DEVELOPMENT OF THE PUPAL PARASITOID *Xanthopimpla stemmator* (THUNBERG) (HYMENOPTERA: ICHNEUMONIDAE) IN VARIOUS CEREAL STEM BORERS (LEPIDOPTERA)

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DECLARATIONS

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

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14th January 2003

Signature Date

We confirm that the work reported in this thesis was carried out by the candidate under our supervision. We have read and approved this thesis for examination.

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DEDICATION

To my family

Dad, mum, Lilian, Jim, my nephew Gitau Jr., George and Carol,

for constant love and support.

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To my family

Dad, mum, Lilian, Jim, my nephew Gitau Jr., George and Carol,

for constant love and support.

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ABSTRACT

Classical biological control (CBC) is a management strategy that employs natural enemies against exotic pests. The method has been used against *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), an introduced pest of maize in Africa, using the introduced larval parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). However, *C. flavipes* is not able to attack all stem borer species in the targeted areas. To complement its work, *Xanthopimpla stemmator* (Thunberg) (Hymenoptera: Ichneumonidae) a solitary pupal endoparasitoid, which attacks pupae of Lepidoptera stem borers was imported from Sri-Lanka via Mauritius and South Africa to Kenya for laboratory trials. *Xanthopimpla stemmator* has successfully been established in Mauritius on *Chilo sacchariphagus* (Bojer) (Lepidoptera: Crambidae).

The objectives of this study were to, examine preference of *X. stemmator* for *C. partellus, Eldana saccharina* Walker (Lepidoptera: Pyralidae), *Busseola fusca* Fuller and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), to assess suitability of the four stem borers for the development of *X. stemmator,* to compare life tables and intrinsic rates of natural increase of *X. stemmator* females reared on *B. fusca* and *C. partellus*, to determine whether *X. stemmator* females mate more than once and whether multiple mating has an effect on longevity and sex ratio of progeny produced.

Xanthopimpla stemmator females emerging from the pupae of the four stem borer species did not discriminate between the four stem borer pupae in dual and four

choice tests. Pre-adult learning experience did not affect choice of the pupae by *X. stemmator* females.

Exposure of *X. stemmator* females to pupae of the four stem borer species showed that all hosts and host ages were acceptable. *Eldana saccharina* had a significantly lower progeny emergence as compared to the other three hosts. Parasitoids emerging from *C. partellus* developed faster while progeny from *B. fusca* had the widest wingspan. Progeny production was significantly higher for *X. stemmator* females emerging from *B. fusca, C. partellus* and *S. calamistis* compared to *E. saccharina* pupae.

Life table studies were carried out at 27 ± 2 °C by giving pupae of *B. fusca* and *C. partellus* every day, to newly emerged *X. stemmator* females until the parasitoids natural death. The intrinsic rates of natural increase, net reproductive rate, mean generation time, finite rate of increase and doubling time of *X. stemmator* female parasitoids were statistically the same. The intrinsic rate of natural increase was 0.105 and 0.106 and the population multiplied 27.9 and 24.0 times in 31.4 and 30.2 days for females emerging from *B. fusca* and *C. partellus* respectively.

Studies on mating frequency revealed that multiple mating is not common in *X. stemmator* females. The number of times females mated did not significantly affect longevity and sex ratio of progeny. Male emergence was significantly high in females mated more than once.

The study shows that *X. stemmator* could be used as a biological control agent against three major Lepidoptera stem borers in Kenya. The parasitoid could play a complementary role to *C. flavipes* but may not establish in areas where *E. saccharina* is the dominant species.

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CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

1.1.1 Stem borers

Cereals, especially maize and sorghum, are the most important field crops in Africa (Kfir, 1998; Seshu Reddy, 1998). The crops are grown primarily for human consumption but surpluses are used as feed for livestock (Sibanda, 1985). In many countries, agricultural practices and food habits have changed in the last century to include maize as an important crop and a major diet component of the human population (Mwangi *et al.,* 1998). A major constraint to an increased production of these crops is herbivory by insects (Youdeowei, 1989; Hassan *et al.,* 1998).

Maes (1998) listed 21 economically important lepidopteran stem borer species of cultivated grasses in Africa, comprising seven noctuids, two pyralids and twelve crambids. Among the noctuids, *Busseola fusca* (Fuller) and six *Sesamia* species are considered economically important. The pyralids *Maliarpha separatella* Ragonot (African white rice borer) and *Eldana saccharina* (Walker), a pest of sugarcane and maize, are serious pests (Kfir *et al.*, 2002).

Most of the stem borer species in Africa are indigenous to the continent, apart from *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Chilo sacchariphagus* (Bojer) (Lepidoptera: Crambidae) (Kfir *et al*, 2002). *Chilo partellus* is an Asian species

(Bleszynski, 1970) that invaded Africa sometime before 1930 when it was first recorded in Malawi (Tams, 1932), but it was not reported again until twenty years later in Tanzania (Duerden, 1953). Previously it was thought that *C. partellus* was restricted to warmer lowland areas (Nye, 1960; Van Hamburg, 1979), but recent evidence suggests that it has moved into cooler high elevation areas (Kfir, 1997, Overholt *et al.*, 2000). The distribution of *C. partellus* now includes Ethiopia, Sudan, Somalia, Kenya, Tanzania, Uganda, Mozambique, South Africa, Swaziland, Lesotho, Zimbabwe, Zambia, Malawi and Botswana (Nye, 1960; Ingram, 1983; CABI, 1989; Harris, 1990). Using Geographic Information Systems (GIS), Overholt *et al.*, 2000 predicted the eventual distribution of *C. partellus* in Africa to locations with similar climates as of locations where it was known to occur. The prediction includes several countries in southern and West Africa (Kfir *et al.*, 2002).

Severity and nature of stem borer damage depends on the borer species, the number of larvae feeding on the plant and stage of plant growth. If infestation occurs in young plants, the growing point may be destroyed, resulting in 'dead heart' and no yield will be obtained. If plants are attacked at a more mature stage, the damage is less devastating. However, the loss could still be 20-60% of the potential yield (Starks, 1969; Warui and Kuria, 1983; Seshu Reddy, 1988; Youdeowei, 1989).

Methods currently used to manage stem borers include chemical control, early planting and intercropping with non-cereals (Minja, 1990). Chemical control is usually

recommended by extension agencies and research has shown that it can be effective in reducing stem borer populations (Mathez, 1972; Warui and Kuria, 1983). Application must therefore be timely and frequent due to continuous infestation and the short time larvae are exposed before they enter into the stem (Mathez, 1972; Ingram, 1983). This method is thus expensive and time consuming and may not be applicable to small-scale farmers in Africa. Chemical control is therefore not appropriate and often not feasible for the majority of small-scale farmers (Bonhof, 2000)

1.1.2 Control measures

Cultural control methods such as intercropping with non-cereals and wild grasses, early planting (Minja, 1990; Waaijenberg, 1993), partial burning or exposing stems to the sun (Gebre-Amlak, 1988; Päts, 1996) and placing ash or soil in the whorl (Grisley, 1997) have been practiced by farmers for centuries for various reasons. Studies have shown that their impact on stem borer populations are limited (Oloo, 1989; Skovgård and Päts, 1996).

Currently at the International Centre of Insect Physiology and Ecology (ICIPE) Nairobi, Kenya, studies have led to the development of a 'push and pull' strategy (Khan *et al.*, 2000) for minimizing stem borer damage to maize and sorghum. This strategy involves combined use of intercropping and trap crop systems. Stem borers are trapped on highly susceptible trap plants (pull) and are driven away from the maize crop by repellant intercrops (push). The plants which are used as trap or repellent plants in a

push-pull strategy are Napier grass *Pennisetum purpureum* Schumach, Sudan grass *Sorghum sudanense* Stapf., Molasses grass, *Melinis minutiflora* Beauv and silverleaf desmodium, *Desmodium uncinatum* (Khan *et al.,* 1997). Napier grass and Sudan grass are used as trap plants whereas molasses grass and silverleaf desmodium repel ovipositiing stem borers. Studies by Khan *et al* (1997) showed that molasses grass when intercropped with maize not only reduced infestation in maize by stem borers, but also increased stem borer parasitism by a natural enemy *Cotesia sesamiae.* All four plants are of economic importance to farmers in eastern Africa as livestock fodder (Kfir *et al.* 2002)

Biological control utilizes natural enemies to reduce the damage caused by noxious organisms to tolerable levels (DeBach and Rosen, 1991). It has the advantage of being safe, with little or no farmer contribution and adverse impacts on the environment. The goal of biological control is not to eliminate the pest but to keep it below economically damaging levels. Under natural conditions, most insect pest populations are controlled by a complex of predators, parasitoids and pathogens that share the same habitat and belong to the same ecological community (Kfir *et al.*, 2002). There is little information available on the occurrence of predators, nematodes and microbial pathogens in sub-Saharan Africa (Bonhof *et al.,* 1997).

Many natural enemies are parasitoids, whose larvae feed on tissues of other arthropods especially insects. Lacewings *Pheidole* sp., ants *amponotus* sp. (Hymenoptera:

Formicidae) and earwigs *Diaperasticus erythrocephala* Olivier and *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae) are believed to cause high mortality on stem borer eggs and young larvae (Bonhof *et al.*, 1997; Oloo, 1989). Nematodes and microbial pathogens have been reported to infect all life stages but their impact is low under natural conditions (Odindo *et al.,* 1990). Generally, indigenous natural enemies are not able to keep stem borer populations below economic injury levels (Oloo, 1989; Overholt *et al.,* 1994).

Classical biological control is typically targeted against exotic pests. It involves the importation and release of an organism outside its natural range from the pest's native home into an area where the pest is invasive (Howarth, 1991). It is a strategy based on the premise that the introduced organism often reaches higher densities in the areas they have invaded than in their native homes due to lack of co-evolved natural enemies that would suppress it (Ngi-Song *et al.*, 1999). The introduced parasitoid attempts to re-establish the same ecological balance as occurs in the native home.

In East Africa, two classical biological control attempts have been made to increase natural mortality of *C. partellus*. In the first 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, ICIPE imported *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), an exotic larval parasitoid from Pakistan in 1991 (Overholt, 1993). *Cotesia flavipes* causes high levels

of parasitization on *C. partellus* in Asia (Nagarkatti and Nair, 1973; Singh *et al.,* 1975) and in the neotropics where it was introduced against *Diatraea saccharalis* (Fabricius (F.)) (Lepidoptera: Pyralidae) in sugarcane (Macedo *et al.,* 1984).

After laboratory studies *C. flavipes* was released in the field at three locations in the coastal area of Kenya in the long rain season of 1993 (Overholt *et al.,* 1994). Studies conducted during the 1996 long rains cropping season showed that *C. flavipes* was the most abundant parasitoid at five of the eleven sites where it was recovered (Overholt *et al.,* 1997). The recovery of stem borers parasitised by *C. flavipes* three years after the release indicated that the parasitoid had established in the coastal area of Kenya. Recent surveys indicated that it had reduced the *C. partellus* population by 53% and the total borer population by 37% (Zhou *et al.,* 2001). Other studies showed that *C. flavipes* was established in many areas of southern Kenya, Uganda, Mozambique, Malawi, and northern Tanzania (Omwega *et al.,* 1995, 1997; Overholt, 1998). *Cotesia flavipes* does not complete development in *Busseola fusca* (Lepidoptera: Noctuidae) (Ngi-Song *et al.*, 1995), an important borer in the highlands. Establishment of the parasitoid may therefore vary and hence the need for complementary parasitoids in such areas.

Xanthopimpla stemmator (Thunberg) (Hymenoptera: Ichneumonidae) was imported into Kenya for laboratory trials in 2000 from SASSEX, South Africa via Mauritius who originally obtained it from Sri-Lanka as a candidate for classical biological control. *Xanthopimpla stemmator* is a pupal parasitoid that drills through the plant stem to attack

the pupa (Smith *et al.*, 1993). It has a broad geographical distribution and is reported across Asia, having been recovered from pyralid and noctuid pupae of numerous lepidopteran stalk borers in India, the Philippines, West Malaysia, Mauritius, Sri-Lanka and Taiwan (Moutia and Courtois, 1952).

Xanthopimpla stemmator is a promising natural enemy and has been released as a bio-control agent against *Chilo sacchariphagus* Bojer (Pylaridae: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) in Mauritius and Reunion (Vinson, 1942; Rao and Ali, 1977; Williams, 1983). It could complement *C. flavipes* in suppressing densities of lepidopteran stem borer species found in Kenya since *C. flavipes* does not develop in all some borer species, especially *B. fusca* (Ngi-Song, 1995) and *E. saccharina* (Overholt *et al.*, 1997). *Xanthopimpla stemmator* can attack the pupae whose larvae escape parasitism by *C. flavipes* and would be applicable in areas where *B. fusca* is predominant. In order to effectively use parasitoids to reduce or control stem borer damage on cereal crops, a detailed knowledge and understanding of the range of hosts suitable for the development of these parasitoids is essential (Overholt, 1997). Intensive studies need to be conducted before the release of biological control agent to examine potential non-target effects.

1.2 Literature review

1.2.1 Cereal stem borers

Within specific crops and geographic regions, stem borer species are considered to be important pests (Table 1.1). *Chilo partellus* has proved to be a good colonizer in many areas it has invaded, often becoming the predominant and most economically important stem borer species in maize and sorghum at elevations below 1800m (Kfir *et al.,* 2002). There is evidence that it is displacing indigenous stem borer species in some areas (Kfir *et al.*, 2002). In Madagascar, it was first recorded in 1972, and by 1975 it was considered to be the most damaging stem borer in maize and sorghum surpassing in importance the indigenous *Chilo orichalcociliellus* (Lepidoptera: Crambidae) (Delobel, 1975).

There is evidence that *C. partellus* has displaced *C. orichalcociliellus* in maize (Overholt *et al.,* 1994) in the Kenyan coast. In the late 1960's, *C. orichalcociliellus* was the most common stem borer in maize (Mathez, 1972). Sampling of maize and sorghum fields in the same area from 1978 to 1981 revealed that *C. partellus* and *C. orichalcociliellus* were more or less equally abundant (Warui and Kuria, 1983). Surveys done in the same area from 1991 to 1992, indicated that *C. partellus* was by far the predominant stem borer accounting for greater than 80% of the total stem borer population in maize and sorghum (Overholt *et al.,* 1994). There is no evidence that total stem borer densities have changed and the apparent shift in the abundance of the two *Chilo* species suggests that the exotic stem borer may be displacing the indigenous

species (Ofomata *et al.*, 2000). *Chilo orichalcociellus* has been reported from coastal East Africa, Madagascar and Malawi (Bleszynski, 1970; Mathez, 1972; Delobel, 1975).

The biology of both *Chilo* species is similar. The females lay batches of 10-80 overlapping eggs on the upper and under side of green parallel leaves, mainly near the midribs (Overholt *et al.*, 2001). Some eggs are also laid on the stem. The eggs hatch and young larvae ascend plants and enter the leaf whorl to feed on the young leaves (Overholt *et al.*, 2001). Older larvae tunnel into stem tissue and in maize ears to feed for 2-3 weeks. They pupate in the stem tunnels after excavating emergence windows to facilitate the exit of adult moths (Kfir, 1988). Pupation takes 5-12 days (Overholt *et al.*, 2001).

Busseola fusca is a serious pest of maize at high elevations in east and southern Africa (Harris and Nwanze, 1992). The pest status of *B. fusca* varies from region to region. In East and southern Africa it is a pest at higher altitudes (>600m) (Nye, 1960; Sithole, 1989), but in West Africa, *B. fusca* occurs from sea level to >2000m (Tams and Bowden, 1953). It is primarily a pest in the dry savanna zone (Harris, 1962) particularly in areas where sorghum is grown. Females lay eggs in batches between the leaf (green or dry) sheath and the stem. Eggs hatch after one week and the larvae feed on young blades of the leaf whorl and then, suspended from silk strands, spread to neighbouring plants (Overholt *et al.*, 2001). The larvae pupate inside the stem and the pupal stage lasts about three weeks (Bosque-Perez and Schulthess, 1998).

Sesamia calamistis Hampson (Lepidoptera: Noctuidae) occurs throughout most of tropical Africa (Overholt *et al.,* 2001). Females lay up to 350 eggs, deposited in batches of 10-40 (Overholt *et al.*, 2001) between the leaf green sheaths of the host plant. Most larvae penetrate the stem after egg hatch and pupate within the base of the stem or in folds of withered leaf sheaths (Bosque-Perez and Schultess, 1998).

Eldana saccharina (Walker) (Lepidoptera: Pyralidae) is found throughout sub-Saharan Africa (Bosque-Perez and Mareck, 1990). Females lay batches of 50-100 eggs on leaves at the base of plants. Eggs hatch after about 6 days and the young larvae feed externally on epidermal tissue before penetrating the stems (Overholt *et al.*, 2001). The length of larval development is variable and may take up to 2 months. Larvae pupate inside a cocoon made of silk and plant debris (Bosque-Perez and Schulthess, 1998). Prior to pupation, the larvae leave an adult exit hole on the plant, which often has a large amount of frass hanging from it.

Table 1.1: Important stem borer species in Africa and the Indian Ocean Islands, their distributions and major host plants

Family	Species	Distribution	Host plants	Reference
Crambidae	*Chilo partellus*	Eastern and southern Africa	maize, sorghum	Harris, 1990
	Chilo orichalcociliellus	Coast east Africa, Malawi, Madagascar, South Africa, Zimbabwe	maize, sorghum	Bleszynski, 1970
	Chilo aleniellus	West and Central Africa	rice, maize	Moyal and Tran, 1992
	Chilo sacchariphagus	Indian Ocean Islands	sugarcane	Williams, 1983
		Mozambique	sugarcane	Van Rensburg *et al*, 1989
	Chilo zacconius	West Africa	rice	Maes, 1998
	Chilo diffusilineus	Tropical Africa	rice	Maes, 1998
	Coniesta ignefusalis	Sahelian Africa	millet	Harris and Youm, 1998
	Scirpophaga spp.	West Africa	rice	Breniere *et al.*, 1962
Pyralidae	*Eldana saccharina*	Sub-Saharan Africa	sugarcane maize, rice	Atkinson, 1979
	Maliarpha separatella	Sub-Saharan Africa Indian ocean Islands	rice	Cook, 1997
Noctuidae	*Busseola fusca*	Sub-Saharan Africa	maize, sorghum	Harris and Nwanze, 1992
	Sesamia calamistis	Sub-Saharan Africa	maize, sorghum, rice	Tams and Bowden, 1953
	Sesamia nonagrioides botanephaga	West Africa, Sudan	maize, sorghum, rice sugarcane	Tams and Bowden, 1953
	Sesamia cretica	Northeast Africa	maize, sorghum	Tams and Bowden, 1953

Source: Kfir *et al.*, 2002

1.2.2 Pupal Parasitoids

The parasitoids of holometabolous insects are classified by the stage they attack. Pupal parasitoids attack the pupal stage. Those that attack stem borers include several genera of the Hymenopteran families such as Eulophidae, Ichneumonidae and Chalcididae (Smith *et al.,* 1993). In contrast to parasitization of early host life stages, the indispensable contribution of mortality by pupal parasitoids could contribute more to intergenerational mortality of the stem borers (Rodriguez-Del-Bosque and Smith, 1997). Stem borer pupae that escape parasitism at the larval stages can be attacked at the pupal stages reducing stem borer pests at the next generation. Pupal parasitoids such as *P. furvus*, *D. busseolae* and *Psilochalcis soudanensis* Steffan (Hymenoptera: Chalcididae) are widespread in East Africa but they are generally not able to keep stem borer populations at a low level (Oloo, 1989).

Pupal parasitoids common in East Africa are *Pediobius furvus* Gahan (Hymenoptera: Eulophidae) and *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae). Studies done at the Kenyan coast showed pupal parasitism levels of 0-10 % (Skovgård and Päts, 1996; Ogol *et al.,* 1998) but up to 58 % in Western Kenya (Oloo, 1989) on gramineous crops. *Xanthopimpla citrina* (Holmgren) (Hymenoptera: Ichneumonidae) is an indigenous pupal parasitoid of Lepidoptera stem borer species in maize and grasses found in Kenya, Tanzania and Mozambique (Lacroix, 1967). For the last 8 years, surveys done in these countries showed seven recoveries of *X. citrina* (Overholt, Pers comm). This parasitoid is rare and leaves a void niche that could possibly be filled

through the introduction of *X. stemmator*. Moreover, parasitoids recorded in East Africa are incidental or of doubtful status (Bonhof, 2000) and have not been able to stop the spread of *C. partellus* since its introduction in the 1930's (Tams, 1932).

