ASSESSMENT OF INFESTATION LEVELS OF *CHILO PARTELLUS* SWINHOE (LEPIDOPTERA: CRAMBIDAE) ON MAIZE AND THE IMPACT OF ITS PARASITOID *COTESIA FLAVIPES* CAMERON (HYMENOPTERA: BRACONIDAE) IN SINAZONGWE DISTRICT OF ZAMBIA.

BY

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements of the degree of Master of Science in Entomology.

DEPARTMENT OF BIOLOGICAL SCIENCES

THE UNIVERSITY OF ZAMBIA

LUSAKA

February 2007

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DECLARATION

I, Miyanda Nzala Moonga hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or any other university.

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APPROVAL

THIS DISSERTATION BY *MIYANDA NZALA MOONGA* ENTITLED: "*ASSESSMENT OF INFESTATION LEVELS OF CHILO PARTELLUS SWINHOE (LEPIDOPTERA: CRAMBIDAE) ON MAIZE AND THE IMPACT OF ITS PARASITOID COTESIA FLAVIPES CAMERON (HYMENOPTERA: BRACONIDAE) IN SINAZONGWE DISTRICT OF ZAMBIA.*" *IS APPROVED AS FULFILING THE PARTIAL REQUIREMENT FOR THE AWARD OF* THE DEGREE OF MASTER OF SCIENCE IN ENTOMOLOGY OF THE UNIVERSITY OF ZAMBIA.

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ABSTRACT

The spotted stem borer, *Chilo partellus* (Swinhoe) is one of the most destructive pests of maize in the warm, low altitude regions of Zambia. A study was carried out in Sinazongwe district, which is located at about 600 metres above sea level in the Southern Province to determine the incidence of *C. partellus,* damage to maize plants and the abundance of its natural enemy, *Cotesia flavipes*. This study was carried out during the winter cropping seasons of 2005 and 2006 in four locations where previous releases of *C. flavipes* had been made. The locations described by intensity of *C. flavipes* released were: locality 1; 500-1000 parasitoids, locality 2; 50,000 parasitoids, locality 3; 100,000 parasitoids and locality 4; non-release. These were classified as low, medium, high and non-release locations respectively. In each location, maize plants were sampled from sixteen farmers' fields at three different growth stages, namely; knee height, tasseling and cob maturity. Ten plants were also randomly collected from each farmer's fields by destructive sampling to assess severity of *C. partellus* damage.

Analysis of variance (ANOVA) was carried out to determine if there were any significant differences across the locations and between plant growth stages. The results showed significant differences ($F=7.74$; d.f.=3; $P<0.001$) in incidence of *C. partellus* across the locations. Similar significant differences were also obtained among the three plant growth stages ($F=63.59$; d.f.=2; $P<0.001$). In addition, significant differences were obtained in respect to the following damage variables; tunnel length ($F=12.04$; d.f.=3;

P<0.001), damage index (F=16.39; d.f.=3; P<0.001) and number of exit holes (F=100.83; d.f.=3; P<0.001) across the locations. Similarly, significant differences were also obtained at the three plant growth stages in tunnel length (F=115.54; d.f=2; P<0.001), damage index (F=12.04; d.f.=2; P<0.001) and number of exit holes (F=7.96; d.f.=2; P<0.001) . There were however, no significant differences in leaf damage when a *t*- test was carried out to compare damage at knee height and tasseling stages across all the locations. Similarly, there were no significant differences in the number of parasitized larvae across the locations and at the three maize growth stages. *C. partellus* constituted 94.5% and 95.6% of the total stem borer larvae collected from maize plants, and the remaining 5.9% and 4.4% were *Sesamia calamistis* in the 2005 and 2006 winter cropping seasons, respectively.

Results of the present study confirm that *C. partellus* is the dominant cereal stem borer in Sinazongwe district and clearly show that *C. flavipes* is fairly well established in this agroecosystem. The results indicate however, that at this level of establishment, *C. flavipes* did not adequately suppress stem borer populations possibly due to low parasitism levels at peak stem borer incidence. Follow up studies might be necessary to clarify this phenomenon.

DEDICATION

To my mother and father for their constant encouragement.

ACKNOWLEGEMENTS

I would like to express sincere gratitude to my supervisor, Dr. P. O.Y. Nkunika, for the encouragement during my research and for all the guidance. I also thank my co-supervisor, Dr. A. J. Sumani for offering me the support and guidance during the field and laboratory work. Special thanks go to the International Centre for Insect Physiology and Ecology (ICIPE) for providing the financial support for my research. I am equally thankful to Messrs. Chipabika G., Zimba. B. and Simfukwe, G., all of ZARI, Mount Makulu, Chilanga for the assistance during the field and laboratory work.

Thanks to Mr. G. Munakango, Agricultural Camp Officer of Sinazongwe district for the assistance during the fieldwork. My sincere gratitude to all the small-scale farmers of Sinazongwe district from whose fields, maize plants were destructively sampled.

I would also like to thank Mr. J. Chalila of the Geography Department, UNZA, for the assistance rendered with the GIS mapping and Mr. D. Simumba of the Biometrics Section, ZARI, for the advice on statistical analysis.

Lastly, but not the least am greatly indebted to all the members of staff in the Department of Biological Sciences at the University of Zambia for their contribution from the time I enrolled on this Programme to date.

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ACRONYMNS

CABI	Centre for Agriculture and Biosciences International
CSO	Central Statistical Office
GPS	Geographical Positioning System
HSD	Honestly Significant Difference test
ICIPE	International Centre for Insect Physiology and Ecology
IITA	International Institute of Tropical Agriculture
IPM	Integrated Pest Management
MACO	Ministry of Agriculture and Co-operatives
RPF	Resource Poor farmers
ZARI	Zambia Agricultural Research Institute
UNZA	University of Zambia

CHAPTER 1: INTRODUCTION

1.1 Background

Maize (*Zea mays* L.) is the most important cereal crop in Zambia because it is the staple food of almost the entire country of 10 million people (Mwale, 1993; CSO, 2003). Insect pests are an important threat to maize production in Africa. Major insect pests include stem and ear borers, armyworms, cutworms, termites, beetles (rootworms and white grubs), and virus vectors (aphids and leafhoppers). A wide range of pathogens, primarily fungi, also damages the maize plant (IITA, 2005).

Lepidopteran stem borers are generally considered to be the most damaging insect pests of maize in Africa (Bosque-Perez and Schulthess, 1998; Seshu Reddy, 1998). The stem borer pest species that are prevalent in all the three agro ecological zones of Zambia are *Busseola fusca* (Fuller), which is predominant in high altitude areas with high rainfall (over 1000 mm annual rainfall), while *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is abundant in the low altitude areas with dry conditions and *Sesamia calamistis* Hampson is prevalent in all areas but at low populations (Sohati *et al.*, 2001). *B. fusca* and *S. calamistis* are thought to be indigenous, while *C. partellus* is an introduced pest from Asia (Bleszynski, 1970; Overholt *et al.*, 1994a).

Of the various insects attacking maize and sorghum (*Sorghum bicolor* (L.) Moench) in southern Africa, the African maize stalk borer, *B. fusca* and the spotted stem borer, *C. partellus* are the most important (Skoroszewski and van Hamburg, 1987). *C. partellus* is also an important pest of other crops such as pearl millet (*Pennisetum glacum* (L.) R.

Br), rice (*Oryza sativa* L.), sugar cane (*Saccharum officinarum* L.) and finger millet (*Eleusine coracana* (L.) Gaertn) in Asia and Africa. Its wild grass hosts include Napier grass (*Pennisetum purpureum* K. Schumach), corn grass (*Rottboellia exaltata* (L.) L.f), guinea grass (*Panicum maximum* L.), common wild sorghum (*Sorghum arundinaceum* (Desv.) Stapf) and thatching grass (*Hyparrhenia rufa* (Nees) Stapf) (Uys, 2000).

Yield losses of maize due to stem borer damage in Zambia have been reported to range from 10 -100 % (Kuhlman and Dedert, 1987; Sithole, 1990). In Kenya, De Groote (2002) reported a crop loss of 12.9 % due to stem borer damage; amounting to 0.39 million tonnes of maize estimated at US $ 76 million. Sithole (1990) reported yield losses of more than 50 % as common in southern Mozambique and 20-30 % in Zimbabwe. Higher losses in resource poor farmers' fields ranging between 50 – 70 % have been recorded at research stations in the absence of chemical control. In neighbouring South Africa, yield losses from stem borer damage range from 10 % to 100 % (Matthee, 1974; van Rensburg and Bate, 1987).

There are six districts in Zambia namely, Sinazongwe, Livingstone, Sesheke, Luangwa, Serenje and Mkushi; where *C. partellus* has been reported to be of economic importance as a cereal stem borer (Sohati *et al.*, 2001). There are indications that the pest could be present in other districts.

Some of the *C. partellus* control methods include:

(i) Cultural control

Several cultural practices have been employed in *C. partellus* control mainly in disrupting or slowing down the population build-up. These include time of planting, spacing, intercropping, removal and destruction of volunteer and alternative host plants, and fertilizer application (Lawani, 1982; Warui and Kuria, 1983; Sétamou *et al.*, 1995; Seshu Reddy, 1998; Schulthess and Jiang, 2005; Wale *et al*, 2006).

(ii) Chemical control

Chemical application of Endosulfan, Cypermethrin and Malathion is effective when used early in the cropping season before caterpillars have penetrated deeply into the maize stems (Kuhlman and Dedert, 1987). However, control of maize and sorghum stem borers exclusively by insecticides by resource-poor small-scale farmers is uneconomical and not practical (Seshu Reddy, 1998).

(iii) Botanical control

Okech *et al.*, (1997) in a field trial found that *Tephrosia volgelii* Hook aqueous extract effectively reduced maize stalk borer (*C. partellus*) numbers and damage symptoms and consequently improved grain yield.

(iv) Host plant resistance

Different maize varieties are yet to be evaluated for resistance to *C. partellus* in Zambia (Sumani, personal communication, 2005). However, stem borer resistant maize varieties are being developed in Kenya and South Africa (Mugo *et al.*, 2005; Gouse *et al.*, 2006).

(v) Biological control

Biological control has a long history of use in pest management and has gained renewed interest because of problems encountered with the use of pesticides such as high costs, pollution of man and the environment, resistance and pest resurgence. The most prevalent use of this term is restricted to use of natural enemies to manage populations of pest organisms (Lewis *et al.*, 1997). In an effort to control the pest by biocontrol means in Zambia, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a gregarious exotic larval endoparasitoid was obtained from ICIPE, Nairobi and released in three pilot districts, namely; Sinazongwe, Sesheke and Luangwa between 1999 and 2004.

The releases were made annually in fields with visibly stem borer infested plants. The cocoons of *C. flavipes* were placed in glass vials tied to maize plants at tasseling stage. A wick of cotton wool soaked in 10% sugar solution was placed above the glass vials as food for the emerging parasitoids (Sohati *et al.*, 2001). *C. flavipes* was selected as the best candidate for introduction because of its history of success outside Africa and its importance as a parasitoid of stem borers in its aboriginal home (Overholt *et al.*, 1994a). However, despite the releases made from 1999-2004, no recoveries of *C. flavipes* were made from any of the three release locations two years after the first year of release (Sohati *et al.*, 2001). This may have been due to temporal failure of recognition of parasitoid establishment (Overholt, 1998). Another possible reason for non-recoveries could have been that the parasitoid may have been difficult to detect during the initial period following a colonization of *C. flavipes* (Alam *et al.,* 1971).

In this research, detailed studies were conducted in Sinazongwe district to;

(i) determine the incidence of *C. partellus* on maize crop, and;

(ii) assess the establishment and the impact (if any) of the released *C. flavipes* on *C. partellus* incidence during the cropping seasons of June to September, 2005 and August to November, 2006.

Sinazongwe district was chosen as the study area because of its location in Region I (less than 600 mm of rainfall per annum) of the agro ecological zones of Zambia where *C. partellus* is the predominant stem borer pest species (Sohati *et al.*, 2001).

1.2 Objectives of the study

The main objective of the study was to assess the incidence of *C. partellus* on maize and evaluate the impact of its parasitoid, *C. flavipes* in Sinazongwe district of Zambia.

1.3 Specific objectives

To assess

i. damage and incidence of *C. partellus* on maize across locations and at different maize growth stages;

ii. establishment of *C. flavipes* Cameron (Hymenoptera: Braconidae) and;

iii. parasitism levels of *C. flavipes* across locations and at different maize growth stages in Sinazongwe district.

1.4 Hypotheses

1. Parasitism levels of *C. flavipes* are directly related to release intensities.

2. Infestation and damage levels of *C. partellus* are the same across *C. flavipes* release locations and at different maize growth stages.

CHAPTER 2: LITERATURE REVIEW

The review of literature on pests of maize particularly *C. partellus* attempts to bring out the existing knowledge and identify gaps of this pest and its impact on maize production in Zambia.

2.1 Insect pests of maize in Zambia

Maize is an important crop in Zambia as it serves as the main source of carbohydrates (Mwale, 1993). Major constraints to increasing production of maize is competition from phytophagous insects among which lepidopterous stem borers are considered to be the most damaging (Nye, 1960, Kfir *et al.*, 2002). The important stem borers in Zambia include the spotted stem borer *C. partellus* (Swinhoe) (Lepidoptera: Crambidae), the African stalk borer *B. fusca* (Fuller) (Lepidoptera: Noctuidae) and the African pink stem borer *S. calamistis* Hampson (Lepidoptera: Noctuidae) (Sohati *et al.*, 2001). Other pests of minor importance are black cutworm *Agrotis segetum* (Denis and Schiffermüller) (Lepidoptera: Noctuidae), maize leafhopper *Cicadulina mbila* Naudé (Homoptera: Cicadellidae), darkling beetles *Gonocephalum* spp. (Coleoptera: Tenebrionidae), African black beetle *Heteronychus arator* (Fabricius) (Coleoptera: Scarabaeidae) and green corn aphid *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae) (CABI, 2003).

