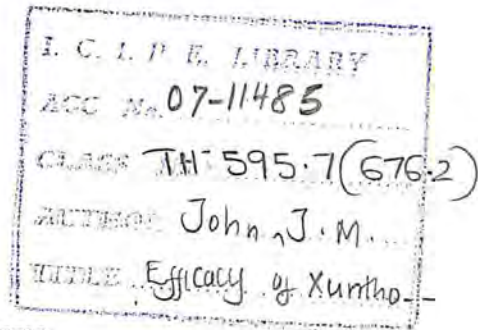


EFFICACY OF *XANTHOPIMPLA STEMMATOR* AGAINST SELECTED
LEPIDOPTERAN PESTS AND ITS INTERACTION WITH PUPAL
ENDOPARASITOID *PEDIOBIUS FURVUS* IN GRAMINACEOUS PLANTS
IN KENYA

BY

JAMLECK MUTURI JOHN

Bed (Sc.) (Kenyatta University)



THESIS SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF
MASTER OF SCIENCE DEGREE IN AGRICULTURAL ENTOMOLOGY OF
KENYATTA UNIVERSITY.

AUGUST 2004

DECLARATIONS

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

Jamleck Muturi John07/10/2004

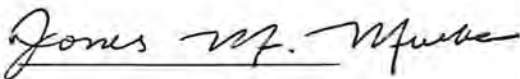
Signature

Date

This thesis has been submitted with our approval as supervisors.

Prof. Jones M. Mueke

Department of Biological Sciences Kenyatta University

12th October, 2004

Signature

Date

Dr. Adele Ngi-Song

International Centre of Insect Physiology and Ecology (ICIPE)

07/10/2004

Signature

Date

DEDICATION

To my family

Wife Eunice, Justice, father, mother, siblings, Kariíthi, Njirû, Karími, Mûchangi, Mûkami, Wanja, Mûthoni, nephew Gítonga and nieces Ngatha, Mwende, Wangûi and Wakio.

ACKNOWLEDGEMENTS

I exalt the Almighty God for His mercies, spiritual and physical providence throughout my physical years. Glory and Honour be to His Holy name. I express special gratitude to ICIPE, DRIP program for granting me the opportunity to undertake this research. I have had the extra ordinary blessing to have Prof. Jones M. Mueke of Kenyatta University and Dr. Adele Ngi-Song of ICIPE as my supervisors. They patiently guided me through my research work with great skill and steadiness. Their advise, criticisms and comments are highly appreciated. I acknowledge the valuable help received from my lecturers Prof. Okelo, Prof. Kokwaro, Dr. Mbugi, Dr. Ogol, Dr. Kairu, Dr. Tengecho, Dr. Syprine and Dr. Ng'ang'a for their academic guidance throughout the course. I sincerely acknowledge the generous support offered by Dr. Sétamou for his enormous input in data analysis, Dr. Schulthess, Dr. Omwega, Dr. Jiang and Dr. Bruno for their constructive research comments. The great technical support I received from Joseph, Julius, Obala, Philip, Mwadime, Gelpas, Mucheru, Pauline, Kiti and Carol is highly appreciated. Thanks to Owour, Mungai and the late Sabaya for their field work support in snake prone areas of Kwale. Thanks to my colleagues Cathrine, Kanya, Jackline, Wanyama, Dr. Kalyebi, Lorna, Dr. Samira, Dr. Waiganjo, Nderitu, Juma, Muiruri, Bruce, Barasa, Joseph, Wilfred, Josephine and Kari for their constant moral and spiritual support. I wish to acknowledge Mr. and Mrs. Minion for their financial, spiritual and moral support throughout the course. I appreciate constant encouragement I received from Dr. Day of C.A.B.I., Prof. Pia Barklund of I.C.R.A.F., Dr. Gethi of K.A.R.I., Mr. and Mrs. Muthui, Heather Mackay and Martins'.

My special gratitude goes to my wife Eunice, my father (John), mother (Grace) and siblings for their understanding and support all through my study period.

ABSTRACT

Chilo partellus (Swinhoe) (Lepidoptera: Crambidae) is a major pest and a threat to cultivated poaceous plants in many parts of Africa. The adverse ecological and environmental effects as well as the cost of chemical insecticides commonly used in its control, besides its limitations makes it necessary to explore environmentally friendly control methods. Classical biological control is one important approach in the control of exotic pests. A larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was introduced in Kenya from Pakistan against *C. partellus*. Though, it has reduced borer density in overall by 32% and by 55% for *C. partellus*, its establishment and effectiveness is limited for it does not complete development in *Busseola fusca* and *Eldana saccharina* which are serious stemborer pests. To increase borer suppression, *Xanthopimpla stemmator* (Hymenoptera: Ichneumonidae) a solitary pupal endoparasitoid which attacks lepidopteran pupae of stemborers was imported from Asia into Kenya for laboratory assessment and future release.

The objectives of this study were, to determine the location of pupae of *C. partellus*, *Sesamia oriaula*, *Busseola fusca* and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) in selected wild and cultivated host plants from different ecological areas, to evaluate host acceptance and preference of *X. stemmator* to *C. partellus*, *S. oriaula* and *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), to assess its developmental suitability in the three borer species and to study the interaction between *X. stemmator* and the indigenous pupal parasitoid, *Pediobius furvus* (Gahan)(Hymenoptera; Eulophidae) in the laboratory.

The results indicated that, there was no significant difference in the tunnel length between *C. partellus* and *Chilo orichalcociliellus* (Strand)(Lepidoptera: Crambidae). However, in the different plant species tested, *B. fusca* and *S. oriaula* had the longest mean tunneling length. Pupal depth and location of *B. fusca*, *C.*

orichalcociliellus, *S. calamistis* showed no significant difference in the different plant species tested. Pupal depth and pupal location for *C. partellus* differed significantly in the different plants. *Chilo partellus* was the most abundant borer species sampled in Kwale whereas, *B. fusca* was the most abundant borer species sampled in Trans Nzoia from the different sampled plant species. Most borer pupae were located in the upper part of the host plant stem except *S. oriaula* whose pupae were exclusively recovered from the lower part of *Pennisetum purpureum*. *Chilo orichalcociliellus* and *S. calamistis* mainly pupated in the cob and upper part of the host plant stem.

Laboratory choice and no-choice tests indicated that female *X. stemmator* did not show any oviposition preference for *C. partellus*, *S. oriaula* and *H. armigera*. The female parasitoid indiscriminately accepted all the hosts. Studies on suitability revealed that the female *X. stemmator* caused high pupal mortality in *H. armigera* and it successfully developed in *C. partellus* and *S. oriaula* but, did not develop in *H. armigera*. *Xanthopimpla stemmator* emerging from *C. partellus* developed faster than those from *S. oriaula*. However, *X. stemmator* emerging from *C. partellus* lived longer than those from *S. oriaula*. *Xanthopimpla stemmator* progeny from *S. oriaula* had a wider wingspan and longer tibia length than those from *C. partellus*. More female *X. stemmator* emerged from both hosts and the probability of getting female progeny increased with increase in host weight.

Studies on multiparasitism showed that *X. stemmator* successfully developed when exposed to *C. partellus* pupae at different time intervals and sequences. Developmental time of *X. stemmator* progeny was slower when it stung pupae alone, but gradually shortened when *P. fuscus* was involved in the stinging of pupae at different sequences. Few *P. fuscus* adults emerged at different sequences and the percent success of *X. stemmator* parasitism increased with time interval in the Xs-Pf

(*Xanthopimpla stemmator*-*Pediobius furvus*) sequence, but decreased in the Pf-Xs (*Pediobius furvus*-*Xanthopimpla stemmator*) sequence.

Male to female proportion of *P. furvus* in both sequences did not differ from that of the control at the four time intervals. More *X. stemmator* progeny were produced in the Xs-Pf sequence than in the Pf-Xs sequence. More male proportions were produced as the time interval of exposure increased in both sequences and progeny production decreased with increase in time interval of exposure.

This study indicates that *X. stemmator* could be used as a biological control agent against major and minor borer species in Kenya. The parasitoid could complement and supplement other borer control strategies.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

1.1.1 Stemborers

Maize (*Zea mays* (L)) and sorghum (*Sorghum bicolor* (L)) Moench are among the most important cereal crops in sub-Saharan Africa (FAO, 1998; Kfir *et al.*, 2002) and are grown for subsistence and commercial purposes. In Kenya about 1.5 million hectares of land has maize under cultivation and about 3.2 million tonnes of maize are produced (FAO, 1999, 2003).

Among several insects that attack maize and sorghum, stemborers constitute one of the major constraints to efficient maize and sorghum production in the developing world. These include the Noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson; the Pyralid, *Eldana Saccharina* (Walker) and the Crambids *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* (Strand) (Overholt *et al.*, 1994).

Twenty-one species of the economically important stemborers belonging to three families; Crambidae, Pyralidae and Noctuidae have been found to attack maize in various parts of Africa (Seshu Reddy, 1983; Bosque-Perez and Mareck, 1990; Khan *et al.*, 1991; Maes, 1998). According to Seshu Reddy (1983) and Warui *et al.* (1986) the most economically important cereal stemborers in Kenya are *B. fusca*, *S. calamistis*, *E. saccharina*, *C. partellus* and *C. orichalcociliellus* (Plate 1.1). Minor borers attacking maize and other graminaceous plants in Kenya include; *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) (Mathews, 1991) and *Sesamia oriaula* (Tams and Bowden) (Lepidoptera: Noctuidae) (Plate 1.1).

Most stemborers in Africa are indigenous including *B. fusca*, *S. calamistis* and *C. orichalcociliellus* (Conlong, 1997; Bosque-Perez and Schulthess, 1998; Seshu-Reddy, 1998; Kfir, 1998; Ofomata *et al.*, 1999a). *Chilo partellus* and *C. sacchariphagus* (Bojer) are exotic species (Kfir *et al.*, 2002). *Chilo partellus* was accidentally introduced from Asia before 1930 (Tams, 1932). It was first reported in Malawi but has since spread in Eastern, Southern and central Africa (Overholt *et al.*, 1994). It is becoming a predominant and serious pest in all the maize and sorghum growing areas, and there is evidence that it is displacing the indigenous stemborer species in some areas (Seshu Reddy, 1983, Kfir, 1997; Overholt, 1998; Ofomata *et al.*, 1999a; 2000)

In coastal Kenya, there is evidence that *C. partellus* is actively displacing the indigenous stemborer, *C. orichalcociliellus* (Ofomata *et al.*, 1999b; 2000). In the late 1960's *C. orichalcociliellus* was more or less equally abundant (Warui and Kuria, 1983). Surveys carried out in coastal Kenya from 1991 to 1992 indicated that *C. partellus* was by far the predominant stemborer accounting for greater than 80% of the total stemborer population (Overholt *et al.*, 1994). *Chilo orichalcociliellus* has been reported from coastal East Africa, Madagascar and Malawi (Bleszynsky, 1970; Delobel, 1975). Recent evidence suggests that *C. partellus* is moving into cooler elevation areas (Overholt *et al.*, 2000). The same author, using geographic information system (GIS) tools predicted distribution of *C. partellus* in Africa to locations with similar climates based on the climate at a location where it was known to occur.

Damage and crop losses due to stemborer injury varies with the growing season, crop species and variety, plant growth stage, geographical region, crop management, climate and stemborer species (Ingram, 1983). The borer larvae cause

extensive foliar damage “windows” on the leaves of the infested plants and they lead to death of the central shoot, a condition referred to as the ‘dead heart’ effect. The stemborer larvae feed on ears, silks, developing seeds and bore into maize cobs, resulting to stunted growth, withering, low farm grain production, disease transmission, weakening and drying of the plants (de Pury, 1968; Harris, 1989 ; Sithole, 1990).

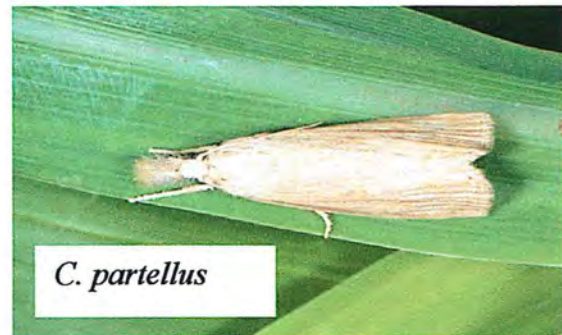
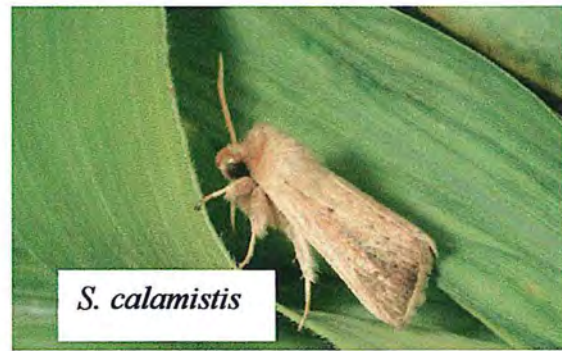




Plate 1.1: Economically important borers of cereal and other graminaceous plants in Kenya

1.1.2 Control measures

In an attempt to avert major losses caused by stemborers various control strategies have been employed. These include application of insecticides (Walker, 1981; Ogunwolu *et al.*, 1981), cultural practices, host plant resistance, genetically modified plants and biological control. These methods have limitations. For instance, the mainstay of control in all cereal growing and subtropical countries is the use of chemical insecticides. Though effective in reducing borer population, chemical control is expensive, environmentally unfriendly, dangerous to user, non target animals and requires timely application to control borers before the larva enters the stalk (Mathez, 1972; Ingram, 1983). This control method though mainly recommended by the Ministry of Agriculture in Kenya is ineffective due to cryptic feeding of the larvae (Khan *et al.*, 2001). Cultural control practices are labor intensive but have little adverse effects on the environment and are readily available without

extra investment in equipment. Therefore, they are the most relevant and economic method of stemborer control available for resource poor farmers in Africa (Kfir *et al.*, 2002). Studies have shown that their impact on stemborer populations is limited due to lack of management capabilities of farmers in areas where farming communities lack the support of an adequate extension service (Harris, 1989).

Classical biological control targets exotic pests that have escaped regulation because they have been separated from their co-evolved natural enemies. It involves importation of exotic natural enemies of the pest from the place of origin of the pest and their establishment in the new habitat where the parasite has invaded. The introduced natural enemies attempt to re-establish the same ecological balance as occurs in the native home.

In East Africa, two biological control attempts have been made to increase the natural mortality of *C. partellus*. In the first biological control attempt, nine parasitoid species were imported from India and released in Kenya, Uganda and Tanzania from 1968 to 1972. None of the parasitoids became established (CIBC, 1968-1972). In the second attempt, International Center of Insect Physiology and Ecology (ICIPE) imported *Cotesia flavipes* Cameron, a gregarious larval endoparasitoid of several noctuid and pyralid stemborer from Pakistan into Kenya in 1991 for laboratory and field releases (Overholt *et al.*, 1994). *Cotesia flavipes* causes high levels of parasitism on *C. partellus* in Asia (Nagarkatti and Nair, 1973; Singh *et al.*, 1975) and in the neotropics where it was introduced against *Diatraea saccharalis* (F) in sugarcane (Macedo *et al.*, 1984).

In Kenya after laboratory studies, *C. flavipes* was released into the Southern coastal region in 1993 (Overholt *et al.*, 1994). Post-release studies conducted in the 1996 long rains cropping season showed that *C. flavipes* has successfully established

(Overholt, 1998; Zhou *et al.*, 2001). Recent investigations of the impact of *C. flavipes* on stemborer populations in coastal Kenya indicate that the parasitoid has established and has reduced stemborer density in overall by 32% and by 55% for *C. partellus* (Zhou *et al.*, 2001). Work done in western Kenya reveals that *C. flavipes* has also established there (Omweya *et al.*, 1995, 1997; Overholt, 1998). This parasitoid has been reported to have established in other countries in Africa like Uganda, Mozambique, Malawi and Tanzania (Matama- Kauma *et al.*, 2001; Cugala and Omweya, 2001; Omweya personal communication). In the laboratory, host range bioassay showed that *C. flavipes* attacks *C. partellus*, *B. fusca*, *E. saccharina*, *C. orichalcociliellus* and *S. calamistis* but does not complete development in *B. fusca* and *E. saccharina* (Ngi-Song *et al.*, 1995). The latter two pests are borers of economic importance with *B. fusca* being an important borer in high elevations. The establishment and effectiveness of the parasitoid varied from region to region due to differences in climatic conditions, stemborer species composition and inability to develop in the above mentioned borers (Ngi-Song *et al.*, 1998). Indigenous pupal parasitoids including *Pediobius furvus* (Gahan), *Dentichasmias busseolae* (Heinrich) and *Psilochalsis soudanensis* (Steffan) are widespread in Kenya but are not effective in controlling stemborers (Oloo and ogeda, 1990; Bonhof *et al.*, 1997). There is, therefore, a need for supplementary or even complementary parasitoids.

Xanthopimpla stemmator (Thunberg) (Hymenoptera: Ichneumonidae) was imported into Kenya by ICIPE from Sri-Lanka via South Africa for laboratory trials. *Xanthopimpla stemmator* is a multivoltine, polyphagous, idiobiont, solitary pupal endoparasitoid widely distributed in Asia/India, having been recovered from numerous pupae of lepidopteran stalk borers in India, the Philippines, West Malaysia, Mauritius, Sri-lanka and Taiwan (Moutia and Courtois, 1952). It attacks by drilling

the stalk and stinging the pupa with its stout ovipositor (Smith *et al.*, 1993). Laboratory host range bioassay at ICIPE, Kenya, revealed that *X. stemmator* would attack and fully develop in all the major borers in Eastern and Southern Africa (Gitau, 2002).

Xanthopimpla stemmator uses 'drill and sting' foraging strategy, a behaviour which is not shared by any common native pupal endoparasitoid. Hence *X. stemmator* can fill the largely vacant niche in Africa. Its ability to develop in many borers makes it a promising natural enemy and can boost other borer control strategies by complementing and increasing population suppression of the borer complex that may have escaped. It would be applicable in areas where *E. saccharina* and *B. fusca* are predominant.

