INTRODUCTION AND ESTABLISHMENT OF
COTESIA FLAVIPES CAMERON
(HYMENOPTERA BRACONIDAE)
AS A BIOLOGICAL CONTROL AGENT
AGAINST CEREAL STEMBORES IN
MOZAMBIQUE

BY
DOMINGOS P. CUCALA

DEPARTMENT OF CROP SCIENCE
FACULTY OF AGRICULTURE
UNIVERSITY OF ZIMBABWE

DECEMBER 2002
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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF 
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Dedication

To my wife Aissa my daughter Fainesse, and my mother Esperança
Cotesia flavipes Cameron (Hymenoptera: Braconidae); a gregarious larval endoparasitoid native to the Indo-Australian region, was imported from Kenya and introduced at two locations in the southern region of Mozambique in 1996 on farmers' fields and in 1999 in the southern and central regions of the country in trial releases to increase the natural suppression of the exotic stemborer, Chilo partellus (Swinhoe) (Lepidoptera: Crambidae). Surveys and experimental studies were conducted in the areas of releases to: 1) evaluate the establishment and spread of C. flavipes from the 1996 release sites, 2) to determine the effect of stemborer species composition on the establishment of the exotic parasitoid and 3) to access the acceptability and suitability of the indigenous and exotic stemborers for oviposition and development of C. flavipes. Three stemborer species occurred, C. partellus and Sesamia calamistis Hampson (Lepidoptera: Noctuidae) in the south and C. partellus, S. calamistis and Busseola fusca Fuller (Lepidoptera: Noctuidae) in the centre. However, C. partellus was the most abundant species at the tow regions. C. flavipes was recovered in the southern part of the country at the two 1996 release sites. However, percent parasitism was low (< 1%). The exotic parasitoid was also recovered from the 1999 release sites during the season of releases and one year after its release. The indigenous parasitoid, Cotesia sesamiae (Cameron) (Hymenoptera: Braconidae), was the most common natural enemy recovered at all sites. Parasitism of the indigenous stemborer, S. calamistis, by C. sesamiae was higher than parasitism of C. partellus at all sites, even though C. partellus was the most abundant species. C. flavipes was recovered from B. fusca; S. calamistis and the exotic stemborer, C. partellus. The recovery of C. flavipes in the areas where it was released in 1996 and 1999 indicated that the parasitoid had become established. However, its impact on the stemborer populations was low. Studies on the acceptability and suitability of the C. partellus and B. fusca, for oviposition and development by C. flavipes reported that C. partellus was significantly more acceptable as host than B. fusca. The parasitoid C. flavipes was capable of developing in both the coevolved and non-coevolved stemborer species. Successful parasitization was higher in the old host/parasitoid association.
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CHAPTER ONE  
INTRODUCTION

Maize (*Zea mays* Linne) and grain sorghum (*Sorghum bicolor* Linne) are the most important cereal crops grown for home consumption or cash income in Mozambique. Maize is the most widely grown crop occupying more than 30% of the land under cultivation (Ministério de Agricultura, 1977), and more than 95% of the annual production is from small scale farmers. Crop yields which are often very low are limited by losses due to pests (Segeren, Oever and Slobbe, 1995).

In Mozambique, stemborer larvae are the most economically important pests of maize and grain sorghum. Among the stemborders 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 in Mozambique (Gonçalves, 1970; Segeren, Rafael and Sitoi, 1991). The pink stalk borer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) is of minor importance. Kfir (1992; 1998) speculated that *S. calamistis* 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 (Davies, Cumbi and Tocoro, 1995; Sithole, 1988; Segeren *et al.* 1995) (Figure 1.1). 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, Overholt, Santos, and Giga, 1999). Yield losses due to stemborer attacks are reported to range from 20 to 40% on research stations, and more than 50% in the small-scale farmers' fields (Segeren *et al.*, 1991).
Fig. 1.1 - Map of Mozambique showing agroecological zones
Control of stemborers by conventional insecticides is difficult because the damaging larvae are protected inside plant stems (ICIPE, 2000). Larvae are vulnerable to pesticides and/or many natural enemies only during the first instar stage, when they are feeding exposed on the leaves before they enter into stems (Davies et al., 1995).

Recommended control methods for reducing stemborer incidence include chemical control, cultural practices, biological control and host plant resistance (Overholt, Ochieng, Lammers and Ogedah, 1994a).

Synthetic insecticides have been shown to be effective for the control of stemborer species at 10% of plant infested when used before young larvae enter into stems and in repeated applications. The timing of insecticidal 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 have been used on large scale farms (Ariyanayagan, 1983; Jimenez and Mugabe, 1990), while there is little or no use of insecticides by small scale farmers (Leeuwen and Zucula, 1987) because of the high cost (Berger, 1994; Kfir, 1995; Overholt, 1995).

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 (Oover, 1990; Seshu Readdy, 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 (Päts, 1996). 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 stemborer (Khan, Chiliswa, Ampong-Nyarko, Smart, Polaszek, Wandera and Mulaa, 1997; Kfir, 1998).

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). Destroying crop residues and wild host plants by burning will suppress off-season survival of stem borers and parasitoids, but results in a loss of nitrogen.

Intercropping maize or sorghum with non-host plants has been recommended to manage stem borer populations. Intercropping is already practised in many low-input agricultural systems in Africa but stem borers are still causing severe losses (Overholt, Ogenda and Lammers, 1994b). Segeren et al. (1995) reported no differences on the stem borer 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. However, sowing date is strongly dependent on rainfall, and cannot be greatly modified.

In Mozambique, biological control is viewed as a potential strategy for the management of the exotic stem borer, *C. partellus*. In general, indigenous natural enemies are not able to keep stem borer populations below economic injury levels (Overholt et al., 1994a; Seshu Reddy, 1998). A parasitoid of *C. partellus* from India and Pakistan, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was introduced into southern Mozambique in 1996. The exotic larval parasitoid, *C. flavipes*, was released in Kenya in 1993 and became permanently established (Overholt, 1998). After establishment it spread from the release sites and colonized new areas. The parasitoid’s impact has recently been evaluated, and a reduction of 37% of the total stem borer population and a reduction of 53% of *C. partellus* population has been observed in some areas (Zhou, Gaumgartner and Overholt, 2000). *C. flavipes* has now also been released in other African countries including Uganda, Zanzibar, Somalia, Malawi, Zambia and Zimbabwe and its establishment has been reported from Uganda and Malawi (ICIPE, 2000).
1.1 Objectives

The following were the objectives of the study:

1.1.1 Overall objective

The main objective of the study was to investigate the ability of the parasitoid C. flavipes to control cereal stem borers in Mozambique.

1.1.2 Specific objectives

a) To determine through extensive surveys the establishment of C. flavipes at the release sites in southern Mozambique;

b) To assess the acceptability and suitability of B. fusca and C. partellus for oviposition and development of C. flavipes; and

c) To determine whether differences in the stem borer species composition influence the establishment of C. flavipes in two locations in Mozambique which have distinct stem borer complexes.
CHAPTER TWO

LITERATURE REVIEW

2.1 Biology and life history of cereal stemborers

The description and biology of the three most common cereal stemborers in Mozambique are well documented (Ingram, 1958; Gonçalves, 1970, 1972; Hill, 1983; Harris, 1962; Harris, 1989a, 1989b; Segeren et al., 1991; Pats, 1992; Berger, 1993; Overholt and Maes, 2000).

2.1.1 Spotted stalk borer, *Chilo partellus* Swinhoe

Adult *C. partellus* emerge from pupae in the afternoon and early evening and are active at night (Gonçalves, 1970; Mathez, 1972; Harris, 1989a). Females lay eggs in batches of 10-80 overlapping on the undersides of leaves. Young larvae climb 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, 2-3 weeks after hatching (Appendix A). 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 go into diapause in stems, stubbles and other crop residues, where they may spend up to 6 months before pupating when favourable conditions reoccur during the next growing season (Harris, 1989a; Kfir, 1992; Segeren et al., 1995). Hill (1983) reported that depending on the geographical location and weather conditions, there are at least six generations per year.

2.1.2 Maize stalk borer, *Busseola fusca* Fuller

The female lays eggs in batches of 30-100, inserted between the sheath and stem (Harris, 1989a, Harris and Nwanze, 1992). 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. The larval development takes between 26 and 33 days under field conditions, then pupate in the tunnel (Appendix A). The larvae are cream to brown coloured (Sithole, 1988, 1989, 1994). According to Sithole (1988), depending on the agroecological conditions and the presence of suitable host plants, two or more generations per year may occur.

2.1.3 Pink stalk borer, *Sesamia calamistis* Hampson

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 to penetrate directly in the stems. Harris (1962), working in Nigeria reported that *S. calamistis* larvae bore directly into stem under the leaf sheaths. Thus, Sithole (1989), reported that *S. calamitis* was a unique stemborer in that its feeding habits are 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, 1989).

2.2 Distribution, species composition and relative abundance

Lepidopteran stemborers occur regularly in maize and grain sorghum with different degrees of importance according to their abundance. 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).

Sithole (1994) reported that the distribution and relative abundance of stemborers were significantly influenced by climatic factors. *C. partellus* was
more abundant at low elevations and high temperature, whereas *B. fusca* was generally considered to be the most damaging stemborer at high elevation (>1500 m above sea-level) and low temperature (Figure 1.1). *Sesamia calamistis* was most abundant at moderate elevations and temperature but rarely reaches damaging levels.

It was observed that *B. fusca* is the dominant stemborer at elevations above 900 m in Botswana, Lesotho, Malawi, Mozambique, South Africa and Swaziland, but also occurs at lower altitudes in those countries and in Zimbabwe (Sithole, 1989). In Mozambique, Davies *et al.* (1995), reported that *B. fusca* was the most abundant stemborer species in the northern province of Niassa at an elevation of 1350 m above sea-level where no *C. partellus* larvae were found.

*Chilo partellus* occurs throughout southern and eastern parts of Asia. It spread into Africa in the early in 1930s. The first record of *C. partellus* in Africa was from Malawi in 1932 (Tams, 1932; Jepson, 1954). In South Africa it first appeared in 1958 (van Hamburg, 1979). It has since spread to most of the lowland areas (below 1500 m above sea-level) of the countries in eastern and southern Africa (CIE, 1988). 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 mainly as a pest of sorghum, later becoming increasingly important in maize (van Rensburg and Bate, 1986; Kfir, 1992).

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 (Davies *et al.*, 1995; Sithole, 1988; Segeren *et al.* 1995). *S. calamistis* is of minor importance at all elevations (Segeren *et al.*, 1995) and cannot be considered a pest species in Mozambique. The three stemborer species were found occurring in the same area at medium to higher elevation zones (500-900 m above sea-level) (Riedel, unpub. data) (Figure 1.1).
2.3 Host plants and damage

2.3.1 Host plants

The original host plants of all cereal stem borers were wild grasses. However, Harris and Nwanze (1992), reported that maize and sorghum were the most important cereal crop hosts and to a lesser extent, pearl millet and sugarcane. While sorghum is indigenous to Africa (De Wet, 1978; Doggett, 1988), maize is an introduced crop from Central America (Harris and Nwanze, 1992). The three stem borer species have different preferences for host plants. For example, Sithole (1989) 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, 1989; Kfir, 1998). *S. calamitidis* was found to be associated with *C. partellus* in the same host plants (Magalhães, 1971).

In addition to the cultivated plants, several non-cultivated wild host plants (*Pennisetum spp.*, *Panicum spp.*, *Sorghum spp.* and *Cyperus spp.*) were recorded as alternative hosts for the stem borers (Ingram, 1958; Gonçalves, 1970; Khan et al., 1997; Ofomata, Overholt, Lux, Huis and Egwuatu, 2000). Some authors have argued that alternative hosts are detrimental in serving as a stem borer reservoir (Ingram, 1958; Harris, 1962; Gebre-Amlak, 1988), whereas others have pointed out that natural enemies can persist and increase their populations during the non-growing season in the alternative stem borers host plants (Overholt, Song, Ofomata and Jeske, 1999). In addition, Khan et al. (1997) suggested that certain wild hosts growing in proximity to maize fields would act as trap plants. Schultighthouse, Bosque-Perez, Chabi-Olaye, Gounou, Ndema and Goergen (1997) found a negative relationship between the abundance of wild hosts and stem borer infestation in maize fields, suggesting that wild hosts may divert stem borers away from maize. For example, Khan et al. (1997), observed that successful colonisation by parasites could depend upon the presence of the appropriate kind and
abundance of primary hosts as well as alternative hosts. The proximity of flowering weeds increased the population of natural enemies.

2.3.2 Damage

The three most important stemborer species attacking maize and grain sorghum in Mozambique produce similar symptoms of damage (Sithole, 1988, 1989; Segeren et al., 1991). Damage is caused by larvae, which at first feed on the young leaves (C. partellus and B. fusca) but soon tunnel into the stems (Seshu-Reddy, 1998; Harris and Nwanze, 1992). S. calamisits bores into stems with little or no leaf feeding, which is quite different from the other borers which leave feeding marks on 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).

