COMPARATIVE EVALUATION OF CERTAIN ASPECTS OF THE BIOLOGY OF *COTESIA FLAVIPES* AND *COTESIA SESAMIAE* (HYMENOPTERA: BRACONIDAE) FOR THE MANAGEMENT OF *CHILO PARTELLUS* (LEPIDOPTERA: PYRALIDAE) IN KENYA.

By

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Kenyatta University.

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DECLARATION

I, Mohamed Nader Said Sallam, declare that this thesis is my original work and has not been presented for a degree in any other university,

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and Dr. William A. Overholt of the International Centre of Insect Physiology and Ecology,

Signature W. A. Overholt Date 26/6/1998

declare that this thesis has been submitted for examination with our approval as supervisors.

DEDICATION

TO MY PARENTS

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ABSTRACT

A comparative study was conducted on two gregarious larval endoparasitoids of lepidopteran stemborers, *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptea: Braconidae). The first parasitoid was imported from Pakistan and released at the Kenya coast during the long rains of 1993 in an attempt to increase the natural control of the spotted stemborer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). The second is indigenous to Africa, and it is the most common parasitoid of gramineous stemborers in Kenya and other parts of East and southern Africa.

The dispersal capacity of the exotic parasitoid was tested in a 100×100 meter maize field at Kilifi, Kenya coast. Results showed that female parasitoids were able to fly at least 64.03 meters downwind before parasitising a host. The highest rate of parasitism was found inside the maize plant where the majority of suitable hosts were located.

A study of the functional response of the two parasitoids on *C. partellus* and an indigenous stemborer, *Sesamia calamistis* (Lepidoptera: Noctuidae), was conducted in large field cages at the Kenya coast and in the laboratory. Results showed that *C. flavipes* had a stronger functional response than *C. sesamiae* when *C. partellus* was the host, while differences were not significant when *S. calamistis* was the host.

Studies on host discrimination indicated that naive as well as experienced females of the two parasitoids oviposited in hosts previously parasitised by the other species. However, experienced

females of the two species were significantly more attracted to non-parasitised host larvae than larvae parasitised by conspecific females.

Fitness consequences of superparasitism were examined for the two parasitoids on both hosts. Progeny production of *C. flavipes* increased gradually with increasing numbers of stings, but leveled off at three stings per *C. partellus* larva, then decreased. Cocoon weight, emergence and sex ratio of progeny were not affected by superparasitism. However, larval development was lengthened, and the longevity and fertility of adult progeny decreased. When *S. calamistis* was the host, no difference was found in the duration of immature stages, emergence rate, progeny production or sex ratio of the progeny, but cocoon weight, adult longevity and fertility were negatively affected.

When *C. partellus* was the host for *C. sesamiae*, low progeny production and poor survival of host larvae were recorded over the different number of stings. Moreover, larvae that were stung more than three times died. Multiple stinging led to a male biased sex ratio and low fertility of female progeny. When *S. calamistis* was the host, progeny production of *C. sesamiae* increased with the number of stings, but leveled off at three stings per *S. calamistis* larva, then decreased. No significant effect of superparasitism was found on the rate of emergence, longevity or sex ratio of adult progeny. However, longer duration of immature stages, lower cocoon weight and lower fertility of adult females were recorded.

Multiple parasitism studies showed that *C. flavipes* was able to eliminate *C. sesamiae* during the

egg stage when *C. partellus* was the host. However, when *S. calamistis* was the host, both *Cotesia* species emerged from host larvae, but with a higher number of *C. flavipes*.

C. flavipes appeared to be competitively superior, both intrinsically and extrinsically, to its African congener, *C. sesamiae*, when *C. partellus* was the host. Both species were equally competitive when the host was *S. calamistis*. A possibility of local displacement of *C. sesamiae* by *C. flavipes* in areas dominated by *C. partellus* is therefore expected. The implication of this displacement on the impact of biological control of stemborers in Kenya is discussed.

CHAPTER I

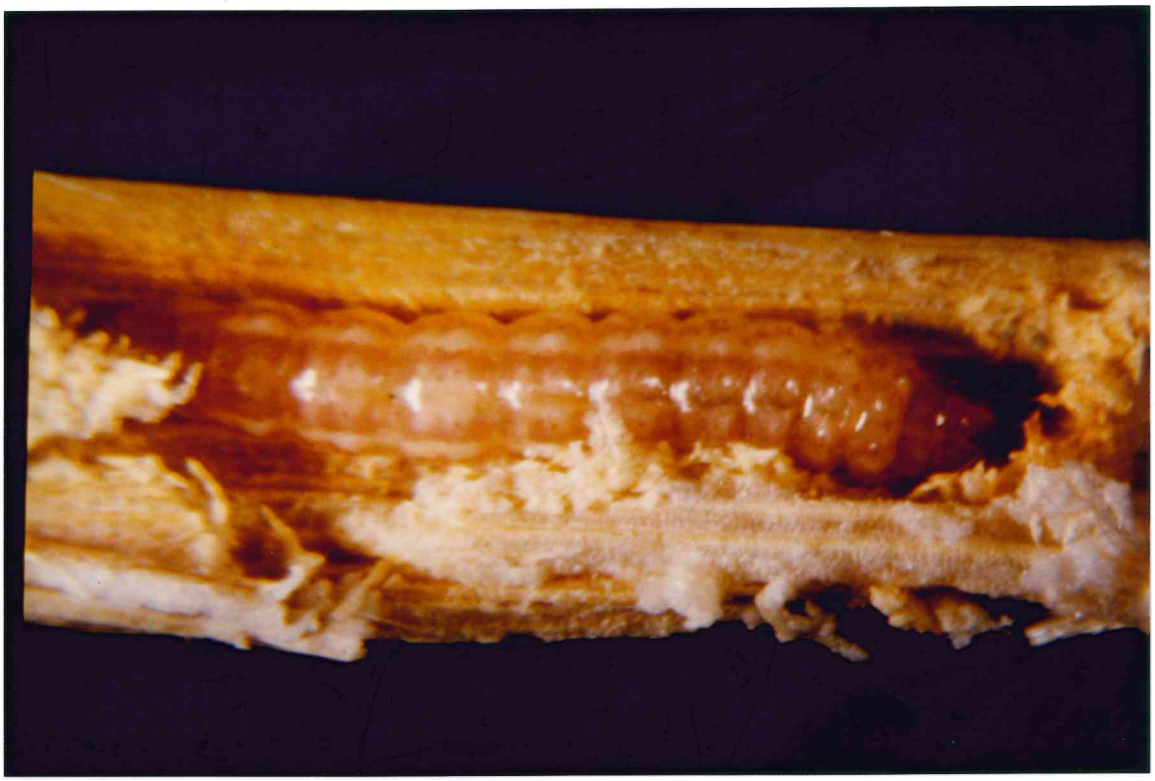
1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Stemborers in Kenya

Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.)) are major food crops in the world. In sub Saharan Africa, where maize and sorghum are widely grown by smallholders, their production is severely reduced by the feeding of lepidopteran stemborers. Estimates of yield losses due to stemborers are in the neighbourhood of 20-40% of the potential yield (Youdeowei, 1989; Seshu Reddy and Walker, 1990). These losses indicate the importance of stemborers as a limiting factor affecting crop productivity. The damage results from larval feeding on young leaves, and older larvae boring into stems. In young plants, infestation can lead to "dead hearts" due to damage to the growing point of the plant. In older plants, stemborer larvae tunnel into stems which weakens the plant growth and results in wind susceptibility and poor yield (Scheltes, 1978; Ajala *et al.*, 1994).

In Kenya, all stemborers, with the exception of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) (Plate 1.1), are thought to be indigenous (Nye, 1960). In the southern coastal area of Kenya, two native stemborers, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) (Plate 1.2) and *Chilo orichalcociliellus* Strand (Lepidoptera: Pyralidae), attack maize and sorghum coincidentally in space and time with *Chilo partellus*. Among the three species, *C. partellus* is the most abundant and serious pest, and there is evidence that it may be displacing the indigenous stemborers (Overholt *et al.*, 1994). Recent surveys indicated that *C. partellus*

A



B

Plate 1.1. *Chilo partellus* 4th instar larva (A) and pupa (B).

A

B

Plate 1.2. *Sesamia calamistis* 4th instar larva (A) and pupa (B).

was by far the most abundant species in maize, sorghum, *Sorghum arundinaceum* and *Panicum maximum* in the coastal area of Kenya and typically accounted for more than 80% of the stemborer population (Overholt *et al.*, 1994).

C. partellus is one of the 41 species in the genus *Chilo* Zincken. Larvae of all *Chilo* species are stemborers that attack gramineous plants (Bleszynski, 1970). The first record of *C. partellus* in Africa was from Malawi in 1932 (Tams, 1932). Since then, *C. partellus* has been recorded in several other countries in Africa, and is now found in most of the lowland areas of the eastern and southern parts of the African continent (Commonwealth Agricultural Bureau, 1989). In West Africa, *C. partellus* has been reported from Togo and Cameroon (Inter-African Phytosanitary Council, 1985).

1.2 Control methods

When selecting a control method to regulate densities of a pest such as *C. partellus*, consideration should be given to its economic importance, and the cost of the management strategy to growers. In addition, consideration must be given to the negative impacts of the selected strategy. Pesticides may be an effective means of controlling stemborer populations. Warui and Kuria (1983) found that properly timed chemical treatments reduced yield losses in maize by about 20%. However, the negative impact of pesticides to the environment and human health is well known. For example, the World Health Organization (1986) estimated that the majority of unintentional human poisonings (56%) and death cases (72%) due to pesticides are taking place in developing countries. Other studies indicated that pesticides do

not always lead to increased grain yields in maize (Cocker, 1956).

Non-chemical pest management methods, such as intercropping or host plant resistance, could reduce production losses. One of the recommended cultural practices is the destruction of crop residues and wild grasses during non-cropping seasons. Nevertheless, this strategy is rarely employed due to labour constraints and alternative uses for crop residues (Scheltes, 1978). Intercropping with non-host plants has shown some success in stemborer management (Minja, 1990), and though widely practiced in Africa, stemborers still continue to cause severe losses (Overholt *et al.*, 1994). Host plant resistance is a promising strategy in pest control, but insect populations are still able to develop biotypes that can attack formerly resistant varieties (Roush and Mckenzie, 1987), and there is evidence that improved varieties tend to perform poorly under low input conditions (International Maize and Wheat Improvement Centre, 1992).

1.3 Biological control

Compared to other control strategies, biological control has the advantage of being a safe approach, with little or no farmer contribution and no adverse impacts on the environment. Biological control, combined with other integrative measures, can solve many pest problems without resorting to polluting chemicals. Compared to other control methods, biological control provides a cheap and adequate alternative for application in the developing countries (Huffaker, 1969).

When starting any biological control programme, there is need to first examine the impact of indigenous natural enemies occurring in the system. A survey of the indigenous natural enemies of stemborers has been carried out in Kenya (Bonhof *et al.*, 1998), and the number of species recovered has been reported to be more than 40. The braconid, *Cotesia sesamiae* (Cameron), was found to be the most common parasitoid of stemborer larvae at the Kenya coast and in other areas of East and southern Africa (Mohyuddin and Greathead, 1970; Mathez, 1972; Kfir, 1992). However, results of field sampling from the Kenya coast have indicated that generational mortality of *C. partellus* due to *C. sesamiae* during 1992 and 1993 was never greater than 3%, and was typically less than 0.5%. Thus, the indigenous parasitoid does not appear to be effectively regulating population densities of the exotic pest at a level acceptable to farmers (Overholt *et al.*, 1994).

In an attempt to increase natural control of *C. partellus*, a classical biological control strategy has been initiated to reestablish the pest/natural enemy relationship that occurs in Asia. Classical biological control refers to an approach whereby natural enemies of a pest in its aboriginal home are introduced to the area the pest has invaded. Classical biological control, when successful, can provide an environmentally and economically suitable means of pest control. In view of this, *Cotesia flavipes* Cameron, a gregarious larval endoparasitoid of *C. partellus* native to south east Asia (Mohyuddin, 1971), was imported from Pakistan and released at the Kenya coast in 1993 (Overholt *et al.*, 1994). Recent reports indicate that *C. flavipes* is now well established at the Kenya coast and in south western Kenya (Omwega *et al.*, 1995; Overholt *et al.*, 1997). Establishment of *C. flavipes* in the Lake Victoria area may

be due to releases made by CIBC in Uganda and Tanzania in 1968-1972, or due to the escape of *C. flavipes* from a laboratory colony that was maintained by ICIPE at Mbita Point Field Station in southwestern Kenya in 1991 (Overholt *et al.*, 1997).

1.4 *Cotesia flavipes* (Plate 1.3)

Cotesia flavipes is a gregarious endoparasitoid which attacks medium and large larvae (third-fifth instars) of gramineous stemborers, belonging mainly to families Pyralidae and Noctuidae (Overholt and Smith, 1990; Overholt *et al.*, 1997). Originally from the Indo-Australian area, it has been introduced into many areas in the tropics for biological control of both old and new association stemborers with varying degrees of success (Polaszek and Walker, 1991). *C. flavipes* is able to successfully parasitise more than 20 host species in more than 15 plant species (Potting, 1996). Successful releases of *C. flavipes* have been documented in sugarcane fields in Barbados (Alam *et al.*, 1971), Brazil (Macedo *et al.*, 1984), and south Texas (Fuchs *et al.*, 1979), against the new world sugarcane borer *Diatraea saccharalis* (F) (Lepidoptera: Pyralidae). On Indian Ocean islands, successful establishments of *C. flavipes* have been reported from Mauritius (Williams, 1983), Reunion (Greathead, 1971) and Madagascar (Appert *et al.*, 1969), where it was imported to control the sugarcane borer *Chilo sacchariphagus* (Lepidoptera: Pyralidae) (Bojer).

Collections of *C. flavipes* from two regions in Pakistan, namely, Rawalpindi (north Pakistan) and Sindh (south Pakistan), were imported into Kenya through the International Centre of

Plate 1.3. *Cotesia flavipes* adult female locating host larva inside a tunnel.

Insect Physiology and Ecology, (ICIPE), in September 1991 and June 1992, respectively. The material from Rawalpindi was used for laboratory experiments and field cage experiments at Mbita Point in south-western Kenya, but was never intentionally released. Materials from Sindh was released during the long rains of 1993 at three sites in the southern coastal area of Kenya (Mtwapa, Kaloleni and Kikoneni). An estimation of the total number of females released in each of the three areas was 20,000 adult wasps. The southern Pakistan material is the one used in this study. Basic studies on the biology of *C. flavipes* indicated that it has a short life span of about 3 days and females have an initial eggload of around 150 eggs. A female *C. flavipes* allocates around 40 eggs in one host larva. Duration of immature stages takes about 16 days and the sex ratio is always female biased (60-70%) (Gifford and Mann, 1967; Wiedenmann *et al.*, 1992; Ngi Song *et al.*, 1995).

C. flavipes is able to develop on the three stemborer species that occur on the Kenya coast; *C. partellus, C. orichalcociliellus* and *S. calamistis*. Moreover, all the three stemborer species are equally accepted for oviposition by *C. flavipes*. A greater number of *C. flavipes* progeny is obtained from *C. partellus* and *C. orichalcociliellus* than progeny of *C. sesamiae*. However, it was found that *Busseola fusca* (Fuller), an indigenous stemborer species that occurs at higher elevations in East Africa, is not a suitable host for the development of *C. flavipes* due to encapsulation of parasitoid's eggs (Ngi-Song *et al.*, 1995).

1.5 Classical Biological Control

The introduction of exotic natural enemies for the control of arthropod pests and weeds has

been practiced for over 100 years (Clausen, 1936). Classical biological control has mainly focused on the introduction of old association natural enemies for the control of exotic pests that were accidentally introduced to other geographical areas of the world. However, it was also shown that classical biological control is applicable to native pests (Carl, 1982). In the latter case, natural enemies that coevolved with hosts ecologically and taxonomically related to the target pest are used, creating "new associations". Hokkanen and Pimentel (1989) suggested that new associations provide more effective means of control. Their argument is based on the assumption that there has not been an opportunity for co-evolution between the pest and the natural enemy, which leads to a reduction of the natural enemy's impact as an evolutionary process, (i.e. hosts developing evading mechanisms). However, It is not possible to tell how realistic this assumption is before an in-depth evaluation of a natural enemy candidate on the target pest has been done. *C. flavipes*, however, is a coevolved natural enemy of *C. partellus* while the African congener, *C. sesamiae,* coevolved with *S. calamistis* and other indigenous stemborers (Fig.1.1). Reuniting the old association parasitoid with its host in a new geographic area will result in new association relationships with native stemborers. *C. flavipes* is ecologically and morphologically similar to *C. sesamiae*. Sharing almost the same ecological niche, the two parasitoids will certainly compete, both extrinsically and intrinsically, over different species of stemborers. It is therefore useful to understand the performance of the two parasitoids on their new and old association hosts to be able to predict their ability to suppress stemborer populations.

The negative impact of classical biological control on native ecosystems has been an area of

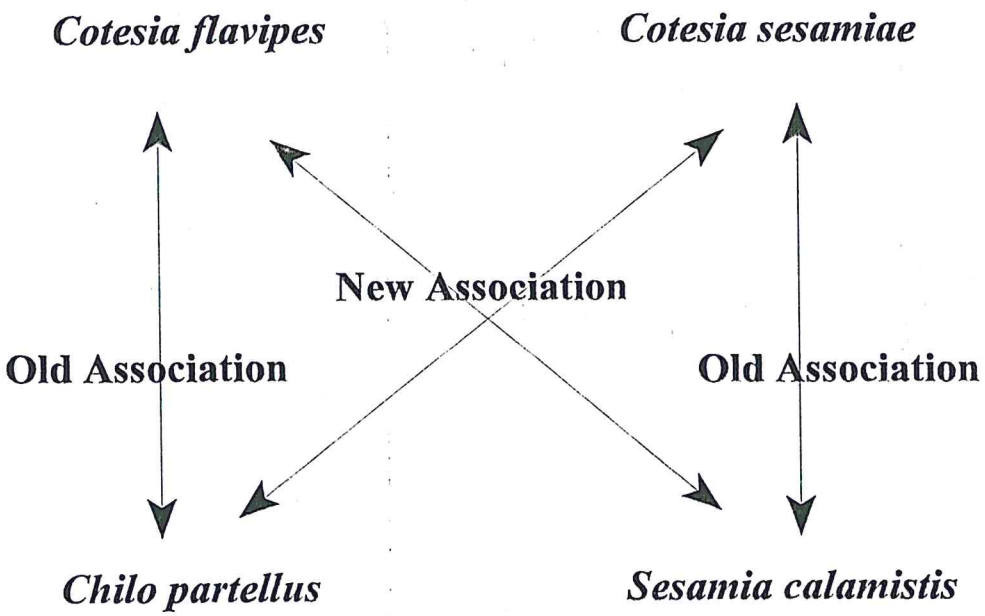


Fig. (1.1) Association patterns among both parasitoids and their hosts.

controversy (Howarth, 1991; Lockwood, 1993). The release of *C. flavipes* in Kenya has been criticised for being destructive to the African continent's ecological balance (Newham, 1993). However, extensive laboratory studies were conducted on *C. flavipes* before any releases were made, and it was found that *C. flavipes* is restricted to later larval instars of certain lepidopteran stemborers found tunneling internally in grasses (Ngi-Song *et al.*, 1995). As mentioned earlier, *B. fusca* is not a suitable host for the development of *C. flavipes*, thus, it is anticipated that *C. flavipes* will only become established in areas where the dominant stemborer species are suitable for its development. Niche differences between *C. flavipes* and *C. sesamiae* will allow the latter to remain active in areas dominated by host species unsuitable for the development of the introduced parasitoid.

1.6 Parasitoid dispersal

The ability of parasitoids to find mates, disperse and search for hosts in the area of release is a vital attribute for successful establishment. One critical aspect in parasitoid dispersal is that an "Allee effect" may drive small, introduced populations to extinction, which could explain cases of failure of establishments in biological control. Allee effect refers to the faliure of parasitoids to find their mates, resulting from dispersal into a new environment, which in turn leads to a male-biased sex ratio and eventually to population extinction (Hopper and Roush, 1993). However, an Allee effect would not be of significant importance in case of gregarious parasitoids since they easily encounter their mates once they emerge. High rate of sib mating soon after emergence of *C. flavipes* has been reported (Arakaki and Gahana 1986).

Another reason of failure of establishment could be a low number of natural enemies collected initially from the area of origin (Hopper and Roush, 1993). In this case, the insects collected may not fully represent the genetic diversity of the natural population. Roush (1990) stated that a sample of 100 individuals theoretically preserves about half of the alleles and 99.5% of the heterozygosity of the original population, and that greater sample sizes will not improve establishment success. On the other hand, Hopper and Roush (1993) showed that, for chalcidoids, ichneumonoids and tachinids, the number of establishments increased significantly with the increase of the numbers collected from the area of origin, which were in many cases more than 10000 individuals. Therefore, it was suggested that if the number of insects available for release is limited, then all should be released at the same time and place. In our case, *C. flavipes* colony reared at the (ICIPE) originated from 50-80 cocoon masses collected from each collection site in Pakistan. This collection size should have been large enough to include more than 99% of the heterozygosity of the source of population (Omwega and Overholt 1996).

Dispersal of the introduced parasitoid, *C. flavipes*, is addressed in chapter 2 of this work, with emphasis on the effect of wind direction and host distribution, in the field and within the same plant, in a 100 × 100 meters maize field in Kilifi, Kenya coast.

1.7 Parasitoid functional response

An important aspect in evaluating the efficiency of a natural enemy is to study its performance as an individual by examining the attack rate on the host or prey. A knowledge of the

functional response is essential for a clear understanding of host-natural enemy interactions. The utilization of hosts for parasitoid population increases, and host finding efficiency have been the foci of host-parasitoid population models over the past half century. The models attempt to express the action and interaction of a number of processes that affect pests and natural enemies populations. One of the first models was the Nicholson-Bailey (1935) host - parasitoid model in which the number of hosts attacked takes the form: -

$$N_a = N_t\,[1-e^{(-aPt)}]$$

where N_a is the number of hosts attacked, N_t is the number of hosts present, Pt is the number of the parasitoids present, a is the proportion of hosts attacked per parasite and e is the base of natural logarithm (=2.718282).

Holling (1959) modified the Nicholson-Bailey model by including the time spent by the parasitoid in "searching" for and "handling" the host: -

$$N_a = T\,a'\,N/1 + a'\,T_h\,N$$

where T is the total time available for search, N is the initial number of prey available, T_h the handling time and a' the coefficient of attack.

Several additional modifications have been incorporated into functional response models. Rogers (1972) model assumes an exponential decay in the number of hosts available for parasitisation: -

$$N_{par} = N_t[1-e^{-(Tt*a'*Pt)/(1+a'*Th*Nt)}]$$

with Tt, a' and Th equaling the total time available, instantaneous search rate and handling time, respectively.

The understanding of functional responses of individual natural enemies is essential for modelling host-parasite or predator-prey interactions (Huffaker *et al.*, 1969). Functional response studies may not explain the role of natural enemies in controlling pest populations (Varley and Gradwell, 1969), however, such studies maybe useful for comparing the efficiency of different biological control candidates (Overholt and Smith, 1990). In view of this, the efficiency of the indigenous and the introduced parasitoids on both the exotic pest *C. partellus* and *S. calamistis* was compared through the understanding of their functional responses on the two hosts (chapter 3).

1.8 Host discrimination

The ability of a female wasp to recognise and avoid oviposition in a previously parasitised host is considered desirable for biological control (van Lenteren, 1981). In earlier studies, host location used to be thought of as a result of random searching (Cushman, 1926; Clausen, 1940). However, in the last 20 years, studies showed that host habitat location, host location and host discrimination is not at all random, and that chemical volatiles, usually referred to as info-chemicals, play an important role in one or more phases in the host selection process (van Lenteren, 1981; Vet Louise and Dicke, 1992; Potting *et al.*, 1997).

The ability of intraspecific host discrimination has been documented in about 200 species of hymenopteran parasitoids (van Lenteren, 1981). Some female parasitoids use an external oviposition marker to identify a successfully parasitised host. The marker is thought to be species-specific and to become less effective with time. Moreover, changes in host quality associated with the developing parasitoid larvae can serve as an internal marker and lead to host

rejection (Chow and Mackauer, 1986).