1.2 LITERATURE REVIEW

1.2.1 Cereal stemborers

Stemborers have been considered to be the most important pests in cultivated poaceous plants in different geographic regions (Table 1.1). Most stemborers in Africa are indigenous with the exception of *C. partellus*, an exotic stemborer from Asia. *Chilo partellus* has proved to be a good colonizer in areas where it has invaded, often dominating and becoming the most economically important borer species in maize and sorghum at elevations below 1800m and sometimes invading cooler elevations (Overholt *et al.*, 2000, Kfir *et al.*, 2002). In Kenya *C. partellus* is becoming an important pest throughout Southern regions at elevations below 1500m and even in high elevations of up to 2300m and the semi-arid areas of Eastern Province (Songa, 1999; Zhou *et al.*, 2001). *Chilo orichalcociliellus* has been reported from coastal East Africa, Madagascar and Malawi (Bleszynsky, 1970; Delobel, 1975). *Chilo*

orichalcociliellus is an important pest in the Coastal area at elevations below 600m and in Taita Taveta (Seshu Reddy, 1983; Zhou *et al.*, 2001). Comparative studies have indicated that *C. partellus* has a competitive advantages over *C. orichalcociliellus*. Population growth rate of *C. partellus* was higher than that of *C. orichalcociliellus* on maize, sorghum and some wild hosts (Ofomata *et al.*, 1999a; 2000). Additionally, diapause terminates faster in *C. partellus* than in *C. orichalcociliellus* (Ofomata *et al.*, 1999b). These differences could explain the already reported displacement of *C. orichalcociliellus* by *C. partellus* in coastal Kenya.

The biology of *C. orichalcociliellus* is similar to that of *C. partellus*. The pre-oviposition period is 24 hours and eggs are oviposited on the upper and underside of leaves, mainly near mid-ribs or on the stems for two to three subsequent nights, in overlapping rows of 10-80 eggs. They are translucent when laid, but turn white on the first day and then become grey when they are about to hatch. They hatch in the early morning hours, 4-8 days after oviposition. Young larvae ascend on plants and enter the leaf whorl where they feed. Old larvae make vertical tunnels into stems and maize ears to feed for two to three weeks (Overholt *et al.*, 2001). Before pupation, larvae make exit holes and pupation takes 5-12 days (Overholt *et al.*, 2001). Adults live for about 2-5 days and do not disperse far from the emergence sites though females fly for a longer time than males (Pats and Wiktellus, 1989). Under favourable conditions, the life cycle is completed in 25-50 days.

Busseola fusca is a pest that is endemic to sub-Saharan Africa and is distributed throughout the maize and sorghum growing areas (Hill, 1975). Distribution varies from region to region. In Eastern and Southern Africa, *B. fusca* is a serious pest in mid-and- high elevation > 600m (Nye, 1960; Sithole, 1990; Harris and Nwanze, 1992). In West Africa, the same species is found predominant at mid and

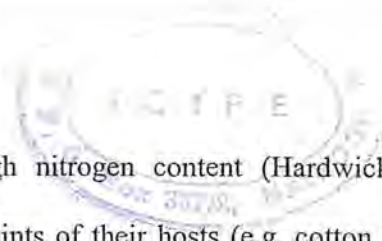
high elevations but, is also abundant in the drier savanna zone (Harris and Nwanze, 1992, Ndemah *et al.* 2001). In Kenya, *B. fusca* is a serious pest in areas above 600m, with its distribution being limited by temperature in warmer lowlands (Nye, 1960; Zhou *et al.*, 2001). According to Harris and Nwanze, (1992), there are local variations in the life cycle depending on the host plant and climate. The pre-oviposition period is 24 hours and female prefers to lay eggs between the leaf sheath and stalk. Oviposition takes place during 3-4 consecutive nights after emergence and eggs are laid in batches of 30-100 under the inner surface of leaf sheaths. Eggs hatch after one week and young larvae feed on young blades of leaf whorl and then spread to neighbouring plants by means of silk strands. Older larvae make vertical tunnels through the stems. Larval period takes 30-45 days in six to eight stages. Prior to pupation, larvae chew an outlet for the adult emergence and pupation takes 10-20 days (Overholt *et al.*, 2001). At the end of rainy season, larvae diapause or migrate to wild hosts.

Sesamia calamistis Hampson occurs throughout most of the tropical Africa and is a serious pest in cultivated cereals in West Africa. In Kenya, *S. calamistis* is distributed in all elevations from sea level to 2400m, from moist coastal regions across the transitional zone to the semi-arid areas of Eastern province (Seshu Reddy, 1983; Zhou *et al.*, 2001). Females lay eggs in two to four contiguous rows inserted between the lower leaf sheath and stems. Oviposition takes place during three to four days after emergence. Eggs hatch after one week and larvae tunnel vertically into the stem. Larval stage lasts between 30-60 days and there are five to six larval stages depending on climatic conditions. Pupation takes place in the stem tunnels or in folds of withered leaf sheaths or cobs. Pupation takes 10-14 days at 25°C (Overholt *et al.*, 2001).

Eldana saccharina occurs throughout sub-Saharan Africa (Overholt *et al.*, 2001). It is a pest of sugarcane in South Africa but also attacks maize usually towards the end of the growing season causing low damage both in East and West Africa (Bosque-Perez and Mareck, 1990). In Kenya, *E. saccharina* is distributed in the lake region area and in mid-elevations of western Kenya (Seshu Reddy, 1983; Zhou *et al.*, 2001). Females lay batches of 50–100 eggs on the dry leaves at the plant bases and on the plant debris on the soil or on the hairy margins of the maize leaf sheaths (Atkinson, 1980; Sampson and Kumar, 1985). They hatch in five to six days and the young larvae feed externally on the epidermal tissues before entering the stems. Larvae may move into ears and feed on the grains. Larval stages vary and may last 60 days. Pupation takes place in a cocoon made of silk and plants debris within the stem or in the cob. Prior to pupation, larvae chew adult exit holes for adults emergence on the plants (Bosque-Perez and Schulthess, 1998). Pupation takes 7-14 days.

1.2.2 Cereal cob borers

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) is a widely distributed pest occurring in Africa, the Middle East, Southern Australia, New Zealand and East Pacific islands. In Kenya, *H. armigera* is distributed in all elevations from sea level to highlands (Van den Berg, 1993). It is a polyphagous pest attacking diverse array of crops including maize, sorghum, cotton, chick pea, pigeon pea, tomatoe, soya bean, sunflower, rape seed and ground nuts (Reed *et al.*, 1982; Fitt, 1999). Larvae causes grain damage in maize and sorghum. Females lay eggs singly on plant structures; leaves, buds, flowers, stem and pre-tasseled maize (Wardlaugh *et al.*, 1980). Females prefer rough surfaces for their oviposition. Eggs are pearly white but turn black towards emergence. They hatch in 2–5 days and the young larvae prefer



feeding on plant structures that have high nitrogen content (Hardwick, 1965), principally the reproductive and growing points of their hosts (e.g. cotton buds and bolls, corn ears, tobacco buds and sorghum heads). Larvae often move about between feeding sites or between adjacent plants. The number of larval instar varies from five to seven, with six being most common (Hardwick, 1965). Developmental time depends on diet, temperature and 'vigour' (Kay *et al.*, 1979). Larval duration varies; in maize it is between 12-22 days at 24°C, and in cotton flower buds it is 21 days at a 21°C-27°C. On completion of growth, the fully fed larva enters the soil to pupate at a depth between 2.5cm-17.5cm depending upon soil hardness, but occasionally in surface litter or at the last feeding site on the plant. In Zimbabwe, pupation was recorded to occur in the tip of maize cob (CABI/EPPO, 1998). Pupation takes 6-14 days for non-diapausing pupae but several months for diapausing pupae. Adult longevity varies between 1-23 days for male and 5-28 days for female depending on availability of food, pupal weight, temperature and activity (Pearson, 1958). Under favorable conditions the life cycle is completed in 25-35 days.

Table 1.1: Important stemborer species in Africa and the Indian Ocean Islands, their distribution and major host plants.

Family	Species	Distribution	Host plant	Reference	
Crambidae	<i>Chilo partellus</i>	Eastern and Southern	maize, sorghum	Harris, 1990	
	<i>Chilo orichalcociliellus</i>	Coastal Eastern, Malawi, Madagascar, South Africa, Zimbabwe	Maize, sorghum	Bleszynsky, 1970	
	<i>Chilo aleniellus</i>	West and Central	rice, maize	Moyal and Tran, 1992	
	<i>Chilo sacchariphagus</i>	Indian Ocean Islands, Mozambique	Sugarcane Sugarcane	Williams, 1983 Van Rensburg, 1992	
	<i>Chilo zacconius</i>	West	Rice	Maes, 1998	
	<i>Chilo diffusilineus</i>	Tropical Africa	rice	Maes, 1998	
	<i>Coniesta ignefusalis</i>	Sahelian Africa	millet	Harris and Youm, 1998	
	Pyralidae	<i>Scirpophaga spp</i>	West	rice	Breniere <i>et al.</i> , 1962
		<i>Eldana saccharina</i>	sub-Saharan	Sugarcane rice, maize	Atkinson, 1979
<i>Maliarpha separatella</i>		sub-Saharan, Indian Ocean Islands	rice	Cook, 1997	
Noctuidae	<i>Busseola fusca</i>	sub-Saharan	Maize, sorghum	Harris and Nwanze, 1992	
	<i>Sesamia calamistis</i>	sub-Saharan	Maize, sorghum	Tams and Bowden, 1953	
	<i>Sesamia nonagrioides</i>	West, Sudan	rice	Tams and Bowden, 1953	
	<i>botanephaga</i>		Maize, sorghum		
	<i>Sesamia cretica</i>	NorthEast Africa	rice, sugarcane Maize, sorghum	Tams and Bowden, 1953	

Source; Kfir *et al.* (2002).

1.3 Parasitoids

Parasitoids of insects are classified by the developmental stage they attack. Using Mill's guild classification scheme, stemborers are attacked by egg endoparasites, egg-larval endoparasites, early-larval endoparasites, late larval endoparasites, larval ectoparasites, prepupal ectoparasites, larval-pupal endoparasites and pupal endoparasites (Smith *et al.*, 1993). Polaszek (1998) reviewed the taxonomic characteristics, distribution and host range of parasitoids of lepidopteran cereal stemborers in Africa. The pupal parasitoid species attacking Kenya's most important stemborers are listed in Table 1.2. Parasitoids contribute significantly to mortality at each of the host's developmental stage. Pupal parasitoids contribute more to intergenerational mortality of the stemborers (Rodriguez-Del-Bosque and Smith, 1997).

Several pupal parasitoids have been recovered from stemborers in East Africa and they contribute to the mortality of these pests. They are mostly indigenous species and cause low mortality. Studies done at the Kenyan coast showed pupal parasitism levels of 0%-10% on maize (Skovgård and Päts, 1996; Ogol *et al.*, 1998) but up to 58% in Western Kenya (Oloo, 1989). These parasitoids include *Pediobius furvus* (Gahan), *Psilochalcis soudanensis* (Steffan) and *Dentichasmias busseolae* (Heinrich) (Bonhof *et al.*, 1997). *Xanthopimpla citrina* (Holmgren) is an indigenous parasitoid found in Tanzania, Mozambique and Kenya (Lacroix, 1967). In surveys carried out in Kenya, only seven individuals of *X. citrina* were recovered (Overholt, unpublished), suggesting that this parasitoid is very rare. *Pediobius furvus* is a pupal parasitoid which is well distributed in varied climatical conditions in Africa (Mohyuddin and Greathead, 1970; Scheibelreiter, 1980), ranging from moist forest zone of Sierra Leone, Ivory Coast and Uganda with an equatorial humid climate to the sub-desert

steppe of the Sudan with a subtropical hot and arid climate to Woodlands, Savannas and forests Savanna of Nigeria, Zimbabwe and Kenya respectively (Nye, 1960). *Pediobius furvus* is a gregarious pupal endoparasitoid of several Pyralid and Noctuid graminaceous stemborers in East and Central Africa (Mohyuddin and Greathead, 1970). Mohyuddin (1968) working in Uganda reported that the parasitoid can cause up to 31% and 16% pupal mortality of *B. fusca* and *C. partellus* respectively. *Pediobius furvus* has been found to co-exist with *D. busseolae*, *Procerochasmias nigromaculatus* (Cam) and *Hyperchalcidia soudanensis* (Steffan) in northern Uganda and Kenya (Mohyuddin, 1968). In these areas, *P. furvus* is relatively common in pyralid and noctuid hosts. The female mates immediately after emergence and oviposition starts a few hours thereafter, and is largely completed within four days. The female drills the host in its habitat and drilling takes up to 15 minutes. The whole of the 0.65-mm-long ovipositor is inserted into the cuticle and oviposition may last up to five hours (Mohyuddin, 1968). The parasitoid is efficient in searching and parasitizing pupae with open adult exit hole and is reported to superparasitise and hyperparasitise parasitized pupae (Milner, 1967; Pfannenstiel, 1992). The developmental period ranges from 18-20 days at 30°C and 25-29 days at 27 ± 1°C.

Pupal parasitoids, like other parasitoids, are attracted to the prey by cues from the habitat itself (host plant), damaged plant volatiles, frass, pupae vibrations or pupae volatiles. Some pupal parasitoids use the 'ingress' and 'sting' foraging strategy. The mature host larvae construct adult moth exit holes (windows) prior to pupation. The pupal parasitoids use these 'open windows' to gain access into the pupal chamber and attack the concealed pupae. With intact windows, parasitoids use their mandible to cut through the window and gain access to the enclosed hosts. Examples of 'ingress and sting' pupal parasitoids include gregarious endoparasite *P. furvus*, *P. soudanensis* and

solitary endoparasite *D. busseolae*. The female *P. furvus* only parasitizes if it gets access to the pupa by entering through open or more fragile moth emergence windows (Pfannenstiel *et al.*, 1992).

Xanthopimpla stemmator is a exotic pupal parasitoid, whose attack strategy is different from those of other parasitoids. It parasitizes by piercing the stem directly with its stout ovipositor and reaching the pupa in the chambers, a method referred to as a 'drill' and 'sting' attack strategy, and which is not shared by any common native pupal endoparasitoid. Its ability to develop in many borers (Gitau, 2002) makes it a promising natural enemy and can boost borer control strategies by complementing and increasing population suppression of the borer complex that may have escaped earlier parasitism. This exotic endoparasitoid can be used in Kenya as an Old World association and New World association control strategy.

Table 1.2: Primary pupal parasitoids of the common cereal stemborers in Kenya.

Parasitoid	Host species	Reference
<u>HYMENOPTERA</u>		
<u>Braconidae</u>		
<i>Chelonus sp</i>	?	Mathez, 1972.
<u>Chalcididae</u>		
<i>Anthrocephalus mitys</i> Walker	Cp	Polaszek, 1995; 1998.
* <i>Brachymeria spp</i>	Co, C. sp	Lacroix, 1967; Mohyuddin and Greathead, 1970.
	Bf, Cp, Es	Overholt <i>et al.</i> , 1997
* <i>Brachymeria olethria</i> Waterson	?	Overholt, unpublished
<i>Psilochalcis</i> <i>soudanensis</i> Steffan	Bf, Cp, Es	Mohyuddin and Greathead, 1970.
Hyperchalcidia <i>soudanensis</i>	?	Ingram, 1958; Oloo, 1989; Minja, 1990 and Overholt, unpublished
<i>Invreia soudanensis</i>	Bf, Co, Cp, Es	Polaszek, 1995; 1998.
<u>Eulophidae</u>		
<i>Pediobius furvus</i> (Gahan)	Bf, Co, Cp, S.sp	Mohyuddin and Greathead, 1970; Mathez, 1972; Oloo, 1989 and Minja, 1990.
<i>Tetrastichus sp</i>	?	Overholt, unpublished
<u>Eurytomidae</u>		
<i>E.oryzivora</i> Delvare	Cp	Overholt, unpublished
<u>Ichneumonidae</u>		
<i>Dentichasmias</i> <i>busseolae</i> Heinrich	Cp, Co, B.sp	Ingram, 1958; Harris, 1962; and Mohyuddin and Greathead, 1970.
<i>Procerochasmias</i> <i>nigromaculatus</i> (Cameron)	Bf, Sc	Mohyuddin and Greathead, 1970
<i>Procerochasmias</i> <i>glaucopterus</i> Morley	Bf, Cp, Es, Sc	Zwart, 1998
<i>Xanthopimpla citrina</i>	Cp	Overholt, unpublished

*Incidental parasitoid or species of doubtful status; Bf = *Busseola fusca*; Co = *C. orichalcociliellus*; Es = *Eldana saccharina*; Sc = *Sesamia calamistis*; B.sp = *Busseola* species; C.sp = *Chilo* species.

Source: Polaszek, (1998); Zhou *et al.* (2003).

Xanthopimpla stemmator is a polyphagous solitary pupal endoparasitoid of lepidopteran stemborers. The adult female locates the habitat where hosts are found and later locates the pupal chamber in the stem. Initially, *X. stemmator* actively antennates at the place where the host is located, and then actively drills through the stalk. It punctures the pupa and deposits several eggs. Multiple parasitism is common and this leads to exude from the pupae, which it feeds on (Moore and Kfir, 1996; Smith and Wiedenmann, 1997). Multiple parasitism leads to deposition of several eggs into the pupa but only one develops into adult wasp. Pupation of the parasitoid occurs in the host chamber and adult parasitoid emerges from the pupal cases after killing the host (Nikam and Basarkar, 1981).

