

HUNTING FOR HIDING HOSTS

the behavioral ecology of the
stem-borer parasitoid *Cotesia flavipes*.

Promotor: dr. J.C. van Lenteren
Hoogleraar in de Entomologie,
in het bijzonder de Oecologie van Insekten

Co-promotor: dr. L.E.M. Vet
Universitair Hoofddocent in de Dieroecologie

N1108201, 2109

Roel P.J. Potting

HUNTING FOR HIDING HOSTS
the behavioral ecology of the
stemborer parasitoid *Cotesia flavipes*.

Proefschrift

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr. C.M. Karssen,
in het openbaar te verdedigen
op maandag 17 juni 1996
des namiddags vier uur in de Aula
van de Landbouwuniversiteit te Wageningen.

ISBN 923339

*Aan mijn ouders
Voor Mariëlle*

BIBLIOTHEEK
LANDBOUWUNIVERSITEIT
WAGENINGEN

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Potting, Roland Peter Joseph

Hunting for hiding hosts: the behavioral ecology of the stemborer parasitoid *Cotesia flavipes* / Roland Peter Joseph Potting. - [S.l. : s.n.]. - Ill. Thesis Wageningen. - With ref. - With summary in Dutch.

ISBN 90-5485-551-7

Subject headings: Hymenoptera / parasitoid / foraging behavior.

The research described in this thesis was carried out at the Department of Entomology, Wageningen Agricultural University, The Netherlands and the International Centre for Insect Physiology and Ecology, Kenya and was funded by the Netherlands Foundation of Scientific Research in the Tropics (WOTRO - W84-325).

Copyright 1996 R.P.J. Potting

Cover design by Mariëlle Wintgens and Piet Kostense

Stellingen

1. Het is gemakkelijker om aan te tonen, dat een bepaald gedragsmechanisme optreedt, dan het bewijs te leveren dat dit gedrag door een bepaalde diersoort niet gebruikt wordt.
Dit proefschrift
2. Het publiceren van negatieve resultaten zou gestimuleerd moeten worden om de kloof tussen theorie en praktijk te verkleinen.
Dit proefschrift
3. Hoewel anekdotes geen definitief bewijs leveren over het belang van gedragsprocessen, kunnen deze een belangrijke bijdrage leveren aan hypothesevorming en toetsing van gedragsoecologische theorieën.
Godfray, H.C.J. 1994. Parasitoids, behavioral and evolutionary ecology. Princeton University Press, New Jersey.
4. Biologische bestrijdingsprojecten, die inzicht verschaffen in het niet slagen van een vestiging van een natuurlijke vijand, zijn ook succesvol.
Mackauer, M., Ehler, L.E. and Roland, J. (eds.) 1990. Critical issues in biological control. Intercept, Andover.
5. Daar verschillende natuurlijke vijanden het sexferomoon van hun gastheer gebruiken als kairomoon, verdient dit fenomeen aandacht bij de toelating van gecombineerde sexferomoon/insecticide produkten, daar de veronderstelde selectieve werking van het attracticide ook de populatie van de natuurlijke vijand kan beïnvloeden.
Minks, A.K. 1990. In: Behavior modifying chemicals for insect management: applications of pheromones and other attractants. Ridgway, R.L. Silverstein, R.M. and Inscoc, M.N. (eds.). Marcel Dekker, New York. pp. 557-568.
6. Het feit, dat het gedrag van natuurlijke vijanden is aangepast aan een puntbronverdeling van attractantia, maakt de praktische uitvoering van gedragsmanipulatie met behulp van chemische signaalstoffen vrijwel onmogelijk.
Lewis, W.J., Beevers, M., Nordlund, D.A., Gross, H.R. and Hagen, K.S. 1979. J. Chem. Ecol. 5: 673-680.
7. Een onderschatte factor in het slagen van bestrijding, gebaseerd op de verwarringstechniek met feromonen, is de rol van natuurlijke vijanden, die door het ontbreken van insecticide-applicaties niet meer onderdrukt worden en dus een belangrijke rol kunnen spelen bij de reductie van plaagpopulaties.
Cardé, R.T. and Minks, A.K. 1995. Annu. Rev. Entomol. 40: 559-585.

-
8. De destructieve werking van de microsporide *Nosema* op insektenkweken is indrukwekkend en verdient meer aandacht.
Walters, H.S. and Kfir, R. 1993. African Entomol. 1: 57-62.
 9. Het enige feit betreffende de origine van de mens dat absoluut zeker is, is dat de discussie erover zal blijven doorgaan.
Avisé, J.C. 1994. Molecular markers, natural history and evolution, Chapman and Hall, New York.
 10. De bureaucratie en geldverspilling van westerse instellingen op het gebied van ontwikkelingssamenwerking doen niet onder voor die van de landen waarvoor ze bedoeld zijn.
Hancock, G. 1989. Lords of poverty. MacMillan, London.
 11. Onder elke klik van de muis zitten het bloed, zweet en tranen van een onbekende programmeur.