It was suggested that the ability to discriminate by wasps against hosts already parasitised by another species is related to the probability of offspring survival. When hosts are scarce, it would be evolutionary advantageous for a female to oviposit in a previously parasitised host if its offspring are likely to win the competition and to survive to the adult stage. However, a number of studies have failed to show interspecific recognition of marked hosts (Mcleod, 1972; Vinson, 1972), though several exceptions have been reported (Wylie, 1970, 1971 a, b; Chow and Mackauer, 1984; Mackauer, 1990). Thus, there is need to investigate if conspecific and heterospecific host discrimination exist in the two *Cotesia* species. A knowledge of these abilities will contribute into our understanding of both intraspecific and interspecific competition possibilities in the two species.

1.9 Parasitoid competition

One important aspect in understanding how an introduced natural enemy fits into a new environment is the knowledge of its interspecific communication and competition with indigenous species naturally occurring in the system. Competition is divided into intraspecific competition, which is the competition between individuals of the same species, and interspecific competition, which is the competition between different species. Competition between introduced natural enemies, or between introduced and native natural enemies, could explain why some species failed either to become established or to successfully control the pest (Mackauer, 1990). Competitive displacement might also occur due to the ability of one species to eliminate another through

competition at any level.

Factors important to successful parasitism were divided by Smith (1929) into extrinsic and intrinsic factors. Extrinsic factors refer to those involved in the host selection process, such as host finding efficiency, competitive displacement and environmental tolerance. Intrinsic factors are the ones affecting the parasitoid ability to possess the host such as host suitability, progeny competition and parasitoids ability to evade host defence mechanisms.

Various mechanisms have been identified which enable "intrinsically superior" species to eliminate the immature stages of potential competitors. Suppression can result from some action or processes between the immature stages or from some action of the adult parasitoid that affects larval survival (Mackauer, 1990). The most important suppression mechanisms may be the physical attack between immature parasitoids of the same age, which is common among solitary parasitoids (Fisher, 1959; Eijsackers and Bakker, 1971; Podler and Mendel, 1979), physiological suppression through lack of nutrients (Klomp and Teerink, 1978), anoxia (Fisher, 1963), or the introduction of a toxic substance or virus particles by one of the parasitoids (Vinson and Ables, 1980). In gregarious parasitoids, physical attack is not known to take place since they frequently contact one another under normal development (Salt, 1961), but in general, parasitoid mortality increases with the number of eggs laid per host (clutch size), which is a common fitness penalty due to superparasitism (Strand and Vinson, 1985; Waage, 1986; Potting *et al.*, 1997).

1.9.1 Superparasitism

The term "superparasitism" was coined by Fiske (1910), who used it to define both super and multiple parasitism (Godfray, 1994). Later in 1987, van Dijken and Waage defined superparasitism as the deposition of a clutch of eggs on a host that has already been parasitised by a member of the same species. The clutch of eggs is the number of eggs laid during a single oviposition bout, which may of course consisit of only one egg (Godfray, 1994). In self-superparasitism, the clutch is laid by the same female rather than a conspecific. Superparasitism may reduce the overall impact of parasitism on the host population (DeBach and Sundby 1963), and it is predicted to be more frequent when rates of host encounter are low (van Lenteren, 1976; Charnov and Skinner, 1985). Studies have shown that, in addition to increasing larval mortality, superparasitism delays the progeny's development (Wylie, 1983; Harvey *et al.*, 1993) and results in a smaller progeny (Vet *et al.*, 1994; Potting *et al.*, 1997). It is generally agreed that the parasitoid should avoid superparasitism if her offspring are unlikely to survive (Mackauer, 1990). On the other hand, Vinson (1972) suggested that a degree of superparasitism may be advantageous specially against active or aggressive hosts. In addition, a solitary parasitoid may benefit from placing two eggs in a host that may saturate the host's defence system (Godfray, 1994). For gregarious parasitoids, however, the probabilities for surviving superparasitism are greater since the number of parasitoids a host can support is somewhat variable (Waage and Godfray, 1985).

1.9.2 Multiple parasitism

Multiple parasitism occures when a parasitoid attacks a host that has previously been parasitised by a diffeerent species (Smith, 1916). Multiple parasitism can lead to competitive displacement

of one species by another, which may happen when an exotic parasitoid species is introduced into a new geographical area (DeBach and Sisojevic, 1960; DeBach and Sunday, 1963). Unlike selective starvation or anoxia, which is equally effective against both conspecific and heterospecific competitors, it is assumed that conspecifics would be more tolerant to venoms or toxins introduced by females of their own species than heterospecific competitors. Little work has been done on interspecific competition between gregarious parasitoid species. However, studies showed that some solitary parasitoids are, interestingly, capable of eliminating gregarious competitors (Laing and Corrigan, 1987; Steven *et al.*,1990). This because first instar larvae in most solitary parasitoids possess sickle-shaped mandibles, while those of gregarious parasitoids are greatly reduced (Crossman, 1922).

1.9.3. Implications on the introduction of natural enemies

Considering the implications of interspecific competition on introduced natural enemies, Ehler and Hall (1982) questioned the empirical approach of releasing all available species of natural enemies, with the hope that the best species or combination of species will establish in the field (Huffaker *et al.*, 1969; Hassell, 1978). The argument is based on the assumption that competitive exclusion of natural enemies might occur whereby the most useful candidates are excluded. Thus, the number of species of natural enemies, released at a given time and place, or the number of exotic incumbent species of natural enemies present in the area of introduction, is considered to negatively affect species establishment. In our case, only *C. flavipes* was released at the Kenya coast, which might be advantageous for its establishment. However, with *C. sesamiae* already in the system, direct competition between the two species is expected. Therefore, it is impo rtant

to study the consequences of super as well as multiple parasitism on the two parasitoid species, and if local displacement may happen due to the superiority of one species over the other. Aspects of competition between the two parasitoids are addressed in chapter 5.

1.10 JUSTIFICATION OF THE WORK

There is a growing concern regarding the impact of exotic natural enemies on native ecosystems. Most classical biological control programmes have mainly focused on the applied aspect of target pest population regulation. Competition between exotic and native natural enemies has been the subject of theoretical discussions, but there is little experimental evidence of the extent nor the result of this competition. In order for classical biological control to become more predictive in nature, and to address the concerns regarding possible negative impacts to non-target species, it is essential to increase our understanding of the interaction between exotic natural enemies and the adopted ecosystem. One important aspect of this interaction is the degree of competition between exotic and indigenous natural enemies. A comparative evaluation of both the indigenous *C. sesamiae* and the exotic parasitoid *C. flavipes* will therefore be useful towards understanding the impact of the establishment of *C. flavipes* on both the target pest and the indigenous parasitoid.

1.11 OBJECTIVES

The overall aim of this study is to increase our understanding of the intra and interspecific relationships between *C. flavipes* and *C. sesamiae* in Kenya. As such, this study will focus on

the following specific objectives: -

1. Measuring the dispersal capacity of *C. flavipes* in the maize agroecosystem at the Kenya coast.

2. Determining the relative host finding abilities and attack rates of *C. flavipes* and *C. sesamiae* through investigation of their functional responses on *C. partellus* and the indigenous stemborer, *S. calamistis*, at the Kenya coast.

3. Examining the role of infochemicals in host recognition in the two parasitoid species.

4. Investigating intra- and interspecific competition in the two species, with particular emphasis on super and multiple parasitism.

CHAPTER 2

2.0 DISPERSAL OF *COTESIA FLAVIPES* (HYMENOPTERA: BRACONIDAE) IN A NEW ECOSYSTEM

2.1 Introduction

The release of *Cotesia flavipes* Cameron in maize fields at the Kenya coast has resulted in establishment, and it is now recovered beyond the areas where it was originally released (Overholt *et al.*, 1997). Releases took place during the long rains of 1993 at three sites in the Coast Province (Mtwapa, Kaloleni and Kikoneni). An estimate of the total number of females released in each of the three areas was 20,000 adult wasps. Monitoring the movements of the parasitoid in Kenya is crrently underway (Overholt *et al.*, 1994).

Unlike augmentation biological control programmes, no further releases are required once the exotic natural enemy is established in the area of introduction. It is assumed that, through uniting the enemy with its coevolved host, the pest /natural enemy relationship is reestablished, and that, over time, a state of equilibrium will be reached. However, for this to be successfully achieved, the natural enemy has to spread first in the new geographical area and reach an equilibrium density for its impact to be realised. Since *C. flavipes* is newly introduced into Kenya, it is useful to investigate its dispersal ability in the new habitat. This study was aimed at determining the dispersal pattern of female parasitoids in one generation, regardless of flight direction, and to examine whether this is influenced by wind direction and host distributuin. This would contribute towards a better understanding of the factors that

influnce the dispersal of the parasitoid in the new ecosystem.

2.2 MATERIALS AND METHODS

2.2.1 Parasitoid release

A 100 ×100 meter maize field was planted in northern Kilifi District on the 16th of April 1995. *C. flavipes* had never been released or recovered in this area. The maize variety used was Coast Composite, planted at a spacing of 100×30 cm between and within rows. Planting was done early in the season to avoid natural parasitisation by indigenous parasitoids, which tend to be rare early in the season (Mathez, 1972; Kfir, 1992).

Insect materials were obtained from a colony maintained at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, where a colony of *C. flavipes* initiated with founders collected from southern Pakistan, was reared on *Chilo partellus* (Swinhoe) larvae.

On the 27th of May 1995, 2500 *C. flavipes* black cocoons (five days old) were placed in a release station in the middle of the field. The release station (Plate 2.1) was a 20-cm^3 perspex chamber with a white roof. Holes (2-mm) were drilled in a 1-cm×1-cm grid pattern on three sides of the chamber to allow parasitoids to exit the station. The outside of the chamber was painted green to darken the interior and stimulate egress of parasitoids which are known to exhibit positive phototropism (Gifford and Mann, 1967). The station was hung to a wooden stand 1.5 meters above the ground. The stand was coated with automotive grease to prevent

Plate 2.1. *Cotesia flavipes* release station in the middle of a maize field.

the entry of ants and other predators (Overholt *et al.*, 1994). The station was left undisturbed for five days to allow the emergence and dispersal of parasitoids. The number of empty cocoons found in the release station after the five days was counted and used to determine the number of adult parasitoids that had emerged.

2.2.2 Sampling method

Four consecutive samples were taken at four days intervals (31st May, 4th, 8th and 12th of June 1995). The field was divided in a grid pattern every 10 meters in two perpendicular directions. From each intersection, the nearest plant exhibiting signs of infestation was uprooted, so that 121 plants were collected on each sampling date. Each plant was given a number corresponding to its location in the field (Fig 2.1). Plants were tagged and later dissected in the laboratory. Stemborer larvae found in each plant were removed and placed in glass vials containing small pieces of maize stems, to serve as food, until the emergence of the moth or adult parasitoids. Plant stage, borer stage and borer location in the plant were recorded. Emerged moths were counted and identified. Emerged parasitoids were counted, sexed and identified. The locations of larvae parasitised by *C. flavipes* were used to estimate the dispersal pattern and capacity of female parasitoids.

An estimation of the number of infested plants in the field was made by counting the number of plants that exhibited symptoms of infestation in every fifth row. This was done on the first sampling date (31st May 1995) prior to sampling. The number of infested plants that had host larvae suitable for parasitisation (medium and large-sized larvae) was estimated by relating the

destructive sampling data of putatively infested plants on the first sampling date to the proportion of plants infested in the field

Wind velocity was measured during the day once in the morning and once in the afternoon using an anemometer throughout the five-day period after the release of parasitoids (since adult parasitoids live for only 2-3 days). Wind direction was determined using a simple hand made wooden vane and a compass.

2.2.3 Data analysis

2.2.3.1 Data modelling

Distances flown by female parasitoids, regardless of the direction, were calculated from the point of release to the locations of parasitised larvae (Fig 2.1). Dispersal data from the four sampling dates were pooled into one data set. This was justified because the life cycle of *C. flavipes* requires 18 days at 28^{0}C (Ngi-Song *et al.*, 1995). The mean temperature during July in Kilifi is 24.2^{0}C (Ministry of Agriculture in Kenya, 1988). Thus, all parasitised stemborers recovered during the four samples (within 16 days of release) were the progeny of the released females. The data were fitted to Taylor's (1978) model using (PROC NLIN METHOD = DUD, SAS Institute, 1988). The model takes the form: $N = \exp(n+bX^c)$

where N = number of individuals dispersing to distance X, whilst n and c are constants. The model assumes randomness if c~2. If $c < 2$, then there is a tendency to aggregation around the point of release, while $c > 2$ indicates repulsion leading to a more uniform distribution.

The coefficient of determination (R^2), goodness of fit (F) and probability levels for the F value (P > F) for the non-linear regression were calculated by regressing the observed numbers of parasitised larvae on the model's predicted values. Linear regression was also performed on the data using the equation ($N=a+bX$), where N = number dispersing to distance X, whilst a and b are constants (PROC REG, SAS Institute, 1988).

2.2.3.2 Effect of wind direction

To examine the effect of wind direction on the spatial frequency of the parasitoids, the study area was divided in two ways:

1. Four equal parts (1, 2, 3 and 4) (Fig 2.2a).
2. Diagonally into two equal parts designated A and B perpendicular to the wind direction (Fig 2.2.b).

A log linear analysis was used to relate numbers of parasitised larvae recovered to wind direction (PROC GENMOD, SAS Institute, 1988).

2.2.3.3 Frequency distributions

Frequencies of plant stage, borer species, borer stage, borer location in the plant and parasitoid species were calculated using PROC FREQ (SAS Institute, 1988).

2.2.3.4 Distribution of parasitisation

To examine if within-plant host distribution affected the distribution of parasitism, plants were

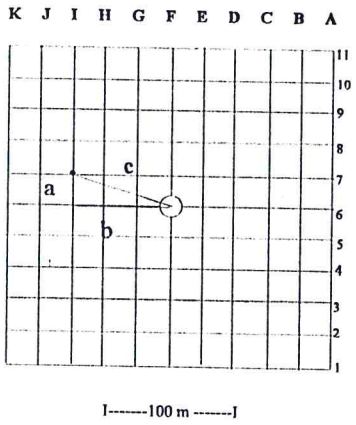


Fig.(2.1) Area of study divided in a grid pattern every 10 meters. Circle in the middle indicates release site. Distances from release point were calculated according to the following equation: $c = \sqrt{(a)^2} + \sqrt{(b)^2}$

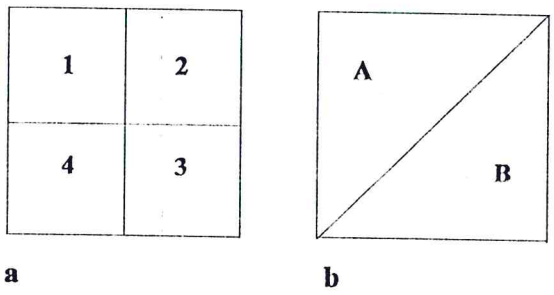


Fig.(2.2) Method of dividing the area of study into four equal parts (1,2,3 and 4) (a) and diagonally into two equal parts (b).

divided into four host density categories: plants with 1-4, 5-8, 9-12 and >12 suitable hosts (medium and large -sized larvae). Parasitisation was compared in the different categories using a χ^2 goodness-of-fit test ($P < 0.05$) (SAS Institute, 1988).

To examine whether the host distribution within the different parts of the same plant (plant stem, leaf, leaf sheath, tassel, ear, shank) influenced parasitism, a χ^2 test of independence was carried out on the proportion of larvae parasitised in the different parts of the plant (SAS Institute, 1988).

2.3 RESULTS

The total number of *C. flavipes* adult parasitoids released as calculated from the number of empty cocoons recovered from the release station was approximately 2437. The dispersal pattern of the parasitoid recoveries in the study area is shown in Fig. 2.3. Females were able to fly as far as 64.03 meters from the point of release. Figure 2.4 shows the numbers of parasitised larvae, as a function of distance from the point of release, as predicted by Taylor's (1978) non-linear model for dispersal and by a linear regression. The data was better described by Taylor's model. The number of adult parasitoids (N) dispersing to distance (X) was described by the following equation: $N=\exp[0.54+(16.819(X^{-1.045}))]$

The model was shown to be significant ($R^2=0.603$, df=3,11, F=18.21, P=0.0011), with the value of c (-1.045) indicating a tendency to aggregation around the point of release.

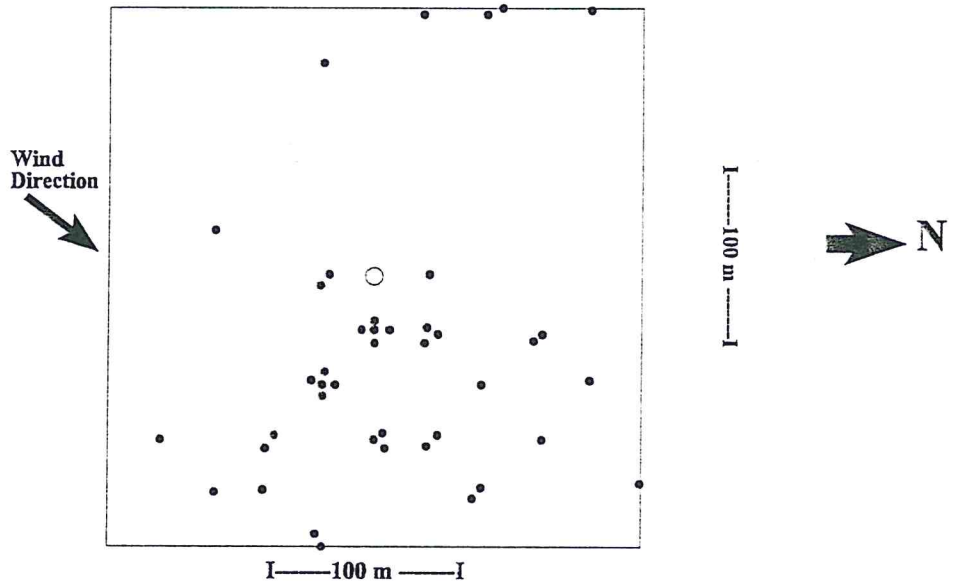


Fig. 2.3. Dispersal pattern of *C. flavipes* in a 100×100 m maize field. Black circles indicate the presence of parasitised stemborer larvae. Release point is indicated by the circle in the middle.

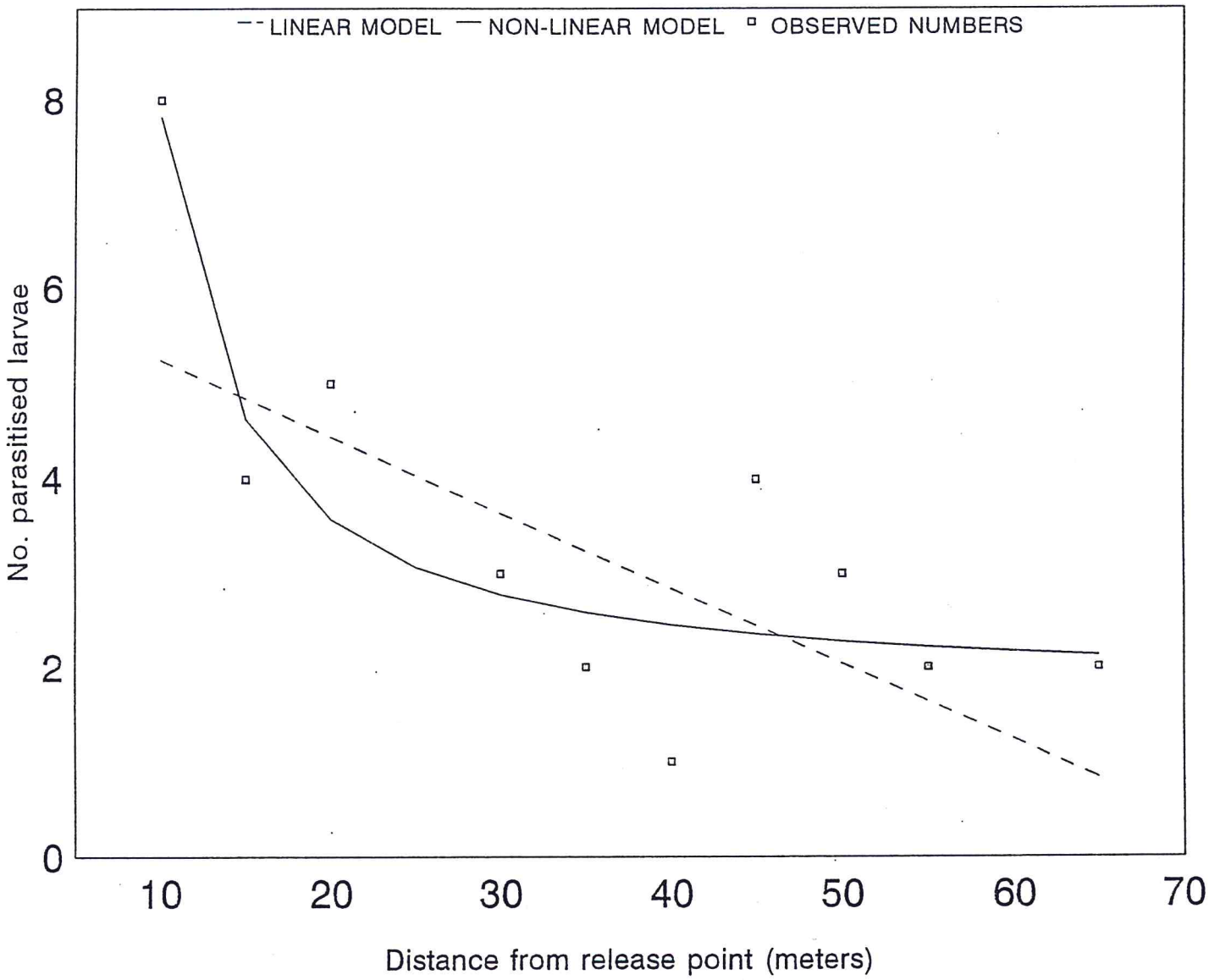


Fig.2.4. Number of parasitised host larvae by *C. flavipes* as predicted by Taylor's (1978) equation of dispersal and a linear regression.

According to the linear regression model, the number of adult parasitoids (N) dispersing to distance (X) was described by the following equation: $N=6.048-[0.0802(X)]$ and it was also significant ($R^2=0.39$, df=1,12, F=7.744, P=0.0166).

A clear trend of dispersal towards the northeastern part of the field was found (Fig 2.3). Wind velocity ranged from 0.0 to 6.0 meter/second during the day. Winds were regularly from the southwest, which is the prevailing direction during the long rains (March-June). There was a significant difference in the number of parasitoids between subdivisions 1,2,3 and 4 (χ^2 = 14.68, df=3, P=0.0021), as well as in subdivisions A and B (χ^2 =11.91, df=1, P=0.0006), which suggests a significant effect of the wind direction on the pattern of parasitoid's dispersal.

Frequencies of plant stage, borer species, borer stage, borer location in the plant and parasitoid species are shown in the frequency tables (Tables 2.1, 2.2, and 2.3). Out of 484 plants collected, a total number of 592 stemborer larvae were found, giving a mean of 1.22 larvae per plant. *C. partellus* represented 80.9% (479 larvae), *Sesamia calamistis* 17.4% (103 larvae) and *Chilo orichalcociliellus* 0.8% (five larvae) of the total stemborer population.

