linear relationship between grain weight and plant growth, damage indicators, stem borer and parasitism levels and that the maize grain weight could be predicted by the following linear function at each site:

1. Chokwe: Gw=-10.9+9.5Pth-7.8Pinod-1.9Stb+1.7Pab

(P=0.0450, N=12, r^2=0.9190)

2.Machipanda: Gw=14.2+12.2Pht-0.6Pinod-1.1stb+5.9pab

(P=0.0467, N=12, r^2=0.7314)

3. Lichinga: Gw=21.7+2.9pht-22.3pinod-0.6stb+0.5pab

(P=0.0027, N=12, r^2=0.9613)

Where: Gw= growth weight, pht = plant height, pinod=proportion internodes bored, stb=stemborers density, pab=parasitism

In general, across the study sites, the grain could be predicted by the following equation:

Gw = 26.6+1.8Pth+2.1sdm-11.0Pinod (P=0.0002, r^2=0.6634)

5.4.5 Economic threshold levels determination

Table 5.5 shows the ETLs at the three study sites. A mean density of about 2.2, 3.8 and 6.9 larvae/plant were estimated to cause yield losses equal to cost of stem borer control at 40 DAE respectively at Chokwe, Machipanda, and Lichinga. The lowest ETL was recorded at Chokwe where *C. partellus* is the most abundant species, followed by Machipanda. At Machipanda both *C. partellus* and *B. fusca* coexist, with *C. partellus* being dominant during the rainy season. The highest value of ETLs was observed at Lichinga where *B. fusca* dominate on more than 90% of the total stemborer populations.

Table 5.4 – Correlation coefficients between plant parameters, damage, parasitism and yield

	1	2	3	4	5	6	7	8	9	10	11
1. Cob weight	**1.000**										
2. Grain weight	0.9065 ***	**1.000**									
3. Plant height	0.5345 ***	0.5812 ***	**1.000**								
4. Stem diameter	0.4026 *	0.4272 **	0.2881 ns	**1.000**							
5. Number internodes	0.4347 **	0.4867 **	0.7279 ***	0.5951 ***	**1.000**						
6. Proportion internodes bored	-0.7743 ***	-0.7479 ***	-0.7391 ***	-0.3730 *	-0.5013 **	**1.000**					
7. Holes	-0.6458 ***	-0.6071 ***	-0.5351 ***	-0.2588 ns	-0.2793 ns	0.7875 ***	**1.000**				
8. Tunnel length	-0.5148 **	-0.5078 **	-0.6212 ***	-0.1000 ns	-0.2759 ns	0.8334 ***	0.7974 ***	**1.000**			
9. Cob damage	-0.5442 **	-0.5606 ***	-0.5423 ***	-04332 **	-0.3977 *	0.7095 ***	0.5698 ***	0.6565 ***	**1.000**		
10. Stem borer abundance	-0.6491 ***	-0.6965 ***	-0.7701 ***	-0.5568 ***	-0.6082 ***	0.7675 ***	0.6225 ***	0.6274 ***	0.6012 ***	**1.000**	
11. Parasitism	0.5056 **	0.3849 *	-0.2303 ns	-0.1250 ns	-0.2158 ns	0.4707 **	0.3303 *	0.2681 ns	0.1915 ns	0.2019 ns	**1.000**

* r - Values significant at $P<0.05$, ns – not significant

Table 5.5 - Economic threshold levels at each study site at 40 DAE

Location	Regression parameters			ETL
	b	P	r^2	
Chokwe	-1.9	0.0450	0.9190	2.2
Machipanda	-1.1	0.3508	0.7314	3.8
Lichinga	-0.6	0.0027	0.9613	6.9

ETL = economic threshold level; DAE = days after emergence

5.5 Discussion

The relative importance of stemborers in the present study corroborates results by Segeren *et al.* (1991) and Cugala *et al.* (1999, 2001) who reported that C. partellus was the most important stem species at lowland areas (<500 m asl) while Davies *et al.* (1995) reported that B. fusca was the most abundant stemborer species at high elevation zones (>1000 m asl). Kfir (1995) suggested that the low numbers of *S. calamistis* in South Africa were due to the action of its indigenous natural enemies and mainly *C. sesamiae*, which was also the most common larval parasitoid in the present study.

The higher stemborer density in the exclusion plots resulted in significantly higher plant damage at all study sites corroborating results by Songa *et al.* (2001), who reported that thc high number of stemborers in infested plants corresponded to a decrease in plant height and grain yield. High stemborer density in exclusion plots and subsequent plant damage was also observed by Seshu Reddy and Sum (1992), Kumar (1997) and Kfir (2002).

Kfir (2002) working in South Africa observed that stemborer populations levels were more abundant in the Dimethoate insecticide sprayed plots when compared to

unsprayed plots and he collected more stemborers from Dimethoate sprayed plots than in unsprayed plots and estimated that the sprayed plants were nearly two to three times more likely to be infested with stemborers than the unsprayed plants and he argued that a partial removal of natural enemies could increase stemborer population numbers. The increase of stem borer population numbers in the plots where activities of natural enemies were excluded was an indication that natural enemies have the ability to suppress stemborer populations. In the present study the significant abundance of stemborer in exclusion plots compared to unprotected plots supports the finding of Kfir (2002).

The abundance of larval and pupal parasitoids in the unprotected plots compared to exclusion plots agree with observations by Kfir (2002), who reported that the chance of stemborer to be parasitized in the sprayed plots was less than stemborers collected from unsprayed plants. Similar observations were made by Lim (1970) in Malaysia who reported severe damage by stemborer in rice where insecticides were used but not where natural enemies remained active.

Eveleens *et al.* (1973), Ehler *et al.* (1973) reported that Dimethoate suppresses predaceous insects but causes no harm to lepidopteran pests in cotton fields. Sinha *et al.* (1990) revealed that by topical application, Dimethoate was less toxic to first instar larvae of the large white butterfly, *Pieris brassica* L. (Lepidoptera: Pieridae) than seven other commonly used insecticides. Also Dimethoate was found to be more toxic to lepidopteran pest when ingested than by topical application, because Dimethoate is not readily absorbed by the lipids in the cuticle but can penetrate faster through the gut wall (Khan, 1993).

Previous works indicate that despite the large numbers of parasitoids and relatively high parasitism levels by indigenous parasitoids of maize stemborers (Berger, 1981; Gonçalves, 1970; Kfir, 1995), the parasitoids are not able to prevent economic damage and/or reduce pest populations to below economic threshold levels because their association with the exotic stemborer *C. partellus* is new (Overholt *et al.*, 1994; Kfir, 1997a; 2000; Overholt, 1998).

Plant height, stem diameter and number of internodes (i.e. number of leaves) tended to be lowest in exclusion plots where stem borers and plant damage were high compared to unprotected plots. Songa *et al.* (2001) working in Kenya observed low plant growth variables on damaged plants compared to un-damaged ones. They further reported that tunnel length and number of holes significantly reduced plant height and that both damage variables had little effect on the number of leaves and stem diameter. These observations may due to stem borer abundance and damage effect. Seshu Reddy and Sum (1992) observed that stemborer damage increased with an increase in stem borer density, while Songa *et al.* (2001) observed that severe stem borer damage resulted in poor maize plant growth.

In the present study, yield losses varied according to location or agro-ecological zone and stemborer species composition. Previous studies by Segeren *et al.* (1991), reported yield losses of 20% at a research station and 40% on small scale farmers' fields. At all the three study sites, yield losses due to stem borer infestation were relatively high in the exclusion plots when compared to plots where natural enemies were present. These results agree with those by Kfir (2002) who stated that without the activities of natural enemies, annual yield losses would be much high. Kfir (1992) speculated that indigenous natural enemies played an important role in

reducing stemborer populations. Thus, the increase of stemborer population abundance in the exclusion plots was attributed to the reduced natural enemy's activities due to their partial elimination by selected pesticides.

Songa *et al.* (2001) in their work in Kenya stated higher stem borer damage, resulted to poor maize plant growth and thus lower maize grain yield. They reported that damage parameters such as tunnel length, stemborer holes had significant negative effect on plant growth (plant height and stem diameter) resulting in a reduction in grain yield to about 30% of the potential yield. These observations are in agreement with the present findings. In Chokwe, for example, low maize grain weight was reported in the exclusion plots where plant damage (tunnel length) was high compared to others plots.

The differences in the stemborer damage and the impact on grain weight may due to the differences in the stemborer species composition and their relative abundance between the three study sites. In Chokwe, *C. partellus* dominated, while in Lichinga, *B. fusca* was the most abundant and in Machipanda both *C. partellus* and *B. fusca* were found coexisting in the same area and/or plant.

van den Berg *et al.* (1991) reported that *B. fusca* caused less stem damage and yield loss than *C. partellus*, either when it occurred singly or in mixed populations with *C. partellus*. In addition, the same authors observed that low numbers of *C. partellus* resulted in high yield loss. These findings suggest that high stemborer damage and yield losses are more likely to occur in the areas where *C. partellus* is the abundant species compared to the areas where *B. fusca* is abundant or where both species occur.

The relationships between grain weight and plant growth and damage have been studied by other researchers (Ndemah and Schulthess, 2002; Songa *et al.*, 2001; Kumar, 1997; Seshu Reddy and Sum, 1992). Songa *et al.* (2001) reported significant high grain weight from taller plants compared to shorter plants. Thick stemmed plants were also observed to produce more grain weight than thin stemmed plants. These findings were similar to the present results which indicated positive significant relationships between grain weight and growth (plant height and stem diameter) at all the study sites.

Correlation analyses undertaken gave significant negative correlations between grain weight and stem damage and stemborer abundance at all study sites. Significant negative correlations between stemborer damage and maize grain yield were also observed by Seshu Reddy and Sum (1991, 1992), Kumar (1997); Ndemah and Schulthess (2002).

The number of stemborers, stemborer holes and tunnel length were thought to be the most important factors affecting grain yield and could explain more than 46% of the yield variation (Songa *et al.*, 2001). Similarly Seshu Reddy and Sum (1992) observed negative regression coefficients between stemborer density and grain yield. They also reported that there was a reduction of yield with increase in stemborer larvae density.

Regression analyses of the effect of stemborer damage on maize grain yield indicated that maize grain weight was not only influenced by stemborer damage parameters and plant growth but also by the interactions between these two factors. These

observations support the significant interactions between the plant growth and damage parameters. The effect of study site was not significant suggesting that location had low effect on the variation of grain weight.

The present study indicated that the higher stemborer density/abundance, damage and low grain weight observed in the exclusion plants were due to removal or exclusion of natural enemies from these plots. The exclusion of natural enemies from maize fields increased the stemborer population. These observations suggest that natural enemies have some impact in the stemborer population. Thus, there is a need and importance to conserve the natural enemies in the agricultural and natural ecosystems to regulate populations of cereal pests.

Economic threshold level estimated the density of *C. partellus* and *B. fusca* larvae expected to cause yield losses equivalent to the cost of their control (Higley and Pedigo, 1993). In the present study the ETLs varied from 2.2 for *C. partellus* at Chokwe to 6.8 larvae/plant for *B. fusca* at Lichinga.

At Chokwe and Machipanda the estimated ETL levels were similar to the values estimated by Seshu Reddy and Sum (1992) for *C. partellus* control in Kenya. They recommended that insecticides should be applied if 3.2 and 3.9 or more active larvae per plant are found at 20 and 40 DAE respectively. van den Berg and Nur (1998) stated that the ETL levels estimated for stem borer control are considered to be very low and are often reached. These observations agree with the fact that all the ETL levels reported in the present study are below the number of larvae per plant observed at each study locations.

The low values of ETL levels suggest the importance of stemborers as pests of maize and sorghum in Southern Africa and Mozambique in particular, thus confirming the significant high damage and yield losses observed in exclusion and unsprayed plots.

The differences in the ETL levels between the study locations may due to the variation in the market value of grain maize that is low in the northern location of Lichinga compared to Chokwe and Machipanda. van den Berg *et al.* (1997) also observed that the ETL models were influenced by several factors including yield potential of the crop, cost of insecticides application and market value of the yield.

CHAPTER SIX

6.0 ESTABLISHMENT OF THE EXOTIC PUPAL PARASITOID *Xanthopimpla stemmator* THUNBERG (HYMENOPTERA: ICHNEUMONIDAE) AT DIFFERENT AGROECOLOGICAL ZONES

6.1 Introduction

Xanthopimpla stemmator Thunberg (Hymenoptera: Ichneumonidae) is a pupal endoparasitoid that attacks pupae of various lepidopteran stem borers that occupy similar cryptic microhabitat in a variety of grass communities including cereal crops such as maize, sorghum and sugarcane, by drilling into the plant's stem. It has a broad geographical distribution on the Asian continent and was recorded from pupae of numerous pyralid and noctuid stem borers species (Moutia and Courtois, 1952).