2.1.2 Lepidopteran Cereal stem borers

Lepidopteran cereal stem and cob borers are generally considered to be the most injurious insect pests of maize, sorghum, millet, rice and sugarcane in Sub-Saharan

Africa (Kfir *et al.*, 2002). Among the stem borers associated with cereal crops and sugarcane, two species are exotic to Africa and thus present traditional targets for classical biological control. These are *C. partellus* (Swinhoe), which attacks maize and sorghum in Eastern and Southern Africa and the sugarcane borer, *C. sacchariphagus* (Bojer) (Lepidoptera: Crambidae) which was recently confirmed to be present in Mozambique (Overholt *et al.*, 2003). *C. partellus* is however, the major stem borer in Zambia (Sohati *et al.*, 2001)

2.2 *Chilo partellus*

This section reviews aspects of *C. partellus*; taxonomy, distribution, lifecycle, feeding habits, damage caused to plants and control.

2.2.1 Taxonomy

C. partellus Swinhoe (Lepidoptera: Crambidae) is known by different names, including 'durra stalk borer', 'maize stalk borer' and 'pink stem borer', but 'spotted stem borer' is now generally more accepted as the common name of this species (Maes, 1997). This pest has often been incorrectly referred to as *C. zonellus* or in earlier literature, as *C. simplex* (Bleszynski, 1970). It is a lepidopteran insect that belongs to the Super family Pyraloidea, Family Crambidae and Subfamily Crambinae. The Pryraloidea larvae are distinguished from other lepidopteran larvae by the presence of large circles or ellipses of crochets on the abdominal prolegs (Maes, 1997). Adults are generally small moths with a wing span of about 1.9 – 2.8 cm and their narrow forewings have a straw to brownish gray colour. However, hindwings tend to have a pale white (Uys, 2000).

2.2.2 Distribution

The immigrant stem borer, *C. partellus,* has proven to be an excellent colonizer in many of the areas it has invaded, often becoming the predominant and most economically important stem borer species in maize and sorghum at elevations below 1800 m (Seshu Reddy, 1983). *C. partellus* occurs throughout the Indian subcontinent and throughout South-East Asia to Indonesia and Taiwan and Southern parts of Asia (Harris, 1989). It invaded Africa from Asia sometime before 1930 when it was first found in Malawi (Tams, 1932; Jepson 1954).

Since arriving in Africa, *C. partellus* has spread to all countries in East and Southern Africa, often becoming the most damaging stem borer of maize and sorghum, particularly in warmer lowland areas as its geographic distribution is thought to be dependent on elevation (Ingram, 1958; Nye, 1960; van Hamburg, 1979; Sithole, 1990; Chinwada *et al.*, 2001; Sohati *et al.*, 2001).

The pest is reported to be expanding to higher elevations in eastern and southern Africa (Sithole, 1990; Kfir, 1997; Overholt *et al.*, 2000; Zhou *et al.*, 2001b). Countries that have reported *C. partellus* as a pest include Kenya, Uganda, Rwanda, South Africa, Ethiopia, Eritrea, Sudan, Somalia, Botswana, Mozambique, Swaziland, Zambia, Zanzibar and Zimbabwe (Ingram, 1983; Harris, 1990; Haile and Hofsvang, 2001; Niyibigira *et al.,* 2001; Sohati *et al,* 2001).

2.2.3 Life cycle of *C. partellus*

Adults of *C. partellus* emerge from pupae in the late afternoon and early evening and are active at night. Females mate soon after emergence and lay eggs on two or three subsequent nights, laying batches of 10-80 overlapping eggs on the underside of leaves, mainly near the midribs. Adults live for about 2-5 days and do not normally disperse far from emergence sites. Eggs hatch in 4-8 days after being laid and young larvae ascend plants to enter the leaf whorls where they start to feed. The larval period lasts for an average of 28 days (Harris, 1989). In southern Africa, 5, 6 and 7 larval instars have been reported but five instars are common (Sithole, 1990). The larvae have a cream colour with a spotted appearance made by four longitudinal stripes along the body. The head capsule and prothoracic shield appear brown. The dorsal surface of the body has four reddish brown or purple longitudinal stripes (Isaac and Rao, 1941; Usua, 1987).

The life cycle is completed in 25-50 days when conditions are favourable, and five or more successive generations may develop during a single maize growing season (Harris, 1989). In cold and/or dry conditions, larvae may enter a resting stage (diapause) in stems, stubbles and other crop residues, where they spend up to six months before pupating when favourable conditions return during the next growing season. However, part of the stem borer population may remain active in wild grasses during dry seasons (Overholt, 1998).

The percentage of larvae surviving on a maize crop is higher when the infestation occurs early in the development stages than when infestation sets in from 35 days after emergence. Clearly, larval survivorship decreases with increases in the age of the host crop. There is also large variation in the duration of different instars, and researchers in the southern Africa subregion ascribe this to differences in the nutritive value of the different parts of the plant (Sithole, 1990).

In regions where there is a diversity of host plants and the climate is warm, *C. partellus* normally develops all-year round. In regions with long dry periods in winter or in summer, the borer enters into a resting period. For example, in India, *C. partellus* was reported to diapause in the dry season (Tams and Bowden, 1953) while populations without a resting period were reported from Uganda (Ingram, 1958). In Sinazongwe area, *C. partellus* has no resting period or no diapause has been observed (Sumani, personal communication, 2005).

2.2.4 Feeding and Damage

Leaf feeding is intense when infestation is made on young plants (Alghali, 1985). The young larvae, after feeding on the leaf lamina, move into the whorl where they cause extensive leaf-feeding injury and can attack the growing tips, causing a condition known as 'dead heart'. Older larvae burrow into the stem and cause extensive damage due to tunneling of internodes (Singh *et al.,* 1983; Kfir, 1992). The damage by tunneling is due to eating through the pith and vascular bundles that constitute the transport system for water and metabolites. Stem tunneling reduces plant vitality, the grain-filling process and promotes lodging of plants as they mature. Damage to the inflorescence often interferes with grain filling (Sithole, 1990). The economic threshold density in maize has been estimated at 2-5 larvae per maize plant or 40 % of plants showing visible damage in South Africa (Bate and van Rensburg, 1992). Peak larval populations occur between six to eight weeks after emergence during the normal rain season (Warui and Kuria, 1983).

Damage parameters such as the number of holes made by stem borers and tunnel length have been used by other workers (eg. Singh *et al.*, 1983; Warui and Kuria, 1983; Ampofo *et al.*, 1986; Haile and Hofsvang, 2001; Songa *et al.*, 2001). Tunnel length has shown to be a more precise and reliable method of yield loss assessment than insect numbers *per se* because by the time the plants are sampled many borers might have already reached adulthood and left the plant or killed by predators or parasitoids (Mgoo, 2005). Kumar (1988) found that there was a significant negative correlation between stem tunneling and yield for susceptible cultivars. It was also predicted that tunnel lengths greater than 20 cm would cause a 40 % reduction of potential yield under natural conditions (Songa *et al.*, 2001).

2.2.5 Control

Some of the control methods for the management of *C. partellus* include, cultural, host plant resistance, biological and chemical.

2.2.5.1 Cultural control

Resource poor farmers (RPF) in Africa rely on the following cultural techniques to manage *C. partellus* because of the difficulties associated with chemical control;

(i) Time of planting

Early planted maize was found to have a lower number of borers per plant (Warui and Kuria, 1983; Shanower *et al.*, 1991; Gounou *et al.*, 1993). This principle is based on the growing of the target crop when the pest is not present or planting at a time that coincides with when the pest is least abundant (Seshu Reddy, 1998).

(ii) Spacing

Increasing the spacing between adjacent plants decreases the chances of the migrating larvae coming into contact with neighbouring plants (Minga, 1990). This may in turn affect the relative rate of development of a plant and its pest population as well as the behaviour of the pest in searching for food or an oviposition site (Lawani, 1982).

(iii) Intercropping

Intercropping cereals with non-cereals has the potential of controlling *C. partellus* (Amoako-Atta and Omolo, 1983; Minga, 1990; Khan *et al.*, 1997). More recent studies however, suggested that intercropping host with non-host plant did not result in pest population decline (Getu *et al.*, 2002; Midega *et al.*, 2005).

A similar method known as the 'push-pull' strategy which involves the combined use of trap crops and repellant fodder plants of economic importance has been developed to reduce stem borer populations on maize. In this strategy, stem borers are repelled from the maize and simultaneously attracted to the trap crops such as Napier grass, Sudan grass, wild sorghum and molasses grass (Khan *et al.*, 2003).

(iv) Removal and destruction of volunteer and alternative host plants

Alternative host plants, such as weeds, and volunteer crop plants play an important role in the carry-over of pest populations from one season to another (van den Berg *et al.*, 1998). Hence, their destruction may often lead to reduced pest populations (Seshu Reddy, 1983).

(v) Removal of Crop residues

It was shown that slashing of maize stubbles destroyed 70 % of *C. partellus* populations and that ploughing and discing destroyed a further 24 % of the pest population in sorghum and 19 % in maize (Kfir, 1989; Kfir, 1990).

(vi) Fertilizer Application

The response by *C. partellus* to plants is generally influenced by the use of fertilizers (van den Berg *et al.*, 1998). Generally, plants that do not receive fertilizer are less preferred by *C. partellus* for oviposition (van den Berg and van Rensburg, 1991).

2.2.5.2 Host plant resistance

Although cultural control is highly recommended for small-scale farmers, it is rarely employed due to labour constraints and destruction of wild grasses which would have deleterious ecological consequences (Scheltes, 1978, Overholt *et al.*, 1994a). As a result, host plant resistance has been identified as a possibility to combat stem borers. Host plant resistance using both conventional and genetic engineered maize in particular *Bt* maize can provide good protection against a range of stem borers but plant breeders have not yet been able to select agronomically acceptable varieties with adequate levels of stemborer resistance (Leuschner *et al.,* 1985; Mugo *et al*., 2005; Gouse *et al*., 2006) and even if they did succeed, experience has shown that insect populations may evolve biotypes capable of attacking formerly resistant varieties and screening for resistance is still in its infancy (Roush and McKenzie, 1987; Sithole, 1990).

2.2.5.3 Biological control

Host plant resistance is a strategy that has not yet been exploited in Zambia and hence the use of biological control is highly recommended. Biological control using several parasitoids and predators are known to suppress the pest density (Teetes and Young, 1977). Methods involved in the biological control of exotic pests like *C. partellus* are augmentation and classical biological control. Augmentation involves actively producing and releasing natural enemies if they cannot persist in the agroecosystem (Debach, 1974; van den Bosch *et al*., 1982). However, this method is costly and mainly preferred for egg parasitoids, nematodes and pathogens. Classical biological control is one of the tools of integrated pest management (IPM) and involves the introduction of a natural enemy into a new area to control a co-evolved invasive species (Flint and van de Bosch, 1981). It is viewed as a potential strategy for stem borer population management particularly against *C. partellus,* because of its status as an introduced pest (David and Easwaramoorthy, 1990; Cugala and Omwega, 2001). Typically in this strategy, a natural enemy is released at a few locations in a new environment, and then relied upon to disperse throughout the environment and colonize other suitable habitats (Sallam *et al*., 2001). Generally, the aim of natural enemy introductions is to reduce the pest populations below economic threshold and maintain them there (Hassel and Waage, 1984).

Natural enemies of *C. partellus* that have been introduced in Zambia include *C. flavipes* and the pupal parasitoid *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae). The indigenous pupal parasitoid *Pediobius furvus* (Gahan) (Hymenoptera: Eulophidae) has been recovered from *C. partellus*, although at very low parasitism levels (Sumani, unpublished report). However, *C. flavipes* had been shown to be a promising biological control agent for *C. partellus* because of its high reproductive potential in comparison to its stem borer host (Skoroszewski and van Hamburg, 1987; Overholt *et al.,* 1994b, Overholt, 1998). Additionally, *C. flavipes* was selected as the best candidate because of its history of success outside Africa and its importance as a parasitoid of stem borers in its Indo-Asian home (Overholt *et al.*, 1994c). *C. flavipes* is an important larval parasitoid in its native home and contributes to the natural regulation of *Chilo* spp. (Neupane *et al.*, 1985; Srikanth *et al.*, 1999).

Some success in reducing *C. partellus* populations by *C. flavipes* has been reported in East Africa. One of these successes is the parasitism levels of up to 62.5 % that have been recorded in western Kenya and Tanzania (Omwega *et al.*, 1997). Furthermore, the mortality rates of *C. partellus* larvae due to *C. flavipes* were in the range of 1.5 – 34.8 % in Uganda (Matama-Kauma *et al.*, 2001; Rwomushana *et al.*, 2005). However, biological control has not been so successful in the southern African region. It was reported that parasitism levels in Mozambique did not exceed 2 % (Cugala and Omwega, 2001). This was attributed to the slow population build up of *C. flavipes* (Cugala *et al.*, 1999). It was reported that no success was achieved in South Africa as the parasitoid failed to establish due to its inability to adapt to the winter conditions

(Skoroszewski and van Hamburg, 1987). Within the same region, suppression of *C. partellus* populations by *C. flavipes* in Zambia has not been reported so far. Therefore, the present study seeks to provide findings on the regulation of *C. partellus* in Sinazongwe district which will be important for future biological control programmes of cereal stem borers.

2.2.5.4 Chemical control

Chemical control using endosulfan, carbaryl and phoskill is practically effective when applied early in the season before caterpillars have penetrated deeply (van den Berg and Nur, 1998). However, variation in time and levels of infestation of this pest between and within season may render chemical control strategies ineffective or uneconomical (Bate *et al.,* 1990; Kfir, 1992). In addition, overlapping generations that result in infestations throughout the season also renders chemical control unsatisfactory (Jotwani, 1983; Kfir, 1988; Kfir *et al.*, 1989). Generally, chemical control of *C. partellus* larvae is particularly difficult because the damage is inside the maize and sorghum stalks where they are protected from insecticides (Warui and Kuria, 1983).