Out of the total number of larvae collected, 46 larvae were found to be parasitised. *C. flavipes* was identified as parasitising 41 *C. partellus* and one *S. calamistis* (Table 2.1). Ten of the parasitised larvae (23.3%) were medium-sized while 32 (74.4%) where large-sized. One *C.*

TABLE 2.1. HOST LARVAE FREQUENCY TABLES

PLST	Frequency	Percent
4	220	37.2
5	123	20.8
6	167	28.2
7	82	13.9

BRSP	Frequency	Percent
1	479	80.9
2	5	0.8
3	103	17.4
6	2	0.3
7	3	0.5

BRST	Frequency	Percent
2	135	22.8
3	145	24.5
4	231	39.0
5	2	0.3
6	60	10.1
7	19	3.2

LOC	Frequency	Percent
1	20	3.4
2	150	25.3
3	227	38.3
4	12	2.0
5	180	30.4
6	3	0.5

INNOD	Frequency	Percent
0	376	63.5
1	16	2.7
2	77	13.0
3	53	9.0
4	31	5.2
5	13	2.2
6	10	1.7
7	8	1.4
8	5	0.8
10	1	0.2
11	2	0.3

PARSP	Frequency	Percent
0	546	92.2
1	3	0.5
13	42	7.1
16	1	0.2

TABLE 2.2.
SUITABLE HOSTS FREQUENCY TABLES (MEDIUM AND LARGE-SIZED HOSTS)

BRSP	Frequency	Percent
1	313	82.8
2	2	0.5
3	59	15.6
6	2	0.5
7	2	0.5

BRST	Frequency	Percent
3	145	38.4
4	231	61.1
5	2	0.5

LOC	Frequency	Percent
1	12	3.2
2	71	18.8
3	160	42.3
4	12	3.2
5	121	32.0
6	2	0.5

INNOD	Frequency	Percent
0	224	59.3
1	10	2.6
2	51	13.5
3	34	9.0
4	22	5.8
5	12	3.2
6	10	2.6
7	7	1.9
8	5	1.3
10	1	0.3
11	2	0.5

PRSP	Frequency	Percent
0	334	88.4
1	2	0.5
13	42	11.1

TABLE 2.3. Cotesia flavipes FREQUENCY TABLES

DIST	Frequency	Percent
10	8	18.6
14.14	4	9.3
22.36	5	11.6
28.28	1	2.3
30	3	7.0
31.62	5	11.6
36.06	2	4.7
41.23	1	2.3
42.43	1	2.3
44.72	4	9.3
50	2	4.7
50.99	3	7.0
53.89	2	4.7
64.03	2	4.7

PLNO	Frequency	Percent
A2	1	2.3
B11	1	2.3
B4	1	2.3
C3	1	2.3
C5	2	4.7
D11	2	4.7
D2	2	4.7
D4	1	2.3
E11	1	2.3
E3	2	4.7
E5	3	7.0
E6	1	2.3
F3	3	7.0
F5	5	11.6
G1	2	4.7
G10	1	2.3
G4	5	11.6
G5	1	2.3
G6	2	4.7
H2	1	2.3
H3	2	4.7
I2	1	2.3
I7	1	2.3
J3	1	2.3

PLST	Frequency	Percent
2	33	76.7
3	10	23.3

BRSP	Frequency	Percent
0	1	2.3
1	41	95.3
3	1	2.3

BRST	Frequency	Percent
3	10	23.3
4	32	74.4
5	1	2.3

LOC	Frequency	Percent
2	4	9.3
3	27	62.8
4	1	2.3
5	11	25.6

INNOD	Frequency	Percent
0	16	37.2
1	2	4.7
2	7	16.3
3	10	23.3
4	6	14.0
5	1	2.3
6	1	2.3

PRSP	Frequency	Percent
13	43	100.0

EMDATE	Frequency	Percent
950613	6	14.0
950614	7	16.3
950615	10	23.3
950616	10	23.3
950617	3	7.0
950618	3	7.0
950619	3	7.0
950620	1	2.3

Key

BRSP = Stemborer species:

1= *Chilo partellus*
2= *Chilo orichalcociliellus*
3= *Sesamia calamistis*
6= Other/ Unknown
7- *Chilo* spp, not identified upto species level

BRST = Stemborer stage:

1= Egg
2= Small larva
3= Medium larva
4= Large larva
5= Parasitoid cocoon without seeing a larva
6= Pupa
7= Empty pupal case

DIST = Distance from release point in meters

EMDATE = Parasitoid's emergence date

INNOD = Internode number:

(If borer found in stem)

LOC = Stemborer location:

1= Leaf
2= Leaf sheath
3= Stem
4= Ear
5= Tassel
6= Shank

PARSP = Parasitoid species:

1- *Cotesia sesamiae*
13- *Cotesia flavipes*
16- *Pseudochalcis soudanensis*

PLNO = Plant number:

(A1..K11)

PLST = Plant stage:

According to plant age, height and maturity (1-9).

flavipes cocoon mass was found without the host larva on the last sampling date (12th of June). One *C. partellus* and two *S. calamistis* were parasitised by *C. sesamiae*.

All *C. flavipes* progeny emerged within one week (13th - 20th of June 1995). As the parasitoid's life cycle requires approximately18 days (Ngi-Song *et al.*, 1995), and *C. flavipes* did not naturally occur in the area, it is nearly certain that parasitoids recovered were the progeny of the adults released on the 27th of May.

Table 2.1 shows frequencies of the distribution of all larvae recovered from the field. The plant stem was the most common location of stemborer larvae (38.3%), followed by the tassel (30.4%) and leaf sheath (25.3%), while leaves, ears and shanks had the lowest abundance (3.4%, 2.0% and 0.5% respectively). Distribution of suitable hosts only (medium and large-sized larvae) is shown in table 2.2. Similarly, plant stem had the highest abundance of suitable hosts (42.3%), followed by the plant tassel (32.0%) and leaf sheath (18.8%), while plant leafs, ears and shanks had the lowest abundance (3.2%, 3.2 % and 0.5% respectively) (Table 2.2). The distribution of parasitism differed significantly among different parts of the plant ($\chi^2=5$, df=5, P =0.032). The highest proportion of larvae parasitised by *C. flavipes* was found in the stem (62.8%), followed by plant tassel (25.6%), leaf sheath (9.3%) and ears (2.3%) (Table 2.2). No parasitised larvae were found on leaves or in the shanks. There was a significant dependence of parasitism on host location whether inside or outside the plant ($\chi^2=5.009$, df=1, P =0.025), whereas 88.37% of the total parasitism took place inside the plant (stems

and tassels) while 74.3 % of the suitable sized larvae are found. Internodes 2, 3 and 4 had the highest larval abundance within the stem (frequency tables 2.1, 2.2 and 2.3-INNOD), and 82.1 % of the larvae parasitised in the stem were found in the same internode. However, aggregation of parasitoids in response to plants with different host densities was not detected. There was no difference in the percentage of larvae parasitised in plants with 1-4, 5-8, 9-12 and >12 suitale larvae (χ^2=4.491, df=3, P=0.213) (Fig 2.5).

Mean total progeny and female progeny of *C. flavipes* were 43.83 and 28.30 per host, respectively, giving a female-male sex ratio of 1.86:1. However, out of the total number of *C. flavipes* cocoons recovered (43), three did not produce females, which suggests that about 92% of female parasitoids had mated before dispersal.

The number of female parasitoids liberated was estimated to be 1574 (considering the resulted sex ratio of 1.86:1), and the total number of parasitised larvae in the field was estimated to be 130, which constitutes about 11% of the estimated number of suitable larvae in the field (1165). This, however, could be an underestimation of the actual number of larvae in the field, since it is based on the assumption that only the plants that exhibited infestation symptoms were infested (5% of the total number of plants in the field). Assuming that 130 is a reasonable estimation, then about 8% (130/1574) of the *C. flavipes* females released successfully found and parasitised hosts in the study field.

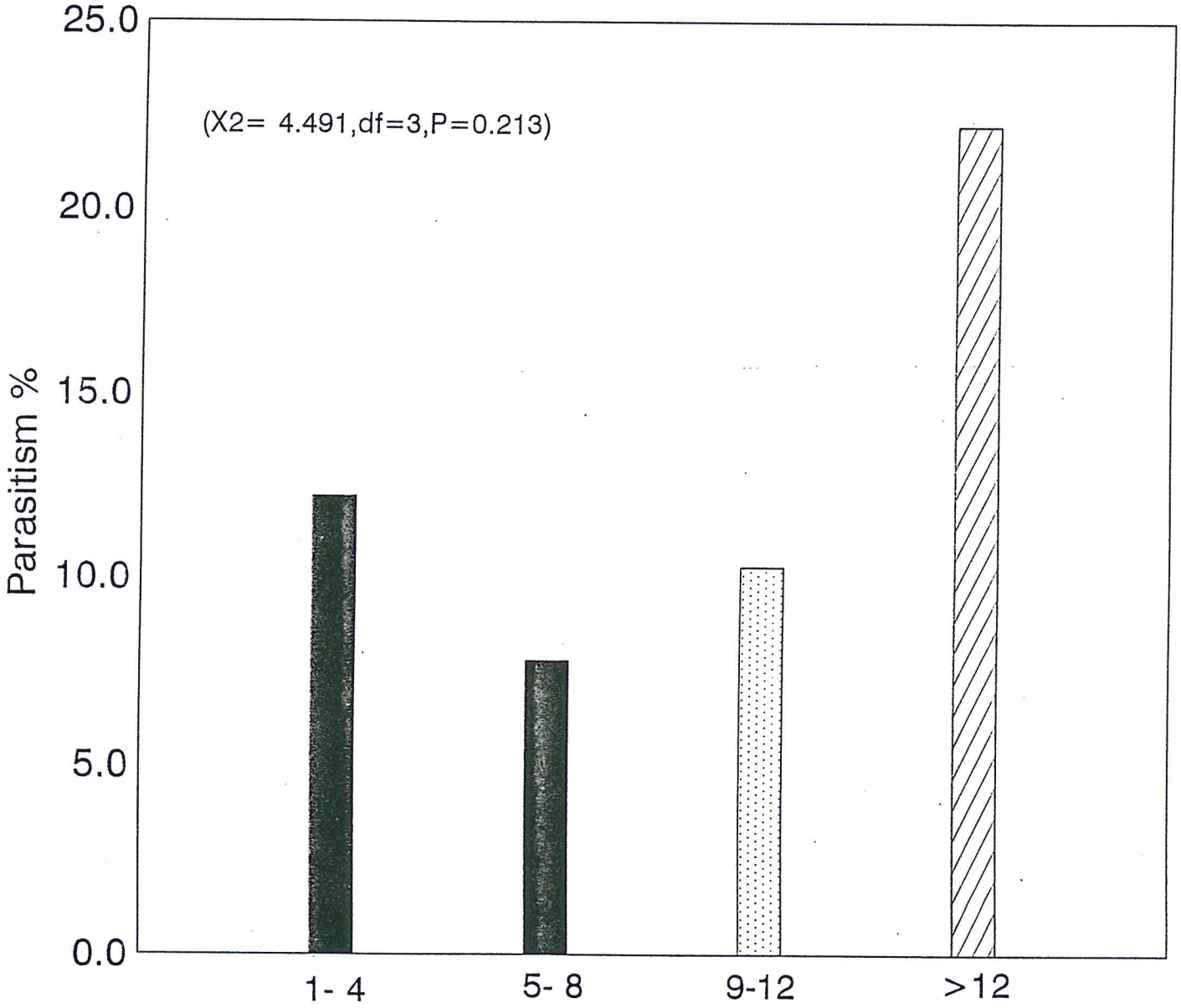


Fig.2.5. Percent parasitism in plants with varying densities of host larvae.

2.4 DISCUSSION

The ability of parasitoids to find mates, disperse and locate hosts in the area of release is an essential attribute for a successful establishment. In classical biological control programmes, where natural enemies are introduced into a new ecosystem, failures of establishment might occur due to different reasons. One reason for failure to establish maybe the numbers released in the area of introduction. In that case, Allee effect may arise if natural enemies fail to find their mates in the new area, which may lead to a male biased sex ratio and eventually to population extinction (Hopper and Roush, 1993). With *C. flavipes* being an arrenhotokus species, this problem will occur if mating does not take place. However, in gregarious parasitoids, which mate with their siblings before dispersal, this restraint may not be an important factor in their establishment. Studies by Arakaki and Gahana (1986) showed that individuals of *C. flavipes* mated with their siblings soon after emergence. This observation is further confirmed by the present study, since at least 92% of the total female population had successfully mated.

Downwind flights are the most spectacular migrations of insects, however, it is difficult to know if insect flight is solely determined by the wind (Mikkola, 1986). Corbet and Rosenheim (1996) discussed the influence of winds on dispersal and cited many cases of upwind dispersal by minute wasps. Keller *et al.*, (1985) showed that upwind movement of *Trichogramma* spp. was not prohibited by moderate wind speeds of less than 3.0 m/s. In a release-recapture study, Hendricks (1967) reported strong downwind displacement at wind

speeds between 3 and 5.5 m/s, but variable directional displacement at wind speeds of 3 m/s or less. Rosenberg (1974) found that, within a crop canopy, wind speed quickly drops and approaches zero near the ground. In the present study, it was shown that female parasitoids can fly as far as 64.03 meters downwind during their life span (2-3 days).

Parasitism was found to be dependant on host location within plants, suggesting that host searching is not random, which is not unexpected for *C. flavipes*. The fact that 88.37% of the total parasitism took place inside the plant (stems and tassels), where 74.3 % of the suitable sized larvae are found, is an indication that the host searching behaviour of *C. flavipes* has evolved to locate host larvae that are suitable for its development. Studies have shown that *C. flavipes* females use infochemicals to locate their hosts. Females are attracted to larval frass, infested maize plants and uninfested plants (Ngi-Song *et al.*, 1995; Potting *et al.*, 1997). Laboratory experiments in Y-Tube olfactometers have shown that *C. flavipes* responds in a dose dependent manner to plants infested with varying numbers of stemborers and different amounts of frass. Plants with a higher number of host larvae and a more copious amount of larval frass attracted more *C. flavipes* female parasitoids (Potting, 1996; Ngi-Song and Overholt, 1997). Thus, it is somewhat surprising that in the present study, though *C. flavipes* females showed a tendency to cause higher levels of parasitisation in plants with >12 suitable host larvae (Fig 2.5), aggregation of parasitism in response to host density was not detected. The small dataset of only 43 parasitised larvae may have been too limited to detect slight differences in response to host density. Alternatively, bioassays in olfactometers measure the response of insects moving upwind towards an odour source, whereas the present dispersal

study clearly showed that most parasitoids moved downwind. Data from olfactometer studies may simply not be a reliable indicator of insect movement in response to more complex natural air currents in the field, particularly in relation to subtle dose dependent responses.

CHAPTER 3

FUNCTIONAL AND NUMERICAL RESPONSES OF *COTESIA FLAVIPES* AND *COTESIA SESAMIAE* ON *CHILO PARTELLUS* AND *SESAMIA CALAMISTIS*

3.1 INTRODUCTION

3.1.1 Parasitoids' functional response

Assaying the performance of a natural enemy requires in depth knowledge of the way it searches for and perceives prey. The understanding of the natural enemy's functional responses is essential for modeling of host-parasite or prey-predator interactions (Huffaker *et al.*, 1969). As early as 1911, Howard and Fiske stated that, a natural balance can only be maintained through the operation of facultative agencies, which affect the destruction of a greater proportionate number of individuals as the insect in question increases in abundance. They asserted that parasitism in the majority of the cases is truly facultative. Smith (1935) changed the word "facultative" to be "density dependent factors". Later in 1964, DeBach stated that, for a natural enemy to be effective and achieve proper control of a certain pest, it should:-

1- Act in a density dependent way.

2- Be host specific.

3- Have a high searching ability for hosts.

4- Have a high reproductive capacity relative to the host pest.

5- Be tolerant to the same environmental conditions as the host.

However, Varley and Gradwell (1969) argued that natural enemies do not act in a density dependent way, since the appropriate test for a density dependent effect of natural enemies is to see if the "percentage mortality" they cause rises with increasing host or prey density, which is not the case when their functional response is considered. Nonetheless, natural enemies do not achieve their major control effect by virtue of the functional response alone. Other parameters can still explain their role as density dependent factors. Numerical response is another aspect that can demonstrate the effective role of natural enemies in pest control. Numerical response refers to parasitoids' progeny produced and the degree of host/parasitoid's synchronisation over time.

The present study was aimed at comparing the efficiency of the indigenous parasitoid (*Cotesia sesamiae*) and the introduced parasitoid (*Cotesia flavipes*) on the exotic stemborer, *Chilo partellus* and the indigenous *Sesamia calamistis* by understanding their functional and numerical responses on the two hosts.

3.2 MATERIALS AND METHODS

3.2.1 Host rearing

C. partellus and *S. calamistis* colonies were maintained at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi. Adult moths originated from immature stemborers collected at the Kenya coast. Adults were placed in oviposition cages lined with folded wax papers and maintained at 25^{0}C, 50-70% RH, and a light/dark regime of 12L:12D.

Eggs were collected daily and transferred to Petri dishes with moist cotton wool, then transferred three to four days later to one litre plastic jars with 125 ml artificial diet (Ochieng *et al.*,1985). Larvae used in this study were removed from the artificial diet as fourth instars and fed on natural diet for at least 24 hours before any field or laboratory experiments.

3.2.2. Parasitoid rearing

A colony of *C. flavipes* was initiated with founders collected from *C. partellus* larvae at Rawalpindi, Pakistan, by the International Institute of Biological Control (IIBC). A colony of *C. sesamiae* was started from material reared from *C. partellus* collected at the Kenya coast. *C. flavipes* and *C. sesamiae* colonies were maintained on *C. partellus* and *S. calamistis* fourth instar larvae, respectively, using the method described by Overholt (1993). Fourth instar larvae were offered individually to one-day-old mated female parasitoids for oviposition. Parasitised larvae were then transferred to vials containing artificial diet until cocoon formation and kept at 28^{0}C and 50-70% RH. Cocoons were collected one or two days prior to emergence and kept in vials containing a strip of cotton wool saturated with a 10% sugar/water solution to serve as a food source. Emerged parasitoids were given 24 hours to mate before they were transferred to 50 cm^{3} perspex oviposition cages.

3.2.3 Laboratory functional response study

Early fourth instar host larvae were placed individually in glass vials (7.5 cm ×2.5 cm) with small pieces of maize stems (2 - 2.5 cm long) for a period of 24 hours. Stems with larvae were then removed from vials and transferred to 15 cm ×15 cm ×20 cm perspex containers.

Stems were fixed vertically to the bottom of the containers with a small piece of masking tape. Host densities were 2, 4, 6 and 8 larvae per container. Three mated female parasitoids were introduced into each container. Ten containers (replicates) were used for each host density/ parasitoid combination with *C. partellus,* while seven replicates were used for *S. calamistis* due to a limited supply of larvae. Containers were kept at 28 ^{0}C for three days which is the average adult parasitoids' longevity at this temperature (Mbapila, 1996). Larvae were then removed and placed in glass vials with artificial diet until the emergence of parasitoids or pupation of the host. The number of progeny was recorded for each parasitized larva. Host larvae which did not pupate were dissected to determine parasitism.

3.2.4 Field functional response study

This study was conducted in the coastal area of Kenya south of Mombasa. Maize was planted inside 2.5 m×2.5 m×2.5 m field cages (Plate 3.1), with 24 plants per cage. Cages consisted of metal tubing covered with a 400-micron polyester screen mesh. Five weeks after planting, plants were artificially infested with 10, 20, 30 or 40 host larvae per cage. Larvae were inserted into holes made with a 5-mm cork borer. For *C. flavipes*, each *C. partellus* density was replicated seven times while each *S. calamistis* density was replicated six times. For *C. sesamiae*, densities of 10, 20 and 30 larvae per cage were replicated six times and the density of fourty was replicated five times due to shortage of larvae. When two larvae were placed in the same stalk (>30 per cage), they were placed 15 and 30 cm above ground level. In each cage, 20 mated female parasitoids were released 48 hours after larval infestation. Plants were excised and dissected after three days. Recovered larvae were kept in single vials with

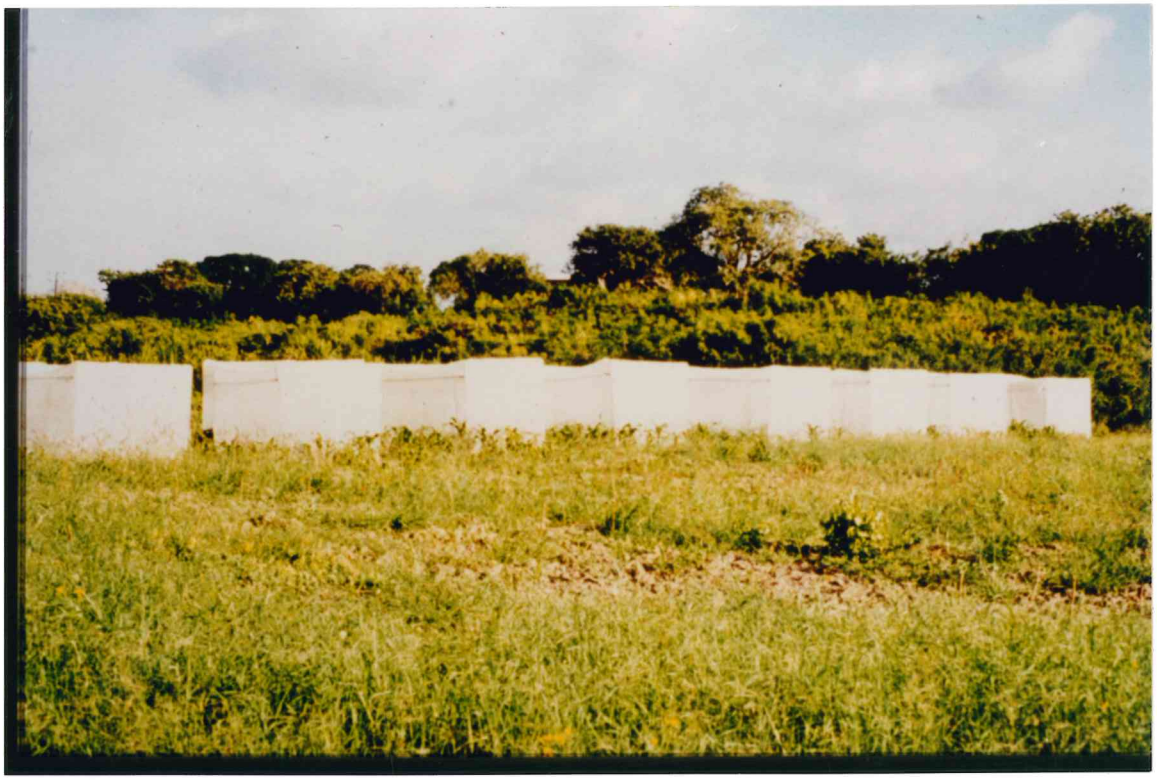


Plate 3.1. 2.5×2.5×2.5 meter field cages. Kilifi, Kenya coast.

artificial diet until emergence of parasitoids or an adult moth. Host larvae which did not pupate were dissected to determine parasitism.

3.2.5 Data analysis

Roger's (1972) random parasite equation was used to relate the total number of hosts attacked and the number of hosts successfully parasitised (those that produced progeny) to host density. The total number of hosts attacked was the number of hosts that produced progeny plus the number of larvae that were found to be parasitised after dissection. The model assumes an exponential decay in the number of hosts available for parasitisation:

$$N_{par} = N[1-e^{-(Tt*a'*p)/(1+a'*b*N)}]$$

where N_{par} is the number parasitised, N is the initial number of prey available, p is the parasitoid density, Tt is the total time available, a' is the instantaneous search rate and b is the handling time. Host density (N) was the number of hosts recovered at the end of the experiment. The total time available (Tt) was three days for field and laboratory experiments. The model was fitted to the data and parameters a' (coefficient of attack) and b (handling time) were estimated by using nonlinear regression (PROC NLIN, method=DUD, SAS Institute, 1988). Linear regression was also performed using the equation ($y=a+bx$) where y was the number attacked and x was the host density (PROC REG, SAS institute, 1988). A t test was carried out to compare the number of hosts attacked by each parasitoid at different densities. The coefficient of determination (R^2) for non-linear regression was calculated by regressing the observed numbers of parasitised hosts on predicted values. The goodness of fit (F) for the non-linear regression was calculated using this formula: F= (CTSS-ESS/RDF)/EMS), where CTSS was the corrected total of sum of squares,

ESS was the error sum of squares, RDF was the regression sum of squares and EMS was the error mean square. The probability levels for the F values were computed using the PROB function of the SAS programme (SAS Institute, 1988). The values of a' and b in all host-parasitoid combinations tested were compared using a t test for independent samples.

The numerical response for each parasitoid on each host was estimated by calculating the means of total progeny, female progeny, total progeny/parasitised host and female progeny/parasitised host. An analysis of variance was performed on each host-parasitoid treatment and means were separated by Student-Newman-Keuls means separation procedure.