2.4 Control methods

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.

2.4.1 Chemical control

The use of pesticides for the control of C. partellus in maize results in significant reduction of infestation, and an increase in yield, when insecticides are applied when the plants are 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 stem borers (Kfir, 1998).

In addition, chemical control must be carefully timed to coincide with the limited period when early instar stem borer larvae are found outside of plant tissues (Mohyuddin and Greathead, 1970; Segeren et al., 1991). Chemical control should be applied if all other integrated pest management tactics are unable to keep an insect pest population below an economic threshold, (Hoffmann and Frodsham, 1993).

2.4.2 Cultural control

There are many agricultural practices that make the environment less favourable to insect pest population. Harris and Nwanze (1992) and Kfir (1998), reported that crop residues are important for carrying over stem borer populations from one growing season to the next. In rural areas where dry stems are used for fencing and building, it may not be possible to reduce carry-over populations.

Khan et al. (1997) found that surrounding maize with Napier grass and intercropping with the legume Desmodium reduced stem borer infestation on maize. Female moths are diverted from the main crop and attracted to susceptible trap plants grown in an adjacent field and at the same time are repelled from the main crop by repellent intercropped plants.

2.4.3 Host-plant resistance

Dabrowiski and Nyangiri (1983) considered host plant resistance as a pest management tactic that was economical and demanded little or no change in farmer practices. Several cultivars of maize and sorghum with some degree of resistance to *C. partellus*, have been identified (van den Berg, 1997).
Host plant resistance offers a long-term solution to the management of stemborers and the most compatible with other components 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 stemborer larvae, making them more susceptible to the insecticides (van de Berg, 1994). Other mechanisms involved in C. partellus and B. fusca resistance in maize and sorghum include non-preference for oviposition, reduced feeding, reduced tunnelling, tolerance of plants to leaf damage, deadheart and stem tunnelling (Seshu Reddy, 1998). However, currently there are no stemborer resistant maize and sorghum varieties that are agronomically acceptable to farmers (van de Berg, 1997).

2.5 Biological control

DeBach (1964) and 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" which 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, their "use" which implies an intervention by man. Some people argue that biological control should include other pest management tactics such as host plant resistance and the release of sterile insects. However, Ajayi (1998), stated that for purpose of consistence and clarity, it was more useful to limit the concept of biological control to the use of natural enemies to regulate pests.
2.5.1 Background to biological control

Biological control is one of the oldest and most effective means of achieving insect control. The earliest record of biological control dates back to the fourth century China, where ants were used to suppress pests in citrus. Many other early attempts were made throughout the world using insects to control other insects, with varying degrees of success (Pedigo, 1989).

However, it was not until 1888 that biological control became permanently established in the United States of America as a significant method, when the introduced predator insect, the vedalia beetle, *Rodolia cardinalis*, controlled the threat to citrus by the cotton cushion scale, *Icerya purchasi*, an introduced insect from Australia. The suppression continues to our days (Pedigo, 1989).

Insect pests have been the most common types of organisms against which biological control has been employed. Natural enemies, the agents used in biological control, are the fundamental resource with which biological control success has been achieved (Driesche and Bellows, 1996). Thus, biological control relies on natural enemies (parasitoids, predators, pathogens and/or parasites) to reduce pest populations or damage to tolerable levels. When successful, biological control agents can provide permanent economic solutions.

2.5.2 Agents of biological control

Many insect and mite species attack crops, forest and livestock, but they occur in very low numbers. Most of these species do not reach pest status because their impact in the crops does not result in an economic loss. Unfavourable weather conditions, lack of food and nesting sites, and very often, natural enemies constitute the main factors that keep these species at low densities (Pedigo, 1989). Many naturally occurring biological control agents, such as parasitoids, predators, pathogens and parasites have been
reported for different growth stages of stem borers of maize and sorghum in Africa (Seshu Reddy, 1998; Overholt, 1998; Bonhof, 1998).

2.5.2.1 Parasitoids

Insect parasitoids have an immature life stage that develops on or within a single insect host, ultimately killing the host, hence the value of parasitoids as natural enemies. Thus, Pedigo (1989) stated that parasitoids were parasitic in immature stages but free-living as adults, which may be predaceous or herbivorous (Appendix C: b).

Cereal stem borer parasitoids of interest in many countries of Africa include the egg parasitoids, such as Trichogramma spp., Telenomus spp., larval parasitoids, C. sesamiae and pupal parasitoids e.g. Pediobius furvus, Denticasmius busseolae and Psilochalcis soudanensis. However, the overall rate of parasitism of stem borers has been found to be low and seldom reach 10-14% (Overholt, 1998; Seshu Reddy, 1998; Bonhof, 1998)

2.5.2.2 Predators

Predators are free-living animals that feed on other animals, their prey, sometimes devouring them completely and rapidly. Major predators of insects include birds, fish, reptiles, mammals and other arthropods (insects, spiders and mites). The most important predators in biological control programs have been insects and mites (Pedigo, 1989). The predaceous habit is extremely widespread among insects and can be found in most orders and a large number of families.

Insect predators have been introduced for control of exotic pests. Indigenous predators are of major importance in the suppression of both native and exotic pests. Insects belonging to the orders Hemiptera, Coleoptera, Diptera and Hymenoptera are among the predators most commonly found preying on pest species in crops (Driesch and Bellows, 1996). However, Bonhof (1998; 2000),
reported that there was little information available on the impact of predators on stemborer populations in Africa, but Bonhof (2000) found that stemborer eggs were among the most vulnerable life stage of stemborers to predatory activities. Mohyuddyn and Greathead (1970) speculated that predation was an important mortality factor for stemborer eggs.

Stemborer larvae were vulnerable to predation, especially while migrating from the egg batch to the leaf whorl (Bonhof, 2000). Disappearance of 90% of first instar larvae was observed in western Kenya and Uganda (Mohyuddyn and Greathead, 1970). Several ant species were mentioned as preying on the eggs as well as on larvae (Mohyuddyn and Greathead, 1970; Bonhof, 2000). Various other predators were observed having some impact on eggs and larvae, including the earwing, coccinellids and spiders (Bonhof, 2000).

2.5.2.3 Pathogens and/or parasites

Insects and mites can be infected by diseases (bacteria, fungi and viruses) and parasitic nematodes. Under high humidity and host abundance, these naturally occurring organisms may multiply rapidly to cause disease or parasite epizootics that can prevent insect populations outbreak (Driesche and Bellows, 1996; Cornell University, 2000).

A parasite feeds on its host usually weakening it and sometimes killing it. Parasites with the greatest impact on insect populations are nematodes (Pedigo, 1989). Several species of nematodes have been recorded from cereal stemborers and some species have potential for biological control.

Some pathogens have been mass produced and are available in commercial formulations as insecticides (microbial insecticide, bio-insecticide). The formulations of the bacterium Bacillus thuringiensis (Bt) are widely used by farmers for the control of lepidopteran insect pests (Cornell University, 2000).
Among the natural enemies of cereal stem borers in Africa, various pathogenic or parasitic organisms may often be found in the field attacking stem borers and several have been the subject of biological control studies (Poinar and Polaszek, 1998). In general, nematodes and pathogens are not of great importance in regulating numbers of stem borers populations because the stem borer life history does not usually contain the predisposing factors for the development of epizootics (Harris and Nwanze, 1992). The stem borer larvae are concealed within the protective stem and thus, are not often in contact with other larvae (Poinar and Polaszek, 1998).

2.6 Approaches to biological control

Biological control includes three approaches: importing exotic natural enemies (classical biological control), increasing the number of natural enemies through mass release of laboratory reared natural enemies (augmentation) and maintaining numbers of natural enemies already present (conservation).

2.6.1 Conservation

Conservation biological control refers to the optimisation of the impact of biotic agents that are already extant in an ecosystem. It can be achieved in several ways. For example, most pesticides are more toxic to natural enemies than to pests, but granular formulations may have less impact on natural enemies, and systemic pesticides will often have a minimal impact (DeBach and Rosen, 1991).

The most common action taken to conserve natural enemies is to reduce the use of pesticides. Pesticides can eliminate important natural enemies of target pests and non-target species, which will become secondary pests (Thomas and Waage, 1996). Natural enemies are more susceptible to insecticides than insect pests due to their small size, greater mobility on the plants, less concealed habitats and their relatively poor or low ability to detoxify chemical poisons (DeBach and Rosen, 1991).
Thus, by reducing pesticides use in such systems to a minimum level, the action of natural enemies is often restored and farmers can reduce the costs of chemical control (Thomas and Waage, 1996). The acceptable approach is to employ selective pesticides that should be used when its found to be absolutely necessary based on economic injury threshold (DeBach and Rosen, 1991; Hoffmann and Frodsham, 1993). If sorghum and maize residues are destroyed after harvest, as is often recommended for the control of *C. partellus* (Päts, 1996; Harris and Nwanze, 1992), there would also be negative impact on natural enemies which are found with the host in the plant stems.

### 2.6.2 Augmentation

Augmentation refers to artificially increasing the numbers of natural enemies in the ecosystem from mass rearing and releases. The mass rearing and release of natural enemies into ecosystem can be divided into inundation and inoculation.

#### 2.6.2.1 Inundation

The inundative approach relies on the release of very large numbers of the biological control agent and an immediate effect is expected, as with pesticides. There is little reliance on a longer term benefit from a numerical increase in the population of the agent in the field (DeBach, 1964; DeBach and Rosen, 1991).

Typically, the agents that are used in the inundative approach are the ones already occurring in the ecosystem, but not in large enough numbers to effectively control pest. Microorganisms (bacteria, fungi and viruses) are the agents most often used in the inundative approach. Insect feeding nematodes are used to a lesser extent. Insect natural enemies are also used with *Trichogramma* egg parasites being the most widely employed.
2.6.2.2 Inoculation

Inoculation refers to the release of relatively small numbers of natural enemies, which are expected to propagate in the target habitat so that their progeny would control the pest for several subsequent generations (DeBach and Rosen, 1991).

2.6.3 Introduction (Classical Biological Control – CBC)

The introduction 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; Thomas and Waage, 1996).

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 natural enemies of the pest 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 regulate the pest at a lower density than would occur in the absence of natural enemy (Debach and Rosen, 1991; Thomas and Waage, 1996).

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. 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, Kimani, Overholt and Ogol, 1995). Recent results from Kenya show that the parasitoid population has increased each year since the first release in 1993. At some
sites in Kenya, parasitism of more than 60% was found in 1997 and a 30 – 50 % decrease in stemborer population has been observed (Zhou et al., 2000).

Classical biological control does not attempt to eradicate pests, but relies on the coexistence of both the pest and natural enemy. When the pest density increases, the natural enemy responds either by increasing its density and attacking more pests (numerical response) or by each natural enemy attacking more pests (functional response), or a combination of both. As the density of the pest decreases, the natural enemy population also decreases and has less impact on the pest population. This “density-dependent” action leads to reciprocal oscillations in the densities of both pest and natural enemy (Dent, 1991; Overholt, 1993).

Most of the emphasis in classical biological control has been against introduced pests and it is better suited to situations in which there is one or only a few major pest species rather than a complex of several pests attacking a crop (Overholt, 1993; 1999) since natural enemies are usually host specific in their activity (Debach and Rosen, 1991; Thomas and Waage, 1996). If pesticides need to be used against one of the pests, this would likely upset biological control of the others.

The control of C. partellus in maize and sorghum in Eastern and Southern Africa appears to fit this criterion, as C. partellus is the major pest species in many areas. Once an exotic natural enemy is established in a new area, it remains and continues to provide control unless the system is disrupted by external factors (pesticide use) (DeBach and Rosen, 1991; Overholt, 1993).

2.7 Releasing parasitoids and establishment

Classical biological control has a long history with many successes. The first planned successful project in classical biological control was in the late 1800s against the cottony cushion scale in California. The cottony cushion scale was first recorded in California in 1868 and rapidly became a very serious
pest of citrus, pear and other plants. Entomologists investigating the new pest problem found that the pest originated in Australia and was accidentally introduced into the United States of America, probably on infested nursery stock. A mission went to Australia in 1868 and found two natural enemies attacking the scale, a parasitic fly and a ladybird beetle. These were imported into California and within months the scale was no longer a problem (Van den Bosch, 1982).

Although most of the emphasis in classical biological control has been against introduced pests, there have been successes against native pests as well. The sugarcane borer, *Diatraea saccharalis*, which is native to the neotropics, became a serious pest with the intensification of sugarcane cultivation in the Caribbean and southern United States of America. *C. flavipes*, the same parasitoid introduced against *C. partellus*, was introduced and provided excellent control of the sugarcane borer (Alam, Bennet and Carl, 1971; Fuchs, Huffman and Smith, 1979). Some authors have suggested that 'new associations' of natural enemy and hosts have a greater potential of success than co-evolved associations (Overholt, 1998).