2.3 Biology and Establishment of *Cotesia flavipes*

This section reviews aspects of *C. flavipes* biology relating to its importance as a biological control agent of *C. partellus.*

2.3.1 Biology

C. flavipes Cameron is a wasp belonging to family Braconidae, which is one of the largest families in the order Hymenoptera. It is one of the species from the subfamily

Microgastrinae known to parasitize cereal stem borers (Walker, 1994). It is a gregarious, larval endoparasitoid of pyralid and noctuid (Lepidoptera) stem borers (Nagarkatti and Nair, 1973; Beg and Inayatullah, 1980; Shami and Mohyuddin, 1987; Polaszek and Walker, 1991). It locates its hosts and deposits about 35-40 eggs in them (Potting *et al.*, 1997).

The egg/larval period lasts ten to fifteen days before emergence from the host. The last larval stage immediately spins cocoons and then pupates. The pupal period lasts about six days, after which adults emerge. The adults are small wasps approximately 3-4 mm in length, which live only a few days (Potting, 1996). The foraging strategy of *C. flavipes* involves entering the holes in the plant stem and searching for the larvae host in the tunnels (Mohyuddin and Inayatullah, 1981; Smith and Wiedenmann, 1997). Plant volatiles and host frass are considered important cues in host finding (van Leerdam *et al*, 1986).

2.3.1.2 Population studies on *C. partellus* and its evaluation of natural enemies

Population studies on *C. partellus* on maize as a model involve estimation of the population densities of the target pest and its natural enemies in relation to crop phenology throughout the season (Oloo, 1989). Phenological relationship of variation in stemborer incidence and damage to crop phenology is crucial in determining economic thresholds and devising management strategies of the pests (Seshu Reddy *et al.*, 1990; Kalule *et al.*, 1997).

To ensure reliable comparisons, it is necessary to quantify pest densities in areas both with and without the natural enemies (Huffaker *et al.,* 1962; Luck *et al.,* 1988). Six approaches for evaluating natural enemies were proposed by Luck *et al.,* 1988. These included: (a) introduction and augmentation, (b) use of cages and barriers, (c) removal of natural enemies, (d) prey enrichment, (e) direct observation, and (f) chemical evidence of natural enemy feeding. Successful control of pests by exotic parasitoid must be determined by quantitative evaluation both before and after the release (Debach *et al.,* 1976). However, the choice of approaches largely depends on the objectives, equipment and technical support available to the researcher (Luck *et al.*, 1988).

2.3.2 Establishment of *C. flavipes*

The ability of parasitoids to find mates, reproduce, disperse and locate hosts in the area of release is an essential attribute and good indicator for a successful establishment (Sallam *et al.*, 2001). Studies indicate that establishment of the species has varied from country to country and within country, suggesting that abiotic factors such as temperature and relative humidity may influence parasitoid performance (Kfir *et al.*, 2002; Getu *et al.,* 2004). In Kenya, *C. flavipes* was released during the long rainy season of 1993 at three locations in the coastal area, over a period of 6-8 weeks (Overholt *et al*, 1994a). Data from the 1996 long rains cropping season showed that number of recoveries increased dramatically (62 recoveries at 27 sampling sites). The increased recovery of stem borers' parasitized by *C. flavipes* three years after the release provided clear evidence that the exotic parasitoid had firmly established itself in the coastal area of Kenya (Overholt *et al*, 1997).

In similar studies, *C. flavipes* was introduced for the first time in southern Mozambique in November 1996 and additional releases were subsequently carried out in several other places in southern and central Mozambique between 1998 and 2000. The parasitoid was recovered from all localities sampled 1-3 years after its introduction, indicating that this exotic parasitoid had established itself in the southern and central regions of Mozambique (Cugala and Omwega, 2001).

Uniquely, *C. flavipes* was found to be widespread in Ethiopia during surveys conducted between 1999 and 2000, even though it had never been released in that country (Emana *et al.*, 2001). Its origin is thought to have been from Somalia where it was released in 1997 near the border with eastern Ethiopia (Getu *et al.,* 2003). Alternatively, the parasitoid could have moved into Ethiopia from Kenya or Uganda in the south where it was released (Overholt, 1998). *C. flavipes* has established itself in the northern area of Tanzania adjacent to southwestern Kenya where the parasitoid is already established (Omwega *et al.*, 1997). The parasitoid has established itself in eastern Uganda comparatively faster than in other places in East Africa (Overholt *et al.*, 1997).

In Zambia, a total of 1000 parasitoid cocoons were released in Sinazongwe district in 1999, while in the year 2000, 1500 cocoons were released in the same area (Sohati *et al,* 2001). Subsequent releases were made between 2002 and 2004 in Sinazongwe district (Sumani, unpublished report) (See App. A). Release of cocoons is considered to be a preferred method as it maximizes the effective life span of the adults in the field (Overholt *et al.*, 1997).

2.4 Justification of Study

To date no proper studies have been conducted to establish the fate of the parasitoid vis-à-vis the status of the stem borer population in the release locations. This study was conducted as a follow-up to the releases of *C. flavipes*, a natural enemy of C. *partellus* by authors who worked earlier in Sinazongwe district. Parameters collected in the present study will be relevant in determining the establishment and effectiveness of the previously released parasitoid. In addition, the data on incidence of the stem borer *populations will be useful for future studies.*

CHAPTER 3: MATERIALS AND METHODS

This chapter presents the geographic description of Sinazongwe district (the study area) in terms of location, size, and physical characteristics such as the relief, soils, climate and vegetation. A section dealing with aspects of the livelihood of the people of the area is included as well as the methods for data collection and analyses.

3.1 Geographical Description of Study Area

This section discusses some aspects of the general physical characteristics of the study area.

3.1.1 Location and size

Sinazongwe district lies at an altitude of about 600 m.a.s.l within the Zambezi Rift Valley Trough. The district is located between latitude 17°09.591′ S and longitude of 27°21.637′ E (see Fig. 3.1) and covers an area of 5,195km² with approximately 13,387 households (Dalal-Clayton *et al.*, 1985; CSO, 2003).

3.1.2 Relief and soils

The Rift Valley Trough, where Sinazongwe district is located, comprises of isolated large hills, ridges, together with some flat plateau areas, dambos, foot slopes and a series of narrow valleys descending to a river terrace that gives way to the river flood plain which presently lies under Lake Kariba. Relief varies between 200-250 m in hills (Dalal-Clayton *et al.*, 1985). Davison (1981) describes this unit as being characterized by many streams and by shallow stony

(Contd. p. 23)

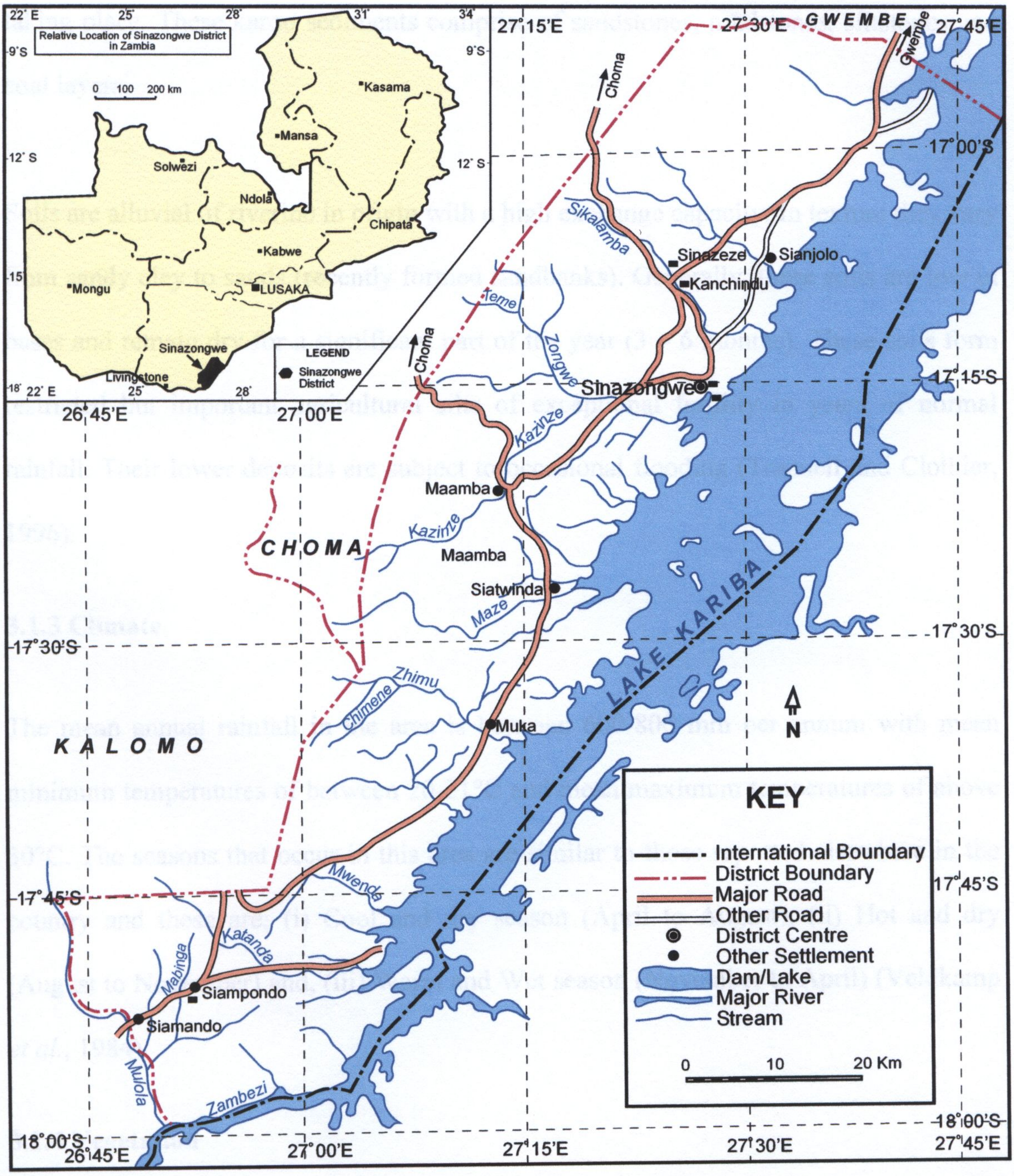

Fig 3.1: General Map of Sinazongwe district

(Source: Geography Department, UNZA)

or rocky soils, and represents areas where active erosion of karoo sediments is still taking place. These karoo sediments comprise of sandstones, mudstones, siltstones and coal layers.

Soils are alluvial of riverine in origin with a high exchange capacity. In texture they vary from sandy clay to sands (recently formed sandbanks). Generally, these soils are low in bases and remain dry for a significant part of the year (3 – 6 months). These soils form restricted but important agricultural silts of exceptional fertility in years of normal rainfall. Their lower deposits are subject to occasional flooding (Trapnell and Clothier, 1996).

3.1.3 Climate

The mean annual rainfall in the area is between 600-800 mm per annum with mean minimum temperatures of between 20-21°C and mean maximum temperatures of above 30°C. The seasons that occur in this area are similar to those reported elsewhere in the country and these are; (i) Cool and dry season (April to August), (ii) Hot and dry (August to November) and, (iii) Warm and Wet season (November to April) (Veldkamp *et al.*, 1984).

3.1.4 Vegetation

The Munga woodland, which is the dominant woodland is an open, park-like 1- storeyed deciduous woodland characterized particularly by species of *Acacia, Combretum* as well as *Adansonia digitata* and the baobab tree. This woodland, like other vegetation types follows a sequence from woodland to shrub to savanna to grassland. Small trees and shrubs are frequent especially where the canopy is open. There appears to be no secondary munga woodland recognized as such, probably because munga is an invasive type (Fanshawe, 1971).

This striking vegetation gives way to stretches of mopane (*Colophospermum mopane*) with a sparse ground cover of ephemeral grasses. The mopane has the appearance of an invader, which occupies unstable and actively eroding soils, some perhaps covered with thicket growth (Trapnell and Clothier, 1996).

Flooding of the alluvial soils and on the flats, assisted by the ravages of fire, leads to purity of stand. When flooding is very slight and very temporary, or even absent in some years, termite mounds are usually absent and shrubs not adapted to flooding are scattered throughout the woodland. Where flooding increases in depth and duration, termite mounds are present and the shrubs, which are not adapted to flooding retreat to the mounds, which may be low or high (Fanshawe, 1971).

3.2 Livelihood of the People in the Area

The main occupation of the people in the area is subsistence farming. Maize is the main staple food crop grown by these RPF farmers. It is grown mainly in the rainy season as well as in the dry season using irrigation from streams and dambos. Relish crops grown include cowpeas, groundnuts, okra and pumpkins. Cattle, goats and chickens are important livestock species kept by farmers in this area (Davies, 1971).

3.3 Study Design

Preliminarily studies were conducted in the 2005 winter cropping season to determine establishment of the parasitoid and stem borer species composition in the area. However, the present study was conducted on farmers' maize fields in four locations to assess incidence of *C. partellus* and abundance of its parasitoid, *C. flavipes* in relation to crop phenology. The locations were categorized on the basis of previous *C. flavipes* releases as follows: locality 1; 500-1000 parasitoids (low intensity-LR), locality 2; 50,000 parasitoids (medium intensity-MR), locality 3; 100,000 parasitoids (high intensity-HR) and locality 4; non-release (NR) location (area where the parasitoid had not been previously released) (Sohati *et al.*, 2001; Sumani, unpublished). The distance between the NR and MR locations was approximately 28 km and that between the NR and LR locations was about 40 km. The four locations (LR, MR, HR & NR) were at elevations of 482, 622, 480 and 482 metres above sea level, respectively.