Xanthopimpla stemmator has been used successfully in classical biological control programs in various countries. In 1987 it was imported from Mauritius into South Africa as a new-association parasitoid against *Eldana saccharina* in sugarcane (Conlong, 1994). *X. stemmator* was also successfully introduced from Sri Lanka into Mauritius and from Mauritius into Reunion, against *C. sacchariphagus* in sugarcane (Caresche, 1962). The same parasitoid was also introduced from Mauritius into Madagascar, where it did not establish (Caresche and Breniere, 1962). Following the success in Mauritius and Reunion Islands, *X. stemmator* was considered for releases against *C. sacchariphagus* in sugarcane in Mozambique (Conlong and Goebel, 2002). *X. stemmator* was first introduced into Mozambique in July 2001 in five sugarcane fields against *C. sacchariphagus* in sugarcane at Mafambisse (19°33.217' S and

34°37.333' E, a lowland area of less than 100 m asl) in central province of Sofala (Conlong and Goebel, 2002).

The parasitoid was recovered in post release surveys conducted during the release season and one year later, from the release fields with a concomitant reduction in *C. sacchariphagus* populations (Conlong and Goebel, 2002). This was clear evidence that the exotic pupal parasitoid was able to successfully adapt and colonize the sugarcane fields. Following this success, it was thought that *X. stemmator* could be a promising candidate for classical biological control against maize and sorghum stem borers and, thus, complement the activities of egg and larval parasitoids.

In subsequent years, a biological control program was developed to import and release *X. stemmator* in small-scale farmers' maize fields in Mozambique. Similar programs were carried out in other countries in the region including Malawi, Zambia, Zimbabwe, Tanzania, Kenya and Uganda. The pupal parasitoid was imported from ICIPE's Insect Mass Rearing Unit in Nairobi into Mozambique. The first releases were conducted in Xai-Xai Southern province of Gaza in 2002. This chapter discusses the results of releases and subsequent sampling to the establishment of *X. stemmator* in small-scale maize fields in three agroecological zones in Mozambique.

6.2 Materials and Methods

6.2.1 Release sites

Parasitoid releases were conducted in maize fields at three agro-ecological zones in Mozambique with distinctly different stemborer complexes: 1) lowland areas at less than 200 m asl where *C. partellus* constitute more that 95% of the total stemborer population (Guija, Magude, Nhamatanda and Xai Xai), 2) mid to high elevation areas

at 800 m asl, where both *C. partellus* and *B. fusca* occur (Manica) and 3) at high elevation area (Lichinga) at about 1400 m asl in the northern province of Niassa where *B. fusca* accounts for more than 90% of the total stemborer population and where *S. calamistis* and *C. partellus* are minor .

6.2.2 *Xanthopimpla stemmator* releases

Parasitoid releases were conducted during the 2001/2002 and 2002/2003 growing seasons in small-scale maize farmers' fields in South, Centre and North Mozambique. The parasitoid was shipped from ICIPE as parasitized *C. partellus* pupae. To increase the probability of its establishment, the parasitized pupae were kept in emergence cages (30x30x30 cm) for adult emergence. After emergence the adult parasitoids were provided a water-honey solution as food until most of the parasitoids emerged and allowed to mate under laboratory conditions.

Whenever possible, the releases were made when host pupae were abundant. Release points were separated by a minimum of 100 km from one another. At each site, infested fields were selected for releases. The release sites consisted of a group of four infested maize fields separated by at least 500m from each other. The emergence cages containing the adult parasitoids were transported to the field and adult wasps liberated from them.

Parasitoid mortality was assessed, after releases were made, counting the number of dead adult parasitoids inside the emergence cage and parasitoids that did not emerge from the host pupae.

The viability of *X. stemmator* received from ICIPE, Nairobi, Kenya was in general high, since more than 90% of adult parasitoid survival was recorded. The highest pre-release mortality of the parasitoid was observed at Lichinga, a high elevation zone, where *B. fusca* was the most abundant stem borer species (Table 6.1). Lichinga, where minimum temperatures during the releasing periods were ranging from 10 to 15°C, is the coolest area in Mozambique.

Table 6.1. Levels of *X. stemmator* survival at the parasitoid release time

Location	Altitude	Number released	Percent survival
Guija	120	2000	93.4
Lichinga	1430	2000	89.3
Magude	140	1500	91.6
Manica	880	1250	90.3
Nhamatanda	460	1500	91.7
Xai-Xai	80	3000	94.6
Total		11250	

6.2.3 Sampling procedures

Post release sampling was conducted at release and non-release sites during the same season and one year after at low, mid and high elevation zones. Lowland sampling sites included Guija, Magude, Nhamatanda, Tica and Xai-Xai, while Manica and Lichinga were situated at mid and high elevations areas, respectively.

Because *X. stemmator* may have spread from sugarcane into other crops , maize fields in close proximity to sugarcane plantations, where *X. stemmator* was previously released in 2001 and 2002, were monitored in Tica to evaluate the spread of *X. stemmator*. Tica is located at approximately 25 Km from sugarcane release fields and about 50 Km from the maize release fields at Nhamatanda.

At each sampling site, 20 farmers' fields within a 25 Km radius from each releasing site were randomly selected when pupae were expected to be abundant to assess if the parasitoid had established at the release areas and whether it spread to other sites. From each field, 20 infested plants were selected and dissected in the field for stem borer pupae. All larvae and pupae were placed individually in glass vials and taken to the laboratory until adult moth or parasitoids emergence. All parasitoids were identified to species.

6.2.4 Data analysis

To evaluate the possible effect of stemborer species composition on the parasitoid establishment, each field was considered a replicate and each release and/or sampling site was used as treatment. Means were calculated for each sampling site. Data were subjected to analysis of variance (ANOVA) ((Proc ANOVA or GLM, SAS Institute, 1999) for each study location and means were separated by Student-Newman-Keuls (SNK) multiple range test when ANOVAs were significant ($P < 0.05$). In order to normalize the data, values for percent parasitism and the number of insects were respectivcly transformed to square root and logarithmic transformation before analysis.

6.3 Results

6.3.1 Stem borer pupae abundance and *Xanthopimpla stemmator* recoveries

Pupae of *C. partellus*, *B. fusca* and *S. calamistis* were collected at all sites. *C. partellus* pupae were abundant in lowland areas whereas pupae of B. *fusca* were found in high proportions at mid to high elevations zones of Manica and Lichinga (Table 6.2). *S. calamistis* was scarce at all sites.

Number of pupae per plant was significantly highest at Tica and Xai Xai ($P<0.05$) followed by Guija and Nhamatanda. The lowest pupa density was recorded at the northern site of Lichinga, a high elevation area dominated *B. fusca* and where *C. partellus* has only been reported since the 2002/2003 growing season.

The number of *X. stemmator* recoveries and levels of parasitism are also presented in Table 6.2. *X. stemmator* was recovered only from *C. partellus* pupae collected mainly from locations where *C. partellus* was dominant. The number of *X. stemmator* recovered was highest at Tica ($P<0.05$) followed by Magude and Xai Xai. *X. stemmator* was not found at Lichinga. In Manica, only two *C. partellus* pupae were found parasitized by *X. stemmator*, a mid elevation zone (Table 6.2). With exception of Lichinga, the levels of parasitism did not vary significantly between sampling sites (Table 6.2). Later sampling at the same sampling locations did not recovery *X. stemmator* at any of the release and/or sampling sites.

Table 6.2 – Number of *C. partellus* and *B. fusca* pupae collected and level of parasitism due to *X. stemmator* on *C. partellus* pupae

Local	*C. partellus* Pupae	*B. fusca* pupae	Pupae per plant	*Number of X. stemmator* recovered	Parasitism on *C. partellus*
Guija	82±0.96a	0	0.21±0.19ab	9±0.51abc	11.0±3.51a
Lichinga	4±0.37b	15±0.65a	0.05±0.12c	0.0±0.0c	0.0±0.0c
Magude	113±0.86a	0	0.28±0.18a	12±0.54ab	10.6±3.28a
Manica	45±0.84a	18±0.69a	0.16±0.17b	2±0.27bc	4.4±1.79b
Nhamatanda	96±0.55a	0	0.24±0.12ab	5±0.40abc	5.2±2.78b
Tica	122±0.60a	0	0.31±0.13a	15±0.55a	12.3±2.95a
Xai Xai	118±0.54a	0	0.30±0.12a	10±0.49ab	8.5±2.36ab
Df	6, 399	6, 39	6, 399	6, 399	6, 399
F	23.8	2.4	12.1	4.6	6.3
P-values	<0.0001	0.1048	<0.0001	<0.0001	<0.0001

Numbers followed by the same letter in the same column are not statistically different (SNK, $P<0.05$)

6.4 Discussion

Xanthopimpla stemmator has been recovered at lowland release sites indicating that this exotic parasitoid was able to locate and sting the host pupae. *X. stemmator* has been previously released in South Africa in maize and sorghum (Moore and Kfir, 1996) and in Mozambique in sugar cane (Conlong and Goebel, 2002). In South Africa, in spite of initial recoveries, the parasitoid did not establish (Kfir, 1997a).

Xanthopimpla stemmator was introduced into Mozambique in 2001 from South Africa. The parasitoid was recovered during the release season (Conlong and Goebel, 2002) and one year after. Similarly, in the present study, *X. stemmator* was recovered during the release season and one year after at some release and non-release locations. *X. stemmator* was not recovered at Lichinga and very few samples were reared from *C. partellus* pupae at Manica. This may due to the stem borer species composition and/or agroecological environmental conditions. Lichinga is one of the cooldest areas in Mozambique dominated by *B. fusca* while Manica is a mid to high elevation zone and is usually cool during winter period where *B. fusca* occurs in mixed populations with *C. partellus*.

In the Northern region of Lichinga *B. fusca* has been reported to undergo diapause in the larval stage during the cold winter dry season (Davies *et al.*, 1995). This may reduce the production and availability of frass, the cues for parasitoid locating the host habitat and attacking it, suggesting that *X. stemmator* could not establish in diapausing stem borer species during the off-season. Hailemichael *et al.* (1994) observed that *X. stemmator* responded to stimuli that resulted from the host's presence or its feeding activities.

In contrast, *C. partellus* does not enter diapause in the lowland areas in southern Mozambique due to continuous maize growing and food availability (Segeren *et al.*, 1991). This may improve host finding by the parasitoid and may explain the high recoveries of the parasitoid in the areas where *C. partellus* was abundant. Laboratory studies showed that *X. stemmator* successfully parasitized and developed in *B. fusca* pupae (Gitau *et al.*, 2005). However, field releases in South Africa did not result in *X. stemmator* establishment (Conlong 1994; Moore and Kfir, 1996). This was attributed to the low temperatures reported during winter that led to host diapausing at the sites where the parasitoid was released against *B. fusca* and *C. partellus* in sorghum (Moore and Kfir, 1996).

The significant high level of parasitism on *C. partellus* at Tica might be due to the parasitoid's spread from the releases sites on sugar cane at Mafambisse Estate made in 2001 against *C. sacchariphagus* (Conlong and Goebel, 2002). *X. stemmator* was reared from *C. partellus* pupae collected on maize surrounding sugar cane fields. Some specimens were collected at Tica, a non-release site located at about 25 Km from the sugar cane release fields or from releases on maize farmers' fields (2002/2003 growing season) at about 50 Km from Tica.

Thus, the recovery of *X. Stemmator* at Tica is an indication that the exotic parasitoid is spreading from the release areas to other locations. It was evident that the parasitoid had spread over 25 Km from the nearest release site at Mafambisse sugarcane state since its first releases in 2001 (three years after its first release). The recovery of *X. stemmtor* from only *C. partellus* pupae may suggest the close association of the parasitoid-host interaction. In Laboratory studies Gitau *et al.*

(2005), argued that this fact might reflect a closer evolutionary relationship between *X. stemmator* and its old-association host *C. partellus*.

In surveys conducted two years after parasitoid releases, *X. stemmator* was not recovered again at any of the previous release sites suggesting that no "permanent establishment" had taken place or that its populations are still too low to be recovered in few samples. Similar results were reported at Mafambisse Sugarcane State where *X. stemmator* was recovered during the release season and one year after (Conlong and Goebel, 2002) but not four years after releases (Conlong Unpubl. Data). Failure of *X. stemmator* to establish in the field has also been reported from South Africa (Moore and Kfir, 1996) and in Madagascar (Caresche and Breniere, 1962).

The *X. stemmator* strategy of drilling through the plant stem to attack the enclosed pupae (Smith *et al.*, 1993) may affect the parasitoid effectiveness because of limited host access. Hawkins *et al.* (1987) reported that *Allorhogas pyralophagus* Marsh (Hymenoptera: Braconidae), a larval parasitoid of stem borers that uses similar strategy of drill-and-sting as *X. stemmator*, was more successful to attack the host in a thin-stemmed grasses such as *Sorghum halapense* (L.) than in sugarcane and they concluded that the plant stem diameter was a factor limiting the success of parasitoid to successful attack the host. Thus, stem borer pupae found in small-stemmed plants should be more accessible to *X. stemmator* than pupae located in larger stemmed plants such as maize and sugarcane.