Females mate shortly after emergence. The male orients the antenna parallel to its body and the antenna releases a pheromone which calms the female as well as orienting the male in order to copulate (Webb, 1997). Females mate once but males can mate several females (Moore and Kfir, 1996). Females can reproduce parthogenetically producing male progeny only. The female has a pre-oviposition period of 3-6 days and an oviposition period of 64 days (Moore and Kfir, 1996). The female can produce 95 offspring during its lifetime with 65% comprising of female progeny. Moutia and Courtois (1952) recorded the life cycle of the parasitoid to be 17 days at 24.5°C and the female lived longer than the male. Moore and Kfir, (1996) found that when *X. stemmator* parasitoids were provided with honey and water without hosts, females lived for about 140 days and males for 87 days at $24 \pm 2^\circ\text{C}$ and 60%-70% relative humidity. Gitau (2002) found that when *X. stemmator* parasitoids were provided with 10% honey and water, at $27 \pm 2^\circ\text{C}$ and 49%-61% relative humidity and 12:12 (L.D.) the female had an average longevity of 44 days. While, Nikam and Basarkar (1981) found the average longevity of the mated females to be

30 days (Maximum 37 days and minimum 22 days) at $22 \pm 1^\circ\text{C}$ and 50%-55% R.H. Life tables and intrinsic rate of natural increase of *X. stemmator* studied using *C. partellus* as a host was carried out in India at $22 \pm 1^\circ\text{C}$ and 50%-55% R.H. The rate of increase was found to be 0.131 and population multiplied 43.43 times in the mean generation time of 28.78. The male to female ratio averaged 1.14:1. Progeny production ranged from 71 to 115 days with an average of 84.5 days (Nikam and Basarkar, 1981). The adult longevity was affected by temperature, availability of water and nutrients. Under natural conditions access to water and sugar solutions accompanied by a combination of rain or dew, honey dew and host feeding provide sustenance for increased female longevity (Hailemichael *et al.*, 1994).

1.3.1 Distribution of *Xanthopimpla stemmator*

Xanthopimpla stemmator is widely distributed across Asia having been recovered from Noctuids and Pyralids in Indonesia, Sri Lanka, Malaysia, Philippines and Taiwan (Moutia and Courtois, 1952). It has been recovered from many lepidopteran stemborers of poaceous plants including *C. partellus* in India (Vinson, 1942), *Chilo infuscatellus* (Snellen), *Eucosoma scistaceana* (Snellen), *Ostrinia nubilalis* (Hubner), *Scirpophaga novella* (F.) and *Sesamia inferens* (Walker) in Taiwan (Sonnan, 1929; Takano, 1934); *Argyroplote rhynchias* (Meyer) in Mauritius (Moutia and Mamet, 1945), *Ostrinia furnacalis* (Guenee) in west Malaysia (Yunus and Hua, 1969) and Philippines (Camarao, 1979; Cartwright, 1993). *Xanthopimpla stemmator* has successfully established in Mauritius after its introduction from Sri Lanka against *Chilo sacchariphagus* and *S. calamistis* (Moutia and Courtois, 1952; Moutia and Mamet, 1945). It was introduced into Reunion from Mauritius with success against *C. sacchariphagus* in sugarcane (Caresche, 1962). It also has been

introduced into South Africa against *E. saccharina* in sugarcane (Conlong, 1994) and against *C. partellus* in maize and sorghum fields (Kfir, 1988). More releases have been made in KwaZulu Natal against *E. saccharina* in sugarcane fields (Moore and Kfir, 1996), maize and sorghum fields between 1987 and 1993 but few recoveries were made within the vicinity of the release sites (Conlong, pers. comm.). In South Africa *X. stemmator* has practically not established itself in all release sites against the target borers. The failure of its establishment was attributed to its failure to diapause during winter and lack of alternative hosts (Kfir, 1988). Recent releases have been made in Mozambique against *C. sacchariphagus* in sugarcane and post-release surveys are going on to evaluate its possible establishment.

1.3.2 Successful parasitization process

Successful parasitization requires a sequence of distinct and consecutive processes that involve the parasite, stemborer host stage and the host plant (Vinson, 1984; Lewis *et al.*, 1990). Initially the adult female parasite locates the habitat that should harbor stemborers, and then she must locate the specific host stage within the habitat. Once the host is located, it must be acceptable for oviposition, be suitable for development of the parasite progeny. The parasite must be able to avoid the host immune system and regulate the host hormonal system. Organizing and implementing biological control of stemborers relies heavily upon an understanding of these general processes.

1.3.3 Habitat finding

Habitat selection is the first of the processes that affect the host utilization. Many parasites use cues from the habitat itself, independent of whether hosts are

present or not. These are long distance cues which are usually visual or volatile odor, that serve to orient a parasite to a habitat in which the host might be found (Smith and Wiedenmann, 1997). The braconid parasite *Asobara tabida* (Nees), is attracted to the odors from either fruit or decaying leaves where the host is found (Vet *et al.*, 1984). Smith and Wiedenmann, (1997) observed that *X. stemmator* drills its ovipositor through the stem of a grass, and oviposits into an enclosed lepidopteran pupa. The parasite is not attracted to naked pupae (Hailemichael *et al.*, 1994). However, it does respond to the presence of a plant stem, especially that of a crop like corn or responds to similarly shaped artificial 'stems' such as a paper straw but less responsive to horizontally orienting 'stems' (Smith and Wiedenmann, 1997; Fischer, *et al.*, 2003). Stemborer feeding may induce plants to release volatile chemicals (infochemicals) that attract stemborer parasitoids (Ngi-Song *et al.*, 1996).

1.3.4 Host finding

The cues responsible for host finding are associated with the cryptic habitat of the stemborer pupae, rather than the particular host species. Hailemichael *et al.*, (1994) examined the host finding behavior of *X. stemmator*. Cues exploited by the parasitoid for host finding include kairomones such as host frass and host odor together with pupal movement or response due to compounds emitted by tunneled/injured plant by stemborer or physical cues such as 'deadheart', host frass, exit window and larval tunnel. Feeding by the hosts stimulate release of synomone that attracts stemborer parasitoid (Vet and Dicke, 1992 ; Potting *et al.*, 1995).

Xanthopimpla stemmator alights on the stems or straws and actively moves up and down the vertical stem, antennating the surface near exit holes, searching cues that indicate host presence. Larval frass helps in guiding *X. stemmator* to the

microhabitat of the host pupa and stimulates ovipositor drilling. Pupal movements, sound, odor and vibrations associated with its activity directs *X. stemmator* to enclosed hosts (Hailemichael *et al.*, 1994; Fischer, *et al.*, 2003). These cues restrict local searching to help pinpoint the location for ovipositor drilling. The actual location of the pupae, stem diameter and rind thickness varies with plant species (Smith and Wiedenmann, 1997; Smith *et al.*, 1987). Most native grasses have small diameters and thin rind but cereal crops and sugarcane have thick stem diameters and thick rind (Smith and Wiedenmann, 1997; Smith *et al.*, 1987). Surveys done indicate that maize stalks have a diameter ranging between 3-5 centimeters while native grasses often have a diameter less than 1 centimeter (Smith and Wiedenmann, 1997; Smith *et al.*, 1987). Stemborer larvae tunneling and pupating in maize stems are 2-5 times far from the stalk periphery than when in native grass stems. However, dimensions of the moth-exit, the width of the tunnel and distance between the moth-exit and head of the pupa are greater for *B. fusca* and *S. calamistis* than for *C. partellus* (Mohyuddin, 1972). For effective parasitism of such borers in pupal stage, ovipositor length of parasitoids using 'drill' and 'sting' attack strategy has to be long enough to reach the host. Effectiveness of *X. stemmator* parasite that uses 'drill' and 'sting' attack strategy may be limited in host pupae in large-stemmed grasses when pupal chambers are constructed near the centre of the stem (Hailemichael *et al.*, 1994). So far there is no published information on the distance between the moth-exit and head of the pupa and pupal depth in relation to stem edge in target gramineous plants.

1.3.5 Host preference

Host species have a substantial influence on whether potential hosts are attacked by parasitoids. *Chilo partellus* is a natural host of *X. stemmator* in its original environment (Vinson, 1942). Gitau (2002) found that *X. stemmator* completed development in *C. partellus*, *C. orichalcociliellus*, *B. fusca* but *E. saccharina* pupae were less suitable for its development. The choice of suitable candidate species or strain is essential for successful biological control programs because the host-specific characteristics of candidate species are among the factors determining their effectiveness as control agents. Use of biological control agents has raised concern on their impact and effect to the environment. Due to these concerns host range tests are carried out to provide a sound basis of predicting the field host range of the organism in its new geographic region (Wapshere, 1974). Organisms introduced in biological control programs have the potential to affect host or affect, either directly or indirectly the associated species, including non target species (Howarth, 1991). The outcome of these encounters range from negligible impacts to extinctions. Roperts and Cameron (1990) established that the decline of some endemic moths in forests was due to the introduction of exotic Lepidoptera parasites, tachinid *Trigonospila brevifavies* and ichneumonid *Glabrodorsum stokesii* into New Zealand. These parasites have spread far from their target habitats on farms and orchards and now occur in native forests competing with native species in North America (Ehler, 1990). Effectiveness is likely to decline if certain minor pest species are preferred over major pest species. Currently, there is no published information on minor host preference by *X. stemmator*.

1.3.6 Host acceptance and suitability

Host acceptance requirements may include host size, shape, age, odor, texture and previous parasitization status. The ovipositing female parasite selects hosts that are suitable to its development and those, which will provide adequate shelter and nutrients for complete development of the parasite. *Xanthopimpla stemmator* readily accepts pupae enclosed in a stem or wrapped tissue but rarely accepts pupae that are naked (Smith *et al.*, 1993). The parasite drills with its stout ovipositor through the stem wall or leaf sheath enclosing the host and parasitizes the pupa. After drilling into the host with the ovipositor, females may accept or reject the host for oviposition (Ueno, 1999). Host acceptance is defined by the presence of external punctures from the parasitoid ovipositor (Hailemichael *et al.*, 1994). Studies done on *X. stemmator* showed that, of the 25 probed hosts dissected, 96% contained at least one parasitoid egg and 88% contained many eggs (Hailemichael *et al.*, 1994, Gitau 2002). Studies done on the parasitoids show that the number of individuals that can survive in a single host is limited because multiparasitism causes larval competition in that host (Vinson and Iwantsch, 1980a; Strand, 1986 ; Mackauer, 1990).

Host suitability is defined as the adequacy of a host, once oviposition has occurred, to successfully support growth and development of the parasitoid progeny. (Vinson and Iwantsch 1980a). Several factors affect the development of parasitoid inside a host, among these include host defense system, competition among parasitoids, presence of toxins, host nutritional inadequacy and host developmental stage (Vinson and Iwantsch 1980a). Presence of competitors inside the host can render a host unsuitable for a parasitoid. High larvae numbers of solitary endoparasitoid are eliminated by physical attack or physiological suppression (Vinson and Iwantsch, 1980b). Gregarious species rarely show inclination to be aggressive

(Waage, 1985). Successful parasitization is defined as the proportion of parasitized pupae yielding adult parasitoids. Multiparasitism is found frequently in nature and in the laboratory (Vinson and ables, 1980 ; Pijls *et al .*, 1995; Ngi-Song *et al .*, 2001). Polyphagus pupal parasitoids will often encounter hosts parasitized by heterospecific parasitoids at different time intervals between encounters, because there are usually several pupal parasitoid species associated with the same host species. No study has so far been published on multiparasitism between *X. stemmator* and *P. furvus* on *C. partellus*.

1.4 RATIONALE OF THE STUDY

Maize and sorghum are major cereal food crops to the majority of households in Kenya and are grown primarily by subsistence small-scale farmers (Pangali, 2001). Lepidopteran cereal stem and cob borers are the major causes of low farm cereal grain yields. Several control strategies have been used to reduce losses by the borers but with limited success. Chemical control which is the mainstay control strategy is effective but due to its continuous use, chemicals have become harmful to human, non-target animals, environment and are also expensive for the resource poor subsistence farmer. Its effectiveness is also limited due to cryptic feeding of the larvae, development of resistance by the pests and protection provided for by the host plant to immature stages (Khan *et al.*, 2001). A biological control program for the cereal stemborers was carried out in Kenya; it used a gregarious larval parasitoid, *C. flavipes* with success (Overholt, 1998). The impact of *C. flavipes* on the stemborer population in Coastal Kenya indicates that the parasitoid has established and has reduced stemborer density in overall by 32% and by 55% for *C. partellus* (Zhou *et al.*, 2001). Laboratory host range tests indicated that *C. flavipes* attacks *C. partellus*, *B. fusca*, *E. saccharina*, *C. orichalcociliellus* and *S. calamistis* but does not complete its development in *B. fusca* and *E. saccharina* (Overholt *et al.*, 1994; Ngi-Song *et al.*, 1995). These two, are borers of economic importance with *B. fusca* being the most important borer at high elevations. Establishment and effectiveness of the parasitoid varies from region to region due to differences in climatic conditions, stemborer species composition and inability to complete development in the above mentioned borers (Ngi-Song *et al.*, 1998).

Xanthopimpla stemmator attacks the borer pupal stage by using 'drill' and 'sting' foraging strategy, behavior not shared by any common native pupal

endoparasitoid. Hence, *X. stemmator* can fill the largely vacant niche in Kenya and increase population suppression of borer complex that may have escaped parasitism in the previous developmental stages.

For effective use of *X. stemmator* in suppressing borer population, knowledge and understanding of its effects on minor lepidopteran pests is needed. Being an introduced natural enemy, interaction with the indigenous pupal endoparasitoid *P. furvus* and information on the actual location of the pupae in the host plant is needed. This will uncover the possible effectiveness of using *X. stemmator* in the borer control.

1.5 NULL HYPOTHESES

1. There is no difference in *Busseola fusca*, *Chilo partellus* and *Sesamia calamistis* pupal position in the gramineous stems.
2. *Xanthopimpla stemmator* has no preference for stem and cob-borers in gramineous plants.
3. *Xanthopimpla stemmator* does not compete with the indigenous pupal parasitoid *P. furvus* for *C. partellus* pupae.

1.6 OBJECTIVES

1.6.1 General objective

The general objective of this work was to evaluate the efficacy of *Xanthopimpla stemmator* for use in the classical biological control program of cereal stemborers in Kenya.

1.6.2 Specific objectives

1. To determine the location of *Busseola fusca*, *Chilo partellus* and *Sesamia calamistis* pupae in different host plants.
2. To examine the preference and acceptance of *X. stemmator* for three borer species.
3. To evaluate the suitability of the minor stemborers for the development of *Xanthopimpla stemmator* in gramineous plants.
4. To study the possible interaction between *Xanthopimpla stemmator* and the indigenous pupal parasitoid *Pediobius furvus* in the laboratory.

CHAPTER TWO

2.0 GENERAL MATERIALS AND METHODS

2.1 Field sites

Field surveys to determine location of stemborer pupae in cultivated host plants (*Z. mays* and *S. bicolor*) and wild host plants (*Pennisetum purpureum*, *Sorghum versicolor* and *Hypperrhenia rufa*) were conducted in Kwale District, Coast Province and Trans Nzoia District, Rift Valley Province (Figure 2.1) of Kenya during long and short rain cropping seasons of year 2002. Kwale district located in the lowland area lies along longitude 0 39° 33.41'E and latitude 0 4° 18.95'S and at 40m above sea level. Rainfall distribution pattern is bimodal occurring between April and August (long rains) and between October and December (short rains). It receives a total annual rainfall of between 700mm–1900mm. Soils are sandy. The average temperature ranges between 24.5°C–27.5°C. The exotic stemborer *C. partellus* and two indigenous stemborer species, *C. orichalcociliellus* and *S. calamistis* are more prevalent in low altitude areas (Mathez, 1972; Warui and Kuria, 1983). Trans Nzoia district, located in the highland area lies in latitude 0° 51N and 1° 18N, longitude 34° 38'E and 35° 23'E and at an altitude of 2000m above sea level (Jaetzold and Schimidt, 1982). Rainfall distribution follows a bimodal pattern with long rains typically occurring from March to June and short rains from July to October. Soils range from mountainous well drained dark reddish brown, rocky and stony clay to bottom land soils that are poorly drained, dark brown clay loam to clay. Average annual precipitation is 1296 mm and temperature varies between 10°C–37°C. *Busseola fusca* is more prevalent at altitudes above 800m (Harris and Nwanze, 1992).

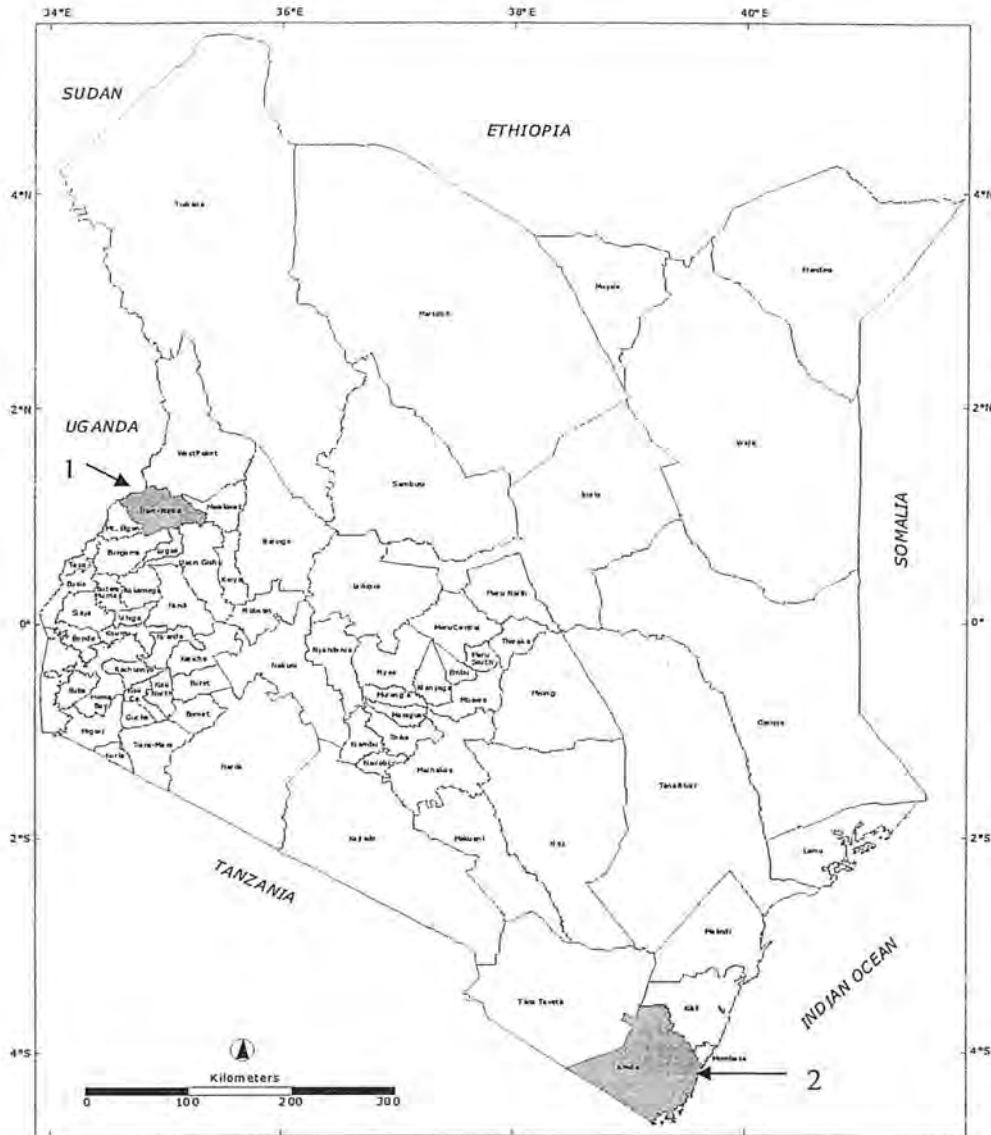


Fig: 2.1 Sampling sites (shaded); (1) in the West Trans Nzoia district

(2) in the East Kwale district

2.2 Experimental insects

2.2.1 Stem and cob-borers

Two species of stemborers *Chilo partellus* and *Sesamia oriaula* and one cob-borer species *Helicoverpa armigera* (Hübner) were used in the laboratory studies. *Chilo partellus* larvae were collected from the *Z. mays* fields while *S. oriaula* larvae were collected from *P. purpureum* fields at Kwale, South Coast. *Helicoverpa armigera* larvae were collected from *Z. mays*, *S. bicolor* and sunflower fields at Mwea, Central Province Kenya. All the collected borers were reared in the Animal Rearing and Quarantine Unit (ARQU) at the International Centre of Insect Physiology and Ecology (ICIPE) Duduville, Nairobi, Kenya. *Chilo partellus* larvae were reared on artificial diet (Ochieng *et al.*, 1985). *Sesamia oriaula* larvae were maintained on *P. purpureum* stems while *H. armigera* larvae were maintained on a natural diet comprising of over-night soaked market grade chickpea seeds (Plate 2.1). All borers were maintained at temperature of $26 \pm 1^\circ\text{C}$, 50% - 80% R.H. and 12:12 (L.D.) h photoperiod until pupation.