The first attempt to introduce *C. flavipes* into Africa was made in 1968 by the Commonwealth Institute of Biological Control (CIBC). Releases in Uganda, Tanzania and Kenya did not result in establishment (CIBC, 1968-72). Later, introductions were made in Ghana, Ivory Coast, Senegal and South Africa, but all reportedly failed to establish *C. flavipes* (Scheibelreiter, 1980; Breniere and Bordat, 1982). However, recent introductions of *C. flavipes* in Kenya resulted in its permanent establishment (Overholt, 1998). One possible explanation for the failures to establish *C. flavipes* in some areas of Africa and the success in others is the suitability of various *C. partellus* populations for the development of *C. flavipes*.

Callan (1969), divided natural enemies into three categories as follows: natural enemies that are pre-adapted and rapidly become established, natural
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Callan (1969), divided natural enemies into three categories as follows: natural enemies that are pre-adapted and rapidly become established, natural
enemies that become established after genetic adaptation through natural selection and natural enemies that can not be established.

Natural enemies that fall into category 1 do not need to be released in large numbers, those in category 2 should be released in large numbers since many will be culled during the natural selection process. Matching the climate in the intended area of introduction with areas of similar climate in the pest's native home will lead to the selection of the natural enemy believed to have the highest potential for establishment (Callan's categories 1 and 2). If more individuals are released, there is a great chance for establishment.

2.8 Factors affecting the establishment of natural enemies

Potting, Overholt, Danso and Takasu (1997) argued that the performance of a released parasitoid population was dependent on its behaviour and physiology. The parasitoid must be able to locate the potential host successfully and be able to develop in these hosts. Suitable host availability and parasitoid adaptability to local conditions may be considered the most important factors that could affect parasitoid establishment.

The failure or success of a parasitoid introduction can thus be dependent on the behaviour of the parasitoids and/or on the physiological compatibility between the introduced natural enemy and the local host population (Potting, Vet and Overholt, 1997; Potting, Osae-Danso, Overholt and Ngi-Song, 1993). For example, in laboratory studies it was shown that the *B. fusca* population in Kenya was not a suitable host for *C. flavipes* development (Ngi-Song, Overholt and Ayertey, 1995).

Effective natural enemies can be rendered ineffective in host population regulation by a variety of other adverse environmental factors, from which pesticides have been considered the most important. The toxicity of a pesticide, its residual life, the frequency and type of application and formulation, all interact to determine the degree of adversity to natural
enemies. (DeBach and Rosen, 1991). The misuse of pesticides will preclude the general possibility of obtaining satisfactory biological control.

Natural enemies were considered to be generally more adversely affected by chemical insecticides than the target pests. Because predators and parasitoids must search for their prey/host, they are generally very mobile and spend a considerable amount of time moving across plant tissue. This increases the likelihood that they will contact the insecticide. When an insecticide is applied, ideally only the target pest(s) should be affected. The goal should be to maximise the pest mortality while minimising harm to natural enemies (Cornell University, 2000).

Cultural pest control practices, such as burning or plowing under stubble or harvest residues, may do more harm than good. Burning crop residues can destroy larval parasitoids found inside hosts that are diapausing in the stems. Other adverse factors on natural enemies and their ability to provide satisfactory pest control include weather extremes, lack of food, lack of alternative host (to provide year-round propagation because of shortage of primary host), and lack of synchronisation between the natural enemy and the host life cycles. In general, not much can be done to minimise the adverse effect of weather extremes (DeBach and Rosen, 1991; Thomas and Waage, 1996).

2.9 The role of biological control in integrated pest management

Biological control should be regarded as the backbone of any IPM program. The optimisation of established natural enemies by conservation practices offers the most immediate and direct means of decreasing insecticide pollution and reducing pest control costs. Chemicals should not be used on routine basis without regard to whether the pest actually constitutes a hazard at the time, or whether natural enemies are in the process of reducing or regulating the pest. If necessary, selective pesticides should be used based on economic injury levels (DeBach and Rosen, 1991).
2.10 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 on *C. partellus* and *B. fusca*. Several other parasitoids were also recorded on stemborers. However, all findings led to the similar conclusion that the native natural enemies are ineffective in controlling stemborer populations. In South Africa, Kfir and Bell (1993), 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, parasitoids did not prevent economically significant damage (Kfir and Bell, 1993). In addition, the following parasitoids were recorded in Mozambique, the egg parasitoids *Trichogramma* sp., the larval parasitoids *Stenobracon (=Euvipio) rufa, Chelonus curvimaculatus*, the pupal parasitoids *Pediobius furvus, Dentichasmias busseolae* and the hyperparasitoids *Aphanogmus faisensis* (Ferrière) (Ceraphronidae) which attack *C. sesamiae* cocoons (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).

In support of current and future programmes on biological control of cereal stemborers in Mozambique, the Plant Protection Department of the Ministry of Agriculture and Rural Development and the Faculty of Agronomy and Forest Engineering at Eduardo Mondlane University conducted a country-wide survey of stemborers in maize during the 1995/96 rainy season. *C. partellus* was the most abundant species at the majority of locations (more than 95% of the stemborer individuals found). *B. fusca* was dominant only at high altitude in the
centre and north of the country, while *S. calamistis* was found at all locations in low densities.

Several parasitoids were reared from the stemborers collected during the survey, but parasitism was generally low. *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).

In Kenya, prior to the introduction of *C. flavipes*, similar levels of parasitism by native parasitoids were observed (Overholt, et al., 1994a). In contrast, during a survey in India conducted by ICIPE, parasitism of *C. partellus* larvae by *C. flavipes* was usually around 30-40% (Omwega, et al., 1995).

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).

As was found in the recent survey in Mozambique, 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, Ngi-Song, Omwega, Kimani, Mbpila, Sallam and Ofomata, 1997). However, *C. flavipes* was only able to successfully develop in the two *Chilo* species and *S. calamistis* (Ngi-Song et al., 1995). In *S. calamistis*, progeny production was reduced.

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 a suitable species (*C. partellus*), and an unsuitable species (*B. fusca*), may
preclude establishment, as many of the parasitoid progeny will be encapsulated inside the non-suitable host (Ngi-Song et al., 1995). However, this is only a hypothesis based on laboratory studies (Ngi-Song et al., 1995). Field investigations where C. flavipes is released in areas where both C. partellus and B. fusca occur may provide greater insight into the potential geographic applicability of C. flavipes for managing stemborer populations in Africa.

There is evidence that the ability of Cotesia spp. parasitoids to develop in a given host varies from location to location. For example, Rajabalee and Govendasamy (1988) and Mohyuddin (1971), reported that C. flavipes could not complete development in S. calamistis, whereas Ngi-Song et al. (1995) found that S. calamistis was a suitable host for C. flavipes. In South Africa, it has been reported that C. flavipes, imported from Pakistan, was able to develop in B. fusca (Skoroszewski and Van Hamburg, 1987). In Kenya, all eggs of C. flavipes were encapsulated in B. fusca (Ngi-Song et al., 1995). Finally, there is evidence that some populations of the native African parasitoid, C. sesamiae, can develop in B. fusca, whereas other populations cannot (Ngi-Song et al., 1995).

It is not as yet clear whether the differences in host suitability are due to genetic variability in the parasitoid populations or in the stemborer populations. Thus, the results of work in Kenya on host suitability of geographically specific populations of stemborers may not be applicable to other areas in Africa.

In Mozambique there is no information on the variability of stemborer populations or their suitability for development of C. flavipes. Thus, it is important to investigate the performance of C. flavipes as a biological control agent against maize and sorghum stemborers in Mozambique.

2.11 The stemborer parasitoids, Cotesia spp.

Insect parasitoids have an immature life stage that develops on or within a single insect host, ultimately killing the host, hence the value of parasitoids as
natural enemies. Adult parasitoids are free-living insects and may be
dreadacious or herbivorous (Hoffmann and Frodsham, 1993) and may attack
more hosts by laying eggs on the host's body. Most insect parasitoids are
wasps (Hymenoptera) or flies (Diptera) and most of them only attack a
particular life stage of one or several related species. The immature parasitoid
develops on or within a host, feeding on body fluids and organs. The life cycle
of the host and parasitoid can coincide, or that of the host may be altered by
the parasitoid to accommodate its development (Cornell University, 2000).

The life cycle and reproductive habits of parasitoids can be very complex. In
some species only one parasitoid will develop in or on each host (solitary
parasitoids) while in others several individuals may develop within the host
(gregarious parasitoids). Parasitoids can be endoparasitoids, or internal
parasitoids, where the parasitoid larva develops within the body of its host. A
parasitoid whose larva feeds on the outside of its host is called an external or
ectoparasitoid. A parasitoid whose host is not another parasitoid is a primary
parasitoid and a hyperparasitoid, or secondary parasitoid, is a parasitoid
whose host is another parasitoid (for example, Aphanogmus fijiensis, a
parasitic wasp that attacks cocoons of the primary parasitoids Cotesia spp.
which in turn are parasitoids of cereal stem borers).

According to LaSalle (1993), hymenopteran insects are the most important
group of biological control agents. They respond to the population size of
their host in a density-dependent manner. The intensity of mortality they
cause to the host increases with an increase in host population, and
decreases when the host population decreases. The two populations fluctuate
between certain upper and lower limits that prevent both a massive increase
in the host population size, or a decrease to the point of extinction.

Cotesia species are among the most important natural enemies of cereal
stem borers (Kimani-Njogu and Overholt, 1997). Three species are considered
to be the most important, C. flavipes from the Indo-Australian region, C.
sesamiae native of Africa and Cotesia chilonis Matsumura, which has only
been recorded from Japan and China (Potting, 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 three species are morphologically similar.

### 2.11.1 Life history

*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.*, 1997a). 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 (Appendix C).

The egg to adult development time is about 20 days (Ngi-Song *et al.*, 1995; Potting, 1997) and the sex ratio is usually female biased (60% - 70%) (Potting, 1997). 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 (Delvare and Polaszek, 1998). 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; Potting *et al.*, 1997a). Fertilised eggs (diploid number of chromosomes) become female and male, and unfertilised eggs (haploid number of chromosomes) become males.
2.11.2 Host finding

Lews, Vet, Tumlinson, van Lenteren and Papaj (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 (Potting *et al.*, 1997a). 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, Overholt, Smith and Vinson, 1999). Other studies demonstrated that larval frass, caterpillar regurgitations (van Leerdam, Smith and Fuchs, 1995; Potting *et al.*, 1997a) and holes in the stem (Potting *et al.*, 1997a) were used in host location by *Cotesia* parasitoids.

Potting *et al.* (1997a) and 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 sometimes has to squeeze through small holes (Potting *et al.*, 1997a). 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 were able to successfully parasitise 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; Potting, 1997). Each female *C. flavipes* has around 150 eggs available for oviposition and can attack 3 to 4 hosts (Potting, 1997).
2.11.3 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 (Turling, Tumlinson and Lews, 1990). However, Potting et al. (1993) and Potting et al. (1997a) did not find differences in C. flavipes searching time on plants infested with C. partellus and B. fusca. C. flavipes did not make distinction between stemborer species feeding on the same plant species. They concluded that C. flavipes was not specific with regards 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.

2.11.4 Species identification

Cotesia flavipes complex has been imported and released in classical biological programmes against cereal stemborers (Polaszek and Walker, 1991). In some cases, releases were made into an area in which one of the three species is native. For example, in Africa where C. sesamiae is indigenous, C. flavipes has been introduced several times in various countries (Overholt et al., 1994a).

Various morphological characters were proposed to separate the Cotesia species complex based on colouration, sculpturing and male genitalia (Rao and Nagaraja, 1967; Nagaraja, 1971; Alam et al., 1971; Sigwalt and Pointel, 1980). However, with exception of male genitalia, all other characteristics have not proven to be completely reliable (Polaszek and Walker, 1991).

Using male genitalia, Polaszek and Walker (1991), separated the species in the Cotesia flavipes complex into two morphospecies; Cotesia sesamiae/Cotesia chilonis subcomplex and Cotesia flavipes. No
morphological characters were found to accurately distinguish *C. sesamiae* and *C. chilonis*.

Kimani-Njogu *et al.* (1998) using electrophoretic and phylogenetic analysis of esterase, hexokinase and sorbital dehydrogenase loci of allopatric populations of *C. flavipes* complex, separated *C. sesamiae* from *C. chilonis*. Due to their morphological similarity, there are doubts as to their taxonomic status as full species rather than geographic races (Polaszek and Walker, 1991).

2.11.4.1 Male genitalia

Nagaraja (1971), separated *C. flavipes* from *C. sesamiae* and *C. chilonis* by the shape of the parameres and the aedeagus, stating that both structures were narrower in *C. flavipes* and wider in the other two species. Sigwalt and Pointel (1980) and Polaszek and Walker (1991) confirmed this statement.

However, there are still difficulties in separating the three species when there are no males in the progeny produced. Attempts to separate the three species have been discussed by various workers (Rao and Nagaraja, 1967; Nagaraja, 1971; Sigwalt and Pointel, 1980) based on the punctuation and pubescence on the mesosoma and metasoma and the shape of the scutellum and they concluded that *C. flavipes* had a sparsely punctuate mesonotum and its scutellum and propodeum were narrow, while *C. sesamiae* differed from *C. flavipes* by having an enlarged propodeum and a uniformly punctate mesonotum. *C. chilonis* had a more pubescent mesonotum than the other two species.