Pupal parasitoids respond to cues associated with plant damage or the pupal chamber (Smith *et al.*, 1993). Most of the East African pupal parasitoids use the "ingress and sting" strategy where the mature host larvae construct a moth-exit window prior to pupation. The pupal parasitoid gains access into the pupal chamber through this window and attacks the enclosed pupa. For windows that remain intact, the parasitoids use their mandibles to cut through the window and gain access to the enclosed host pupa (Smith *et al.*, 1993). Examples of pupal ingress and sting parasitoids include the gregarious endoparasitoid *P. furvus* and the solitary endoparasitoid *P. soudanensis* (Smith *et al.*, 1993).

1.2.3 *Xanthopimpla stemmator*

Xanthopimpla stemmator is a solitary pupal endoparasitoid of lepidopteran stem borers (Smith *et al.*, 1993). The adult female locates the pupal chamber in the stem and actively drills through the plant rind (Smith *et al.*, 1993). The female initially punctures the pupa several times with her ovipositor and feeds on liquid expelled from the pupa (Moore and Kfir, 1996). It is thus clear that females may get access to nutrients while ovipositing through host feeding (Moutia and Courtois, 1952). Eggs are laid inside the pupa but only one develops into the subsequent larval stages and then to the adult wasp

(Smith *et al.*, 1993). Pupation takes place in the host pupa and the parasitoid emerges from the pupal case (Nikam and Basarkar, 1981). The adult wasp uses the moth exit window to egress (Smith *et al.*, 1993).

Temperature, availability of water and nutrients affect adult longevity. Under natural conditions, access to water and sugar solutions accompanied by a combination of rain or dew, honeydew and host feeding provide sustenance for increased female longevity (Hailemichael *et al.,* 1994). Studies on longevity of *X. stemmator* show variable results depending on temperature and relative humidity. When provided with honey and water, females lived for about 140 days and males for 87 days at 24 ± 2°C and 60-70% relative humidity on *C. partellus* pupae (Moore and Kfir, 1996). Nikam and Basarkar (1981) found the average longevity of mated females to be 30 days (maximum 37 days and minimum 22 days) at 22±1°C and 50-55% relative humidity.

The pre-oviposition period of females from studies by Moore and Kfir (1996) was 3-6 days and oviposition period 64 days on *C. partellus*. The females produced an average of 95 offspring with females comprising 64 % of the progeny. The presence of hosts shortened the lifespan of both sexes but was more significant in the females (Moore and Kfir, 1996). Studies by Hailemichael and Smith (1994) showed that egg-to-adult developmental time was 42 days at 20°C and 15 days at 28°C. The number of ovarioles per female was found to vary from four to six and the number of mature eggs present in them at any one time rarely exceeded eight (Moutia and Courtois, 1952).

Parasitization by *X. stemmator* is achieved by piercing the stem directly with a stout ovipositor and reaching the pupa within the chamber, a strategy referred to as a "drill and sting" strategy (Smith *et al.*, 1993). Due to its different attack strategy, *X. stemmator* could complement the pupal parasitoids found in Africa.

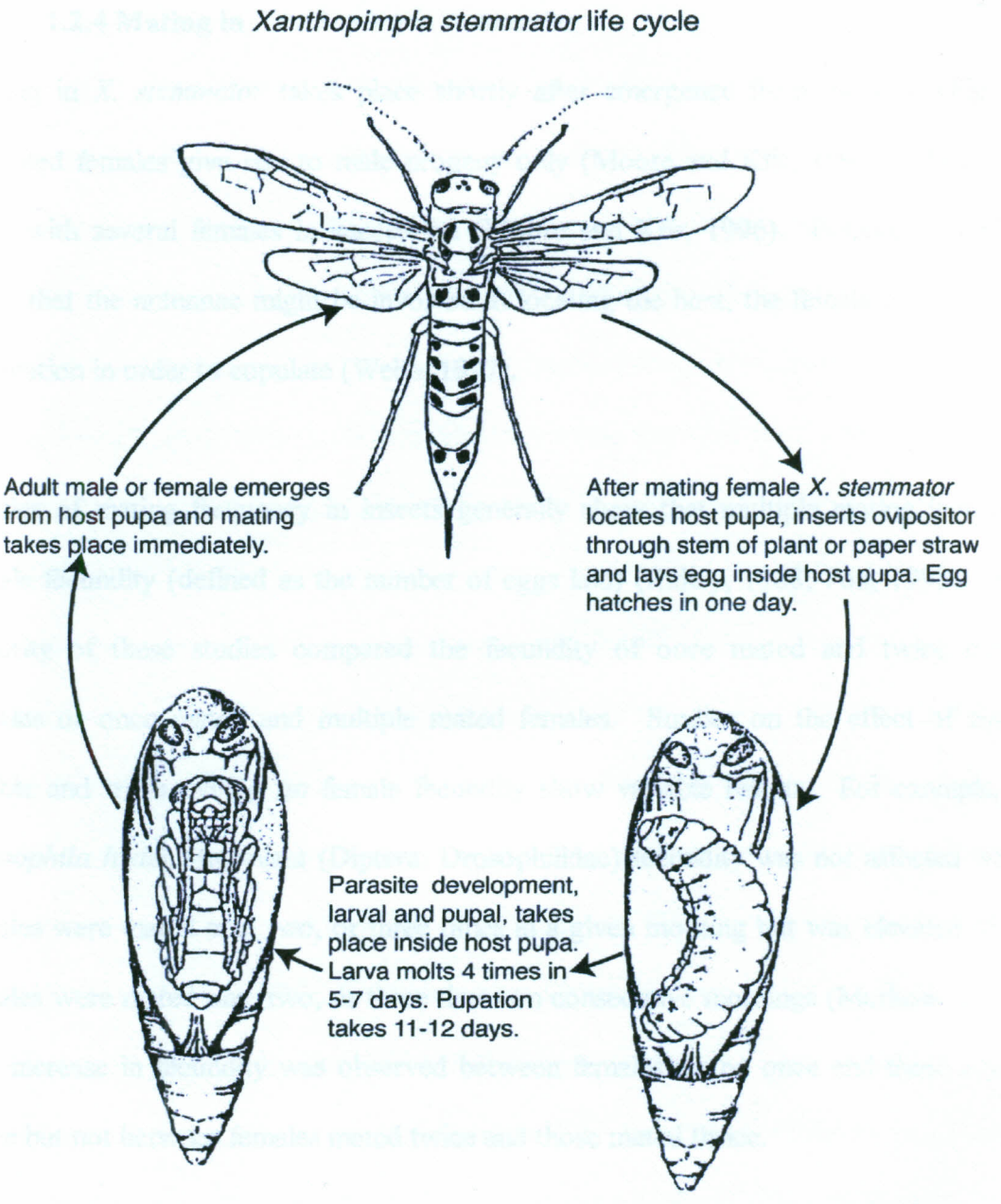


Fig 1.1. Life Cycle of *Xanthopimpla stemmator* (Conlong and Graham, 1988).

1.2.4 Mating in *Xanthopimpla stemmator*

Mating in *X. stemmator* takes place shortly after emergence from the host (Fig 1). Unmated females give rise to male progeny only (Moore and Kfir, 1996). Males can mate with several females in succession (Moore and Kfir, 1996). Behavioral studies show that the antennae might be involved in locating the host, the female and for male orientation in order to copulate (Webb, 1997).

Studies of mating frequency in insects generally show that multiple mating increases female fecundity (defined as the number of eggs laid) (Ridley, 1988; Fox, 1993). The majority of these studies compared the fecundity of once mated and twice mated females or once mated and multiple mated females. Studies on the effect of single double and triple mating on female fecundity show variable results. For example, in *Drosophila hydei* Sturtevant (Diptera: Drosophilidae) fecundity was not affected when females were mated one, two, or three times in a given morning but was elevated when females were mated one, two, or three times on consecutive mornings (Markow, 1985). The increase in fecundity was observed between females mated once and those mated twice but not between females mated twice and those mated thrice.

No studies on mating frequency in *X. stemmator* have been documented. Multiple mating in social Hymenoptera species is rare (Strassmann, 2001). In most Hymenoptera species, sex is determined by haplodiploidy, that is unfertilized (haploid) eggs develop into males and fertilized eggs (diploid) develop as females (Holloway *et al.*, 1999).

1.2.5 Distribution of *Xanthopimpla stemmator*

Xanthopimpla stemmator has a broad geographical distribution and attacks stem borers found in gramineous plants (Table 1.2). It was introduced into Mauritius from Sri-Lanka in 1939 (Jepson, 1939). It became established against the lepidopteran sugarcane borers *C. sacchariphagus* and *S. calamistis* (Vinson, 1942; Moutia and Mamet, 1945; Moutia and Courtois, 1952). It was introduced into Reunion from Mauritius in 1962 and successfully established on *C. sacchariphagus* in sugarcane (Caresche, 1962).

Xanthopimpla stemmator was introduced into South Africa from Sri-Lanka via Mauritius against *E. saccharina* in sugarcane in 1987 (Carnegie, 1991; Conlong, 1994) and against *C. partellus* in maize and sorghum (Moore and Kfir, 1996) between 1987 and 1993. Recoveries of parasitoids were made at the release sites but its establishment was not noted. In maize and sorghum fields, a total of 1600 parasitoids were released in 1987, 1900 in 1989, 1970 in 1990 and about 800 parasitoids in 1993. Of all released parasitoids, 69 % were females. The parasitoid was released each season in January, the period when pupae of *C. partellus* normally start to appear in the field (Kfir, 1992). A small number was recovered from the field in the vicinity of the release sites during February and March. In 1987, four parasitoids were recovered, 12 parasitoids in 1989 and six in 1990, no parasitoid was recovered after 1991 (Kfir, 1994), which indicated that *X. stemmator* failed to establish on the stem borers in South Africa on *C. partellus* (Moore and Kfir, 1996).

Several factors were identified that may have hindered establishment of the parasitoid. *Xanthopimpla stemmator* occurs in warm low altitude areas and the release sites in the Transvaal are at high altitudes with frequent sub-zero temperatures at night and during winter (Moore and Kfir, 1996). *Chilo partellus,* one of the targeted hosts, diapauses as a mature larva in the lower part of the dry stalk in winter (April to October), which is the dry season (Kfir, 1988; Kfir *et al.,* 1989; Kfir, 1991). The host stage is thus available in crops for a short period during January to March (Kfir, 1992). Large numbers of *X. stemmator* were also released in KwaZulu Natal against *E. saccharina* in sugarcane fields but it failed to establish (Moore and Kfir, 1996).

Xanthopimpla stemmator has recently been released in Mozambique against *C. sacchariphagus* in sugarcane (Conlong and Goebel, 2002). Surveys about its effectiveness are still in operation.

Table 1.2: Distribution of *X. stemmator,* host range and previous successful introductions

Location	Host attacked	Host plant	Reference
India	*Bissetia (=Acigona) steniella* (Hampson)	Grasses	Vinson, 1942
India	*Chilo partellus* (Swinhoe)	Sorghum	Vinson, 1942
Taiwan	*Chilo infuscatellus* (Snellen)	Grasses	Sonan, 1929
Taiwan	*Ostrinia nubilalis* (Hubner)	Grasses	Cartwright, 1933
Taiwan	*Scirpophaga nivella* (F.)	Grasses	Takano, 1934
Taiwan	*Sesamia inferens* (Walker)	Grasses	Sonan, 1929
West Malaysia	*Ostrinia salentialis* (Snellen)	Maize Sorghum	Yunus and Hua, 1969
Philippines	*Ostrinia furnacalis* (Guenee)	Maize	Camarao, 1979
West Malaysia	*Ostrinia furnacalis* (Guenee)	Millet	Yunus and Hua, 1969
*Mauritius	*Chilo sacchariphagus* (Bojer)	Sugarcane	Moutia and Mamet, 1945
Sri-Lanka	*Chilo sacchariphagus* (Bojer)	Sugarcane	Vinson, 1942
*Reunion	*Chilo sacchariphagus* (Bojer)	Sugarcane	Caresche, 1962

**Xanthopimpla stemmator* was introduced and is established in these areas against the stem borers indicated.

1.2.6 Habitat and host finding, host preference, acceptance, suitability and regulation

For any natural enemy to be a successful biological control agent it must follow the consecutive processes that include habitat finding, host location, acceptance, suitability and regulation (Salt, 1935; Doutt, 1959). The continuum of processes begins with the parasitoid reacting to stimuli like host plant odour, and progressively becoming more interactive with the host itself as the sequence progresses (Hailemichael *et al.,* 1994).

1.2.6.1 Habitat finding

The process requires the female parasitoid to locate the habitat that her host has colonized. Cues for locating an appropriate habitat are usually emitted by the host plant community in which the stem borer resides (Smith *et al.*, 1993). The parasitoid most likely responds to long distance cues emanating from the habitat of their hosts like wild grasses and cultivated grasses (Smith *et al.*, 1993). Plants may be induced by the stem borer feeding to release volatile chemicals that attract stem borer parasitoids (Ngi-Song *et al.*, 1996). No studies have been done on habitat finding in the field in *X. stemmator*. The female successfully parasitises the host when she receives the proper cues that lead to finding the hosts in a particular habitat (Smith *et al.,* 1993).

1.2.6.2 Host finding

Some aspects of host finding behaviour of *X. stemmator* have been examined (Hailemichael *et al.,* 1994). Cues that can be exploited by the parasitoid for host finding include chemical cues such as by-products of normal host activity or a response by the plant to host attack, or physical cues such as discoloration or deadheart of stems, host frass, larval tunnels or emergence window or the host itself.

Xanthopimpla stemmator females alight on stems and move rapidly up and down the stem, palpating the surface with their antennae, apparently searching for cues that denote that a host is present (Hailemichael *et al.,* 1994). Attraction of *X. stemmator* females to larval frass helps guide it to the microhabitat of the host pupa and stimulates ovipositor drilling. Sound and/or vibration associated with host activity guide *X. stemmator* to concealed hosts (Hailemichael *et al.,* 1994). Host odours and pupal movements are close range attractants that retain searching females and stimulate them to drill frequently into host pupae. They restrict local searching to help pinpoint the location for ovipositor drilling (Hailemichael *et al.,* 1994).

1.2.6.3 Host preference

The choice of suitable candidate species or strains is essential for successful biological control programs because the host-specificity characteristics are among the factors determining their effectiveness as control agents (Pak, 1988). Effectiveness is likely to

be diminished if certain host ages or species are preferred over others because non-preferred stages or species have an increased probability of remaining unparasitised (Ehler and Van den Bosch, 1974). No study has so far been published on host preference by *X. stemmator*.

1.2.6.4 Host acceptance

Requisites for host acceptance may include host size, shape, texture, age, odour, behaviour and previous parasitization status. The ovipositing female parasitoid must select hosts that are the correct life stage and age to support successful progeny development (Poppy, 1997). *Xanthopimpla stemmator* readily accepts pupae enclosed in a stem or wrapped tissue but rarely accepts pupae that are naked (Smith *et al.,* 1993). The parasitoid drills with its stout ovipositor through the stalk wall or leaf sheath enclosing the host and parasitises the pupa constrained in the cryptic microhabitat (Smith *et al.,* 1993). Host acceptance is defined by the presence of external punctures from the parasitoid ovipositor (Hailemichael *et al*, 1994) here referred to as probe wounds.

1.2.6.5 Host suitability and regulation

Host suitability is defined as the adequacy of a host, once oviposition has occurred to successfully support growth and development of the parasitoid progeny (Hailemichael *et al.,* 1994). Successful parasitization is evidenced by parasitoid emergence. Moth

emergence from hosts with ovipositor probe wounds indicates unsuccessful parasitization. Host suitability studies done on the parasitoid showed that host suitability decreases with host age (Hailemichael *et al.*, 1994). One to five-day-old *D. saccharalis* pupae averaged 31-37% suitability whereas only 19% of six-day-old pupae were suitable (Hailemichael *et al.*, 1994). For laboratory rearing, the appropriate host age for each parasitoid is one that is co-adapted with the parasitoid (Smith *et al.*, 1993). A suitable host provides adequate shelter and nutrients for complete development of the parasitoid.

1.2.7 Life-tables and intrinsic rates of natural increase of *Xanthopimpla stemmator*

Fertility life tables are appropriate to study of the dynamics of animal populations, especially arthropods, as an intermediate process for estimating parameters related to population growth potential (Hulting *et al.*, 1990). The population growth potential of insects can be used as an indicator in studies that aim to assess environmental effects of agricultural technologies and practices (Stark and Wennergren, 1995; Nascimento *et al.*, 1998) such as the assessment of potential biological control agents.

The parasitization potential of a natural enemy is assessed by comparing the fertility (cumulative and daily), progeny production, and oviposition period including the proportion of days spent for oviposition and adult longevity. The age specific life tables

involve counting individuals in a single cohort over time. To associate variation in the intrinsic rate of natural increase and the net reproductive rates (r_m and R_o), the jackknife method (Meyer *et al.*, 1986; Wermelinger *et al.*, 1991) may be used. The sex ratio, the standard error and the confidence interval of the mean are then calculated as elaborated by Hulting *et al.* (1990).

Life tables and intrinsic rate of natural increase of *X. stemmator* have been studied using *C. partellus* as a host in India at 22 ±1°C and 50-55% relative humidity (Nikam and Basarkar, 1981). The rate of increase was found to be 0.131 and the population multiplied 43.43 times in the mean generation time of 28.78 days. The male to female ratio averaged 1.14:1. Progeny production ranged from 71 to 115 with an average of 84.50 (Nikam and Basarkar, 1981). Such a study needs to be conducted under tropical conditions.

1.3 Rationale of the study

Pesticides are widely used for pest control. They have harmful effects to the environment, affect non-target organisms, are costly to farmers and some pests develop resistance to them (Bonhof, 2000). An alternative strategy is biological control. A successful classical biological control is underway in Kenya in which *Cotesia flavipes,* a larval parasitoid of stem borers has been released in the field (Overholt, 1998). However, the level of control provided by this natural enemy varies from area to area

(Overholt *et al.*, 1997). It is now becoming clear that its effectiveness will not be uniform in all regions of the country. One of the reasons is that *C. flavipes* is not able to develop in *B. fusca* (Ngi-Song *et al.*, 1998), one of the indigenous stem borer species found in the mid and high elevation areas of >600m in eastern and southern Africa (Overholt *et al.*, 2001). *Xanthopimpla stemmator* attacks the pupal stage of the stem borers and could therefore provide complimentary mortality to *C. flavipes.* It uses the "drill and sting" attack strategy hence it is not likely to compete with other indigenous pupal parasitoids.

Effective use of *X. stemmator* for control of stem borers on cereal grain crops requires a detailed knowledge on the biology of the parasitoid and an understanding of the host range suitable for parasitoid development. Information on host acceptability and suitability are necessary to gain insight into parasitoid-host interactions because of the absence of a co-evolutionary history with native African stem borers. Information on the hosts that are most preferred will be useful in determining the ecological application of *X. stemmator.* Being a new parasitoid, its reproductive biology needs to be studied before it is released into the field. Over-production of males is a disadvantage in a biological control program (Waage and Greathead, 1985; Waage, 1986). A study of mating behavior and effect on sex ratio may uncover any problem associated with biased sex ratios in laboratory colonies. This will enhance efficient techniques for mass-rearing of the parasitoids at particular times for field releases.

1.4 Hypotheses

1. *Xanthopimpla stemmator* has no preference for any of the Kenyan common stem borer species
2. *Xanthopimpla stemmator* accepts and develops in the major stem borers found in Kenya.
3. The intrinsic rate of natural increase is the same in *C. partellus* and *B. fusca*.
4. *Xanthopimpla stemmator* females mate once and progeny production and sex ratio are independent of number of mating.

1.5 General Objective

To assess the potential of *X. stemmator* for use as a biological control agent of stem borers in Kenya.

1.5.1 Specific objectives

1. To examine the preference of *X. stemmator* for *C. partellus, B. fusca, S. calamistis, E. saccharina*
2. To assess the suitability of the four major stem borers found in Kenya for the development of *X. stemmator.*
3. To compare the life tables and intrinsic rate of natural increase of *X. stemmator* on *B. fusca* and *C. partellus*.
4. To determine the effects of multiple mating on fecundity in female *X. stemmator.*

CHAPTER TWO

GENERAL MATERIALS AND METHODS

2.1 Insects

2.1.1 Stem borer species

Four stem borer species were used in this study. Two of the borers, *C. partellus* and *S. calamistis,* were collected from maize fields at the Kenyan coast. A colony of *B. fusca* and *E. saccharina* was initiated with material collected from western Kenya. All stem borers were reared in the Animal Rearing and Quarantine Unit (ARQU) at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. *Chilo partellus* and *E. saccharina* were reared on a diet described by Ochieng *et al.* (1985) while *S. calamistis* and *B. fusca* (Lepidoptera: Noctuidae) were reared according to the method described by Onyango and Ochieng-Odero (1994).