Four farmers' fields with maize crop of the same variety (var. Omatamba) at three growth stages, knee height, tasseling and maturity were randomly selected from each locality. A total of forty four fields, instead of the selected forty eight were sampled across the four localities because four fields at maturity stage in the MR location had been sprayed with pesticides. To be able to relate to the sites of previous *C. flavipes* releases described above, GPS (Geographic Positioning System) instrument was used and recorded the exact location of each farmer's field before sampling.

3.4 Field sampling

The sampling unit was a standing maize plant. Assessment of stem borer incidence, damage on plants, maize yield and level of *C. flavipes* parasitism are described in the following subsections.

3.4.1 Stem borer incidence

To determine the incidence of stem borers, a destructive sampling method was used. In each farmer's field, ten maize plants were randomly sampled. The plant stems were split open using a knife from the base to the apical end in order to expose the stem borer larvae. Altogether, 440 maize stems were dissected in the same manner. The collected larvae were identified on the basis of body pigmentation and abdominal crochets to species level (Meijerman and Ulenberg, 1998).

3.4.2 Stem borer damage

Severity of leaf damage on maize plants at knee height and tasseling stages in the field was assessed before the destructive sampling. This was done by scoring damage on leaves using a scale of 1-5 on ten randomly selected plants based on the amount of feeding on the four uppermost leaves (1= 1-20%, 2=21-40 %, 3= 41-60%, 4=61-80%, 5= over 81%) (see App. C). This rating scale was adopted from work reported by Kalule *et al.*, (1997) and Singh *et al.*, (1983).

The extent of stem borer damage on maize stems was assessed by measuring lengths of the tunnels using a 30 cm rule on the interior of the dissected plants. Prior to splitting the stem, the number of holes created by stem borer feeding on all the maize stalks were counted and recorded. In addition, the height on all plant stems was also measured and recorded. A damage index determined as the ratio of tunnel length to plant stem height was established. Data were recorded on data sheets pre-designed for each field (see App. D).

3.4.3 Rearing of stem borer in the laboratory for assessment of *C. flavipes* abundance

Stem borer larvae collected during field sampling were kept in glass vials corked with cotton wool and later brought to the Mount Makulu laboratory for rearing. Larvae collected from the different localities were separated and individually reared in glass vials at the laboratory at temperatures of 25 – 30 °C and relative humidity of 60 –70 %. Fresh maize stems provided a natural diet (see Plate 3.1). Sections of maize cuttings used as feed were changed every two days until the larvae pupated or parasitoids emerged.

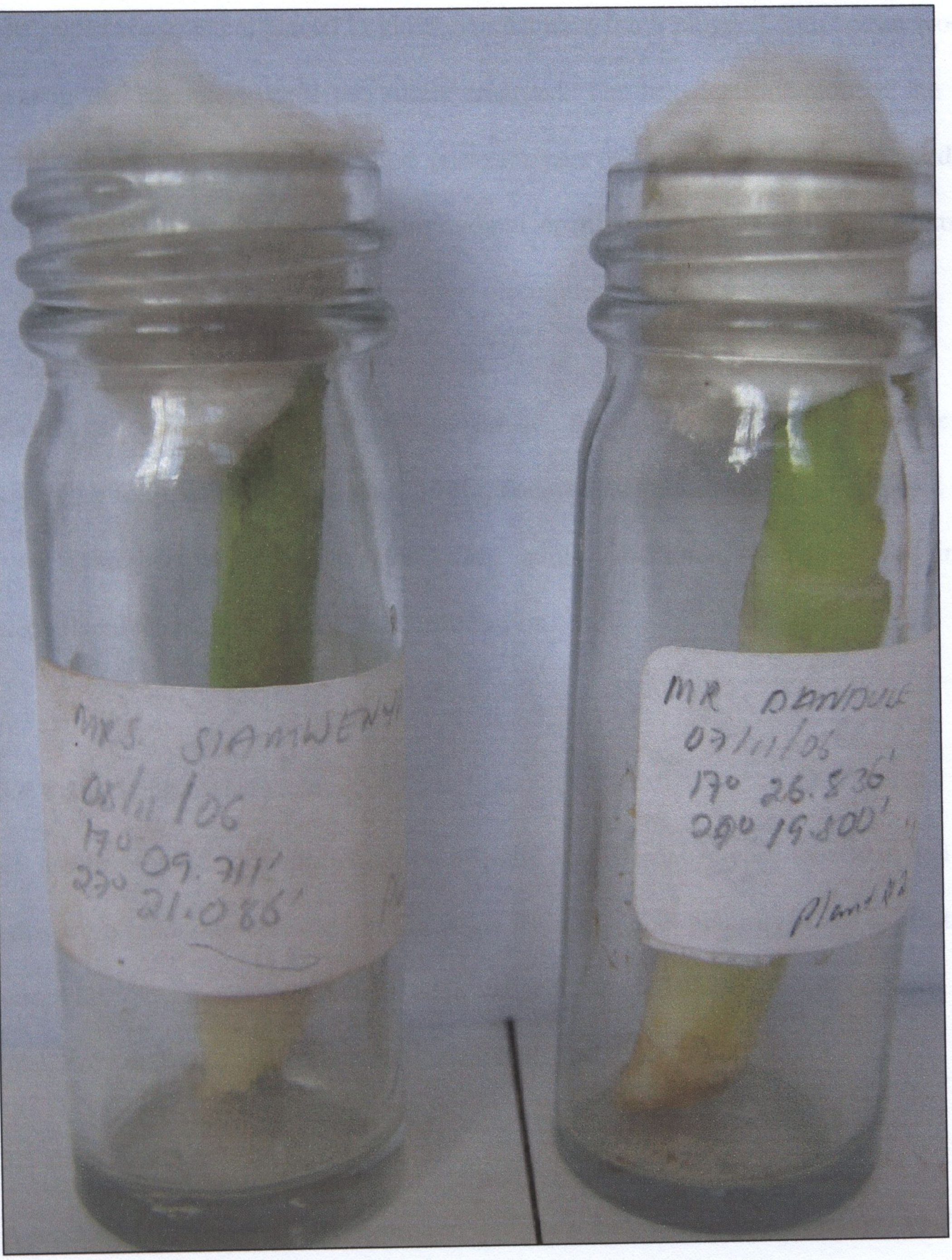

Plate 3.1: Rearing of stem borers in the laboratory: Glass vials containing a 3 – 5 cm section of green maize stem as food source for the larvae.

The pupae were sexed, placed in glass vials without food, plugged with cotton wool and kept in the laboratory until the adults emerged. The sex determination of the pupae involved examination of the ventral posterior part of the pupal abdomen according to Bleszynski (1970). Males have longitudinal openings on segment ix and segment x. Females on the other hand, have openings on segment viii and segment x.

Soon after emergence from the host larvae, the adult parasitoids were identified according to Polaszek and Walker (1991), counted and sexed using the antennal length (van Achterberg and Walker, 1998). The adult parasitoids were preserved in 70% alcohol and later sent to ICIPE for confirmation.

3.4.4 Assessing maize yield

To assess crop performance in terms of yield in different release locations, ten maize cobs were randomly collected in the fields from plants at maturity stage by destructive sampling. The maize cobs were later weighed on a balance (Globe Brand) in the laboratory to obtain the wet weight for yield analysis.

3.5 Data Analysis

A randomized block design was used in this study and means were separated by Tukey's studentised range test (HSD) (JMP, 2003). Insect counts were subjected to log (x+1) transformations before statistical analysis. This method normalizes data particularly when recorded values are small numbers including zero (Zar, 1984; Southwood, 1996), as was the case in this study.

i. Mortality due to parasitoids was obtained by the total number of parasitized larvae divided by the total number of susceptible larvae in farmers' fields where *C. flavipes* was previously released (Skovgård and Päts, 1996).

ii. The average leaf damage scores, incidence of stem borers, tunnel length, damage index, number of holes and cob weight were summarized in the form of graphs using Microsoft Excel®.

iii. A *t*- test using JMP™ was carried out to compare leaf damage between the knee height and tasseling stages.

iv. Analysis of Variance (ANOVA) using JMP™ was carried out to determine if there were significant differences in (a) maize yield (b) leaf damage (c) number of stem borers (d) tunnel length (e) damage index (f) number of parasitized larvae and (g) number of holes caused by stem borers across the four release locations.

v. Analysis of Variance (ANOVA) using JMP™ was carried out to determine if there were significant differences in (a) maize yield (b) leaf damage (c) number of stem borers (d) tunnel length (e) damage index (f) number of parasitized larvae and (g) number of holes caused by stem borers among the three different growth stages, that were, at knee height, tasseling and maturity.

vi. Correlation analysis was carried out to determine the relationship between maize cob weight and presence of *C. partellus* using JMP™.

CHAPTER 4: RESULTS

4.1 Stem borer Species Composition

A total of 359 and 1003 *C. partellus* larvae were collected during the 2005 and 2006 winter cropping seasons, respectively (App. E & F). It was the dominant stem borer species accounting for 94.5% and 95.6% during the 2005 and 2006 study periods, respectively. *S. calamistis,* the only other stem borer species recorded accounted for 5.9% and 4.4% during the two seasons. Larval densities were 1.2 – 2.3 and 0.25 - 0.07 larvae per plant for *C. partellus* and *S. calamistis,* respectively.

4.1.1 Incidence of *C. partellus* larvae

Numbers of *C. partellus* were highest in the high release (HR) location than in the other locations (Fig 4.1.1). *C. partellus* ranged from 1.2 – 2.3 larvae per plant across the locations with the non-release (NR) location having had the lowest. There were significant differences in the mean number of *C. partellus* larvae across the four locations sampled ($F=7.74$; d.f.=3; $P<0.001$), although incidences from the low (LR) and medium release (MR) locations were statistically similar (Fig. 4.1.1).

Numbers of *C. partellus* larvae at knee height stage were highest across locations when compared to the two other plant growth stages (Fig. 4.1.2) and the differences were significant ($F=63.59$; d.f.=2; $P<0.001$). The numbers of larvae at tasseling and maturity stages across release locations were not statistically different.

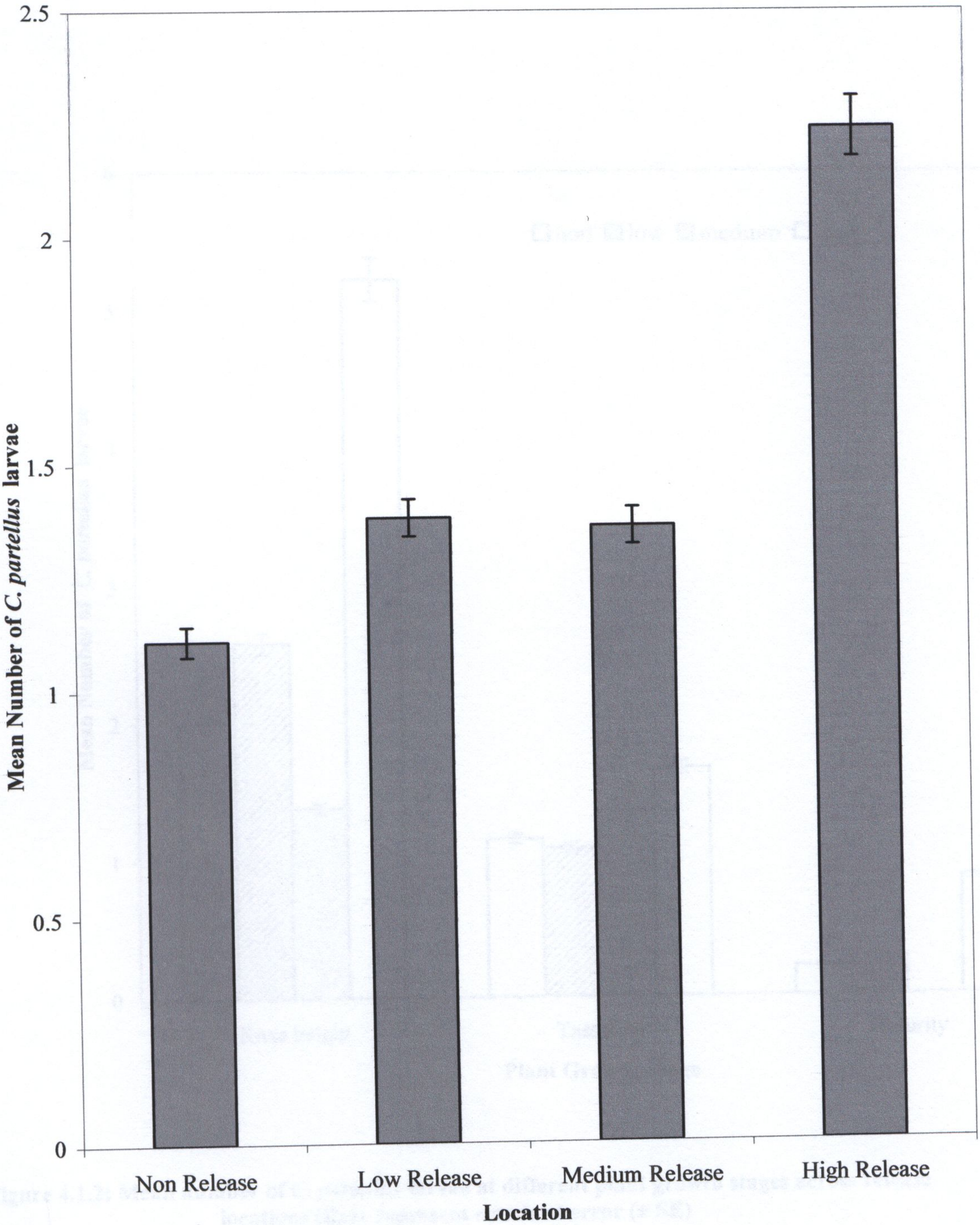

Figure 4.1.1: Mean number of *C. partellus* larvae across release locations (Bars represent standard error (± SE)