Kimani-Njogu and Overholt (1997), stated that the scuto-scutellar sulcus was straight in *C. chilonis* and curved in the other two species, more than 20 hairs on the scutellum of *C. chilonis* and less than 20 in the *C. sesamiae* and *C. flavipes* were found. *C. sesamiae* was separated from *C. flavipes* by the
rugosity of the propodeum. While in the C. sesamiae the propodeum was less rugose in the basal half it, is fully rugose in the C. flavipes and C. chilonis.

2.11.5 Success of Cotesia species as biological control agents

C. flavipes has been introduced into more than 40 countries in the tropics and subtropics for biological control of pyralid stemborers in the genera Chilo and Diatraea (Polaszek and Walker, 1991). Whereas in Mauritius, where C. flavipes may have been accidentally introduced, parasitism of 50% of larvae of the introduced Chilo sacchariphagus Bojer has been reported (Rajabalee and Govendasamy, 1988). In Madagascar, C. flavipes was introduced in 1960, and parasitism of 60% of C. sacchariphagus larvae has been reported (Betbeder-Matibet and Malinge, 1968). This parasitoid has also been introduced into several other countries in the neotropical region for biological control of Diatraea saccharalis (F.) in sugarcane and substantial control has been reported in many areas (Alam et al., 1971; Fuchs et al., 1979). The success of C. sesamiae is limited to its introduction and establishment in Mauritius, Reunion and Madagascar against S. calamistis.

Several factors could be responsible for success of the two Cotesia species parasitoids. In their areas of endemism, the Cotesia spp. parasitoids attack several species of pyralid and noctuids (Mohyuddin, 1971). The relatively wide taxonomic range of suitable hosts, coupled with narrow habitat specificity, may favour its establishment. Stemborers often occur as species complexes and a parasitoid that could exploit more than one host may be better able to colonise a new area than a monophagous parasitoid, due to a more constant availability of hosts (Overholt et al., 1999).

Another factor may be the high reproductive potential of C. flavipes and C. sesamiae in relation to most stemborers. Both C. flavipes and C. sesamiae have short life cycle (18 to 20 days) in comparison to their stemborer hosts (30 to 50 days) and a fairly high fecundity (30 to 40 progeny per oviposition) (Overholt, 1998). A high host searching ability may also be involved.
Wiedenmann and Smith (1993) demonstrated that even at low host densities, *C. flavipes* was able to successfully locate stemborer hosts. The high searching ability of *C. flavipes* may in part, be due to its behaviour of entering tunnels in plant stems to attack stemborer larvae.

Overholt *et al.* (1999) stated that many other larval parasitoids of stemborers remain on the outside of the stem and parasitize larvae by drilling through the stem with their ovipositor. This strategy may be effective for attaching stemborers in wild grasses, such as *Panicum maximum*, but in relatively larger-stemmed cultivated grasses, the length of the ovipositor may limit the number of hosts susceptible to attack.

In a review of biological control of stemborers in Africa, Overholt (1998) found that old parasitoid-host associations were responsible for most of the successful establishments. All of the establishments on the Indian Ocean Islands and the only known establishment on mainland Africa are probably old associations. The behavioural and physiological compatibility of old association parasitoids and their hosts are implicit, whereas in new associations compatibility cannot be assumed. The necessary cues for host finding in new associations may be missing, or the host may not be suitable for parasitoid development (Overholt, 1998). Host suitability was particularly considered important for endoparasitoids, which have a more intimate relationship with their hosts than ectoparasitoids. Old associations are more likely to result in establishment and suppression of the target host population (Waage, 1991). However, the greater likelihood of success with old associations should not preclude efforts against native pests. One of the greatest successes in stemborers biological control was against *D. saccharalis* with the new association parasitoid, *C. flavipes* (Overholt *et al.*, 1999).
CHAPTER THREE

EVALUATION OF THE ESTABLISHMENT AND SPREAD OF COTESIA FLAVIPES CAMERON (HYMENOPTERA: BRACONIDAE) AS A BIOLOGICAL CONTROL AGENT FOR CEREAL STEMBORERS IN MOZAMBIQUE

3.1 INTRODUCTION

Classical biological control is viewed as a potential management strategy for stemborer population, particularly against C. partellus, because of its status as an introduced pest. In support of current and future programmes on biological control of cereal stemborders in Mozambique, a country-wide survey of stemborders species composition and their indigenous natural enemies in maize was carried out during the 1995/96 rainy season to augment data collected earlier by other workers (Gonçalves, 1970; Segeren et al., 1991; Davies et al., 1995; Riedel, unpub. data). However, all surveys led to similar conclusions that C. partellus was the most abundant stemborer at most locations and that the rate of parasitism was very low (<5%) (Segeren et al., 1991; Cugala et al., 1999). It was found in some areas that C. partellus, B. fusca and S. calamistis coexisted in the same area and/or plant (Cugala, et al., 1999; Riedel unpub. data).

Based on the finding that C. partellus was the predominant stemborer in many areas of Mozambique, and that parasitism by indigenous parasitoids was generally 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 February-March 1999 in the southern and central regions of Mozambique.
3.1.1 Specific objectives

a) To evaluate the establishment and spread of *C. flavipes* from the 1996 release sites; and

b) To evaluate the effect of stemborer species composition on the establishment of *C. flavipes* from the 1999 release sites.

3.2 MATERIALS AND METHODS

3.2.1 Establishment and spread of *C. flavipes* from the 1996 release sites

During 1996/97 growing season (early November), the exotic parasitoid, *C. flavipes*, was introduced for the first time into Moamba and Marracuene Districts, Maputo Province, in the southern region of Mozambique. About 100 cocoon masses were released at each site. The parasitoids were placed in a releasing cage and then left in the farmers' field (Overholt et al., 1994a). Both sites have similar environmental conditions; they are lowland areas (<200 m altitude) associated with high temperatures. The two *Cotesia* parasitoids (*C. flavipes* and *C. sesamiae*) were considered as treatments and replicated 20 times (each farmers' field) at each site.

During the 1998/99 growing season, 20 farmers' fields located in Marracuene and Moamba Districts in Maputo Province (southern Mozambique), within a radius of 20 km of each of the 1996 release sites, were randomly selected at the tasselling stage (Figures 3.1 and 3.2). As much as possible, the fields were evenly distributed in all directions from the release fields. The exact location (latitude and longitude) of the fields was recorded using a GPS (global positioning system) and the distance from the release site was estimated using Arc-View 3.2 software (ArcView Institute, 1999) and the GPS.

In each field, 20 plants of maize were randomly selected, inspected and the presence/absence of symptoms of stemborer infestation recorded (for stemborer infestations). If the randomly selected plant exhibited signs of
infestation (leaf feeding, entrance holes in the stem), it was removed from the field and dissected. If the selected showed no evidence of infestation, the nearest plant showing symptoms of stemborer infestation was removed. Sampling was conducted twice at each location during the 1998/1999 growing season.

As *C. flavipes* attacks medium (third to fourth) to large (fifth to sixth instar larvae) sized larvae (Ngi-Song *et al*., 1995), only larvae meeting these size categories were retained. Larvae were held individually in glass vials (2.5 cm diameter and 7.0 cm high or 3.5 cm x 8.0 cm) covered with cotton wool, provided with fresh pieces of maize or sorghum stem and then taken to the laboratory for observation. Stemborers were identified and reared until death, pupation or parasitoid emergence. Pupae were individually kept in glass vials without food, covered with cotton wool, and reared under conditions of temperature and relative humidity until death, adult emergence or parasitoid emergence.

All emerging parasitoids were identified. Adults *Cotesia spp.* were identified to species by the shape of male genitalia (Polaszek and Walker, 1991). Adult wasps from the same cocoon mass were sexed, counted and sex ratios (proportion of females) calculated. To determine the sex ratio, females were distinguished from males by the length of male antennae (male antennae are longer than of female). Progeny produced is the sum of males and females.

### 3.2.2 Establishment of *Cotesia flavipes* from the 1999 release sites

Releases were made in two ecologically different areas, selected according to differences in the stemborer species complex. One location was Nhacoongo village (24°19’41”S; 35°12’82”E, elevation 40 m), a warm, lowland area in the Southern Province of Inhambane where previous work had shown that *C. partellus* was the dominant stemborer (>90% of the population), followed by *S. calamistis* (<10% of the population) (Segeren *et al*., 1991; Riedel unpub. data).
Fig. 3.1 - Maputo province showing sampling fields at Marracuene
Fig. 3.2 – Maputo province showing sampling fields at Moamba district
B. fusca has not been recorded from this area. The second area was Machipanda (18°52'16"S; 32°47'96"E; elevation 800 m above sea level), a medium to high elevation, cooler zone located in the Central Province of Manica. In Machipanda, both B. fusca and C. partellus occurred with nearly equal frequency (40% and 60% of the population for B. fusca and C. partellus; respectively) (Riedel, unpub. data).

Factorial combination of two crops (maize and grain sorghum) and two (Nhacoongo) or three (Machipanda) stemborer species in Randomized Complete Block Design with four replications was used.

Four plots of about 625 m² each were prepared during the 1998/99 rainy season at each site. Each plot was divided into 4 sub-plots of about 100 m² each; two for maize and two for grain sorghum. Sub-plots were monitored for stemborer infestation during the growing season (November-April) of 1998/99. Insects were allowed to infest plants naturally and no insecticides were applied.

About 2,000 cocoon masses (approximately 60,000 individuals) of C. flavipes were released three times in Nhacoongo in February, and about 700 cocoon masses (approximately 21,000 individuals) were released once in Machipanda in March, according to the method described by Overholt et al. (1994a). Releases were made when the presence of suitable larval instars (third and later) were expected to be abundant. Parasitoids were released both as cocoons and adults. Releasing cocoons is a preferred method as it maximises the effective life span of the adults in the field (Overholt et al., 1997). To protect the cocoons from predators and rainfall, they were placed in a 'releasing cage' (Overholt et al., 1994a). The cocoons which were released were shipped to Mozambique by DHL from ICIPE’s Rearing Unit, Nairobi, Kenya.
Two weeks after release, all cocoon masses were collected and the number of adults that did not emerge was estimated by counting the number of dark cocoons or adults dead inside the release station. In the following growing season (1999/2000), similar plots were established and sampled, but no parasitoids were released. At about 60 days after crop emergence, samples of 20 plants were systematically selected from each sub-plot at each site during the two growing seasons. From each of seven rows (out of seven), one plant was selected every two meters for a total of 3 plants per row. Only two plants were removed from the last row sampled. The plants were dissected in the field, and all stemborer larvae and pupae were removed and mass reared in the laboratory.

3.2.3 Data analysis

Comparisons between the abundance of the two *Cotesia* parasitoids (percent parasitism) at the two study sites were performed by t-test (PROC TTEST) (SAS Institute, 1996). Any larva which died (before parasitoid emergence), aestivated, escaped or was injured was excluded from the analyses. Percent parasitism and the number of insects were transformed to square root while the proportions of females were transformed to arcsine square root before being subjected to t-test.

3.3 RESULTS

3.3.1 Establishment and spread of *C. flavipes* from the 1996 release sites

Two stemborer species were found at each of the 1996 release sites, *C. partellus* and *S. calamistis* (Appendix B; a and c). Table 3.1 shows stemborer density, abundance and mean of larvae collected at each location. *C. partellus* was the most abundant stemborer at both sites constituting more than 98 and 96 percent of stemborers at Marracuene and Moamba; respectively.
Significantly more plants were infested by stemborers at Marracuene than at Moamba \( (F=3.35, \text{ df}=38, \text{ P}>0.0114) \). Despite the high number of larvae collected and high proportion of plants infested at Marracuene, there were no differences between the two locations on the number of stemborer larvae collected and stemborer density (Table 3.1).

The two stemborer species found at each site were parasitised by *Cotesia* spp. parasitoids. *C. flavipes* was recovered from *C. partellus* at both locations, but not from *S. calamistis* (Table 3.2). *C. flavipes* was recovered more than 10 Km from the release field at Marracuene (Appendix D) and about 6 Km from the release field at Moamba (Appendix E). At both sites, *C. sesamiae* parasitised significantly more *C. partellus* larvae than *C. flavipes* at Marracuene \( (F=3.22, \text{ df}=38, \text{ P}>0.0585) \) and at Moamba \( (F=4.00, \text{ df}=38, \text{ P}>0.0040) \).