2.2.2 Parasitoids

The exotic pupal endoparasitoid *X. stemmator* was imported from Sri Lanka via South Africa reared at the ARQU, ICIPE. Rearing was done in clean Perspex cages (15cm x 15cm x 15cm) fitted with mosquito net on one side, to allow free air circulation (Plate 2.2). Parasitized *C. partellus* pupae were then kept in petri dishes (8cm in diameter) until adult parasitoid emergence (Plate 2.3). Since *X. stemmator* females do not attack naked pupae, they were offered pupae in paper straws smeared with frass from respective stemborer species to enhance acceptance.(Plate 2.4)

The indigenous pupal endoparasitoid *P. furvus* from *C. partellus* collected from Kwale was reared on the same host in ARQU. *Pediobius furvus* was offered naked *C. partellus* pupae in a clean Perspex vial 10cm x 2.5cm (Plate 2.5). Upon emergence, the adult parasitoids were kept in ARQU and provided with 20% sugar-water solution dispensed on cotton wool for nourishment. Parasitoids were maintained and used for experiments at the temperature of $26 \pm 1^\circ\text{C}$, 50% - 80% R.H. and of 12:12 (L: D) h photoperiod.

2.3 Host plants

Maize (hybrid 512) and Napier grass (originary from Kwale) were grown in the fields at ICIPE Duduville farm, while chickpea seeds were bought from a super market. These were used to produce frass whenever required during the experiments.



Plate 2.1 : *H. armigera* larvae with chickpea seeds in Petri-dishes



Plate 2.2 : *X. stemmator* adult parasitoids in rearing cage (15cm x 15cm x 15cm)



Plate 2.3 : Parasitized *C. partellus* pupae in Petri-dishes



Plate 2.4 : Paper straw smeared with frass and attached to base of cage with clay substrate and a female *X. stemmator* searching for the pupae.



Plate 2.5 : *Chilo partellus* pupa in Perspex vial with *P. furvus* inside.

CHAPTER THREE

3.0 LOCATION OF CEREAL STEMBORER PUPAE IN THE SELECTED HOST PLANTS

3.1 Introduction

Location of pupae in relation to stem periphery and exit hole may influence effective parasitization by *X. stemmator* which uses 'drill' and 'sting' attack. Silica and lignin contents influence the hardness of stems (Sétamou *et al.*, 1993). Stems with high content of silica and lignin are harder than those with low contents. For effective parasitization of stemborers, pupae need to be near the edge of the stem, and the stem should be soft for easy penetration by the pupal parasitoid's ovipositor. The parasitoid's ovipositor also needs to be long enough to reach the host. The effectiveness of *X. stemmator* parasitization may be limited in large-stemmed grasses when pupal chambers are constructed near the centre of the stem and when stems are very hard (Hailemichael *et al.*, 1994). The objective of this study was to determine the location of *B. fusca*, *C. partellus* and *S. calamistis* pupae in the selected host plants so as to predict *X. stemmators*' effectiveness in the field.

3.2 MATERIALS AND METHODS

3.2.1 Field surveys

Field surveys were carried out in Kwale and Trans Nzoia districts (Fig. 2.1) during the long and short rains of 2002. *Zea mays* fields were established at each sampling site in the two districts. Using a plant spacing of 90 cm between the rows and 30 cm between the plants, *Sorghum bicolor* was intercropped with *Z. mays*, while *P. purpureum* was planted in strips around the farm. *Sorghum versicolor* and *H. rufa*

grew naturally near the cultivated fields (Plates 3.1-3.4). Participating farmers planted *P. purpureum* two weeks before planting. *Zea mays* and *S. bicolor*. *Zea mays* variety 614 was planted in Trans Nzoia while coast composite variety was planted in Kwale. Trans Nzoia has only one cropping season that extends the two rain seasons. All the recommended agronomic practices for the crops were followed with no insecticide application.



Plate 3. 1 : *S. bicolor* intercropped with *Z. mays*

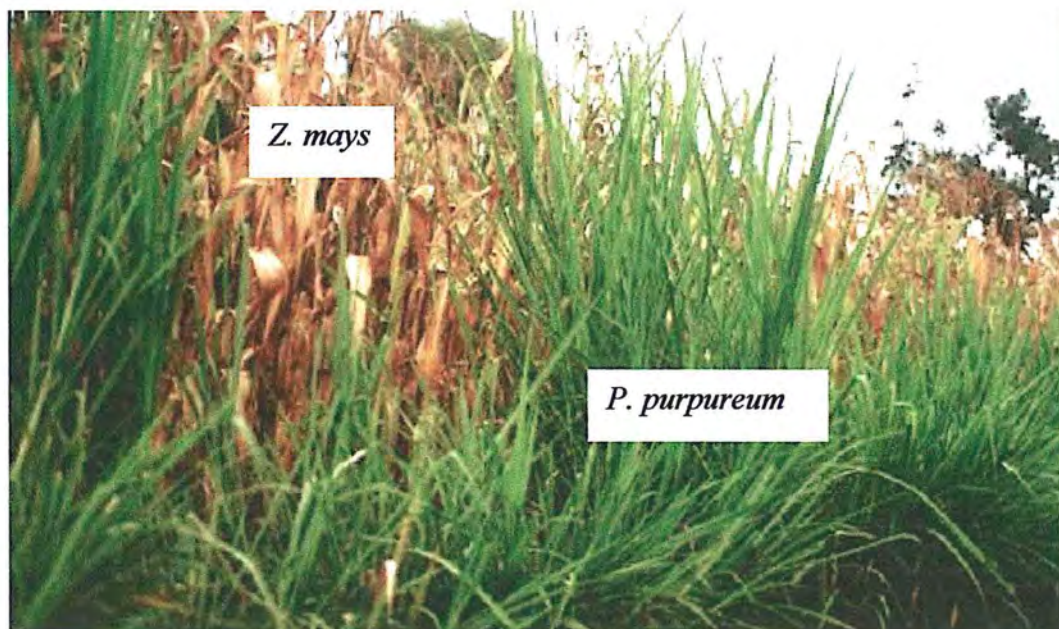


Plate 3.2 : *P. purpureum* planted in strips near *Z. mays* fields.



Plate 3.3 : *S. versicolor* growing near *Z. mays* fields.



Plate 3 .4 : *H. rufa* growing near maize fields.

3.3.2 Host plants

Five poaceous plants were sampled for pupae. In Kwale, the sampled plants included, *Z. mays*, *S. bicolor*, *S. versicolor*, and *P. purpureum* while in Trans Nzoia they included, *Z. mays*, *S. bicolor*, *P. purpureum* and *H. rufa*.

3.3.3 Sampling protocol

The same sampling protocol was used during the two seasons of this study. Ten fields were randomly surveyed along the road at 10 ± 1 km interval. Twenty plants of each species were randomly selected from one field. A total of four hundred plants of each plant species were sampled during the two cropping seasons. Systematic sampling method was used for small plots while stratified systematic

sampling method was applied for large plots (Southwood, 1995). Destructive sampling technique was used whereby plants were uprooted. Stems' length was divided into three proportions, top, middle and bottom from their bases. The sheaths silk and ears were checked for borer pupae. Each plant stem was carefully dissected from the base of the stem to the top most part to recover pupae inside. Depth (pupa position in relation to edge of stem), location (pupa distance between the moth-exit and head of the pupa), tunneling length (length of tunnel from moth-exit to the base of the tunnel) and position of the pupa in relation to the height of the stem were measured for every pupa found in the different plant species (Fig 3.1). Each pupa dead or alive and pupal cases were put in individual vials and taken to the laboratory. Live pupae were maintained in the incubators at $26^{\circ} \pm 1^{\circ}\text{C}$, 50% - 80% R.H. and 12 : 12 (L:D.) photoperiod until emergence. All the pupal cases recovered and emerged adults were recorded and taken to BioSystematics Unit at ICIPE Duduville for identification.

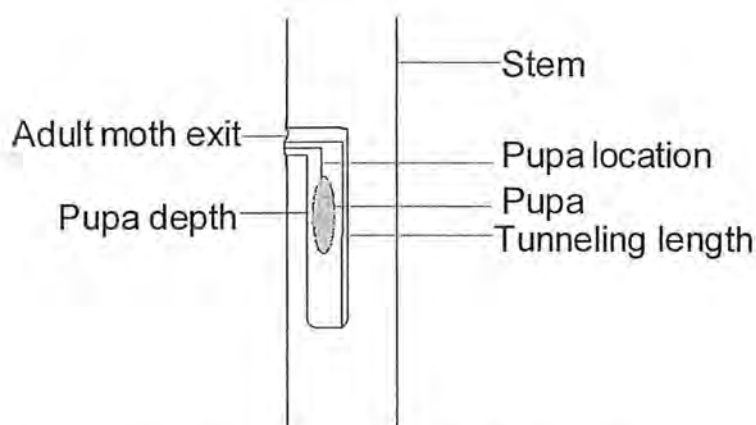


Fig 3.1 : Location of stem borer pupa in the host plant

3.3.4 Data analysis

Data obtained on the locations of pupae were averaged for each field and pooled together for the two seasons. Prior to analysis, data were log-transformed for normalization. Analysis was done using one way analysis of variance (ANOVA) (PROC GLM, SAS Institute, 2000). Means were compared using the multiple comparison LSD Bonferroni test when ANOVAs were significant.

3.4 Results

Chilo orichalcociliellus, *C. partellus*, *S. oriaula* and *S. calamistis* were the borer species recovered from the four plant species in Kwale district during the long and short rain cropping seasons. During both cropping seasons in Kwale, *C. partellus* was the most abundant borer species found in the four host plants (Figure 3.2 and 3.3). Few *C. orichalcociliellus* and *S. calamistis* were recovered from the sampled plant species. *Sesamia oriaula* pupae were only collected from *P. purpureum*. In Trans Nzoia, only *B. fusca* was recovered from *H. rufa*, *S. bicolor* and *Z. mays* and no pupa was collected from *P. purpureum* (Fig. 3.4). Most pupae were located in the middle to upper part of the stem except in *P. purpureum*. On maize and sorghum 59% and 43% of *S. calamistis* and *C. orichalcociliellus* pupated in the maize cob, respectively. *Sesamia oriaula* pupae were exclusively recovered from the lower part of *P. purpureum* stems (Fig. 3.5-3.9).

There was no significant difference in the tunneling length for *C. orichalcociliellus* in *Z. mays* and *S. bicolor* (Table 3.1). *Chilo partellus* pupae which were collected from three plants species did not show any significant difference in tunneling length (Table 3.2). *Sesamia oriaula* pupae were only collected from *P. purpureum* in Kwale and had a mean tunneling length of 5.7 cm hence not included in

the analysis. Few *S. calamistis* pupae were collected from *S. bicolor* and *Z. mays* (results not shown). There was no significant difference in tunneling length in *S. bicolor* and *Z. mays* (Table 3.3). Tunneling length made by *C. orichalcociliellus*, *S. calamistis* and *C. partellus* in *S. bicolor* did not show any significant difference (Table 3.4). However, these same borers' tunneling lengths were significantly different in *Z. mays* (Table 3.5). *Chilo partellus* pupae were the only borer pupae found in *S. versicolor* and had a mean tunneling length of 4.3 ± 0.3 cm.

The pupal depth and pupal location of *C. orichalcociliellus* in *Z. mays* and *S. bicolor* also did not show any significant difference (Table 3.1). For *C. partellus*, pupal depth in *Z. mays* was significantly deeper (Table 3.2) than that of the borer in *S. bicolor* and *S. versicolor*. Location of pupae showed similar trend (Table 3.2). Similarly, *S. calamistis* pupal depth and pupal location in *S. bicolor* and *Z. mays* showed no significant differences (Table 3.3). Pupal depth and pupal location of *C. orichalcociliellus*, *S. calamistis* and *C. partellus* in *S. bicolor* did not also show any significant difference respectively. Pupal depth for the three borers in *Z. mays* showed similar trend (Table 3.5). However, pupal location for the three borers in *Z. mays* was significantly different (Table 3.5). *Chilo partellus* pupae were the only borer pupae recovered in *S. versicolor* and had a mean pupal depth of 0.1 centimeter.

Results obtained during the long and short rains cropping seasons in Trans Nzoia showed that *B. fusca* was the only borer species collected from three of the four plant species sampled (*H. rufa*, *S. bicolor* and *Z. mays*). Tunneling length resulting from this borer species was significantly longer (Table 3.6), in *S. bicolor* than in other plant species, while *H. rufa* had a significantly shorter tunneling length than the rest (Table 3.6). Pupal depth in the three plant species showed no significant difference.

Pupae found in *H. rufa* had a significantly shorter pupal location than in *S. bicolor* and *Z. mays* which showed no significant difference (Table 3.6).

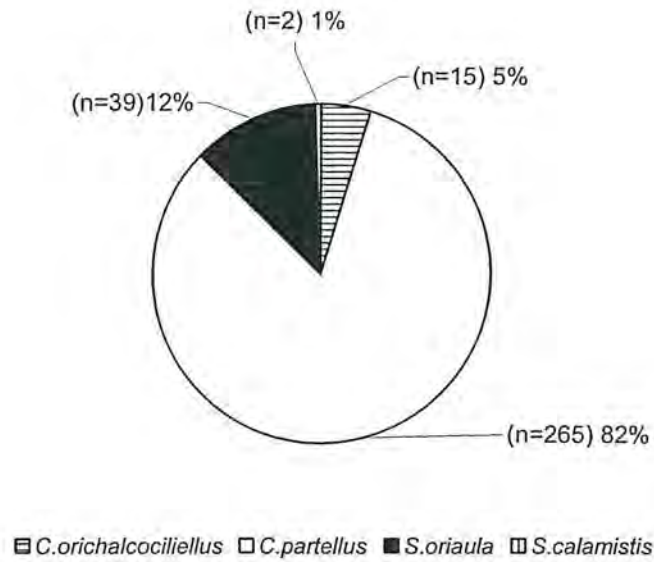


Fig. 3.2: Relative abundance of pupal stemborers in different plants in Kwale (long rains)

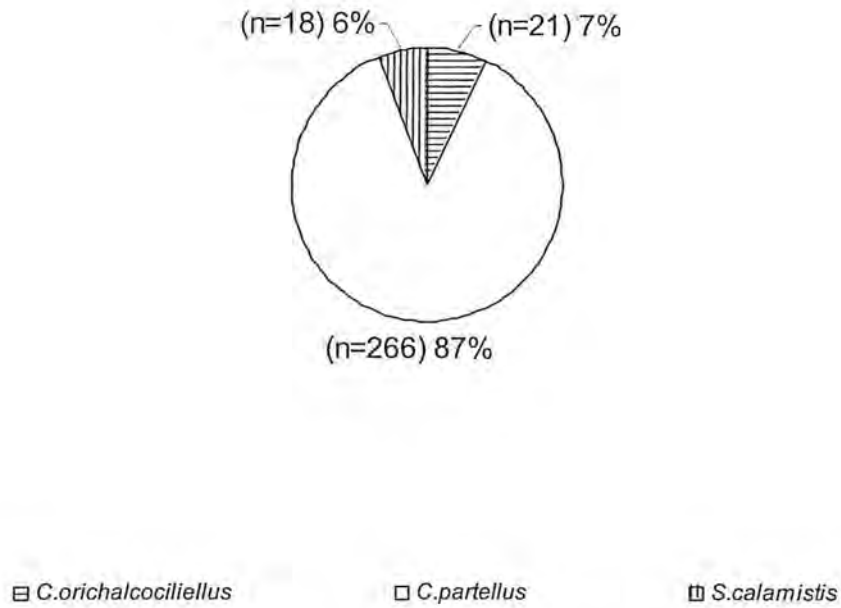


Fig. 3.3: Relative abundance of pupal stemborers in different plants in Kwale (Short rains)