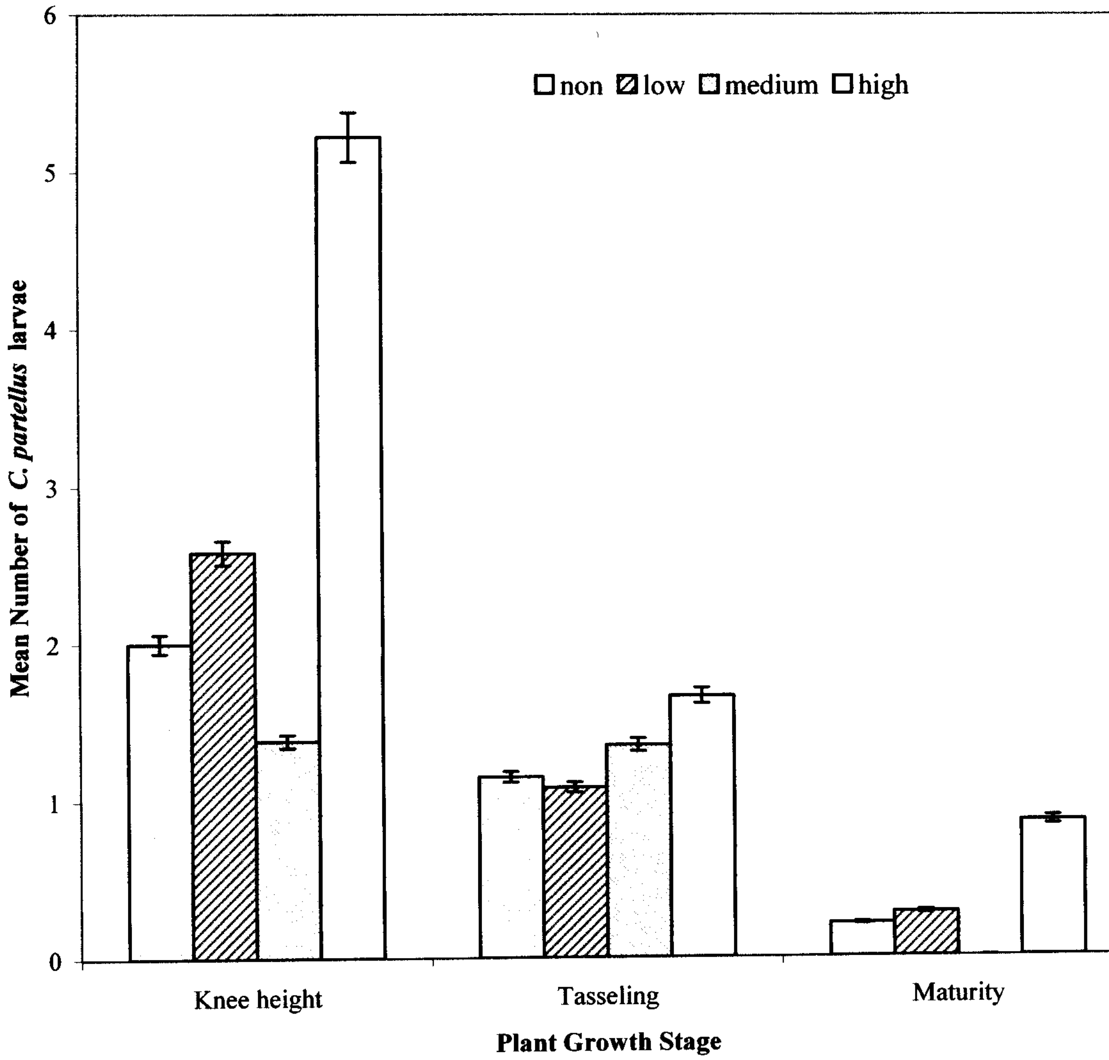

Figure 4.1.2: Mean number of *C. partellus* larvae at different plant growth stages across release locations (Bars represent standard error (± SE)

4.2 Plant Damage

The stem borer damage observed in the farmers' fields ranged from slight leaf injury (in the form of pin-holes) to 'dead-heart' conditions. The leaf injury shown was as a result of first instar larval feeding on the leaves before they tunneled into the stem. The 'dead-heart' condition resulted from the destruction of the growing point in the maize plant as the larvae fed in the stem (see Plate 4.2.1 and Plate 4.2.2).

Plate 4.2.1: 'Pin holes' caused by *C. partellus* on maize plant (see arrow).

Plate 4.2.2: 'Dead-heart' condition caused by *C. partellus* on maize plant (see arrow).

4.2.1 Leaf damage scores

Mean leaf damage scores were relatively low with a range of between 1.09 – 1.17 across the locations. The highest scores were obtained in the NR location while the lowest were from the LR location. The differences between the two locations was however, not statistically different (Fig. 4.2.1).Similarly, the differences between NR, LR and the other two locations were not significant (F=0.92; d.f.=3; P=0.43).

In addition, leaf damage scores were similar between the two growth stages, although scores obtained at the tasseling stage were slightly lower compared to the knee height stage (Fig.4.2.2). Generally, differences between the two maize plant growth stages were not significant (t=1.69; d.f.=383; P=0.08).

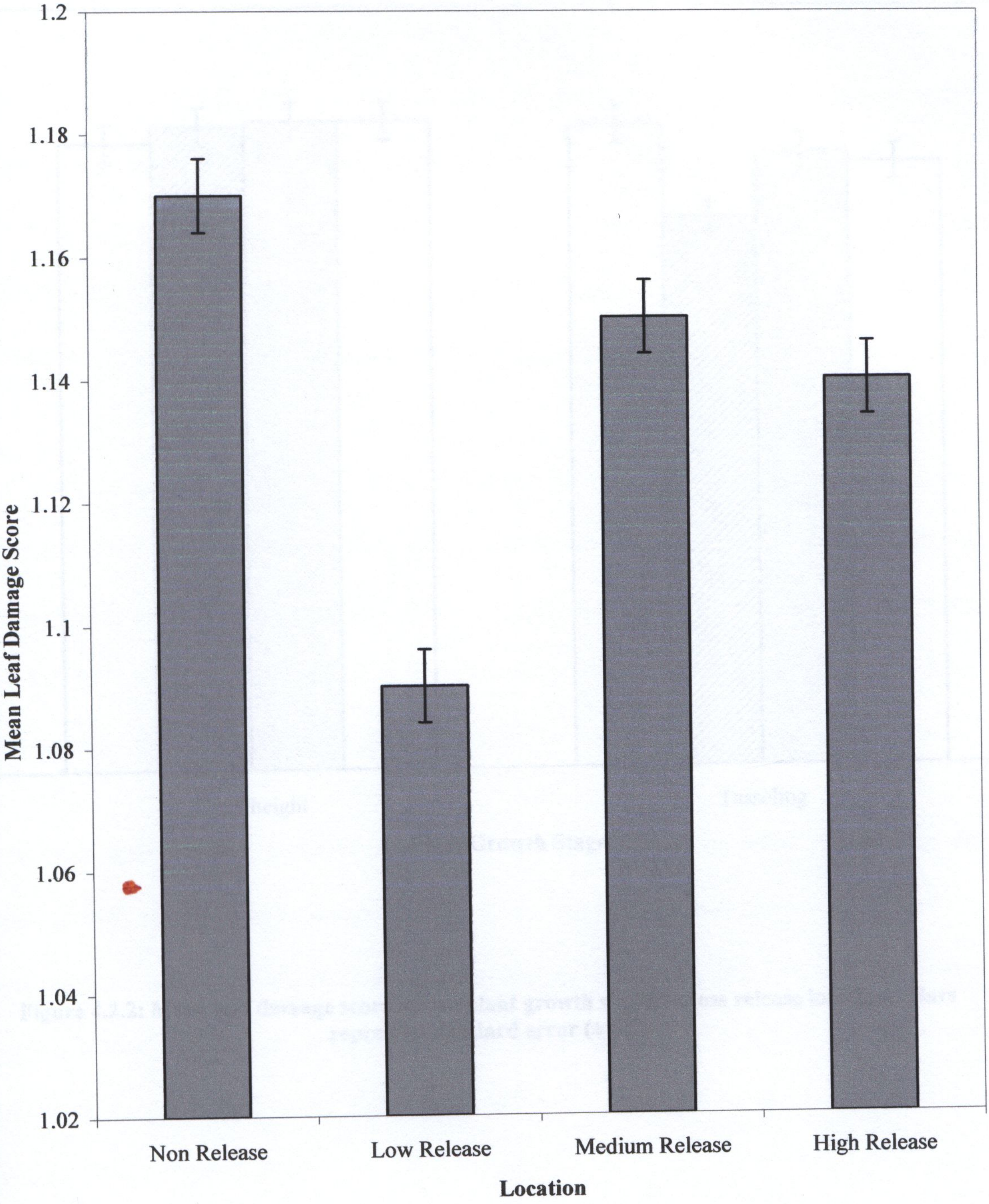

Figure 4.2.1: Mean leaf damage score across release locations (Bars represent standard error (± SE)

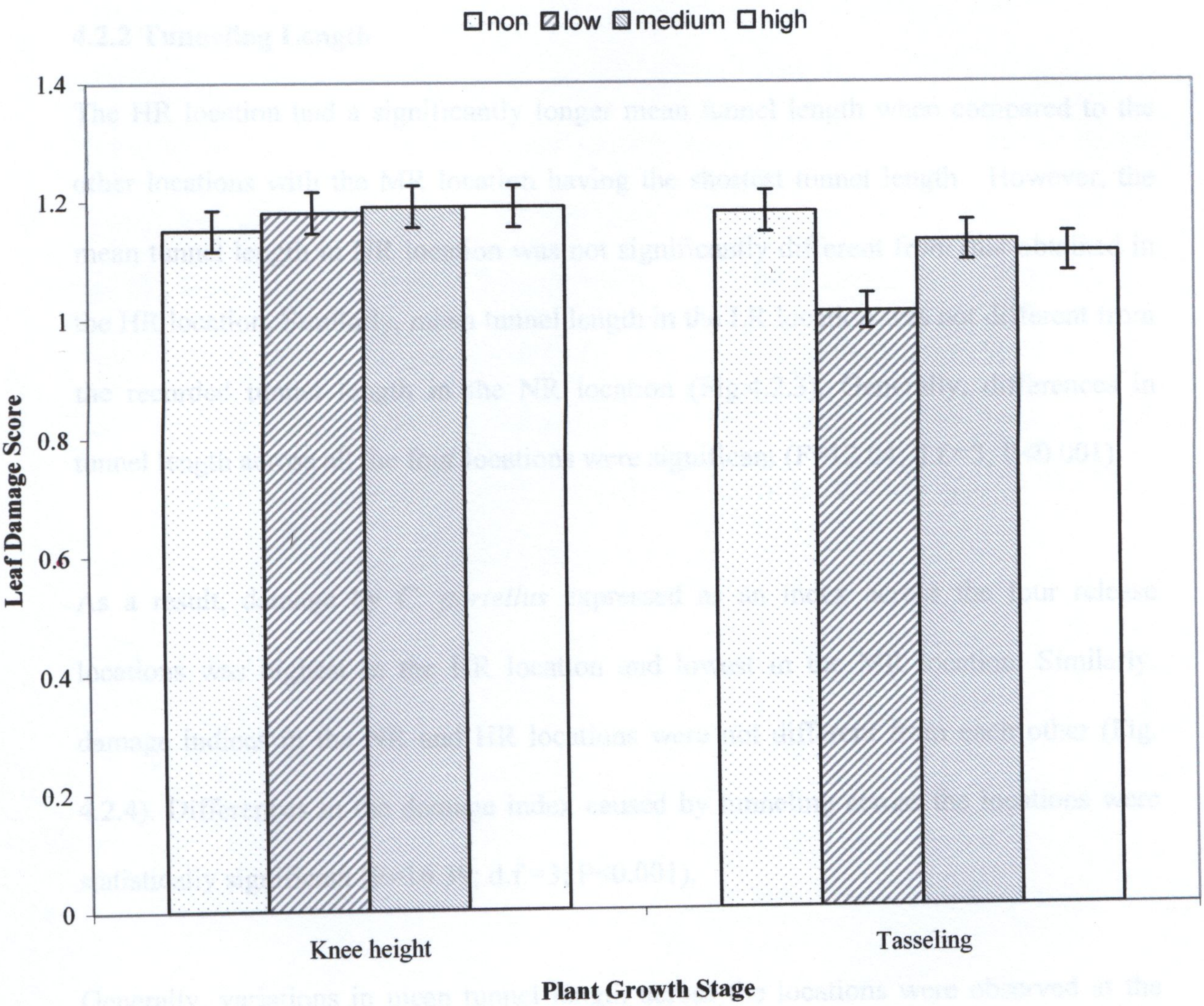

Figure 4.2.2: Mean leaf damage score at two plant growth stages across release locations (Bars represent standard error (± SE)

4.2.2 Tunneling Length

The HR location had a significantly longer mean tunnel length when compared to the other locations with the MR location having the shortest tunnel length. However, the mean tunnel length in NR location was not significantly different from that obtained in the HR location. Similarly, mean tunnel length in the LR location was not different from the recorded tunnel length in the NR location (Fig.4.2.3). Generally, differences in tunnel length across all the four locations were significant ($F=12.04$; d.f.=3; $P<0.001$).

As a result, damage by *C. partellus* expressed as an index across the four release locations was highest in the HR location and lowest in the MR location. Similarly, damage indices in the NR and HR locations were not different from each other (Fig. 4.2.4). Differences in the damage index caused by tunneling across the locations were statistically significant ($F=16.39$; d.f.=3; $P<0.001$).

Generally, variations in mean tunnel length across the locations were observed at the three growth stages. Plants at the maturity stage had the longest mean tunnel length as compared to the knee height and tasseling stages. However, the HR location had a slightly longer mean tunnel length at each plant growth stage (Fig. 4.2.5). Differences in the mean tunnel length were statistically different at the three plant growth stages ($F=115.54$; d.f.=2; $P<0.001$).