The exotic pupal parasitoid, *X. stemmator* released in maize fields was able to successful adapt to the local environmental field conditions and colonized stemborer pupae in the release field sites. Parasitoid samples recovered at non-release site

indicated the ability of parasitoid to spread from the release sites to other areas where it was not released.

If successful established, *X. stemmator* will provide stem borer mortality jointly with the previously released and established larval endoparasitoid, *C. flavipes* in the areas where *C. partellus* is the most abundant stem borer species. However, observations made by Gitau *et al.* (2005) that *X. stemmator* may be able to establish in areas dominated by *B. fusca*, contradicts the present results which indicated that the parasitoid failed to parasitize and develop in *B. fusca* and colonize the areas where this stem borer was dominant. These observations suggest that the parasitoid may not be able to establish in the areas where *B. fusca* is the most abundant stem borer species, and thus its release should be restricted to the areas dominated by its old-association host, *C. partellus*.

CHAPTER SEVEN

7.0 EFFECT OF TEMPERATURE AND HOST SPECIES ON THE DEVELOPMENT OF PUPAL PARASITOIDS, *Dentichasmias busseolae* (HEINRICH) AND *Xanthopimpla stemmator* (THUNBERG) (HYMENOPTERA: ICHNEUMONIDAE)

7.1 Introduction

Xanthopimpla stemmator a solitary pupal parasitoid was imported from Mauritius and released at sugar cane state of Mafambisse in Mozambique in 2001 (Conlong and Goebel, 2002). Other releases were made in three other different agro-ecological zones for the control of maize and sorghum stem borers. *D. busseolae* is the most abundant indigenous pupal parasitoid of cereal stem borers in many areas of Mozambique (Segeren *et al.*, 1991; Cugala *et al.*, 2001).

In the areas where *X. stemmator* was released, the native pupal parasitoid, *D. busseolae* does occur. Both parasitoids are solitary pupal parasitoids that have similar host range. Thus, there is a possibility that both parasitoids will reside in the same area at the same time and attack the same host species. In its original habitat, *X. stemmator* occurs in warm low-altitude areas (Moore and Kfir, 1996). The three agro-ecological zones occurring in Mozambique have distinct temperature and stem borer species composition. Pak (1986), stated that host species and temperature have substantial influence on the successful attack and development of parasitoids.

The ability of each parasitoid to develop in different stem borer species and at different temperature ranges may influence the predominance or success of both parasitoids. However, there is no information available on the development of *X.*

stemmator on the different hosts at different temperatures. This study was proposed to evaluate the effect of temperature and host species on the development of *X. stemmator* and *D. busseolae*.

7.2 Materials and methods

The successful development and emergence of *D. busseolae* and *X. stemmator* were evaluated in the laboratory on three stem borer species (*C. partellus*, *B. fusca* and *S. calamistis*) and five constant temperatures (15, 20, 25, 30 and 35°C). The five temperatures were chosen because they are experienced in the majority of agro ecological regions of Mozambique. A total of 160 host pupae of each stem borer species were exposed at each temperature to each of the parasitoids.

7.2.1 Colony of stem borer pupae

A colony of *C. partellus*, *B. fusca* and *S. calamistis* pupae was established at ICIPE's Rearing Unit. Three stem borer species, *C. partellus*, *B. fusca* and *S. calamistis* were used as hosts replicated 4 times at 5 different temperatures 15, 20, 25, 30 and 35°C at a range of 60-70% relative humidity (RH). Factorial combination of five different temperatures and the 3 common stem borer species were arranged in Randomised Complete Block design. At each replication, 40 pupae of each stemborer species were exposed to mated female parasitoids for oviposition at each temperature as described by Bahana (1990).

7.2.2 Host exposure to *Dentichasmias busseolae*

Full grown larvae of each stemborer species were placed in field collected maize stems measuring 10 cm in length. The larvae were allowed to tunnel into the stems and to pupate and make the moth exit holes. Ovipositing female parasitoids are able

to reach the pupae through the moth exit holes opened by larvae prior to pupation (Bahana, 1990). The pupae were then offered to mated female parasitoids in oviposition cages for 24 hours during which time they were expected to have been parasitized.

The exposed pupae were removed from the stems by splitting them in such a way that moth exits remain intact. The split stems were used several times, placing pupae into the pupal chamber, where the previous larva had pupated. The two halves of the stems were then held together with tape. Fresh larval frass of fifth instar larvae of each stem borer species was sprinkled around the pupae before offering them to the female parasitoids.

7.2.3 Exposure to *Xanthopimpla stemmator*

Adult parasitoids were released in a clean cage and provided with a 20% honey water solution for food. The host pupae were placed in paper straws and then placed in the cage containing adult parasitoids for oviposition for 1 hour due to the female parasitoid aggressive behavior. The straws were smeared with fresh larval frass from the respective stem borer species to simulate stalks of cereal crops and to enhance acceptance (Gitau, 2002). The pupae contained in paper straws were placed in an oviposition cage and then offered to mated female parasitoids for oviposition. Parasitized pupae were kept in Petri dishes at the five different temperatures until either adult parasitoid or moth emergence.

7.2.4 Developmental time and adult longevity of parasitoids

After exposure, the pupae were removed from the stems or paper straws and placed on moistened filter paper in petri dishes. The petri dishes containing pupae, were

kept in a controlled temperature chambers at 15, 20, 25, 30 and 35 °C and 60-70% RH. The petri dishes were inspected for emergence of either moths or adult parasitoids at least twice per day. Host pupae dying without producing adult parasitoids were dissected to evaluate the presence of parasitoids inside.

Adult parasitoids, which emerged, were provided a 20% honey-water solution as food. The developmental time of the parasitoid (period between oviposition and adult parasitoid emergence) was evaluated by observing daily the emergence of adult parasitoids at each temperature and from each host. To estimate the longevity (time between adult parasitoid emergence and its death), adult parasitoids mortality was recorded daily until all adult parasitoids died.

7.3 Data analysis

Data were subjected to analysis of variance (ANOVA) (Proc ANOVA, SAS Institute, 1999). Means were separated using the Student Newman Keuls multiple range test when ANOVA was significant ($P < 0.05$). The relationships between temperatures, parasitoids performance and host species were assessed using correlation matrix and regression analysis (PROC CORR and REG, SAS). For data normalization and homogeneity, data on proportion of hosts surviving, dying and parasitoids emergence were arcsine transformed before being subjected to ANOVA. While developmental time and adult parasitoid longevity were transformed using square root (n+1). Paired comparisons between the two parasitoids and between males and females were carried out using t-test (PROC TTEST SAS Institute, 1999)

The effect of temperature on the developmental rate was estimated only for *X. stemmator* reared from *C. partellus* as host species. For estimation of the lower

temperature threshold ($T\ell$) and the thermal constant (k=the number of degree-days) data on developmental rates plotted against the tested temperatures were fitted to a single regression model ($Rt=a+bT$ where Rt=developmental rate, T=temperatures, a and b are regression parameters) as described in Daane *et al.* (2004) and Chabi-Olaye *et al.* (2004). The lower development threshold ($T\ell$) was estimated as $T\ell= a/b$ and the thermal constant as $K=1/b$.

Life table parameters such as net reproductive rate (Ro), intrinsic rate of increase (r_m), mean generation time (T), finite rate of increase (λ) and doubling time (Dt) of each parasitoid on each host species at different temperatures were estimated using the SAS program (Life table SAS) according to the protocol of Maia *et al.* (2000). Differences in Ro, r_m, T, λ and Dt values at the different temperatures were calculated using Jacknife method and compared with Student Newman Keuls based on Jacknife estimates of variance.

Due to nil or low numbers of adult parasitoid emergence from the three host stem borer species at some temperatures, life table parameters were estimated at 20°C, 25°C and 30°C for *X. stemmator* in all the three host species and 15°C, 20°C and 25°C for *D. busseolae* on *C. partellus* only.

7.4 Results

7.4.1 Effect of temperature and host on the parasitoid emergence

The exotic, *X. stemmator* and the native *D. busseolae* pupal parasitoids of cereal stem borers both were able to successfully parasitize and complete their development in all three stem borer species at four of the five temperatures tested in this study (15, 20, 25 and 30°C). Adult parasitoid emergence was directly proportional to

temperature in the range of 15 to 25°C. Complete failure of parasitoid emergence in all host species was reported at 35°C (Table 7.1).

Both host species and temperature significantly affected the emergence of *D. busseolae* at 15, 20 and 25 °C. Significantly ($P<0.05$) more *D. busseolae* emerged from *C. partellus* pupae than from *B. fusca* and *S. calamistis* at the same temperatures ($P<0.05$). However, *X. stemmator* adult emerged at 15, 20, 25 and 30 °C but not at 35 °C. Significantly ($P<0.05$) more *X. stemmator* emerged from *C. partellus* at 15°C and from *B. fusca* at 25 °C (Table 7.1).

The highest and the lowest values of *X. stemmator* emergence were recorded on *B. fusca* at 25 °C (53.1±0.6 individuals) and 35 °C (0.0) respectively. However, on *B. fusca* and *S. calamistis X. stemmator* was significantly ($P<0.05$) superior in relation to *D. busseolae* at 20, 25 and 30 °C. No development was observed at 30 and 35 °C

7.4.2 Effect of temperature and host on parasitoids developmental time

The mean developmental time, from oviposition to parasitoid adult emergence, at each tested temperature, host and parasitoid are shown on Table 7.2. The developmental time of both parasitoids was slow at longer temperatures and shorter when temperatures increased.

D. busseolae developed significantly faster only on *C. partellus* and *S. calamistis* at 15°C ($P<0.05$) than on *B. fusca*. No significant differences were found on the *D. busseolae* developmental time at 25°C for all hosts (Table 7.2). *X. stemmator* developed significantly ($P<0.05$) faster on *C. partellus* than on *B. fusca* and *S. calamistis* at 20 °C and on *B. fusca* at 25°C than on *C. partellus* and *S. calamistis*

(Table 7.2). However, at 30°C there were no significant differences on the *X. stemmator* development in all the three host species used. No development was observed at 35 °C.

The range of suitable temperatures for the development of *D. busseolae* varied from 15 to 25°C (on *C. partellus*, *B. fusca* and *S. calamistis*), while for *X. stemmator* the suitable temperatures varied from 15 to 30°C on *C. partellus*

Table 7.1 – The effect of temperature on the performance of *D. busseolae* and *X. stemmator* in three stem borer species

Temperature	Percent parasitoid dying inside host					Percent host producing parasitoids				
D. busseolae										
	C. partellus	*B. fusca*	*S. calamistis*	*Df*, F	*P*	*C. partellus*	*B. fusca*	*S. calamistis*	*Df*, F	*P*
15	16.9±0.37aA	1.9±0.14aB	3.7±0.17aB	2, 17.2	0.0001	46.0±0.50bA	1.3±0.11aC	12.5±0.33aB	2, 70.1	0.0001
20	3.1±0.22bA	1.2±0.11aB	0.0±0.00bB	2, 5.4	0.0001	62.5±0.48aA	0.0±0.00aB	0.0±0.00cB	2, 265.8	0.0001
25	15.0±0.35aA	0.6±0.08aB	1.3±0.11abB	2, 20.6	0.0001	44.4±0.50bA	0.6±0.08aB	5.0±0.22bB	2, 93.2	0.0001
30	0.0±0.00bA	0.0±0.00aA	0.0±0.00bA	-	-	0.0±0.00cA	0.0±0.00aA	0.0±0.00cA	-	-
35	0.0±0.00bA	0.0±0.00aA	0.0±0.00bA	-	-	0.0±0.00cA	0.0±0.00aA	0.0±0.00cA	-	-
Effect T*H	***	ns	**			***	ns	***		
Fdf	4, 15.9	4, 21.9	4, 13.9			4, 90.1	4, 1.3	4, 15.3		
P	0.0001	0.0001	0.0001			0.0001	0.2547	0.0001		
X. stemmator										
15	26.2±0.44aA	21.9±0.42aAB	13.8±0.34bB	2, 4.0	0.0190	3.8±0.19cdA	0.0±0.00cB	0.6±0.08cB	2, 4.5	0.0112
20	13.1±o.34cA	7.5±0.26bA	11.2±0.31bA	2, 1.4	0.2522	44.4±0.90aA	47.5±0.50aA	33.1±0.47aA	2, 2.1	0.1200
25	6.2±0.24cdA	5.6±0.23bA	12.5±0.32bA	2, 2.8	0.0607	28.8±0.45bB	53.1±0.64aA	33.8±0.48aB	2, 97.0	0.0001
30	16.2±0.38bA	28.1±0.45aA	26.8±0.44aA	2, 3.0	0.0503	15.6±0.34cA	10.6±0.32bA	10.6±0.31bA	2, 0.4	0.7071
35	0.0±0.00dA	0.0±0.00bA	0.0±0.00cA	-	-	0.0±0.00dA	0.0±0.00cA	0.0±0.00dA		
Effect T*H	***	***	***			***	***	***		
Df, F	4, 15.9	4, 21.9	4, 13.9			4, 23.0	4, 71.3	4, 41.5		
P	0.0001	0.0001	0.0001			0.0001	0.0001	0.0001		

Means in a column followed by the same lower case letter and means within a row followed the same upper case letter are not significantly different (SNK, $P<0.05$), T=temperature, H=host species

and *S. calamistis* and 20 to 30°C on *B. fusca*. At 15°C *X. stemmator* took about 68±12.9 and 66.0±12.0 days to complete its development respectively on *C. partellus* and *S. calamistis*, while at the same temperature, *D. busseolae* took approximately 42,6±24.4, 95±0.42 and 42.3±1.2 days respectively on *C. partellus*, *B. fusca* and *S. calamistis* (Table 7.2).