<table>
<thead>
<tr>
<th>Location</th>
<th>Percent Infestation</th>
<th>Percent Stemborer density (larvae/plant)</th>
<th>Chilo partellus</th>
<th>Sesamia calamistis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marracuene</td>
<td>77.8±0.2a</td>
<td>2.9±0.1a</td>
<td>2109(98.9%)±0.5a</td>
<td>24 (1.1%)±0.2a</td>
<td>2133</td>
</tr>
<tr>
<td>Moamba</td>
<td>81.8±0.3b</td>
<td>1.8±0.1a</td>
<td>676(96.7%)±0.4b</td>
<td>23 (3.3%)±0.2a</td>
<td>699</td>
</tr>
</tbody>
</table>

Numbers followed by the same letter in a column are not statistically different \( (P>0.05) \) 
( ) = stemborer relative abundance

At Marracuene *C. flavipes* produced significantly more progeny than *C. sesamiae* \( (F=14.60, \text{ df}=39, \text{ P}>0.0000) \), but *C. sesamiae* produced more females than *C. flavipes* \( (F=28.03, \text{ df}=38, \text{ P}>0.0000) \). At Moamba, there were no differences between the two *Cotesia* parasitoids in progeny production or number of females produced (Table 3.2).

*C. flavipes* produced more individuals and females at Maracuene than at Moamba. In general, more *C. flavipes* were recovered at Maracuene than at Moamba (Table 3.2).
Table 3.2. Means percent parasitism and progeny produced by *Cotesia* spp. from stemborer species at the two 1996 release sites (±SE)

<table>
<thead>
<tr>
<th>Location</th>
<th>Stemborer species</th>
<th>Parasitoid species</th>
<th>N</th>
<th>Percent parasitism</th>
<th>Progeny produced</th>
<th>Proportion of females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maracuene</td>
<td><em>C. partellus</em></td>
<td><em>C. sesamiae</em></td>
<td>452</td>
<td>21.5±0.2a</td>
<td>30.9±0.2b</td>
<td>0.78±0.0a</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. flavipes</em></td>
<td>14</td>
<td>0.7±0.1b</td>
<td>33.5±0.7a</td>
<td>0.77±0.0b</td>
</tr>
<tr>
<td></td>
<td><em>S. calamistis</em></td>
<td><em>C. sesamiae</em></td>
<td>6</td>
<td>25.0±6.2a</td>
<td>40.2±6.3a</td>
<td>0.83±1.2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. flavipes</em></td>
<td>0</td>
<td>0.0b</td>
<td>0.0b</td>
<td>0.0b</td>
</tr>
<tr>
<td>Moamba</td>
<td><em>C. partellus</em></td>
<td><em>C. sesamiae</em></td>
<td>161</td>
<td>23.8±0.3a</td>
<td>25.5±0.2a</td>
<td>0.73±0.0a</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. flavipes</em></td>
<td>3</td>
<td>0.5±0.2b</td>
<td>21.7±0.2a</td>
<td>0.68±0.0a</td>
</tr>
<tr>
<td></td>
<td><em>S. calamistis</em></td>
<td><em>C. sesamiae</em></td>
<td>2</td>
<td>8.7±5.4a</td>
<td>50.5±4.9a</td>
<td>0.87±8.3a</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. flavipes</em></td>
<td>0</td>
<td>0.0b</td>
<td>0.0b</td>
<td>0.0b</td>
</tr>
</tbody>
</table>

Numbers followed by the same letter in a column are not statistically different (P>0.05)
N = number of recoveries of *Cotesia* spp.

3.3.2 Establishment of *Cotesia flavipes* from the 1999 release sites

Adult parasitoids successfully emerged from more than 95% of the cocoons at both release sites. All stemborer species found at each release site, *C. partellus* and *S. calamistis* at Nhacoongo, and *C. partellus*, *B. fusca* and *S. calamistis* at Machipanda, were parasitized by *Cotesia* spp.

Table 3.3 and Appendix F show the numbers of stemborer larvae of the different species found at each release site during two sampling periods. *C. partellus* larvae collected in maize were significantly more than in grain sorghum at Nhacoongo during the release season (1998/99) (F=5.32, df=14, P>0.04).

*C. partellus* was the dominant stemborer species at the two study sites constituting more than 90% of all collected larvae at Nhacoongo during 1998/1999 and 1999/2000 growing seasons. At Machipanda, *C. partellus* accounted for 61% of borers during 1998/1999 growing season and 98% the following season (Table 3.3). The proportion of plants infested was significantly higher in maize than in grain sorghum at Nhacoongo during the release season (F=7.42, df =14, P>0.02), but there were no differences in the
proportion of infested plants in maize and sorghum at Machipanda during 1999/2000 growing season. The stemborer density did not differ between the two crops at either site for two seasons (Table 3.3).

Table 3.4 shows the numbers of *Cotesia* spp. recovered during the two seasons of sampling at the two study sites. All stemborer species found at Nhacoongo were parasitised by both species of *Cotesia* while at machipanda, only *C. partellus* and *B. fusca* were parasitized. At Nhacoongo, parasitism of *C. partellus* by *C. sesamiae* in both crops was significantly higher than parasitism by *C. flavipes* during the first year of parasitoid release (*F* =4.24, df =14, *P > 0.04*). At Machipanda, *C. sesamiae* parasitized significantly more *B. fusca* than *C. flavipes* on grain sorghum during the first season of release (*F* =29.21, df =14, *P > 0.02*) (Table 3.4).

Small numbers of *C. flavipes* were recovered at the two study locations on both crops during the seasons 1998/1999 and 1999/2000. Parasitism due to *C. flavipes* was very low, with the highest parasitism of 3.2% observed on *S. calamistis* in grain sorghum at Nhacoongo. At Machipanda, *B. fusca* parasitism by *C. flavipes* was 2.6% in sorghum (Table 3.4).

In general, the two *Cotesia* species parasitoids produced similar numbers of cocoons on *C. partellus* on maize during the period of release (at Nhacoongo) and during the following season (at both study areas). However, in general, *C. sesamiae* produced more females than *C. flavipes* when *C. partellus* was the host at Nhacoongo in maize (Table 3.4 and Appendix G). The largest broods of *C. flavipes* were reared from *S. calamistis* larvae collected from sorghum at Nhacoongo one year following its introduction. In general, parasitism rates due to the exotic parasitoid were low and ranged from 0 to 1.9% on *C. partellus* and 2.6% to 3.2% on indigenous stemborers, *B. fusca* and *S. calamistis*; respectively (Table 3.4).
Table 3.3. Stemborer density and relative abundance (%) from the 1999 trial release sites (±SE)

<table>
<thead>
<tr>
<th>Location/Crop</th>
<th>Percent Infestation</th>
<th>No. Larvae per plant</th>
<th>Stemborer species and their relative abundance (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. partellus</td>
<td>S. calamistis</td>
</tr>
<tr>
<td>1998/1999 growing season:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At Machipanda:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>27.5±5.1</td>
<td>1.5±7.2</td>
<td>147 (61.0±2.8)</td>
<td>17 (7.0±3.4)</td>
</tr>
<tr>
<td>At Nhacoongo:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>89.4±0.1a</td>
<td>4.0±0.1a</td>
<td>562 (94.6±0.2a)</td>
<td>32 (5.4)±0.3a</td>
</tr>
<tr>
<td>Sorghum</td>
<td>62.5±0.3b</td>
<td>2.8±0.1a</td>
<td>420 (97.0±0.6b)</td>
<td>13 (3.0)±0.3a</td>
</tr>
<tr>
<td>1999/2000 growing season:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At Machipanda:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>41.9±0.5a</td>
<td>1.8±0.1a</td>
<td>163 (100)±0.4a</td>
<td>0.0</td>
</tr>
<tr>
<td>Sorghum</td>
<td>56.3±0.3a</td>
<td>1.7±0.1a</td>
<td>213 (98.2)±0.4a</td>
<td>1 (0.5)±1.3</td>
</tr>
<tr>
<td>At Nhacoongo:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>56.3±0.4a</td>
<td>3.2±0.1a</td>
<td>483 (98.6)±0.5a</td>
<td>7 (1.4)±0.3a</td>
</tr>
<tr>
<td>Sorghum</td>
<td>66.9±0.4a</td>
<td>3.2±0.1a</td>
<td>452 (93.6)±0.6a</td>
<td>31 (6.4)±0.4a</td>
</tr>
</tbody>
</table>

Numbers followed by the same letter in a column are not statistically different (P>0.05)
() = Stemborer relative abundance; - = Species not recorded
Table 3.4 Percent parasitism and progeny produced by *Cotesia* spp. from different stemborer species in different crops at the two locations of trial releases (±SE)

<table>
<thead>
<tr>
<th>Location/Crop Parasitoid Species</th>
<th>Mean percent parasitism</th>
<th>Mean progeny produced</th>
<th>Mean proportions of females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Cp</td>
<td>Sc</td>
</tr>
<tr>
<td>1998/1999 growing season</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At Machipanda</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorghum <em>C. sesamiae</em></td>
<td>26</td>
<td>10.2±7.6</td>
<td>50.0±1.4</td>
</tr>
<tr>
<td><em>C. flavipes</em></td>
<td>2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Maize <em>C. sesamiae</em></td>
<td>56</td>
<td>9.1±0.2a</td>
<td>15.6±9.7</td>
</tr>
<tr>
<td><em>C. flavipes</em></td>
<td>8</td>
<td>1.4±0.3b</td>
<td>0.0</td>
</tr>
<tr>
<td>Sorghum <em>C. sesamiae</em></td>
<td>80</td>
<td>17.6±0.4a</td>
<td>46.2±1.6</td>
</tr>
<tr>
<td><em>C. flavipes</em></td>
<td>8</td>
<td>1.9±0.4b</td>
<td>0.0</td>
</tr>
<tr>
<td>1999/2000 growing season</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At Machipanda</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize <em>C. sesamiae</em></td>
<td>17</td>
<td>10.4±0.6a</td>
<td>0.0</td>
</tr>
<tr>
<td><em>C. flavipes</em></td>
<td>3</td>
<td>1.8±0.6a</td>
<td>0.0</td>
</tr>
<tr>
<td>Sorghum <em>C. sesamiae</em></td>
<td>21</td>
<td>9.9±0.6a</td>
<td>0.0</td>
</tr>
<tr>
<td><em>C. flavipes</em></td>
<td>2</td>
<td>0.9±0.3a</td>
<td>0.0</td>
</tr>
<tr>
<td>Maize <em>C. sesamiae</em></td>
<td>28</td>
<td>5.8±0.5a</td>
<td>0.0</td>
</tr>
<tr>
<td><em>C. flavipes</em></td>
<td>3</td>
<td>0.6±0.3a</td>
<td>0.0</td>
</tr>
<tr>
<td>Sorghum <em>C. sesamiae</em></td>
<td>28</td>
<td>5.8±2.86</td>
<td>6.5±0.7a</td>
</tr>
<tr>
<td><em>C. flavipes</em></td>
<td>1</td>
<td>0.0</td>
<td>3.2±0.9a</td>
</tr>
</tbody>
</table>

- = species not recorded.

*Cp* = *Chilo partellus*, *Sc* = *Sesamia calamistis*, *Bf* = *Busseola fusca*.

N = number of recoveries of *Cotesia* spp.

Numbers followed by the same letter in a column are not statistically different (P > 0.05)
In maize, *C. partellus* larvae were significantly parastised by *C. flavipes* at Machipanda more than at Nhacoongo during the 1999/2002 season (F=4.16, df=14, P>0.05), but there were no significant differences between the two study areas on the proportion of *C. partellus* larvae attacked by *C. flavipes* in grain sorghum. At both study sites the indigenous *C. sesamiae* parasitised more larvae than exotic *C. flavipes* when the exotic species, *C. partellus* and indigenous species *B. fusca* and *S. calamistis* were hosts (Table 3.4).

During the two sampling seasons, several other parasitoids were reared from stemborers including the egg parasitoid *Trichogramma* sp., the larval parasitoids *Stenobracon (=Euvipio) rufa* Szepligeti (Hymenoptera: Braconidae), *Goniozus indicus* Ashmead (Hymenoptera: Bethylidae), *Chelonus curvimaculatus* Cameron (Hymenoptera: Braconidae), *Dolichogenidea polaszeki* (Hymenoptera: Braconidae) and *Sturmiopsis sp.* (Diptera: Tachinidae) and the pupal parasitoids, *Pediobius furvus* (Gahan) (Hymenoptera: Eulophidae), and *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae). The hyperparasitoid *Aphanogmus Fijiensis* (Ferriere) (Hymenoptera: Ceraphronidae) was reared from cocoons of *Cotesia spp.* collected at both places.

### 3.4 DISCUSSION

#### 3.4.1 Establishment and spread of *C. flavipes* from the 1996 release sites

Previous surveys indicated the occurrence of *C. partellus* and *S. calamistis* in the warm low-altitude (0 to 200 m altitude) southern region of Mozambique. *C. partellus* was the most abundant stemborer constituting more than 90% of the total stemborer population, with *S. calamistis* accounting for the remainder (Gonçalves, 1970; Berger, 1981; Segeren *et al.*, 1991; 1995). Present results show that *C. partellus* accounted for more than 98% of stemborer population in Marracuene and about 96.7% in Moamba. Sithole (1994) working in Zimbabwe reported that the distribution and relative abundance of stemborer
species were influenced by climatic factors with *C. partellus* being the abundant species at low elevations and higher temperature zones.