2.1.2 Parasitoids

A colony of *X. stemmator* was initiated from material imported from South Africa. They were offered pupae of *C. partellus* (ratio 20 *X. stemmator* females to 100 pupae) two times a week for oviposition in clear and clean Perspex cages (15cm x 15cm x 15cm) (Plate 2.1). The parasitized pupae were kept in Petri dishes, 8 cm in diameter (Plate 2.2) after each exposure until adult parasitoids emerged. On emergence, the adults were released in a clear Perspex cages (Plate 2.1) and provided with 10 % honey and water solution as diet. Since *X. stemmator* females do not attack naked pupae, they

were offered the pupae in paper straws (Plate 2.3). Ten pupae were placed in each of the straws. Preliminary tests showed that they could equally attack pupae inserted in stems of maize and Napier grass (*Pennisetum purpureum* Schumach). The straws were smeared with frass from the respective stem borer species to simulate stalks of gramineous crops and to enhance acceptance (Plate 2.4). All the experiments were carried out at 27 ± 2°C, 49-61% RH and 12: 12 (L: D) h photoperiod in the ICIPE quarantine laboratory.

2.2 Plants

Maize (*Zea mays* L., (hybrid 5-12)) and the Napier grass were grown in the fields at ICIPE. Larvae were given stems of maize and the grass and used to produce frass whenever required during the experiments.

Plate 2.1: 15cm x 15cm x 15cm Perspex cages used for rearing the parasitoids

Plate 2.2: Petri dishes where parasitized pupae were kept until adult *X. stemmator* emergence.

Plate 2.3: Pupae in paper straws smeared with frass attached to clay at the base

Plate 2.4: Straw smeared with frass from the respective stem borer species and a female *X. stemmator* searching for the pupae

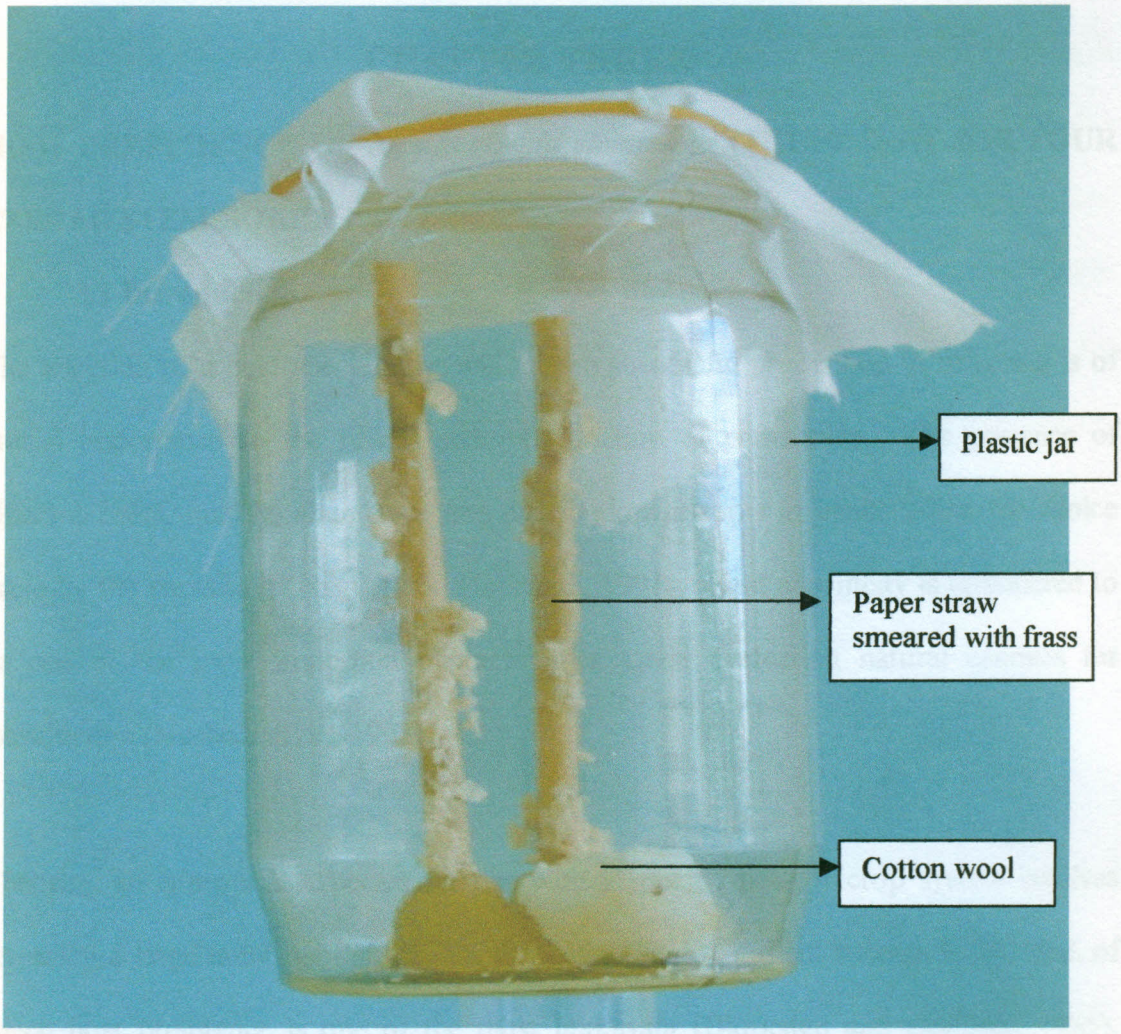


Plate 2.5: Plastic jar 9 cm by 5 cm used for exposing pupae to females until their natural death. The paper straws were smeared with respective stem borer frass. Cotton wool soaked in 10 % honey was put inside the jar for the parasitoids' nourishment. The jar was covered with a polyester net to prevent the parasitoid from escaping.

CHAPTER THREE

HOST PREFERENCE OF *Xanthopimpla stemmator* (THUNBERG) FOR FOUR CEREAL STEM BORERS

3.1 Introduction

The selection of a host species that will ensure successful production of offspring is of crucial importance to the fitness and reproduction of parasitoids. The presence of different hosts, quality, abundance and distribution patterns in plants affect the choice made by the parasitoid (Hagvar and Hofsvang, 1991). Host specificity is considered to be one of the most important characteristics when evaluating natural enemies for introduction (Nechols *et al.*, 1992).

Choosing an appropriate species for use with a specific pest or crop system involves screening a large number of strains for characters likely to affect success in the field, of which host preference is one of the most important (Bjorksten and Hoffman, 1995). Since screening is time consuming, several workers have tried to devise the simplest and most time efficient method (Dijken *et al.,* 1986; Hassan, 1991; Scholz, 1991). A number of methods have been reported in literature, including both choice (Dijken *et al.*, 1986; Pak *et al.*, 1990; Hassan, 1991) and non-choice (Scholz, 1991) tests.

Immature parasitoids can gain experience through developing inside hosts (Vinson *et al.*, 1977; Drost *et al.*, 1988; Sheehan and Shelton, 1989). This type of pre-adult learning may be important in the use of *X. stemmator* as a candidate for classical biological control since the wasp can be mass reared on a factitious host. Pre-adult experience and host preference have not been studied in *X. stemmator*. Several studies have revealed the presence of multiple stem borer species in gramineous plants (Girling, 1978; Hughes *et al.*, 1982; Kaufmann, 1983; Seshu Reddy, 1983 and Ofomata *et al.*, 1999). There is therefore need to establish whether *X. stemmator* discriminates between stem borer species in the laboratory so as to predict its behaviour in the field.

3.2 Objectives

1. To determine the effect of pre-adult experience on the choice of hosts attacked by *X. stemmator* females.
2. To determine host preference of *X. stemmator* for *B. fusca, C. partellus, E. saccharina* and *S. calamistis*

3.3 Materials And Methods

3.3.1 Insects

Pupae of *B. fusca, C. partellus, E. saccharina* and *S. calamistis* were all reared as described in chapter two. One to five day old stem borer pupae were used in this study. The study was carried out with female parasitoids that emerged from the four stem borer species.

3.3.2 Larval frass

Ten fourth instar larvae of *B. fusca, C. partellus, E. saccharina*, and *S. calamistis* were given fresh maize-stalk sections to feed on in order to produce frass.

3.3.3 Bioassays

Two tests were conducted, the dual and four choice tests.

3.3.3.1 Dual choice test

Two three or four-day-old pupae from each of the four stem borer species were presented to *X. stemmator* female in dual choice tests. A pupa of one stem borer species was inserted into the lower half of an eight cm long paper straw, which was secured upright with clay bases (Plate 2.3). A little frass of the corresponding stem borer species was smeared around the tip of each end of the straw (Fig 3.1). A piece of cotton wool was squeezed halfway down the straw to prevent movement of pupal odour inside

the straw. A second pupa from a different stem borer species was inserted into the upper half of the straw (Fig 3.1). Frass of the respective borer species was smeared at the tip of the end. The straw containing both pupae was put in a plastic jar measuring 9.5 cm wide and 16.5 cm high. A five or six-day-old mated *X. stemmator* female that emerged from one of the two species was released into the jar.

Observations were made for one hour or until the female made two choices within the one-hour period. Choice was recorded if a host pupa was probed. Observations taken included first and second choice made by a given female. No choice was recorded if after one-hour the parasitoid did not probe either of the two pupae. The observations were carried out thirty times and the pupae were interchanged in fifteen of thirty observations to avoid any positional bias. A total of twelve tests were conducted with 30 observations for each dual test and with a female parasitoid reared on each of the stem borer species. The following were the dual combinations tested.

B. fusca vs. *C. partellus*

B. fusca vs. *E. saccharina*

B. fusca vs. *S. calamistis*

C. partellus vs. *E. saccharina*

C. partellus vs. *S. calamistis*

E. saccharina vs. *S. calamistis*

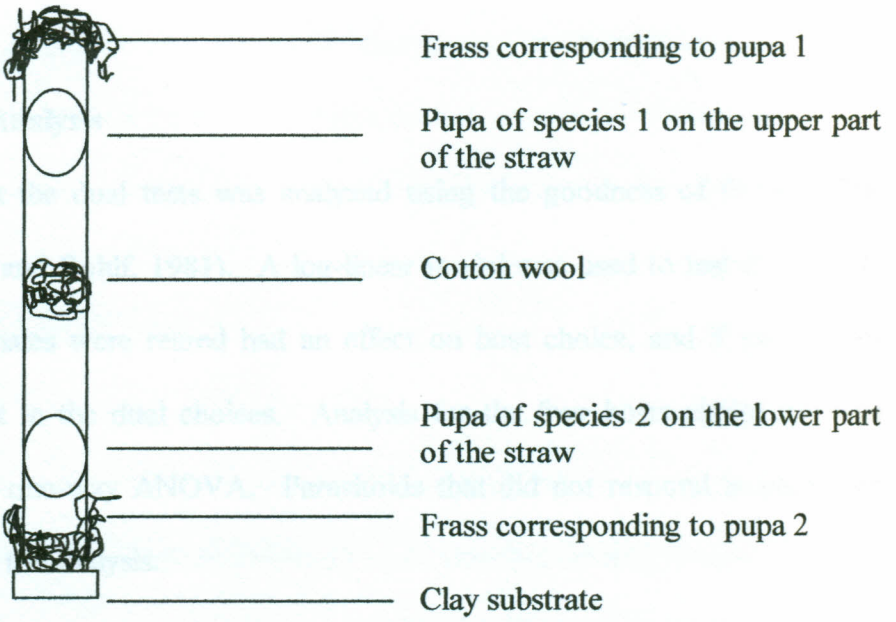


Fig 3.1: Set up for the dual choice test

3.3.3.2 Four choice tests

A single pupa of each of the four stem borer species was placed in a five cm paper straw, which was secured with a clay base. The straws were smeared and sealed with frass made from the corresponding species. The four straws were placed 5cm from the corners of a clear Perspex cage (Plate 2.1). A five of six-day-old mated *X. stemmator* female was released into the cage. A total of ninety females were used. The positions of the straws containing the different stem borers species were rotated regularly within the cage to avoid any positional bias. The first and second choices made by the female

wasps were recorded within a one-hour period in the same way as in the dual tests. Females reared from the four stem borer hosts were used.

3.3.4 Data Analysis

Data obtained from the dual tests was analyzed using the goodness of fit test (Chi-square test) (Sokal and Rohlf, 1981). A log-linear model was used to test if the hosts from which the females were reared had an effect on host choice, and if the choices made were different in the dual choices. Analysis for the four hosts choice test was carried out using a one-way ANOVA. Parasitoids that did not respond in both tests were excluded from the analysis.

3.4 Results

Since a few females made a second choice in a different host pupa (26.4 % of the observations), analysis was carried out for the first choice the females made. First and second choices were different in the dual tests carried out (χ^2 = 69.59; df = 1; P = 0.0001). The parasitoids always attempted to oviposit on the first host that they encountered. The mean time females took to make a choice was 2.6 ± 0.5 minutes (range 0-10 minutes).

Females did not discriminate between pupae they were reared on and those they were not. Stimuli perceived during development or upon eclosion do not seem to condition *X. stemmator* females to respond to odours associated with the host from which they emerged. The choice of host pupae was independent of the host species the female was reared on (χ^2 = 2.11; df = 9; P = 0.9896). Therefore, analysis for choice was carried out ignoring the source of the female parasitoid. Dual tests carried out in this study showed no discrimination between host species. Dual choices were not different in all the six pairs tested (χ^2=5.85; df = 4; P = 0.2108; Fig 3.2).

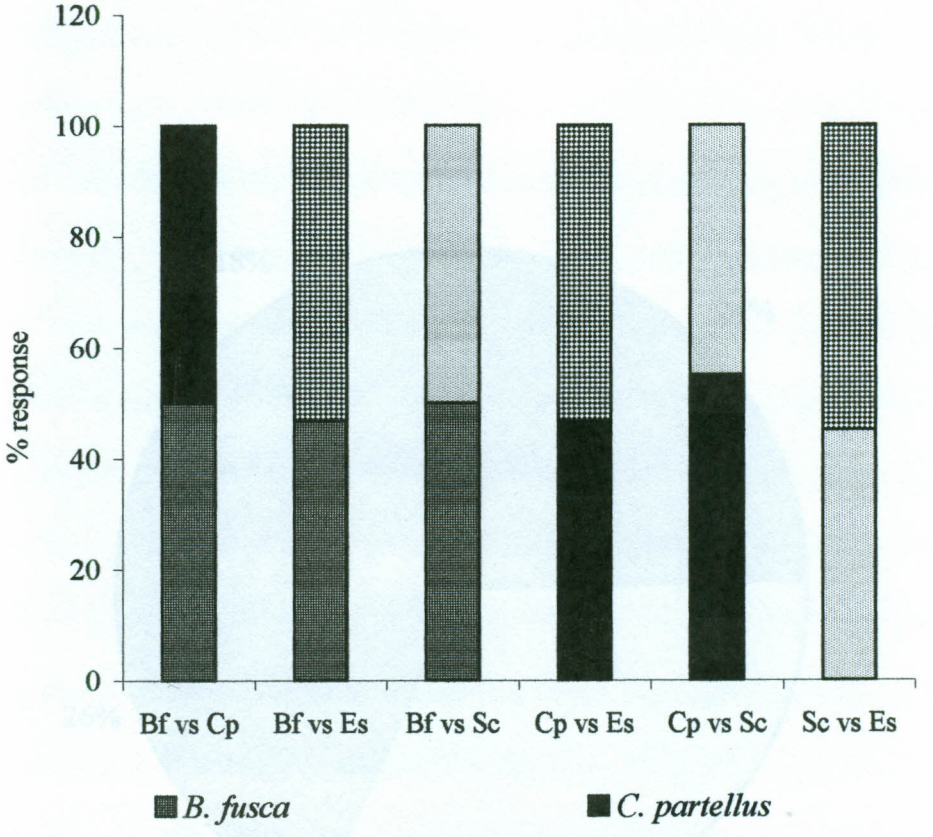


Fig 3.2: Response of naïve *X. stemmator* females to pupae of four stem borer species in dual choice tests (n = 60 for each dual test).

In the tests where all four-host pupae were presented to the females, host pupae from which females emerged did not influence choice ($F = 0.48$; $df = 3$; $P = 0.7029$). The females also did not discriminate between the four host pupae provided ($F = 1.28$; $df = 3,12$; $P = 0.3262$) (Fig 3.3).

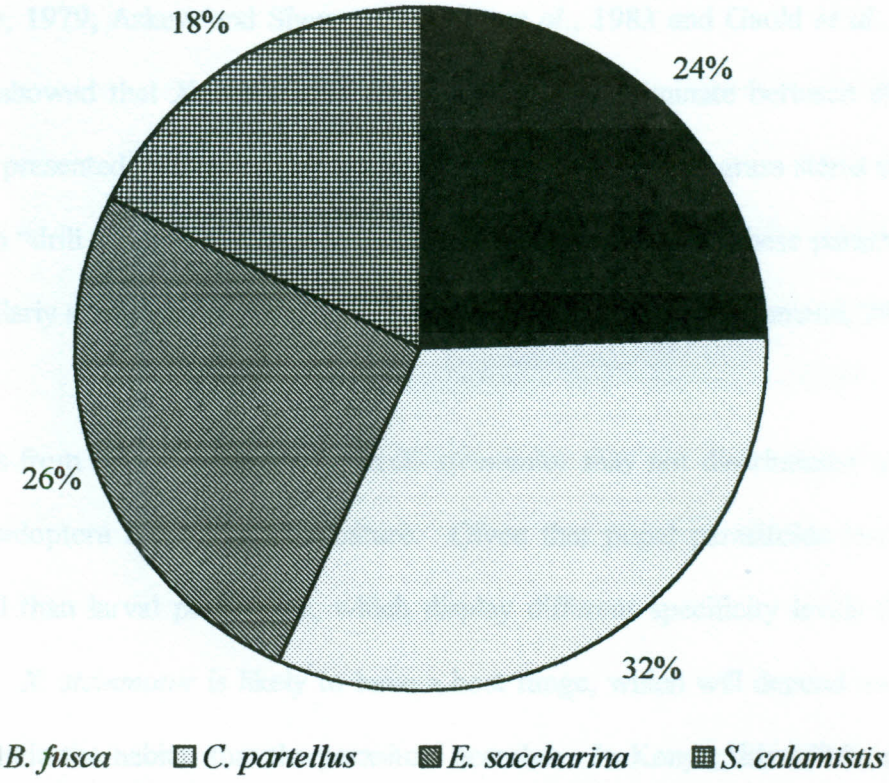


Fig 3.3: Percentage response of *X. stemmator* females to four stem borer species in four choice tests (n = 90 for each test).

3.5 Discussion

Parasitoids can generally be categorized as koinobiont or idiobiont. Koinobiont parasitoids allow hosts to grow after oviposition. Idiobiont parasitoids kill or paralyze hosts and avoid internal host defenses (Askew and Shaw, 1986). *Xanthopimpla stemmator* is classified as an idiobiont parasitoid. Because koinobionts parasitoids must coexist with their hosts, they tend to be more specialized than idiobionts (Salt, 1968; Waage, 1979; Askew and Shaw, 1986; Vet *et al.*, 1983 and Gauld *et al.*, 1992). This study showed that *X. stemmator* females did not discriminate between the stem borer pupae presented as hosts. They probe any pupae enclosed in grass stems such as maize, using a "drill and sting" attack strategy (Smith *et al.*, 1993). These parasitoids respond to similarly shaped artificial "stems" such as a paper straw (Weidenmann, 2000).

Results from this study suggest that *X. stemmator* may not discriminate between pupae of Lepidoptera stem borers in nature. Given that pupal parasitoids tend to be more general than larval parasitoids, which display different specificity levels (Fleury *et al.*, 2001), *X. stemmator* is likely to have a host range, which will depend on the diversity of hosts in the habitat that the parasitoid searches. In Kenya, this will be probably four or five stem borer species. Host finding studies by Hailemichael *et al.* (1994) showed that the *X. stemmator* females begin by searching straws in the laboratory or grass stems

in nature. The presence of larval frass, host odour and movement of host pupae stimulate this host seeking activity.

However, laboratory host preference tests have some limitations and may not be fully trusted (Simmonds, 1944). For example, Sands (1993) states, "insect enemies of arthropod pests, when confined in cages, can sometimes utilize hosts or prey not normally attacked in the field." Applicability of results obtained from the laboratory tests need to be validated in the field. It has been argued (Salt, 1935) that a better picture of host preference would be gained if hosts were offered in combination so that the parasitoid has a choice between hosts. In this study, as with other studies of host preference in parasitoids using laboratory choice tests, (Cornell and Pimentel, 1978; Dijken *et al.*, 1986; Mandeville and Mullens, 1990), females tend to continue to parasitise the host species that was encountered first. Recent studies have emphasized the relative importance of parasitoid foraging behaviour as opposed to immunological and physiological constraints, in the evolution of parasitoid host range (Vet and Dicke, 1992; Wiskerke and Vet, 1994; Brodeur *et al.*, 1996).