However, at 30°C the developmental time of both parasitoids dropped to 14,9±0.5, 9.1±0.5 and 17.8±0.8 for *X. stemmator* respectively on *C. partellus*, *B. fusca* and *S. calamistis*. In the same order of host species, the developmental time of *D. busseolae* at 25 °C decreased to 18.8±0.8, 20.0±0.4 and 19.9±0.3 days to complete development in the three hosts above mentioned (Table 7.2).

Comparisons between the two parasitoids shows that *X. stemmator* developed significantly slowly at 15, 20 and 25°C on *C. partellus* and *S. calamistis*, but was significantly faster on *B. fusca* than *D. busseolae*. At the temperatures between 15 and 25°C, the developmental time was a positive linear function with temperature, particularly on *C. partellus* ($y= -14.4x+69.9$, $r^2 = 0.874$), but there was no development at temperature above 30 °C.

With exception of 20°C, there were no significant differences on the effect of sex on *X. stemmator* developmental time at tested temperatures in all host species. Only at 20°C did males develop significantly faster than females of *X. stemmator* on *S. calamistis*. The development of both males and females *X. stemmator* increased linearly within the tested temperature range.

7.4.3 Effect of temperature on the development rate of *X. stemmator* reared from *C. partellus*

The effect of temperature on the developmental rate of *X. stemmator* in *C. partellus* is shown in Fig 7.1. At temperatures between 15 to 30°C, the development rate was a positive linear function of temperature. The developmental rate plotted against temperature on a nonlinear regression model provided the optimal and maximum temperatures of 29.5 and 35°C respectively. The lower temperature threshold estimated from linear regression of temperature and developmental rate (Rt= -0.0322 + 0.0033T, r^2=0.9670, P=0.0166) of 9.76°C and the thermal constant of 303.0 DD were above the lower developmental threshold.

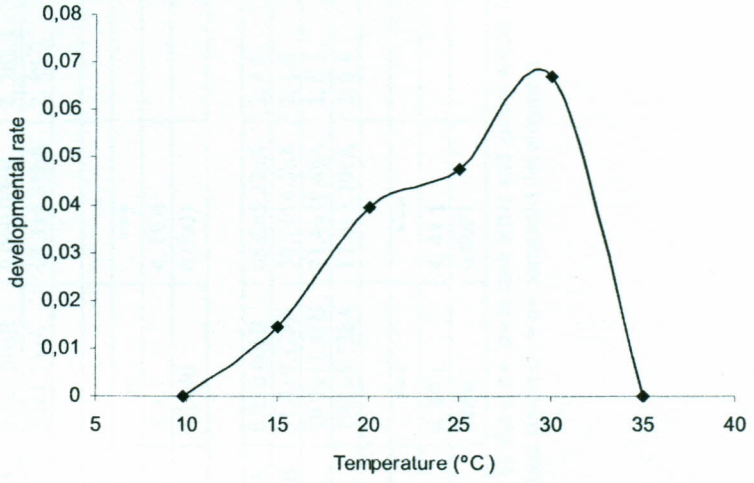

Fig 7.1 Effect of temperature on the developmental rates (1/developmental time in days) of *X. stemmator* reared from *C. partellus*

Table 7.2 – Mean parasitoids developmental time, longevity in relation to host and temperature and host survivorship and death

Temperature	Developmental time (±SE) (days)					Parasitoid longevity (±SE) (days)				
D. busseolae										
	C. partellus	*B. fusca*	*S. calamistis*	Df, F	*P*	*C. partellus*	*B. fusca*	*S. calamistis*	Df, F	*P*
15	42.6±21.4aB	95±5.32aA	42.3±14.8aB	2, 70.6	0.0001	25.6±12.4cA	11.0±0.82aB	28.8±17.7bA	2, 26.8	0.0001
20	23.3±11.3bA	0.0±0.00aB	0.0±0.00cB	2, 262.3	0.0001	99.3±21.3aA	0.0±0.00aB	0.0±0.00cB	2, 86.5	0.0001
25	18.8±9.4cA	20±1.58aA	19.3±4.35bA	2, 89.9	0.0001	55.8±8.5bB	137±10.8aA	52.3±15.2aB	2, 34.4	0.0001
30	-	-	-			-	-	-		
35	-	-	-			-	-	-		
Effect T*H	***	ns	***			***	ns	***		
Fdf	4, 90.3	4, 1.7	4, 18.4			4, 52.5	4, 0.9	4, 6.0		
P	0.0001	0.1440	0.0001			0.0001	0.4295	0.0001		
X. stemmator										
15	68.0±12.9aA	0.0±0.00cB	66.0±5.22aA	2, 4.6	0.0110	17.8±4.63cB	0.0±0.00dC	30.0±2.37bA	2, 2.1	0.1234
20	25.4±14.2bB	31.4±9.12aA	30.1±14.3bA	2, 4.8	0.0083	57.4±12.4aA	46.7±8.31aA	45.5±31.4aA	2, 9.1	0.1551
25	21.1±9.2bA	19.2±11.4bB	21.4±10.4bA	2, 6.5	0.0017	44.1±8.6bA	30.1±12.4bA	43.5±32.0aA	2, 0.5	0.6146
30	14.9±5.7cA	19.1±5.73bA	17.0±5.30cA	2, 0.4	0.6925	10.9±6.7cA	13.2±6.61cA	11.1±5.42bA	2, 0.3	0.7481
35	-	-	-			-	-	-		
Effect T*H	***	***	***			***	***	***		
Df, F	4, 30.9	4, 89.1	4, 44.1			4, 29.4	4, 40.4	4, 25.8		
P	0.0001	0.0001	0.0001			0.0001	0.0001	0.0001		

Means in a column followed by the same lower case letter and means within a row followed the same upper case letter are not significantly different (Anova, P>0.05), T=temperature, H=host species, - = no parasitoid development

Table 7.3 – Mean percentage of host survivorship and death at different temperature under the two parasitoid parasitization levels

D. busseolae										
Temperature	Host surviving*					Host dying**				
D. busseolae	*C. partellus*	*B. fusca*	*S. calamistis*	Df, F	*P*	*C. partellus*	*B. fusca*	*S. calamistis*	Df, F	*P*
15	9.4±0.29aC	86.9±0.33aA	64.4±0.48cB	2, 176.6	0.0001	27.5±0.49bA	11.3±0.32cB	19.4±0.38bcB	2, 19.1	0.0001
20	5.6±0.20abC	93.8±0.24aA	83.1±0.38abB	2, 470.5	0.0001	28.8±0.45cA	5.0±0.22cC	16.9±0.37cB	2, 17.2	0.0001
25	7.5±0.26aB	89.4±0.32aA	85.6±0.36aA	2, 344.2	0.0001	33.1±0.47bcA	9.3±0.30cB	8.1±0.27dB	2, 24.2	0.0001
30	0.6±0.08bC	21.2±0.41bB	73.8±0.44bA	2, 2.0	0.1348	99.4±0.08aA	78.8±0.41bB	26.2±0.44bC	2, 187.4	0.0001
35	0.0±0.00bA	0.0±0.00cA	1.2±0.11dA			100±0.00aA	100±0.00aA	98.8±0.11aA	2, 2.0	0.1348
Effect T*H	***	***	***			***	***	***		
Df, F	4, 6.7	4, 356.0	4, 133.2			4, 157.4	4, 400.2	4, 187.0		
P	0.0001	0.0001	0.0001			0.0001	0.0001	0.0001		
X. stemmator										
15	23.8±0.43bC	60.0±0.49aA	48.1±0.50aB	2, 24.7	0.0001	46.2±1.24cA	18.1±0.48cB	37.5±0.50bcB	2, 13.7	0.0001
20	12.5±0.33cA	20.6±0.40bA	11.3±0.32cA	2, 2.8	0.0613	30.0±0.48dA	24.4±0.43dB	44.4±0.49bA	2, 7.3	0.0008
25	40.6±0.49aA	26.9±0.44bB	21.3±0.41bB	2, 7.6	0.0006	24.4±0.43dA	14.4±0.41dA	31.9±0.46dA	2, 2.1	0.1195
30	16.9±0.38bcA	1.2±0.11cB	2.5±0.15dB	2, 20.5	0.0001	51.2±0.50bA	60.0±0.49bA	60.0±0.49bA	2, 1.5	0.2152
35	3.1±0.17dA	0.0±0.00dB	0.0±0.00dB	2, 5.1	0.0063	96.9±0.17aB	100±0.00aA	100±0.00aA	2, 5.1	0.0063
Effect T*H	***	***	***			***	***	***		
Df, F	4, 22.3	4, 79.3	4, 56.5			4, 33.6	4, 103.3	4, 56.2		
P	0.0001	0.0001	0.0001			0.0001	0.0001	0.0001		

Means in a column followed by the same lower case letter and means within a row followed the same upper case letter are not significantly different (SNK, $P<0.05$), T=temperature, H=host species, * = host producing adult moth, ** = host dying without producing parasitoid

7.4.4 Adult parasitoid longevity

Table 7.2 shows the adult parasitoid longevity of both parasitoids in relation to temperature and host species. With the exception of *X. stemmator* at 15°C, there were no significant ($P<0.05$) differences on the adult *X. stemmator* longevity at 20, 25 and 30°C between the host species. At 15°C *X. stemmator* longevity was significantly longer on *S. calamistis* than on *C. partellus*.

D. busseolae adult lived significantly ($P<0.05$) much longer on *C. partellus* and *S. calamistis* than on *B. fusca* at 15°C, but its longevity was significantly longer on *B. fusca* than on *C. partellus* and *S. calamistis* at 25°C (Table 7.2). On *C. partellus*, the parasitoid longevity increased with temperature on the range of 15 to 20 °C and then decreased when temperature increases to 25 °C. The highest period of *D. busseolae* longevity was recorded at 25 °C on *B. fusca* (137±10.8 days) (Table 7.2).

When comparing both parasitoids, the present results indicate that *D. busseolae* longevity was significantly higher than of *X. stemmator* when *C. partellus* and *B. fusca* were the hosts. However, on *S. calamistis X. stemmator* adult lived longer than *D. busseolae* adults at 15°C ($P<0.05$), but at 25°C *D. busseolae* lived significantly much longer than those from *X. stemmator*.

When *C. partellus* was the host, *X. stemmator* female adult longevity was significantly longer than males at all tested temperatures. However, on *B. fusca* males, longevity was significantly higher at 20°C, while at 30°C females lived much longer than males. No significant differences were found on males and females longevity at 25°C.

When *S. calamistis* was the host, males and females of *X. stemmator* exhibited similar longevity at 20, 25 and 30°C.

Using data from the temperature tested, the longevity of both males and females was first positively affected by temperature in the range between 15 to 25°C and then negatively affected from 25 to 35°C. In general, adult parasitoids longevity was inversely proportional to temperature. Linear regression equations were obtained for developmental time and adult longevity for both males and females of *X. stemmator* at all temperatures and host species (Table 7.4).

Table 7.4 – Regression equations for *X. stemmator* developmental time and adult longevity on different hosts and different temperatures

Host species	Developmental time		Adult longevity	
	Males	Females	Males	Females
C. partellus	y= -15,7x+74.7 r^2 = 0.8359	y= -15,6x+69.9 r^2 = 0.874	y= -10,8x+55.7 r^2 = 0.7686	y= -8.5x+56.5 r^2 = 0.2024
B. fusca	y = -1.3x+18.1 r^2 = 0.0221	y= -1,3x+18.3 r^2= 0.022	y= -6.9x+43.2 r^2 = 0.1175	y= -2.2x+24.3 r^2 = 0.0352
S. calamistis	y= -1.8x+17.7 r^2 = 0.048	y= -1.9x+18.6 r^2 = 0.0521	y= -3.9x+35.6 r^2 = 0.0562	y= -2.6x+28.3 r^2 = 0.0362

Where y = developmental time and longevity (days) of both males and females (dependent variable);
x = is the temperature (°C) (independent variable)

7.4.5 Proportion of parasitoids found dead inside host

Host pupae dissections revealed that some parasitoids died inside host as adult that failed to emerge at 15, 20, 25 and 30°C. Significantly more adults of *D. busseolae* died in *C. partellus* at 15, 20 and 25°C than on *B. fusca* and *S. calamistis* ($P<0.05$). However, *X. stemmator* significantly failed to emerge from *C. partellus* than from *B. fusca* and *S. calamistis* at 15°C ($P<0.05$), but there were no significant differences between hosts on the *X. stemmator* mortality inside host at 20, 25 and 30°C (Table 7.1).