Since more damage by *C. partellus* was caused at the maturity stage, it consequently resulted in a higher damage index at this stage. However, differences across the locations were observed at each plant growth stage. The HR location had a higher damage index compared to the other three locations at the knee height stage. Similarly, the HR location had a higher damage index at tasseling stage, although it was not statistically different from the NR and LR locations. The damage index at the maturity stage was also highest in the HR location and this was not statistically different from the NR (Table 4.2.1), although significant differences were obtained among the three growth plant stages ($F=12.10$; $d.f.=2$; $P<0.001$).

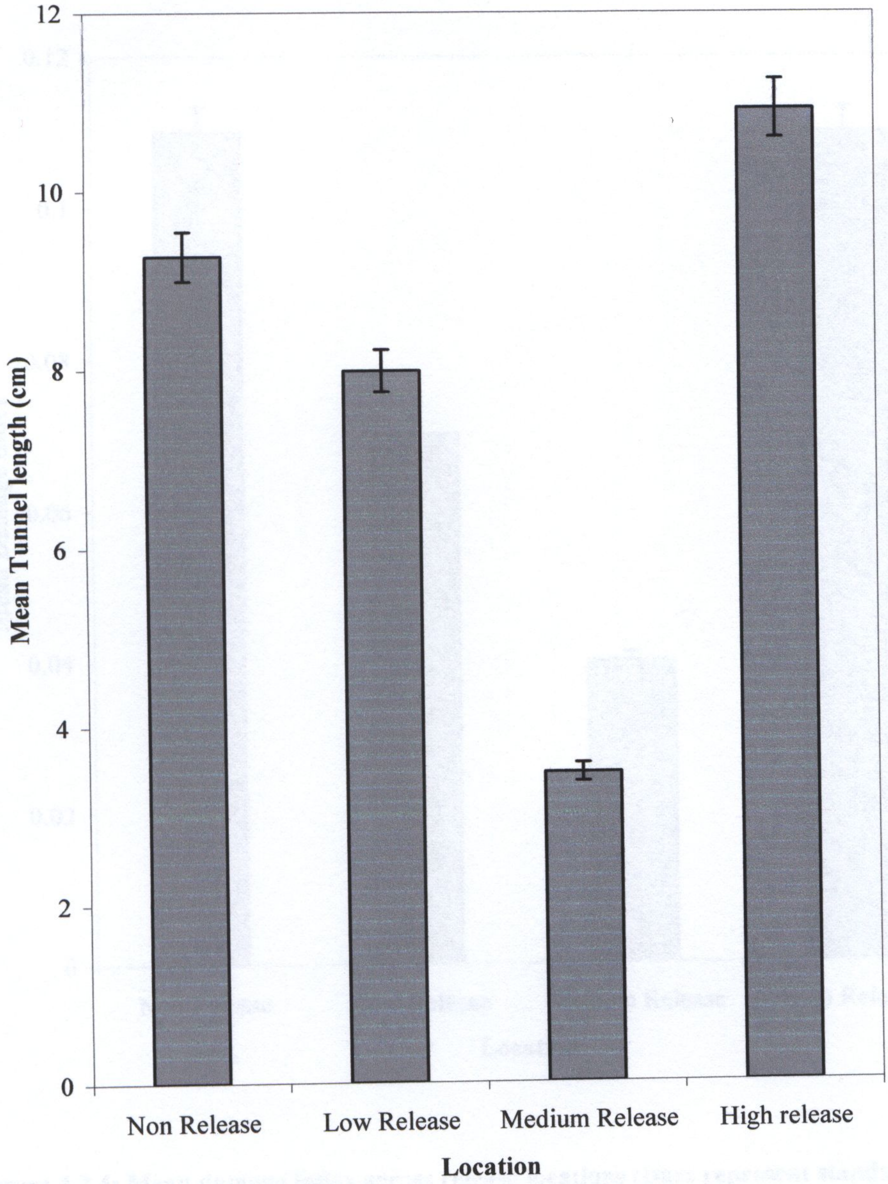

Figure 4.2.3: Mean tunnel length (cm) across release locations (Bars represent standard error (± SE)

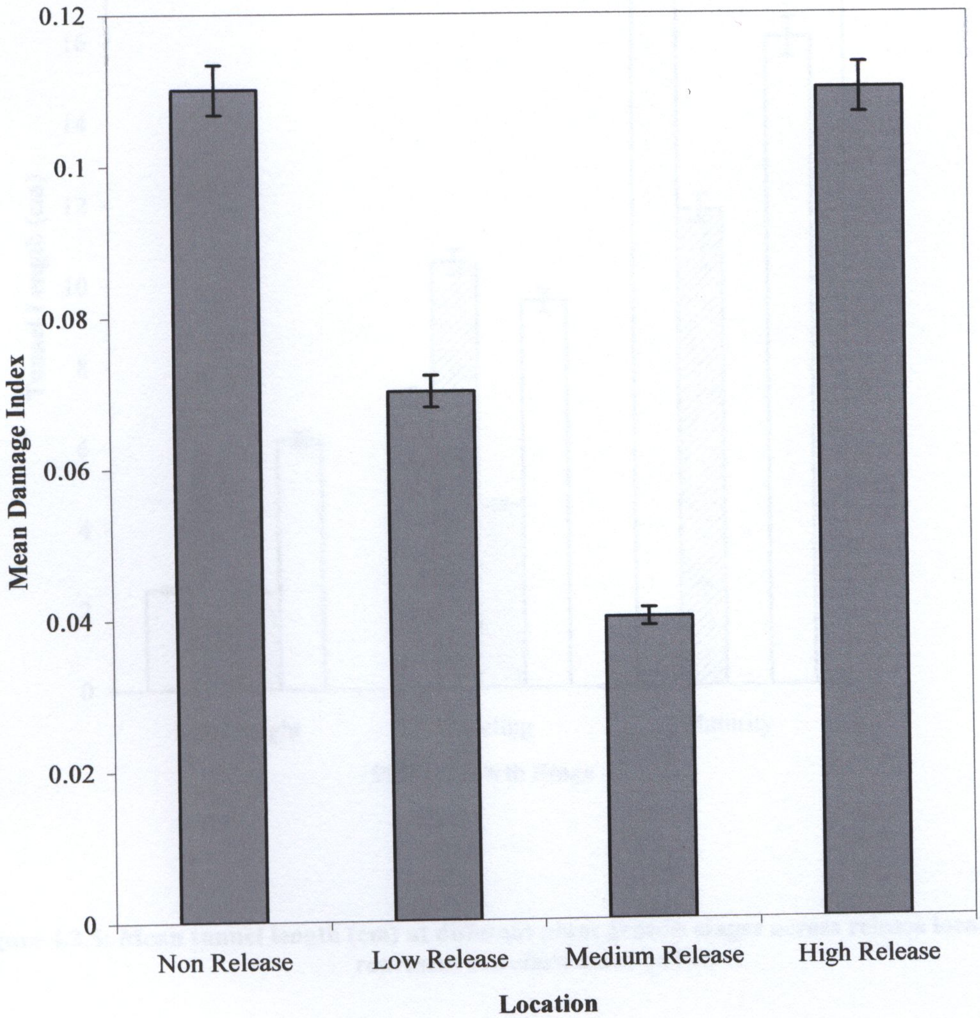

Figure 4.2.4: Mean damage index across release locations (Bars represent standard error (± SE)

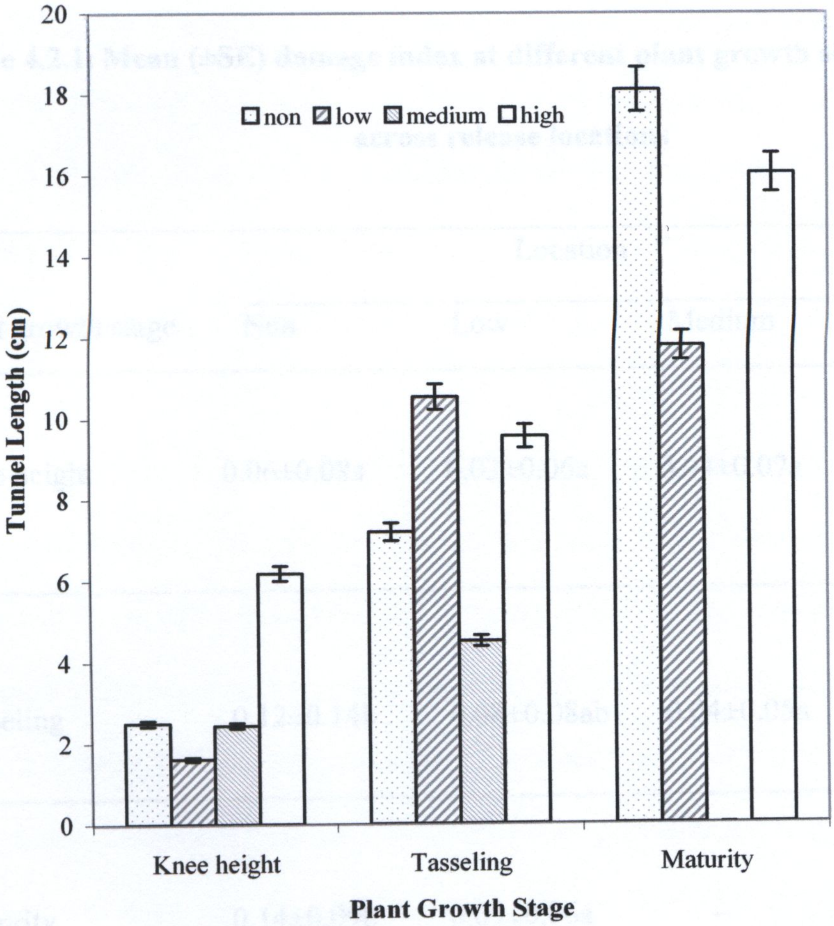

Figure 4.2.5: Mean tunnel length (cm) at different plant growth stages across release locations (Bars represent standard error (± SE)

Table 4.2.1: Mean (±SE) damage index at different plant growth stages across release locations

	Location			
Plant growth stage	Non	Low	Medium	High
Knee height	0.06±0.08a	0.03±0.06a	0.04±0.07a	0.15±0.11b
Tasseling	0.12±0.14b	0.08±0.08ab	0.04±0.05a	0.15±0.11b
Maturity	0.14±0.09b	0.09±0.06a	-	0.11±0.09b

Means in the same row followed by the same letter (s) are not significantly different at 5 % level using Tukey's studentised range test (HSD).
-, Data not collected for that location

4.2.3 Number of exit holes

A high number of stem borer exit holes were present on maize stems in the NR and HR locations. However, the number of holes in the LR location was not statistically different from those obtained in the two locations above. The MR location recorded the lowest number of holes (Fig.4.2.6). There were significant differences in the number of exit holes made on stems across all release locations ($F=100.83$; d.f.=3; $P<0.001$).

There were variations in the mean number of exit holes across the locations at the three plant growth stages. Plants in the HR location had a higher number of exit holes when compared to the other three locations at the knee height stage. However, there were no statistical differences in the number of exit holes at the tasseling stage. On the contrary, the NR location had the highest number of exit holes at the maturity stage (Table 4.2.2). Significant differences were also observed across the locations since the means for each plant growth stage were different from each other ($F=7.96$; d.f.=2; $P<0.001$).

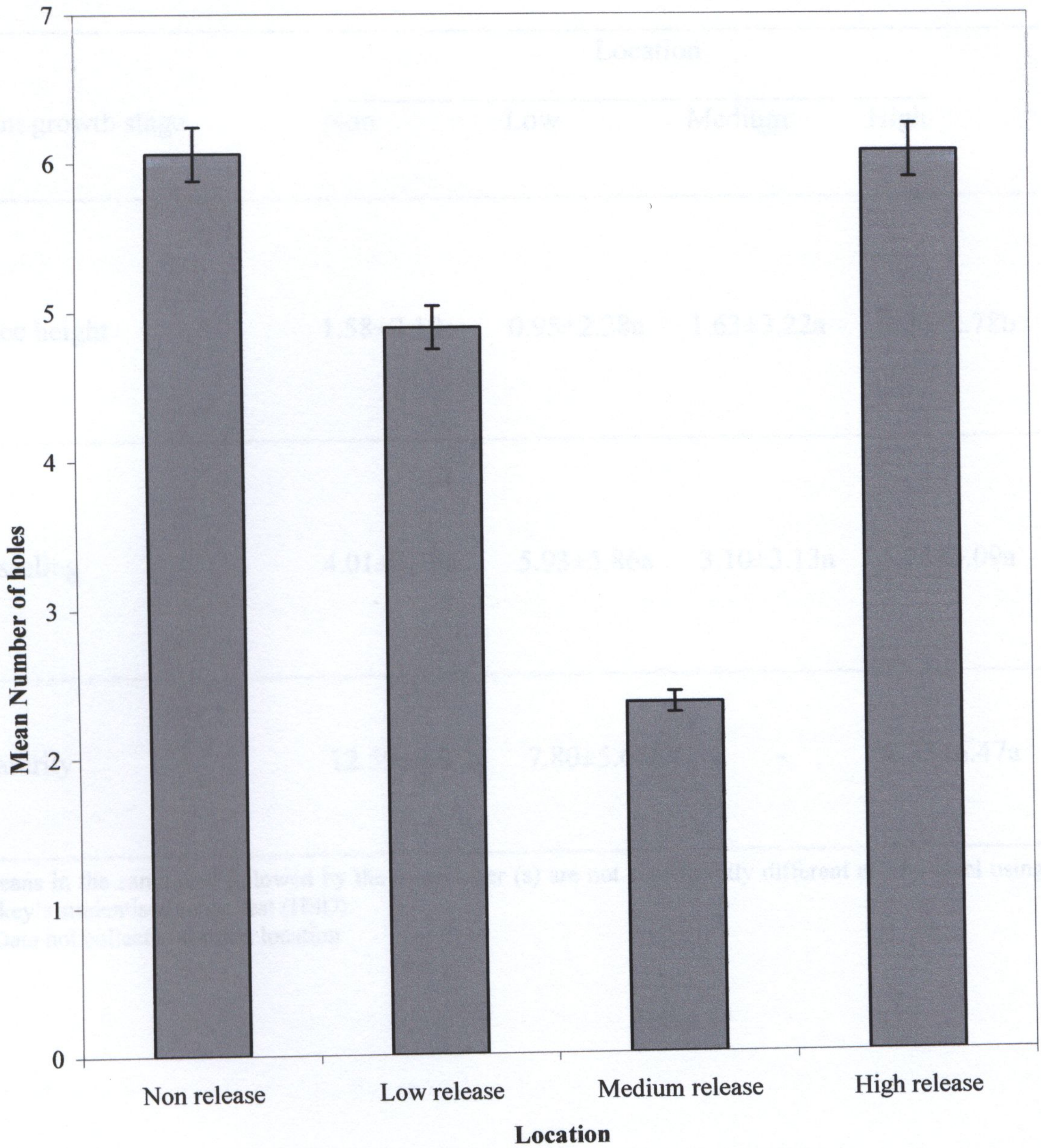

Figure 4.2.6: Mean number of holes across release locations (Bars represent standard error (± SE)

Table 4.2.2: Mean (±SE) number of holes at different plant growth stages across release locations

	Location			
Plant growth stage	Non	Low	Medium	High
Knee height	1.58±2.19a	0.95±2.28a	1.63±3.22a	4.36±2.78b
Tasseling	4.01±4.79a	5.93±5.86a	3.10±3.13a	5.26±5.09a
Maturity	12.59±9.95b	7.80±5.68a	-	8.33±6.47a

Means in the same row followed by the same letter (s) are not significantly different at 5 % level using Tukey's studentised range test (HSD).
-, Data not collected for that location

4.3 Abundance and parasitism levels of *C. flavipes* across the non-release, release locations at the three plant growth stages.