Host preference studies need to be conducted in the field since laboratory results may not completely reflect the behaviour of *X. stemmator* in the field. For example, the experimental set-up had no airflow and the odours from the different frass samples may have intermixed in the glass jars. Searching *X. stemmator* females may not have been

able to discriminate between the odours. The females may have used frass to detect the presence of pupae in the straws and this may have made them select the first host they encountered, a second time. The role of tactile and visual cues in locating the hosts also needs to be examined.

Host preference can also be modified through adult learning (Vet and Dicke, 1992) and this has far reaching implications on biological control due to its effect on the females' searching efficiency. The phenomenon of associative learning (establishment through experience of an association between two stimuli or between a stimulus and a response) in parasitoids may be exploited for purposes of biological control (Prokopy and Lewis, 1993). Further behavioural studies are needed to determine whether *X. stemmator* females are able to learn to discriminate between pupae of different borer species as they gain experience and age. The results of this study suggest that *X. stemmator* females will attack the four stem borers if introduced to areas where all the four borers are present coincidentally in time and space. It is predicted that any stem borer species in the habitat where the parasitoids search which were not included in this study, would be attacked. The advantage of this non-discrimination of hosts by *X. stemmator* includes rapid spread and increased survival when some host species are rare.

Studies on non-target Lepidoptera should be carried out to establish whether the parasitoid might have any adverse effect on other insect species, once its released into

the fields. However, any laboratory non-target tests should be conducted in an ecologically relevant context. *Xanthopimpla stemmator* may be able to attack and develop in non-target hosts in the laboratory, especially if the non-target hosts were concealed in straws on grass stems. In nature however, *X. stemmator* would never search the habitat where such hosts are found.

CHAPTER FOUR

HOST SUITABILITY OF FOUR GRAMINEOUS STEM BORER SPECIES FOR THE DEVELOPMENT OF *Xanthopimpla stemmator* (THUNBERG)

4.1 Introduction

The International Centre of Insect Physiology and Ecology (ICIPE) is searching for additional natural enemies of stem borers to complement the ones already existing in eastern and southern Africa. *Xanthopimpla stemmator* is a promising candidate (Hailemichael *et al.*, 1994). *Xanthopimpla stemmator* was imported into Kenya from a laboratory colony in South Africa, where it had been previously introduced from Mauritius. The Mauritius population originated from insects introduced from Sri-Lanka. In its original habitat, *X. stemmator* occurs in warm low-altitude areas (Moore and Kfir, 1996). Kenyan temperatures in areas where pests of gramineous crops are found may favour the establishment of the parasitoid.

Host species and host age have a substantial influence on whether potential hosts are attacked successfully by parasitoids (Pak, 1986). Physiological suitability of the host is necessary for the successful development of parasitoid progeny (Hailemichael *et al.*, 1997; Wiedemann and Smith, 1997). The aim of this study was to investigate acceptance and suitability of both indigenous and exotic Kenyan stem borers for the development of *X. stemmator*. The suitable pupal host age was also examined to determine the optimal age for the purpose of mass rearing.

4.2 Objectives

1. To determine whether *X. stemmator* accepts and successfully develops in *Chilo partellus, Busseola fusca, Eldana saccharina* and *Sesamia calamistis*.
2. To determine the suitable pupal age of the four stem borer species for successful development of *X. stemmator*.
3. To determine fitness of parasitoids emerging from the four stem borer species.

4.3 Materials And Methods

4.3.1 Insects

The four stem borer species, *C. partellus, B. fusca, E. saccharina* and *S. calamistis* and the parasitoid *X. stemmator* were reared and maintained as described in chapter two.

To establish whether the size of host affected acceptance, developmental time, longevity and wing length of parasitoids, at least 225 individual pupae from each of the four host species were weighed.

4.3.2 Host species and host age acceptance

Mated naïve *X. stemmator* females (those that had not oviposited) between five to nine day old emerging from *C. partellus* were used in these experiments. One to six day old pupae of *B. fusca, C. partellus, E. saccharina* and *S. calamistis* were each exposed to female *X. stemmator.* An average of 100 pupae less than 24 hours old were collected from the insectary daily. Tests began when all day one to six old host ages needed were obtained. Four pupae of the same host and host age 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 (Plate 2.3) and placed in a vial 2cm in diameter and 12cm height.

One mated *X. stemmator* female aged between five and nine days old was given the four pupae of the same host and age to test acceptability. The female was released into the vial and a piece of cotton wool soaked in 10% honey / water solution was attached to

the surface of the vial as diet. The vial was plugged with clean cotton wool to prevent the parasitoid from escaping.

The female and pupae were removed after 6 hours exposure. A minimum of 60 pupae (15 replicates) were exposed for each host and host age combination. Individual parasitoids and stem borers were used only once. Each pupa was removed and the numbers of probe wound (s) on its cuticle counted and recorded. A sample of 64 probed pupae was dissected. Dissection was carried out under a dissecting microscope. The number of eggs found inside the pupal case were counted and recorded.

4.3.3 Host species and host age suitability

All the stem borer pupae that were exposed in the above experiment were placed individually in vials, plugged with clean cotton wool and maintained at 27 ± 2° C, 49-61% RH and 12: 12 (L: D) photoperiod. The host pupae were inspected daily for moth emergence, parasitoid emergence 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 given a 10% honey / water solution and confined in a vial until their natural death. The diet was changed every day and longevity and left upper wing length of each adult parasitoid recorded upon its death.

Progeny produced by F1 generation was determined to assess and compare the fitness of the offspring. 30 mated females emerging from *B. fusca, C. partellus, S. calamistis* and 27 emerging from *E. saccharina* were given 10 pupae every day after the first day of emergence until their natural death. Records were taken for the length of time the female lived and the number of male and female progeny each female produced in its lifetime.

A control group was monitored. For each sample of four host pupae provided to naïve females, four pupae of the same host and age were kept as control. They were not offered to the females for oviposition and were kept in the quarantine laboratory for 30 days or until all the pupae emerged adult moths. Moth emergence or death of the host pupae was recorded.

4.3.4 Data analysis

Data on acceptance, fate of parasitized pupae, developmental time, longevity, wing length, host mortality and progeny produced per female were analyzed using the General Linear Model procedure (proc GLM) SAS version 8.1 system (SAS Institute, 2000). Since data were not normally distributed, percentage data on acceptance (probe wounds) were transformed to arcsine (arcsine+0.5) before being subjected to ANOVA. Longevity, wing length and developmental time were transformed to square root (sqrt (n+0.5)) before ANOVA. Acceptance, parasitoids and female emergence data were

presented as proportions while longevity, wing length and developmental time were presented as means. Means were compared using the Student Newman-Keul's (SNK) test when ANOVAs were significant.

Data on fate of pupae and progeny produced per female were arcsine transformed before being subjected to ANOVA. Effect of pupal weight on developmental time, longevity and wing length was assessed using a correlation analysis. Mortality of exposed pupae was adjusted for mortality of control pupae using Abbot's (1925) formula. A linear regression model for the number of probe wounds on pupae of all ages and species combined, and eggs laid was drawn but the R^2 from this regression was low, hence a non-linear statistical procedure was used to describe this relationship. Following the method of Neter *et al.* (1989) the least squares estimator,

$Y = 2.2(1-\exp(-0.15X))$ was fitted to the data using Microsoft excel for windows (1995).

4.4 Results

4.4.1 Host and host age acceptance

All pupae of the six age classes of the four stem borer species exposed to female parasitoids received ovipositor probe wounds (Table 4.1). Out of 63 pupae selected and dissected within one day of female exposure, 94% contained at least one parasitoid egg. The number of eggs laid approached an asymptotic value of 2.2 as the numbers of probe wounds on the pupae increased ($R^2 = 0.776$; $F = 12.10$; $P = 0.008$) (Fig 4.1).

Comparisons were made for the six host ages within and across the host species. Acceptance was not different across the host species except for two-day-old *C. partellus* pupae (Table 4.1). There was no difference when comparison was made in the six age classes within the four host species (Table 4.1).

Multiple probing was consistently dominant across all hosts (χ^2=52.63; $P = 0.0001$) and it seemed to enhance successful parasitization. Sixty-four percent of hosts probed more than once produced parasitoid progeny, as compared to 35.5% of hosts probed only once. Multiple probing was not significantly affected by weight of pupae ($F = 3.01$; df = 1, 1031; $P = 0.083$)

Table 4.1: Acceptability of the four stem borer species measured by the presence of female *X. stemmator* probe wounds. Acceptance was compared across host species and host ages within the species.

Age	*Percentage of hosts probed (% ± SE)				Statistical Parameters		
(Days)	*B. fusca*	*C. partellus*	*E. saccharina*	*S. calamistis*	F	df	P
1	76.7 ± 0.06a (N = 60)	58.3 ± 0.06a (N = 60)	64.1 ± 0.07a (N = 64)	71.7 ± 0.05a (N = 60)	1.68	3,57	0.181
2	66.7 ± 0.05b (N = 60)	81.7 ± 0.05a (N = 60)	63.3 ± 0.04b (N = 60)	60.0 ± 0.04b (N = 60)	5.90	3,56	0.001
3	71.7 ± 0.06a (N = 60)	65.0 ± 0.05a (N = 60)	70.6 ± 0.06a (N = 60)	57.8 ± 0.05a (N = 60)	1.31	3,59	0.280
4	61.7 ± 0.06a (N = 60)	69.4 ± 0.05a (N = 60)	63.3 ± 0.06a (N = 60)	62.5 ± 0.06a (N = 60)	0.54	3,58	0.656
5	81.3 ± 0.04a (N = 60)	67.3 ± 0.07a (N = 60)	80.0 ± 0.06a (N = 60)	73.3 ± 0.05a (N = 60)	1.37	3,55	0.263
6	76.7 ± 0.06a (N = 60)	68.3 ± 0.07a (N = 60)	62.5 ± 0.07a (N = 60)	69.1 ± 0.05a (N = 60)	0.64	3,57	0.589
F	2.00	2.12	1.49	1.37			
df	5, 85	5, 85	5, 86	5, 86			
P	0.087	0.071	0.202	0.243			

*Figures are mean percentages ± standard errors. Percentages are number of pupae probed divided by the total number exposed to *X. stemmator* females for each host and age grouping. Means followed by the same letters in the same row are not significantly different at P = 0.05 (Student-Newman-Keuls multiple comparison test).

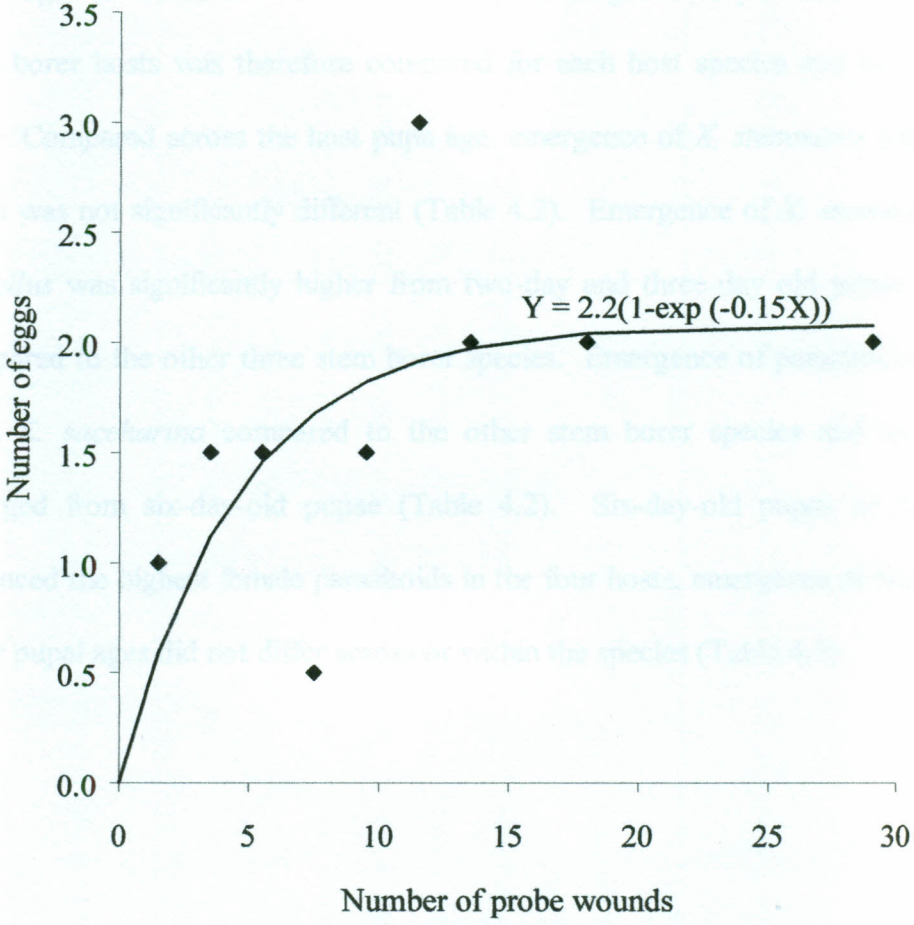


Fig 4.1: Non-linear response for the numbers of probe wounds for all pupae combined and number of eggs laid in pupae by *X. stemmator* females dissected 24 hours after oviposition ($R^2 = 0.78$; F = 12.10; P=0.008).

4.4.2 Host species and age suitability

Analysis of variance indicated significant interaction between the hosts and the host pupal ages (F=3.43; df = 15; P = 0.0001). Emergence of parasitoids from the four stem borer hosts was therefore compared for each host species and host age (Table 4.2). Compared across the host pupa age, emergence of *X. stemmator* adults from *B. fusca* was not significantly different (Table 4.2). Emergence of *X. stemmator* from *C. partellus* was significantly higher from two-day and three-day old pupae (Table 4.2) compared to the other three stem borer species. Emergence of parasitoids was lowest from *E. saccharina* compared to the other stem borer species and no parasitoids emerged from six-day-old pupae (Table 4.2). Six-day-old pupae of *S. calamistis* produced the highest female parasitoids in the four hosts, emergence of females for the other pupal ages did not differ across or within the species (Table 4.3).

Table 4.2: Mean percentage emergence (± S.E) of adult *X. stemmator* from pupae of four stem borer species of six different ages. Four pupae were put in pape straws in a vial and one mated naïve *X. stemmator* female released into the vial at 27 ± 2° C, 49-61% RH and 12: 12 (L: D) photoperiod.

Age	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Statistical parameters		
Host	N		N		N		N		N		N		F	df	P
Bf	15	36.6 ± 7ABa	15	35.0 ± 7Ba	15	45.0 ± 6Aa	15	41.7 ± 7Aa	16	46.9 ± 6Aa	15	40.0 ± 5Aa	0.68	5,85	0.6380
Cp	15	31.7 ± 7ABb	15	60.0 ± 7Aa	15	55.0 ± 7Aa	18	36.1 ± 5Ab	15	26.7 ± 6Bb	15	30.0 ± 8Ab	5.31	5, 87	0.0003
Es	16	23.4 ± 5Ba	15	28.3 ± 6Ba	17	13.2 ± 4Bab	15	20.0 ± 6Aa	15	5.0 ± 3Cbc	15	0	6.59	5, 87	0.0001
Sc	15	50.0 ± 4Aa	15	31.7 ± 5Bab	16	26.6 ± 5Bab	15	31.7 ± 7Aab	15	41.7 ± 7ABab	17	23.5 ± 7Ab	2.36	5, 87	0.0464
Statistical Parameters	F	3.56		5.24		11.98		2.30		11.97		13.31			
	df	3, 57		3, 56		3, 59		3, 59		3, 57		3, 58			
	P	0.0196		0.0030		0.0001		0.0868		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 th same row are not significantly different (Student-Newman-Keuls multiple comparison test, P<0.05). Bf, Cp, Es and Sc are *B. fusca, C. partellus, E. saccharin* and *S. calamistis* respectively.

Table 4.3: Mean percentage female progeny (± S.E) of *X. stemmator* emerging from pupae of four stem borer hosts of six different ages. Four pupae were pu in paper straws in a vial and one mated naïve *X. stemmator* female released into the vial at 27 ± 2°C, 49-61% RH and 12: 12 (L: D) photoperiod.

Age	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Statistical parameters		
Host	N		N		N		N		N		N		F	df	P
Bf	12	70.8 ± 11Aab	12	50.0 ± 12ABb	14	77.0 ± 9Aab	13	92.3 ± 5Aa	15	50.0 ± 11Ab	14	41.7 ± 11Bb	3.60	5, 74	0.0058
Cp	10	66.7 ± 15Aa	15	73.9 ± 9Ba	15	56.1 ± 12Aa	16	56.3 ± 11Aa	10	50.0 ± 13Aa	12	26.4 ± 12Ba	1.95	5, 72	0.0966
Es	10	80.0 ± 11Aa	11	95.4 ± 5Aa	8	87.5 ± 13Aa	9	87.0 ± 9Aa	3	66.7 ± 33Aa	0	0	0.67	4, 36	0.6155
Sc	15	47.8 ± 11Aa	13	70.5 ± 12ABb	12	86.1 ± 10Ab	10	90.0 ± 10Aa	12	70.8 ± 10Ab	10	82.5 ± 11Aa	2.25	5, 66	0.0593
Statistical	F	1.38		3.14		2.13		3.84		0.77		5.75			
Parameters	df	3, 43		3, 47		3, 45		3, 44		3, 36		2, 33			
	P	0.2618		0.0338		0.1091		0.0159		0.5208		0.0072			

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 (Student-Newman- Keuls multiple comparison test, $P<0.05$). Bf, Cp, Es and Sc are *B. fusca, C. partellus, E. saccharina* and *S. calamistis* respectively.

4.4.3 Fate of pupae exposed to *X. stemmator*

Emergence of adult *X. stemmator* and death of pupae in all four stem borers species was significantly different (F = 225.48; df = 3, 1475; P = 0.0001) but not moth emergence (F = 1.52; df = 3, 1475; P = 0.2081) (Table 4.4). Mortality in *B. fusca* and *S. calamistis* did not differ significantly across the six host ages (F = 0.42; df = 5, 85; P = 0.8308 and F = 1.93; df = 5, 87; P = 0.0971; Fig 4.2a) respectively. Mortality differed across age classes for *C. partellus* and *E. saccharina* (F = 4.28; df = 5, 87; P = 0.0016 and F = 3.64; df = 5, 87; P = 0.0049) respectively (Fig 4.2a). *Eldana saccharina* had the highest death when mortality was compared across the pupal age (Fig 4.2a).

Adjusted proportion mortality of all ages of pupae offered to females differed significantly (F = 18.44; df = 3, 20; P = 0.0001) and so was mortality of the pupae that were not exposed to females (control) (F = 10.50; df = 3, 20; P = 0.0002; Fig 4.2b). Mortality of pupae that were offered to the females was therefore not attributed to parasitization. Sixty-five percent of pupae probed more than once died in comparison to 22.5% of hosts probed only once. Host death increased as the number of probe-wounds on pupae increased.

Table 4.4: Fate of pupae offered to *X. stemmator* females after 25 days of exposure at 27 ± 2° C, 49-61% RH and 12: 12 (L: D) photoperiod 27°C.

		Fate of pupae offered to females (%)		
	N	*X. stemmator*	Moth	Dead
B. fusca	364	40.93a	43.13a	15.93b
C. partellus	372	39.78a	45.69a	14.53b
E. saccharina	372	15.05b	40.05a	44.89a
S. calamistis	371	33.87a	47.31a	18.82b
Statistical parameters F		25.48	1.52	46.27
df		3, 1476	3, 1476	3, 1476
P		0.0001	0.2081	0.0001

Means within the same column with different letters are significantly different at P<0.05 (Student-Newman-Keuls multiple comparison test).