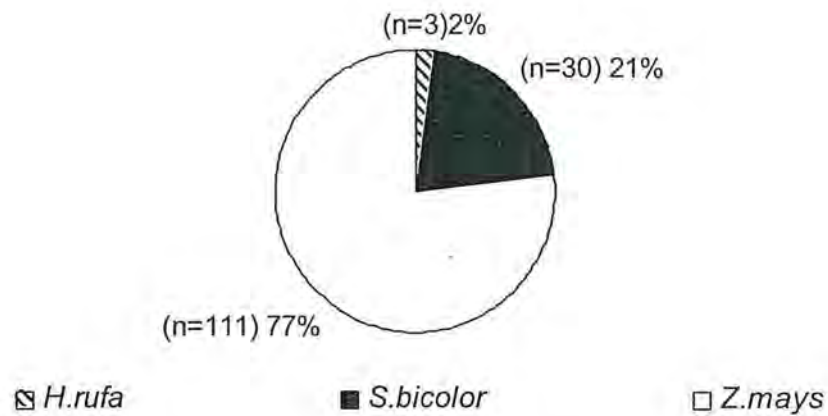


Fig. 3.4 : Relative abundance of *Busseola fusca* in different plants in Trans Nzoia (Short and long rains)

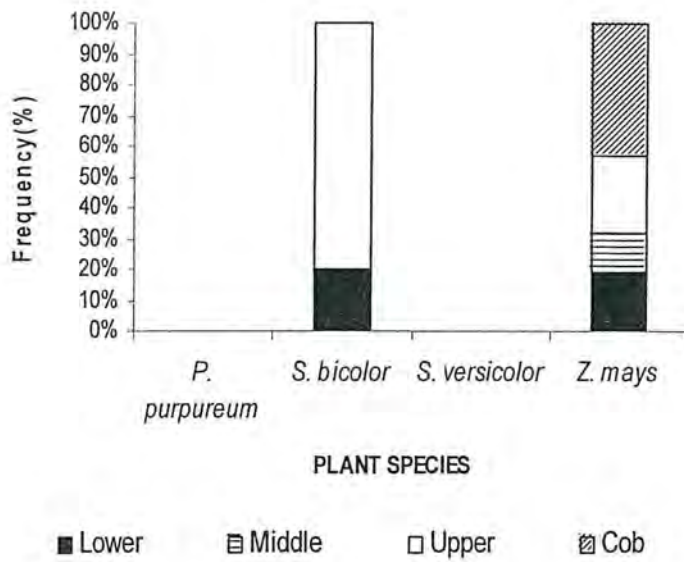


Fig. 3.5 : Frequency of *Chilo orichalcociliellus* pupal locations in different plants parts in Kwale

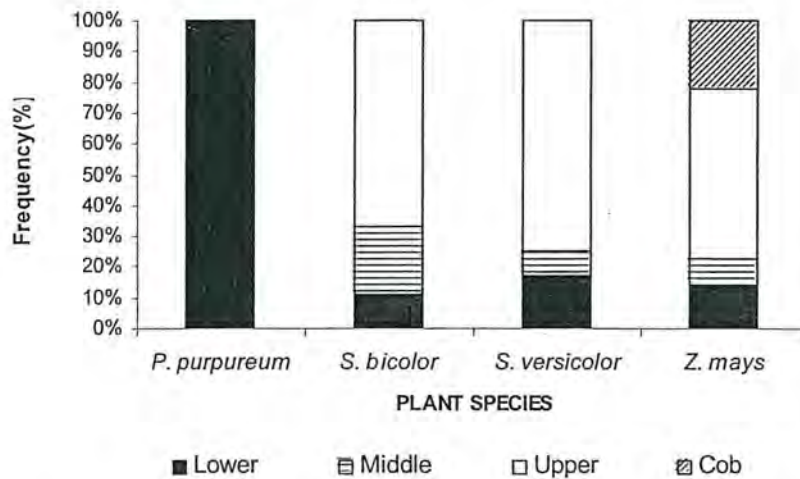


Fig. 3.6 : Frequency of *Chilo partellus* pupal locations in different plants parts in Kwale

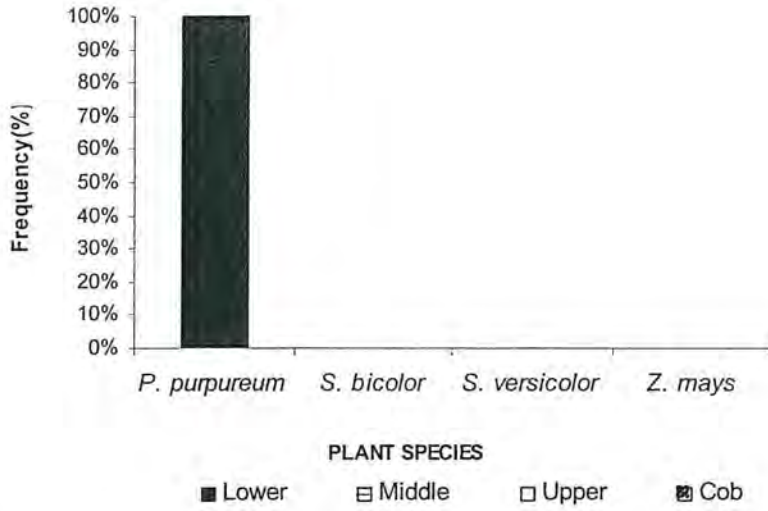


Fig. 3.7 : Frequency of *Sesamia oriaula* pupal locations in different plants parts in Kwale

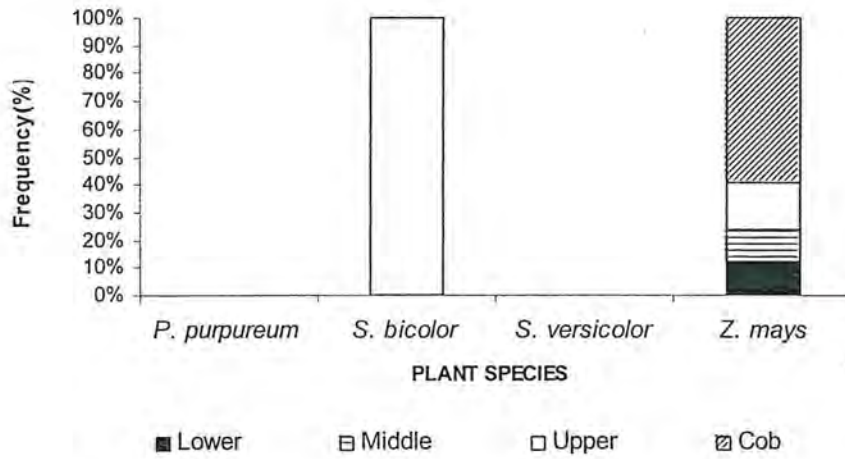


Fig. 3.8 : Frequency of *Sesamia calamistis* pupal locations in different plants parts in Kwale

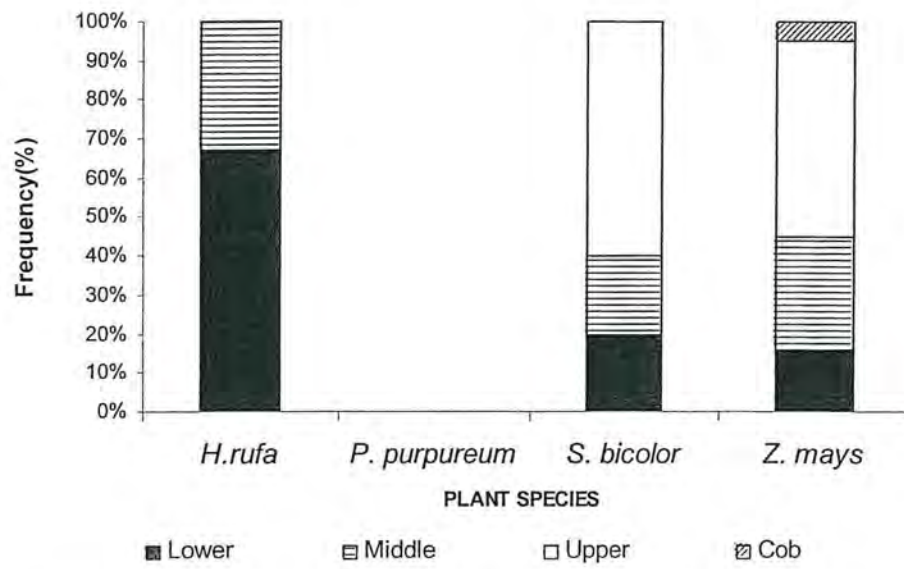


Fig. 3.9 : Frequency of *Busseola fusca* pupal location in different plants parts in Trans Nzoia

Table 3.1: Position (cm) of *C. orichalcociliellus* (Co) pupae in stems of two host plant species in Kwale

Borer species	Plant sp	N	Tunneling length	Pupal depth	Pupal location.
Co	<i>S. bicolor</i>	10	4.9±0.7a	0.16±0.01a	1.2±0.2a
	<i>Z. mays</i>	32	5.7±0.4a	0.2 ±0.03a	1.7±0.2a
F-value			0.49	1.11	0.93
Df			1,40	1,40	1,40
P-value			0.4891	0.2975	0.3404

Means in the same column followed by the same letter are not significantly different at $P < 0.05$ by Bonferroni.

Table 3.2: Position (cm) of *C. partellus* (Cp) pupae in stems of three host plant species in Kwale

Borer species	Plant sp	N	Tunneling length	Pupal depth	Pupal location
Cp	<i>S. bicolor</i>	232	4.1 ±0.1a	0.2±0.01b	0.7±0.04b
	<i>S. versicolor</i>	24	4.3±0.3a	0.1±0.01b	0.7±0.1b
	<i>Z. mays</i>	274	4.1±0.1a	0.24±0.01a	0.9±0.06a
F-value			1.11	4.59	4.33
Df			3,528	3,528	3,528
P-value			0.3464	0.0035	0.005

Means in the same column followed by the same letter are not significantly different at $P < 0.05$ by Bonferroni.

Table 3.3: Position (cm) of *S. calamistis* (Sc) pupae in stems of two host plant species in Kwale

Borer species	Plant sp	N	Tunneling length	Pupal depth	Pupal location
Sc	<i>S. bicolor</i>	2	4.8±0.0a	0.2±0.0a	2.0±0.0a
	<i>Z. mays</i>	34	4.2±0.3a	0.2±0.03a	1.3±0.2a
F-value			0.40	0.04	1.14
Df			1,34	1,34	1,34
P-value			0.5315	0.8505	0.2928

Means in the same column followed by the same letter are not significantly different at $P < 0.05$ by Bonferroni.

Table 3.4: Position (cm) of *C. orichalcociliellus*, *S. calamistis* and *C. partellus* in stems of *S. bicolor* in Kwale.

Plant species	Borer sp	N	Tunneling length	Pupal depth	Pupal location
<i>S. bicolor</i>	<i>C. orichalcociliellus</i>	10	4.9±0.7a	0.16±0.1a	1.2±0.2a
	<i>S. calamistis</i>	2	4.8±0a	0.2±0a	0.4±0a
	<i>C. partellus</i>	232	4.1±0.08a	0.2±0.1a	0.7±0.4a
F-value			1.19	0.09	3.50
Df			2,241	2,241	2,241
P-value			0.3051	0.9154	0.0619

Means in the same column followed by the same letter are not significantly different at $P < 0.05$ by Bonferroni.

Table 3.5: Position (cm) of *C. orichalcociliellus*, *S. calamistis* and *C. partellus* in stems of *Z. mays* in Kwale.

Plant species	Borer sp	N	Tunneling length	Pupal depth	Pupal location
<i>Z. mays</i>	<i>C. orichalcociliellus</i>	32	5.7±0.5a	0.2±0.03a	1.7±0.2a
	<i>S. calamistis</i>	34	4.2±0.3b	0.2±0.03a	1.3±0.2a
	<i>C. partellus</i>	274	4.1±0.1b	0.2±0.02a	0.9±0.05b
F-value			7.24	0.46	9.19
Df			2,337	2,337	2,337
P-value			0.0008	0.6313	0.0001

Means in the same column followed by the same letter are not significantly different at $P < 0.05$ by Bonferroni.

Table 3.6: Position (cm) of *B. fusca* pupae in stems of three host plant species in Trans Nzoia

Borer species	Plant sp	N	Tunneling length	Pupal depth	Pupal location
<i>B. fusca</i>	<i>H. rufa</i>	3	2.8±0.7c	0.15±0.03a	0.13±0.01b
	<i>S bicolor</i>	30	7.6±0.6a	0.32±0.05a	2.0±0.2a
	<i>Z mays</i>	111	5.6±0.2b	0.35±0.02a	1.6±0.1a
F-value			8.74	1.46	6.71
Df			2,141	2,141	2,141
P-value			0.003	0.2354	0.0016

Means in the same column followed by the same letter are not significantly different at $P < 0.05$ by Bonferroni.

3.5 Discussion

The studies indicated that the tunneling length made by each borer depended on the plant species and to a lesser extent on borer species. These findings concur with the work reported by Smith and Wiedenmann, (1997) and Smith *et al.*, (1987) that stemborers tunnels in *Z. mays* are longer than that in the native grass, *Sesamia oriaula*, *C. orichalcociliellus* and *B. fusca* had the longest mean tunneling lengths. Noctuid larvae are generally large while, crambids larvae are small. This may explain the findings in the current study where tunnel lengths made by *B. fusca* and *S. oriaula* were longer than those made by *C. partellus* in the same host plant. This implies that noctuids would cause more damage to the host plant than crambids. However, the overall damage caused by *C. partellus* would be more due to its high numbers in the host plants. Ofomata *et al.* (2000), working on *C. partellus* and *C. orichalcociliellus* in the laboratory found that the former consumes more maize and sorghum stems than the latter. Thus *C. partellus* may cause greater damage to these host crops than *C. orichalcociliellus*.

The results obtained in the current study indicated that the pupae of *C. partellus* were found far from plants' edge and the adult exit hole in *Z. mays* and *S. bicolor* than in *S. versicolor*. This suggests that stem thickness may influence the location of the pupae. This concurs with the work reported by Smith and Wiedenmann, (1997) and Smith *et al.*, (1987) that location of pupae, varies with plant species. The study also found that pupal depth of *C. orichalcociliellus*, *C. partellus*, *S. calamistis* and *B. fusca* was less than 0.3 cm in the host plants sampled. But, pupal depths of *C. partellus* and *B. fusca* in the wild hosts *S. versicolor* and *H. rufa* were about 0.1 centimeter. Probably, this may be due to difference in rind and stem thickness of the hosts' stem. These findings are in agreement with the work reported

by Smith and Wiedenmann, (1997) and Smith *et al.*, (1987) that stemborers pupating in *Z. mays* stems are 2-5 times farther from the stem periphery than in the native grass stems. More than 40% *C. orichalcociliellus* and *S. calamistis* pupated in the maize cob sheaths.

Xanthopimpla stemmator has an ovipositor of about 0.522cm and attacks its host by drilling the stem and stinging the pupa with its' stout ovipositor (Smith *et al.* 1993). In the present study, the greatest pupal depth was about 0.35 cm for *B. fusca* on maize but mostly less than 0.25 cm for the other species. Since pupae of these borers are found pupating a distance shorter than the length of the parasitoids' ovipositor, it is expected that *X. stemmator* would easily reach and parasitize the borer pupa found pupating between sheaths, inside husks of maize ears and close to the stem edge. Ovipositors' length determines how deep the host can be reached (Hanks *et al.*, 2001). *Xanthopimpla stemmator* ovipositor is long, thick, robust and has serrated tip hence adaptable for piercing hard stems (Fischer *et al.* , 2003). This is supported by the findings of Moutia and Courtois (1952) who reported that *X. stemmator* has successfully established in sugarcane fields in Mauritius and Reunion against *C. sacchariphagus* and *S. calamistis* which pupate inside the sheaths. Length of the tunnels made and the location of *B. fusca* varied in *H. rufa*, *S. bicolor* and *Z. mays*. Probably due to difference in stem thickness and hardness suggesting that the host stems' nature may influence the pupal location.

Results obtained from the current study showed that of the total number of stemborer pupae recovered in coastal Kenya, 82% were *C. partellus*. Similar findings were reported by Overholt *et al.* (1994) and Ofomata *et al.* (2000). They indicated that *C. partellus* was the predominant stemborer species accounting for over 80% of the total borer population in maize and sorghum in coastal Kenya. Results in Trans Nzoia

indicated that *B. fusca* was the predominant borer species. To manage the borers in these areas, a more efficient and environmentally friendly control strategy would be required to increase pest suppression. This calls for application of biological control method with the use of exotic parasitoids. Works by Gitau (2002) reported that *X. stemmator* completed development in the four main borers. *Xanthopimpla stemmator* is an indigenous natural enemy of *C. partellus* in Asia (Moutia and Courtois, 1952). The parasitoid could be introduced as an old world association biological control strategy against *C. partellus* and this would increase population suppression by causing intergeneration mortality. The introduction of *X. stemmator* for the control of cereal borers would simply attempt to balance the disturbed system following the arrival of *C. partellus* in Africa without its coevolved natural enemies. This study suggests that the introduction of *X. stemmator* would result in increased suppression of stemborer population.

These results highlight the importance of further research to determine the effect of hardness of the host plant to the efficacy of *X. stemmator*. Pilot bioassays should be conducted in screen houses to evaluate the efficacy of *X. stemmator* in parasitizing stem and cob borers on different host plants in order to predict its possible field effectiveness.

CHAPTER FOUR

4.0 HOST ACCEPTANCE AND PREFERENCE OF *X. STEMMATOR* TO *C. PARTELLUS*, *H. ARMIGERA* AND *S. ORIAULA*

4.1 Introduction

In East Africa two biological control attempts have been made against both exotic and indigenous stemborers (CIBC, 1968-1972). Use of exotic organisms as biological control agents, raises several concerns due to their ecological and environmental effects (Howarth, 1991). To address these concerns, host specific tests are carried out (Wapshere, 1974). For example insects are screened using both choice (Dijken *et al.*, 1986; Pak *et al.*; 1990; Hassan, 1991; Barrat *et al.*, 1997a) and non-choice tests (Scholz, 1991; Barrat *et al.*, 1997a). *Xanthopimpla stemmator* was imported into Kenya in the year 2000 for the control of *C. partellus*. Before the release of *X. stemmator* in the field for the control of borers, host specific tests are necessary in order to predict the field host range of the parasitoid. There is a need to establish whether *X. stemmator* discriminates between borer species in the laboratory. The objective of this study was to determine host acceptance and preference of *X. stemmator* to *C. partellus*, *H. armigera* and *S. oriaula*.