High stemborer infestation levels have been documented in the areas where *C. partellus* is the dominant species (Berger, 1981; Segeren et al., 1991). In this study, fields with more than 60% of plants infested were reported in both areas. Since its first record in Africa, *C. partellus* has been regarded as the most destructive pest of maize and grain sorghum in the warm and low-altitude zones in southern Africa (van Hamburg, 1979).

The number of *C. flavipes* cocoon masses released at each site in November 1996 (100 cocoon masses) was low. The probability of establishment increases as more natural enemies are released (DeBach, 1964 and DeBach and Rosen, 1991). *Cotesia spp.* parasitoids are natural enemies that should be released in large numbers because many individuals will die during the natural selection process of adaptation to the field environmental conditions (Callan’s category II) (Callan, 1969).

This was the first time that *C. flavipes* was recovered from the area where it was released in 1996, and clearly indicated that this exotic parasitoid has been established in southern Mozambique. The low levels of parasitism are not unexpected. In Kenya, where *C. flavipes* was released in 1993, the population density remained very low for the first 4 years, and then increased dramatically to more than 30% parasitism (Overholt, 1998) and at some sites parasitism of more than 60% was found in 1997 and a 30-50% decrease in stemborer populations has been observed (Zhou et al., 2000). The same phenomenon has been reported after the release of *C. flavipes* in Barbados where it was released against *D. saccharalis* in sugarcane, it was not recovered for more than one year after the releases in spite of intensive surveys, but then parasitism rose steadily during the next few years (Alam et al. 1971) and in Madagascar where *C. flavipes* was released against *C. sacchariphagus* in sugarcane, levels of 60% of parasitism were not reached until 6 years after the release (Greathead, 1971). It appears that the population simply needs time to build up and spread from the release sites.
The majority of recoveries at Marracuene were made in fields located more than 10 Km from the release sites. However, the indigenous larval parasitoid, *C. sesamiae*, was far more abundant than the introduced species at both sites. Parasitism by the indigenous species was 21.5% on *C. partellus* and 25.0% on *S. calamistis* at Marracuene and 23.8 and 8.7% on *C. partellus* and *S. calamistis* at Moamba; respectively. The levels of parasitism by *C. sesamiae* found in this study were considerably higher than the 5% previously reported by Segeren *et al.* (1991). However, despite the relatively high parasitism on stemborer larvae, *C. sesamiae* was not able to reduce stemborer infestation above economy injury levels that were estimated to be 10% of plants infested for *B. fusca* and 40% for *C. partellus* on maize in South Africa (van den Berg, 1998).

The number of progeny emerging from parasitized larvae varied according to host and parasitoid species; more *C. sesamiae* cocoons were recorded from *S. calamistis* larvae than from *C. partellus* larvae at both places. One possible explanation is that higher numbers of progeny emerged from *S. calamistis* because it is larger than *C. partellus*. Mohyuddin (1971) reported an average of 105 and 101 *C. sesamiae* adults emerging from *B. fusca* and *S. calamistis*, respectively, and that early larval instars produced few cocoons. Ngi-Song *et al.* (1995), reported the highest *C. flavipes* progeny (36.5) production from *C. partellus* and 35.2 *C. sesamiae* from *S. calamistis*.

### 3.4.2 Establishment of *Cotesia flavipes* from the 1999 release sites

Fields with 100% plants infested with stemborers have been reported in Maputo province (Berger, 1981). The present study reported higher infestations in Nhacoongo than in Machipanada. An average of stemborer density of 4.0 larvae per plant was observed in Nhacoongo. The higher infestation levels in the southern region may have been due to the continuous cultivation of maize in this area. Gonçalves (1970) and Segeren *et al.* (1991), reported a mean of 7 to 10 larvae per plant in the areas where *C. partellus* is the most abundant stemborer species.
According to Debach and Rosen (1991), the numbers of *C. flavipes* cocoon masses released at Machipanda (700 cocoon masses) was not sufficient for natural enemy establishment. Skoroszewski and van Hamburg (1987), did not recover any *C. flavipes* when small numbers were released. However, in spite the low numbers released at this site, *C. flavipes* was recovered during the release period and the following year. The higher number released at Nhacoongo (2,000 cocoon masses) associated with the continuous maize growth that makes host available throughout the year and the parasitoid adaptability to local conditions may generally be considered to improve the possibility of establishment (Beirne, 1975; DeBach and Rosen, 1991).

In general, more *C. flavipes* were recovered at Nhacoongo than at Machipanda during the two growing seasons. This may have been due to differences in the stemborer species composition at the two sites. The high abundance of *C. partellus* at Nhacoongo, which is an old association host of *C. flavipes*, may have favoured its establishment. Moreover, the higher number of parasitoids released at Nhacoongo is likely to have resulted in a higher number of parasitized stemborers in the field.

*C. flavipes* was recovered from all stemborer species found at each study site in both maize and sorghum; *C. partellus* and *S. calamistis* at Nhacoongo and *C. partellus, B. fusca* and *S. calamistis* at Machipanda. The recoveries of *C flavipes* from *B. fusca* is quite surprising as it contradicts the results of laboratory studies conducted in Kenya which concluded that *B. fusca* was not a suitable host for the development of *C. flavipes* (Ngi-Song et al., 1995), except in cases where a *B. fusca* larva was already attacked by another natural enemy. Hailemicheal, Schultess, Smith and Overholt (1997), working in Benin also found that *B. fusca* was not a suitable host for *C. flavipes*. Because of these results, Overholt (1998), speculated that *C. flavipes* would not be able to establish itself in areas of Africa where *B. fusca* is abundant. However, Skoroszewski and van Hamburg (1987), reared *C. flavipes* from field collected *B. fusca* larvae in South Africa.
The parasitoids that were used in the Kenyan study (Ngi-Song et al., 1995) and in this study both originated from the same cultures, so it seems unlikely that there were any genetic differences between the parasitoids, which could explain the variance in host compatibility. It is believed that the most likely explanation is that the immune system of \textit{B. fusca} populations in southern Africa may be sufficiently different from the Kenyan population to the extent of allowing parasitoid development. Alternatively, the \textit{B. fusca} larvae from which \textit{C. flavipes} were recovered in this study may have previously been parasitised by another natural enemy (Ngi-Song, Kimani, and Overholt, 2001).

Differences in compatibility have also been found between \textit{C. flavipes} and \textit{S. calamistis}. Mohyuddin (1971), working in Uganda, and Rajabalee and Govendasamy (1988) working in Mauritius, reported that \textit{S. calamistis} was not a suitable host for \textit{C. flavipes}, whereas laboratory studies by Ngi-Song et al. (1995), found that \textit{S. calamistis} was a suitable host. The suitability of \textit{S. calamistis} for development of \textit{C. flavipes} was later confirmed in field studies (Sallam, Overholt and Kairu, 1999).

Thus, the compatibility between \textit{C. flavipes} and various potential host populations should be investigated before or during release programmes. Moreover, the ability of \textit{C. flavipes} to develop in southern African populations of \textit{B. fusca} suggests that the parasitoid may have a potential to control indigenous stemborers in Africa than previously thought (Overholt, 1998).

Skoroszewski and van Hamburg (1987), Sithole (1989) and Ebenebe, van den Berg and van der Linde (1999), reported that although \textit{C. partellus} causes severe losses to maize, it prefers sorghum as host plant. In contrast, we found that the \textit{C. partellus} infestation was high in both crops, which agrees with a study by Overholt et al. (1994b) in Kenya, which showed no major differences in stemborer infestation in maize and sorghum. After the invasion of \textit{C. partellus} in South Africa, it was first considered to be primarily a pest of sorghum, but later became increasingly important in maize (van Rensburg and Bate, 1986; Kfir, 1992). It is possible that differences in the varieties of maize and sorghum planted in the various studies may have influenced the relative
attractiveness of the two crops. However, it may be probable that populations of *C. partellus* from different geographic areas, respond differently to maize and sorghum, and that the response may vary over time.

The recoveries of *C. flavipes* a year after its release at Machipanda indicated that this exotic parasitoid had successfully survived the cool winter conditions at this site where the mean minimum temperature in July is 7.3 °C and the mean maximum is 30.9°C in October (FAO, 1984). Skoroszewski and van Hamburg (1987), reported that *C. flavipes* could not survive the dry winter period in South Africa, where the mean minimum temperature varies from -0.8°C to 2.5°C (FAO, 1984). The recoveries of *C. flavipes* at both study sites provide clear evidence that this exotic parasitoid was able to adapt successfully to the field conditions and had established at both study sites. However, based on experience from Kenya (Overholt, 1998), it may take a few years for the parasitoid population to increase and have a significant impact on the stemborer populations in Mozambique.

At both study areas the indigenous parasitoid, *C. sesamiae* was still the most abundant larval parasitoid. *C. sesamiae* parasitised more stemborer larvae than *C. flavipes*. However, Omwega and Overholt (1997) and Sallam et al., (1999) working in Kenya reported that in case of multiple parasitism by the two *Cotesia* species, the exotic *C. flavipes* emerged when *C. partellus* and indigenous stemborers were hosts. Thus, *C. sesamiae* was regarded as a relatively poor natural enemy of the cereal stemborers in Kenya. However, in South Africa *C. sesamiae* was reported as an important mortality factor of the indigenous stemborer, *B. fusca* (Kfir and Bell, 1993; Kfir, 1998).

### 3.5 CONCLUSIONS

Recoveries of *C. flavipes* from the 1996 release sites indicated that this parasitoid had established in southern Mozambique. However, the population numbers and percent parasitism were very low and the indigenous parasitoid, *C. sesamiae* was still the most abundant. *C. flavipes* was also recovered at the trial release sites during the release period and the year that followed
indicating that this exotic parasitoid has become established at the two locations in the southern and central regions of Mozambique in spite of differences in stemborer species composition. However, it seems premature to evaluate the impact of C. flavipes on the stemborer populations. Based on experience from Kenya, Barbados and Madagascar (Zhou et al., 2000; Alam et al., 1971; Betbeder-Matibet and Malinge, 1968) it may take a few years for the parasitoid to produce significant impact on the stemborer populations present at each location.
CHAPTER FOUR

ACCEPTABILITY AND SUITABILITY OF STEMBORERS FOR COTESIA FLAVIPES CAMERON (HYMENOPTERA: BRACONIDAE) OVIPOSITION AND DEVELOPMENT

4.1 INTRODUCTION

Traditionally, classical biological control has emphasised the control of introduced pests through the introduction of coevolved natural enemies from the pest’s indigenous home (Huffaker, Messenger and DeBach, 1971), based on the assumption that coevolved natural enemies are best adapted to locating and successfully attacking the target host (Ngi-Song et al., 1999). Following this approach, Cotesia flavipes Cameron (Hymenoptera: Braconidae) was introduced into Africa for biological control of the exotic cereal stemborer, Chilo partellus Swinhoe (Lepidoptera: Crambidae).

Cotesia flavipes, a gregarious larval endoparasitoid of C. partellus and other stemborers in the Indo–Australian region (Kimani-Njogu and Overholt, 1997), was imported from The International Centre of Insect Physiology and Ecology (ICIPE), Kenya and released in Mozambique to increase natural suppression of C. partellus. ICIPE obtained C. flavipes from collections made in India and Pakistan. In some cases, releases were made into areas in which the native stemborer species as well as the exotic stemborer occur.

In laboratory studies, it was shown that C. flavipes was not highly host specific (Ngi-Song et al., 1995, Potting et al., 1993; Potting et al., 1997a, Overholt et al. 1997), and will attack not only the exotic target stemborer (C. partellus), but also several indigenous stemborers which reside in the same habitat, including the noctuids Busseola fusca Fuller and Sesamia calamistis Hampson (Lepidoptera: Noctuidae), and the pyralids Chilo orichalcociliellus Strand and Eldana saccharina Walker (Lepidoptera: Pyralidae).
Studies from Kenya (Ngi-Song et al., 1995) and Benin (Hailemichael et al., 1997), showed that although B. fusca was an attractive host for oviposition for C. flavipes, it was not suitable for development of the parasitoid, which led Overholt (1998) to speculate that the probability of establishment of C. flavipes would be low in areas where B. fusca, or other unsuitable hosts were present. However, Skoroszewski and van Hamburg (1987), working in South Africa, reported that B. fusca was a suitable host for C. flavipes, suggesting that various populations of B. fusca may have different immune responses to parasitoid attack.

In some areas of Mozambique, both C. partellus and B. fusca occur coincidentally in time and space (Cugala et al., 1999; Riedel, unpub. data). The likelihood of the C. flavipes establishment will depend on its ability to successfully parasitize both hosts. As C. partellus is an old association host of C. flavipes, a compatible host/parasitoid relationship can be assumed. However, there is no information on the suitability of the B. fusca population in Mozambique for the development of C. flavipes.