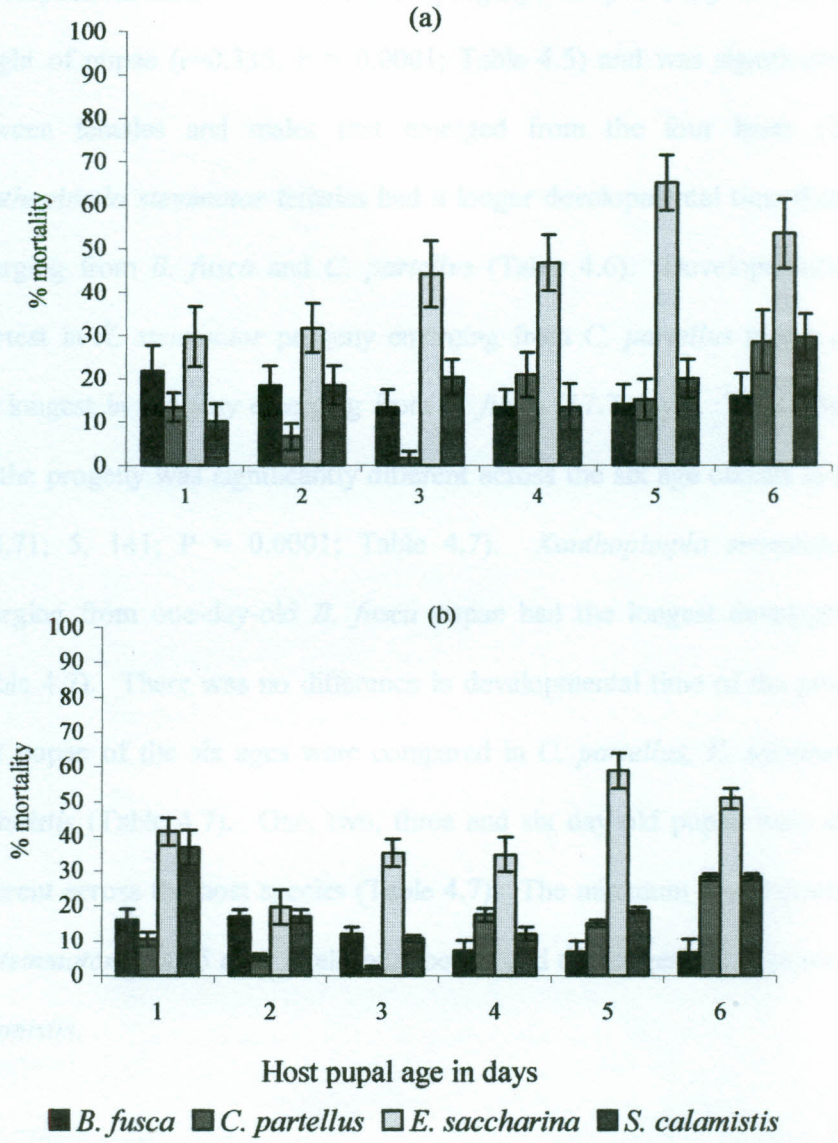


Fig 4.2: (a) Mean percentage (± S.E) mortality of pupae of four stem borer species exposed to *X. stemmator* females at different pupal ages. (b) Mean percentage (± S.E) mortality of similar ages and species of stem borer pupae not offered to females (control).

4.4.4 Developmental time of F1 *X. stemmator* progeny

Developmental time of *X. stemmator* progeny was positively correlated with the weight of pupae (r=0.335, P = 0.0001; Table 4.5) and was significantly different between females and males that emerged from the four hosts (Table 4.6). *Xanthopimpla stemmator* females had a longer developmental time than the males emerging from *B. fusca* and *C. partellus* (Table 4.6). Developmental time was shortest in *X. stemmator* progeny emerging from *C. partellus* pupae (16.8 days) and longest in progeny emerging from *B. fusca* (17.7 days). Developmental time for the progeny was significantly different across the six age classes in *B. fusca* (F = 8.71; 5, 141; P = 0.0001; Table 4.7). *Xanthopimpla stemmator* progeny emerging from one-day-old *B. fusca* pupae had the longest developmental time (Table 4.7). There was no difference in developmental time of the progeny when host pupae of the six ages were compared in *C. partellus, E. saccharina* and *S. calamistis* (Table 4.7). One, two, three and six day old pupae were significantly different across the host species (Table 4.7). The minimum developmental time of *X. stemmator* was 15 days in all four species and the longest 23 days recorded in *S. calamistis*.

4.4.5 Wing length of F1 *X. stemmator* progeny

Wing length of *X. stemmator* offspring that emerged was positively correlated with the weight of pupae (r = 0.442; P = 0.0001) (Table 4.5) and was different in females and males compared across the host species (Table 4.6). Wing length for parasitoids emerging from two to six day old pupae differed significantly across the host species (Table 4.8). However, when host age was compared across the

individual hosts there was no significant difference in the hosts apart from *S. calamistis* (Table 4.8). The minimum wing length was 0.6cm in *C. partellus* while the longest was 1.2cm recorded in *B. fusca* and *C. partellus*.

4.4.6 Longevity of F1 *X. stemmator* progeny

Longevity was not significantly different between *X. stemmator* females and males nor was there any difference in this parameter when longevity was measured for *X.* stemmator progeny from pupae of the six host ages and host species (Table 4.6 and 4.9).

Table 4.5: Mean weight of host pupae of four stem borer species

Host species		N	Mean weight in Grams
B. fusca		228	0.17 ± 0.0002a
C partellus		263	0.09 ± 0.0019d
E. saccharina		244	0.11 ± 0.0017c
S. calamistis		301	0.12 ± 0.0015b
Statistical	F		320.28
parameters	df		3, 1032
	P		0.0001

Means within the same column followed by different letters are significantly different (Student-Newman-Keuls multiple comparison test $P<0.05$).

Table 4.6: Mean developmental time, longevity and wing length (+SE) of *X. stemmator* progeny compared within sexes in the four host species

Hosts	N	Developmental time ♀ (in days)	N	Developmental time ♂ (in days)	N	Longevity ♀ (in days)	N	Longevity ♂ (in days)	N	Wing length ♀ (in cm)	N	Wing length ♂ (in cm)
B. f	88	18.1 ± 0.1a	58	17.2 ± 0.1ab	81	25.5 ± 0.9a	54	24.9 ± 1.0a	81	1.0 ± 0.01a	54	1.0 ± 0.01a
C. p	86	17.2 ± 0.1b	62	16.5 ± 0.2b	81	27.5 ± 1.2a	57	26.7 ± 1.3a	81	0.9 ± 0.01b	57	0.8 ± 0.01b
E. s	45	17.5 ± 0.1b	*9	17.4 ± 0.4a	39	26.4 ± 1.5a	*8	25.8 ± 3.1a	41	0.9 ± 0.01b	*9	0.9 ± 0.02b
S. c	88	17.5 ± 0.1b	38	16.5 ± 0.1b	75	28.9 ± 1.2a	41	26.1 ± 1.4a	75	0.9 ± 0.01b	37	0.9 ± 0.02b
Statistical parameters		F = 8.28		F = 4.77		F = 1.67		F = 0.40		F = 31.34		F = 24.8
		df = 3, 306		df = 3, 163		df = 3, 299		df = 3, 163		df = 3, 298		df = 3,163
		P = 0.0001		P = 0.0032		P = 0.1744		P = 0.7566		P = 0.0001		P = 0.0001

Means followed by the same lower case letter(s) in the same column are not significantly different (Student-Newman-Keuls multiple comparison test, $P<0.05$). *B. f, C. p., E. s.* and *S. c.* are *B. fusca, C. partellus, E. saccharina* and *S. calamistis* respectively. *Few male parasitoids emerged from *E. saccharina* out of total of 360 pupae exposed.

Table 4.7: Mean developmental time (in days) (± SE) of F1 *X. stemmator* offspring emerging from four stem borer pupae of 6 host ages

Age		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	Statistical parameters		
Host	N		N		N		N		N		N		F	df	P
B. f	22	19.1 ± 0.4Aa	21	17.8 ± 0.3Ba	27	17.8 ± 0.2Ba	25	17.5 ± 0.1Ba	30	17.1 ± 0.2Ba	24	17.3 ± 0.2Bab	8.71	5,141	0.0001
C. p	19	16.9 ± 0.3Ab	36	16.8 ± 0.2Ab	33	16.9 ± 0.3Aa	26	17.0 ± 0.3Aa	16	17.2 ± 0.3Aa	18	16.7 ± 0.2Ab	0.35	5,142	0.880
E. s	15	17.2 ± 0.2Ab	17	18.0 ± 0.2Aa	8	17.1 ± 0.3Aa	12	17.7 ± 0.4Aa	3	17.0 ± 1.2Aa	0	0	1.57	4,51	0.197
S. c	30	17.1 ± 0.2ABb	19	16.8 ± 0.3Bb	17	17.0 ± 0.2ABa	19	17.4 ± 0.3ABa	25	17.2 ± 0.2ABa	16	17.8 ± 0.5Aa	1.7	5,120	0.132
Statistical Parameters	F	13.12		6.59		3.70		1.12		0.12		3.85			
	df	3, 82		3,88		3,82		3,78		3, 70		2,54			
	P	0.0001		0.0005		0.015		0.3461		0.9491		0.028			

Means followed by the same upper case letter(s) in the same row are not significantly different; means followed by the same lower case letter(s) in the same column are not significantly different (Student-Newman-Keuls multiple comparison test, $P<0.05$). *B. f., C. p., E. s.* and *S. c.* are *B. fusca, C. partellus, E. saccharina* and *S. calamistis* respectively. N are the number of *X. stemmator* adults that emerged from at least 60 pupae exposed for each age class and host species.

Table 4.8: Mean length of the left upper wing (in centimeters ± S.E) of *X. stemmator* F1 offspring that emerged from 6 ages of pupae in four stem borer species

Age	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6				
Host	N		N		N		N		N		N		Statistical parameters F	df	P
B. f	22	1.0 ± 0.02Aa	20	1.0±0.02Aa	27	1.0± 0.01Aa	25	1.0±0.02Aa	30	1.0 ± 0.01Aa	24	1.0± 0.02Aa	0.71	5,141	0.618
C. p	19	0.9± 0.03Aa	36	0.9±0.02Ba	33	0.9± 0.02Ba	26	0.8± 0.02Ba	16	0.8 ± 0.02Ba	18	0.8± 0.03Ba	2.15	5,141	0.063
E. s	15	0.9± 0.03Aa	17	0.8± 0.01Ba	8	0.9± 0.02Ba	12	0.8± 0.02Ba	3	1.0 ± 0.01ABa	0	0	1.42	4,51	0.240
S. c	30	0.9± 0.02Aa	19	0.9± 0.02ABa	17	0.8±0.02Bab	19	0.8± 0.02Bb	25	0.9 ± 0.02Bab	16	0.8± 0.03Bab	3.95	5,118	0.002
Statistical Parameters	F	2.21		9.14		15.77		17.75		15.91		14.02			
	df	3, 82		3,88		3,81		3,76		3, 70		2,54			
	P	0.0931		0.0001		0.0001		0.0001		0.0001		0.0001			

Means followed by the same upper case letter (s) in the same row are not significantly different; means followed by the same lower case letter(s) in the same column are not significantly different (Student-Newman-Keuls multiple comparison test, $P<0.05$). *B. f, C. p., E. s.* and *S. c.* are *B. fusca, C. partellus, E. saccharina* and *S. calamistis* respectively. N are the number of *X. stemmator* adults that emerged from at least 60 pupae exposed for each age class and host species.

Table 4.9: Mean longevity ± SE (in days) of F1 *X. stemmator* offspring that emerged from six ages of pupae of four stem species. The adults were fed on 10 % honey/water solution until their natural death.

Age	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6				
Host	N		N		N		N		N		N		Statistical parameters F	df	P
B. fusca	22	23.9 ± 0.8	20	22.8±1.7	27	25.4 ± 1.5	25	25.3 ± 1.6	30	28.2 ± 1.8	23	24.9 ± 1.6	0. 87	5,141	0.506
C. partellus	19	30.4 ± 2. 8	36	26.9±1.3	32	29.8 ± 2.3	26	25.7 ± 1.6	16	22.1 ± 2.0	18	26.2 ± 2.9	1.39	5,141	0.233
E. saccharina	15	26.3 ± 3.5	17	25.2±2.1	9	29.7 ± 2.4	12	23.9 ± 3.2	3	32.0 ± 4.2	0	-	1.05	4,51	0.390
S. calamistis	30	29.6 ± 1.2	19	24.9± 2.0	17	26.7 ± 3.1	17	32.9 ± 3.0	25	27.3 ± 2.1	16	24.8 ± 2.4	1.69	5,118	0.141
Statistical Parameters F		2.41		0.87		0.89		2.54		1.57		0.01			
df		3, 82		3, 88		3, 81		3, 76		3, 70		2, 54			
P		0.0732		0.4583		0.4503		0.0624		0.2049		0.9876			

Means were not significantly different (Student-Newman-Keuls multiple comparison test, $P<0.05$). N are number of *X. stemmator* parasitoids that emerged from at least 60 pupae exposed for each age class and host species.

4.4.7 Progeny production

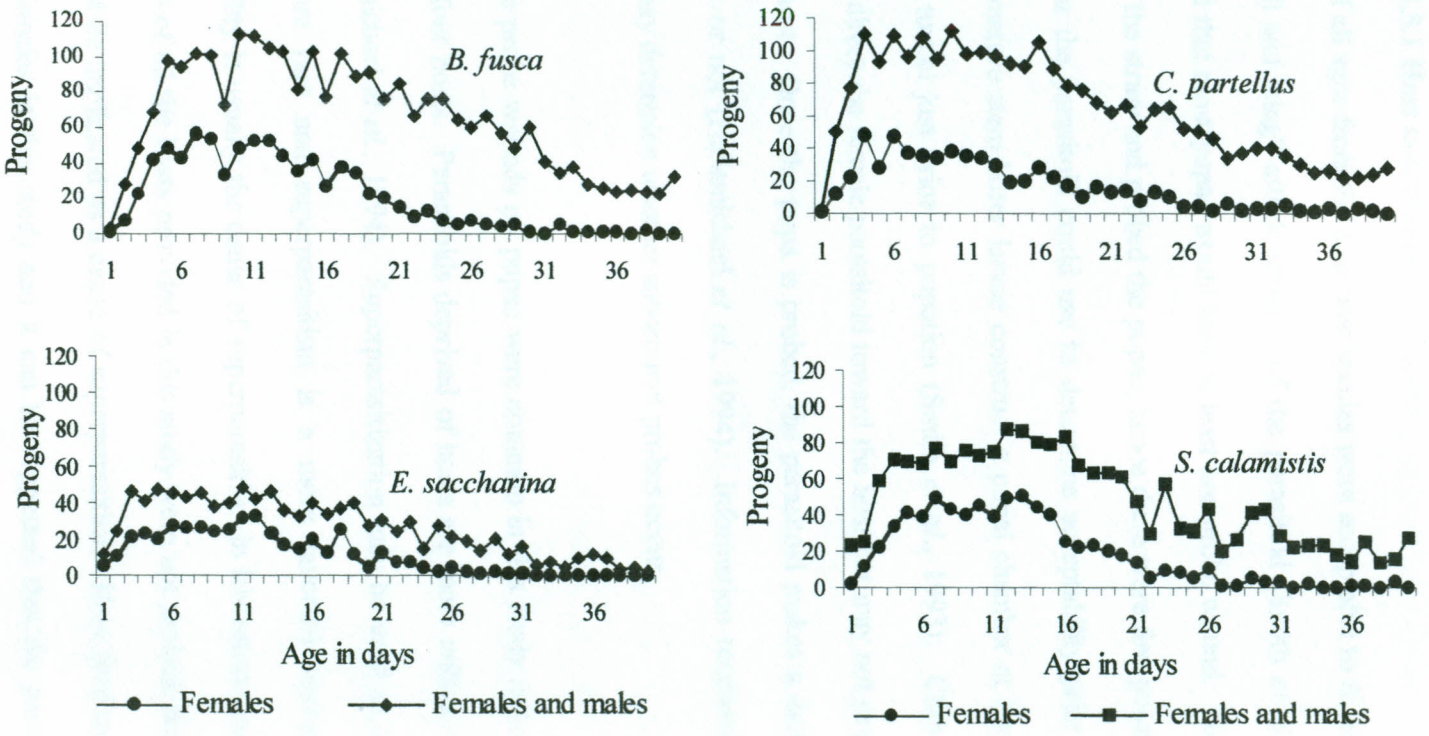
Progeny produced by F1 females was compared across the four stem borer species. Total females and males produced was significantly different across the four stem borer species ($F = 6.17$; $df = 3, 112$; $P = 0.0006$) and so was the number of males ($F = 7.11$; $df = 3, 112$; $P = 0.0002$). *Eldana saccharina* had the lowest progeny production (Table 4.10). Longevity of the ovipositing female parasitoids reared from pupae of different hosts was not different ($F = 1.16$; $df = 3, 112$; $P = 0.3293$: Table 4.10). Sex ratio was male biased as parent females that emerged from the host pupae aged. Emergence of female progeny in females that emerged from all the four host species was highest from pupae exposed during the first twenty days (Figure 4.3).

Table 4.10: Progeny production and longevity of ovipositing F1 *X. stemmator* females that emerged from the four stem borer species

Host	N	Females (Mean ± SE)	Males (Mean ± SE)	Total progeny of F1 parents (Mean ± SE)	Average longevity (Mean ± SE)	Sex ratio F: M ± SE
B. fusca	30	27.1 ± 4.8a	66.4 ± 9.3a	93.5 ± 10.7a	32.1 ± 3.4a	1: 1.6 ± 0.11a
C. partellus	30	22.6 ± 4.3a	72.4 ±10.8a	95.0 ± 11.5a	35.0 ± 3.7a	1: 2.0 ± 0.11a
E. saccharina	27	15.7 ± 3.1a	20.6 ± 3.8b	36.3 ± 5.7b	26.4 ± 3.5a	1: 1.1 ± 0.23a
S. calamistis	29	25.5 ± 3.6a	45.2 ± 7.2a	70.7 ± 8.5a	39.5 ± 4.6a	1: 1.0 ± 0.19a
Statistical Parameters	F	1.16	7.11	6.17	1.51	2.33
	df	3, 112	3, 112	3, 112	3, 112	3, 112
	P	0.3293	0.0002	0.0006	0.2146	0.0783

Means followed by the same letter(s) in the same column are not significantly different; (Student-Newman-Keuls multiple comparison test, $P<0.05$). F= female: M=male.

Fig. 4.3: Number of females emerging as a function of age of mother for the 1st 40 days



4.5 Discussion

4.5.1 Host acceptance

Pupae of all ages from the four host species were acceptable to *X. stemmator.* Given the "drill and sting" attack strategy of the parasitoid (Smith *et al.*, 1993), it was expected that most pupae would have at least one probe wound. The females drilled through the straw and probed the pupae, hence there were few possible host-specific cues that the parasitoid could use to determine acceptability prior to probing. In nature, mature stem borer larvae construct a pupal chamber at the terminus of the feeding tunnel just prior to pupation (Smith *et al.*, 1993). Cues from the larval activity direct the female parasitoid toward the host but may not provide recognition of the host. Once the pupa is probed, the parasitoid makes a decision whether to oviposit or not (Hailemichael *et al.*, 1994). Information received from the initial probe may determine whether subsequent probes occur.

Multiple probe wounds on pupae were common in this study and were observed in all the four hosts. Parasitoids deprived of hosts are more willing to superparasitise (Hailemichael *et al.*, 1994). Superparasitization may be used as an indication that hosts are rare and superparasitism is a more favorable strategy. Low host availability is usually the cause of superparasitism in laboratory cultures. However, 32.25% of all the hosts provided in this study were not probed, hence host scarcity, may not be implicated as a cause of superparasitism. Host feeding was observed in some females in this study and it can be suggested that the parasitoids may have probed to establish if the host could be used for food or oviposition site. Pupal

movement inside the straws may also have caused repeated probing as the females tried to gain access to the pupa. Hailemichael *et al.* (1994) also observed multiple probe wounds and suggested that nearly any potential host encountered offered a good probability of successful parasitization, which is enhanced by superparasitisation.

Xanthopimpla citrina (Holmgren) (Hymenoptera: Ichneumonidae) superparasitised hosts in the laboratory but deposited one egg per host under natural field conditions (Moutia and Courtois, 1952). No studies under field conditions with *X. stemmator* have been documented. It appeared that females refrained from laying eggs in pupae that were drilled more than five times (Fig 4.1). Dissection of probed pupae showed that oviposition stabilized at just above 2 eggs per host despite the fact that the numbers of probe wounds on the pupae increased (Fig 4.1). Hailemichael *et al.* (1994) in a similar study on *X. stemmator* reared on *Diatraea saccharalis* (F.)(Lepidoptera: Pyralidae) found that successful parasitization appeared to level off at greater than four probes. It would be interesting to investigate if naïve *X. stemmator* could tell the difference between parasitised and unparasitised hosts.

4.5.2 Host suitability

Host suitability was influenced by age. There was a general decline in emergence of parasitoids from five and six day-old pupae, which could be explained by the fact that females may have allocated fewer eggs to hosts that were less suitable for progeny development (van Alphen and Theunnissen, 1983). As host pupae age, they

change both physiologically and morphologically. Studies by Hailemichael *et al.* (1994) on *X. stemmator* reared on *D. saccharalis* attributed the decrease in suitability of six-day-old pupae to the presence of well-developed appendages of the imago. They speculated that insufficient food resources for development of parasitoids as the pupae grew older could be a limiting factor. Host acceptance was directly related to host suitability for *C. partellus* pupae but not the other three stem borer pupae. Two-day-old pupae of *C. partellus* were more acceptable and were the most suitable for development. It is not clear why this trend is not the same for the other three hosts.

Total progeny produced by the individual females reared on *B. fusca*, *C. partellus* and *S. calamistis* was higher than in *E. saccharina*. *Chilo partellus* and *S. calamistis* are old association hosts of *X. stemmator*, which may explain the better performance as compared to *E. saccharina*, which is a new association. High emergence of progeny from *B. fusca* was attributed to large pupae size. Given that naïve females may or may not allocate eggs depending on the size of the host (Mackauer, 1986), *B. fusca* pupae were on average heavier than the other 3 hosts (Table 4.5) hence could have provided sufficient resources for complete development of the parasitoids.