4.2 Materials and methods

4.2.1 Insects

Larvae of *C. partellus*, *H. armigera* and *S. oriaula* were all reared as described in chapter 2 section 2.2 until pupation. Two-day-old borer pupae were used in this study. The study was carried out with *X. stemmator* female parasitoids also reared as described in chapter 2 section 2.2.

4.2.2 larval frass

Fourth instar larvae of *C. partellus* were given fresh maize-stalk sections to feed on in order to produce frass while *H. armigera* and *S. oriaula* were given fresh natural diets as described in chapter 2 section 2.3.

4.2.3 Bioassays

Two tests were conducted; the dual and three no choice tests.

4.2.3.1 Dual choice test.

One two-day-old pupa from each of the three borer species was presented to *X. stemmator* in dual choice tests. One pupa of each borer species was inserted into the lower half of an eight centimeter long paper straw, which was secured upright with clay bases (Fig 4.1). A little frass of the corresponding borer species was smeared round the surface of the straw (Fig 4.1). A similar set of other borer species was prepared. A pair of each paper straw with different borer species was put in 30cm x 30cm x 30cm clear Perspex cage. A 5-day-old mated female *X. stemmator* was released into the cage.

Observations were made for 30 minutes until the female made two choices within a period of 30 minutes. Choice was recorded if a host pupa was probed. The positions of the straw containing different stemborer pupae species were interchanged regularly within the cage to avoid any positional bias. Each pupa was observed under a dissecting microscope for probe wounds. A total of forty observations for each dual test were made. The following pairs were observed; *C. partellus* vs *S. oriaula*, *C. partellus* vs *H. armigera*, *S. oriaula* vs *H. armigera*

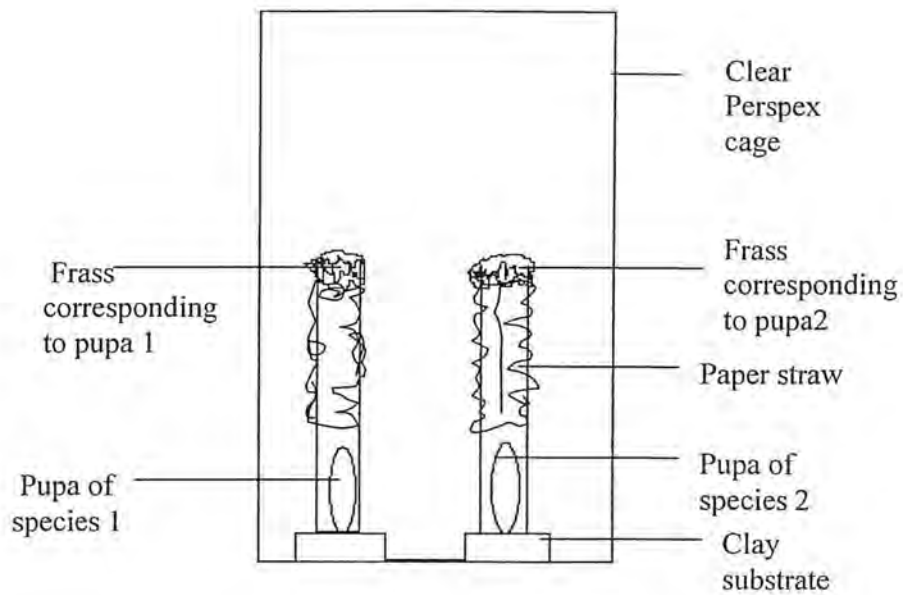


Fig 4.1: Straw arrangement in the Perspex cage

4.2.3.2 No choice tests

Four two-day-old pupae of one borer species were inserted in 8cm long and 5mm wide paper straw with respective frass spread on the surface. The same was repeated for the other borer species. One straw, containing four pupae of each borer species were placed in 30cm x 30cm x 30cm clear Perspex cage, with one end of the straw attached to a clay substrate to secure the straw vertically (Plate 2.4). A 5-day-old mated *X. stemmator* female was released into the cage and a piece of cotton wool soaked in 20% honey/water solution was provided as diet.

The female and pupae were removed after 6 hours of exposure. 60 pupae (15 replicates) were exposed for each host and each pupa was examined under a dissecting microscope for probe wounds.

4.2.4 Data analysis

Data obtained from dual tests were analyzed using the goodness of fit test (Chi-square test) (Sokal and Rohlf, 1981). Log-linear model was used to test if the choices made were different in the dual choice. Data analysis for the three hosts (no choice test) were carried out using a one way ANOVA. Prior to analysis, data were log-transformed for normalization. Parasitoids that did not respond in both tests were excluded from the analysis.

4.3 Results

In the no choice test where the three host pupae were presented to the female parasitoids, the hosts were accepted as indicated by the presence of probe wounds on the cuticle. The females did not show any discrimination between the three hosts (Table 4.1). 80% of the *C. partellus* pupae exposed to *X. stemmator* were accepted, while 77% and 75% of *H. armigera* and *S. oriaula* pupae respectively were accepted (Table 4.1).

Table 4.1: Mean number of punctures and percentage (\pm SE) of host pupae accepted by *X. stemmator*

Borer sp	N	Mean no. punctures	%hosts accepted
<i>C. partellus</i>	48	1.8 \pm 0.2a	80 \pm 5a
<i>H. armigera</i>	46	1.7 \pm 0.2a	77 \pm 5a
<i>S. oriaula</i>	45	1.5 \pm 0.2a	75 \pm 5a
F-value		0.54	0.22
Df		2,177	2,177
P-value		0.6176	0.8044

Means with the same letter in the same column are not significantly different (SNK)

at $P < 0.05$

In the dual choice test, where a few females made a second choice in a different host pupa (26.4% of the observations) analysis was carried out using the first choice the females made. First and second choices were different in the dual tests carried out ($\chi^2 = 33.34; df=1; P < 0.0001$). Dual choice tests showed no discrimination between the borer host species. Host choice by the females differed significantly within each dual choices in Cp vs Ha and Cp vs So sequences ($\chi^2 = 3.025; Df=1; P=0.04$; $\chi^2 = 5.625; Df = 1; P < 0.05$) but, did not differ within So vs Ha sequence ($\chi^2 = 0.225; Df=1; P > 0.1$)(Fig 4.2)

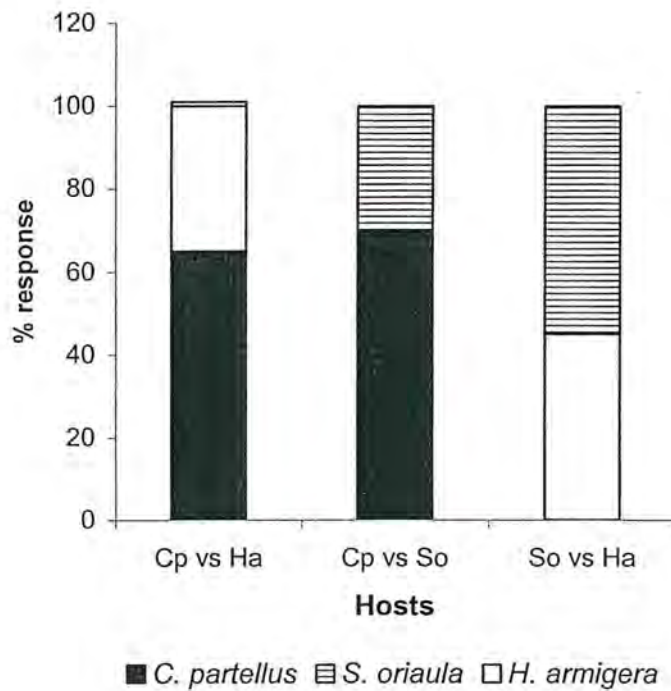


Fig 4.2: Response of *Xanthopimpla stemmator* females to pupae of the three stemborer species in dual choice tests (N=40)

4.4 Discussion

In this study the three host borer species that were exposed to *X. stemmator* (i.e. *H. armigera*, *C. partellus* and *S. oriaula*) were accepted for oviposition. Moore and Kfir (1996) reported similar results while testing *H. armigera* vs *C. partellus* pupae with *X. stemmator*. Being a polyphagous pupal parasitoid and its unique attack strategy, *X. stemmator* may accept a wide range of host borer species. Gitau (2002), working on the four major East African lepidopteran stemborers found that *X. stemmator* accepts all the borers. Females drill through the plant stem (or the straw) and probe the pupae. There are few probable host-specific cues that the parasitoid could use to determine the acceptability before probing the pupae. Host-frass and movements will attract the parasitoid closer to the host. Once the pupa is probed, then the decision on whether to oviposit or not is made. Information received from the initial probe may determine whether subsequent probes would occur.

Acceptance is evident by the presence of external punctures from the parasitoids' ovipositor (Hailemichael *et al.*, 1994). This study found that the mean number of punctures made on the three borers were not significantly different. These results suggest that *X. stemmator* may not discriminate between pupae of lepidopteran stemborers in graminaceous plants and may accept non target lepidopteran species. *Xanthopimpla stemmator* is a solitary endoparasitoid widely distributed in Asia/India, having been recovered from numerous pupae of lepidopteran stalk borers in India, the Philippines, West Malaysia, Mauritius, Sri – Lanka and Taiwan (Moutia and Courtois, 1952). *Chilo partellus* is its main host in the aboriginal home. Results obtained from this study showed that, though the parasitoid attacked the non target borers, it had a higher inclination to *C. partellus* its' co-evolved host.

Hence, this exotic endoparasitoid can be used in Eastern and Southern Africa as an Old and New World association control strategy.

Post release field trials need to be conducted to determine the actual host range of *X. stemmator* and also to confirm the accuracy of the laboratory predictions of the host ranges. Parasitoids' may have a wider laboratory host range compared to that of the field host range. For example, Balciunas *et al.* (1996), while evaluating the actual field host range of the weevil *Bagous hydrillae* O'Brien in Australia found the weevil to feed only on seven out of the sixteen host species it had fed on in the laboratory experiments. There is some evidence that suggests that parasitoids may oviposit on species outside their normal host ranges when presented with hosts and non-hosts in the choice tests. This may occur because specific chemical signals from hosts stimulate oviposition behaviors which are unleashed against both species present (Sands, 1993). In the current dual choice experimental set-up investigated, there was a limited airflow and the cues from the different frass samples may have intermixed in the Perspex cages, thus interfering with the parasitoids' searching ability. The females may have used previous cues encountered from the first host to select it a second time. The role of other cues used in locating a host by *X. stemmator* needs to be evaluated.

The polyphagous nature of *X. stemmator* may be desirable, for alternate hosts would enable it to maintain its population when the target moth pupae are scarce. *Xanthopimpla stemmator* may develop in other borer species in the field especially those in the same micro-habitat and order with the target hosts.

CHAPTER FIVE

5.0 HOST SUITABILITY OF *C. PARTELLUS*, *S. ORIAULA* AND *H. ARMIGERA* SPECIES FOR THE DEVELOPMENT OF *X. STEMMATOR* (THUNBERG).

5.1 Introduction

Host suitability for parasitoids is the ability of a host once oviposition has occurred to support successful growth and development of the parasitoid progeny (Hailemichael *et al.*, 1994). The International Centre of Insect Physiology and Ecology (ICIPE) is searching for additional natural enemies of stemborers to complement the ones already existing in Eastern and Southern Africa. In the year 2000 *Xanthopimpla stemmator* was imported into Kenya from Sri Lanka via South Africa. *Xanthopimpla stemmator* has a broad geographic distribution and a broad host range, having been recovered from pyralid and noctuid of lepidopteran stalk borers. Laboratory host range tests indicated that *X. stemmator* can attack all the five major stemborers of maize and sorghum in Kenya (Gitau, 2002). However, there is no information on the effect of *X. stemmator* on other potential lepidopteran hosts found in the maize agroecosystem. The objective of this study was therefore to investigate the suitability of *C. partellus*, *S. oriaula* and *H. armigera* borer species for the development of *X. stemmator*.

5.2 Materials and methods

5.2.1 Insects

The three borer species *C. partellus*, *S. oriaula* *H. armigera* and parasitoid *X. stemmator* were reared and maintained as described in chapter2 section 2.2.

5.2.2 Host suitability

Five-day-old mated naïve *X. stemmator* females emerging from *C. partellus* were used in this experiment. Two-day-old pupae of *C. partellus*, *S. oriaula* and *H. armigera* were each exposed to *X. stemmator* female.

Four pupae of the same host were inserted into paper straws smeared with frass obtained from the respective host species (Plate 2.4). One straw containing the four pupae was secured firmly onto a clay substrate and placed in a Perspex cage 30cm x 30cm x 30cm. One 5-day-old mated *X. stemmator* female was given the four pupae of the same host for oviposition. The female was released into a Perspex cage and provided with 20% honey/water solution soaked in cotton wool as diet.

The female and pupae were removed after 6 hours exposure. Individual parasitoids and borers were used only once. Each pupa was removed and the numbers of probe wound(s) on its cuticle counted and recorded. A total of 80 pupae (20 replicates) of each species were exposed to the parasitoids. All borer pupae were each placed in individual vials, plugged with clean cotton wool and maintained at $26 \pm 1^\circ\text{C}$, 50% - 80% R.H. and 12 :12 (L.D.) photoperiod until adult emergence. The host pupae were inspected daily for moth emergence, parasitoid or pupal mortality. The fate of pupae offered to the females (either emergence of adult parasitoids or moths, or death of pupae), developmental time of the parasitoid and sex of offspring were recorded. The emerged adult parasitoids were maintained on 20% honey/water solution soaked in cotton wool and confined in Petri-dishes until their natural death. The Petri-dishes were cleaned and diet changed daily. Longevity, left upper wing length and left hind tibia length of each adult parasitoid were recorded upon its death.

A control group of pupae was monitored. They were not offered to the females for oviposition and were kept in the quarantine laboratory until all the pupae emerged into moths. Moth emergence or death of the host pupae were recorded.

5.2.3 Data analysis

Data on acceptance, developmental time, longevity, left upper wing length, left hind leg tibia length were analyzed using the General Linear Model procedure (proc GLM) (SAS Institute 2000). Logistic model was used to evaluate influence of hosts' weight to female production. Data were log-transformed for normalization before being subjected to ANOVA. Means were compared using the Student - Newman - Kuel's (SNK) test when ANOVAs were significant.

5.3 Results

5.3.1 Host suitability

Xanthopimpla stemmator females indiscriminately attacked pupae of *C. partellus*, *S. oriaula* and *H. armigera*. 95% of the *C. partellus* pupae exposed to *X. stemmator* were attacked, while 88% and 86% of *H. armigera* and *S. oriaula* pupae respectively were attacked (Table 5.1). *Chilo partellus* and *S. oriaula* were successfully parasitized with 91% and 78% success respectively ($\chi^2 = 157.3587$, Df=2, $P < 0.0001$) (Table 5.2). The parasitoid did not complete development in *H. armigera*.

5.3.2 Development time

Xanthopimpla stemmator development time was not significantly different among females and males offsprings that emerged from the *C. partellus* and *S.*

oriaula, respectively (Table 5.1). The minimum developmental time of *X. stemmator* was 15 days in the two borer species and the longest was 23 days in both borer species. No *X. stemmator* progeny emerged from *H. armigera*.

5.3.5 Longevity

Longevity for males across the two borer host species was not significantly different while, the female longevity differed significantly across the two host species (Table 5.1). The earliest death recorded was of the *X. stemmator* female at 16 days in *S. oriaula* while, the oldest *X. stemmator* female died at 98 days in *C. partellus*.

5.3.3 Wing length

Wing length of *X. stemmator* was significantly different in the adult females and males compared across the two borer host species (Table 5.1). The minimum wing length of *X. stemmator* was 0.6 cm in *C. partellus* while, the longest was 1.5 cm recorded in *S. oriaula*.

5.3.4 Tibia length

Length of the left tibia of *X. stemmator* was significantly different in both females and males compared across the two borer host species (Table 5.1). The minimum tibia length recorded was 16.74 μm in *C. partellus* while the longest was 31.97 μm in *S. oriaula*.

Table 5.1: Mean developmental time, longevity, wing length, tibia length and percentage attacked (+SE) of progeny compared within sexes in the four borer host species.

Host	N	Dvt♀	N	Dvt♂	N	Lvt♀	N	Lvt♂	N	Wl♀	N	Wl♂	N	Tl♀	N	Tl♂	N	%par.
Cp	42	17.5±0.2a	31	17.3±0.2a	42	63.8±0.3a	31	49.9±0.3a	42	0.78±0.01b	31	0.78±0.02b	42	23.8±0.8b	31	23.2±0.7b	80	95±2a
Ha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80	88±3a
So	44	18.1±0.2a	17	17.6±0.3a	44	48.4±0.2b	17	45.1±0.4a	44	0.89±0.02a	17	0.91±0.04a	44	34.4±0.5a	17	26.6±0.1ba	80	86±3a
F-Value		3.15		0.19		8.24		0.76		19.42		7.65		7.63		8.20		2.237
Df		1,84		1,46		1,84		1,46		1,84		1,46		1,84		1,46		2,236
P-Value		0.0795		0.6690		0.0052		0.3864		0.0001		0.0081		0.0007		0.0063		0.1479

Means followed by the same letter (s) in the same column are not significantly different (SNK test, $P < 0.05$). C.p., H.a., S.o, Dvt♀, Lvt♀, Lvt♂, Wl♀, Wl♂, Tl♀, Tl♂ and %par. Are *C. partellus*, *H. armigera*, *S. oriaula*, Developmental time females, Developmental time males Longevity females, Longevity males, Wing length females, wing length males, Tibia length females, Tibia length males and Percentage parasitism respectively. 80 pupae of each host were exposed in each set up.

5.3.6 Sex proportion

Chilo partellus and *S. oriaula* were successfully parasitized by *X. stemmator* with 91% and 78% success (Table 5.2). *Chilo partellus* and *S. oriaula* produced high percentage of *X. stemmator* females than males. *Sesamia oriaula* produced 69% females while, *C. partellus* produced 58% females of the total progeny. (Table 5.3), but the proportion of females to males produced by *C. partellus* pupae did not differ (Table 5.3). More females emerged from heavier hosts. In both borer hosts, there was significant association in the sex of the progeny and the weight of the host ($\chi^2 = 5.2173$, Df=1, P=0.0224). The probability of getting a female *X. stemmator* progeny increased with increase in the weight of the host (Fig 5.1).