When *C. partellus* was the host, there was significantly higher mortality of *X. stemmator* at 15 and 20°C ($P<0.05$) compared to *D. busseolae* at the same temperatures. However, *D. busseolae* had significantly higher mortality at 25°C compared to *X. stemmator*. The proportion of *X. stemmator* adult that failed to emerge from *B. fusca* pupae was significantly higher compared to *D. busseolae* at all tested temperature, except at 35°C on which no individuals of either parasitoid emerged.

7.4.6 Host stem borer species survival

The number of host that survived or produced moth (i.e. escaped the parasitism or parasitoid did not develop inside host) producing adult moth was recorded from all five tested temperatures and host species. The highest proportion of host survival found on *D. busseolae* parasitism was reported at 20°C from *B. fusca* (93.8±0.2%) and the lowest from the same parasitoid at 25°C on *S. calamistis* (1.2±0.1%). Significantly more *B. fusca* pupae produced adult moth at 15°C ($P<0.05$) than *C. partellus* and *S. calamistis*, while at 25°C both *B. fusca* and *S. calamistis* similarly survived the parasitism of *D. busseolae* when compared with *C. partellus* pupae. At 30°C *S. calamistis* survived better than the other two hosts (Table 7.3).

When *X. stemmator* was the parasitoid, both the highest and lowest percentage of host survival was reported on *B. fusca* at 15°C (60.0±0.5%) and at 30°C (1.2±0.1%) respectively. At 15°C *B. fusca* pupae produced more adult moth when parasitised by *X. stemmator* than *C. partellus* and *S. calamistis* ($P<0.05$). However, at 25°C, 30°C and 35°C the survival of *C. partellus* pupae was significantly higher than other hosts. All the three host species survived similarly at the temperature of 20°C. In general,

for the three stem borers the proportion of host surviving decreased as the temperature increased (Table 7.3).

7.4.7 Host mortality

Table 7.3 shows the percent of host dying without producing parasitoids. Host death increased as temperature increased to a level of 100% when temperature was 35°C. Host exposure to 35°C was completely lethal to all host pupae of *B. fusca*, but only a 1.2% of *S. calamistis* (on *D. busseolae*) and 3.1% of *C. partellus* (on *X. stemmator*) survived at this temperature. When *X. stemmator* was the parasitoid, the proportion of host dying without producing parasitoids increased from 24.4±0.4% (at 20°C) to 96.9±0.2% (at 35°C), from 14.4±0.0 (at 25°C) to 100% at 35°C and from 31.9±0.5% (at 25°C) to100% (at 35°C) for *C. partellus*, *B. fusca* and *S. calamistis* respectively (Table 7.3).

At 15, 20, 25 and 30°C significantly more *C. partellus* died due to *D. busseolae* parasitism ($P<0.05$), while at 35°C there were no significant differences among the hosts. However, when *X. stemmator* was the parasitoid, *C. partellus* pupal death was significant higher compared to *B. fusca* and *S. calamistis* at 15°C. At 20°C, *C. partellus* and *S. calamistis* pupae mortality was significantly ($P<0.05$) higher in relation to *B. fusca* pupae. However, at 25°C there were no significant differences between host pupae death (Table 7.3).

7.4. 5 Life table parameters estimates of the parasitoids

The effect of temperature and host species on the life table parameters including net reproductive rate (Ro), intrinsic rate of increase (r_m), mean generation time (T), finite rate of increase (λ) and doubling time (Dt) of *D. busseolae* on *C. partellus* and *X.*

stemmator on *C. partellus*, *B. fusca* and *S. calamistis* at different temperatures are presented in Tables 7.5 and 7.6 respectively. The finite rate of increase indicate that *D. busseolae* population could increase similarly at all the three temperature used for life table analysis.

There were no significant differences between the different temperatures on the *D. busseolae* net reproductive rate (Ro), finite rate of increase and doubling (Dt). However, the mean generation time was significantly greater at 15°C (52.69 days) compared to the 33.72 and 31.98 days at 20°C and 25°C respectively (Table 7.5), and thus, decreased with increasing temperature. The intrinsic rate of increase was significantly ($P<0.05$) higher at 20 °C than at 15 and 25 °C. The intrinsic rate of increase doubled from 0.06 at 15°C to 0.123 at 20°C (Table 7.5).

Table 7.5 – Life table parameters of *D. busseolae* reared on *C. partellus* at different temperatures and constant RH

Life table parameters	Temperature			P
	15°C	20°C	25°C	
Net reproductive rate (Ro)	30.77	32.96	20.6	ns
Intrinsic rate of increase (r_m)	0.06	0.123	0.09	*
Mean generation time (T) (days)	52.69	33.72	31.98	*
Finite rate of increase (λ)	1.11	1.06	1.10	ns
Doubling time (Dt) (days)	6.70	10.63	7.32	ns

* significant; ns = not significant ($P<0.05$, SNK)

Xanthopimpla stemmator net reproductive rate as well as the mean generation time were significantly high at 20°C compared to 25 and 30°C in all the three stem borer species. However, the intrinsic rate of natural increase (r_m) was similar for females reared from *B. fusca* and on *S. calamistis*, but on *C. partellus* the intrinsic rate of

increase was significantly greater at 30°C than at 20 and 25°C. On *C. partellus*, intrinsic rate of increase doubled from 0.10 at 20°C to 0.22 viable females per female/day at 30°C (Table 7.6)

The doubling time (Dt) in Table 7.6 shows that in general, *X. stemmator* took longer to double its generation at lower temperatures (<20 °C) when compared to higher temperatures (>25 °C) in all host species. Thus, the doubling time of *X. stemmator* decreased with increasing temperature. The finite rate of increase indicated that *X. stemmator* population could multiply or increase faster at high temperatures in all the three stem borer species.

Table 7.6 – Life table estimates for *X. stemmator* reared on *C. partellus*, *B. fusca* and *S. calamistis* at different temperatures

Life table parameters	C. partellus				B. fusca				S. calamistis			
	20ºC	25ºC	30ºC	P	20ºC	25ºC	30ºC	P	20ºC	25ºC	30ºC	P
Net reproductive rate (Ro)	72.00	26.53	20.57	*	43.72	63.48	6.28	*	27.91	20.97	5.68	*
Intrinsic rate of increase (r_m)	0.10	0.10	0.22	*	0.13	0.18	0.12	ns	0.11	0.11	0.13	ns
Mean generation time (T) (days)	43.53	33.86	13.50	*	28.92	23.06	15.33	ns	29.65	27.86	13.06	*
Finite rate of increase (λ)	1.10	1.10	1.25	*	1.14	1.19	1.12	*	1.12	1.12	1.14	ns
Doubling time (Dt) (days)	7.06	7.16	3.09	ns	5.31	5.85	3.78	ns	6.17	6.35	5.21	ns

* significant; ns = not significant ($P<0.05$, SNK)

7.5 Discussion

The parasitoids emerged from all the three host species, *C. partellus*, *B. fusca* and *S. calamistis*. Similar resulats have been reported by Hailemichael *et al.* (1994) and Gitau (2002). Hailemichael *et al.* (1994) and Moore and Kfir (1996), stated that *X. stemmator* was a pupal parasitoid with a broad range of Lepidopteran hosts in the family Pyralidae, Crambidae and Noctuidae. The present study indicated that *X. stemmator* could probably parasitise pupae of the three stem borer species (*C. partellus*, *B. fusca* and *S. calamistis)* occurring in maize fields in Mozambique. However, the interaction between *B. fusca* (new association) and low temperatures would limit the effectiveness of *X. stemmator* since no individuals of *X. stemmator* adult emerged from *B. fusca* at low temperatures (15°C). Thus, in the areas with lower temperatures (<15°C) where *B. fusca* is dominant, the chance of *X. stemmator* establishment is low.

The emergence of a parasitoid is an indication of successful parasitoid development and host suitability. Although all hosts used in the present study were acceptable and suitable for the development of both parasitoids, there was a general decline in the emergence of parasitoids at very high temperatures (>30 °C), which may be explained by the fact that survival of parasitoids was affected by temperature.

In the present study, the proportion of hosts producing parasitoids was high at temperatures between 20 and 25°C and declined at 30°C reaching zero at 35°C. Similar results have been obtained in experiments with other parasitoids. Foerst and Butnariu (2004) reported that *Telenomus cyamophylax* adult emergence was directly proportional to temperature in the range of 15 and 25°C followed by a drop at 30°C.

In the present study, the highest progeny production of *X. stemmator* was from *B. fusca* contradicts results from Gitau (2002) who reported better performance of *X. stemmator* on *C. partellus* and *S. calamistis*. The pupal size of *B. fusca*, compared to that of *C. partellus* may have supported better the development of *X. stemmator*.

Both parasitoids tested in the present study successfully completed development at 20, 25 and 30°C. This finding proves that the exotic parasitoid, *X. stemmator* may colonize or get established at release sites where temperatures are above 15°C. No *X. stemmator* emerged on *B. fusca* at 15°C, suggesting that the interaction between *B. fusca* and low temperature (most common in the areas where *B. fusca* is abundant) may not be suitable for the development of this parasitoid.

Generally, the developmental time of both parasitoids decreased when temperatures increased from 15 to 30°C in all hosts. This is an indication of faster rate of development at higher temperatures compared to low temperatures. The faster development of *X. stemmator* on *C. partellus* at high temperatures represents a competitive advantage in relation to *D. busseolae* that parasitizes the same hosts. These observations corroborate with findings by Muli and Schulthess (Unpubl. Data) who found that *X. stemmator* was competitively superior to *D. busseolae* at 25°C and 50-60% RH. This is a clear evidence that the exotic parasitoid will perform better in areas with higher temperatures where *C. partellus* is the most dominant stem borer species.

Similar results have been reported by other researchers on the effect of host and temperature on the development of both parasitoids. Mohyuddin and Greathead (1970) reported a 18-20 days developmental time for *D. busseolae* at 24°C. similarly

Muli and Schulthess (Unpubl. Data) reported a developmental period ranging from 16-17 days at 25°C. Gitau (2002) recorded faster *X. stemmator* development (16.8 days) from *C. partellus* and slowest from *B. fusca* (17.7 days) at 27 °C and 60 to70% RH.

The decrease in developmental time of both parasitoids at high temperatures agrees with observations reported by other researchers (Bahana, 1990; Foerst and Butnariu, 2004; Jiang *et al.*, 2004). Mbapila and Overholt (2001) also reported a decrease on the developmental time of *C. flavipes* as the temperatures increased from 22 to 28°C. Bahana (1990), reported shorter developmental period of *D. busseolae* at high temperatures (20°C) and higher development periods at low temperatures (15°C).

In the present study, there was an indication that in general, males and females developed in a similar period in all hosts species at four of five tested temperatures. A faster development of *X. stemmator's* males compared to females on *S. calamistis* at 20°C was observed. Gitau (2002) found short developmental period of *X. stemmator* males than females. Godfray (1994), stated that males of solitary parasitoids usually emerge several days before the females to ensure that females become fertilized immediately after emergence. Thus, the interaction between temperature, host and parasitoids sex appears to have no apprcciablc cffcct on the development of *X. stemmator* males and females as mentioned by Godfray (1994).

Temperature and host quality have been considered as some of the factors affecting the parasitoid adult longevity (Foerst and Butnariu, 2004; Jiang *et al.*, 2004). Moore and Kfir (1996), working in South Africa reported that when provided with food and water, female *X. stemmator* lived for about 140 days and males for 87 days at 24°C

and 60-70% RH on *C. partellus* pupae as host. These results differed from those by Nikan and Basarkar (1991), who recorded the average longevity of *X. stemmator* on mated females to be 30 days with maximum of 37 days and minimum of 22 days at 22°C and 50-55% RH. Similarly, in the present study, longevity of *X. stemmator* females was longer than of males at all tested temperatures when *C. partellus* was the host. The highest mean females longevity was 70.7±8.19 days at 20°C.

The inverse relationship between temperature and longevity of parasitoids has been reported in previous studies in other insect parasitoids (Mbapila and Overoholt, 2001; Jiang *et al.*, 2004; Foerster and Butnariu, 2004). This fact was attributed to the negative effect of temperature in insect longevity when approaching lethal temperatures (Daane *et al.*, 2004).

The number of parasitoid individuals that failed to emerge from the host pupae were affected by both temperature and host species. A significant large proportion of *D. busseolae* died on *C. partellus* at lower temperatures this might have been due to the fact that both the host and the parasitoid usually occur at warm low altitude areas associated with high temperatures (Bahana, 1990; Moore and Kfir, 1996) so that the low temperatures may affected the development of both.