An increase in female fitness with adult size has been reported in many studies of parasitoid biology. Jervis and Copland (1996) presented a list of parasitoid and predator species in which a positive correlation had been recorded between the body size or weight and reproductive ability of parasitoids and predators. Ngi-Song *et al.*

(1995) reported higher progeny production in larger instars of *C. partellus* when they were parasitised by *C. flavipes*. Other similar studies by Mochiah *et al.* (2001) showed that *B. fusca* and *S. calamistis* had a higher progeny in the larval parasitoid *C. sesamiae* (Cameron) (Hymenoptera: Braconidae) than did *C. partellus* and *C. orichalcociliellus*.

Laboratory studies clearly show a correlation between adult size and several components of fitness (Godfray, 1994). Larger females of species that attack protected hosts may find it easier to oviposit through the protective covering (Salt 1940, 1941). This is probably true for *X. stemmator* females, which have to drill through the plant rind to locate the pupae. In this study, the parasitoid's size was determined by the left upper wing length. The largest parasitoids emerged from *B. fusca* pupae, which were much heavier and larger than the other three hosts. Many of the parasitoids consume virtually all host tissues prior to pupation and thus the size of the host may profoundly influence host quality and hence parasitoid fitness; with larger hosts producing larger parasitoids (Harvey *et al.*, 1999).

Males had a shorter developmental time than females in this study. Males of solitary wasps usually emerged several days before the females, a phenomenon called protandry (Godfray, 1994). Doutt (1964) suggested that it is quite common for males of parasitic Hymenoptera to have a shorter developmental time than females. The shorter developmental time serves to prevent inbreeding and ensures that the females become fertilized immediately after eclosion. Males that emerge before the

females stand a greater chance of mating with one or several females than do males emerging later (Godfray, 1994). The advantage of early reproduction in growing populations with overlapping generations is a potent evolutionary force favoring early reproduction (Fisher, 1930; Lewontin, 1965).

Developmental time in *B. fusca*, with the heaviest pupae, was longest compared to the other three hosts. Late emergence of parasitoids from bigger hosts probably reflects a longer feeding period. Feeding and development of parasitoid larvae is extended in hosts that provide an abundance of food and arrested in hosts with less available food (Godfray, 1994). Developmental time in one day-old *B. fusca* pupae was longer than in other pupal ages. A relatively shorter developmental time in older hosts reflects a response to a reduction in available food as pupal fluids are converted into sclerotized adult structures (Lashomb *et al.,* 1983) and waste products of pupal development accumulate (Chapman, 1971). Fast development of parasitoids is favoured because the sooner offspring become sexually mature, the sooner they themselves can reproduce (Godfray, 1994).

Ovipositing females lived longer than non-ovipositing ones in this study. This differs from studies by Moore and Kfir (1996) where *X. stemmator* females lived for a shorter time when they were provided with hosts. This could be attributed to confinement of the non-ovipositing females in the present study to small vials (2.5 x 7.5 cm) till their natural death while the ovipositing females were confined and given hosts in a bigger and wider cylinder (Plate 2.5).

4.5.3 Fate of pupae

Like many pupal parasitoids, *X. stemmator* is an idiobiont parasitoid, which kills its hosts immediately after oviposition as opposed to koinobionts, which allow hosts to grow before eventually killing them. Because koinobionts must coexist with their hosts, they have to overcome specialized immunological and chemical defenses as opposed to idiobionts (Salt, 1968; Askew and Shaw, 1986; Gauld and Bolton, 1988; Gauld *et al.*, 1992). Eggs and pupae are less protected (or even not protected) by immune responses than the larval stages (Wiedenmann, 1998). Hence involvement of the immune system cannot be implicated as a cause of mortality in this study.

Oviposition trauma or some other consequences of parasitoid attack might have caused incidental mortality in hosts exposed to the female parasitoids. Hosts that were not drilled served as controls because they were handled the same as those attacked by the parasitoids. Incidental mortality was much higher in hosts that had been drilled than in those which had not. Some incidental mortality may be ascribed to premature death of parasites in early stages of development. Of dead pupae, 27 % of those dissected had dead adult or larvae of parasitoids inside the pupal case. Mortality was also particularly high in hosts that received multiple probe wounds. The increased mortality as the number of probes increased may reflect increased damage to the developing pupa, causing death. Hailemichael *et al.*, (1994) in a similar study reported increased mortality with number of probes on *D. saccharalis* pupae.

4.5.4 Conclusion

Xanthopimpla stemmator was able to develop in at least four of the common stem borer pests found in Kenya. If released in the field, it is expected to compliment *C. flavipes*, a larval parasitoid that is not able to develop in *B. fusca,* in the highland areas where *C. flavipes* is not established. *Xanthopimpla stemmator* does not hibernate in its original habitat and reproduces throughout the year as hosts are always available (Moore and Kfir, 1996). In regions where there is an abundance of host plants and the climate is warm, stem borers normally develop continuously all year round. Kenya is located near the equator and stem borers are not affected by day length and temperature, which would cause them to diapause (Kfir *et al.*, 2002).

Mass rearing should be based on the suitable host in terms of developmental time, pupal age and proportion of females that emerge from the exposed pupae. *Chilo partellus* and *B. fusca* pupae can be used effectively for mass rearing due to shorter developmental time in *C. partellus* and large host size in *B. fusca. Xanthopimpla stemmator* is a synovigenic species, a phenomenon where the eggs mature with time, after emergence of the females. Females that can be used for the rearing purposes must be less than 20 days. After this age, the females tend to lay male eggs probably due to depletion of sperms from the spermatheca (van den Assem, 1986; King, 1987). There is no literature on effect of age on female reproductive potential in the field.

This study has shown that *E. saccharina* is a poor host for the development of *X. stemmator* suggesting that it may not establish in areas of Kenya where *E. saccharina* is an abundant species. *Xanthopimpla stemmator* is reared on *E. saccharina* in South Africa and releases have been done against this borer (Conlong pers comm.). It is not clear why this host showed a poor suitability and a low progeny production in Kenya. Probably biotypes of *E. saccharina* exist and this needs further investigations (King *et al.*, 2002).

CHAPTER FIVE

LIFE TABLES AND INTRINSIC RATE OF NATURAL INCREASE OF *Xanthopimpla stemmator* (THUNBERG) POPULATIONS ON *Chilo partellus* AND *Busseola fusca* PUPAE

5.1 Introduction

A crucial component in the study of insects is the determination of reproductive capabilities. Life tables are one of the most useful numerical aids in studying population biology (Southwood, 1978). While it is important to study reproduction at the level of the individual, it is often desirable to calculate a standardized estimate of the growth rate of insect populations (Southwood, 1978). One such estimator, the intrinsic rate of increase (r_m) has been used for many years by insect ecologists. It is useful for population growth potential, as a bioclimatic index and as a natural enemy rating parameter under given climatic and food conditions (Messenger, 1964; Watson, 1964).

Intrinsic rate of natural increase has been demonstrated to be both a predictive and comparative measure of population growth potential (Messenger, 1964). The r_m is useful for comparing beneficial species and biotypes of the same species and also for comparing population growth of natural enemies and their prey or hosts (Orphanides and Gonzalez, 1971). It is a true measure of the reproductive potential of an organism.

Other parameters used to estimate fertility life tables are the net reproductive rate (R_o); the mean generation time (T); the doubling time (Dt) and the finite rate of increase (λ). Life table statistics may provide insight into the potential outcome of competition between ecologically similar exotic and native natural enemies (Mbapila and Overholt, 2001).

This study may provide such information for *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) and the natural enemies found in the intended area of release such as other pupal parasitoids like *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae) and *Pediobius furvus* Gahan (Hymenoptera: Ichneumonidae). The main aim of this study was to assess the reproductive potential of *X. stemmator* when reared from two stem borer species, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) and *Busseola fusca* Fuller (Lepidoptera: Noctuidae).

5.2 Objective.

To determine and compare the intrinsic rates of natural increase, net reproductive rates, the mean generation time, finite rate of increase and doubling time of *X. stemmator* female parasitoids, when reared on *C. partellus* and *B. fusca* at 27 ± 2° C, 12:12 L: D and 49-61 % RH.

5.3 Materials and methods

5.3.1 Stem borer species

Life table studies were carried out with parasitoids reared on *B. fusca* and *C. partellus.* They were reared as described in chapter two. Two-day old *C. partellus* and three or four-day old *B. fusca* pupae were used.

5.3.2 Parasitoids

One day old mated *X. stemmator* females emerging from *B. fusca* and *C. partellus* were reared as described in chapter two.

5.3.3 Bioassay

At least 100 pupae of *C. partellus* and *B. fusca* were collected from the insectary at the International Centre of Insect Physiology and Ecology (ICIPE) daily. The pupae were offered to mated *X. stemmator* females in the morning (between 0900hrs and 1000 hrs) (ratio 100: 20 pupae: female) in cages measuring 15 x 15 x 15 cm (Plate 2.1). The pupae were exposed for six hours and thereafter kept in polystyrene Petri dishes, 8 cm in diameter (Plate 2.2) at a temperature of 27 ± 2°C, 49-61% RH and 12: 12 (Light: Day) photoperiod until adult parasitoid emergence.

Twelve adult females were randomly selected from adults emerging from parasitized *C. partellus* and *B. fusca* pupae within 24 hours of emergence. Six adult females that emerged from *C. partellus* and six from *B. fusca* pupae were mated and placed singly in

clean plastic cylinders of height 9cm and width 5cm (Plate 2.5). The top of the cylinder was covered with a polyester cloth to prevent the parasitoid from escaping. Each female was provided with a 10 % honey-water solution soaked in clean cotton wool for nourishment.

Five *C. partellus* pupae were placed inside paper straws measuring 8cm each. Two of the paper straws were smeared with frass from *C. partellus* larvae to simulate bored stalks of gramineous crops and enhance acceptance of the pupae by the *X. stemmator* females. The two straws were thereafter placed in the cylinder with a female parasitoid such that each female was exposed to ten pupae. Pupae and diet were removed after 24-hours and replaced with ten fresh pupae and diet. This was repeated every day until the female died.

Six parasitoids from *B. fusca* were similarly treated. This experiment was replicated six times so that a total of 36 females from each of the stem borer species were used. Parasitized pupae were kept in Petri dishes measuring 4cm in diameter and monitored until emergence of the parasitoids. Daily emergences of *X. stemmator* adults and other biological parameter, such as sex of progeny and number of female survivors were recorded. Moth emergence and mortality of parasitized pupae were also recorded.

5.3.4 Estimation of life table parameters

The parasitization potential of *X. stemmator* was assessed by comparing (cumulative and daily) fertility, progeny production and adult longevity in mated *X. stemmator* females reared on *C. partellus* and *B. fusca.* The span between two consecutive time periods was represented by its midpoint (female age plus 0.5) called the pivotal age (x). It was not possible to dissect host pupae for the presence of eggs, as this would have killed the pupae. Thus, it was assumed that all eggs laid developed into adults. Total number of eggs laid by all females of each group at the pivotal age (x) was therefore calculated as the total males and females that emerged.

Sex ratio (percentage of *X. stemmator* female eggs) was calculated by dividing the total number of females that emerged by total eggs laid (counted as total adults emerging from parasitized pupae). Pre-imaginal survivorship was 100% for adults emerging from both *C. partellus* and *B. fusca* pupae. A jackknife method proposed by Meyer *et al.* (1986) was used to estimate the variance and bias of estimators. The algorithms used to calculate associated fertility life table parameters were performed using the iterative method. It was assumed that the total population had an exponential growth described in the model:

$$N(t) = N_o e^{r_m x t}$$

where N (t) is the size of the population at time (t), N_o is the initial size of the population, r_m is a parameter related with the rate of population growth called the intrinsic rate of increase and e is a constant.

5.3.4.1 Intrinsic rates of natural increase (r_m)

Intrinsic rates of natural increase were assessed by analyzing the fecundity of ovipositing *X. stemmator* females based on emergence of adult offspring. This rate was obtained by iteratively solving the equation:

$$\sum_{x=x0}^{\Omega} e^{-rx} l_x m_x = 1$$

5.3.4.2 Net reproductive rate (Ro)

The net reproductive rate (Ro) is the mean net contribution per female to the next generation, expressed as total of *X. stemmator* female offspring per female during the entire oviposition period. It was calculated by summation over all females in each group of *C. partellus* and *B. fusca* of the number of female offspring produced in the pivotal age "x" multiplied by cumulative female survivorship:

$$R_o = l_x\, m_x \text{ where:}$$

l_x = female survivorship at each pivotal age

m_x = number of female offspring emerging from all females reared from *C. partellus* and *B. fusca* at each pivotal age "x".

5.3.4.3 Mean generation time (T)

Mean generation time (T) is the mean time span between the birth of individuals of a generation and that of the next generation. It was calculated from the exponential growth model, considering $t = T$ and $R_o = N_T / N_o$

$$T = \frac{Ln\ (Ro)}{r_m}$$

5.3.4.4 Doubling time (Dt)

Doubling time (Dt) is the time span necessary for doubling the initial population and was calculated from the exponential growth model, considering $R_o = N_T / N_o$:

$$Dt = \frac{ln\ (2)}{r_m}$$

where ln is the natural logarithm

5.3.4.5 Finite rate of increase (λ)

Finite rate of increase (λ) is a multiplication factor of the original population at each time period. The decimal part of the finite rate of increase corresponds to the daily rate of increase expressed as a percentage. The rate of increase for each female emerging from *B. fusca* and *C. partellus* was calculated as follows:

$$\lambda = e^{r_m}$$

5.3.5 Data analysis

Analysis was performed using the Statistical analysis system (SAS) program, Lifetable SAS (Maia *et al.*, 2000). Comparison of parameters between *X. stemmator* females that emerged from *C. partellus* and *B. fusca* was performed using the Student t-test for independent samples with different variances. The Welch correction for degrees of freedom was according to Zar incorporated, (1984). Longevity, developmental time of the parent *X. stemmator* females, sex ratio and total progeny produced was compared using analysis of variance (ANOVA) by SAS (SAS Institute Inc, 2000).

5.4 Results

Longevity of *X. stemmator* females given pupae until their natural death ranged from 6 to 71 days for those females that emerged from *B. fusca* and 2 to 82 days for those that emerged from *C. partellus*. Average life span in days was 45 and 44 days respectively. Longevity did not differ between parasitoids reared from the two stem borer species ($F = 2.36$; $df = 1, 2447$; $P = 0.1247$; Table 5.1).

Developmental time for *X. stemmator* females that emerged from *B. fusca* was significantly higher that those females that emerged from *C. partellus* ($F = 28.74$; $df = 1,294$; $P = 0.0001$; Table 5.1).

The total progeny produced ranged from 2 to 198 in females from *B. fusca* pupae and 8 to 244 in females from *C. partellus* pupae. Total progeny produced was not different for the females emerging from the two stem borer species ($F = 0.88$; $df = 1, 2447$; $P = 0.3495$; Table 5.1). The female/male ratio was male biased when total progeny produced by *X. stemmator* females emerging from the two stem borer species was assessed (Table 5.1).

Maximum mean female progeny production per day, (m_x), was 1.20 on the 12th, 18th, 20th 22nd and 28th day for *B. fusca* (Appendix 1). The m_x was 1.736 on the 78th day for *C. partellus* (Appendix 2). The gross reproductive rate was 42.9 in *B. fusca* and 47.5 in *C. partellus* females per female.

Population parameters of the *X. stemmator* females emerging from the two stem borer species did not differ (Table 5.2). The finite rate of increase of 1.11 per female per day in each of the stem borer species shows that each female contributed an average of 103.2 and 96.3 individuals (Table 5.1) to the population over a mean generation time of 31.4 and 30.3 for females from *B. fusca* and *C. partellus* pupae, respectively (Table 5.2) (as per the definition of Siddiqui *et al.*, 1973). The r_m was the same for females reared on both stem borer species (Table 5.2).

Survival of the adult females (l_x), number of females in the reproductive period (l_xr) and the mean fertility (m_x) for females emerging from *C. partellus* and *B. fusca* is summarized in figure 5.1.

The population doubling time (Dt) was 6.53 and 6.60 days in females emerging from *B. fusca* and *C. partellus* pupae respectively (Table 5.2).

Table 5.1 *Xanthopimpla stemmator* adult longevity, oviposition days and progeny produced by females that emerged from *B. fusca* and *C. partellus* pupae at 27 ± 2 ° C, 49-61% RH and 12: 12 (L: D) h photoperiod. Figures are mean ± SE

Host species	N	Female longevity in days	Oviposition period (days)	Female developmental time in pupa	Parasitoid progeny			Sex-ratio
					female	male	Total	Female: Male
B. fusca	36	44.2 ± 0.49	28.9 ± 0.41	17.3 ± 0.105	27.9 ± 1.44	75.0 ± 2.05	103.22 ± 2.16	1: 3 ± 0.03
C. partellus	36	45.4 ± 0.53	29.4 ± 0.40	16.4 ± 0.109	24.0 ± 1.08	72.2 ± 1.8	96.39 ± 2.16	1: 3 ± 0.02
Statistical		F = 2.36	F = 0.83	F = 0.0001	F = 0.1329	F = 0.00	F = 0.00	F = 0.00
Parameters		df = 1, 2447	df =1, 2447	df = 1, 2447	df =1, 2447	df = 1, 2447	df = 1, 2447	df = 1, 2447
		P = 0.1247	P = 0.3620	P = 0.001	P = 0.1329	P = 0.9971	P = 0.9971	P = 0.9963

Table 5.2 Associated life table estimates for *X. stemmator* females reared on *B. fusca* and *C. partellus* pupae at 27±2°C, 49-61 % RH and 12:12 (L: D) photoperiod (n=36). Jackknife estimates (means) and associated 95% confidence limits.

Life table	*B. fusca*		*C. partellus*		
Population parameters	Mean	95% CL	Mean	95% CL	P>0.05
Intrinsic rate of increase (r_m)	0.106	0.10 – 0.11	0.105	0.09-0.11	0.802 ns
Net reproductive rate (R_o)	27.9	22.3 – 29.5	23.9	18.3-29.5	0.312 ns
Finite rate of increase (λ)	1.11	1.11–1.12	1.11	1.10 – 1.11	0.801 ns
Mean generation time (T_c)	31.4	29.9 – 33.0	30.3	27.6 – 33.0	0.467 ns
Doubling time (Dt)	6.53	6.22 – 6.85	6.60	6.11 – 7.1	0.816 ns

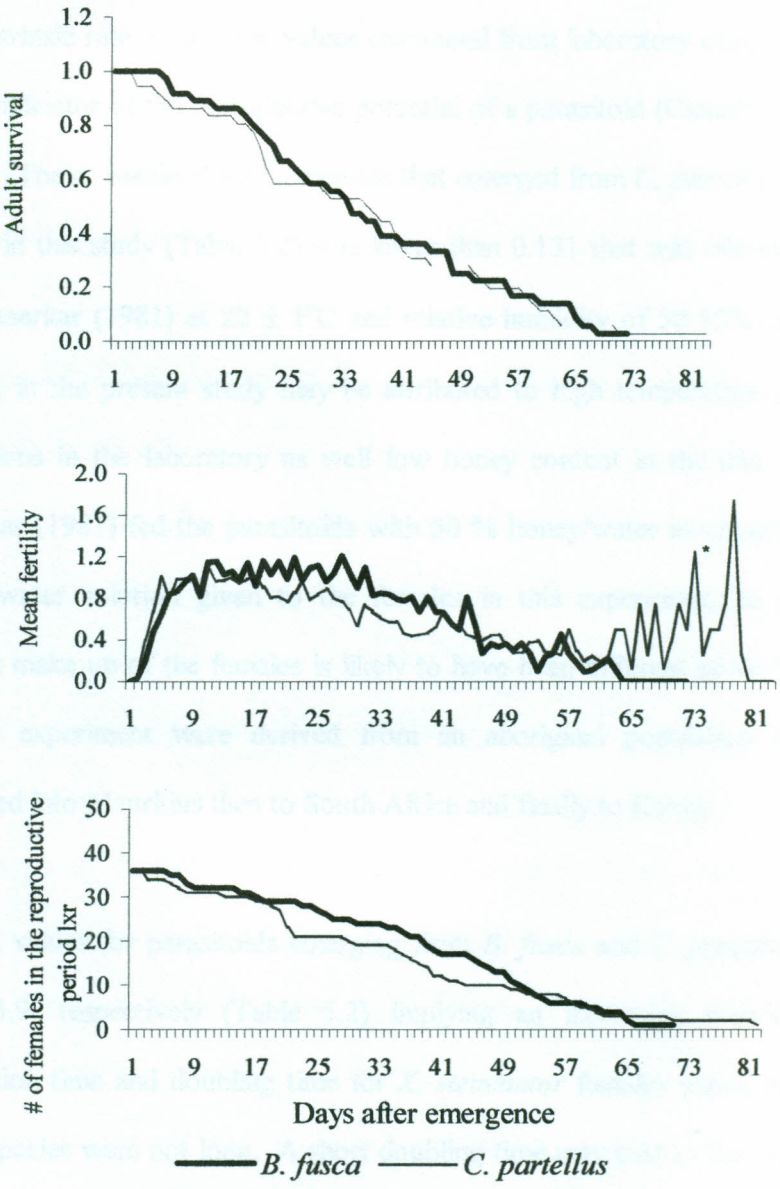


Fig 5.1: Adult survival (l_x), number of females in the reproductive period (l_xr) and fertility rate (m_x) of female *X. stemmator* adults emerging from *B. fusca* and *C. partellus*. * A female that laid female eggs towards the end of her life.