3.3 RESULTS

The total number of hosts exposed, number of hosts recovered, total number of hosts attacked and the number of hosts successfully parasitised for all host-parasitoid combinations tested are shown in table 3.1. Observed and predicted values for Roger's (1972) random parasite equation for field and laboratory studies are shown in figures 3.1-3.4. In both laboratory and field studies, *C. flavipes* attacked significantly higher numbers of *C. partellus* when host densities were four ($t=2.75$, $df=20$, $P=0.0224$), six ($t=2.80$, $df=20$, $P=0.020$) and eight ($t=2.50$, $df=18$, $P=0.083$) but no significant difference was detected at the lowest host density (two) ($t=0.00$, $df=18$, $P=1.00$) (Fig 3.1, 3.3). In the field, *C. flavipes* attacked significantly higher numbers of *C. partellus* when host densities were 10 ($t=3.21$, $df=16$, $P=0.014$), 30 ($t=3.77$, $df=12$, $P=0.012$) and 40 ($t=3.43$, $df=12$, $P=0.018$). No significant difference was detected when the

Table 3.1. Total number of hosts exposed, attacked and successfully parasitised in field and laboratory studies [1].

Treatment [2]	Total exposed	Total recovered (%)	Total attacked (%)	Successfully parasitised (%)
		Field		
C. f. × C. p.	700	496 (70.8)	111 (22.3)	105 (21.1)
C. s. × C. p.	700	434 (62.0)	52 (11.9)	47 (10.8)
C. f. × S. c.	600	384 (64.0)	45 (11.7)	29 (7.55)
C. s. × S. c.	560	298 (53.2)	36 (12.1)	26 (8.72)
		Laboratory		
C. f. × C. p	200	199 (99.5)	85 (42.7)	63 (31.6)
C. s. × C. p.	200	193 (96.5)	41 (21.2)	19 (9.84)
C. f. × S. c.	140	80 (57.1)	29 (36.2)	18 (22.5)
C. s. × S. c.	140	86 (61.4)	27 (31.4)	22 (25.5)

[1]Percentage of total number attacked and successfully parasitised hosts is calculated out of the total number of hosts recovered.

[2]C. f.= *Cotesia flavipes* , C. s.= *Cotesia sesamiae,* C. p.= *Chilo partellus*, S. c.= *Sesamia calamistis*

Table 3.2. Parameters of the random parasite equation for *C. flavipes* and *C. sesamiae* attacking *C. partellus* and *S. calamistis* in laboratory cages.

Host	*C. partellus*		*S. calamistis*	
	a'	*b*	*a'*	*b*
Number attacked				
C. flavipes	0.082	0.577	0.074	3.120
C. sesamiae	0.032	1.150	0.230	4.730
Successfully parasitised				
C. flavipes	0.106	2.610	0.057	6.790
C. sesamiae	0.018	5.770	1.520	7.340

Table 3.3 Parameters of the random parasite equation for *C. flavipes* and *C. sesamiae* attacking *C. partellus* and *S. calamistis* in field cages.

Host	*C. partellus*		*S. calamistis*	
	a'	*b*	*a'*	*b*
Number attacked				
C. flavipes	0.0065	3.31	0.0024	2.89
C. sesamiae	0.0037	9.93	0.0023	2.21
Successfully parasitised				
C. flavipes	0.0060	3.330	0.0015	3.73
C. sesamiae	0.0032	10.57	0.0016	2.43

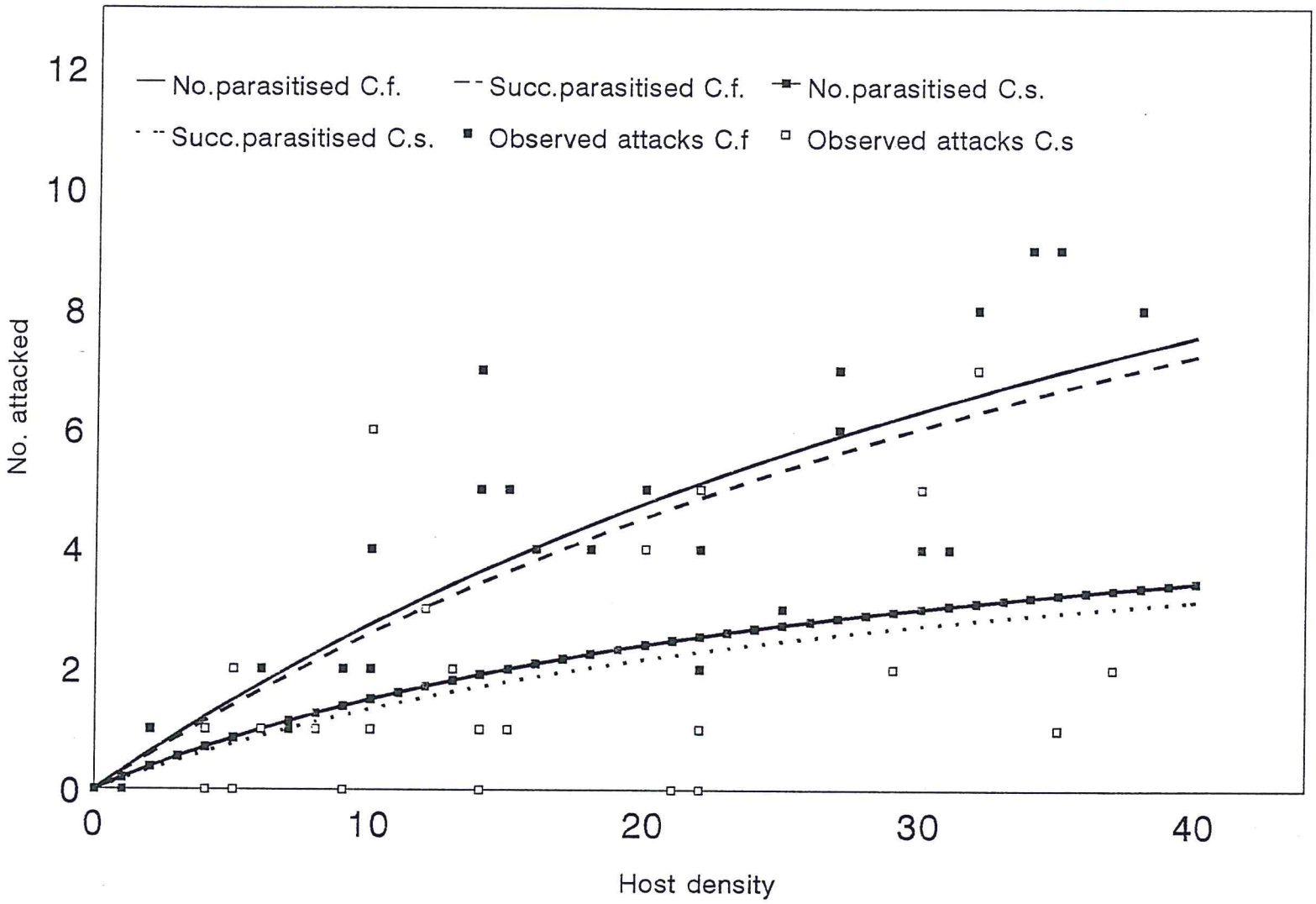


Fig. 3.1. Functional responses of *C. flavipes* (C.f) & *C.sesamiae*(C.s) on *C. partellus* in the field. Succ. parasitised= successfully parasitised.

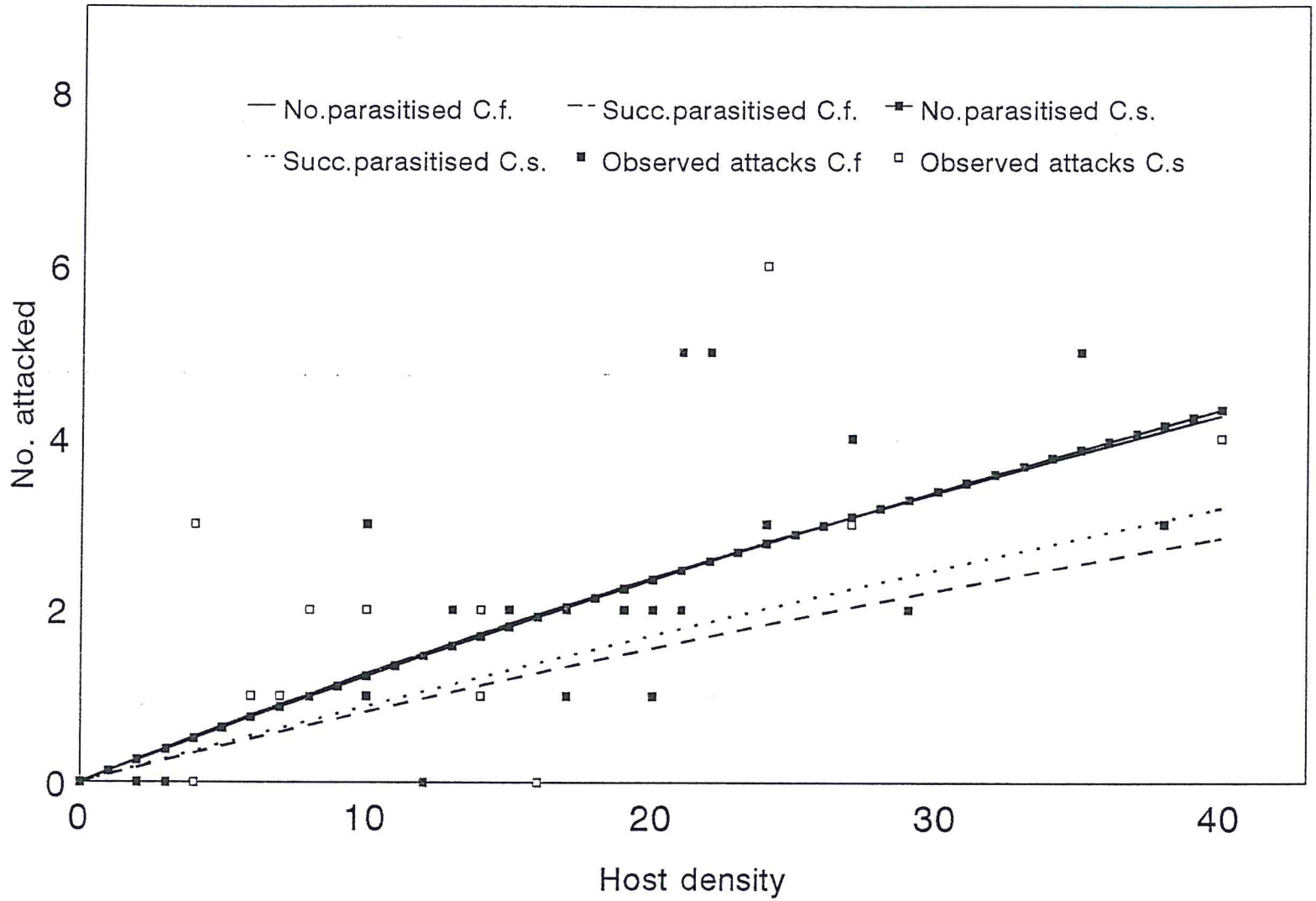


Fig. 3.2. Functional responses of *C. flavipes*(C.f) & *C. sesamiae*(C.s) on *S. calamistis* in the field. Succ. parasitised= successfully parasitised.

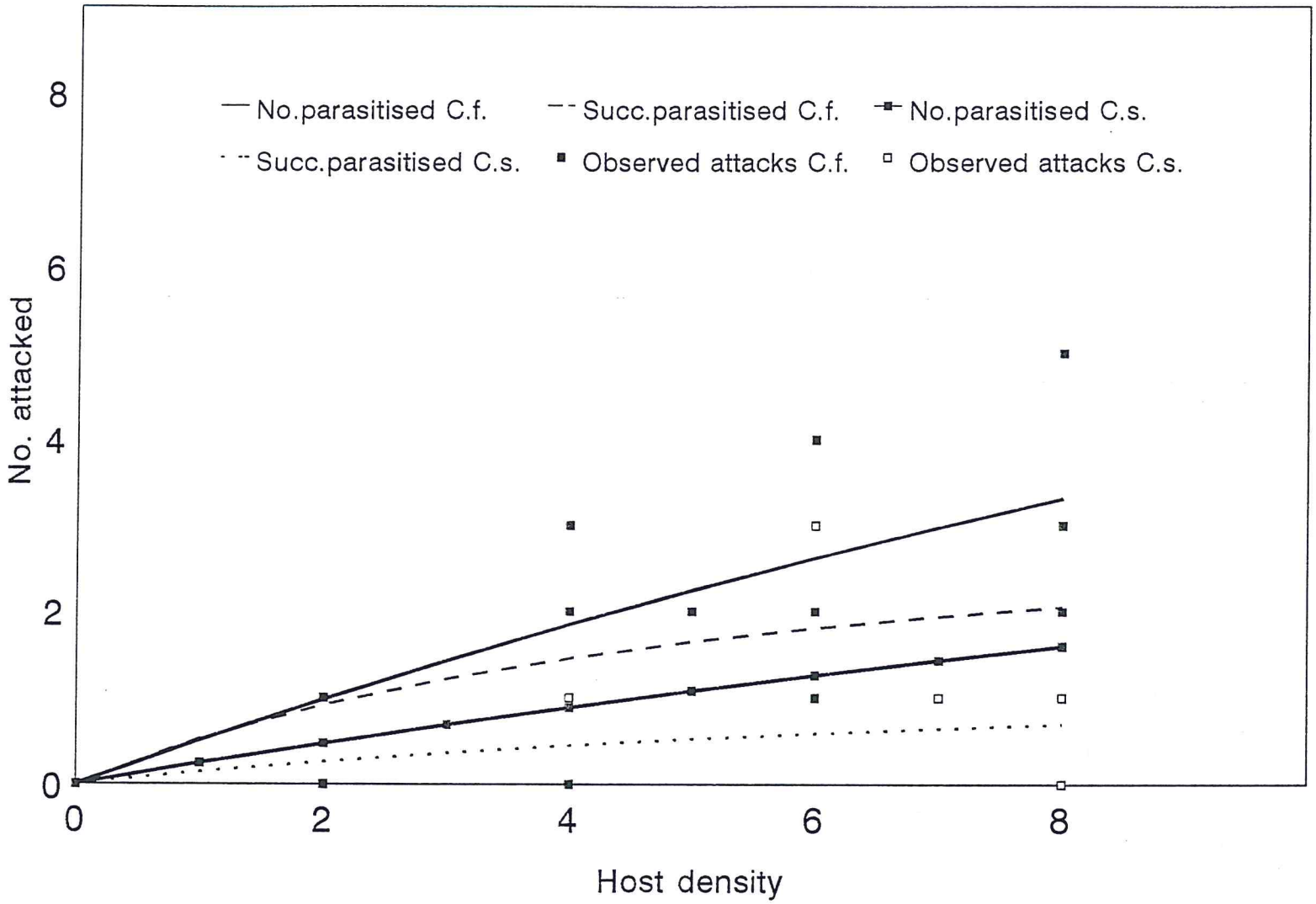


Fig. 3.3. Functional responses of *C. flavipes*(C.f) & *C.sesamiae*(C.s) on *C. partellus* in the laboratory. Succ parasitised= successfully parasitised.

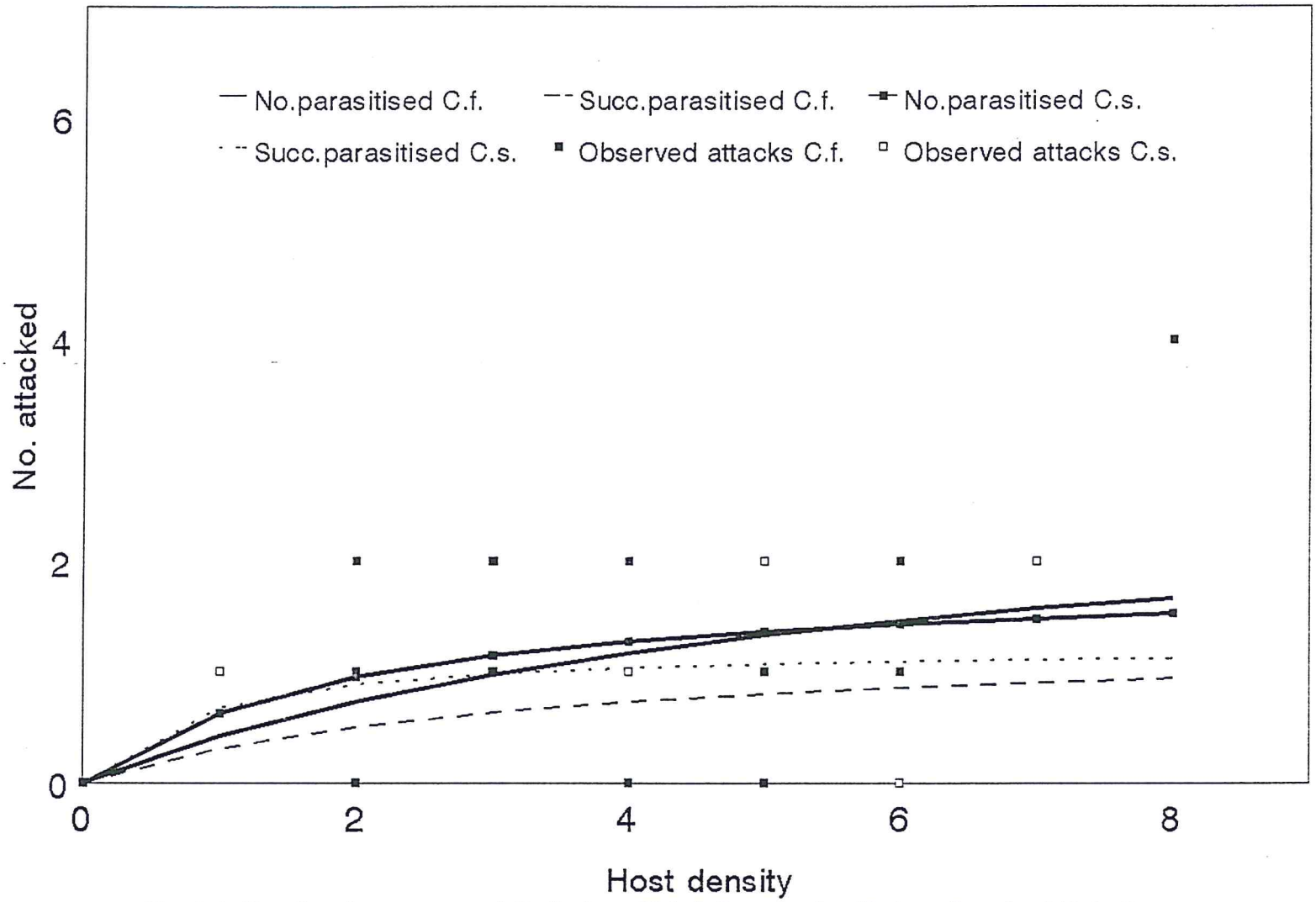


Fig. 3.4. Functional responses of *C. flavipes* (C.f) & *C. sesamiae* (C.s) on *S. calamistis* in the laboratory.
Succ. parasitised= successfully parasitised.

host density was 20 (t= 1.53, df=12, P= 0.18) (Fig 3.1).

When *S. calamistis* was the host, there was no significant difference in the number of hosts attacked by the two parasitoids at all host densities tested (t=0.34, df=11, P=0.7 for the host density of two; t= 0.49, df=10, P=0.63 for the host density of four; t= 0.48, df=10, P=0.63 for the density of six ; t= 0.27, df=11, P=0.79 for the host density of eight) in the laboratory (Fig 3.4). In the field, no significant difference was detected at any host density (t=0.21, df=10, P=0.830 for the host density of 10; t=0.22, df=9, P=0.83 for the density of 20; t=1.17, df=9, P=0.27 for the host density of 30; t=0.37, df=11, P=0.71 for the host density of 40) (Fig 3.3).

In the laboratory, the coefficient of attack (*a'*) of *C. flavipes* was significantly higher than *C. sesamiae* on *C. partellus* (t=1.47, df=78, P<0.05). However, the values of *a'* for the two parasitoids on *C. partellus* in the field were not different (t=0.9, df=54, P>0.05). When *S. calamistis* was the host, there was no significant difference between the two parasitoids in thenumber of larvae attacked in the laboratory (t=0.13, df=47, P>0.05) or in the field (t=0.18, df=42, P>0.05) (Tables 3.2 and 3.3).

The numerical response of *C. flavipes* was markedly higher than *C. sesamiae* on *C. partellus* in field and laboratory studies. In the laboratory, *C. flavipes* produced more females on the two hosts than did *C. sesamiae*. On *S. calamistis*, there were no differences in the total progeny or female progeny per introduced female by either parasitoid (Tables 3.4 and 3.6). In the laboratory, *C. flavipes* produced significantly more total progeny (F= 4.51; df=3,119; P=0.0049) and

female progeny (F= 4.73; df=3,119; P=0.0037) per host than *C. sesamiae* on the two hosts (Table 3.5). In the field, there was no significant difference in the total progeny per host between the two parasitoids on the two hosts (F=2.38; df=3,209; P=0.0703), however, *C. flavipes* produced the highest female progeny per host on *C. partellus* (F=7.12; df=3,209; P=0.0001) (Table 3.7).

Table (3.8) shows the regression formulas and goodness of fits as predicted by Roger's random parasite equation and by a linear regression. Values of R^2, Prob > F and goodness of fit (F) are also shown. Functional response data was well described by the two equations. However, linear regression seems to describe the data better than Roger's equation over the range of host densities used in both the field and laboratory.

Table 3.4. Number of total progeny and female progeny per female parasitoid (means±SD) at varying host densities in the laboratory.

		Host	*C.partellus*		*S. calamistis*	
	Initial host density	Parasitoid	Total progeny/♀	Female progeny /♀	Total progeny/♀	Female progeny /♀
	2	*C. flavipes*	1.83 ± 2.69 a	1.13 ± 1.93 a	6.43 ± 10.7 a	3.20 ± 7.02 a
		C. sesamiae	1.16 ±2.66 a	0.56 ±1.25 a	8.88 ±7.69 a	6.88 ± 6.00a
	4	*C. flavipes*	21.3 ± 13.8 a	13.9 ± 9.88 a	7.19 ± 9.14 a	3.23 ± 4.33a
		C. sesamiae	2.93 ±3.92 b	1.63 ±2.29 b	9.33 ± 7.92 a	4.23 ± 4.93 a
	6	*C. flavipes*	24.9 ± 8.18 a	15.4 ± 6.03 a	14.4 ± 12.5 a	10.4 ± 8.84 a
		C. sesamiae	3.85 ± 2.99 b	2.07 ± 2.27 b	19.6 ± 19.9 a	0.66 ± 0.88 a
	8	*C. flavipes*	11.2 ± 8.02 a	7.70 ± 6.45 a	3.77 ± 7.60 a	2.88 ± 5.73 a
		C. sesamiae	3.13 ± 3.61 b	1.10 ± 1.79 b	9.22 ± 8.91 a	2.66 ± 4.18 a

Means with the same letter for the same initial host density in the same column are not significantly different.

Table 3.5. Means of total progeny and female progeny per parasitized host in the laboratory (means ± SD)

Parasitoid × Host	Total progeny /♀ ± SD	Female progeny /♀ ± SD
C. flavipes × *C. partellus*	28.25 ±14.41 a	18.19 ± 12.56 a
C. flavipes × *S. calamistis*	33.10 ±21.13 a	19.57 ± 18.61 a
C. sesamiae × *C. partellus*	16.89 ± 7.75 b	8.15 ± 6.02 b
C. sesamiae × *S. calamistis*	31.90 ±16.82 a	10.40 ±12.03 b

Means with the same letter within the same column are not significantly different.

Table 3.6. Number of total progeny and female progeny per female parasitoid (mean ± SD) at varying host densities in the field.