Dentichasmias busseolae has been reared mostly from *C. partellus* pupae in maize in many places of Africa. Mohyuddin and Greathead (1970), reared it only from *C. partellus* even at localities where both *C. partellus* and *B. fusca* were abundant in the same crop in East Africa. In South Africa and Mozambique *D. busseolae* was also reared mainly from *C. partellus* (Kfir, 1995; Cugala *et al.*, 1999; 2001).

The high proportion of *X. stemmator* individuals dying in *B. fusca* pupae may be explained by the fact that *X. stemmator-B. fusca* interactions are new association host parasitoid interactions. The increased mortality as temperature increased may reflect the negative effect of high temperatures on both host and parasitoids physiological processes and consequently causing their death (Ruberson *et al.*, 1995).

The highest proportion of host surviving was reported at low temperatures. *C. partellus* exhibited lower survivorship compared to *B. fusca* and *S. calamistis*. The host survival is an indication that the host pupae escaped the parasitism of the parasitoids during the period of exposure or they survived the feeding activities of the parasitoids inside. The interactions between host species, temperature and parasitoids revealed that host mortality was high at high temperatures. These observations suggest that the establishment and activities of *X. stemmator* as biological control agent against cereal stem borer in the areas where the maximum temperatures are more than 30 °C may be limited.

The life table parameters of *D. busseolae* have been a subject of study by other authors. Bahana (1990) reported an intrinsic rate of increase of (0.131) and that its population multiplied about 24 times with a mean generation time of 28 days. In the present study an intrinsic rate of natural increase of 0.123 was observed. Both values were low compared to an intrinsic rate of increaseof 4.76 per week of its host *C. partellus* which was reported by Bains and Shukla (1976). The low rates of increase of *D. busseolae* in relation to the rate of increase of its host indicates that the host population will increase faster than that of the parasitoid, and this may suggest that the parasitoid could not control the population of its host.

Despite the observations that, *D. busseolae* was not effective in controlling *C. partellus* populations in Eastern and southern Africa (Bahana, 1990; Kfir, 1998), the net reproductive rate (mean net contribution per female to the next generation i.e. the number of females offspring produced/female) greater than 1 indicated that the population of *D. busseolae* used in the present experiments could develop in *C. partellus* as host.

The net reproductive rate (Ro) values observed for *X stemmator* emerging from *C. partellus*, *B. fusca* and *S. calamistis* indicated that the parasitoid population could increase in those host species. Similar observations were made by Gitau *et al.* (2005) who found an increasing population of *X. stemmator* reared from *C. partellus* and *B. fusca* at a constant temperature of 27±2°C and 49-61% RH. The short doubling time on *C. partellus* reported in this study was similar to 6.6 days required to double its population reported by Gitau *et al.* (2005), and they suggested that the short doubling time may lead to faster population growth and consequently a suppression of the target pest *C. partellus* and *B. fusca*.

In a life table analysis, Gitau (2002) estimated a r_m of 0.106 and 0.105 viable females/female/day on *B. fusca* and *C. partellus* respectively. These results corroborates with findings reported in the present study at 25°C, which were 0.10, 0.18 and 0.11 on *C. partellus*, *B. fusca* and *S. calamistis* respectively.

Temperature and host species should be taken into consideration as important factors affecting survival, development and longevity of both parasitoids. If released in the field, it is expected that the exotic parasitoid *X. stemmator* may compliment the activities of *C. flavipes* and other parasitoids in controlling maize stem borers in the

areas where *C. partellus* is abundant. However, these observations are based only from laboratory results where host availability is not a problem and natural enemies can utilize hosts that are not normally attacked in the field.

These results provide valuable information to improve classical biological control programs conducted in Mozambique against *C. partellus* and *C. sachariphagus* and may contribute to predict and explain the success or failure of *X. stemmator* establishment in the areas of its release in Mozambique according to their prevalent environmental conditions and stem borer species composition. At high altitude zones of Mozambique in June–August temperatures are often below 15°C and where *B. fusca* is usually the most abundant species it would limit the establishment or success of *X. stemmator*.

The analysis of life table parameters (Ro, r_m, T, Dt and λ) indicated that the exotic pupal parasitoid, *X. stemmator* could be adapted and establish in the warm lowland areas dominated by *C. partellus* and also suggested that this parasitoid could breed and increase its population numbers under Mozambican environmental conditions and that it should able to effectively suppress the target stem borer species.

CHAPTER EIGHT

8.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General discussion

8.1.1 Establishment and spread of *C. flavipes* and *X. stemmator*

Three stem borer species associated with maize were recorded from study sites and these were *C. partellus*, *B. fusca*, and *S. calamistis*. *C. partellus* was the most abundant species at warm lowland areas whereas *B. fusca* dominated the high elevation altitude and *S. calamistis* was reported in low numbers at all study sites. Previous studies in Mozambique as well as in Southern Africa indicated similar stem borer species abundance and distribution (Overholt *et al.*, 1994, Davies *et al.*, 1995; Kfir, 1998; Segeren *et al.*, 2001).

Reports from South Africa indicate that *C. partellus* is already occurring in cool high elevation zones at the same pest status as in warm lowland areas on maize indicating that this species is displacing the indigenous species *B. fusca* (Kfir, 1997b). These observations support the reported occurrence of *C. partellus* at the cool high elevation area (>1400 m asl) in the present study. This was the first record of this species at this location because Davies *et al.* (1995) using *C. partellus* phcromone traps as well as field surveys did not find this pest in the area suggesting that this species had not yet invaded the area at that time.

It is plausible that *C. partellus* is spreading its ecological niche to colonize cooler areas, or the species is adapting itself to new niche conditions. In South Africa, *C.*

partellus was first considered as a pest of maize at lowland areas, but later became an important pest of maize at high elevation zones (Kfir, 1992).

The findings in the present study demonstrated that the extent to which maize was damaged by stem borers varied according to stem borer species composition and study locations. High damage levels were reported in the areas where *C. partellus* was the dominant species. After its introduction in 1930 (Tams, 1932), *C. partellus* has been regarded as the most damaging species at low to mid elevation zones (Gonçalves, 1970; van Hamburg, 1979).

Several parasitoids were recovered from three of the four stem borer developmental stages including egg, larval and pupal parasitoids. These were, the egg parasitoid, *Trichogramma bournieri,* larval parasitoids, *Cotesia sesamiae* and *Cotesia flavipes* and pupal parasitoids, *Dentichasmias busseolae and Procerochasmias nigromaculatus.* More than 80% of egg parasitism has been reported on *C. partellus* eggs whereas no egg parasitism was reported from *B. fusca* eggs. The *Trichogramma bournieri* an egg parasitoid was also reported in *C. sacchariphagus* egg batches in sugarcane in Mozambique (Conlong and Goebel, 2002).

Two exotic parasitoids; the larval parasitoid, *C. flavipes* and pupal parasitoid *X. stemmator* were released in the study areas during the years 1996 to 2002 and 2002 and 2003 respectively. The pupal parasitoid was recorded during the releasing period and a year after its releases only on *C. partellus* pupa in maize, but it was not recovered in subsequent seasons. Similar observations have been made on *C. sacchariphagus* in sugarcane at Mafambisse state in the central province of Sofala (Conlong and Goebel, 2002; Conlong personal communication). Cugala (2002) was

able to recover *C. flavipes* from 1996 and 1999 release sites at Southern and Central regions of Mozambique from all stem borer species occurring in maize and sorghum. Most recoveries were made from *C. partellus* and particularly in lowland areas, an indication that *C. flavipes* has been able to successfully adapt to the environmental field conditions and establish in Mozambique. However, the rates of parasitism were generally low <10%). Pre-release surveys conducted (Segeren *et al.*, 1991; Davies *et al.*, 1995; Cugala *et al.*, 1999) recorded a percent parasitism due to indigenous parasitoids of less than 5%. It was therefore concluded that indigenous parasitoids were not able to reduce stem borer populations below economic injury levels accepted by the farmers

In some of the *C. flavipes* release sites, *C. flavipes* is increasingly becoming the most abundant larval parasitoid than its African congeneric species, *C. sesamiae.* Cugala *et al.* (2003) reported a 40.8% stem borer larval parasitism due to *C. flavipes* at one of the 1996 release sites in the Southern lowland region Mozambique. The parasitoid's spread was also evaluated by Cugala (2002) who recovered it at more than 20 Km from the release fields. This was an indication that the introduced parasitoid is spreading from the release fields to new areas where releases were never made.

8.1.2 Impact of natural enemies on stem borer populations and maize yield

Several methods for evaluating the impact of natural enemies on pest populations have been stidied and include methods such as introduction and augmentation, cages and others barriers, removal or elimination of natural enemies. However, Kfir (2002) considered that most of these methods are specific to a particular natural enemy-host interaction, for example evaluating the effect of sessile insects (such as scales, aphids,

mealybugs) or those that form large colonies, but are not suitable for parasitoids of Lepidopteran pests due to their relatively low densities and high mobility.

DeBach and Rosen (1991) recommended that disruption, removal or elimination and/or exclusion with insecticides is the best experimental techniques for evaluation of the impact of natural enemies in Lepidopteran pests, as this method can provide measurement of the amount of control due to natural enemies.

Thus, in the present study, field experiments were conducted at three agro ecological zones to evaluate the impact of natural enemies by exclusion methods using insecticides. The results indicated that natural enemies play an important role in reducing stem borer populations and increasing maize yield and their impact varied from location to location according to the dominant stem borer species present. Significantly high impact was observed in lowland areas where *C. partellus* is the dominant species followed by the mid elevation zones and then high altitude areas dominated by *B. fusca.*

In general, the number of stem borer per plant as well as damage levels were high in the exclusion sprayed plots compared to unprotected control. However, parasitoids were more abundant in the unprotected control plots than the exclusion ones. Kfir (2002) argued that these observations may be due to partial removal or elimination of parasitoids in exclusion plots leading to an increase in stem borer densities and a decrease in maize grain yield.

8.1.3 Effect of temperature on development of *X. stemmator* and *D. busseolae*

Laboratory experiments were conducted to evaluate the effect of host and temperature in *X. stemmator* and *D. busseolae* life table parameters and developmental time. Both parasitoids performed better on *C. partellus* than from *B. fusca* or *S. calamistis*. The fact that few *X. stemmator* emerged from *B. fusca* in the laboratory might explain the no recovery from field releases at cool high altitude zones where *B. fusca* is dominant. The low suitability of *B. fusca* for the development of *X. stemmator* suggests that this exotic parasitoid may not establish in the low temperature agro ecological zones of Mozambique where *B. fusca* is the dominant species.

In its original habitat, *X. stemmator* occurs in warm lowland areas where if reproduces throughout the year and does not hibernate when hosts are available (Moore and Kfir, 1996). Ngi Song *et al.* (1999) stated that the common obstacle in new parasitoid-host relationship was the physiological suitability of the host for the development of the parasitoids. Parasitization is considered successful if the host is acceptable and suitable for the development of the parasitoid. Thus, although *B. fusca* was accepted for parasitization by *X. stemmator* in the laboratory, it was only partially suitable for the development of the parasitoid. This may explain why *X. stemmator* was not recovered in the release sites where *B. fusca* is the dominant stem borer species.

Life table studies conducted in the laboratory showed that the Net reproductive rate (Ro) and Intrinsic rate of increase (r_m) values observed for *X stemmator* emerging from *C. partellus* indicated an increasing population. The short doubling time reported in this study suggests that it may lead to rapid population growth and consequently a suppression of the target pest *C. partellus*. The optimal and maximum temperatures for the developmental rate of *X. stemmator* reared from *C. partellus* were 29.5 and

35°C respectively. The lower temperature threshold was estimated as 9.76°C and the thermal constant as 303.0 DD above the lower developmental threshold. The lower temperature threshold of 9.76°C suggests that the pupal parasitoids *X. stemmator* may survive in cooler zones.

8.2 Conclusions

1. Two stem borer species, *C. partellus* (at low, mid elevations and few at high altitude zones) and *B. fusca* (at mid and high altitude zones) were the most frequently collected from field sampling sites with varying densities according to their agro ecological zones. Infestation levels of stem borer on maize were as high as reported previously.
2. Several parasitoids were recovered from stem borers in farmers' fields' surveys as well as from experimental sites including both indigenous and the exotic ones. Indigenous parasitoids including the egg parasitoid *T. bournieri*, the larval parasitoid *C. sesamiae*, *S. parasitica*, the pupal parasitoids *D. busseolae*, *P. nigromaculatus* and *P. furvus* were commonly recovered from the study sites. Exotic parasitoids recovered included the larval parasitoid, *C. flavipes*.
3. The introduced larval endoparasitoid *C. flavipes* was found to be more abundant than the indigenous *C. sesamiae* in some areas with concomitant reduction of stem borer density, suggesting that the parasitoid has firmly established at the release sites and is spreading to surrounding areas since it was recovered up to 50 Km from the release sites. The level of parasitism due to the exotic larval parasitoid, *C. flavipes* are increasing in some of its release sites while stem borer densities are decreasing in those areas.
4. Under environmental field conditions, the natural stem borer infestation may cause 34.5, 31.2% and 28.9 yield losses at mid, high and low altitude areas respectively.