5.5 Discussion

The intrinsic rate of increase values calculated from laboratory experiments is used as an indicator of the reproductive potential of a parasitoid (Chaudhari and Nikam, 2001). The r_m obtained for parasitoids that emerged from *C. partellus* and *B. fusca* pupae in this study (Table 5.2) was lower than 0.131 that was obtained by Nikam and Basarkar (1981) at 22 ± 1°C and relative humidity of 50-55% in India. The low r_m in the present study may be attributed to high temperature and humidity conditions in the laboratory as well low honey content in the diet. Nikam and Basarkar (1981) fed the parasitoids with 50 % honey/water as compared to 10 % honey/water solution given to the females in this experiment. In addition, the genetic make up of the females is likely to have been different as the females used in this experiment were derived from an aboriginal population in Sri-Lanka imported into Mauritius then to South Africa and finally to Kenya.

The R_o values for parasitoids emerging from *B. fusca* and *C. partellus* were 27.9 and 23.9, respectively (Table 5.2) implying an increasing population. Mean generation time and doubling time for *X. stemmator* females reared on both stem borer species were not long. A short doubling time may lead to fast building up of the *X. stemmator* population and consequently a suppression of the target pests, *C. partellus* and *B. fusca* in a relatively short time. Female parasitoids that emerged from *B. fusca* had a longer developmental time inside the pupa than those emerging from *C. partellus* compared to observations in the host suitability study (Chapter four). The longer developmental time is attributed to the large size of *B. fusca* pupae. Mean longevity in this study recorded for parasitoids emerging from

C. partellus was higher than that recorded by Nikam and Basarkar (1981) in India. This was probably because of the higher humidity in the quarantine unit and the diet. Life span of *X. stemmator* females used in this study was higher compared to non-ovipositing females as discussed in host suitability studies (Chapter Four). Long life span may have been due to confinement in larger cylinders.

The pattern of daily oviposition rate in *X. stemmator* was similar to that found in most parasitoids with an increase to peak fecundity followed by a gradual decline as the insect ages (Fig 5.1). The preoviposition period was 2 days and the *X. stemmator* females tended to produce a greater proportion of males as they grew old. This trend was also observed for many other parasitoids including *Trichogramma species. nr. mwanzai* and *T. bounieri* (Abera, 2001), *Anagyrus pseudococci* (Girault) (Avidov *et al.*, 1967), *Apoanagyrus lopezi* De Santis (both Hymenoptera: Encyrtidae) (Odebiyi and Bokonon- Ganta, 1986), *Tetrastichus incertus* (Ratzeburg) (Hymenoptera: Eulophidae) (Pitcairn and Gutierrez, 1992) and *Catolaccus grandis* (Burks) (Hymenoptera: Pteromalidae) (Morales-Ramos and Cates, 1992).

If parasitoids are supplied with an unlimited number of hosts in the laboratory, the female to male sex ratio often becomes increasingly male biased with time, presumably because of sperm depletion (Van den Assem, 1986; King, 1987). The females were given pupae every day until their natural death. In this study, *X. stemmator* females were probably given more pupae than they would ever encounter in the field. The problem of sperm depletion may not arise in the field.

The increased longevity should be matched by greater fecundity if *X. stemmator* is to be reckoned of good quality in biological control. The sex ratio of the progeny in this case becomes a secondary attribute. High fertility and progeny production of female adults would more than offset the male biased sex ratio in parasitoids emerging from *B. fusca* and *C. partellus*. In the field, it is unlikely that *X. stemmator* females will find hosts in large numbers and on a daily basis and thus the issue of males emerging at the later days as the females age remain to be studied.

The life table study showed that there were no differences in the main population parameters (r_m and R_o) between *X. stemmator* females emerging from *C. partellus* and *B. fusca* pupae. This indicates that no preference need be given to the two host species for mass rearing (Abera, 2001). Optimal values for the life table parameters are expected to depend upon environmental conditions (Johansson *et al.*, 2001). This study was carried out on females fed on a 10 % honey-water solution and maintained at a temperature of 27° C. However, comparative studies on the growth rates of both *X. stemmator* and its stem borer hosts at different temperatures and diet are necessary to be able to speculate on its potential for population growth in specific host-parasitoid communities, taking into account other important factors such as effect of competition as discussed by Force (1970, 1973).

Life table parameters obtained under laboratory conditions are typically higher than those found in the field. This is because, survival rate is typically higher

under laboratory conditions and host finding is not an issue. The present study provides complementary data on the parasitoid, which may prove useful in planning its utilization in classical biocontrol programmes.

CHAPTER SIX

MATING FREQUENCY AND EFFECTS ON SEX RATIO IN *Xanthopimpla stemmator* (THUNBERG) FEMALES

6.1 Introduction

Some of the most important aspects of female reproductive biology are age at mating, the frequency of mating and in cases of multiple mating, whether the first or last male's sperm fertilizes the egg (Godfray, 1994). *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) is an arrhenotokous (haplo-diploid) species with unfertilized eggs developing into male progeny and fertilized eggs into female progeny (Poppy, 1997). Assuming that there is no environmental influence over the sex ratio, arrhenotokous species will produce both sexes from a mated female (Poppy, 1997). Mating therefore becomes important to the parasitoid population at some time and scale. This has a bearing in the parasitoid population since the females will determine the size of the population. This study was undertaken to investigate the relationship between multiple mating and sex ratio of *X. stemmator* progeny.

6.2 Objectives

1. To determine whether *X. stemmator* females mate more than once
2. To determine whether the sex ratio of progeny is affected by multiple mating in female *X. stemmator*

6.3 Materials and methods

6.3.1 Insects

The study was carried out using parasitoids that emerged from *Sesamia calamistis* (Lepidoptera: Noctuidae) and *Chilo partellus* (Lepidoptera: Crambidae) pupae. They were reared as described in chapter two. The experiments were carried out at 27 ± 2°C, 49-61% RH and 12:12 (Light: Day).

6.3.2 Bioassay

6.3.2.1 Mating Interval

At least 100 two-day-old *Chilo partellus* pupae were collected from the insectary at the International Centre of Insect Physiology and Ecology (ICIPE) daily. Ten pupae were put inside a straw, which was then smeared with frass formed from damage by *C. partellus* larvae. A total of 50 pupae, in five of the straws were exposed for four hours to 20 *X. stemmator* females in cages measuring 15 x 15 x 15 cm (Plate 2.1). The pupae were thereafter removed and kept in Petri dishes, 8 cm in diameter (Plate 2.2) until *X. stemmator* adult emergence.

A batch of 160 *X. stemmator* females that emerged from the parasitised pupae was randomly selected within 12 hours of emergence. The females were paired with males in a vial (2.5 x 7.5 cm) and mating was observed. Time taken for start of courtship (when the pair was put together in the vial and when the male mounted the female) was recorded for first and any subsequent mating. Similarly, time taken for copulation (when the male inserted his aedeagus in the female genitalia) was also recorded. Copulation that lasted for 29 seconds was considered successful

insemination since previous preliminary observations showed that females that copulated for this duration or more produced female progeny (Personal observations). Females that mated for less than 29 seconds were discarded. All the females that mated were placed in a rearing cage and provided with a diet of 10 % honey/ water solution soaked in clean cotton wool.

After 24 hours, 51 *X. stemmator* females were randomly selected from the cage and paired with naïve one or two day old males to mate a second time. Those that accepted mating a second time were all paired again with naïve one or two day old males for mating after another 24-hour period so that this batch had a chance to mate three times.

A batch of 54 females was randomly selected from the 107 females that were mated once after emergence and left in the cage. They were put in vials (2.5 x 7.5 cm) with a naïve one or two day old male to mate after 72 hours such that this batch also had a chance to mate three times after a 72-hour interval.

The remaining 53 females were paired to mate 3 times after every 168 hours the same manner as above. Pairs that did not mate within one hour they were put together in the vial were discarded.

6.3.2.2 Progeny production in females mated more than once

At least 100 two-day-old pupae of *S. calamistis* were collected from the insectary at ICIPE daily. These pupae were used to produce *X. stemmator* females to be used to investigate if multiple mating affects overall sex ratio of total progeny produced by the females in her lifetime. The *S. calamistis* pupae were available in the required amounts from the Animal Rearing and Quarantine Unit (ARQU) at this time. Moreover it had been observed in the previous study that the host pupae on which females are reared does not affect development of offspring and egg laying in *X. stemmator* (Chapter 3). The pupae were offered to *X. stemmator* females in the ratio of 100 pupae to 20 *X. stemmator* females for oviposition in cages measuring 15 x 15 x 15 cm (Plate 2.1) for four hours. Thereafter the exposed pupae were kept in Petri dishes, 8 cm in diameter (Plate 2.2) until *X. stemmator* adults emerged.

Twenty-five females that emerged were randomly selected within 12 hours of emergence and placed singly in clean plastic cylinders measuring 9 x 5 cm (Plate 2.5). A one-day-old male that had not mated was introduced into each of the cylinders with the females. The mating process was observed and time taken for each mating pair recorded as described in section 6.3.2.1. Each female was mated once and the males removed from the cylinder thereafter. The females were provided with a diet of 10 % honey/water solution soaked in cotton wool. After 24 hours, all the previously once mated females were paired to mate with naïve males and mating observed. Females that did not mate after one hour were discarded. The males were removed after the second mating and female given fresh diet.

After 72 hours, twenty males that had not mated with any females were introduced into twenty cylinders and left with the females that had mated twice, two days consecutively. Ninety-six hours post emergence of the female parasitoids, five *C. partellus* pupae were inserted in each paper straw and the straws smeared with frass made from *C. partellus* larvae. Two straws with pupae were introduced into each of the 20 plastic cylinders containing the male and female for oviposition such that each female was exposed to ten pupae. Pupae and honey soaked in cotton wool were removed after 24-hour of exposure every day and replaced with fresh pupae and 10 % honey. A male that died in the course of the experiment was replaced. All females that mated twice and thereafter left with males were assumed to have mated more than once. *Chilo partellus* was used as the host provided to the females everyday in this experiment because of its continuous availability from ARQU in large numbers.

The pupae were offered to the females for 32 days after which males and females that were still alive were discarded. Ten and six females that mated once and more than once, respectively, died before the last day of exposure. All exposed pupae were kept in Petri dishes measuring 4cm in diameter and monitored till emergence of the parasitoids.

A control group was maintained with the same number of females. They were given the same treatment except that they were paired to mate only once after emergence. Mating was also observed and duration recorded. Daily emergence, sex of progeny and adult female longevity was recorded.

6.3.3 Data analysis

Data on courtship, first, second and mating frequency were square root transformed and analyzed using ANOVA (SAS institute, 2000). Sex ratio was analyzed by ANOVA. The results of females that mated at 24, 72 and 168-hour interval were presented as proportions. Data on longevity of multiple mated ovipositing females was analyzed by studentized test (Proc Ttest) (SAS institute, 2000).

6.4 Results

6.4.1 Mating interval

Courtship was 48 times longer for pairs that mated a second time than with naïve ones ($F = 189.0$; $df = 1,60$; $P = 0.0001$) (Table 6.1). Time taken for copulation was not significantly different between first and second mating ($F = 0.45$; $df = 1,60$; $P = 0.5042$). All females mated readily on the first day of emergence (Fig 6.1). Few females readily mated a second and third time as the mating interval increased from 24 hours (Fig 6.1). None of the females mated a third time for the females that mated at 168 hours intervals. First, second and third mating frequency varied significantly at 24, 72 and 168 hours interval ($F = 125.03$; $df = 2, 6$; $P = 0.0001$; $F = 121.7$; $df = 2, 6$; $P = 0.0001$; $F = 283.49$; $df = 2, 6$; $P = 0.0001$; respectively) (Fig 6.1).

Table 6.1: Mean duration (seconds) spent in courtship and copula (± S.E) by 25 *X. stemmator* females mated after emergence with naïve males at 27 ± 2°C, 49-61% RH and 12:12 (L: D) h.

	N	Courtship	Copulation
1st mating	31	27.3 ± 3.0a	35.3 ± 1.4a
2nd mating	31	1327.4 ± 139.5b	34.1 ± 1.62a
3rd mating	-	-	-
Statistical	F	189.0	0.45
Parameters	df	1, 60	1, 60
	P	0.0001	0.5042

Means followed by the same letter in the same column are not significantly different (Student–Newman-Keuls multiple comparison test) $P < 0.05$

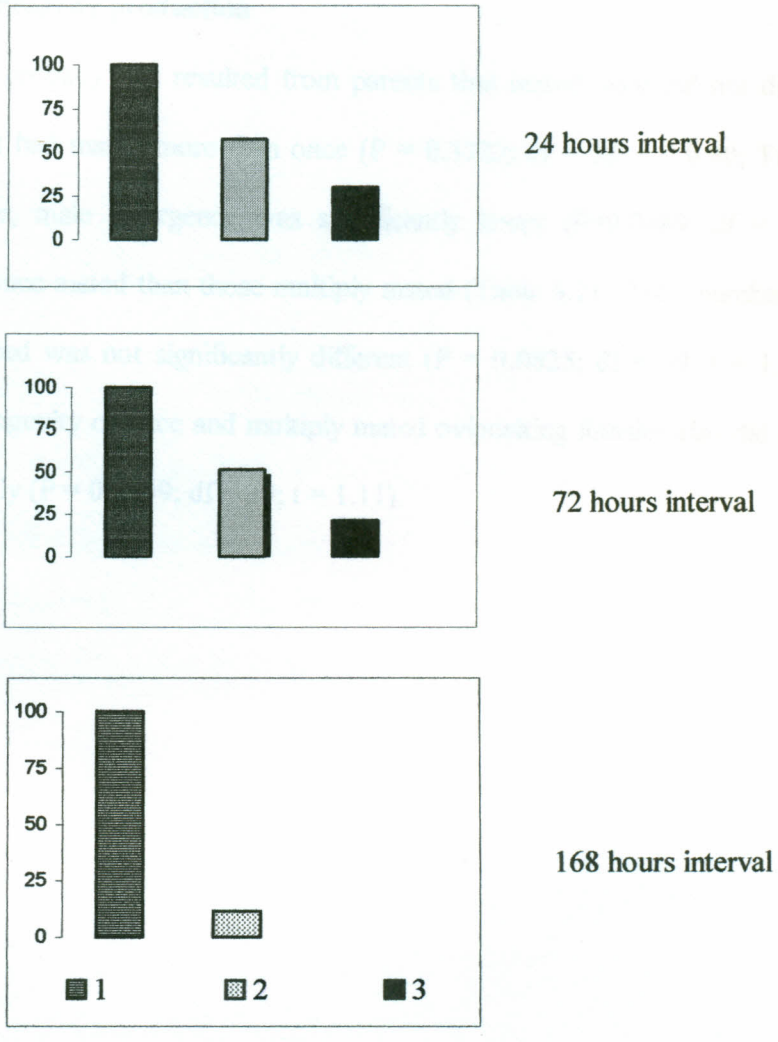


Fig 6.1 Mean percentage (± S.E) of *X. stemmator* females that mated at 24, 72 and 168-hour interval at 27 ± 2°C, 49-61% RH and 12:12 (L: D).
1. Females that mated once 2. Females that mated twice 3. Females that mated three

6.4.2 Progeny production

Overall female progeny that resulted from parents that mated once did not differ from those that had mated more than once (P = 0.3722; df = 39; t = 0.90; Table 6.2). However, male emergence was significantly lower (P=0.0489; df = 39; t=2.03) in the once mated than those multiply mated (Table 6.2). Total number of progeny produced was not significantly different (P = 0.0825; df = 39; t = 1.77; Table 6.2). Longevity of once and multiply mated ovipositing females also did not differ significantly (P = 0.2759; df = 39; t = 1.11).

Table 6.2 Mean progeny production, sex ratio and longevity of female *X. stemmator* mated once and those mated more than once

Parameter	Mated > once (N = 20)	Mated once (N = 20)	Statistical parameters
Female Progeny	27.6 ± 4.4	24.6 ± 4.0	t = 0.90 ns
Male Progeny	43.6 ± 6.3	28.7 ± 6.0	t = 2.03 *
Total progeny	71.2 ± 7.7	53.3 ± 8.0	t = 1.77 ns
Longevity	29.2 ± 2.1	25.9 ± 2.5	t = 1.11 ns
Sex- ratio (F: M)	1: 1.05 ± 0.18	1.5: 1 ± 0. 4	F=1.45 df = 1,39 P = 0.24

* = significant
ns = not significant

6.5 Discussion

6.5.1 Mating interval

Results obtained in the present study indicate that *X. stemmator* females can copulate more than once. However this is rare and insignificant. *Xanthopimpla stemmator* females were receptive to mating immediately after emergence. This supports work documented by Moore and Kfir (1996). The mating behaviour of *X. stemmator* females in the field is not known. *Xanthopimpla stemmator* females were observed to mate more than once in the laboratory but they became reluctant to mate a second time as they became older. This suggests that in nature *X. stemmator* females copulate once in their lifetime within the first day of emergence. This agrees with what is observed in other Ichneumonidae, particularly *Bathyplectes curculionis* (Thompson) (Dowell and Horn, 1978) and *B. anurus* (Thompson) (Bartell and Pass, 1980). Simmonds (1954) also found that in the ichneumonid *Trachysphyrus inornatus* (Platt) mating did not occur if females were several days old.

Xanthopimpla stemmator males that had previously mated were observed to court unwilling females in the course of the experiment especially in the second and third mating (Personal observations). This phenomenon of males courting unreceptive females has been observed in other studies. Van den Assem *et al.* (1984) found that *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) males courted unreceptive females for longer periods of time when females were rare. This seemed true for females in this study as a non-receptive female avoided an approaching male or used

her wings and legs to prevent the male from mounting on her. This behaviour was commonly observed for many of the females during the second and third mating.

Studies by Moore and Kfir (1996) on *X. stemmator* showed that males usually mated with several females in succession. The results in this study showed that half of the females willingly or unwillingly mated a second or third time if the interval between matings did not exceed 72 hours. If a population of *X. stemmator* has more males than females at a particular time, chances are that the females will be mated more than once in succession. This multiple mating does not affect sex ratio according to results obtained in this study. It was observed that males attempted to mount and mate with unwilling females and even dead ones in the laboratory. Males may mount dead females, but if females' antennae or wings are removed, the male is apt to mount at the wrong end (Godfray, 1994). Hymenoptera female parasitoids are receptive to mating immediately after emergence. It was also observed that some males ignored the females during the second and third time the parasitoids were paired to mate as the females aged. Similar studies on mating of the pupal parasitoid *Dentichasmias busseolae* (Hymenoptera: Ichneumonidae) by Mohyuddin (1972) showed that newly emerged females were more attractive to males than old ones.

6.5.2 Progeny production

The present study reveals that the frequency of mating does not influence sex ratio of the parasitoid population. In other parasitoids, it has been reported that multiple

mating may cause clogging of the spermathecal ducts leading to the oviposition of unfertilized eggs (Godfray, 1994). Given that *X. stemmator* is a synovigenic species (ovigenesis occurs during adulthood), excessive early mating may not have been favored, as the sperm may die in the spermathecal ducts.

For haplodiploid species, there is enforced production of male offspring referred to as constrained sex allocation (Godfray, 1994). Constrained oviposition leads to a male-biased population sex ratio (Godfray, 1994). The presence of some females in the population producing only male offspring will lead to selection of unconstrained females to produce more daughters. *Xanthopimpla stemmator* is a haplodiploid species and previous laboratory studies showed that females have a highly male biased sex ratio in the later days of their lives (Chapter 5). It is not known whether this phenomenon exists in the field and if unconstrained females may be selected over the constrained females in cases where most of the females give a highly biased male sex ratio.

Stouthamer *et al.* (1992) hypothesized that the high rate of biocontrol failure with Ichneumonidae and Braconidae may result from inbreeding, loss of genetic diversity during culturing and the subsequent production of diploid males. The production of diploid males will negatively influence establishment and population growth rates (Stouthamer *et al.* 1992). This is more so with monandrous (mate only once) females. This study reveals that *X. stemmator* females are monandrous. When mated to diploid

males, they would subsequently not remate and hence become constrained to produce only haploid sons, leading to male-biased sex ratios and decreased population growth rates (Stouthamer *et al.* 1992).