Initial host density	Parasitoid	Host *C.partellus* Total progeny/♀	Host *C.partellus* Female progeny/♀	Host *S. calamistis* Total progeny/♀	Host *S. calamistis* Female progeny/♀
10	*C. flavipes*	3.19 ± 2.05 a	2.26 ± 1.45 a	1.59 ± 2.49 a	1.04 ± 1.67 a
	C. sesamiae	0.48 ± 0.70 b	0.40 ± 0.59 b	0.43 ± 0.67 a	0.18 ± 0.26 a
20	*C. flavipes*	8.55 ± 2.12 a	6.95 ± 1.54 a	1.05 ± 1.21 a	0.68 ± 0.79 a
	C. sesamiae	4.52 ± 3.73 a	3.83 ± 3.18 a	1.99 ± 2.10 a	1.11 ± 1.12 a
30	*C. flavipes*	8.26 ± 2.70 a	6.05 ± 1.85 a	1.17 ± 0.26 a	0.40 ± 0.32 a
	C. sesamiae	2.66 ± 3.81 b	1.96 ± 2.78 b	1.47 ± 1.61 a	0.45 ± 0.58 a
40	*C. flavipes*	13.5 ± 6.74 a	10.3 ± 5.35 a	4.23 ± 2.78 a	2.60 ± 2.21 a
	C. sesamiae	4.62 ± 4.01 b	3.73 ± 3.84 b	3.72 ± 1.67 a	1.49 ± 0.97 a

Means with the same letter for the same initial host density in the same column are not significantly different.

Table 3.7. Number of total progeny and female progeny per parasitised host in the field (means ±SD)

Parasitoid × Host	Total progeny /♀ ± SD	Female progeny/♀ ± SD
C. flavipes × *C. partellus*	40.10 ±16.70 a	30.27 ± 15.57 a
C. flavipes × *S. calamistis*	37.07 ±25.34 a	22.00 ± 23.30 a
C. sesamiae × *C. partellus*	32.38 ± 7.75 a	26.22 ± 15.37 a
C. sesamiae × *S. calamistis*	33.50 ±14.24 a	14.53 ± 13.87 b

Means with the same letter within the same column are not significantly different.

Table 3.8. Non-linear and linear regression equations of the functional responses of *C. flavipes* and *C. sesamiae* on *C. partellus* and *S. calamistis* in the field and laboratory.

Treatment[1]	regression equation	R^2	F-value	Prob>F	Linear regression	R^2	F-value	Prob>F
Field								
C. f. × C. p.	$y= x[1-e^{-(0.39/1.021x)}]$	0.63	21.01	0.0001	$y= 0.890+0.183x$	0.63	43.17	0.0001
C. s. × C. p.	$y= x[1-e^{-(0.18/1.036x)}]$	0.18	2.690	0.0874	$y= 0.586+0.083x$	0.16	4.900	0.0360
C. f. × S. c.	$y= x[1-e^{-(0.144/1.007x)}]$	0.46	8.027	0.0029	$y= 0.027+0.115x$	0.46	15.60	0.0009
C. s. × S. c.	$y= x[1-e^{-(0.138/1.005x)}]$	0.45	8.691	0.0017	$y= 0.168+0.107x$	0.45	17.43	0.0004
Laboratory								
C. f. × C. p.	$y= x[1-e^{-(0.738/1.047x)}]$	0.44	14.85	0.0001	$y= 0.447x - 0.103$	0.43	28.92	0.0001
C. s. × C. p.	$y= x[1-e^{-(0.288/1.037x)}]$	0.35	10.12	0.0041	$y=0.027+0.206x$	0.34	19.66	0.0001
C. f. × S. c.	$y= x[1-e^{-(0.666/1.23x)}]$	0.18	1.093	0.3507	$y=0.457+0.166x$	0.11	3.128	0.0890
C. s. × S. c.	$y= x[1-e^{-(2.079/2.092x)}]$	0.17	0.694	0.5112	$y=0.630+0.216x$	0.33	9.870	0.0051

[1]C. f. = *Cotesia flavipes* C. s. = *Cotesia sesamiae* C. p. = *Chilo partellus* S.c. = *Sesamia calamistis*

3.4 DISCUSSION

The release of *C. flavipes* in maize fields at the Kenya coast has resulted in establishment, and it is now recovered beyond the areas where it was originally released (Overholt *et al.*, 1997). In this study, *C. flavipes* proved to be a more efficient searcher of *C. partellus* than *C. sesamiae* in field and laboratory experiments. When *S. calamistis* was the host, no significant difference was found between the two parasitoids in the number of hosts attacked.

Functional response studies have been criticised by several workers. For example, Kareiva (1990) argued that functional responses measured by confining a known number of hosts and parasitoids are unnatural because insects are forced to stay in a confined arena. However, the stemborer larval stages used in the present study were not highly mobile. In nature, fourth instar larvae reside in plant stems, so confinement in plant stems inside cages probably had little effect on their behaviour or accessibility to parasitoids. A previous study on *C. flavipes* showed that once a searching female landed on an infested plant, she crawled on the plant until finding the entrance to a stemborer tunnel (Potting *et al.*, 1993). Thus, placing a female in a caged environment with infested plants or plant stems is likely to have had minimal effect on parasitoid searching.

O'Neill (1989) suggested that functional response studies may be misleading because they usually involve prey densities that are far in excess of those encountered in natural situations. However, host densities used in the present field study ranged from 0.41 to 1.66 borers/plant which are representative of the average stemborer densities found in the field in Kenya (0.2 to 1.8) (Overholt

et al., 1994).

Functional response studies may not explain the role of natural enemies in controlling pest populations (Varley and Gradwell, 1969), however, such studies maybe useful for comparing the efficiency of different natural enemies (Overholt and Smith, 1990). The present study shows that functional and numerical responses found in the laboratory gave the same ranking of the two parasitoids on the two hosts as in the field. Laboratory studies in a simplified environment may exaggerate the effect of natural enemies (Munyaneza and Obrycki, 1997), but still provide a valid means of comparing the host finding abilities of candidate natural enemies.

Field functional response data was well described by both non-linear and linear regressions, which may be an indication that the host densities used were less than densities needed to show a typical (type II) functional response. A linear response is not unexpected over a range of low host densities (Wiedenmann and Smith, 1993). In the laboratory, the functional response of *C. sesamiae* on *S. calamistis* was better described by a linear regression. However, the functional response of *C. flavipes* on *S. calamistis* in the laboratory was highly variable and not well described by either model, which may have been due to the low number of larvae recovered. *S. calamistis* larvae are known to be cannibalistic which may provide an explanation for their disappearance (Bosque-Perez and Dabrowski, 1989).

The handling times derived from Roger's random parasite equation, which ranged from 2.21 to 10.57 days in the field and from 0.577 to 7.34 days in the laboratory, are biologically unrealistic.

Potting *et al.* (1993) directly measured the time taken by *C. flavipes* to enter a maize stem, parasitise a *C. partellus* larva, and then exit the stem, and found a mean "handling time" of 34.6 minutes. Alebeek (1996) also found that handeling times estimated from Roger's equation were far greater than the values found by direct observation. Fernando and Hassell (1980) argued that both the instantaneous search rate and handling time in the Roger's equation should be regarded as mathematical, rather than biological parameters. This may be particularly true in the present experimental design where the total time was set at three days. *C. flavipes* is a short lived parasitoid with a mean longevity of only about three days at the temperatures experienced in the coastal area of Kenya.

The difference between observed and calculated handling times may also be due to Roger's assumption that a constant number of parasitoids are searching throughout the duration of the experiment. It is almost certain that, in the present experiment, the parasitoid density decreased over time. Even if the Roger's parameters have no well-defined biological meanings, however, they may still be useful for comparing the functional response curves of two ecologically similar parasitoids examined under identical conditions.

Though a non-coevolved natural enemy of *S. calamistis*, *C. flavipes* was able to attack *S. calamistis* at similar levels to *C. sesamiae* at all host densities. Moreover, *C. flavipes* produced more female progeny than *C. sesamiae* at all densities of *C. partellus* in both field and laboratory studies. These findings agree with the higher intrinsic rate of increase of *C. flavipes* over *C. sesamiae* found by Mbapila (1996), who also reported a higher percentage parasitism of *C.*

flavipes than *C. sesamiae* in field cages when *C. partellus* was the host (Mbapila and Overholt 1997). It was also found that *C. flavipes* produced more progeny on *C. partellus* than *C. sesamiae* in the field, but there were no differences in numbers of males, females or total progeny when *S. calamistis* was the host. The poor performance of *C. sesamiae* on *C. partellus* in the field was due to the low numbers of female progeny produced per female and the lower coefficient of attack on that host.

The ratio of successful attacks to the total number of attacks may provide a reliable estimate of physiological host-parasitoid compatibility. A higher number of hosts producing progeny relative to the total number attacked indicates a more compatible natural enemy. The proportion of hosts successfully parasitised is a useful measure for estimating the long-term impact on the host (Wiedenmann and Smith, 1993). In the present study, it was shown that *C. flavipes* successfully parasitised a higher proportion of *C. partellus* than *C. sesamiae*, while there was little difference in parasitism of *S. calamistis*.

There is obvious niche overlap between *C. flavipes* and *C. sesamiae* (Ngi-Song *et al.*,1995). After the invasion of *C. partellus* into Africa, native stemborer parasitoids expanded their host ranges to include the exotic pest (Oloo and Ogedah 1990, Kfir, 1992). However, recent surveys indicated that *C. partellus* was by far the most abundant species in maize, sorghum, *Sorghum arundinaceum* and *Panicum maximum* in the coastal area of Kenya and typically accounted for >80% of the stemborer population (Overholt *et al.*, 1994). Thus, *C. flavipes* may have an

important advantage over *C. sesamiae* due to the dominance of its coevolved host.

Of 7932 *C. partellus* larvae collected from 28 sites at the Kenya coast during 1996, 36 were parasitised by *C. flavipes* and 42 were parasitised by *C. sesamiae*. (Overholt, unpublished). Considering that *C. sesamiae* has been the most common parasitoid of *C. partellus,* and that *C. flavipes* was only recently introduced into the new ecosystem at the coast, this level of parasitism is promising in this early stage of introduction.

The results of this study suggest that *C. flavipes* is extrinsically superior to *C. sesamiae* when *C. partellus* is the host, and equally competitive when *S. calamistis* is the host. Studies in chapter 5 investigate the competitiveness of the two parasitoids within the same host (multiple parasitism). If *C. flavipes* proves to be intrinsically superior to *C. sesamiae*, then competitive displacement may eventually occur, particularly in areas where *C. partellus* is the dominant host.

CHAPTER 4

INTRASPECIFIC AND INTERSPECIFIC HOST DISCRIMINATION IN *COTESIA FLAVIPES* AND *COTESIA SESAMIAE* (HYMENOPTERA: BRACONIDAE)

4.1 INTRODUCTION

4.1.1 Parasitoids' searching behaviour

Parasitoids are faced with a heterogeneous environment in which they have to make particular decisions to achieve successful parasitism. Due to experiencing several fitness consequences, parasitoids evolved certain strategies towards optimising their resources. Steps of a successful parasitism were divided by Vinson (1976) into (1) host-habitat location, (2) host location, (3) host acceptance, (4) host suitability and (5) host regulation. During the process of searching, volatiles from infested plants act as long-range attractants for parasitoids (Vinson, 1985; Lewis *et al.*, 1990; Ngi-Song *et al.*, 1996). When the plant is infested, it may release chemical signals known as herbivore-induced synomones attractive to natural enemies. The compunds may not be restricted to only the infested parts (Dicke, 1994; Potting, 1996). For example, Turlings *et al.*,(1991a) found that the braconid *Cotesia marginiventris* responded strongly to herbivore damaged maize leaves. The wasp responded less strongly to artificially damaged leaves. *Cotesia rubecula* was also attracted to plants damaged by its host (*Pieris rapae*), but artificial damage failed to elicit the same response (Nealis, 1986). However, Turlings *et al.*, (1990) showed that if artificially damaged leaves are treated with host saliva, female parasitoids responded as if to host damaged leaves.

Parasitoids of stemborers search for the host-microhabitat using short range olfactory stimuli from frass (Leerdam *et al.*, 1985), vibration from the host (Hailemichael *et al.*, 1994) or can be stimulated by holes in the plant stem in its search for the damaged parts (Pfannenstiel *et al.*, 1992). Short range volatiles appear to orient the parasitoid when it is a few centimeters away from its target (Schmidt, 1974). Once a female *C. flavipes* has located the host's tunnel entrance, she ingresses through the larval frass until she reaches the host larva (Smith *et al.*, 1993).

4.1.2 Host discrimination in parasitoids

The ability of a female wasp to recognise and avoid ovipositing in a previously parasitised host is considered desirable for biological control (van Lenteren, 1981). Salt (1937) was the first to report that a parasitoid marked a host to inhibit further attack. These inhibitory factors were termed spoor factors (Flanders, 1953), trail odours (Rogers and Hassell, 1974), deterrent pheromones (Greany and Oatman, 1972), search deterrent substances (Matthews, 1974) and host marking pheromones (Vinson, 1972). There are several studies showing that most parasitoids are capable of some degree of host discrimination. For example, it was found that both mated and unmated females of *Cotesia glomerata* discriminated between parasitised and unparasitised larvae of *Pieris rapae* and strongly preferred to oviposit in the later. Moreover, females laid smaller clutch size in previously parasitised hosts (Ikawa and Suzuki, 1982; Tagawa, 1992). Potting (1996) showed that *C. flavipes* females that had a previous oviposition experience refrained from entering a tunnel with a *Chilo partellus* larva parasitised by herself or by another female. Other solitary parasitoids had a higher mean number of

ovipositions in unparasitised hosts than in hosts previously parasitised by a conspecific. This was shown for *Cotesia kazak*, a larval endoparasitoid of *Heliothis virescens* (Tillman and powell, 1992). However, Potting *et al.*, (1997) found no significant difference between the first and the second clutch size deposited by *C. flavipes* in superparasitised *C. partellus* larvae.

Some parasitoid species are not only able to discriminate between parasitised and unparasitised hosts, but also between parasitised hosts containing eggs of a conspecific female from hosts containing their own eggs (McBrien and Mackauer, 1991). Parasitoids may mark the host itself or the searched substrate (Vinson, 1976). Godfray (1994) suggested four reasons for the selection of marking in parasitoids: (1) avoidance of females attacking the same host twice (self-superparasitism) (2) enhancement of parasitoid efficiency by increasing the evenness of host attacks (3) as an altruistic act to help related females to forage efficiently (4) to alert other females that the host has already been attacked (thus avoiding conspecific superparasitism).

Intraspecific host discrimination is a well documented behaviour in insect parasitoids (Mackauer, 1990). Probably less common, but also widely reported, is interspecific host discrimination. According to Salt (1961), the first example of interspecific discrimination was due to Lloyd (1940) who showed that the ichneuomonid *Diadegma eucerophaga* (=*Angitia cerophaga*) avoided the larvae of the diamond back moth, *Plutella xylostella* previously attacked by the braconid *Cotesia* (*Apenteles*) *plutella*. One hypothesis to explain why interspecific discrimination is less common is that a parasitoid is more likely to encounter hosts parasitised by conspecifics than heterospecifics, thus, selective pressure for intraspecific

host discrimination is likely to be higher (Godfray, 1994). Interspecific host discrimination is not expected to evolve in allopatric species.

Studies on *C. flavipes* and the African congener, *C. sesamiae* (Cameron), showed that the two parasitoids responded to volatiles emanating from various grasses infested by the different stemborer species found in Kenya (Ngi-Song and Overholt, 1997). Sharing almost the same ecological niche, the two parasitoids are expected to compete, both extrinsically and intrinsically, for their stemboring hosts. The aim of this study is to examine the role of infochemicals in host recognition in the two parasitoid species, and to determine whether females of one species can detect a previously parasitised host by a conspecific or heterospecific female. A study of the intra- and interspecific host discrimination will provide insight to understanding the interactions between the two species in the field.

4.2 Materials and Methods

4.2.1 Host rearing

The exotic stemborer, *Chilo partellus* (Lepidoptera: Pyralidae) and the indigenous stemborer, *Sesamia calamistis* (Lepidoptera: Noctuidae) were used in this study. Stemborer colonies were maintained at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi. Adult moths of the two species originated from immature stemborers collected at the Kenya coast. Rearing techniques are explained in detail in chapter 3.

4.2.2 Parasitoid rearing

A colony of *C. flavipes* was initiated with founders collected from *C. partellus* larvae at Rawalpindi, Pakistan, by the International Institute of Biological Control (IIBC). A colony of *C. sesamiae* was started from material obtained from *C. partellus* collected at the Kenya coast. *C. flavipes* and *C. sesamiae* colonies were maintained on *C. partellus* and *S. calamistis* fourth instar larvae, respectively, using the method described by Overholt (1993). (See chapter 3 for more details).

4.2.3 Behaviour assays

4.2.3.1 Experimental procedures

A dual choice test was conducted to determine whether female parasitoids could discriminate between a healthy and a parasitised host larva. Host larvae were introduced individually to 7.5 cm ×2.5 cm glass vials containing small pieces of maize stems (3-4 cm long) 48 hours before starting any experiments. During this time, larvae bored a small tunnel into the stem and pushed out some frass. Larvae were then divided into three groups. Each larva in the first group was exposed to one *C. flavipes* female parasitoid, while each larvae in the second group was exposed to one *C. sesamiae* female parasitoid. Larvae in the third group remained unparasitised. For the first and the second group, host larvae in stems were placed individually in glass vials. The stems were partially cut longitudinally using a sharp blade to enable observation of the larvae. A one-day old fed and mated female parasitoid was introduced into the vial and given ten minutes to locate the tunnel, sting the host larva and leave the tunnel. Larvae that killed the parasitoid during stinging were not used in the

experiment. Pieces of stems with larvae were then used for the dual choice test. Odour sources used in this experiment were:

1- Unp C.p (Unparasitised fourth instar *C. partellus* larva in a fresh tunnel).

2- Unp S.c (Unparasitised fourth instar *S. calamistis* larva in a fresh tunnel).

3- C.p * C.f (Parasitised fourth instar *C. partellus* in a tunnel visited by one *C. flavipes* female parasitoid).

4- C.p * C.s (Parasitised fourth instar *C. partellus* in a tunnel visited by one *C. sesamiae* female parasitoid).

5- S.c * C.f (Parasitised fourth instar *S. calamistis* in a tunnel visited by one *C. flavipes* female parasitoid).

6- S.c * C.s (Parasitised fourth instar *S. calamistis* in a tunnel visited by one *C. sesamiae* female parasitoid).

4.2.3.2 The Y-tube setup

The response of female parasitoids towards volatiles emanating from tunnels made by either healthy or parasitised host larvae was investigated in a Y-tube olfactometer (Steinberg *et al.*, 1992) (Fig 4.1). Air was pumped through an activated charcoal filter into the two arms of the olfactometer. The airflow was set at 1.5 litres/minute for each arm. Female parasitoids were introduced in the Y-tube and given a maximum of five minutes to make a choice for one of the arms. Four dual choice tests were conducted as follows:

1- C.p * C.f versus Unp C.p

2- C.p * C.s versus Unp C.p

3- S.c * C.f versus Unp S.c

4- S.c * C.s versus Unp S.c

Each choice was repeated four times with a different category of female parasitoids as follows:

1- Naive *C. flavipes* female parasitoid

2- Naive *C. sesamiae* female parasitoid

3- Experienced *C. flavipes* female parasitoid

4- Experienced *C. sesamiae* female parasitoid

Naive females had no stinging experience, while experienced ones had a previous experience in entering a tunnel, stinging the host larva and successfully exiting the tunnel. Therefore, 16 dual choice tests were conducted. Number of female parasitoids used for each test varied from 45 to 65 parasitoids due to availability of insect materials. A choice was recorded if the female stayed for more than 15 seconds beyond the finish line (4 cm past the intersection). The odour sources (stems with larvae) were exchanged after testing five parasitoids or after every 30 minutes to avoid the effect of any asymmetrical bias in the olfactometer or its surroundings. A cream-white curtain was used to separate the experimental area from the surroundings. Tests were conducted at 26-28 ^{0}C and 65-75% relative humidity, and light intensity of 350-450 lux.

Parasitised larvae (in their tunnels) were used for the dual choice tests immediately after they had been stung, and renewed every two hours with stems containing recently parasitised larvae to insure the existence of any marking pheromones left by the stinging female. Data were analysed using the log likelihood ratio test (*G* test) for goodness of fit (Sokal and Rohlf, 1981).

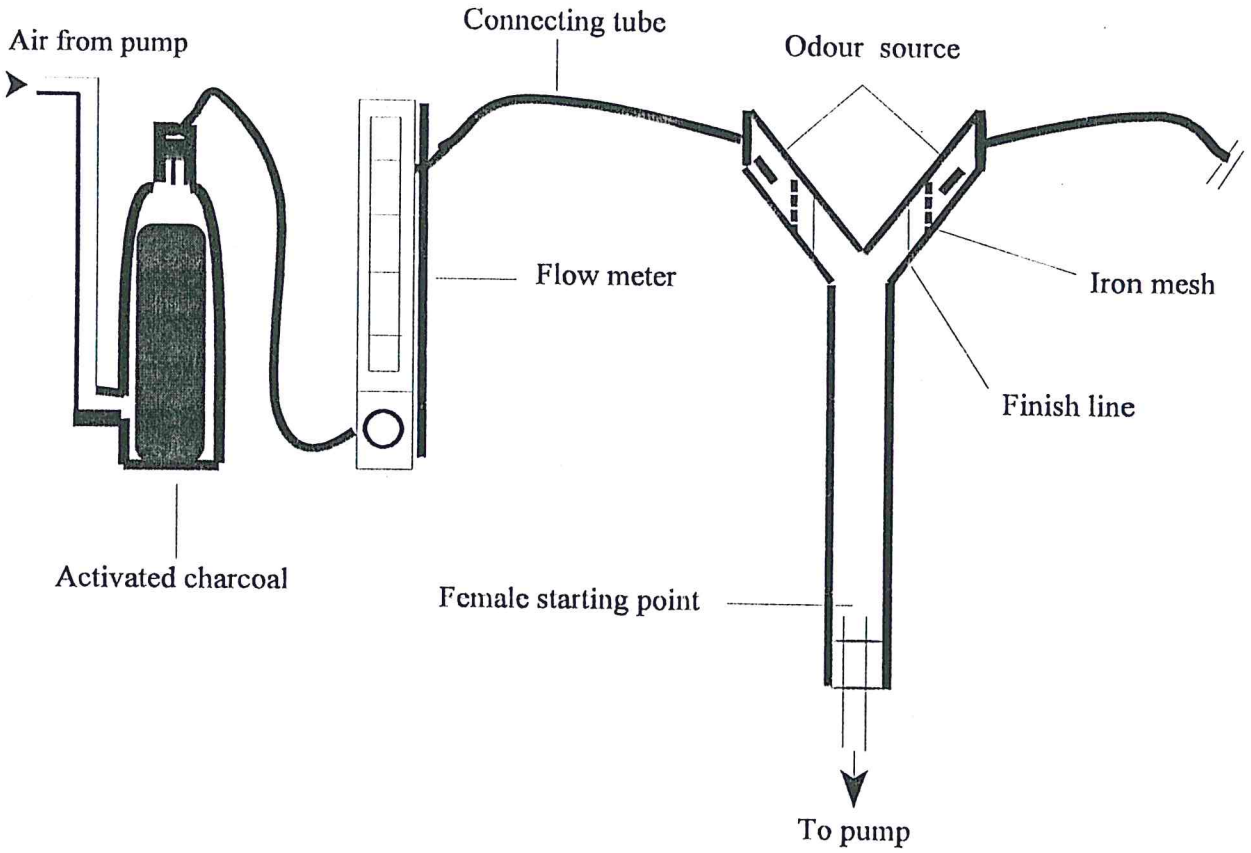


Fig. (4.1) A schematic diagram of the Y-tube olfactometer

4.3 RESULTS

Host discrimination results are summarised in figures 4.2,4.3,4.4 and 4.5. Neither naive nor experienced females of the two species exhibited interspecific host discrimination ability. However, experienced females of the two species were significantly more attracted to healthy host larvae in fresh tunnels than larvae parasitised by conspecific females.