Table 5.2 : Percentage of pupae successfully parasitized by *X. stemmator*.

Host	N	Success Parasitism (%)	χ^2 - Value	Df	P-Value
<i>S. oriaula</i>	80	78			
<i>C. partellus</i>	80	91	157.3587	1	<0.0001

Table 5.3: Sex proportion of *X. stemmator* in different borer hosts

Host	N	Sex		Proportion (%)	χ^2 - Value	Df	P-Value
		Females	Males				
<i>S. oriaula</i>	43	69	31	19	9.2903	1	0.0023
<i>C. partellus</i>	42	58	52	31	1.6575	1	0.1979

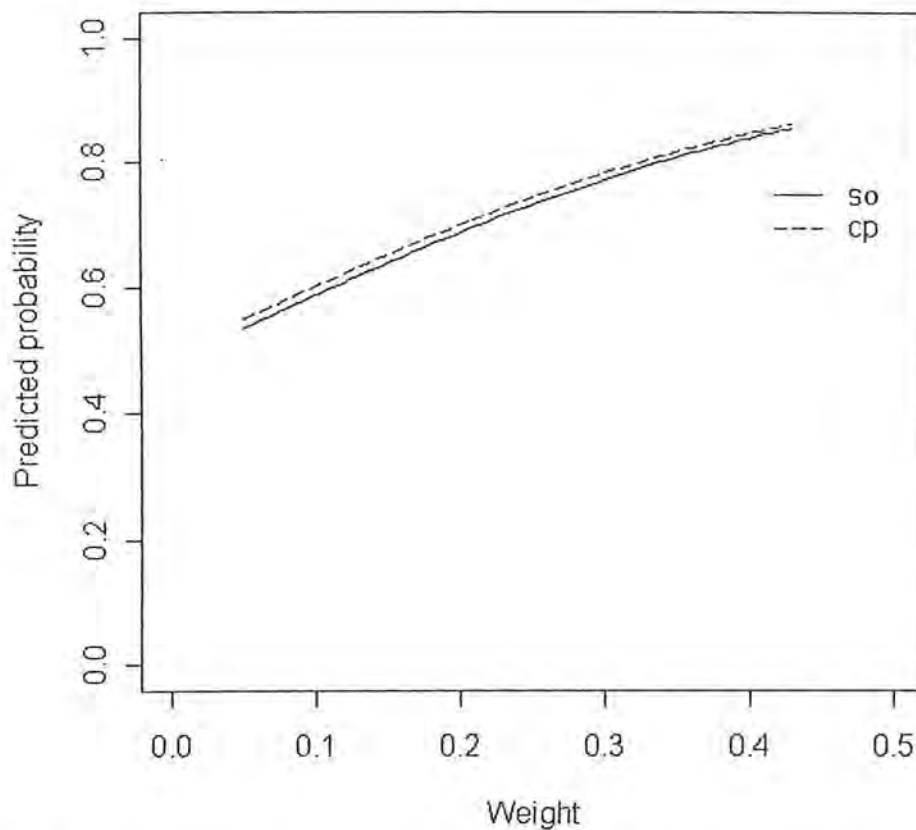


Fig 5.1 : Association between the host weight and the probability to produce *X. stemmator* female progeny from the different borer hosts. NB- so and cp are *Sesamia oriaula* and *Chilo partellus*

5.3.7 Fate of pupae

Emergence of *X. stemmator* adults and death of pupae in all the three borer hosts was significantly different, however, moth emergence was not (Table 5.4). Mortality differed across the three hosts (Table 5.4). Mortality in the control (pupae not exposed to female *X. stemmator*) for the two hosts was less than 5% in *C. partellus* and *S. oriaula*, while it was 0% in *H. armigera*. Hence, mortality of pupae that were offered to *X. stemmator* the females was attributed to parasitization.

Table 5.4: Fate (%) of pupae offered to *X stemmator* females.

Host	N	<i>X. stemmator</i> produced	Moth emerged	Pupa dead
<i>C. partellus</i>	80	91±4a	7.5±4a	1.25±1b
<i>H. armigera</i>	80	0±0c	13.75±8a	86.25±3a
<i>S. oriaula</i>	80	77±6b	16.25±3a	6.25±2b
F-value		4.06.73	1.30	7.94
Df		2,57	2,57	2,57
P-value		<0.0001	0.2806	0.0009

Means within the same column with different letter(s) are significantly different at $P < 0.05$ (SNK test) (Eighty pupae were used for each host)

5.4 Discussion

Xanthopimpla stemmator females accepted the three borer hosts as evidenced by the presence of punctures on the cuticle. *Xanthopimpla stemmator* attacks concealed hosts using 'drill and sting' attack strategy (Smith *et al.*, 1993). Due to its unique attack strategy, females may be unable to discriminate between hosts, for there are few host specific cues that guide parasitoid to determine acceptance before probing. *Chilo partellus* and *S. oriaula* proved to be suitable hosts for *X. stemmator* as they supported complete development of the parasitoid. On the other hand, the parasitoid did not complete its development in *H. armigera*. More *C. partellus* pupae were parasitized than those of *S. oriaula*. *Chilo partellus* is the natural host of *X. stemmator* in its aboriginal home (Vinson and Iwantsch, 1980a), and this may explain the better performance of *C. partellus* compared to *S. oriaula* which is a new world association.

Helicoverpa armigera was accepted by *X. stemmator* females for oviposition but did not support full development of the parasitoid. Host mortality which was inflicted by *X. stemmator* was high (86%). Dissections of the 20 parasitized pupae from day one to day four showed encapsulated parasite eggs and first instar larvae. These findings concur with studies previously conducted by Moore and Kfir, (1996) using *H. armigera* exposed to *X. stemmator* females. These authors found the borer to be an unsuitable host and the parasitoid caused 85% mortality to the borer pupae. The similar life histories and cryptic microhabitats of stemborer pupae lead to prediction that *X. stemmator* may be able to locate, find and attack a broad spectrum of stemborer species in gramineae species in Eastern and Southern Africa. Stemborers pupate in a cryptic microhabitat and share behavioral and physiological similarities. The current study revealed that *X. stemmator* could effectively develop in *S. oriaula*, a

borer found pupating in similar ecological environment like its main host *C. partellus*. This implies that, *X. stemmator* has high potential to attack other borers sharing similar ecological and physiological similarities. Its ability to develop in *C. partellus* and other major and minor borers makes it a good candidate for release as a biological control agent. Increased attack on minor borer hosts has costs as well as benefits. Increased attack on unsuitable hosts would reduce its effectiveness as it would result to wastage of eggs, while increased attack on suitable minor hosts would boost its effectiveness as these hosts would sustain its population when the target hosts population are low during the off cropping season.

The proportion of females emerging from the two hosts did not differ significantly. However, the sex was female biased in both hosts with more female emergences being realized from *S. oriaula*. Moore and Kfir (1996) and Gitau (2002) found that when hosts of *X. stemmator* are exposed to a single female, the sex ratio was female biased. The current study found that more females emerged from heavier hosts and this observation concurs with a general trend for gender survival in parasitic hymenopteras where males do better than females as parasite survival decreases as a result of a decline in host suitability (Waage, 1985). Males superiority in survival over females in gregarious species is due to their high competitiveness when resources are limited (Waage, 1985). Adult parasitoid females prefer to allocate male eggs to hosts for oviposition that are a less suitable for progeny development to ensure species survival (Van Alphen and Theunissen, 1983; King, 1989)

The current studies predict successful establishment of *X. stemmator* in Eastern and Southern Africa because high female progeny production from large hosts and more production of males from smaller hosts would increase chances of progeny production at varying host sizes. However, further research needs to be carried out to evaluate the

potentiality and effectiveness of males emerging from smaller hosts as it is known that large males have greater mating success (Godfray, 1994). Biotic fitness influencing offspring fitness includes host size, age and species (Godfray, 1994). Indexes of fitness includes development time from egg to adult (wasp) emergence, fore wing length and hind tibia length of the emerging wasp (Ueno, 1999). Size of the emerging parasitoids is an indicator of hosts suitability for parasitoid development. Studies have shown that female fitness increases with the adult size (King, 1989), for large individual may have a higher potential fecundity (Vet *et al.*, 1984), a greater longevity (Harvey and Gols, 1998), and better searching capacities (Visser, 1994). Similarly, the male adult size may influence longevity, ability to locate females and intersexual competition for mates, thereby determining the number of the mating achieved (Godfray, 1994)

Many studies have reported increased female fitness with the adult size (Jervis and Copland (1996). Studies by Ngi-Song *et al.*, (1995) on *C. flavipes* exposed to *C. partellus* found correlation between progeny production and host larvae instar size with higher progeny production in large hosts. In the current study, parasitoids fitness was determined by the left hind tibia length and left upper wing length. *Sesamia oriaula* produced larger parasitoids compared to *C. partellus* which correlated with the hosts size. In both hosts, more females were produced from heavier pupae. Dissections of pupal cases from which parasitoids emerged revealed that the cases were virtually empty. Parasitoids larvae consume hosts content for their nutritional requirement, thus the size of the host may influence host quality and hence parasitoid fitness (Harvey *et al.*, 1998). Observations made from the current study suggest that large males and females may have a better searching ability, greater longevity and a higher reproductive potential. More studies need to be carried out to evaluate whether

the size of *X. stemmator* has influence on its fitness under laboratory and field conditions.

In the current study, females had a longer developmental time than males. Protandry is the emergence of male organisms in a given species prior to female emergence, a common phenomenon to solitary wasps (Godfray, 1994). Early male emergence ensures female fertilization immediately after emergence as they are usually ready for mating. Such males have high chances of multiple mating with females (Godfray, 1994). This reproductive adaptation serves to prevent inbreeding. In this study *S. oriaula* had heavier pupae and, *X. stemmator* emerging from these pupae had a slightly longer developmental time compared to those of *C. partellus*. Growth and development may be arrested in hosts with less food reserve. Probably, longer development time in *S. oriaula* may be due to longer feeding period. Generally, *X. stemmator* could develop well in *C. partellus* because of their long association.

Though, parasitoids from *C. partellus* had a shorter developmental time compared to those from *S. oriaula*, they had longer lifespan. Fast development is important in the fitness of parasitoid for it ensures early reproduction (Godfray, 1994). This study found that non-ovipositing unmated fed females lived longer from both hosts compared to their males counterparts. Life span of *X. stemmator*, as recorded in the current study, was shorter than previously recorded by Moore and Kfir (1996) but longer than the previous record of Vinson, (1942), Hailemichael *et al.*(1994) and Gitau (2002). This could be attributed to high temperatures and humidity in the laboratory, as well as the diet provided. In this study, the emerged parasitoids were maintained at $26\pm 1^{\circ}\text{C}$, 50-80 R.H., 12 :12 (L.D.). and provided with 20% honey/water solution. The oldest female was recorded for *C. partellus* and died at 98 days,

implying that the host is more suitable compared to female from *S. oriaula* whose longevity was 92 days. *Xanthopimpla stemmator* eggs mature with time after female emergence. Increased longevity of females may be beneficial, for this could increase chances of reproduction and higher number of offspring per females as they remain productive for over 70 days (Gitau, 2002)

Xanthopimpla stemmator is an idiobiont pupal parasitoid which kills its hosts after oviposition. Normally, eggs and pupae are less protected by immune responses (Wiedemann, 1998). All the pupae that died had punctures from the parasitoid. Hence, the mortality of these pupae is attributed to punctures and other factors introduced by the parasitoid to the host system. Moore and Kfir, (1996) in a similar study reported high mortality in *H. armigera* on pupae with probes.

The current study found that *X. stemmator* is able to develop in *C. partellus* and *S. oriaula*, a new host. Successful release and establishment of *X. stemmator* could therefore increase borer suppression in areas dominated by *C. partellus*. In East Africa, there are diverse host plant species and several borer species (Polaszek, 1998; Overholt *et al.*, 2001). *Xanthopimpla stemmator* would therefore establish very well because there would always be some borers feeding on different plant species hosts during the cropping and non cropping seasons. As climatic conditions in the region is similar to that of its aboriginal home, the parasitoid would not be adversely affected by climatic changes.

Xanthopimpla stemmator is a multivoltine and polyphagous pupal endoparasitoid which attacks a wide range of lepidopteran pests. Its wide host range may contribute significantly to the reduction of the varied borer species in graminaceous plants but, this may affect its effectiveness on major hosts in the field. Field releases need to be carried out to evaluate its effectiveness in different Eastern

African regions. Post release studies need to be conducted to confirm whether *X. stemmator* is as polyphagous as the laboratory data has indicated in the current study.

CHAPTER SIX

6.0 INTERACTION BETWEEN *XANTHOPIMPLA STEMMATOR* AND THE INDIGENOUS PUPAL PARASITOID (*PEDIOBIUS FURVUS*) IN THE LABORATORY.

6.1 Introduction

Successful parasitization may be defined as the proportion of parasitized larvae or pupae yielding adult parasitoids. Among the factors affecting the development of a parasitoid inside a host include competition among parasitoids (Vinson and Iwantsch, 1980a). Presence of competitors inside the host can render a host unsuitable for a parasitoid survival. Multiparasitism is found frequently in nature and in the laboratory (Vinson and Ables, 1980; Pijls *et al.* , 1995; Ngi-Song *et al.*, 2001, Utsunomiya and Iwabuchi, 2002). Polyphagus pupal parasitoid will often encounter hosts parasitized by heterospecific parasitoid at different time intervals between encounters because there are usually several pupal parasitoid species associated with the same host species. This study was conducted to investigate multiparasitism interaction between *Xanthopimpla stemmator* and the indigenous pupal parasitoid *Pediobius furvus* in the laboratory.

6.2 MATERIALS AND METHODS

6.2.1 Study organisms

The host *C. partellus* and the two parasitoids *X. stemmator* and *P. furvus* adults were reared and maintained as explained in chapter 2 section 2.2.

6.2.2 Experimental layout

Two-day-old *C. partellus* pupae were used in the tests. Two sets of experiments were conducted each using 120 *C. partellus* pupae with one replication of each set. The hosts were sexed and weighed prior to the test. Prior to each test, two-day-old mated naïve *P. fuscus* and 5-day-old mated naïve *X. stemmator* females (no oviposition experience) were selected from the colonies. In the first set, pupae were first exposed to *P. fuscus* and then to *X. stemmator* for parasitization, while in the second set, pupae were first exposed to *X. stemmator* and then to *P. fuscus*. For both experiments, the 120 pupae were classified into six sub-groups of 20 pupae each. One sub-group of 20 pupae was used as control to evaluate the natural pupal mortality. A second sub-group of 20 pupae was exposed to either of the parasitoid. Pupae exposed to *P. fuscus* remained in vials with the parasitoid for 12 hours before the next exposure. The remaining four sub-groups in each experiment were exposed to both parasitoids at four different time intervals as follows

6.2.2.1 Experiment set up 1

Sub-group 3 (Pf-Xs 0hr); twenty pupae were exposed to *P. fuscus* and then to *X. stemmator* (Same time exposure). Subgroup 4 (Pf-Xs 3hrs); twenty pupae were exposed to *P. fuscus* and then to *X. stemmator* (three hours later). Subgroup 5 (Pf-Xs 24hrs); twenty pupae were exposed to *P. fuscus* and then to *X. stemmator* (twenty-four hours later). Subgroup 6 (Pf-Xs 48hrs); twenty pupae were exposed to *P. fuscus* and then to *X. stemmator* (forty-eight hours later).

6.2.2.2 Experiment set up 2

Sub-group 3 (Xs-Pf 0hr); twenty pupae were exposed to *X. stemmator* and then to *P. furvus* (Same time exposure). Subgroup 4 (Xs-Pf 3hrs); twenty pupae were exposed to *X. stemmator* and then to *P. furvus* (three hours later). Subgroup 5 (Xs-Pf 24hrs); twenty pupae were exposed to *X. stemmator* and then to *P. furvus* (twenty-four hours later). Subgroup 6 (Xs-Pf 48hrs); twenty pupae were exposed to *X. stemmator* and then to *P. furvus* (forty-eight hours later).

6.2.3 Experimental procedure

One 2-day-old *C. partellus* pupa was put in a 5cm long and 5mm wide paper straw with its maize frass smeared on the surface for the attraction of the parasitoid. The paper straw was put in 10 cm by 2.5 cm vial with one end of the straw attached to a clay substrate to vertically maintain its position. A mated naïve 5-day-old *X. stemmator* female was introduced in each vial to give a 1:1 host-parasitoid ratio. In the other set, one 2-day old *C. partellus* pupa was put in the base of 10 cm by 2.5 cm vial and a single mated naïve 2-day-old *P. furvus* female was introduced into the vial. Pupae exposed to *X. stemmator* were removed from the paper straw once attacked and put in the vial for *P. furvus* exposure and vice versa. Parasitoids were maintained as described in chapter 2 section 2.2. Pupae were removed after they were stung and examined under a dissecting microscope to detect puncture on the cuticle. All the pupae were each kept in a vial and maintained as described in chapter 2 section 2.2. Data on development time and production of progeny of parasitoids was recorded.

6.2.4 Data analysis

Data collected were subjected to analysis of variance (ANOVA) using the GLM procedure (SAS Institute 2000). Means were separated by SNK test when ($P < 0.05$). Data on parasitism and progeny production were analyzed using the goodness of fit test (Chi square test, Sokal and Rohlf (1981)). Insect counts were log transformed to normalize the data before they were subjected to analysis (Sokal and Rohlf, 1981).