Yield losses increased by 43.3, 40.8 and 36.4% at low, mid and high altitude areas respectively when the activities of the natural enemies were excluded using insecticides.

5. The fact that higher maize yield losses were reported on exclusion plots than on unprotected control plots, indicated that natural enemies played an important role in reducing stem borer infestation and have led to increased maize yields. According to Kfir (2002) this was an indication that without their activities, annual yield losses would be much higher than observed.
6. The impact of parasitoids on stem borer populations and maize yield were high on experiments conducted at *C. flavipes* release sites than at non-release sites. These observations coupled with the increasing percent parasitism due to *C. flavipes* in some areas may suggest that the exotic parasitoid is beginning to have an impact on stem borer populations. It should also be noted that it is not only the parasitoids that were excluded from the exclusion plots, but other natural enemies such as predators as well.
7. The introduced pupal parasitoid *X. stemmator* was able to adapt successfully to the field conditions at warm lowland areas dominated by *C. partellus*. However, it was not recovered at high elevation zone dominated by *B. fusca*, suggesting that the host suitability played an important role on its establishment.
8. Despite the high numbers of *X. stemmator* released, apparently this parasitoid has not able to establish in maize at high elevation zones. Furthermore, *C. flavipes* is not a suitable candidate for the control of *B. fusca*, therefore considerations should be given to the importation and release of parasitoids of other life stages of *B. fusca* such as egg parasitoids to enhance mortality of *B. fusca* beyond the suppression due to indigenous parasitoids.

9. Results from laboratory studies indicated that the exotic pupal parasitoid, *X. stemmator* successfully emerged from all the three stem species. The analysis of life table parameters (Ro, r_m, T, Dt and λ) indicated that *X. stemmator* can breed and increase its population numbers under environmental conditions tested and that it should able to effectively suppress the pupation of *C. partellus*. The lower temperature threshold of *X. stemmator* was 9.76°C and the maximum was 35°C.

8.3 Recommendations

1. A comprehensive monitoring of the effect of egg parasitoids on *B. fusca* and *C. partellus* eggs should be conducted to provide an accurate estimate of egg parasitism in the country;
2. The establishment and spread of the pupal parasitoid *X. stemmator* should be evaluated in order to access its levels of parasitism in the release sites;
3. Laboratory studies should be conducted on the suitability of *B. fusca* for the development of *X. stemmator* using Mozambican *B. fusca* populations, since there may be differences between Mozambican stem borer populations and those from other Eastern and Southern African countries;
4. Studies to evaluate the factors (stem borer species composition, maize varieties) that may have affected the establishment of *X. stemmator* in Mozambican high elevation zones should be conducted;
5. More detailed field surveys on stem borer and parasitoids population dynamics, particularly of the *C. flavipes* on stem borer populations and maize yield in the areas where it is performing well should be conducted;
6. Studies on the cost benefit of the stem borers biological control programs in Mozambique should be conducted;

7. Studies should also be conducted to determine the stem borer economic threshold in Mozambique since data on this aspects are so far lacking;
8. On farm experiments should be considered also to evaluate yield losses more accurately under small-scale farmers' situations.

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APPENDICES

Appendix 1. a) – Correlation coefficients between plant parameters, damage and parasitism and/or yield in Chokwe

	1	2	3	4	5	6	7	8	9	10	11
1. Cob weight	**1.000**										
2. Grain weight	0.7858 0.0024	**1.000**									
3. Plant height	0.7889 0.0023	0.9277 <0.0001	**1.000**								
4. Stem diameter	0.5018 0.0964	0.5720 0.0520	0.4935 0.1030	**1.000**							
5. Number internodes	0.8105 0.004	0.7638 0.0038	0.7773 0.0029	0.4771 0.1168	**1.000**						
6. Proportion internodes bored	-0.7900 0.0022	-0.9063 <0.0001	-0.9384 <0.0001	-0.6325 0.0273	-0.8005 0.0018	**1.000**					
7. Holes	-0.8003 0.0018	-0.8080 0.0015	-0.8968 <0.0001	-0.4727 0.1207	-0.7755 0.0030	0.8766 0.0002	**1.000**				
8. Tunnel length	-0.7135 0.0092	-0.8779 0.0002	-0.9682 <0.0001	-0.5713 0.0523	-0.8030 0.0017	09461 <0.0001	0.8715 0.0002	**1.000**			
9. Cob damage	-0.5104 0.0899	-0.8144 0.0013	-0.7467 0.0053	-0.7729 0.0032	-0.5080 0.0917	0.7527 0.0047	0.6101 0.0351	0.7474 0.0052	**1.000**		
10. Stemborer abundance	-0.6057 0.0368	-0.8629 0.0003	-0.9336 <0.0001	-0.4010 0.1964	-0.6466 0.0231	0.8025 0.0017	0.7188 0.0084	0.9012 <0.0001	0.7776 0.0029	**1.000**	
11. Parasitoids abundance	-0.3094 0.3277	-0.1631 0.6124	-0.2842 0.3705	0.1544 0.6318	-0.3006 0.3424	0.3213 0.3084	0.3445 0.2728	0.3436 0.2741	-0.2140 0.5042	0.1230 0.7031	**1.000**

Appendix 1.b) – Correlation coefficients between plant parameters, damage and parasitism and/or yield in Machipanda

	1	2	3	4	5	6	7	8	9	10	11
1. Cob weight	**1.000**										
2. Grain weight	0.9531 <0.0001	**1.000**									
3. Plant height	0.2867 0.3662	0.2064 0.5197	**1.000**								
4. Stem diameter	0.6683 0.0175	0.7544 0.0214	0.6451 0.0235	**1.000**							
5. Number internodes	0.2649 0.4052	0.2727 0.3910	0.6738 0.0163	0.6730 0.0164	**1.000**						
6. Proportion internodes bored	-0.7249 0.0076	-0.6596 0.0385	-0.6920 0.0126	-0.8555 0.0004	-0.6322 0.0274	**1.000**					
7. Holes	-0.8330 0.0008	-0.6733 0.0164	-0.3055 0.3342	-0.7868 0.0024	-0.3194 0.3116	0.8219 0.0010	**1.000**				
8. Tunnel length	-0.4922 0.1040	-0.3336 0.2893	-0.7768 0.0030	-0.8206 0.0011	-0.6966 0.0118	0.8474 0.0005	0.6861 0.0137	**1.000**			
9. Cob damage	-0.5626 0.0569	-0.4111 0.1843	-0.5426 0.0683	-0.8523 0.0004	-0.5868 0.0449	0.7936 0.0021	0.7581 0.0043	0.9111 <0.0001	**1.000**		
10. Stemborer abundance	-0.6343 0.0267	-0.6858 0.0125	-0.8124 0.0013	-0.7136 0.0092	-0.5868 0.0449	0.8026 0.0017	0.6233 0.0303	0.8400 0.0006	0.6767 0.0156	**1.000**	
11. Parasitoids abundance	0.5828 0.0467	-0.4179 0.1764	0.0415 0.8981	-0.6184 0.0321	-0.1481 0.6458	0.5914 0.0428	0.8463 0.0005	0.4540 0.1381	0.6233 0.0303	0.2277 0.4766	**1.000**

Appendix 1. c) – Correlation coefficients between plant parameters, damage and parasitism and/or yield in Lichinga

	1	2	3	4	5	6	7	8	9	10	11
1. Cob weight	**1.000**										
2. Grain weight	0.9908 <0.0001	**1.000**									
3. Plant height	0.7838 0.0026	0.7870 0.0024	**1.000**								
4. Stem diameter	0.4239 0.1696	0.4398 0.1525	0.8187 0.0011	**1.000**							
5. Number internodes	0.5452 0.0668	0.5690 0.0535	0.8818 0.0001	0.8004 0.0018	**1.000**						
6. Proportion internodes bored	-0.9631 <0.0001	-0.9652 <0.0001	-0.8252 0.0009	-0.5261 0.0789	-0.6081 0.0359	**1.000**					
7. Holes	-0.8457 0.0005	-0.8450 0.0005	-0.7942 0.0020	-0.4694 0.1236	-0.5955 0.0411	0.9233 <0.0001	**1.000**				
8. Tunnel length	-0.7884 0.0023	-0.7781 0.0029	-0.7817 0.0027	-0.6100 0.0352	-0.6473 0.0229	0.8875 0.0001	0.8626 0.0003	1.000			
9. Cob damage	-0.5365 0.0721	-0.5148 0.0868	-0.5835 0.0464	-0.3576 0.2538	-0.5660 0.0550	0.5139 0.0874	0.5447 0.0671	0.3426 0.2755	**1.000**		
10. Stemborer abundance	-0.8043 0.0016	-0.8152 0.0012	-0.9439 <0.0001	-0.7681 0.0035	-0.7653 0.0037	0.8868 0.0001	0.8380 0.0007	0.8618 0.0003	0.4497 0.1424	**1.000**	
11. Parasitoids abundance	-0.7625 0.0039	-0.7384 0.0061	-0.3708 0.2354	0.2746 0.3593	-0.0402 0.9012	0.7733 0.0032	0.8006 0.0018	0.6175 0.0324	0.3509 0.2634	0.4887 0.1069	**1.000**

Appendix 2 - Paired comparisons on parasitoids performance at different temperatures and three host species a) *C. partellus*, b) B. fusca and c). *S. calamistis*

a) Host stem borer: *C. partellus*

	%. host surviving	% host dying	% parasitoid dying inside host	% host producing parasitoids	Parasitoid longevity (d)	Developmental time (d)	Sex ratio	Proportion of females
At temperature: 15								
D. busseolae	9.4±0.29b	27.5±0.48b	16.9±0.38b	46.0±1.48a	25.6±10.2a	42.6±21.4b	1:3	0.76±0.48a
X. stemmator	23.8±0.42a	46.2±1.23a	26.2±0.44a	3.8±0.19b	17.8±4.9b	68.0±12.9a	1:2	0.67±0.16b
At temperature: 20								
D. busseolae	5.6±0.19b	28.8±0.45a	3.1±0.21b	62.5±0.48a	99.9±15.5a	23.3±11.2b	1:6	0.85±0.50a
X. stemmator	12.5±0.33a	30.0±0.48a	13.1±0.34a	44.4±0.90b	57.4±16.7b	25.4±14.2a	1:4	0.80±0.89b
At temperature: 25								
D. busseolae	7.5±0.02b	33.1±0.04a	15.0±0.02a	44.4±0.04a	55.8±3.7a	18.8±0.75b	1:1	0.59±0.04a
X. stemmator	40.6±0.04a	24.4±0.03a	6.2±0.01b	28.8±0.04b	44.1±2.5b	21.1±0.76a	1:2	0.67±0.03a
At temperature: 30								
D. busseolae	0.6±0.01b	99.4±0.01a	0.0±0.00b	0.0±0.00b	0.0±0.00b	0.0±0.00b	0.0	0.0±0.00b
X. stemmator	16.9±0.03a	51.2±0.04b	16.2±0.42a	15.6±0.03a	10.9±0.54a	14.9±0.45a	1:2	0.64±0.02a
At temperature: 35								
D. busseolae	0.0±0.00b	100±0.00a	0.0	0.0	0.0	0.0	0.0	0.0
X. stemmator	3.1±0.01a	96.9±0.01a	0.0	0.0	0.0	0.0	0.0	0.0

Means in a column followed by the same letter are not significantly different (T-test, P>0.05)

b) Host stem borer: *B. fusca*

	%. host surviving	% host dying	% parasitoid dying inside host	% host producing parasitoids	Parasitoid longevity (d)	Developmental time (d)	Sex ratio	Proportion of females
At temperature: 15								
D. busseolae	86.9±0.03a	11.3±0.03b	1.9±0.01b	1.3±0.01a	11.0±0.07a	95.0±0.42a	2:0	0.00
X. stemmator	60.0±0.04b	18.1±0.04a	21.9±0.03a	0.0±0.00a	0.0±0.00b	0.0±0.00b	0:0	0.00
At temperature: 20								
D. busseolae	93.8±0.02a	5.0±0.02b	1.2±0.01b	0.0±0.00b	0.0±0.00b	0.0±0.00b	0:0	0.00±0.00b
X. stemmator	20.6±0.03b	24.4±0.03a	7.5±0.02[a]	47.5±0.04a	46.7±2.91a	31.4±1.25a	1:3	0.71±0.04a
At temperature: 25								
D. busseolae	89.4±0.02a	9.3±0.02b	0.6±0.01b	0.6±0.01b	137±0.86a	20.0±0.13a	0:1	1.00±0.01a
X. stemmator	26.9±0.05b	14.4±0.03a	5.6±0.02a	53.1±0.05a	13.2±2.37b	19.1±0.90b	1:2	0.71±0.04b
At temperature: 30								
D. busseolae	21.2±0.03a	78.8±0.03a	0.0±0.00b	0.0±0.00b	0.0±0.00b	0.0±0.00b	0:0	0.00±0.00b
X. stemmator	1.2±0.01b	60.0±0.04b	28.1±0.04a	10.6±0.02a	13.2±0.52a	9.1±0.45a	1:2	0.71±0.02a
At temperature: 35								
D. busseolae	0.0	100±0.00a	0.0	0.0	0.0	0.0	0:0	0.00
X. stemmator	0.0	100±0.00a	0.0	0.0	0.0	0.0	0:0	0.00