4.4 DISCUSSION

There was a clear tendency for experienced females to avoid superparasitism, while this ability was absent in naive females. This finding agrees with Potting (1996), who found that *C. flavipes* females leave a mark in the tunnel after parasitism. However, the observation of superparasitism does not necessarily mean that a parasitoid is unable to discriminate between parasitised and unparasitised hosts (van Lenteren, 1981). The short life span of the two *Cotesia* species and the high risk involved in the process of parasitism may provide an explanation. Females of the two species live for 2-3 days which is a relatively short time. Moreover, stemborer larvae defend themselves by biting the parasitoid during ovipositing (Takasu and Overholt, 1996). Almost 40% of *C. flavipes* females were killed inside the tunnel during parasitism (Potting *et al.*, 1993). It was also realised that parasitised larvae were more aggressive than unparasitised ones. A low frequency of host encounters in a limited life span may increase the range of hosts acceptable to a parasitoid. Moreover, the fact that the two parasitoid species are proovigenic (limited with a certain amount of eggs in the ovary) may

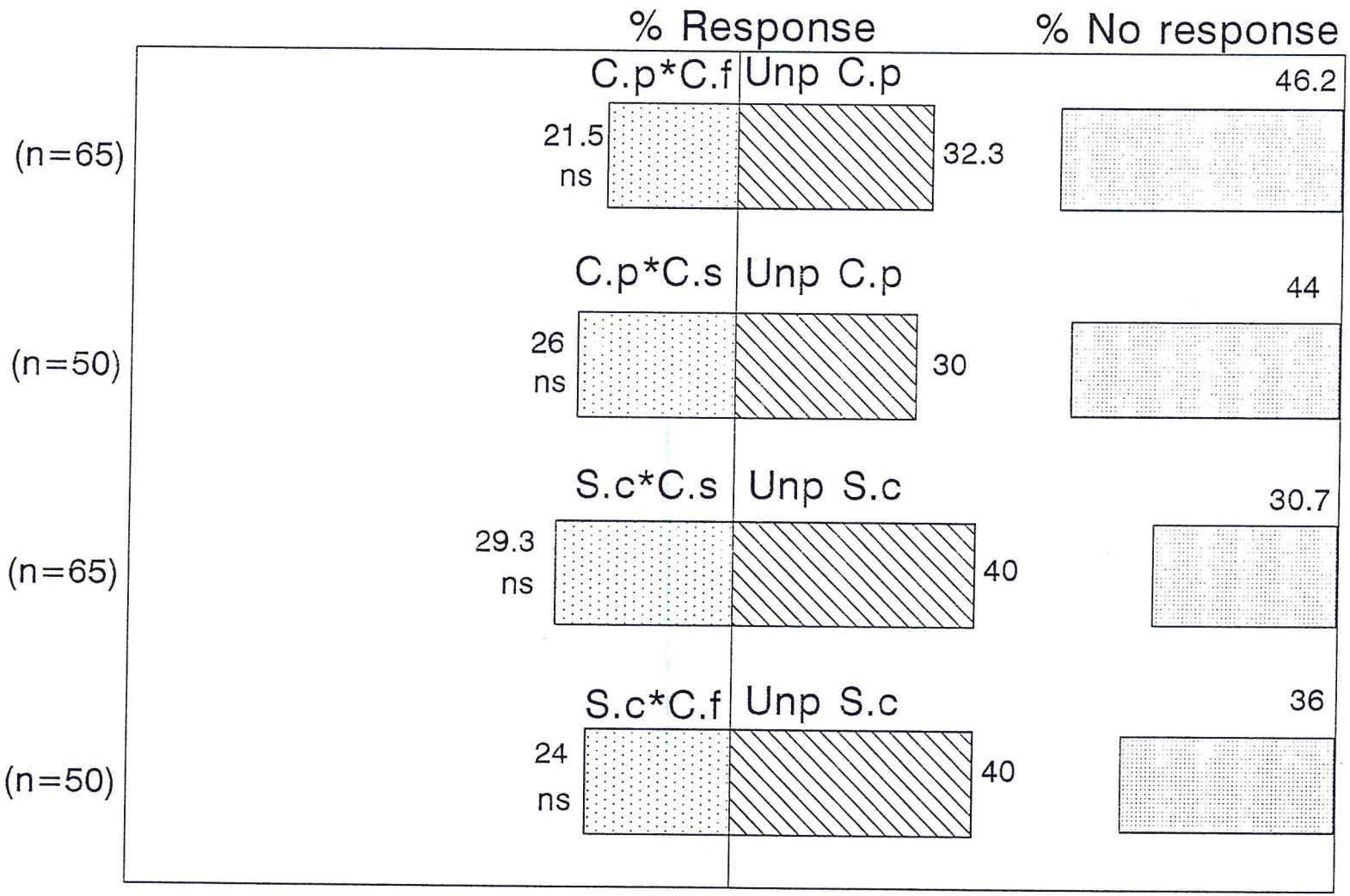


Fig.4.2. Response of *C. flavipes* naive females in a Y-tube olfactometer. n=no. females tested. (**)indicate a significant difference within the choice test (P<0.001). ns=not significant. *G* test for goodness of fit.

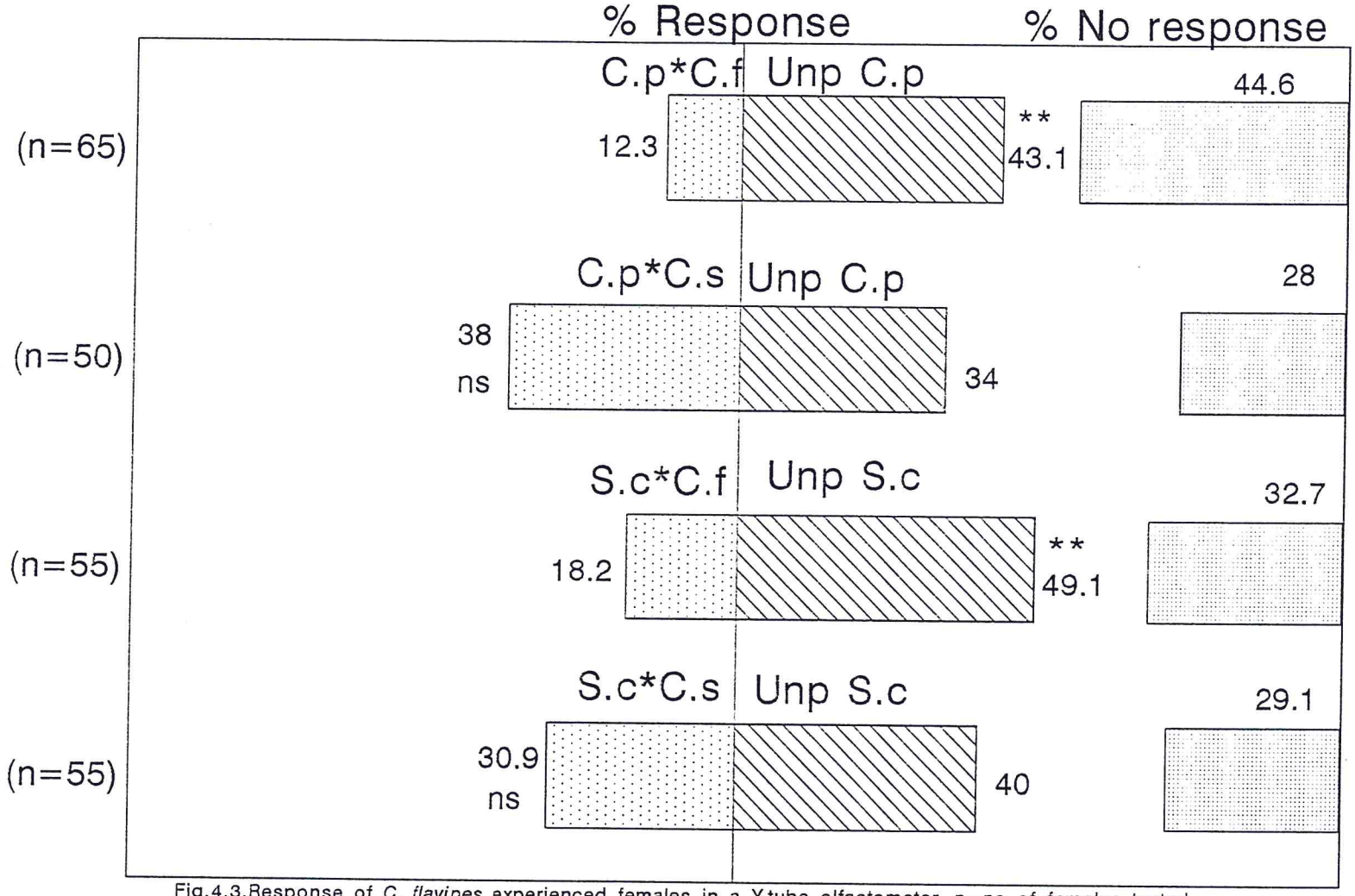


Fig.4.3.Response of *C. flavipes* experienced females in a Y-tube olfactometer. n=no of females tested. (**) indicate a significant difference within the choice test (P<0.001). ns=not significant. *G* test for goodness of fit.

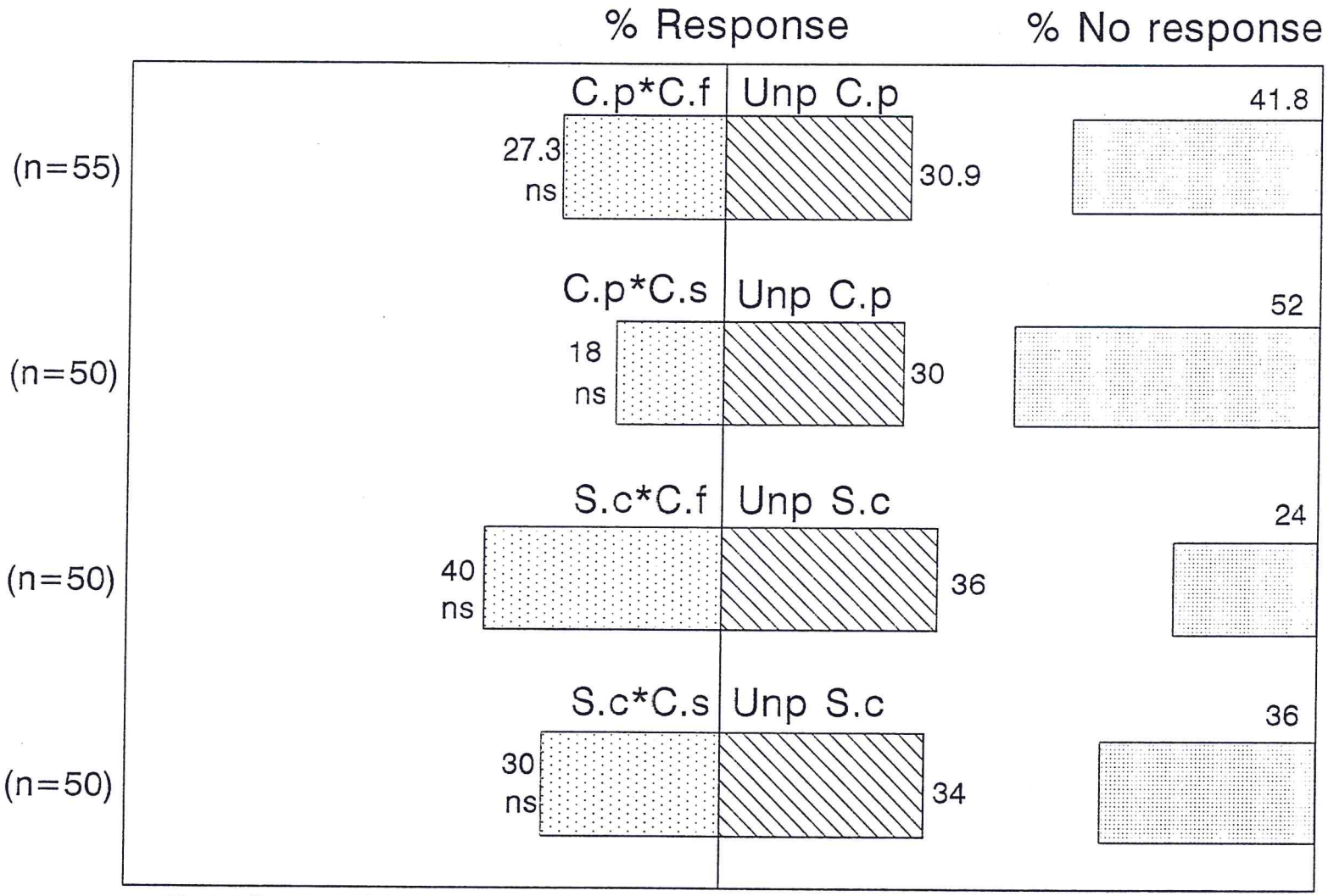


Fig.4.4. Response of *C. sesamiae* naive females in a Y-tube olfactometer. n=no. of females tested. (**) indicate a significant difference (P<0.001). ns=not significant. *G* test for goodness of fit.

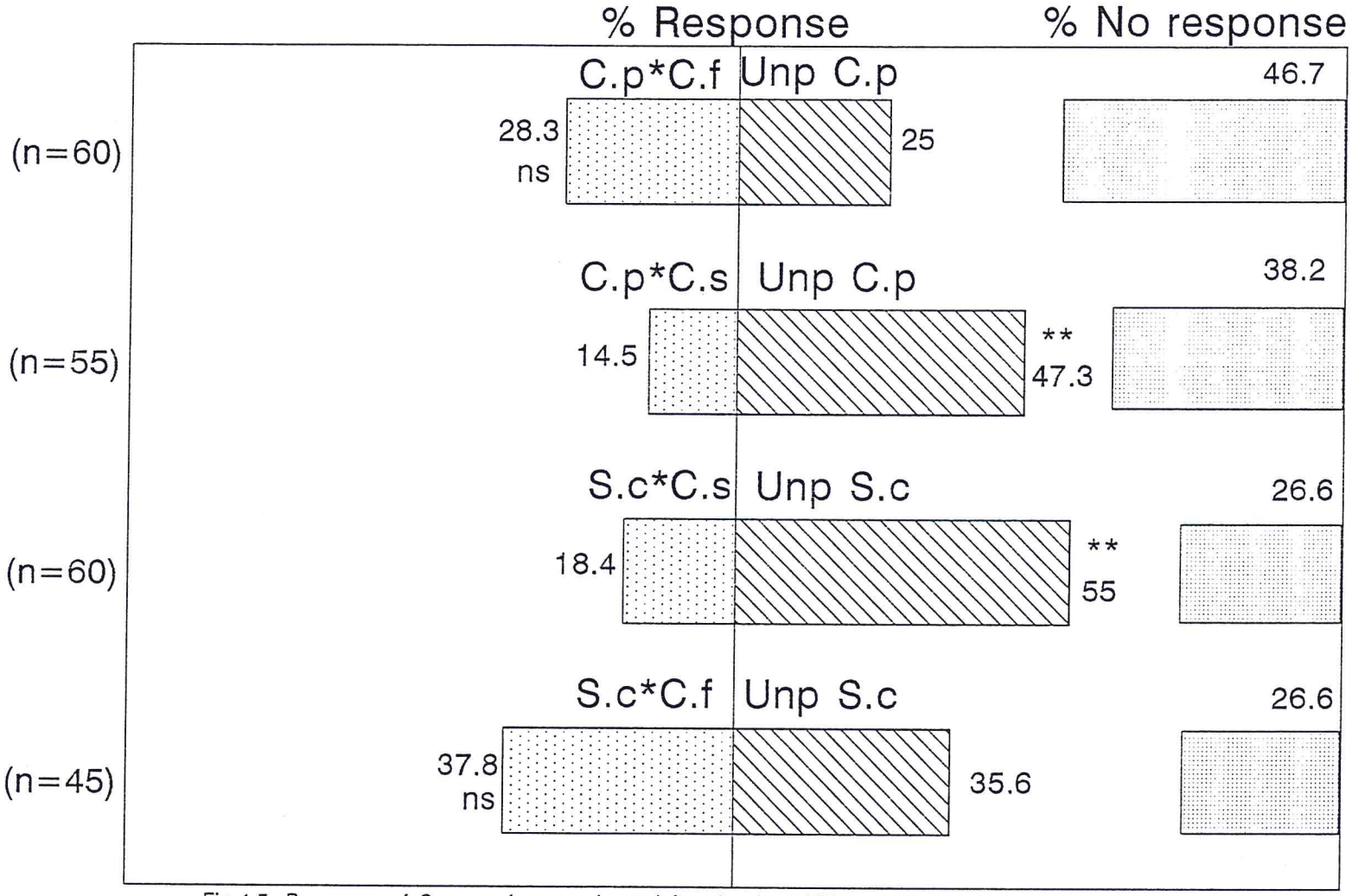


Fig.4.5. Response of *C. sesamiae* experienced females in a Y-Tube olfactometer. n=no. females tested, (**) indicate a significant difference within the choice test (P<0.001). ns=not significant. *G* test for goodness of fit.

also contribute to allowance of superparasitism against the chance of finding other hosts, which is a substantially difficult and risky task. Mangel (1987) developed a host-acceptance model taking into account different assumptions about insect oviposition. The model predicts that a pro-ovigenic parasitoid should attack a more restricted set of hosts when it begins to run short of eggs. In addition, the parasitoid should attack a wider set of hosts when the risk of mortality is high. Thus, naive females of the two *Cotesia* species may readily accept any quality of host larva in the first ovipositing trial, then become more selective if it escapes death. A degree of superparasitism is therefore expected in the field depending on the availability of the hosts. In the absence of interpecific discrimination, multiple parasitism would be more frequent than superparasitism wherever the two species occur sympatrically. Consequences of such phenomenon are discussed in chapter five.

Experience gained by insect parasitoids is an important factor inducing variability in foraging behaviour (Vet *et al.*, 1995). For example, *Cotesia marginiventris* females responded more strongly to odours from the cabbage looper (*Trichoplusia ni*) and the fall armyworm *Spodoptera frugiperda* after they had oviposited in either of the hosts (Turlings *et al.*, 1991a,1991b). However, Rosenheim (1993) noted that it is difficult to distinguish learning from the effects of changes in eggload on behaviour. In the two *Cotesia* species, a significantly high proportion (30%) of the total egg load is allocated in each parasitised larva, therefore, the increased selectivity after the first oviposition experience might be due to the reduction in eggload (Potting, 1996).

The realised avoidance of previously parasitised hosts by a conspecific in experienced females is interesting, though in gregarious parasitoids, a host larva may be able to support the development of variable numbers of parasitoids, this possibility is studied in the two stemborer species in the following chapter.

CHAPTER 5

INTRASPECIFIC AND INTERSPECIFIC COMPETITION BETWEEN *C. FLAVIPES* AND *C. SESAMIAE* (HYMENOPTERA: BRACONIDAE)

5.1 INTRODUCTION

5.1.1 Superparasitism

It is generally agreed that superparasitism will be more frequent when rates of host encounter are low or when rates of encounter with parasitised hosts are high (van Lenteren, 1976; Werren, 1980). In addition, parasitoids species with large egg reserves are unlikely to favour avoidance of superparasitism (Liu and Morton, 1986). The study on host discrimination in chapter 4 indicated that naive females of the two *Cotesia* species accepted previously parasitised hosts, either by conspecifics or heterospecifics, for oviposition. Considering the relatively large eggload (150 eggs), short life span (3 days) and the high risk involved in the process of parasitism by the two species, a degree of superparasitism in the field is therefore expected.

Little work has been done on the effect of superparasitism on the progeny of *Cotesia flavipes*. Beg and Inayatullah (1980) recorded a progressive increase in cocoon production and adult emergence of *C. flavipes* in superparasitised *Chilo partellus*, but with a steady decrease in sex ratio and progeny's body weight. On the other hand, Potting *et al.*, (1997) found no significant increase in the number of *C. flavipes* adults emerging from superparasitised hosts,

however, larval developmental times were longer and progeny size was significantly smaller than for singly parasitised hosts. The present study investigated the fitness consequences due to superparasitism by the two *Cotesia* species on the two stemborers, *C. partellus* and *Sesamia calamistis*, with emphasis on the host/parasitoid's compatibility and parasitoids' intrinsic efficiency on the two hosts.

5.1.2. Multiple parasitism

As two allopatric species, *C. flavipes* and *Cotesia sesamiae* are not expected to develop an interspecific host discrimination ability. This was confirmed by the study in chapter 4, where it was shown that naive as well as experienced females of one species accepted previously parasitised larvae by the other species, which may be an indication that multiple parasitism is likely to occur in the field. The fact that *C. flavipes* is a coevolved natural enemy of *C. partellus* while the African congener, *C. sesamiae,* coevolved with *S. calamistis* might induce variability in the host suitability and physiological compatibility with the two parasitoids, which may lead to one species having a consistent advantage over the other on its coevolved host. As gregarious parasitoids, the elimination of one species by the other would be attributed to selective starvation, anoxia or the introduction of a toxic substance by the parasitoid female. Overholt *et al.*, (1997) found that when *C. partellus* was stung by both parasitoids, *C. flavipes* emerged in nearly all cases. However, there is need to investigate the nature of this interaction on the indigenous stemborer, *S. calamistis*, for a clear understanding of the potential for the two parasitoids to coexist in the field.

C. flavipes is ecologically and morphologically similar to *C. sesamiae*. The degree of stemborer population regulation may be affected by this interspecific competition in the area of the parasitoids' suitable habitat. The aim of this study was to understand the nature of inter- and intraspecific relationships between individuals of the two species. Therefore, it is important to study the consequences of super as well as multiple parasitism on the parasitoids progeny, and to speculate on the possibility of local displacement due to the superiority of one species over the other.

5.2 MATERIALS AND METHODS

5.2.1 Intraspecific competition study

To study the effect of superparasitism on the subsequent parasitoid generation, a total of 100 4th instar host larvae, from the two stemborer species (*C. partellus* and *S. calamistis*), were obtained from the Animal Rearing and Quarantine Unit at the ICIPE (Refer to 3.2.1 for details on host rearing). The larvae were divided into five groups of 20 individuals each. Host larvae in each group were placed in glass vials and exposed to 1,2,3,4 and 5 newly emerged, mated and fed female parasitoids respectively. Each female was allowed to oviposit only once, and then removed from the experimental arena. Parasitised larvae were then kept in vials containing artificial diet until the emergence of the adult parasitoids. Due to high rates of larval mortality, some treatments had to be repeated more than once to obtain enough replicates. Parasitoid cocoons emerging from each larva were counted, weighed and then placed in vials containing small droplets of honey on the walls to serve as a food source for the

emerging adults, and kept at 28^{0}C and 50-60% RH. Duration of the parasitoids immature stages, percentage of emergence, number of adult progeny, adult longevity and sex ratio of the emerged parasitoids were recorded. Five vials were chosen randomly from each group and a total number of twenty five female parasitoids, five from each, were dissected and eggs in the ovaries counted (Plate 5.1).

5.2.2 Interspecific competition study

Sixty 4th instar host larvae from the two species (*C. partellus* and *S. calamistis*) were put in glass vials and exposed, individually, to *C. flavipes* female parasitoids. Females were allowed to sting only once and then removed. Larvae were then divided into three groups with twenty larvae in each group. Each larva in the first group was stung immediately with one *C. sesamiae* female parasitoid. Each larva in the second and the third was stung with one *C. sesamiae* female after 24 and 48 hours respectively. Parasitised larvae were kept in glass vials containing artificial diet until the emergence of the adult parasitoids. Adults were then identified, sexed and counted. The same experiment was repeated with females of *C. sesamiae* introduced initially then *C. flavipes* stinging immediately, 24 and 48 hours later. 20 *C. partellus* and 20 *S. calamistis* 4th instar larvae were left unstung and used as control for the experiment. Larvae were fed on artificial diet until pupation. Mortality of the host larvae was recorded for the two species.