4.1.1 Specific objective

To evaluate the acceptability and suitability of the native and exotic African stemborers for oviposition and development of C. flavipes

4.2 MATERIALS AND METHODS

4.2.1 Stemborer larvae

C. partellus and B. fusca larvae were collected from small scale farmers' fields at Machipanda village (18° 52.16' S; 32° 47.96' E, elevation 800 m above sea level) in the central province of Manica. Small-sized larvae were collected from maize. The larvae were placed individually in glass vials, and provided with fresh cut maize as food. The larvae were identified and separated according to their respective species, and brought to the laboratory for mass rearing.
In the laboratory, the larvae were reared on the natural diet (fresh cut maize stems) temperature 28°C –30°C until they attained third instar or later. The food was changed every 3 to 4 days or whenever it was necessary. Two maize plots were planted as a source of food for the host larvae at the Experimental Field of the Faculty of Agronomy and Forest Engineering, at the University Eduardo Mondlane, in Maputo.

4.2.2 Parasitoids, *Cotesia flavipes*

*Cotesia flavipes* cocoon masses of (700 masses, ca., 21,000 individuals) were shipped to Mozambique from cultures maintained at ICIPE, Nairobi, Kenya. The cocoons were placed in glass cages for adult emergence. After emergence, adult parasitoids were provided a honey/water solution as food and set aside to mate for 24 hours.

4.2.3 Host acceptability

The experiment was conducted using the Randomised Complete Block design with two treatments (stemborer species) and three replications (days of exposure).

Acceptability of the two stemborer species for parasitization by *C. flavipes* was assessed by offering individual *C. partellus* or *B. fusca* larvae collected from the field to individual female parasitoids following the method described by Ngis-Song et al. (1995).

A one-day-old female parasitoid was randomly selected from the cage and introduced into a glass vial. The vial was then inverted and placed on a flat surface, and the female allowed climbing to the top (that is the bottom of the inverted vial). After the female reached the top, a randomly selected stemborer larva was introduced into the vial. Each female parasitoid was allowed 5 minutes in the vial to oviposit in the host larva. The parasitoid and the stemborer larva were maintained in the vial together until the parasitoid
female stung the larva, or for 5 minutes (Ngi-Song et al., 1995). An observation was completed when the female parasitoid stung the host larva or after 5 minutes.

A total of 85 C. partellus and 90 B. fusca larvae were exposed in 3 days. The exposures were made in 3 days because the adult parasitoids did not all emerge on the same day. Each female parasitoid and each stemborer larva were used only once. The number of stemborer larvae successfully parasitized and the time spent by each female to oviposit (in seconds) were recorded.

A total of 199 C. partellus and 248 B. fusca unexposed larvae were kept in the laboratory as a control to provide information on the levels of parasitism and/or mortality in the field. The larvae were reared until pupation, death for more than 25 days after oviposition experiment. The proportion of larvae that pupated, died or survived was estimated.

4.2.4 Host suitability

The stemborer larvae stung in the acceptability experiment were placed individually in their respective glass vials on natural diet and reared at ambient laboratory conditions (28°C – 30°C). The stemborer larvae were inspected every two days for mortality (during the first two weeks after parasitization) and daily for mortality and cocoon parasitoid emergence (from 16 days to 23 days).

The time of parasitoid development (in days) inside the host, the number of stemborer larvae dying, pupating, numbers of hosts that produced cocoons, proportion of hosts that did not produce cocoons and still alive at 23 days after parasitization, were recorded. Progeny produced and proportions of female progeny were also calculated.
4.2.5 Data analysis

The data were subjected to t-test (PROC TTEST) (SAS Institute, 1996) analysis to compare the time spent by a female parasitoid on the vial until oviposition on the two stemborer species. Parasitoid developmental time, percent of hosts producing cocoons, progeny produced per host larva and the proportion of females to males were subjected to t-test analysis. Developmental time and number of progeny produced were transformed to square root and proportion of females was transformed to arcsin square root before being subjected to t-test.

4.3 RESULTS

The two stemborer species, the exotic stemborer, C. partellus and the indigenous stemborer B. fusca exposed to C. flavipes for oviposition were accepted for oviposition and suitable for development of C. flavipes.

4.3.1 Host stemborer species acceptability

Table 4.1 shows the acceptability of C. partellus and B. fusca for C. flavipes oviposition. The two stemborer species were suitable for C. flavipes oviposition. However, C. partellus was significantly more acceptable than B. fusca (F=2.55; df=1, 173; P>0.0001). The time spent by each female parasitoid until oviposition varied according to stemborer species. It was significantly longer for B. fusca than for C. partellus (F=1.65; df=1, 173; P>0.0205). However, there were no significant differences on the proportion of hosts dying, pupating or surviving between the two stemborer species (Table 4.1).

Of the larvae parasitized by C. flavipes, 9.4% C. partellus and 16.7% B. fusca died of unknown causes before producing cocoons; 38.8% of C. partellus and 32.2% of B. fusca pupated, while 22.3% C. partellus and 32.2% B. fusca were alive with no sign of parasitism at the end of the experiment (23 days after oviposition).
Table 4.1. Means of hosts exposed to C. flavipes for oviposition (±SE)

<table>
<thead>
<tr>
<th>Host species</th>
<th>N</th>
<th>percent larvae attacked</th>
<th>time to oviposition (sec)</th>
<th>percent host dead</th>
<th>percent host pupated</th>
<th>percent host survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. partellus</td>
<td>85</td>
<td>95.7±0.17a</td>
<td>47.6±0.03b</td>
<td>9.4±0.30a</td>
<td>38.8±0.37a</td>
<td>22.3±0.25a</td>
</tr>
<tr>
<td>B. fusca</td>
<td>90</td>
<td>86.8±0.11b</td>
<td>57.7±0.06a</td>
<td>16.7±0.26a</td>
<td>44.4±0.48a</td>
<td>32.2±0.24a</td>
</tr>
</tbody>
</table>

Numbers followed by the same letter in a column are not significantly different (P>0.05); N = number of stemborer species exposed

The proportion of larvae that were not exposed and pupated, died or were alive at 25 days are shown in Figure 4.1. No parasitoids emerged from the larvae that were not exposed to C. flavipes.

![Figure 4.1 Proportion of host that pupated, survived and dead in the control](image)

4.3.2 Host stemborer species suitability

Stemborer larvae that produced parasitoid cocoons were considered as being suitable for parasitoid development. Both C. partellus and B. fusca collected in Machipanda and exposed to C. flavipes for oviposition were suitable for development of this parasitoid. The parasitoid emerged from both stemborer species. There was a significant difference between the two stemborer species on the proportion of hosts that produced cocoons. The number of C. partellus larvae that produced cocoons were significantly more than B. fusca larvae (F=3.78; df=1, 173; P>0.0000) (Table 4.2).
Table 4.2. Means host stemborer species suitability for the development of *C. flavipes* (±SE)

<table>
<thead>
<tr>
<th>Stemborer species</th>
<th>N</th>
<th>percent host producing cocoons</th>
<th>development time (days)</th>
<th>progeny produced</th>
<th>proportion females</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. partellus</em></td>
<td>85</td>
<td>29.4±0.05a</td>
<td>17.9±0.56a</td>
<td>26.8±0.17a</td>
<td>0.68±0.18a</td>
</tr>
<tr>
<td><em>B. fusca</em></td>
<td>90</td>
<td>6.7±0.03b</td>
<td>18.5±0.33a</td>
<td>7.2±0.18b</td>
<td>0.46±0.09a</td>
</tr>
</tbody>
</table>

Numbers followed by the same letter in a column are not significantly different (P>0.05). N = Number of stemborer species exposed

*C. partellus* larvae produced significantly more adult parasitoid progeny than *B. fusca* larvae (F=12.11; df=1, 29; P>0.0113). Although *C. partellus* produced more female parasitoids than *B. fusca*, there was no significant difference between the two stemborer species in the proportion of females (F=1.33; df=1, 29; P>0.648). The developmental time of *C. flavipes* (egg-adult emergence) did not differ from that of *B. fusca* (F=1.44; df=1, 29; P>0.732) (Table 4.2)

4.4 DISCUSSION

4.4.1 Host stemborer species acceptability

The indigenous African stemborer, *B. fusca*, and the exotic Asian stemborer, *C. partellus*, were both acceptable for *C. flavipes* oviposition with a large proportion of the larvae attacked. However, *C. partellus* was slightly more acceptable for oviposition than *B. fusca*, contradicting results from Ngi-Song *et al.* (1995) who found that both *C. partellus* and *B. fusca* were equally acceptable. The present results agree with Potting *et al.* (1993) and Potting *et al.*, (1997b), who reported that *C. flavipes* did attack all stemborer species feeding on the same host plant and, therefore, *C. flavipes* was not specific with regard to the host species it attacked on a particular plant species.

Potting *et al.* (1993) and Potting *et al.*, (1997b), did not find any differences in *C. flavipes* searching time in *C. partellus* and *B. fusca* infested maize plants.
Ngii-Song et al. (1995) reported that C. flaviges spent similar time to oviposit, 52.6 seconds on C. partellus and 45.2 seconds on B. fusca. However, in the present study, C. flaviges took significantly longer to oviposit on B. fusca (57.7 seconds) than on C. partellus (47.6 seconds). Ngii-Song et al. (1999) speculated that coevolved natural enemies were best adapted to successfully locating and attacking the target host.

4.4.2 Host stem boring species suitability

The levels of mortality of the control larvae were low. Parasitism of early instar larvae is typically low. Cotesia spp. attack only third and large instar larvae (Smith, Wiedmann and Overholt, 1993). Therefore, there was little possibility that the collected larvae were already parasitized before collection from the field. Mohyuddin and Greathead (1970), Kfir (1992) and Kfir and Bell (1993) reported that the most common indigenous larval parasitoid C. sesamiae attacks medium and large-sized stem boring larvae.

The physiological suitability of the stem boring species for the development of C. flaviges varied. The old parasitoid/host association with C. partellus was the most suitable. The new association of B. fusca and C. flaviges was partially suitable with 6.7% of larvae successfully parasitized. The noctuid, B. fusca is a new host for C. flaviges and it was shown that B. fusca was totally an unsuitable host for C. flaviges development in Kenya and in Benin (Ngii-Song et al., 1999; Hailemichael et al., 1997). Because there has been no evolutionary association between B. fusca and C. flaviges, the parasitoid has been under no selection pressure to develop mechanisms to evade the B. fusca immune system and parasitoid eggs and first instar larvae were encapsulated (Ngii-Song et al., 1995). However, at least some C. flaviges were inherently capable of evading the immune system and develop in B. fusca in Mozambique.

Ngii-Song et al. (1999), considered the physiological suitability of the host for development of parasitoids as an obstacle in new parasitoid/host relationships. Similarly, Gifford and Mann (1967), working in the United States
on the acceptability and suitability of several noctuid and crambid larvae for C. flavipes, found that parasitoid development was only completed in the crambid stemborers. The developmental time from egg to adult emergence was no different in the two stemborer species. Results from Ngi-Song et al. (1999) led to the same conclusion that C. flavipes developed slightly faster in C. partellus (coevolved or old association) than in B. fusca (non-coevolved or new association).

Ngi-Song et al. (1999) reported some new associations produced small or no broods due to encapsulation of eggs and/or first instar larvae of parasitoids by their hosts as compared to old associations. Equally, they observed higher C. flavipes larvae mortality in new association hosts and lower in old associations. In our study, the new association host, B. fusca, produced an average of only 7.2 adult C. flavipes while C. partellus produced 26.8 individuals. It is likely that many C. flavipes eggs and early instar larvae were encapsulated in B. fusca.

Despite the low numbers of C. flavipes cocoons observed in the new association, the proportion of females was not different in the two hosts. These results agree with those reported by Ngi-Song et al. (1995) who observed no differences on the proportion of female C. flavipes between new and old association hosts. Generally, these results support the contention of Wiedenmann and Smith (1993) that before field colonization, physiological compatibility between the target host and the novel association natural enemy needs to be evaluated. This will not only increase the understanding of the successes and failures in biological control, but also avoid wasted effort in rearing and releasing parasitoids incompatible with the target hosts.

4.5 CONCLUSIONS

The parasitoid C. flavipes was capable of developing in both the coevolved and non-coevolved stemborer species. However, successful parasitization was higher in the old host/parasitoid association. C. flavipes could extend its host range to include the indigenous species, B. fusca, a important African
stemborer in some ecological zones. *C. flavipes* will contribute to natural suppression of both exotic species *C. partellus* and the indigenous stemborer, *B. fusca* occurring in the same habitat. The ability of *C. flavipes* to oviposit and develop in *B. fusca* is an encouraging result as it opens opportunities for the regulation of indigenous pest species and may constitute an important means of increasing the natural suppression of indigenous stemborer populations in Mozambique.
CHAPTER FIVE

DISCUSSION

Three stemborer species associated with maize and sorghum were recorded from the areas of study confirming previous studies that reported the occurrence of *C. partellus*, *B. fusca* and *S. calamistis* in Mozambique (Gonçalves, 1970; 1972; Berger, 1981; Segeren et al., 1991; Davies et al., 1995; Sithole, 1988; 1989), often found in the same field and/or plant. The exotic stemborer *C. partellus*, was the most abundant at all study sites. (Overholt et al., 1994a; Kifir, 1998) reported that *C. partellus* is displacing indigenous stemborer species and that is is the most damaging species.