6.3 Results

6.3.1 Parasitism

Chilo partellus was successfully parasitized by *X. stemmator* stinging alone (sequence Xs alone) with 90% parasitism (Table 6.1). However, there was no significant difference in successful parasitization of *C. partellus* by *X. stemmator* at the different time intervals in the Xs-Pf sequence ($\chi^2 = 6.8825$, $df = 4$ $P = 0.1422$). Among the successfully parasitized pupae in this sequence and at 3-hours interval, 86% of the pupae produced *X. stemmator*. But, none produced *P. furvus* alone in sequence Pf (Table 6.1) respectively. 86% and 100% pupae produced *X. stemmator* progeny at the 24 and 48-hour intervals at the Xs-Pf sequence (Table 6.1). No pupae produced *P. furvus* or *X. stemmator* when exposed alone at these time intervals and sequences (Table 6.1-6.2). Both parasitoids emerged from 28% of the pupae in the same sequence (Xs-Pf) at the 3-hour interval (Table 6.3). None of the pupae produced both parasitoids in the Xs-Pf sequence at 24-hours and a similar trend was observed at 48-hours (Table 6.3). There was a significant difference in successful parasitization by *X. stemmator* in the Pf-Xs sequence at all the four time intervals (0, 3, 24 and 48) hours (Table 6.1). However, success in parasitism declined with increase in time

interval. At 0-hours and 3-hours interval, 100% of the pupae were successfully parasitized. Nevertheless, 55% and 63% pupae were successfully parasitized at 24-hours and 48-hours

Pediobius furvus had a lower successful parasitization rate compared to *X. stemmator* when it stung *C. partellus* pupae alone (Pf sequence only) (Table 6.2) with only 52% success. There was a significant difference in successful parasitism at all the four time intervals (0, 3, 24 and 48 hours) (Table 6.2) in the Xs-pf sequence. However, no *P. furvus* progeny was produced in the same sequence and at 24 and 48-hour intervals. In the Pf-Xs sequence at the four time intervals, there was significant difference in successful parasitism (Table 6.2). However, the successful parasitization declined with increase in time interval. At 3 hours interval, only 27% of the *C. partellus* pupae were successfully parasitized and similarly low parasitism trend followed at 24 and 48-hour interval (Table 6.2)

At 0-hour and 3-hours interval in the Xs-Pf sequence, both progeny emerged with 15% and 28% success parasitism, respectively. However, only 15% of the pupae which produced both progeny at the 0-hours interval in the Pf-Xs sequence (Table 6.3). None of the parasitized pupae produced both parasitoids in the Xs-Pf sequence at 24 and 48-hours intervals (Table 6.3).

Table 6.1 : Percentage of pupae (%(n)) successfully parasitized by *Xanthopimpla stemmator* in different sequences and at different time intervals.

Sequence	Time				Chi-value	df	P-value
	0 hrs	3 hrs	24 hrs	48 hrs			
<i>X. stemmator</i> only	90(36)	-	-	-	-	-	-
<i>X. stemmator-P. furvus</i>	85(34)	86(37)	86(37)	100(40)	6.8825	4	0.1422
<i>P. furvus</i> only	0	-	-	-	-	-	-
<i>X. stemmator-P. furvus</i>	100(40)	100(40)	55(22)	63(25)	42.2620	3	0.0001

Table 6.2 : Percentage of pupae (%(n)) successfully parasitized by *Pediobius furvus* in different sequences and at different time intervals.

Sequence	Time				Chi-value	df	P-value
	0 hrs	3 hrs	24 hrs	48 hrs			
<i>X. stemmator</i> only	0	-	-	-	-	-	-
<i>X. stemmator-P. furvus</i>	18(7)	0	0	0	29.0155	4	0.0001
<i>P. furvus</i> only	52(21)	-	-	-	-	-	-
<i>X. stemmator-P. furvus</i>	15(6)	27(11)	3(1)	3(1)	43.75	4	0.0001

Table 6.3 : Percentage of pupae (%(n)) successfully parasitized by both *Xanthopimpla stemmator* and *Pediobius furvus* in different sequences and at different time intervals.

Sequence	Time				Chi-value	df	P-value
	0 hrs	3 hrs	24 hrs	48 hrs			
<i>X. stemmator</i> only	0	-	-	-	-	-	-
<i>X. stemmator-P. furvus</i>	15(4)	28(11)	0	0	31.8869	4	0.0001
<i>P. furvus</i> only	0	-	-	-	-	-	-
<i>X. stemmator-P. furvus</i>	15(6)	0	0	0	24.7423	4	0.0001

6.3.2 Parasitoid Progeny

When pupae were exposed to Pf only, high *P. furvus* progeny were obtained than in the Pf-Xs and Xs-Pf sequences. Proportion of *P. furvus* males and females obtained in the Pf-Xs and Xs-Pf sequences was significantly different compared to that of the control (Pf sequence alone) at the four time intervals ($\chi^2 = 11.9502$, $df = 2$, $P = 0.0025$) (Table 6.4). There were very few *P. furvus* progeny obtained in the Xs-Pf sequence at 0-hour time interval and with a sex ratio of almost 1 male: 2 females.

Xs-Pf sequence produced more *X. stemmator* progeny than Pf-Xs sequence at the four time intervals. However, maximum production of *X. stemmator* progeny was realized in the Pf-Xs sequence at 0 and 3-hours. Progeny production decreased with increase in time interval of exposure (Table 6.5). There was no significant difference in the proportions of *X. stemmator* female progeny ($\chi^2 = 4.9368$, $df = 4$, $P = 0.2949$) (Table 6.5) in the Xs-Pf sequence at the four different time intervals, but significant difference in the proportions of *X. stemmator* female progeny was observed ($\chi^2 = 45.6577$, $df = 3$, $P = 0.0001$) (Table 6.5). In the Pf- Xs sequence at the four different time intervals, more male proportions were produced as the time interval of exposure increased with the highest proportion being realized at the 48-hour interval in the Pf-Xs sequence.

Table 6.4 : Sex proportion (%) of *Pediobius furvus* in different sequences and at different time intervals

Sequence	Time								Chi-value	df	P-value
	0 - hrs N	hrs %(f)	3 - hrs N	hrs %(f)	24 - hrs N	hrs %(f)	48 - hrs N	hrs %(f)			
<i>X. stemmator</i> only	0	0	-	-	-	-	-	-	-	-	-
<i>X. stemmator-P. furvus</i>	155	64	0	0	0	0	0	0	-	-	-
<i>P. furvus</i> only	841	62	-	-	-	-	-	-	-	-	-
<i>P. furvus-X. stemmator.</i>	40	65	93	78	-	-	-	-	11.95	2	0.0025

Table 6.5 : Sex proportion (%) of *Xanthopimpla stemmator* in different sequences and at different time intervals

Sequence	Time								Chi-value	df	P-value
	0- hrs N	hrs %(f)	3- hrs N	hrs %(f)	24- hrs N	hrs %(f)	48- hrs N	hrs %(f)			
<i>X. stemmator</i> only	24	67	-	-	-	-	-	-	-	-	-
<i>X. stemmator-P. furvus</i>	15	47	15	41	13	37	14	35	4.93	4	0.2949
<i>P. furvus</i> only	0	0	-	-	-	-	-	-	-	-	-
<i>P. furvus-X. stemmator</i>	31	79	31	78	5	23	3	12	45.66	3	0.0001

6.3.3 Development Time

Xanthopimpla stemmator development time was significantly longer ($P < 0.0001$) in the control (when Xs alone oviposited on the pupae) than in the other two sequences that produced progeny (i.e. Pf-Xs and Xs-Pf) at the 0-hour interval (Table 6.6). Development time was different at 24 hour and 48 hour interval in (Pf-Xs and Xs-Pf) sequences. However, the development time was shorter at 3-hour interval in Pf-Xs and Xs-Pf sequences ($P=0.0714$). The development time was significantly slower ($P < 0.0001$) within the sequences at the 3-hours interval than that of the control. However, at the 24 and 48 hour intervals, development was significantly

shorter (by about 2 days; $P=0.0001$) in the Xs-Pf sequence than in the Pf-Xs sequence compared to that of the control.

Pediobius fuscus development time was significantly longer ($P = 0.1859$) in the control (Pf alone sequence) than in the other sequences that produced progeny (i.e. Pf-Xs), but almost the same in the Xs-Pf sequence at the 0-hour interval (Table. 6.7). No *P. fuscus* progeny developed from the pupae parasitized at the 3-hours, 24-hours and 48-hours in the Xs-Pf sequence. Few *P. fuscus* progeny developed from the pupae parasitized at the 3-hours, 24-hours and 48-hours in the Pf-Xs sequence, with development time increasing as time of exposure increased (Table. 6.7). *Xanthopimpla stemmator* developed at each time interval and each sequence. The development time of *X. stemmator* was significantly different in each sequence and time interval except at 3-hours interval (Table 6.6).

Table 6.6: Mean development time of *Xanthopimpla stemmator* in different time intervals and sequences

Sequence	N	0-hour	N	3- hours	N	24- hours	N	48- hours	F- value	Df	P- value
<i>P. furvus</i> - <i>X. stemmator</i>	40	17.5±0.1bB	40	16.0±0.0cA	22	19.0±0.0aA	25	18.9±0.1aA	224.4	3,58	<0.0001
<i>X. stemmator</i> - <i>P. furvus</i>	34	15.6±0.3bC	37	15.6±0.3bA	37	15.3±0.1bB	40	16.7±0.1aB	8.2	3,67	0.0001
<i>X. stemmator</i>	36	18.4±0.2aA	-	-	-	-	-	-			
F- value		33.62		3.38		393.28		100.81			
Df		2,50		1,35		1,27		1,30			
P- value		0.0001		0.0714		0.0001		0.0001			

Means followed by the same upper case letter(s) in the same column are not significantly different; means followed by the same lower case letter(s) in the same row are not significantly different (SNK test, P<0.05)

Table 6.7: Mean development time of *Pediobius furvus* in different time intervals and sequences

Sequence	N	0-hour	N	3- hours	N	24- hours	N	48- hours	F- value	Df	P- value
<i>P furvus-X stemmator</i>	11	15.8±3bB	6	20.0±0a	1	24.0±0a	1	20.0±0a	0.89	3,9	0.0405
<i>X stemmator-P furvus</i>	12	20.4±0.4A	-	-	-	-	-	-	-	-	-
<i>P furvus</i>	22	19.0±0A	-	-	-	-	-	-	-	-	-
F- value		1.85									
Df		2,18									
P- value		0.0309									

Means followed by the same upper case letter in the same column are not significantly different; means followed by the same lower case letter in the same row are not significantly different (SNK test, $P < 0.05$)

6.4 Discussion

In this study, *Xanthopimpla stemmator* and *Pediobius furvus* had a successful parasitization when each parasitoid stung *C. partellus* pupa alone. However, *X. stemmator* had a higher percentage success than *P. furvus*. This was in agreement with the earlier works reported by Smith *et al.* (1993) and Bonholf *et al.* (1997) that *X. stemmator* is a natural enemy of *C. partellus* in its original home while *P. furvus* attacks the indigenous *B. fusca* and the exotic *C. partellus* in East and Central Africa. Low percentage mortality caused by *P. furvus* reported in this study concurs with earlier report by Mohyuddin (1968) that *P. furvus* causes low pupal mortality of upto 16% in *C. partellus* in maize in Uganda. The current study found that percentage success of *X. stemmator* parasitism increased with time interval in the *X. stemmator* - *P. furvus* sequence but decreased in the *P. furvus* - *X. stemmator* sequence. In kionobionts, hosts grow after parasitism. The physiological development of the host and its immune can affect success of parasitism (Vinson and Iwantsch, 1980a; Mellini, 1990). Low percentage of *X. stemmator* parasitism with increase in time interval in the *P. furvus* - *X. stemmator* sequence could have been due to physiological development of the host. In this study *P. furvus* had low success percentage parasitism when exposed to pupae before or after they were exposed to *X. stemmator*. Low success parasitism of *P. furvus* could be complemented by the introduction of *X. stemmator* in areas where these borers are prevalent.

In the current study less than 28% of the pupae produced both parasitoids at 0 and 3-hours interval in the *X. stemmator* - *P. furvus* sequence while only 15% in the *P. furvus* - *X. stemmator* sequence with highly reduced *P. furvus* progeny production. The high parasitization success of *X. stemmator* at different time intervals and in different sequences, and the low *P. furvus* success in this study indicates competitive

superiority of the latter parasitoid. During the study, dissections were made to a set of 20 pupae stung by both parasitoids at different time intervals and sequences upto the 10th day after oviposition. Observations made revealed that, *P. furvus* oviposits many eggs in a pupa while, *X. stemmator* oviposits a few eggs. Only one egg of *X. stemmator* which develop to adult. Many *P. furvus* larvae hatched but larval number decreased as development time increased in those pupae that had been stung by the two parasitoids. Larvae of *X. stemmator* were found chewing the small larvae of *P. furvus*. This was in agreement with the findings reported by McBrien and Mackauer (1991) that solitary hymenopteran parasitoids have free-roaming larvae adapted for fighting. Pupal cases where *X. stemmator* emerged were completely empty while those where *P. furvus* emerged had tissue residues. This implies that *X. stemmator* larvae used their mandibles to feed, consuming the content and in the process feeding on *P. furvus* larvae. Hence, this resulted to the low emergences of *P. furvus* with increase in time interval.

The results indicated that the sex of progeny was male biased for *X. stemmator* in the *P. furvus* - *X. stemmator* sequence as time interval increased while the reverse of this was realized in the *X. stemmator* - *P. furvus* sequence. Sex proportion of *P. furvus* remained relatively the same in the two sequences and at different time intervals. This concurs with the findings reported by Charnov and Stephens (1988); King (1989) and Reece *et al.*, (2004) that, females show host preference and choose high quality hosts usually depositing male eggs in small hosts, while female eggs are laid in large hosts. Hence, host's resource may influence oviposition decision by the female parasitoids. This may therefore, explain sex progeny biasness observed in the study.

The study showed that development time of *X. stemmator* progeny was longer when *X. stemmator* stung pupae alone but gradually shortened when the two parasitoids stung pupae at different time intervals and in different sequences. The shortened development time of *X. stemmator* would be of adaptational importance because more generations of the parasitoid would develop within a short time. This would help population build up of the parasitoid and increase chances of establishment. Multiple parasitism can affect development of the emerging parasitoid. In idiobiont solitary parasitoids such as *X. stemmator*, the host arrests its development after parasitism because of paralysis, hence influence resources but reduces its defense system. This may explain these studies findings that the development time of *X. stemmator* was longer when pupae were stung at zero time interval than when pupae was exposed at 24 and 48-hours after the previous exposure in either of the sequence.

The current study indicates that *X. stemmator* is able to develop in *C. partellus* pupae parasitized by *P. furrus*. *Xanthopimpla stemmator* appears to be intrinsically superior to *P. furrus* in a *C. partellus* pupa attacked by both parasitoids. Low parasitism of stemborers by native parasitoids has been identified as a gap which the introduction of exotic pupal endoparasitoid *X. stemmator* could fill. *X. stemmator* may be a good candidate for biological control of stemborers in Africa and could boost borer population suppression.

CHAPTER SEVEN

7.0 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 General discussion

This study has shown that tunneling length depended on the plant species and to a lesser extent on borer species. Pupal depth and pupal location on the other hand depended on plant. Thus abundance of the borer influenced the overall damage caused to the host plant. *Chilo partellus* was found to be the most abundant borer in coastal Kenya while *B. fusca* was dominant in Trans Nzoia. Results obtained from this study showed that these two borers pupated at a depth close to the periphery of host stems. On the other hand, *C. orichalcociliellus* and *S. calamistis* were found pupating in sheathes and cobs of the maize plant. *Xanthopimpla stemmator* has a long, stout ovipositor with serrated end that could reach these borer species in their pupating sites. This parasitoid could be released in the field where these borers are predominant to complement naturally occurring natural enemies.

Host acceptance, preference and suitability experiments indicated that *X. stemmator* females did not discriminate between *S. oriaula*, *C. partellus* and *H. armigera* pupae in the laboratory. The parasitoid attacked and developed in the minor borer *S. oriaula*, but had a higher inclination to *C. partellus*. Hence, this exotic endoparasitoid can be used in Eastern and Southern Africa against the major and minor borers species. The presence of alternative host pupae in varied host plants may be desirable to enable *X. stemmator* maintain its' population when target moth pupae are scarce. Although, it effectively attacked other minor borers example *S. oriaula*, in the laboratory, it may not do so in the field because these borers pupate in lower parts of the stems. Parasitism in the field may even be lower than in the laboratory since

hosts are scattered and host finding may not be effective due to lack of proper cues. However, parasitism caused to major and minor borer hosts by the parasitoid would contribute significantly to the borer mortality.

The current study found that *Xanthopimpla stemmator* is superior to *P. furvus* when both parasitoids are exposed to *C. partellus* at different time intervals and sequences. *Xanthopimpla stemmator* developed faster in the hosts which were previously attacked by *P. furvus* and generally produced more progeny. This suggests that if *X. stemmator* were to be introduced in areas where *P. furvus* is present, it would complement its borer suppression activity and establish faster due to the shortened development period. However, population of *P. furvus* is bound to decrease though the two parasitoids may still co-exist due to diversity in stemborer populations to which *P. furvus* could develop, use of different attack strategy and ability to reach pupae in their oviposition sites. The overall interaction would lead to increased borer mortality.

7.2 CONCLUSION

1. Stemborers are the major constraints to maize and sorghum production in Kenya.
2. *Busseola fusca* and *C. partellus* are the most important stemborer species in highland and lowland areas respectively in Kenya, though other three borer species were recorded.
3. Pupal depth and location for *C. partellus* depended on plant species and to a lesser extent borer species.
4. Most borer species pupate in the upper part of cultivated host plants.
5. *Xanthopimpla stemmator* accepted all hosts and attained development in *S. oriaula* and *C. partellus*.

6. More female *X. stemmator* parasitoids emerged from large hosts.
7. *Xanthopimpla stemmator* developed faster in all hosts which were previously attacked by *P. furvus*, hence it is more intrinsically superior.
8. *Xanthopimpla stemmator* is predicted to develop and establish in areas where *C. partellus* and *B. fusca* are predominant

7.3 RECOMMENDATIONS

Further research is required in the following areas to provide more information on the effectiveness of *X. stemmator* as biological control agent.

1. To determine the effect of host plant hardness in relation to the efficacy of *X. stemmator*.
2. To conduct pilot bioassays in screen houses and evaluate efficacy of *X. stemmator* in controlling stemborers in different host plants.
3. To evaluate the effect of commercial insecticides on *X. stemmator*.
4. To evaluate the interaction between *X. stemmator* and a solitary indigenous pupal parasitoid (*Dentichasmias busseolae*)
5. To evaluate host-searching and foraging behavior of *X. stemmator* in semi field conditions.
6. To evaluate the cues used in locating hosts by *X. stemmator*.

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