In order to investigate the physiological suitability of *C. partellus* for the two *Cotesia* species, three groups of 40 host were exposed to one or both parasitoids. Each larva in the first group

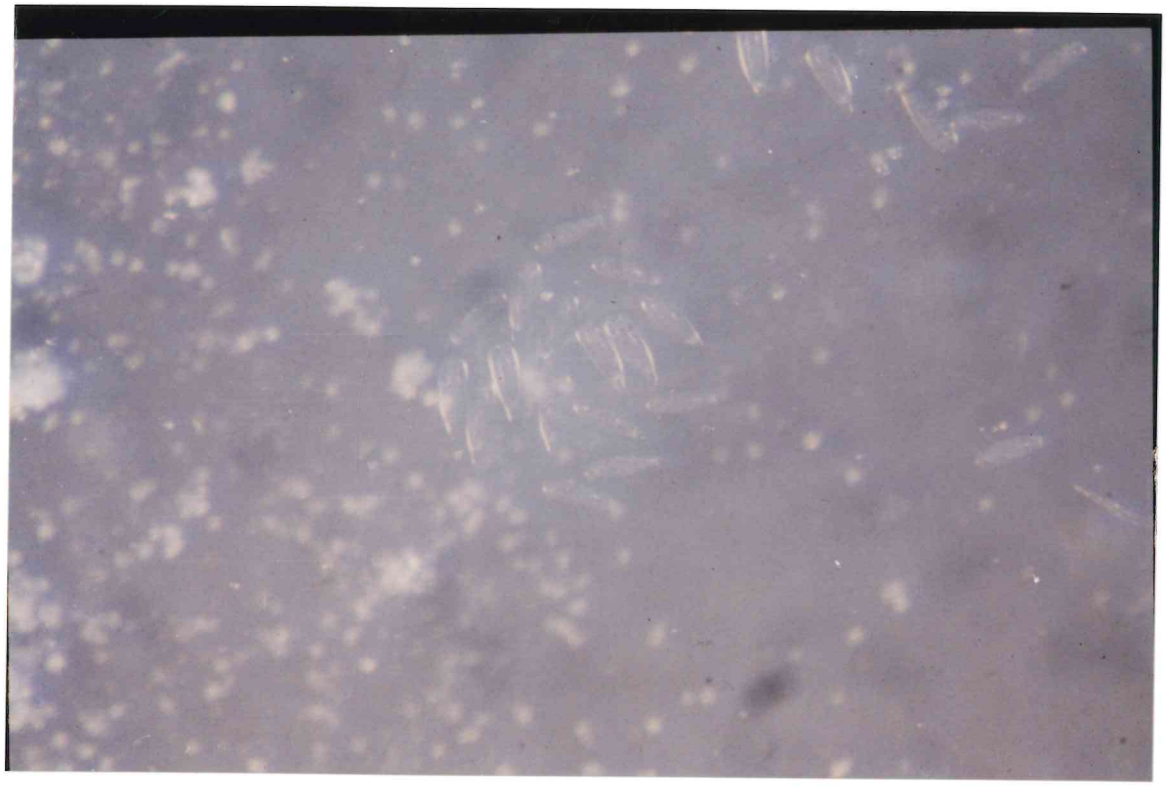


Plate 5.1. *Cotesia flavipes* ovarian eggs (100 X).

was stung once by a *C. flavipes* female parasitoid, while each larva in the second group was stung once by a *C. sesamiae* female parasitoid. Each larva in the third group was stung by the two parasitoids at the same time (< five minutes between ovipositions). In each of the three groups, 20 larvae were randomly selected and dissected four days after exposure. The number of parasitoid eggs in each larva was recorded. This was repeated on the remaining larvae in each group after nine days. The number of immature parasitoid larvae in each host larva was recorded. Results of this experiment were used to determine the intrinsic superiority and timing of elimination of one species by the other in *C. partellus* larvae.

5.2.3 Statistical analysis

The effect of different numbers of stings by both parasitoids on both hosts on the number of cocoons, cocoon weight ,adult progeny, duration of immatures, sex ratio, adult longevity, adult emergence and female fertility was compared within the different numbers of stings and between the two parasitoid species by analysis of variance. Means were separated by Student-Newman-Keuls means separation procedure (SAS Institute, 1988).

5.2.4 Insect identification

Male parasitoids were identified through dissection of male genitalia (Polaszek and Walker, 1991). Initial identification of adult females was done using the sculpturing terga I and II of the propodeum (Nagaraja, 1971). Further confirmation was done through isoelectric focusing using the Phastsystem (Pharmacia). Thin layer starch gel electrophoretic techniques were used to assay three polymorphic loci. The buffer system was tris-citrate buffer: 0.22 M tris and

0.08 M citric acid, electrode buffer ph 6.3 and gel buffer ph 6.7 (Pasteur *et al.*, 1988).

5.3 RESULTS

5.3.1 Superparasitism

Table 5.1 shows the mean numbers of cocoons, cocoon weight, adult progeny, immature developmental time, sex ratio, longevity, emergence and potential fecundity of the two parasitoids, resulting from different numbers of stings (1, 2, 3, 4, and 5), on *C. partellus* fourth instar larvae. The numbers of *C. flavipes* cocoons and adult progeny resulting from two and three ovipositions were significantly higher than the number of cocoons and adult progeny emerging from host stung one, four and five times ($F=7.33$; $df=4, 63$; $P=0.0001$). Superparasitism did not significantly affect the proportion of adults successfully emerging from cocoons ($F=2.22$; $df=4, 63$; $P=0.077$).

For *C. sesamiae*, there was no significant difference in the number of progeny resulting from one to three stings ($F=1.39$; $df=2, 13$; $P=0.28$). No parasitoids emerged from larvae stung four or five times. More *C. flavipes* cocoons ($F=17.91$; $df=1, 82$; $P=0.0001$) and adult progeny ($F=20.63$; $df=1, 82$; $P=0.0001$) were produced over all numbers of stings than *C. sesamiae*.

Duration of *C. flavipes* immature stages ranged from 15.9 to 16.7 days and was not different between one, two or three ovipositions, but longer in hosts stung four and five times (18.1 and

Table 5.1. Number of cocoons, adult progeny, cocoon weight, duration of immature stages, sex ratio, longevity, emergence rate and potential fecundity of *C. flavipes* and *C. sesamiae* (means±SD) as a result of different numbers of ovipositions on *C. partellus* fourth instar larvae.

Number of stings	Parasitoid species	Number of cocoons	Adult progeny	Cocoon weight (mg)	Duration (Days)	Sex ratio (♀♀/Total progeny)	Longevity (Days)	%Emergence	Potential Fecundity (No.eggs)
1	*C. flavipes*	38.8 (10.5) a	37.5 (12.6) a	0.085 (0.015) a	15.9 (1.12) a	0.65 (0.22)a	3.3 (0.4) a	95.4 (16.3) a	178.8 (12.4)a
	C. sesamiae	16.6 (5.62) b	14.4 (6.34) b	0.060 (0.027) b	16.9 (1.07) a	0.54 (0.21)a	2.7 (0.5) b	84.1 (20.9) a	136.0 (20.5)b
2	*C. flavipes*	57.2 (14.6) a	52.7 (13.6) a	0.069 (0.018) a	15.6 (0.72) b	0.65 (0.21) a	2.7 (0.6) a	92.6 (7.50) a	150.9 (18.1) a
	C. sesamiae	24.8 (4.35) b	20.8 (5.74) b	0.049 (0.023) a	18.8 (1.26) a	0.08 (0.15) b	2.8 (0.5) a	83.4 (17.1)a	120.1 (9.96) b
3	*C. flavipes*	58.5 (31.7) a	55.1 (30.3) a	0.064 (0.018) a	16.7 (1.79) a	0.73 (0.12) a	2.4 (0.5) a	94.3 (4.61) a	141.3 (25.2) a
	C. sesamiae	18.8 (11.8) b	11.0 (14.2) b	0.050 (0.033) a	19.3 (4.93) a	0.23 (0.35) b	2.3 (1.2) b	37.4 (44.8) b	103.5 (13.4) b
4	*C. flavipes*	31.1 (10.7)	27.4 (10.1)	0.068 (0.030)	18.1 (2.84)	0.59 (0.20)	1.8 (0.7)	88.1 (11.5)	122.4 (12.9)
	C. sesamiae	–	–	–	–	–	–	–	–
5	*C. flavipes*	25.4 (23.7)	22.1 (22.5)	0.033 (0.031)	19.3 (1.66)	0.51 (0.29)	1.7 (0.7)	82.1 (18.2)	116.8 (14.8)
	C. sesamiae	–	–	–	–	–	–	–	–

Means with the same letter for the same number of stings within the same column are not significantly different (SNK).

19.3 days, respectively) (F=9.60; df=4, 63; P=0.0001). For *C. sesamiae*, there was no difference in the duration of immature development (F=1.52; df=2, 11; P=0.26). The sex ratio (proportion female) of the progeny of *C. flavipes* ranged from an average of 0.73 to 0.51 and was not significantly affected by the number of ovipositions (F=1.89; df=4, 63; P=0.12), while for *C. sesamiae*, the mean proportion of female progeny decreased significantly when the number of ovipositions was increased to three (F=4.83; df=2, 13; P=0.027). The proportion of female progeny was significantly higher for *C. flavipes* than *C. sesamiae*'s (F=22.82; df=1, 82; P=0.0001) over the different number of ovipositions.

The adult longevity of *C. flavipes* progeny significantly decreased with increasing numbers of ovipositions, from 3.3 days as a result of one oviposition, to 1.7 days as a result of five ovipositions (F=14.61; df=4, 63; P=0.0001). For *C. sesamiae*, mean adult longevity ranged from 2.7 to 2.3 days and was not significantly affected by superparasitism (F=0.42; df=2, 11; P=0.67).

Total potential fecundity of *C. flavipes*, as determined by dissecting adult female progeny, decreased with increasing the number of oviposition, from an average of 178.8 eggs from one oviposition, to 116.8 eggs from five oviposition (F=30.59; df=4, 70; P=0.0001). The mean potential fecundity of *C. sesamiae* decreased from 136.0 from one oviposition, to 103.5 eggs for three ovipositions (F=16.96; df=2, 42; P=0.0001). Female *C. flavipes* emerging from hosts stung 1-5 times were significantly more fecund than *C. sesamiae* (F=21.64; df=1, 118;

P=0.0001).

Table 5.2 shows the mean numbers of cocoons, adult progeny, cocoon weight, immature developmental time, sex ratio, longevity, emergence and potential fecundity of the two parasitoids, resulting from 1, 2, 3 and 4 stings, on *S. calamistis* fourth instar larvae. No progeny of either parasitoid emerged when hosts were stung five times. Neither the average number of *C. flavipes* cocoons nor adults progeny were significantly affected by multiple ovipositions (F=1.50; df=3, 39; P=0.229, F=1.19; df=3, 39; P=0.327), respectively. For *C. sesamiae*, the average number of cocoons increased significantly from 32.1 from one sting to 66.0 cocoons from two stings, then decreased to 59.6 and 56.7 as a result of three and four stings (F=4.46; df=3, 42; P=0.0083). A similar trend was found for adult progeny (F=3.94; df=3, 42; P=0.0145). The mean number of cocoons did not differ significantly between *C. flavipes* and *C. sesamiae* (F=0.73; df=1, 90; P=0.39), nor did the mean number of adults (F=0.05; df=1, 90; P=0.819). The duration of immature *C. flavipes* was not significantly affected by superparasitism (F=1.04; df=3, 39; P=0.387), while immature development time became significantly longer in the case of *C. sesamiae* (F=3.36; df=3, 42; P=0.027).

In the two parasitoids, *C. flavipes* and *C. sesamiae*, sex ratio was not significantly affected by superparasitism (F=1.91; df=3, 39; P=0.144; F=0.73; df=3, 42; P=0.539) respectively, and sex ratios were not different between the two parasitoids (F=0.66; df=1, 90; P=0.418).

Table 5.2. Number of cocoons, adult progeny, cocoon weight, duration of immature stages, sex ratio, longevity, emergence rate and potential fecundity of *C. flavipes* and *C. sesamiae* (means ± SD) as a result of different numbers of ovipositions on *S. calamistis* fourth instar larvae.

Number of stings	Parasitoid species	Number of cocoons	Adult progeny	Cocoon weight (mg)	Duration (Days)	Sex ratio (♀♀/Total progeny)	Longevity (Days)	% Emergence	Potential fecundity (No.eggs)
1	*C. flavipes*	34.3 (17.2) a	32.0 (17.6)a	0.106 (0.011) a	17.2 (3.0) a	0.53 (0.26) a	3.6 (0.7) a	92.9 (8.9) a	203.6 (8.7) a
	C. sesamiae	32.1 (12.6) a	26.8 (14.2)a	0.098 (0.023) a	16.5 (0.7) a	0.59 (0.32) a	3.1 (1.0) a	81.1(18.5) a	120.3 (8.4) b
2	*C. flavipes*	49.6 (24.8) a	45.9 (23.5) a	0.082 (0.007) a	17.1 (3.2) a	0.75 (0.21) a	3.6 (0.5) a	92.8 (5.3) a	188.2 (10.4) a
	C. sesamiae	66.0 (25.2) a	60.5 (28.1) a	0.068 (0.016) b	19.5 (4.8) a	0.49 (0.22) b	3.1 (0.8) a	88.2 (17.3) a	100.6 (7.4) b
3	*C. flavipes*	46.2 (22.3) a	41.3 (23.2) a	0.069 (0.017) a	15.7 (0.7) b	0.61 (0.26) a	2.5 (0.7) a	86.4 (9.8) a	179.3 (13.6) a
	C. sesamiae	59.6 (39.3) a	52.8 (39.7) a	0.069 (0.027) a	17.0 (1.3) a	0.57 (0.28) a	2.2 (0.8) a	79.1 (24.0) a	116.7 (21.7) b
4	*C. flavipes*	51.9 (20.9) a	47.8 (22.3) a	0.068 (0.029) a	16.0 (1.0) b	0.68 (0.22) a	2.3 (0.5) a	90.0 (10.9) a	161.1 (26.8) a
	C. sesamiae	56.7 (22.5) a	43.4 (19.4) a	0.086 (0.030) a	17.7 (1.4) a	0.67 (0.15) a	2.4 (1.2) a	76.2 (16.5) a	91.10 (14.7) b

Means with the same letter for the same number of stings within the same column are not significantly different (SNK).

The proportion of cocoons from which adults successfully emerged was not significantly affected by superparasitism for the two parasitoids. More *C. flavipes* adults successfully emerged from cocoons than *C. sesamiae* ($F=9.73$; $df=1, 90$; $P=0.0024$).

The number of ovarian eggs declined in both *C. flavipes* ($F=17.34$; $df=3, 56$; $P=0.0001$) and *C. sesamiae* ($F=13.9$; $df=3, 56$; $P=0.0001$) as a result of superparasitism. Female progeny of *C. flavipes* were more fecund than *C. sesamiae* over all the number of ovipositions ($F=413.59$; $df=1, 118$; $P=0.0001$).

Figures 5.1 and 5.2 show the percentages of *C. partellus* and *S. calamistis* larvae surviving from superparasitism, respectively. Survival of *C. partellus* larvae superparasitised by *C. flavipes* was not affected until five ovipositions. However, when *C. partellus* was superparasitised by *C. sesamiae*, very poor survival of host larvae was recorded in the first three treatments (one, two and three ovipositions/larva) and 100% larval mortality occurred after four and five ovipositions/host . In contrast, superparasitised *S. calamistis* larvae were able to support the development of the two parasitoids up to four stings/host larva. However, no hosts survived after five ovipositions by either parasitoid.

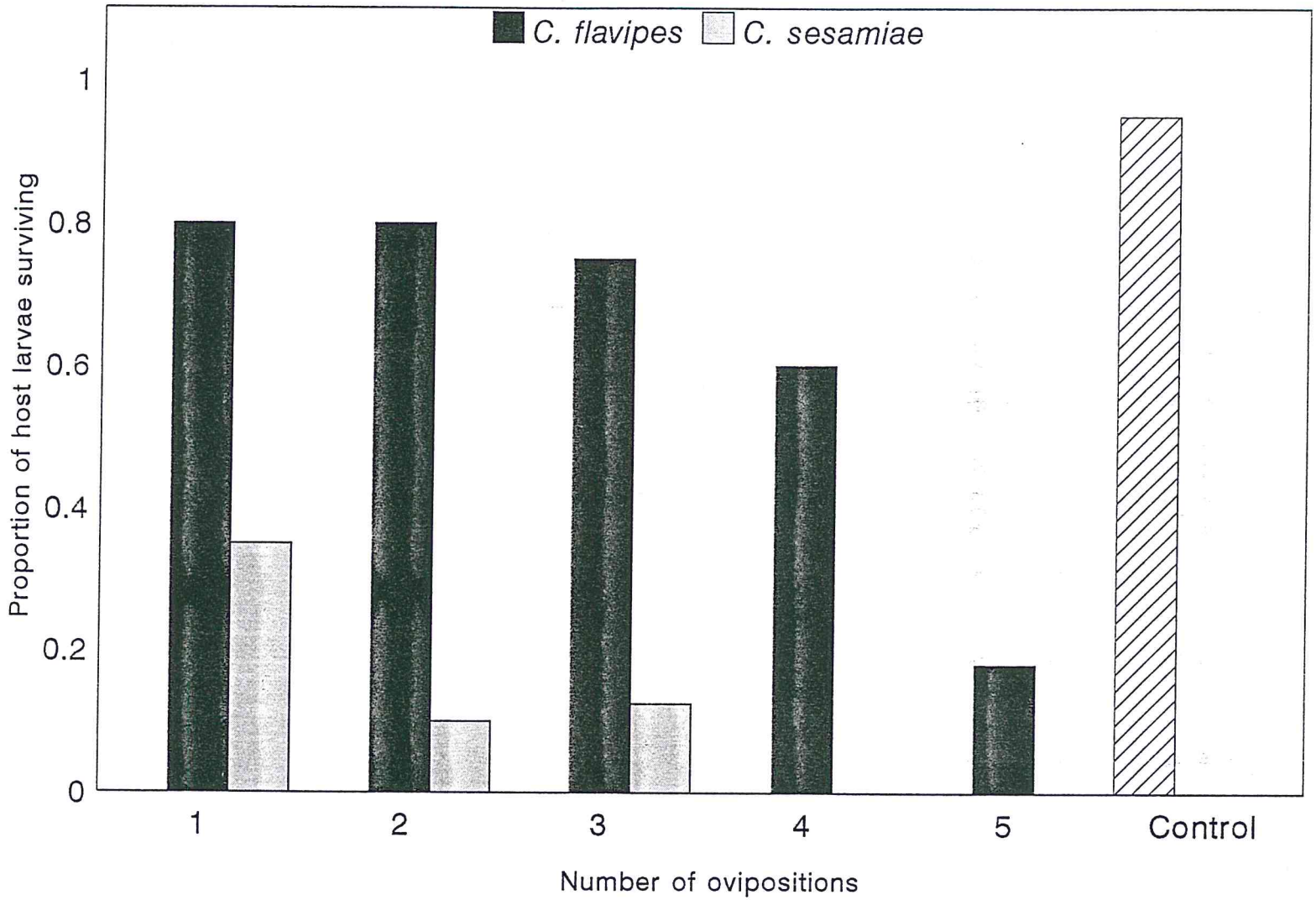


Fig. 5.1. Survival of *C. partellus* 4th instar larvae at different number of stings by the two *Cotesia* species.

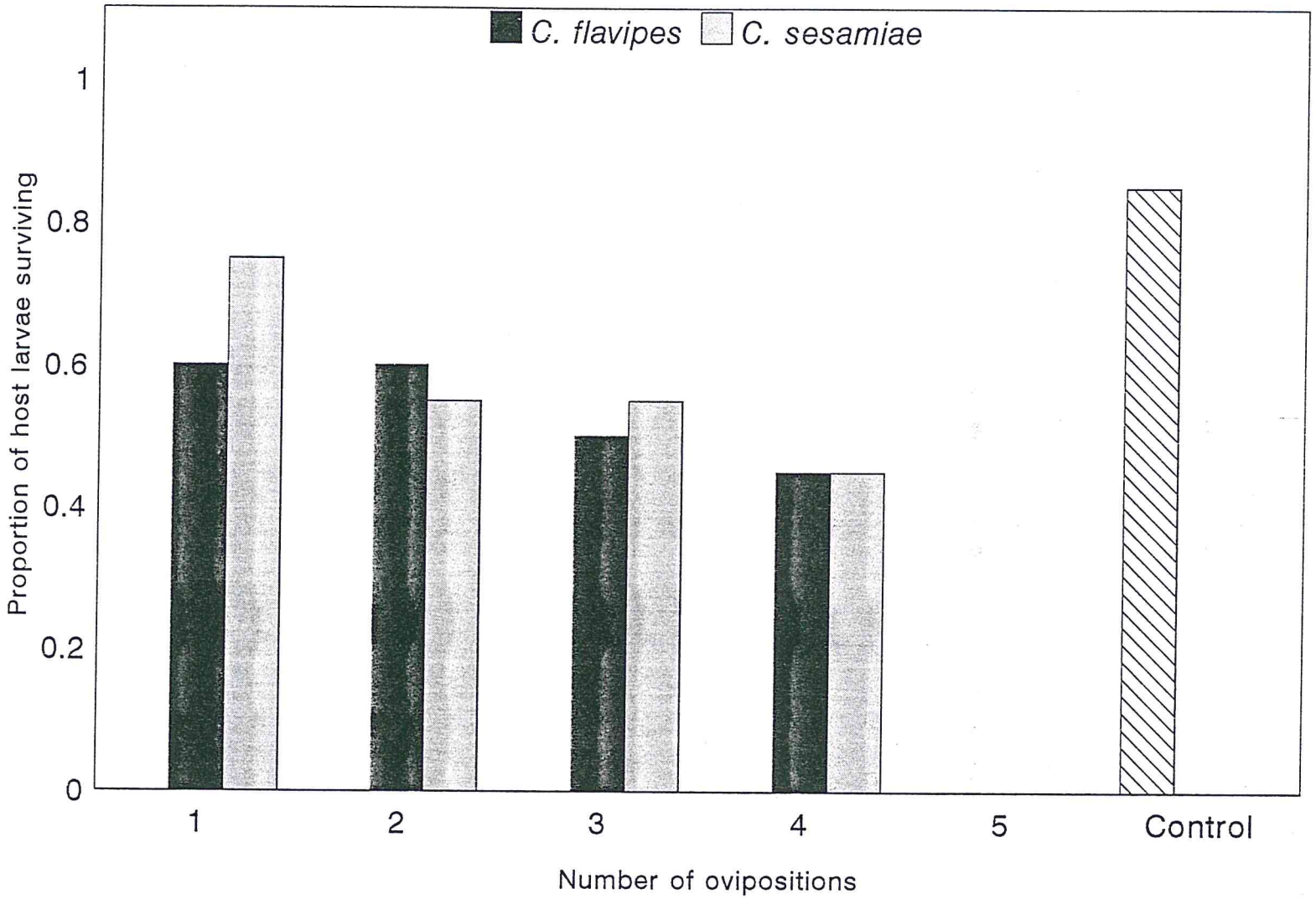


Fig. 5.2. Survival of *S. calamistis* 4th instar larvae at different number of stings by the two *Cotesia* species.

5.3.2 Multiple parasitism

Tables 5.3 and 5.4 show the number of progeny of the two *Cotesia* species resulting from multiple parasitism *C. partellus* and *S. calamistis* 4th instar larvae, respectively, at different time intervals between the first and second sting. When both parasitoids stung *C. partellus* larvae at the same time, only *C. flavipes* emerged in nearly all cases with a higher average female and total progeny than *C. sesamiae*. No *C. sesamiae* progeny emerged from *C. partellus* larvae when *C. sesamiae* stung the host larvae 24 or 48 hours after *C. flavipes*. When *C. flavipes* stung *C. partellus* one day after *C. sesamiae*, a high rate of host larval mortality was recorded (90%), but *C. flavipes* emerged in three cases out of four. Only one *C. partellus* larva produced *C. sesamiae* progeny, and all progeny were males. No progeny of neither parasitoids was obtained when *C. flavipes* stung *C. partellus* two days after *C. sesamiae* as 100% of host larvae died.

When *S. calamistis* was the host, all larvae that survived produced both parasitoids species, but interestingly, with a higher number of *C. flavipes* female progeny and total progeny than the number of *C. sesamiae* female and total progeny when both parasitoids stung the host larvae on the same time. *C. sesamiae* emerged when it stung *S. calamistis* larvae one day after *C. flavipes*. However, when *C. sesamiae* females stung *S. calamistis* larvae two days later than *C. flavipes*, one third of the total number of larvae that produced progeny only produced *C. flavipes*, and two third produced both species with a higher *C. flavipes* female progeny and total progeny than *C. sesamiae*.