6.5.3 Conclusion

Multiple mating does not seem to occur in *X. stemmator* and when it does it is purely accidental and inconsequential. In a review of mating behaviour among parasitoid wasps, Gordh and DeBach (1978) found that multiple mating by males occurred in 48 cases studied where mating behaviour had been described, while multiple mating by females occurred in only 5 out of 34 cases studied. Ridley (1988) also collected information from literature on the female mating behaviour of 99 species of parasitoid wasps of which nearly 80% were reported to mate once. The unusual cases of multiple mating in the parasitoids are most likely to be selected for because they increase genetic diversity in the brood though support for specific genetic diversity hypotheses has proved to be elusive. What is clear is that single mating is predominant in the Hymenoptera, evolutionary and ecologically successful group, (Strassmann, 2001). It is therefore not necessary to leave males and females together during rearing. The females after emergence should be mated and separated from the males and given hosts for oviposition.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

The introduced lepidopteran stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) is a major constraint to maize and sorghum production in Kenya (Kfir *et al.*, 2002). The common indigenous natural enemies, including the pupal parasitoid of *C. partellus*, *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae), are unable to maintain the population of stem borers at a level acceptable to farmers (Mathez, 1972; Skovgård and Päts, 1996; Zhou and Overholt, In press). *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a larval parasitoid of Lepidoptera stem borers has been introduced in parts of East and southern Africa but *C. flavipes* does not complete development in *B. fusca* (Ngi-Song *et al.* 1995).

A programme has been initiated in Eastern and southern Africa to introduce the exotic parasitoid *X. stemmator* from Sri-Lanka, to reduce damage caused by the major stem borer pests such as *C. partellus, B. fusca*, *S. calamistis*, *E. saccharina* and *C. sacchariphagus* (Kfir *et al.*, 2002). The use of "new associations" opens opportunities for the regulation of indigenous pest species by using natural enemies from closely related hosts from other areas of the world (Hokkanen and Pimentel, 1984; Waage, 1990).

Before introduction of biological control agents of gramineous stem borers are released, studies on oviposition behaviour of parasitoids are necessary, as hosts and their parasitoids occupy a specialized habitat and different host species show variation in their feeding and pupation behaviour (Mohyuddin, 1972). Some parasitoid species may accept and develop on species other than their normal hosts in the laboratory but may not parasitise them in the field because of the parasitoids specialized oviposition behaviour and /or the presence of chemical and/or physical factors that stimulate oviposition (Mackauer, 1986).

Studies reported here have examined host preference (Chapter 3), host acceptance and suitability (Chapter 4), life tables and intrinsic rates of natural increase (Chapter 5) and mating behaviour (Chapter 6) of *X. stemmator.* Host preference experiments indicated that *X. stemmator* females do not discriminate between different host pupae in the laboratory. The hosts on which *X. stemmator* females were reared on (pre-adult experience) did not influence choice of host pupae. *Xanthopimpla stemmator* does not respond to pupae that are naked that is, have no cocoon or are not enclosed in a stem (Smith *et al.*, 1993). However, it does respond to the presence of a plant stem, especially that of a grass crop like maize (Weidenmann, 2000). The parasitoid apparently is less responsive to horizontally oriented "stems" than to vertical stems (Weidenmann, 2000).

The obstacle often encountered in new parasitoid/host relationships is the physiological suitability of the host for development of parasitoids (Ngi-Song *et al*, 1999). Parasitization is successful if an accepted host is suitable for development. *Xanthopimpla stemmator* accepted all the hosts and the six pupal ages used in this study. It completed development in the four stem borer species and the six pupal ages apart from six-day-old *E. saccharina* pupae. Five-day-old *E. saccharina* pupae were partially suitable. Surprisingly, *E. saccharina* showed an overall low suitability as compared to the other three species. *Xanthopimpla stemmator* is reared on *E. saccharina* in South Africa (Conlong pers comm) and has been released against this borer species in sugarcane in KwaZulu-Natal (Moore and Kfir, 1996).

Suitability studies on *E. saccharina* in South Africa showed a 40.00 % parasitoid emergence (Moore and Kfir, 1996) as compared to 15.05 % in Kenya. The population of *E. saccharina* found in South Africa is probably different from that in Kenya. This study suggests that if *X. stemmator* is released in areas of Kenya where *E. saccharina* is the dominant species, this parasitoid may not establish. Sex ratio of the progeny emerging from females reared from *B. fusca*, *C. partellus* and *S. calamistis* the four stem borer species studied was male biased. The sex ratio became increasingly male biased as the females aged. Inbreeding in the laboratory colonies may be the cause of this occurrence. The founders obtained from South Africa had a minimum of 220-laboratory generations (Conlong pers comm.) plus 16-laboratory generations here at the International Centre of Insect Physiology and Ecology (ICIPE) quarantine unit

where the parasitoids were reared. There may have been genetic changes with time causing inbreeding and probably production of diploid males. The colony needs to be strengthened with *X. stemmator* from other parts of the world where the parasitoid is native to increase the genetic diversity of the laboratory colony. Younger females should be used for mass rearing and during releases since a high male progeny is produced as the females age. It will be interesting to examine in the laboratory if sex ratio changes when hosts are scarce.

Parasitism of pupae in this study was less than 50 % for those that were exposed to females in the four hosts. Though this parasitism compares with emergence of parasitoids from *C. partellus* and *E. saccharina* by Moore and Kfir (1996), better methods to improve parasitism in the laboratory need to be investigated. Parasitism in the field may be lower since hosts are scattered and host finding may be an issue. However, even if a low level of parasitism does occur in grasses infested by any of the four stem borer species, the potential benefits could outweigh the risk of the damage that these borers cause.

A life table study was conducted to determine if reproductive capabilities of *X. stemmator* differed when the parasitoids were reared on two serious pests of gramineous crops, *C. partellus* and *B. fusca* under laboratory conditions (27°C ± 2, 49-61 % RH and 12: 12 (L: D)). The population parameters of the *X. stemmator* females did not differ significantly hence the female parasitoids have the same fertility.

It is a common phenomenon for haplo-diploid parasitoids to give rise to male progeny when insemination does not take place (Doutt, 1964). Mating becomes important to the parasitoid population since a male biased sex ratio can bring about collapse of the population. *Xanthopimpla stemmator* females showed a tendency to mate once. Multiple mating by females in the present study did not have any significant effect on either sex ratio or longevity. Multiple mating in female Hymenoptera is not a common occurrence (Godfray, 1994).

The success of *X. stemmator* as a biological control agent depends on its ability to attack stem borers enclosed in the stems of gramineous plants. *Xanthopimpla stemmator* should be able to attack stem borers in wild grasses throughout the year and move to cultivated gramineous plants during the cropping seasons. In its original habitat, *X. stemmator* occurs in warm low-lying areas, reproduces throughout the year and does not hibernate, as hosts are always available (Moore and Kfir, 1996). Due to its "drill and sting attack" strategy, stem borer pupae that are deeply located in the stems may not be accessible.

In Mauritius where *X. stemmator* was released and is established on *C. sacchariphagus* and *S. calamistis* (Vinson, 1942, Moutia and Mamet, 1945), the pupae are accessible. *Chilo sacchariphagus* pupates in litter and dry leaf sheaths (Williams, 1983), while *S. calamistis* sometimes pupates in the stems or between the stems and the leaf sheath (Smith *et al.*, 1993).

7.2 Major conclusions

1. *Xanthopimpla stemmator* does not show preference for pupae of different stem borer hosts and the adults completed development in *B. fusca, C. partellus, E. saccharina* and *S. calamistis*.
2. The stem borer species *X. stemmator* are reared from does not affect host species choice of subsequent generations of *X. stemmator* females in the laboratory.
3. Two-day-old *C. partellus* pupae were parasitized most and were thus more suitable in terms of parasitoid emergence. Hence two-day-old *C. partellus* are more appropriate to use during mass rearing.
4. *Eldana saccharina* is a poor laboratory host for *X. stemmator* in Kenya.
5. When provided with hosts every day in the laboratory, *X. stemmator* females tended to have a male biased sex ratio as they advance in age except for those reared from *E. saccharina.*
6. The intrinsic rates of natural increase and the net reproductive rates were not different for parasitoids emerging from neither *C. partellus* nor *B. fusca*, hence both can be used during mass rearing and *X. stemmator* can be released against both species non-preferentially.
7. Multiple mating is not a common occurrence in *X. stemmator* females and has no effect on sex ratio.

7.3 Recommendations

More information on natural pupal parasitism of target Lepidoptera pests in Kenya and other East and southern African countries is required. The biology of *X. stemmator* has also not been well studied or documented. Further research in the following areas will be useful for designing a biological control programme.

1. The oviposition behaviour of *Xanthopimpla stemmator* and number of eggs laid per host pupae under natural conditions.
2. Whether *X. stemmator* completes development in other lepidopteran stem borers and non-target Lepidoptera species that are likely to be attacked by this parasitoid.
3. The host-searching behaviour of *X. stemmator* in the field and semi-field condition.
4. The infochemicals that are emitted by plants damaged by stem borer feeding larvae that are highly attractive to *X. stemmator* females.
5. Factors that may have caused poor parasitoid emergence in *E. saccharina* in Kenya.
6. Basic reproductive biology of *X. stemmator* females.
7. Present genetic status of *X. stemmator* compared to parasitoids in the original habitat
8. Methods to improve parasitism in the laboratory for the purpose of mass rearing and establish whether host scarcity affects sex ratio in female *X. stemmator.*

9. Effect of temperature and diet on life table parameters of *X. stemmator* females.
10. The pupal depth of target stem borer species in cereal crops and the depth, which *X. stemmator* can parasitise hosts in stems.
11. *Xanthopimpla stemmator* may be released in fields against the tested Lepidoptera stem borers and surveys conducted on its biology in a field context.

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APPENDIX

Appendix 1: Fertility life table at 27±2° C, 49-61 % RH and 12:12 (L: D) photoperiod (N=36) of females emerging from *B. fusca*

age	surv	l_x	m_x	l_xm_x	x l_xm_x
17.5	1	1.00000	0.00000	0.0000	0.0000
18.5	1	1.00000	0.03000	0.0300	0.5550
19.5	1	1.00000	0.21000	0.2100	4.0950
20.5	1	1.00000	0.48750	0.4875	9.9938
21.5	1	1.00000	0.63750	0.6375	13.7063
22.5	1	1.00000	0.84000	0.8400	18.9000
23.5	1	1.00000	0.87000	0.8700	20.4450
24.5	1	0.97222	0.95657	0.9300	22.7850
25.5	1	0.91667	1.02273	0.9375	23.9063
26.5	1	0.91667	0.81818	0.7500	19.8750
27.5	1	0.91667	1.14545	1.0500	28.8750
28.5	1	0.88889	1.15594	1.0275	29.2838
29.5	1	0.88889	1.02938	0.9150	26.9925
30.5	1	0.88889	1.06313	0.9450	28.8225
31.5	1	0.86111	0.97548	0.8400	26.4600
32.5	1	0.86111	1.14097	0.9825	31.9313
33.5	1	0.86111	0.91452	0.7875	26.3813
34.5	1	0.86111	1.15839	0.9975	34.4138
35.5	1	0.83333	1.02600	0.8550	30.3525
36.5	1	0.80556	1.15448	0.9300	33.9450
37.5	1	0.77778	0.98357	0.7650	28.6875
38.5	1	0.75000	1.19000	0.8925	34.3613
39.5	1	0.72222	0.97615	0.7050	27.8475
40.5	1	0.66667	1.09125	0.7275	29.4638
41.5	1	0.66667	1.11375	0.7425	30.8137
42.5	1	0.63889	0.96261	0.6150	26.1375
43.5	1	0.61111	1.04318	0.6375	27.7313
44.5	1	0.58333	1.20857	0.7050	31.3725
45.5	1	0.58333	1.06714	0.6225	28.3238
46.5	1	0.58333	0.90000	0.5250	24.4125
47.5	1	0.55556	1.09350	0.6075	28.8563
48.5	1	0.55556	0.82350	0.4575	22.1888
49.5	1	0.52778	0.85263	0.4500	22.2750
50.5	1	0.47222	0.84176	0.3975	20.0738
51.5	1	0.47222	0.79412	0.3750	19.3125
52.5	1	0.44444	0.77625	0.3450	18.1125

53.5	1	0.44444	0.81000	0.3600	19.2600
54.5	1	0.38889	0.67500	0.2625	14.3063
55.5	1	0.38889	0.81000	0.3150	17.4825
56.5	1	0.38889	0.67500	0.2625	14.8313
57.5	1	0.36111	0.66462	0.2400	13.8000
58.5	1	0.36111	0.56077	0.2025	11.8463
59.5	1	0.36111	0.49846	0.1800	10.7100
60.5	1	0.36111	0.81000	0.2925	17.6963
61.5	1	0.33333	0.56250	0.1875	11.5313
62.5	1	0.33333	0.27000	0.0900	5.6250
63.5	1	0.33333	0.36000	0.1200	7.6200
64.5	1	0.25000	0.33000	0.0825	5.3213
65.5	1	0.25000	0.36000	0.0900	5.89500
66.5	1	0.25000	0.30000	0.0750	4.98750
67.5	1	0.22222	0.37125	0.0825	5.56875
68.5	1	0.22222	0.27000	0.0600	4.11000
69.5	1	0.22222	0.10125	0.0225	1.56375
70.5	1	0.22222	0.23625	0.0525	3.70125
71.5	1	0.22222	0.27000	0.0600	4.29000
72.5	1	0.16667	0.45000	0.0750	5.43750
73.5	1	0.16667	0.27000	0.0450	3.30750
74.5	1	0.16667	0.18000	0.0300	2.23500
75.5	1	0.16667	0.09000	0.0150	1.13250
76.5	1	0.13889	0.10800	0.0150	1.14750
77.5	1	0.13889	0.16200	0.0225	1.74375
78.5	1	0.13889	0.21600	0.0300	2.35500
79.5	1	0.13889	0.10800	0.0150	1.19250
80.5	1	0.13889	0.00000	0.0000	0.00000
81.5	1	0.08333	0.00000	0.0000	0.00000
82.5	1	0.05556	0.00000	0.0000	0.00000
83.5	1	0.05556	0.00000	0.0000	0.00000
84.5	1	0.02778	0.00000	0.0000	0.00000
85.5	1	0.02778	0.00000	0.0000	0.00000
86.5	1	0.02778	0.00000	0.0000	0.00000
87.5	1	0.02778	0.00000	0.0000	0.00000
88.5	1	0.02778	0.00000	0.0000	0.00000
			42.8733	27.8775	1050.387

Appendix 2: Fertility life table at 27±2° C, 49-61 % RH and 12:12 (L: D) photoperiod (N=36) of females emerging from *C. partellus*

age	surv	l_x	m_x	l_xm_x	x l_xm_x
16.5	1	1.00000	0.00000	0.00000	0.0000
17.5	1	1.00000	0.04822	0.04822	0.8439
18.5	1	1.00000	0.39956	0.39956	7.3918
19.5	1	0.94444	0.67106	0.63378	12.3587
20.5	1	0.94444	1.00659	0.95067	19.4887
21.5	1	0.94444	0.80235	0.75778	16.2922
22.5	1	0.91667	0.98448	0.90244	20.3050
23.5	1	0.88889	0.98425	0.87489	20.5599
24.5	1	0.86111	1.01600	0.87489	21.4348
25.5	1	0.86111	0.88000	0.75778	19.3233
26.5	1	0.86111	1.08800	0.93689	24.8276
27.5	1	0.86111	0.96800	0.83356	22.9228
28.5	1	0.86111	0.97600	0.84044	23.9527
29.5	1	0.83333	1.00027	0.83356	24.5899
30.5	1	0.83333	0.94240	0.78533	23.9527
31.5	1	0.83333	0.98373	0.81978	25.8230
32.5	1	0.83333	1.00027	0.83356	27.0906
33.5	1	0.80556	0.90648	0.73022	24.4624
34.5	1	0.80556	0.80386	0.64756	22.3407
35.5	1	0.77778	0.81486	0.63378	22.4991
36.5	1	0.72222	0.77262	0.55800	20.3670
37.5	1	0.63889	0.85183	0.54422	20.4083
38.5	1	0.58333	0.95657	0.55800	21.4830
39.5	1	0.58333	0.79124	0.46156	18.2314
40.5	1	0.58333	0.83848	0.48911	19.8090
41.5	1	0.58333	0.88571	0.51667	21.4417
42.5	1	0.58333	0.80305	0.46844	19.9089
43.5	1	0.58333	0.76762	0.44778	19.4783
44.5	1	0.58333	0.69676	0.40644	18.0868
45.5	1	0.55556	0.53320	0.29622	13.4781
46.5	1	0.52778	0.74400	0.39267	18.2590
47.5	1	0.52778	0.67874	0.35822	17.0156
48.5	1	0.52778	0.61347	0.32378	15.7032
49.5	1	0.52778	0.57432	0.30311	15.0040
50.5	1	0.52778	0.57432	0.30311	15.3071
51.5	1	0.50000	0.46844	0.23422	12.0624
52.5	1	0.47222	0.43765	0.20667	10.8500
53.5	1	0.44444	0.44950	0.19978	10.6881
54.5	1	0.44444	0.46500	0.20667	11.2633

55.5	1	0.38889	0.53143	0.20667	11.4700
56.5	1	0.33333	0.66133	0.22044	12.4551
57.5	1	0.30556	0.54109	0.16533	9.5067
58.5	1	0.30556	0.54109	0.16533	9.6720
59.5	1	0.30556	0.45091	0.13778	8.1978
60.5	1	0.27778	0.42160	0.11711	7.0852
61.5	1	0.27778	0.39680	0.11022	6.7787
62.5	1	0.27778	0.44640	0.12400	7.7500
63.5	1	0.27778	0.44640	0.12400	7.8740
64.5	1	0.27778	0.34720	0.09644	6.2207
65.5	1	0.27778	0.27280	0.07578	4.9634
66.5	1	0.25000	0.27556	0.06889	4.5811
67.5	1	0.22222	0.37200	0.08267	5.5800
68.5	1	0.19444	0.38971	0.07578	5.1908
69.5	1	0.19444	0.28343	0.05511	3.8302
70.5	1	0.19444	0.28343	0.05511	3.8853
71.5	1	0.19444	0.24800	0.04822	3.4479
72.5	1	0.19444	0.49600	0.09644	6.9922
73.5	1	0.16667	0.28933	0.04822	3.5443
74.5	1	0.13889	0.19840	0.02756	2.0529
75.5	1	0.11111	0.24800	0.02756	2.0804
76.5	1	0.11111	0.18600	0.02067	1.5810
77.5	1	0.08333	0.24800	0.02067	1.6017
78.5	1	0.05556	0.49600	0.02756	2.1631
79.5	1	0.05556	0.49600	0.02756	2.1907
80.5	1	0.055556	0.124	0.006889	0.55456
81.5	1	0.055556	0.744	0.041333	3.36867
82.5	1	0.055556	0.248	0.013778	1.13667
83.5	1	0.055556	0.744	0.041333	3.45133
84.5	1	0.055556	0.124	0.006889	0.58211
85.5	1	0.055556	0.372	0.020667	1.76700
86.5	1	0.027778	0.744	0.020667	1.78767
87.5	1	0.027778	0.496	0.013778	1.20556
88.5	1	0.027778	1.240	0.034444	3.04833
89.5	1	0.027778	0.248	0.006889	0.61656
90.5	1	0.027778	0.496	0.013778	1.24689
91.5	1	0.027778	0.496	0.013778	1.26067
92.5	1	0.027778	0.744	0.020667	1.91167
93.5	1	0.027778	1.736	0.048222	4.50878
94.5	1	0.027778	0.248	0.006889	0.65100
95.5	1	0.027778	0.000	0.000000	0.00000
96.5	1	0.027778	0.000	0.000000	0.00000
97.5	1	0.027778	0.000	0.000000	0.00000

98.5	1	0.027778	0.000	0.000000	0.00000
		47.54	23.9044	867.097	

Appendix 3: List of acronyms and abbreviations

1. ANOVA = Analysis of variance
2. Bf = *Busseola fusca*
3. cm = centimeters
4. Cp = *Chilo partellus*
5. Es = *Eldana saccharina*
6. GLM = General linear model
7. L: D = Light and dark
8. RH = relative humidity
9. SAS = Statistical Analysis Systems
10. Sc = *Sesamia calamistis*
11. WAU = Wageningen Agricultural University
12. df = degrees of freedom
13. SE = Standard error
14. N = sample size
15. ln = Natural logarithm
16. R_o = Net reproductive rate
17. r_m = Intrinsic rate of natural increase
18. λ = finite rate of increase
19. T = mean generation time
20. ICIPE = International Center of Insect Physiology and Ecology
21. ARQU = Animal Rearing and Quarantine Unit