C. flavipes was the only parasitoid recovered from the stem borer larvae during the two winter maize cropping seasons of 2005 and 2006 (see App. E, F and G). It was the only parasitoid recovered from *C. partellus* larvae in three of the locations, i.e., NR, LR and HR locations. However, parasitism in the NR was very low because only one parasitized larva was recovered.

The overall parasitism of *C. partellus* due to *C. flavipes* was 10.8% and 2.7% in the 2005 and 2006 cropping seasons, respectively. The HR location had a higher mean number of parasitized larvae with an average of 0.03 than the two other locations where *C. flavipes* was recovered (Fig. 4.3.1). There were significant differences in the number of parasitized larvae in the three locations where *C. flavipes* was recovered ($F=9.71$; d.f. $=2$; $P<0.001$).

However, there were no significant differences in the mean number of parasitized larvae among the three plant growth stages ($F=1.92$; d.f. $=2$; $P=0.14$). The average number of parasitized larvae ranged from 0 – 0.2 across the plant growth stages with the maturity stage having the highest number (Fig. 4.3.2).

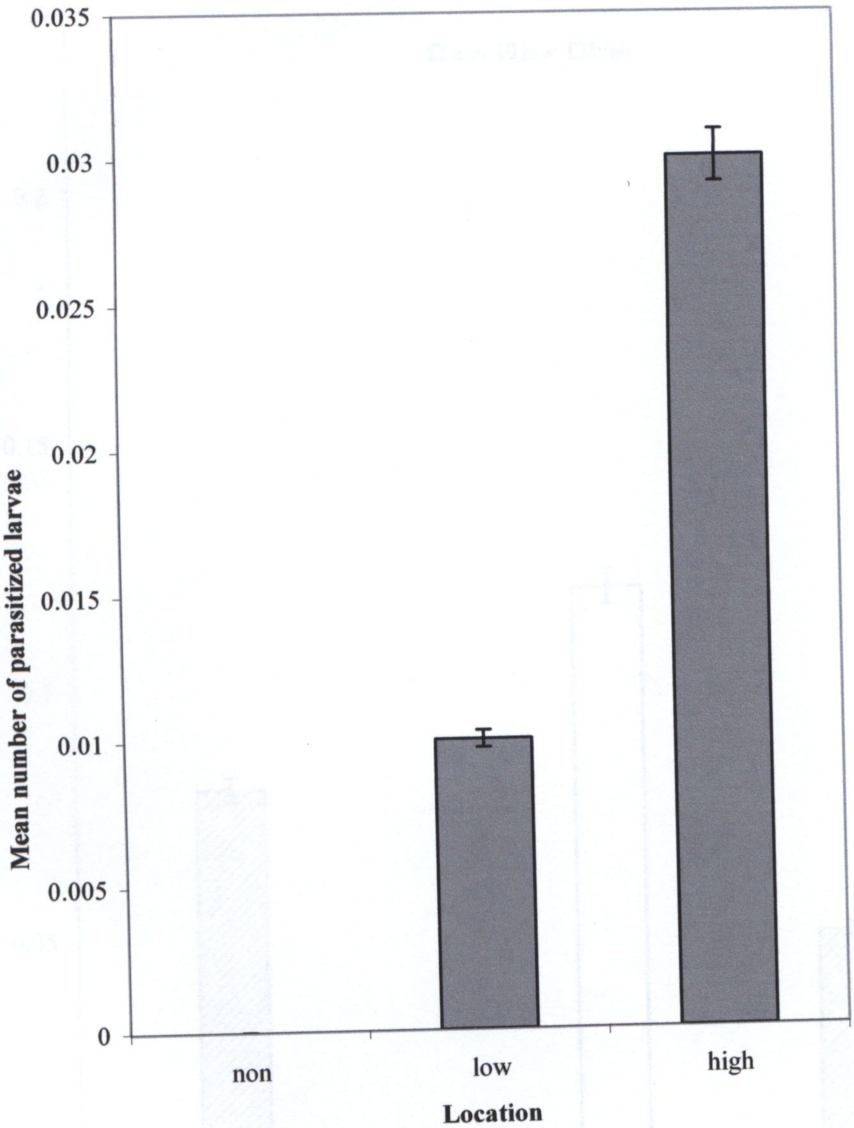

Figure 4.3.1: Mean number of parasitized larvae across release locations (Bars represent standard error (± SE)

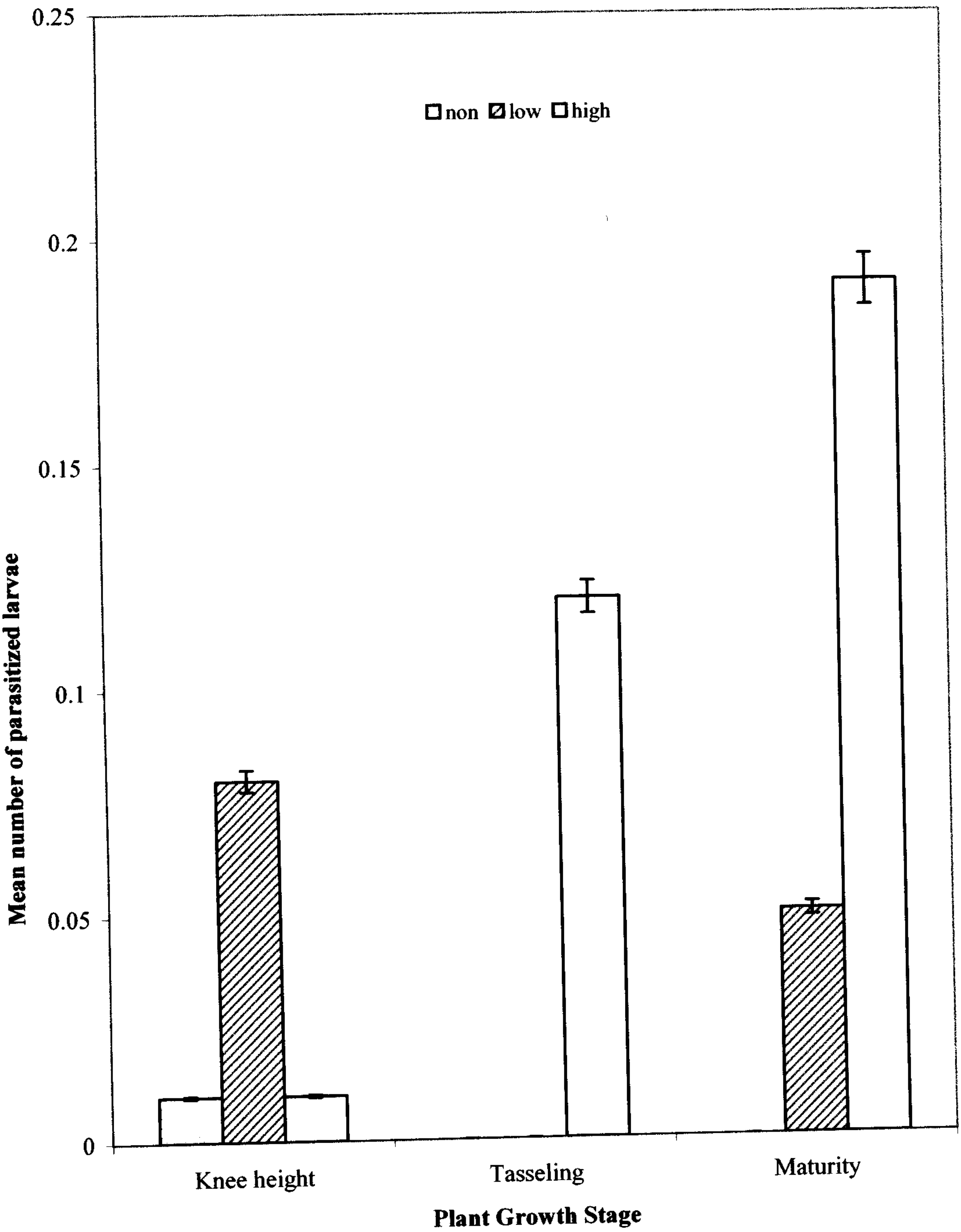

Figure 4.3.2: Mean number of parasitized larvae at different plant growth stages across release locations (Bars represent standard error (± SE)

4.4 Yield assessment

Weight of maize cobs from the LR location was higher than in the two other locations with the HR location having the lowest mean weight (Table 4.4.1). Significant differences in cob weights were observed across the release locations (F=10.15; d.f.=2; P<0.001).

Damage parameters were generally not significantly correlated with maize cob weight. The only parameter that yielded a significant correlation (P<0.05) was the number of *C. partellus* found in the NR location (Appendix H).

Table 4.4.1: Mean (±SE) cob weight (g) across locations

Location	Cob weight (g)
Non	73.13±8.63a
Low	99.13±12.21a
Medium	-
High	33.53±9.23b

Means in the same column followed by the same letter (s) are not significantly different at 5 % level using Tukey's studentised range test (HSD).
-, Data not collected for that location

4.5 Summary of results

There were significant differences in the incidence of *C. partellus* across the release locations and at the three plant growth stages. Clearly, incidences of *C. partellus* were higher in the HR location and at the knee height stage (see Tables 4.5.1 and 4.5.2). Similarly, more leaf injury in the form of 'pin-holes' and 'dead-heart' conditions caused to plants were common in the HR and NR locations. However, the there were no significant differences in leaf damage across the release locations and at the three plant growth stages. On the contrary, stem damage parameters on maize stems such as tunnel length, damage index and number of exit holes caused by *C. partellus* were significantly different across the release locations and at the three plant growth stages.

The HR location had the highest tunnel length, which was not significantly different from that obtained in the NR location. Similarly, the damage index was highest in both the HR and NR locations. The number of exit holes was also highest in the HR location and was not statistically different with that obtained in the NR location (see Table 4.5.1). Damage due to *C. partellus* on maize stems by tunneling, its damage index and number of exit holes was higher at the maturity stage as compared to the two other plant growth stages (see Table 4.5.2). Correlation of maize cob weight with the number of *C. partellus* and damage parameters was generally not significant across the locations (Appendix H). However, the number of *C. partellus* and cob weight were negatively correlated in the NR location.

Table 4.5.1: Means (± SE) of damage variables, abundance of *C. partellus* and parasitized larvae in non-, low, medium and high release locations in Sinazongwe district during the 2006 cropping season.

Release Location	Damage variables				Number of *C. partellus*	Number of parasitized larvae
	Leaf damage	#holes	Tunnel length	Damage Index		
Non	1.17±0.03a	6.06±0.41a	9.27±0.62ab	0.11±0.01a	1.11±0.02b	0.00±0.00b
Low	1.09±0.03a	4.89±0.58a	7.98±0.88b	0.07±0.01b	1.38±0.03b	0.01±0.01ab
Medium	1.15±0.04a	2.36±0.71b	3.47±1.08c	0.04±0.01b	1.36±0.03ab	-
High	1.14±0.04a	6.07±0.44a	10.91±0.66a	0.11±0.01a	2.24±0.02a	0.03±0.00a

Means in the same column followed by the same letter (s) are not significantly different at 5 % level using Tukey's studentised range test (HSD).
-, Data not collected for that location

Table 4.5.2: Means (± SE) of damage variables, abundance of *C. partellus* and parasitized larvae at different plant growth stages in non-, low, medium and high release locations in Sinazongwe district during 2006 cropping season.

Plant Stage	Damage variables				Number of *C. partellus*	Number of parasitized larvae
	Leaf damage	#holes	Tunnel length	Damage Index		
Knee height	1.17±0.02a	2.13±0.39c	3.19±0.58c	0.07±0.01b	0.46±0.02c	0.02±0.00a
Tasseling	1.12±0.02a	4.62±0.36b	8.15±0.53b	0.09±0.01b	1.35±0.01b	0.01±0.01a
Maturity	-	10.01±0.41a	16.01±0.61a	0.12±0.01a	2.75±0.02a	0.02±0.01a

Means in the same column followed by the same letter (s) are not significantly different at 5 % level using Tukey's studentised range test (HSD).
-, Data not collected for that location

CHAPTER 5: DISCUSSION

The present study was aimed at estimating the incidence of *C. partellus*, establishment of *C. flavipes* and its impact across release locations and at different maize plant growth stages in Sinazongwe district. This chapter discusses the results obtained from the study.