Table 5.3 Resulted *C. flavipes* and *C. sesamiae* progeny from multiple parasitised *C. partellus* 4th instar larvae at different time intervals

Simultaneous ovipositions by *C. f*[1], *C. s*[2]	*C. f* stinging first			*C. s* stinging first	
	Time interval between 1st and 2nd sting				
	Same time	24 hrs	48 hrs	24 hrs	48 hrs
No. larvae producing *C.f* only (%)	16 (40%)	13 (32.5%)	16 (40%)	3 (7.5%)	0
No. larvae producing *C.s* only (%)	1 (2.5%)	0	0	1 (2.5%)	0
No. larvae producing both *C.f* & *C.s* (%)	1 (2.5%)	0	0	0	0
No. dead larvae (no progeny produced) (%)	22 (55%)	27 (67.5%)	24 (60%)	36 (90%)	60 (100 %)
Mean *C.f* female progeny/host larva (±SD)	22.9 (±11.9)	23.7 (±13.7)	25.9 (±12.8)	9.0 (±9.9)	0
Mean *C.f* total progeny/host larva (±SD)	30.0 (±15.6)	35.5 (±18.2)	38.1 (±11.8)	12.5(±11.3)	0
Mean *C.s* female progeny/host larva (±SD)	0.22 (±0.73)	0	0	0	0
Mean *C.s* total progeny/host larva (±SD)	0.33 (±0.97)	0	0	0.75 (±1.5)	0

1. *C. f = Cotesia flavipes*
2. *C. s = Cotesia sesamiae*

Table 5.4 Resulted *C. flavipes* and *C. sesamiae* progeny from multiple parasitised *S. calamistis* 4th instar larvae at different time intervals

	Simultaneous ovipositions by *C. f*[1], *C. s*[2]	*C. f* stinging first		*C. s* stinging first	
	Time interval between 1st and 2nd sting				
	Same time	24 hrs	48 hrs	24 hrs	48 hrs
No. larvae producing *C.f* only (%)	0	0	4 (10%)	1 (2.5%)	0
No. larvae producing *C.s* only (%)	0	2 (5%)	0	11(27.5%)	16 (40%)
No. larvae producing both *C.f* & *C.s* (%)	14 (35%)	14 (35%)	8 (20%)	2 (5%)	0
No. dead larvae (no progeny produced) (%)	26 (65%)	24 (60%)	28 (70%)	26 (65%)	24(60%)
Mean *C.f* female progeny/host larva (±SD)	15.7 (±7.13)	6.19 (±6.5)	13.2 (±4.4)	0.14 (±0.5)	0
Mean *C.f* total progeny/host larva (±SD)	30.0 (±9.12)	18.3 (±13.8)	22.0 (±9.0)	1.14 (±3.5)	0
Mean *C.s* female progeny/host larva (±SD)	3.64 (±4.18)	9.13 (±8.9)	2.42 (±2.9)	1.85 (±2.9)	3.0(±2.9)
Mean *C.s* total progeny/host larva (±SD)	8.86 (±4.64)	15.6 (±11.6)	6.5 (±8.18)	6.7 (±6.3)	8.1(±3.9)

1. *C. f = Cotesia flavipes*
2. *C. s = Cotesia sesamiae*

Progeny production of the two species was markedly affected when *C. flavipes* females stung *S. calamistis* larvae 24 hours after *C. sesamiae*, with a *C. sesamiae* male biased sex ratio. No *C. flavipes* progeny was obtained from *S. calamistis* when *C. flavipes* females stung the host larvae 48 hours after *C. sesamiae*, and both female progeny and total progeny of *C. sesamiae* were very low.

Dissection of *C. partellus* 4th instar larvae four days after oviposition by the two *Cotesia* species showed no significant difference in the number of parasitoid eggs in hosts parasitised by one or both of the two species (fig 5.3). When *C. partellus* 4th instar larvae were dissected nine days after oviposition, no significant difference was found in the number of parasitoid immatures between hosts parasitised by both parasitoids and *C. flavipes* only, but were significantly higher than in hosts parasitised by *C. sesamiae*, suggesting a temporal decline in the number of *C. sesamiae* immatures inside *C. partellus* host larvae.

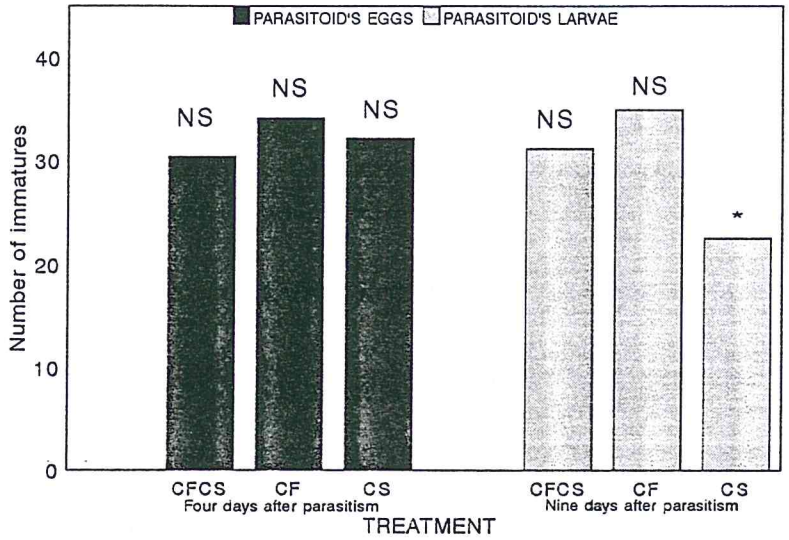


Fig.3. Average number of parasitoid immatures in 4th instar *C. partellus* larvae after 4 and 9 days of stinging respectively.

CFCS4 = Multiple parasitised *C. partellus* 4th instar larva by *C. flavipes* and *C. sesamiae* 4 days after parasitism

CF4 = Single parasitised *C. partellus* larva by *C. flavipes* after 4 days of parasitism

CS4 = Single parasitised *C. partellus* larva by *C. sesamiae* after 4 days of parasitism

CFCS9 = Multiple parasitised *C. partellus* by *C. flavipes* and *C. sesamiae* 9 days after parasitism

CF9 = Single parasitised 4th instar *C. partellus* by *C. flavipes* 9 days after parasitism

CS9 = Single parasitised 4th instarr *C. partellus* by *C. sesamiae* 9 days after parasitism

* indicates a significant difference in treatment (F=5.89,df=5,P=0.0001)

5.4 Discussion

Data in the present study agrees with other studies on fitness consequences of superparasitism which found that superparasitism results in delayed development of parasitoid progeny (Harvey *et al.*, 1993; Vet *et al.*, 1994). In the present study, different host/parasitoid combinations resulted in variable outcomes. For example, the sex ratio of progeny and the percentage of cocoons from which adults emerged was negatively affected only in case of *C. partellus* superparasitised by *C. sesamiae*. Moreover, *C. flavipes* immature stages generally completed their development in a shorter period than *C. sesamiae* in superparasitised *C. partellus* larvae. Superparasitism of *S. calamistis* by *C. sesamiae* delayed parasitoid pupation, but pupation of *C. flavipes* was not affected. It also seems that *S. calamistis*, being a larger size host than *C. partellus*, was able to support the development of variable numbers of *C. flavipes* as no significant difference was recorded in their cocoon weight in response to the number of ovipositions. Moreover, *C. flavipes* adult females emerging from superparasitised hosts were more fecund than *C. sesamiae* over the different numbers of ovipositions not only on its coevolved host, *C. partellus*, but also on *S. calamistis*, suggesting a successful new association between the exotic parasitoid and the indigenous host. The higher potential fecundity of *C. flavipes* could result in a higher population growth rate and confer a competition advantage over *C. sesamiae* in areas where *S. calamistis* is common. Moreover, previous results have shown that when *C. partellus* is the host, *C. flavipes* has a strong functional response (Chapter 3) and a higher rate of intrinsic increase (Mbapila, 1996).

The number of *C. flavipes* progeny emerging from *C. partellus* larvae increased from one to three ovipositions. This finding generally agrees with Beg and Inayatullah (1980) who recorded a progressive increase in cocoon production and adult emergence of *C. flavipes* in *C. partellus* larva stung up to five times. The present study showed a decline in progeny production in hosts stung four or five times. The discrepancy in these results maybe due to the low number of replicates used in their study (five host larvae only). On the other hand, results in the present study disagree with Potting *et al.*, (1997) who did not find any increase in adults production in *C. partellus* larvae superparasitised . In their study, Potting *et al.*, (1997) recorded a significant increase in cocoon production due to superparasitism, but this increase was not reflected in adult production. However, their study agrees with the present study that superparasitism did not significantly affect the resulted progeny's sex ratio, which might be expected in gregarious parasitoids since the larvae do not fight, physiological suppression usually does not occur, and the number of parasitoids a host can support may be variable provided that the time between ovipositions does not give the first clutch too much of a developmental advantage (Strand, 1985). Other studies confirmed the possibility of increasing the number of adult progeny in gregarious parasitoids through superparasitism, as was shown for *Cotesia chilonis* superparasitising *Diatraea saccharalis* (Wiedenmann and Smith, 1995). It can be generally stated that a degree of superparasitism in the field may not negatively influence the impact of *C. flavipes* on stemborer populations. The decrease in the fecundity due to superparasitism may be compensated by the increase in progeny production.

Results of the study on multiple parasitism suggested that *C. flavipes* was intrinsically superior

to *C. sesamiae* when *C. partellus* was the host, which is expected as *C. partellus* is a coevolved host of this parasitoid. However, *C. flavipes* proved to be equally competitive to *C. sesamiae* on *S. calamistis* when hosts were parasitised at the same time or at a one day interval. The relatively higher female and total progeny of *C. flavipes* resulting from *S. calamistis* parasitised by both parasitoids at the same time suggest that *C. flavipes* is a highly competitive parasitoid, and may eventually dominate the ecological niche occupied by *C. sesamiae* in the new ecosystem.

The fact that, no significant difference was found in the number of parasitoid eggs in either multiple parasitised or single parasitised *C. partellus* larvae four days after oviposition by the two species, suggests a possibility of early elimination of *C. sesamiae* by *C. flavipes* during the egg stage, since almost all larvae parasitised by both species produced only *C. flavipes* progeny. Early elimination in this case may be due to a toxic substance or viral particles injected by adult females while stinging the host larvae (Vinson and Ables, 1980). Moreover, the average number of *C. sesamiae* progeny (32.2 parasitoid egg/host larva) four days after stinging declined to 22.5 parasitoid larvae/host nine days after parasitism, confirming that *C. partellus* is not as suitable for the development of *C. sesamiae* as for *C. flavipes*. It remains to be investigated if the factor inducing this early elimination of *C. sesamiae* can be isolated and identified to increase understanding of the host parasitoid interactions in this host/parasitoid system.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

The present study was a part of a collaborative project between the International Centre of Insect Physiology and Ecology (ICIPE) in Kenya, and Wageningen Agricultural University (WAU), the Netherlands. The project's major aim is to suppress population densities of certain lepidopteran gramineous stemborers in Kenya. A classical biological control programme was initiated to introduce *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a gregarious larval endoparasitoid from southeastern Asia, where the pest originated, into Kenya. The exotic parasitoid is now established in Kenya and northern Tanzania. The purpose of the present study was to first compare the efficiency and host finding ability of *C. flavipes*, with that of its African congener, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), which is the most common parasitoid of stemborer larvae in Kenya and other parts of East and Southern Africa, and second, to investigate competition between the two species.

In sub Saharan Africa, maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.)) production is severely reduced by the feeding of lepidopteran stemborers. In Kenya, all stemborers, with the exception of the spotted stemborer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae), are thought to be indigenous (Nye, 1960). Two native stemborers, *Chilo orichalcociliellus* Strand (Lepidoptera: Pyralidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), attack maize and sorghum coincidentally in space and time with *C. partellus* in the southern coastal area of Kenya. Among the three species, *C. partellus* is the most abundant and serious pest,

and there is evidence that it may be displacing the indigenous stemborers (Overholt *et al.*, 1994).

More than 40 parasitoid species are recorded from *C. partellus* in Africa (Bonhof *et al.*,1998) The braconid, *C. sesamiae*, is the most common parasitoid of stemborers in most of East and southern Africa (Mohyuddin and Greathead, 1970; Mathez, 1972; Kfir, 1992). However, in coastal Kenya, generational mortality of *C. partellus* due to *C. sesamiae* during 1992 and 1993 was never greater than 3%, and was typically less than 0.5% (Overholt *et al.*, 1994). Therefore, *C. flavipes*, a coevolved natural enemy of *C. partellus* in Asia, was imported from Pakistan and released at the Kenya coast in 1993 to reestablish the pest/natural enemy relationship that occures in the aboriginal home of the target pest (Overholt *et al.*, 1994).

Collections of *C. flavipes* from two region in Pakistan, Rawalpindi (north Pakistan) and Sindh (south Pakistan), were imported into Kenya in September 1991 and June 1992, respectively. Material from Sindh was released during the long rains of 1993 at three sites in the southern coastal area of Kenya. Recent reports confirmed that *C. flavipes* is now well established at the Kenya coast and in southwestern Kenya (Omwega *et al.*, 1995; Overholt *et al.*, 1997). The present study was a contribution towards understanding the ecological implications of the introduction of *C. flavipes* into East Africa, particularly with regards to competition with its African homologue, *C. sesamiae*, for the two stemborers, *C. partellus* and *S. calamistis*.

6.1 Dispersal of *C. flavipes*

Successful dispersal of an introduced natural enemy is a key factor in its establishment in a new ecosystem. A study of the dispersal ability of *C. flavipes* was conducted in a 100 × 100 m maize field in northern Kilifi district on the Kenya coast. Results of this study are presented in Chapter 2. In this study, it was shown that female parasitoids can fly as far as 64.03 meters downwind during their life span (2-3 days), and that parasitoid attack is dependant on host location within the plant. The majority of parasitised hosts (88.37%) were found inside the plant (stems and tassels), where 74.3 % of the suitable sized larvae are found. However, aggregation of parasitoids in response to plants with different host densities was not detected. The present study used a single plant spatial scale to measure aggregation, which may not have been appropriate (Walde and Murdoch 1988). Aggregation could have occured on a larger scale, such as groups of plants, but gone undetected. A more detailed study with a larger dataset and a range of spatial scales may provide more insight into the response of *C. flavipes* to host density. However, it can be anticipated that *C. flavipes* will continue to disperse and colonise areas where the dominant stemborer species is suitable for its development. In the present study, *C. flavipes* females were able to parasitise 11% of the estimated total number of suitable larvae in the field. This level of parasitism is considered promising, and can be used to estimate the number of parasitoids needed in future releases to obtain high levels of parasitism. Further studies can be designed to investigate if *C. flavipes* acts in a density dependent manner. This will provide important knowledge to better predict the parasitoid's impact on the stemborer population in the field.

6.2 Functional responses of *C. flavipes* and *C. sesamiae*

Functional and numerical response studies were conducted in the field and in the laboratory, to compare the performance of the two *Cotesia* species on two of the stemborers occuring at the Kenya coast, *C. partellus* and *S. calamistis*. Functional response studies measure the number of hosts attacked as a function of host density, while numerical response studies measure the generational increase in the natural enemy population in response to increasing host density. Such studies are useful for comparing the efficiencies and host finding abilities of natural enemies. Results presented in Chapter 3 indicate that *C. flavipes* had a higher searching ability when *C. partellus* was the host. When *S. calamistis* was the host, there was no significant difference between the numbers of stemborers attacked by the two parasitoids. Numerical response studies on *C. partellus* showed that *C. flavipes* produced more total progeny and female progeny per female parasitoid than *C. sesamiae*. No significant difference in progeny production was detected between the two parasitoids on *S. calamistis*. Functional and numerical responses tested in the laboratory gave the same ranking of the two parasitoids on the two hosts as in the field. This suggests that laboratory studies may be useful tool to compare the efficiency of natural enemies. In this study, *C. flavipes* proved to be a more effecient parasitoid than *C. sesamiae* on its coevolved host, *C. partellus*, and equally effecient to *C. sesamiae* on *S. calamistis*. With *C. partellus* being the most abundant stemborer in much of Kenya and other parts of East and southern Africa (Overholt *et al.*, 1994), *C. flavipes* may have an advantage over *C. sesamiae*.

6.3 Host discrimination in *C. flavipes* and *C. sesamiae*

It is generally agreed that a host previously parasitised by a conspecific parasitoid is a less suitable host for parasitisation, therefore, female parasitoids are expected to develop an ability to discriminate between parasitised and non parasitised hosts, thus avoiding superparasitism. However, in gregarious parasitoids, optimal foraging theory suggests that parasitoids should maximise their rate of fitness gain and to add eggs to a host until their gain of fitness drops below the maximum achievable average rate (Godfray, 1994). Adults of the two *Cotesia* species are limited with a short life span of about 2-3 days and a fixed eggload of about 150 eggs. In addition, female parasitoids find their host larvae inside tunnels made in plant stems. This process can be time consuming as females must move through larval frass to access the host. Moreover, parasitising a host is not without risk, since almost 40% of female parasitoids are killed during stinging as a result of aggressive host behaviour (Potting, 1996). Thus, it is expected that *Cotesia* female parasitoids may be less selective and accept previously parasitised hosts. The ability of female parasitoids to distinguish between healthy and parasitised host larvae attacked by either of the two species was examined in Chapter 4, and it was shown that naive females did not exhibit any host discrimination ability. However, experienced females of both species were significantly more attracted to healthy host larvae in fresh tunnels than larvae parasitised by conspecific females. Thus, a degree of superparasitism is expected to take place in the field depending on host availability.

The two *Cotesia* species are allopatric (originally from two different geographical zones), therefore, they are not expected to develop the ability to discriminate between unparasitised

hosts and those parasitised by the other species (Godfray, 1994). In the present study, it was shown that the two parasitoids do not avoid multiple parasitism. Therefore, the theory that allopatric species are not expected to develop an interspecific host discrimination ability (Godfray, 1994) is valid in this case. No selective pressures acted on the two species to avoid multiple parasitism which is likely to happen when two species are sympatric. In conclusion, it can be stated that experienced females of the two *Cotesia* species avoid superparasitism, but do not appear to avoid multiple parasitism. The factor involved in discriminating between hosts parasitised by conspecifics and non-parasitised hosts is volatile, as it was detected from a distance in an olfactometer. The nature of the marking pheromone, and its persistence remain to be investigated.

6.4 Intraspecific and interspecific competition between *C. flavipes* and *C. sesamiae*

As was discussed in Chapter 4, superparasitism as well as multiple parasitism, is expected in the field due to the degree of niche overlap between the two species. The main aim of the study in Chapter 5 was to investigate the fitness consequences of superparasitism among individuals of the two parasitoid species on progeny production, and to explore the intrinsic superiority of one species over the other on the two host species for better understanding of the possibility of their coexistence in nature.

6.4.1 Fitness consequences of superparasitism

The progeny production of *C. flavipes* in *C. partellus* larvae increased gradually as the number of ovipositions increased from one to three, but then decreased as a result of four and five

ovipositions. Cocoon weight, sex ratio and emergence of the progeny were not affected by superparasitism. However, prolonged immature development, shorter longevity of adult progeny and lower potential fecundity of female progeny were found as a result of increasing the number of ovipositions. For *C. sesamiae*, low progeny production and poor survival of *C. partellus* host larvae were found over the different numbers of ovipositions. Larvae that were stung more than three times died. Multiple ovipositions led to a male biased sex ratio and low potential fecundity among female progeny.

When *S. calamistis* was the host, the duration of immature stages, parasitoid emergence, progeny production and sex ratio of the progeny of *C. flavipes* did not differ significantly between the numbers of ovipositions. However, cocoon weight, adult longevity and the potential fecundity of adult females were negatively affected. For *C. sesamiae*, progeny production increased with increasing numbers of ovipositions. No significant effect of superparasitism was realised on emergence, longevity or sex ratio of adult progeny. However, longer duration of immatures, lower cocoon weight and lower potential fecundity of adult females were recorded. In general, *C. flavipes* had a higher progeny production, emergence, sex ratio and higher potential fecundity than *C. sesamiae* when *C. partellus* was the host. When *S. calamistis* was the host, no significant difference was detected in most of the aspects studied between the two parasitoid species. Nevertheless, *C. flavipes* had a higher proportion of emerged adults from cocoons and, interestingly, higher potential fecundity of female progeny than *C. sesamiae* over the different numbers of ovipositions.

It can be concluded that a degree of superparasitism might not significantly reduce the overall impact of *C. flavipes* on stemborer population in the field. The present study agrees with other studies by Waage and Godfray (1985) and Wiedenmann and Smith (1995), who showed that, in other gregarious parasitoids, the number of parasitoids a host can support is somewhat variable, and that a gregarious parasitoid may benefit from superparasitising the same host (Strand, 1985).

6.4.2 Multiple parasitism

C. flavipes proved to be intrinsically superior to *C. sesamiae* when *C. partellus* was the host, and it was capable of eliminating *C. sesamiae* during the egg stage. On *S. calamistis*, *C. flavipes* proved to be equally competitive to *C. sesamiae* when hosts were parasitised at the same time or at a one day interval. Based on these results, it can be concluded that *C. flavipes* is a highly competitive parasitoid. Local displacement of *C. sesamiae* is therefore likely to happen in areas dominated by *C. partellus*.

6.5 Conclusion

An improved predictability of natural enemy behaviour will stimulate the application of biological control (Lewis *et al.*, 1990). The present study provides basic knowledge of the interspecific relationships between the exotic parasitoid, *C. flavipes* and its African homologue, *C. sesamiae*. This knowledge may facilitate prediction of their coexistence in nature. For example, it was shown that *C. flavipes* is able to eliminate *C. sesamiae* during an

early stage of development when *C. partellus* was the host. If any of the two parasitoids is not able to avoid previously parasitised hosts by the other species, then a strong possibility of outcompeting *C. sesamiae* is therefore expected. Competitive displacement of *C. sesamiae* is likely in areas dominated by *C. partellus*, which includes many lower elevation maize growing areas in East Africa.

A controversial question is: Would the displacement of *C. sesamiae* by *C. flavipes* be considered a "negative impact" on the environment?. The present study suggests that *C. flavipes* is a more efficient parasitoid, and therefore is likely to result in increased suppression of stemborer populations. It is generally agreed that the displacement of a natural enemy by a second can only occur if the second one is more effective, and therefore should produce better host population regulation. Moreover, competition between natural enemies is not detrimental to host regulation (Debach and Rosen, 1991).

True ecological homologues cannot coexist in the same habitat (Debach and Rosen, 1991). *C. flavipes*, though capable of parasitising a fairly wide host range of noctuid and pyralid stemborers, does not produce progeny on the indigenous stemborer, *Busseola fusca*, which is a suitable host for the development of *C. sesamiae* occurring in western Kenya (Ngi-Song *et al.*, 1995). Moreover, Mohyuddin (1971) found that *C. flavipes* is better adapted to drier climates then *C. sesamiae*. Thus, complete eradication of *C. sesamiae* is highly unlikely to happen as a result of the introduction of *C. flavipes*. Such differences in suitable habitats will enhance

stability of the parasitoid community in Kenya.

Another important aspect that can be concluded from the present study, is the successful new association relationship that was demonstrated between *C. flavipes* and the indigenous stemborer, *S. calamistis*. Wiedenmann and Smith (1995) stated that host-parasitoid physiological interactions can be predicted for old associations, however, they are less predictable in novel associations because of no coevolutionary history between the pest and the natural enemy. When a natural enemy is introduced into a new region, new associations can develop when a related host species occupies a niche similar to that of the original host species (Hokkanen and Pimentel, 1989). This seems to be the case with *C. flavipes* on *S. calamistis*. The fact that *C. flavipes* females emerging from *S. calamistis* were more fecund than the ones obtained from *C. partellus* suggests that, in some cases, classical biological control may also be applicable for native as well as exotic pests (Carl, 1982). It can therefore be anticipated that *C. flavipes* will contribute to the control of the indigenous stemborer as well as the exotic one.

It is proably still early to notice the impact of *C. flavipes* on stemborer populations in Kenya. During early stages of initial colonisation, dispersal of a natural enemy may have a counteracting influence on increases in density. Adequate levels of parasitism might not be realised before several years (Betbeder-Matibet and Malinge, 1967; Alam *et al.*, 1971). However, the superiority of *C. flavipes* to its African congener, *C. sesamiae*, may result in a higher level of pest regulation.

The invasion of *C. partellus* may have led to partial displacement of some indigenous stemborers (Overholt *et al.*, 1994). If *C. flavipes* attacked only the exotic stemborer, then it is likely that native stemborer populations would expand to fill a partially empty ecological niche. Thus, the ability of *C. flavipes* to utilize other stemborers may increase its colonising ability and provide an overall suppression of the stemborer complex. Therefore, based on the results from the present study, and the success of *C. flavipes* against various tropical stemborers, it can be generally stated that *C. flavipes* will contribute to the natural control of *C. partellus* and other, less important, native stemborers in East Africa.

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