The three stemborer species have different host plant preferences. Some authors have reported that although *C. partellus* prefers sorghum as its host plant, it may also cause severe losses to maize (Sithole 1989; 1994; Ebenebe et al., 1999). In southern Mozambique, 100% infestation of plants by *C. partellus* has been observed. This observation agrees with Berger, (1981); Segeren et al., (1991); Cugala et al., (1999), who reported infestation levels of 100% in the southern region.

In the southern region (low altitude), the high stemborer infestation, may be due to continuous maize cultivation (Berger, 1981; Segeren et al., 1991; 1995). Stemborer density (larvae per plant) varied according to prevalent agroecological conditions at each study area and host plant. High stemborer density was observed on maize in the areas where *C. partellus* was the most abundant species. Similar observations have been made by Gonçalves (1970) and Segeren et al (1991). Three stemborer species recorded in the areas of study were *C. partellus*, *B. fusca* and *S. calamistis*. *C. partellus* was the most abundant species in low elevation areas whereas, *B. fusca* was observed occurring in medium to high elevations areas. Low densities of *S. calamistis* were found at all sites.
Previous studies reported that *C. partellus* was the most abundant species at low altitude areas (Segeren *et al.*, 1991; 1995; Berger, 1981; Sithole, 1988; 1989; 1994; Kfir, 1992). The findings of this study agree with Davies *et al.* (1995), Sithole (1988; 1989) that *B. fusca* could occur at medium to high elevation zones in Mozambique. *S. calamistis* occurred throughout all the study areas, but it was found in highest densities in areas where *C. partellus* was abundant. This distribution patterns confirms finding from other authors that *S. calamistis* was more often found in association with *C. partellus* than with *B. fusca* (Gonçalves, 1970; 1972; Overholt *et al.*, 1994b; Sithole, 1988; 1989). In this study, *S. calamistis* and *C. partellus* were often observed in the same plant, but *S. calamistis* was rarely found in the same plant with *B. fusca*.

Post-release surveys recovered *C. flavipes* from the 1996 and 1999 release sites. *C. flavipes* was reared from all the three stemborer species *C. partellus*, *B. fusca* and *S. calamistis* in both maize and sorghum. Most *C. flavipes* were recovered from *C. partellus* at all release sites, particularly from the lowland areas where maize is cultivated throughout the year. It was also observed that when the three stemborer species were found in the same plant, almost, *C. partellus* larvae were parasitized by *C. flavipes*, proably due to fact that *C. partellus* was the most abundant stemborer species at all study sites. The parasitoid is spreading and colonizing new areas, as it was recovered up to 14 km from the release sites in the Southern Mozambique. However, the rates of parasitism were generally low and it varied according to location and stemborer species.

The recovery of *C. flavipes* from *B. fusca* in the field as well as in the laboratory probably is probably clear evidence that this exotic parasitoid is able to evade the immune system of the native stemborer. Similar observations were made in South Africa where *C. flavipes* was reared from field collected *B. fusca* larvae (Skoroszewski and van Hamburg 1987) and in Uganda (Matamá-Kauma 1999). However, in Kenya, *B. fusca* was found to be unsuitable for the development of *C. flavipes* (Ngí-Song *et al.*, 1995) and there was a similar finding in Benin (Hailemichael *et al.*, 1997).
The laboratory study showed that most parasitoids did not emerge from *B. fusca* larvae indicating that *B. fusca* encapsulated most of the parasitoid eggs and/or first instar larvae. However, the ability of *C. flavipes* to emerge from *B. fusca* indicated that this parasitoid may be able to establish in areas where *B. fusca* is an abundant species. Moreover, the proportion of *B. fusca* successfully parasitized by *C. flavipes* may increase in the future due to natural selection.

It is believed that there are three main alternative hypotheses which could explain the variance in host-parasitoid compatibility (1) the immune system of southern African *B. fusca* populations may be sufficiently different from the Kenyan and Benin populations as to allow parasitoid development (2) the *B. fusca* larvae from which *C. flavipes* were recovered in the field may have previously been parasitized by other natural enemies and (3) the *C. flavipes* colony used in the laboratory studies in Kenya and the one that was released in Mozambique may have originated from different locations in the national home of *C. flavipes* in Asia.

The parasitoids that were used in the laboratory studies in Kenya (Ngi-Song et al., 1995) and used in the laboratory studies as well as released in the field in Mozambique originated from the same cultures at ICIPE, so the third hypothesis, that genetic differences between the parasitoids could explain the variance in the host-parasitoid compatibility is unlikely to be true.

The recovery of *C. flavipes* from *S. calamistis* did confirm the results from Ngi-Song et al. (1995) and field studies by Sallam et al. (1999) who reported that *S. calamistis* was a suitable host for *C. flavipes* development. However, *S. calamistis* and *C. flavipes* were found to be incompatible in Uganda (Mohyuddin, 1971) and in Mauritius (Rajabalee and Govendasamy, 1988). Thus the compatibility between *C. flavipes* and various stemborer species populations occurring in the target area should be investigated for before introducing classical biological control programs.
The recoveries of *C. flavipes* from the areas where it was released in 1996 and 1999 may indicate that the exotic parasitoid was able to adapt successfully to the field conditions and that it had become established in the southern and central regions in Mozambique.

Despite of differences in the stemborers species composition between the lowland and highland, *C. flavipes* was able to establish in both agro-ecological zones where *C. partellus* I is dominant as well as where *B. fusca* is abundant. However, Ngi-Song *et al.* (1995), Overholt (1998), speculated that *C. flavipes* would not be able to establish in areas of Africa where *B. fusca* is abundant.

As is evident from the levels of parasitism due to the exotic parasitoid in the farmers' field, *C. flavipes* does not yet appear to be an important mortality factor of stemborers. Based on the experience from Kenya (Overholt, 1998), it will probably take some years for parasitoid population to increase and produce a significant impact on the stemborer populations in Mozambique.

*Cotesia flavipes* and *C. sesamiae* both can oviposit and complete development in *C. partellus* and indigenous stemborers, *S. calamistis* and *B. fusca*. The indigenous parasitoid, *C. sesamiae* parasitised more *C. partellus* larvae than *C. flavipes* and the indigenous parasitoid produced relatively more progeny and females, compared to the exotic one. However, studies from Kenya by Omwega and Overholt (1997) and Sallam *et al.* (1999), reported that the exotic parasitoid, *C. flavipes* was superior on its coevolved host, *C. partellus*, compared to the indigenous, *C. sesamiae* on progeny produced and proportion of females.

Kfir (1992) argued that after the introduction of *C. partellus* into Africa, indigenous parasitoids expanded their host ranges to include the exotic pest. *C. flavipes* may have an advantage over *C. sesamiae* and could constitute an important mortality factor of stemborers due to the fact that *C. partellus* is the most abundant stemborer species at majority of locations.
CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Three stemborer species, *C. partellus*, *B. fusca* and *S. calamistis* occur at varying density and relative abundance in Maracuene, Moamba, Nhacoongo and Machipanda in Mozambique.

*Chilo partellus* was the most abundant stemborer species at all study sites. *B. fusca* was found at medium and high elevation areas while *S. calamistis* occurred found at all altitudes, but infestations are low.

*Cotesia flavipes*, released in Mozambique was able to adapt successfully to the field conditions in Mozambique. The recoveries of *C. flavipes* from the areas where it was released in 1996 and 1999 indicated that this exotic parasitoid had become established in the Southern and Central parts of Mozambique, and it is spreading and colonizing new areas where it was not released.

The exotic parasitoid will encounter and attack the three commonly found stemborer species and will oviposit and develop in all the three stemborer species, but *C. partellus* is the most preferred. *C. flavipes* was released and established in the areas where both *C. partellus* and *B. fusca* occur. Thus, stemborer species composition had no influence on parasitoid establishment. Over time, it is likely that *C. flavipes* will oviposit and develop on the indigenous stemborers.

The percent parasitism due to *C. flavipes* and parasitoid abundance are still low. *C. sesamia* was by far the most abundant parasitoid. Probably the *C. flavipes* population needs time to build up and spread from the release sites.
The abundance of *C. flavipes* and its impact on stemborer populations is likely to increase in the near future.

The information obtained from the current investigations may eventually help to explain the success or failure of the biological control programs in Mozambique and in the Southern Africa.

6.2 Recommendations

Continue monitoring stemborers and their parasitoids to evaluate host-parasitoid population dynamics and the spread of *C. flavipes* to new areas.

Evaluate the impact of exotic and indigenous natural enemies on the stemborer populations and yield losses.

Implement the natural enemies conservation measures to augment the impact of indigenous and exotic natural enemies on the stemborer populations.

Conduct more and detailed laboratory studies on the host-parasitoid compatibility and parasitoid behaviour.

Conduct studies to determine whether any non-target insects are attacked by *C. flavipes*. 
REFERENCES


CIBC (1968-72). Annual reports of the Commonwealth Institute of Biological Control, Farnham Royal, UK.


APPENDICES

Appendix A: General stemborer life cycle

Key:

a - eggs
b - larva
c - pupa
d - adult
Appendix B: Stemborer species and damage

Key:

C. partellus  B. fusca  S. calamistis

a - adult;  b - adult;  c - adult,
d - larva  e - larva  f - larva
 g-i - damages  g-i - damages  h-i - damages
Appendix C: General insect larval parasitoid life cycle

Key:

a – adult female laying eggs
b – parasitoid larvae inside host larva
c – parasitoid larvae emerging from host larva
d – adult parasitoid emerging from the cocoons
Appendix D: Number of stemborer larvae and *Cotesia spp.* parasitoids collected from Marracuene district, 1996 release site

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<th>Stemborer larvae per plant</th>
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<th><em>Sc</em> larvae</th>
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Key: *Cp* - *Chilo partellus*; *Sc* - *Sesamia calamistis*
Appendix E: Number of stemborer larvae and *Cotesia spp.* parasitoids collected from Moamba district, 1996 release site

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Key: Cp = *Chilo partellus*; Sc = *Sesamia calamistis*
Appendix F: Stemborers density and relative abundance (%) from two 1999 trial release places (±SE)

<table>
<thead>
<tr>
<th>Location/Crop</th>
<th>Percent Infestation</th>
<th>No. Larvae per plant</th>
<th>Stemborer species</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. partellus</td>
<td>B. fusca</td>
</tr>
<tr>
<td>Machipanda</td>
<td></td>
<td></td>
<td>S. calamistis</td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>41.9±6.6a</td>
<td>1.8±0.2a</td>
<td>163 (100)±3.4a</td>
<td>0.0</td>
</tr>
<tr>
<td>Sorghum</td>
<td>56.3±4.9a</td>
<td>1.6±0.3a</td>
<td>360 (78.6)±6.3a</td>
<td>18 (3.9)±1.1</td>
</tr>
<tr>
<td>Nhacoongo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>89.4±1.9a</td>
<td>3.6±0.3a</td>
<td>945 (96.0)±10.3a</td>
<td>.39 (4.0)±1.1</td>
</tr>
<tr>
<td>Sorghum</td>
<td>66.9±5.0b</td>
<td>3.0±0.2a</td>
<td>872 (95.2)±8.8a</td>
<td>44 (4.8)±1.3</td>
</tr>
</tbody>
</table>

Numbers followed by the same in a column are not significant (P>0.05)
Appendix G: Percent parasitism and progeny produced by *Cotesia* *spp.* from different stemborer species in different crops at the two locations of trial releases (±SE)

<table>
<thead>
<tr>
<th>Location/Crop</th>
<th>Parasitoid species</th>
<th>Mean percent parasitism</th>
<th>Mean progeny produced</th>
<th>Mean proportions of females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cp</td>
<td>Sc</td>
<td>Bl</td>
</tr>
<tr>
<td>Machipanda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td><em>C. sesamiae</em></td>
<td>10.4±2.6a</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td><em>C. flavipes</em></td>
<td>1.8±2.3a</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sorghum</td>
<td><em>C. sesamiae</em></td>
<td>10.0±2.2a</td>
<td>22.2±1.6</td>
<td>1.3±4.0a</td>
</tr>
<tr>
<td></td>
<td><em>C. flavipes</em></td>
<td>0.6±0.4b</td>
<td>0.0</td>
<td>2.5±1.5b</td>
</tr>
<tr>
<td>Nhacoongo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td><em>C. sesamiae</em></td>
<td>8.4±1.2a</td>
<td>12.8±4.6</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td><em>C. flavipes</em></td>
<td>1.2±0.3b</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sorghum</td>
<td><em>C. sesamiae</em></td>
<td>11.5±1.9a</td>
<td>18.2±8.3a</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td><em>C. flavipes</em></td>
<td>0.9±0.4b</td>
<td>2.3±6.3</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Key: - = species not found  
*Cp*—*Chilo partellus*  
*Sc*—*Sesamia calamistis*  
*Bl*—*Busseola fusca*

Numbers followed by the same in a column are not significant (P>0.05)