5.1 Incidence of *C. partellus* and its damage to maize

It was clear from this study that there was variability in the incidence of *C. partellus* at the three maize plant growth stages in the study area. Clearly, these results did not support the hypothesis that infestation levels of *C. partellus* were the same at different maize growth stages. It was further observed that the incidence of stem borers was affected by age of the maize crop. There were comparatively more *C. partellus* larvae collected at knee height stage than at maturity stage. These results concurred with findings by other authors in other study areas who did similar work (Seshu Reddy *et al.*, 1990; Bonhof, 2000; Ndemah *et al.*, 2001; Emana *et al.*, 2002). The stem borer densities in the study area decreased at tasseling and maturity stages. Kalule *et al.*, (1997) obtained similar results in Uganda after monitoring fluctuations of stem borers at different plant growth stages in maize.

Furthermore, results also indicated that previous release intensities of *C. flavipes* in the study area did not suppress populations of *C. partellus*. This was because the high intensity release location recorded the highest incidence of *C. partellus*. The non-release location, on the other hand, had the lowest incidence of stem borers. It was evident that these results did not validate the second hypothesis regarding incidence of *C. partellus* across locations.

Stem tunneling is a good indicator of the degree of plant damage and thus, yield loss. The more extensive the tunneling, the higher the yield loss (Bosque-Perez and Mareck, 1991; Kalule *et al.*, 1994). Extensive damage by way of tunneling into the maize plant stems and holes made on the internodes varied at the three maize plant growth stages across the locations in this study. Despite a higher incidence of *C. partellus* recorded at knee height, more damage was observed at the cob maturity stage. The damage would probably have been as a result of two or more generations of the pest infesting the crop. Kfir (1992) reported that *C. partellus* completed two and a half generations per season.

5.2 Establishment of *C. flavipes*

Dispersal is an essential attribute of parasitoid establishment (Sallam *et al.*, 2001). The recovery of *C. flavipes* from the non-release location was an indication that the parasitoid had dispersed. *C. flavipes* is likely to establish itself in areas where the suitable stem borer host species are present (Overholt *et al.*, 1994b; Ngi-Song *et al.*, 1995; Emana *et al.*, 2002). The relatively high incidence of C. *partellus* in the study area may explain its establishment. The entire *C. flavipes* cocoon masses were recovered from *C. partellus* during 2005 and 2006 cropping seasons.

Preliminary results from the 2005 cropping season suggested that the establishment of *C. flavipes* in Sinazongwe district was as a result of the high intensity of parasitoid release in the study area after 2000 (see App. A). Establishment of this parasitoid in Coastal Kenya was only observed after 18,000 to 24,200 female parasitoids were released (Overholt *et al.*, 1997). However, this aspect could be further studied in the future.

5.3 Parasitism levels of *C. flavipes* on *C. partellus*

Estimating levels of parasitism is one way of determining the overall impact of parasitoids upon their host populations (Hassel and Waage, 1984). The current overall parasitism levels in the study area were lower than those observed in the 2005 cropping season. These results were a more reliable estimate since a larger number of stem borer larvae was collected in the 2006 cropping season as compared to the previous season. It may take a few more years before the parasitoid population increases in order to reduce the stem borer populations in Sinazongwe district.

Several authors had suggested that the population build up of *C. flavipes* is not spontaneous (Omwega *et al.*, 1995; Overholt *et al.*, 1997; Cugala *et al.*, 1999; Zhou and Overholt, 2001). This is because during colonization, dispersal has a counteracting effect on increase in parasitoid density. This phenomenon was observed in Kenya where *C. flavipes* was released in 1993 and the population density remained low for the first four years and then increased dramatically (Overholt, 1998; Zhou *et al.*, 2003). In this study, it could be speculated that *C. flavipes* in Sinazongwe district was in the initial stages of population build-up. However, this will need further study.

It is therefore possible that parasitism levels in the study site may increase with time. This was observed in Madagascar, where *C. flavipes* was released against *C. sacchariphagus* in sugarcane, maximum levels of parasitism (60%) were not reached until six years after release (Betbeder-Matibet and Malinge, 1968, cited by Overholt, 1998). The ultimate impact of *C. flavipes* on *C. partellus* in Africa cannot be measured until the population stabilizes at an 'equilibrium' density, which may take several years (Overholt *et al.,* 1997). The effect of *C. flavipes* usually appears in the following season (Zhou *et al.*, 2001a).

Results on parasitism levels across release locations supported the first hypothesis that parasitism levels of *C. flavipes* are directly related to release intensities. It was further observed that the parasitism levels were significantly different across the release locations (Fig. 4.3.1). A high number of parasitized *C. partellus* larvae were recovered in areas of high *C. flavipes* releases. Similar results were obtained by other authors in Ethiopia (Emana *et al.*, 2002).

An interesting observation in this study was the pest and parasitism level pattern at the three maize plant growth stages (Figs. 4.1.2 and 4.3.2). Parasitism levels were lower when pest populations were high at knee height stage. As the maize plant growth stage advanced, larvae populations became lower at maturity stage. On the other hand, parasitism levels were higher at the maturity stage as compared to the early plant growth stages. In a related study carried out in Ethiopia, Degaga, (2002) also observed that parasitism of *C. partellus* by *C. flavipes* seemed to increase between maturity and reproductive stages. Some authors have argued that low parasitism is likely to occur when the host is abundant and vice versa (Mikkola, 1976; Várkonyi *et al.*, 2002). The results obtained from this study showed that *C. partellus* could have been engaged in a similar host-parasitoid interaction with *C. flavipes* in Sinazongwe district during the winter cropping season. It is also possible that a different situation could be obtained during summer cropping season. However, this needs to be validated by a follow up study.

5.4 Yield assessment

Cob weight basically showed negative and insignificant correlations between most damage parameters and cob weight across the locations with the exception of the non-release location. Similar results were obtained by other authors elsewhere when stem borer tunnel length and other damage parameters were correlated with yield (Ogwaro, 1983; Ajala and Saxena, 1994; Songa *et al.*, 2001).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Results from the present study indicated that the incidence of *C. partellus* larvae was at its peak during the early stages of the maize crop and the lowest incidence occurred at maturity stage. Variability in the incidence of the pest was also observed across locations with the high release location having the highest incidence as compared to the other locations.

Other information obtained in this study was the recovery of *C. flavipes*. There was clear evidence that the parasitoid had established itself in the study area. However, *C. flavipes* was not an important factor in suppressing stem borer populations. This was probably because the parasitism levels were low at knee height stage when stem borer populations were at their peak.

Furthermore, the study confirmed that *C. partellus* was the most abundant cereal stem borer in the study area. The presence of an indigenous stem borer species, *S. calamistis* was also reported, although *C. flavipes* did not parasitize it. It is hoped that the results from this study will significantly contribute to the future studies of biological control of cereal crop pests in Zambia within the context of integrated pest management (IPM).

6.2 Recommendations

1) Future studies could possibly be carried out in the study area to compare incidence of *C. partellus* among several growing seasons. These studies will also be important in determining the dispersal pattern of *C. flavipes* in Sinazongwe district by sampling as many fields as possible in the different release locations and comparing the parasitism levels among different seasons.

2) Yield losses at harvest (dry weight) of maize should also be assessed in the study area to establish implications of this parasitoid on the pest.

3) A follow-up study to assess the behaviour of *C. flavipes* on *C. partellus* on the summer maize crop will be necessary to compliment the current study.

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APPENDICES

APPENDIX A: Date, location and numbers of *C. flavipes* cocoons released in Sinazongwe District from 1999-2004.

NO.	DATE	ALTITUDE (m)	LATITUDE	LONGITUDE	NUMBER RELEASED
1	12/03/99	528	17°07.497′ S	27°32.086′ E	1000
2	03/03/00	528	17°04.788′ S	27°31.499′E	1000
3	22/09/00	528	17°06.844′ S	27°30.609′E	500
4	06/10/02	528	17°10.526′ S	27°30.501′E	800
5	14/09/03	-	17°09.641 ′ S	27°21.627′ E	50,000
6	18/09/03	-	17°12.651′ S	27°20.250′ E	50,000
7	14/07/04	491	17°16.229′ S	27°21.902′ E	100,000
8	06/08/04	-	17°21.287′ S	27°20.412′ E	100,000
9	08/09/04	485	17°11.65′ S	27°30.323′ E	100,000

- no records available

(Source: Entomology Section, ZARI, Chilanga)

APPENDIX B: Exact location of sampled fields in Sinazongwe district

Release location	Altitude(m)	Latitude (S)	Longitude (E)
Non	477	17°27.042′	27°19.653′
Non	476	17°26.836′	27°19.800′
Non	475	17°26.984′	27°19.710′
Non	479	17°26.831′	27°19.772′
Non	481	17°27.052′	27°19.673′
Non	482	17°27.078′	27°19.617′
Non	482	17°27.041′	27°19.665′
Non	479	17°26.938′	27°19.787′
Non	494	17°27.103′	27°19.576′
Non	481	17°26.829′	27°19.635′
Non	484	17°27.344′	27°16.689′
Non	479	17°26.938′	27°19.787′
Low	491	17°07.604′	27°32.505′
Low	482	17°07.565′	27°32.731′
Low	488	17°07.643′	27°32.540′
Low	476	17°07.600′	27°32.770′
Low	483	17°06.643′	27°32.078′
Low	497	17°07.599′	27°32.490′
Low	484	17°07.672′	27°32.622′
Low	480	17°07.673′	27°32.805′
Low	487	17°06.628′	27°32.055′
Low	481	17°07.650′	27°32.657′
Low	488	17°06.670′	27°31.437′
Low	484	17°07.697′	27°32.505′
Medium	621	17°09.711′	27°21.086′
Medium	629	17°09.704′	27°21.079′
Medium	622	17°09.696′	27°21.102′
Medium	626	17°09.694′	27°21.022′
Medium	615	17°09.681′	27°21.059′
Medium	627	17°09.722′	27°21.020′
Medium	625	17°09.731′	27°21.073′
Medium	621	17°09.721′	27°21.092′
High	481	17°16.497′	27°23.892′
High	482	17°12.020′	27°31.445′
High	485	17°11.944′	27°31.355′
High	485	17°11.951′	27°31.349′
High	479	17°16.412′	27°23.924′
High	490	17°11.911′	27°31.133′
High	479	17°16.448′	27°23.861′
High	482	17°16.575′	27°23.885′

High	480	17°16.479′	27°23.864′
High	489	17°11.933′	27°31.241′
High	479	17°16.561′	27°23.888′
High	477	17°16.519′	27°23.886′

APPENDIX C: Leaf Damage Score Sheet

Release Location (LR/MR/HR): ………………………………………….

Farmer Name/ Plot No.: ………………………………………………….

Date sampled:…………………………………………………………….

Plant Stage:……………………………………………………………….

Plant No.	Damage Score	Stem borer species			Natural enemies recovered
		Cp	*Sc*	*Bf*	
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Key:

Release Locations

LR = Low Release Location (< 1000 parasitoids); MR = Medium Release Location (50, 000 parasitoids); HR = High Release Location (100, 000 parasitoids)

Damage Score

1= 1-20%, 2=21-40 %, 3= 41-60%, 4=61-80%, 5= over 81%

Stem borer species

Cp = Chilo partellus; *Sc = Sesamia calamistis*; *Bf = Busseola fusca*

APPENDIX D: Stem borer infestation levels and damage parameters data sheet

District:………………. Province:………………….. Altitude:……………… Latitude:…………..
Longitude:……………..

Farmer:…………………………..Date Sampled:…………………… Plant Stage:…………………...

Plant#	#holes	Tunnel length (cm)	*Cp*	*Sc*	*Bf*	#larvae	#pupae	Total stem borers
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

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

APPENDIX E: Numbers of *C. flavipes* emergences from *C. partellus* attacking maize recovered from Sinazongwe district during the 2005-winter season

Release location	No. of larvae	% successfully reared	% parasitized	No. of parasitoid progeny	Sex ratio (Female to Male)
Non	313	79.6	-	-	-
High	46	78.3	10.87	59	3:1

APPENDIX F: Numbers of *C. flavipes* emergences from *C. partellus* attacking maize recovered from Sinazongwe district during the 2006 winter season

Release location	No. of larvae	% successfully reared	% parasitized	No. of parasitoid progeny	Sex ratio (Female to Male)
Non	267	85.4	0.00	22	10:1
Low	157	87.9	0.03	87	5:1
Medium	109	86.2	-	-	-
High	470	87.7	0.04	540	7:1

APPENDIX G: Adult *C. flavipes* after emergence in the laboratory

APPENDIX H: Correlation coefficients between maize cob weight and abundance of *C. partellus* and damage variables in three release locations.

Location	Variable	r
Non	Number of holes	0.095(F=0.16, P=0.69)
	Tunnel length	0.161(F=3.57, P=0.06)
	Damage Index	0.114(F=3.54, P=0.07)
	Number of *C. partellus*	-0.047(F=8.61, P<0.05)*
Low	Number of holes	0.197(F=3.82, P=0.26)
	Tunnel length	-0.007(F=0.00, P=0.95)
	Damage Index	-0.000(F=1.31, P=0.26)
	Number of *C. partellus*	-0.157(F=0.07, P=0.80)
High	Number of holes	-0.092(F=0.46, P=0.50)
	Tunnel length	-0.023(F=0.86, P=0.36)
	Damage Index	0.005(F=1.21, P=0.28)
	Number of *C. partellus*	-0.050(F=2.44, P=0.13)

*Significant at P< 0.05