Means in a column followed by the same letter are not significantly different (T=test, P>0.05)

c) Host stem borer: *S. calamistis*

	%. host surviving	% host dying	% parasitoid dying inside host	% host producing parasitoids	Parasitoid longevity (d)	Developmental time (d)	Sex ratio	Proportion of females
At temperature: 15								
D. busseolae	64.4±0.04a	19.4±0.44b	3.7±0.20b	12.5±0.03a	28.8±1.18b	42.3±1.11b	1:1	0.55±0.02a
X. stemmator	48.1±0.04b	37.5±0.56a	13.8±0.40a	0.6±0.19a	30.0±0.42a	66.0±2.04a	1:0	0.00±0.00b
At temperature: 20								
D. busseolae	83.1±0.03a	16.9±0.03b	0.0±0.00b	0.0±0.00b	0.0±0.00b	0.0±0.00b	0:0	0.00±0.00b
X. stemmator	11.3±0.02b	44.4±0.04a	11.2±0.03a	33.1±0.04a	46.5±2.48a	30.1±1.13a	1:3	0.72±0.03a
At temperature: 25								
D. busseolae	85.6±0.03a	8.1±0.02b	1.3±0.01b	5.0±0.02b	52.3±1.20a	19.9±0.34b	1:3	0.75±0.02a
X. stemmator	21.3±0.03b	31.9±0.04a	12.5±0.03a	33.8±0.04a	43.5±2.54b	21.4±0.83a	1:2	0.67±0.03b
At temperature: 30								
D. busseolae	73.8±0.03a	26.2±0.03b	0.0±0.00b	0.0±0.00b	0.0±0.00b	0.0±0.00b	0.0	0.00±0.00b
X. stemmator	2.5±0.01b	60.0±0.04a	26.8±0.04a	10.6±0.02a	11.1±0.43a	17.0±0.42a	1:5	0.82±0.02a
At temperature: 35								
D. busseolae	1.2±0.01a	98.8±0.01a	0.0	0.0	0.0	0.0	0.0	0.0
X. stemmator	0.0±0.00a	100±0.00a	0.0	0.0	0.0	0.0	0.0	0.0

Means in a column followed by the same letter are not significantly different (T-test, P>0.05)

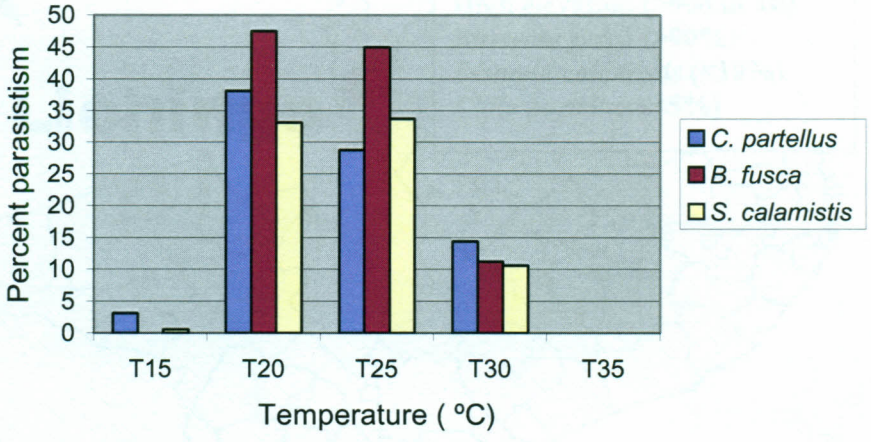
Appendix 3 – Male and female developmental time and adult longevity of *X. stemmator* in relation to temperature (° C) and host species

	Developmental time					Adult longevity			
Sex									
	15	20	25	30		15	20	25	30
				Chilo partellus					
Males	72.0±4.00a	29.1±0.55a	20.9±0.53a	16.2±0.40a		34.0±13.0a	48.6±13.42b	25.1±8.30b	8.1±3.93b
Females	66.0±2.00a	29.1±0.12a	21.3±0.20a	16.2±0.35a		15.0±5.56a	70.7±8.19a	55.1±8.93a	15.1±4.06a
				Busseola fusca					
Males	0.0	31.0±0.45a	22.2±0.40a	18.0±0.52a		0.0	73.0±9.40a	35.3±7.81a	3.8±2.33b
Females	0.0	31.1±0.62a	22.9±0.15a	18.0±0.55a		0.0	37.4±4.47b	35.6±5.10a	15.7±5.37a
				Sesamia calamistis					
Males	0.0	28.9±0.21b	21.7±0.34a	11.0±0.33a		0.0	54.8±9.55a	48.6±10.16a	15.3±9.02a
Females	0.0	30.6±0.56a	21.9±0.15a	11.1±0.37a		0.0	43.2±6.51a	42.2±6.82a	17.4±3.57a

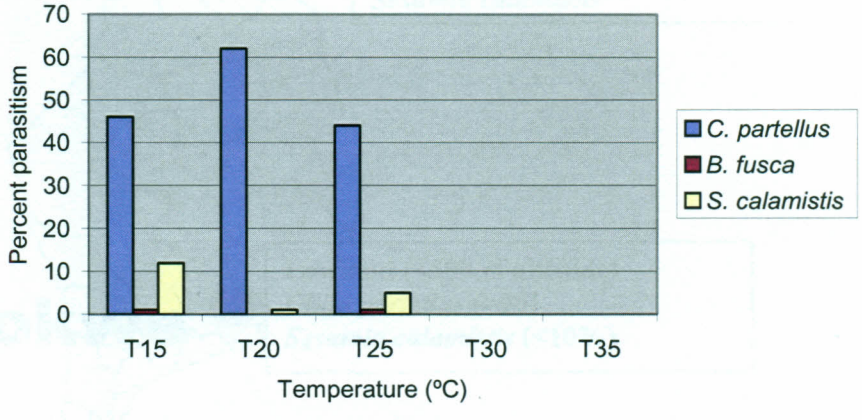
Means in a column followed by the same letter are not significantly different (T-test, P>0.05)

Appendix 4 – Percent parasitism due to the two pupal parasitoids at the tested temperatures on the three stemborer species

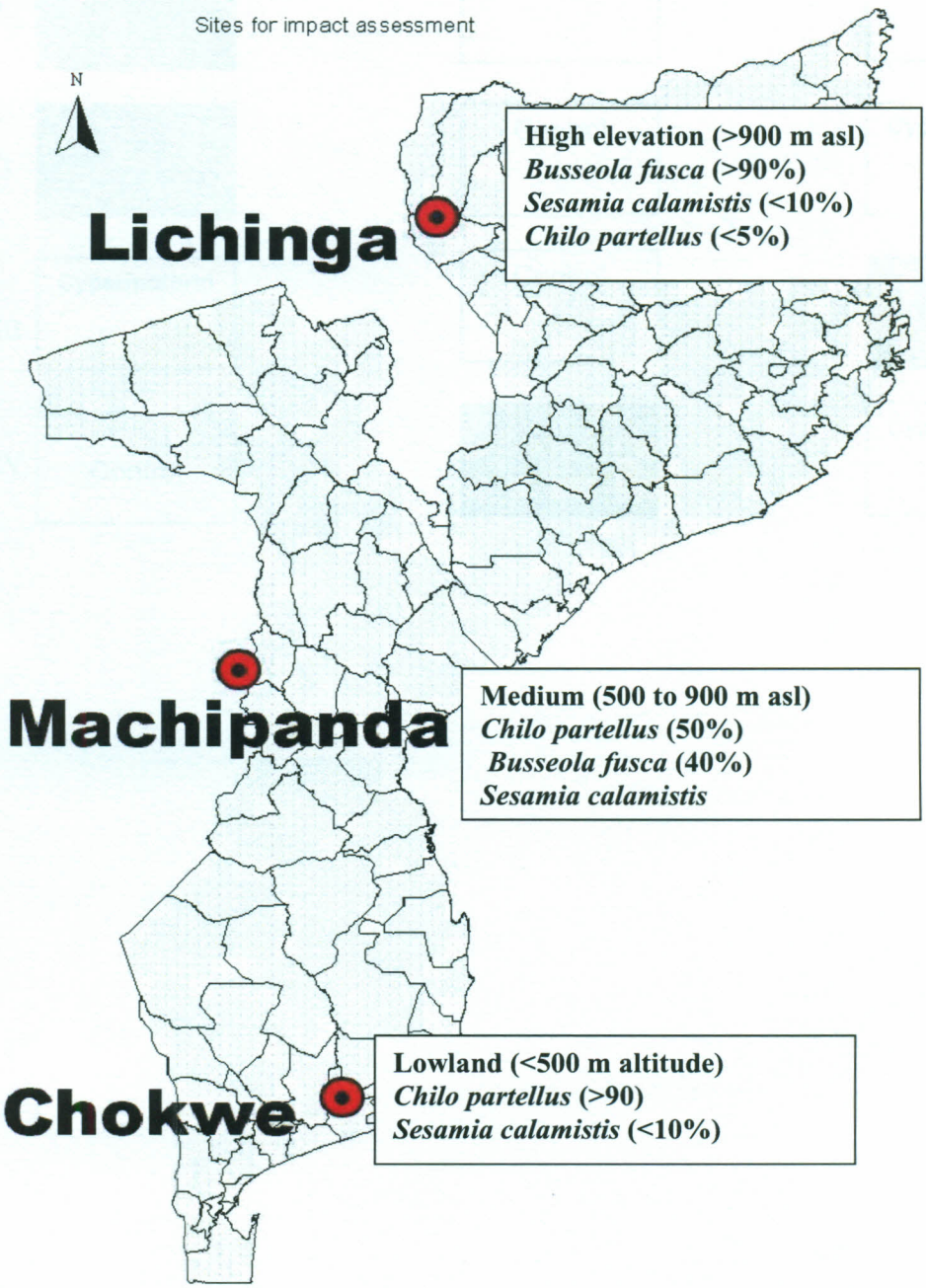
a) *X. stemmator*



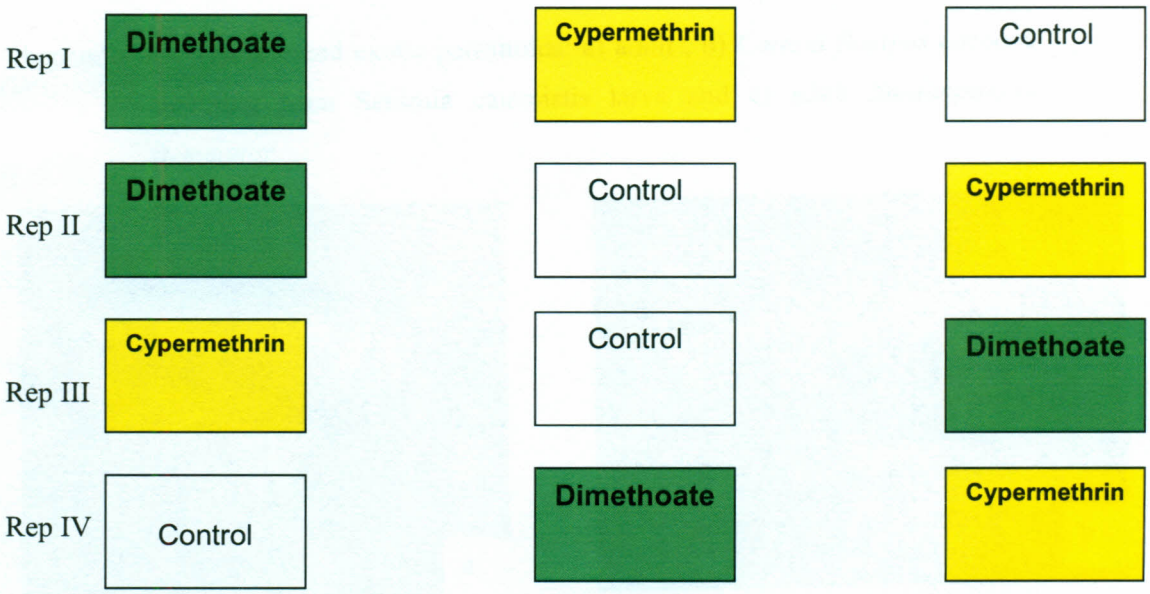
b) *D.busseolae*



Appendix 5 – Locations where impact assessment experiments were conducted



Appendix 6 – Impact assessment experiment layout



Appendix 7 – The released exotic parasitoids: a) adult , b) *Cotesia flavipes* cocoons emerging from Sesamia calamistis larva and c) adult *Xanthopimpla stemmator*