Stellingen behorend bij het proefschrift:

*Hunting for Hiding Hosts:
the behavioral ecology of the stemborer parasitoid Cotesia flavipes.*

Roel Potting

Wageningen, 17 juni 1996.

Chapter 1	
General introduction	1
Chapter 2	
Host microhabitat location by the stemborer parasitoid <i>Cotesia flavipes</i> : the role of locally and systemically induced plant volatiles.	23
Chapter 3	
Foraging behavior and life history of the stemborer parasitoid <i>Cotesia flavipes</i> .	39
Chapter 4	
Fitness consequences of superparasitism and the mechanisms of host discrimination in the stemborer parasitoid <i>Cotesia flavipes</i> .	55
Chapter 5	
The relation between parasitoid ecology and learning: the absence of learning in the stemborer parasitoid <i>Cotesia flavipes</i> .	71
Chapter 6	
Geographic variation in host selection behaviour and reproductive success in the stemborer parasitoid <i>Cotesia flavipes</i> .	89
Chapter 7	
Summarizing Discussion	109
Samenvatting	117
Afterword	122
List of publications	124
Curriculum vitae	125

1

General Introduction

BEHAVIORAL ECOLOGY OF PARASITIDS AND BIOLOGICAL CONTROL

Behavioral ecology is concerned with the evolution of adaptive behavior in relation to ecological circumstances (Krebs and Davies, 1981). Because an individual's success at surviving and reproducing depends on its behavior, natural selection will favor animals with efficient foraging strategies. Insect parasitoids forage for hosts that directly support the development of their progeny. The lifetime reproductive success of a female parasitoid is depended on the number and quality of hosts located and utilized in her lifetime. Thus, host foraging in parasitoids is closely linked to fitness, which make parasitoids ideal organisms to study the adaptive value of foraging decisions (Godfray, 1994). These foraging decisions include for example where to search for hosts and for how long (e.g. Van Alphen and Vet, 1986; Stephens and Krebs, 1986; Vet et al., 1995). Once a host is located the female has to decide whether the host should be accepted and if so, how many eggs to lay and in which sex ratio (e.g. Waage, 1986; Godfray, 1987; Van Alphen and Visser, 1990). The behavioral ecology of insect parasitoids is not only interesting from a theoretical evolutionary perspective, but also from an applied perspective, because parasitoids are frequently used in biological control of insect pests.

In biological control parasitoid establishment and parasitism rates are often variable. For biological control to be a reliable and efficient method insight is needed in the foraging behavior of the natural enemy used. The ability of parasitoids to locate and attack hosts is a key determinant of how well a given parasitoid population performs. Thus variability in this host-location ability can be a major source of inconsistent results in biological control with parasitoids.

The need to improve the scientific basis of biological control is increasingly being expressed (Van Lenteren, 1980; Mackauer et al., 1990). A more complete understanding of the ecology and behavior of natural enemies is critical when we want to increase the reliability and predictability of biological control with insect parasitoids (Lewis et al., 1990; Luck, 1990). Behavioral ecology theory forms a suitable framework to design research programs for biological control (Waage, 1990; Luck, 1990), such as the development of selection criteria for biological control agents, the improvement of mass-rearing of selected natural enemies and the

evaluation of success or failures of released natural enemies.

VARIATION IN PARASITOID BEHAVIOR

Intraspecific variation in parasitoid physiology and behavior has long been speculated to be one of the factors responsible for variability in effectiveness of biological control. For instance, biological control workers frequently refer to races, strains or biotypes that differ in biological traits considered to be important for successful biological control (e.g. González et al., 1979; Caltagirone, 1985). Failure of a biological control project may result from an incorrect identification of the species involved or a selection of an inappropriate strain.

Especially in augmentive (seasonal) releases of laboratory reared parasitoids it is essential that a strain is selected with the highest efficiency against the target pest species in a given environment to minimize seasonal production costs. Among the criteria used for this selection are traits related to reproductive capacity and searching activity (e.g. Chambers, 1977; Bigler, 1989; van Lenteren, 1991). Several researchers have explored the possibilities to manipulate the behavioral plasticity of parasitoids (Lewis and Martin, 1990), either before release (e.g. Gross et al., 1975) or in the field (e.g. Lewis and Martin, 1990), especially for parasitoids used in inundative and seasonal programs. In classical biological control, where a new natural enemy is introduced in a particular region without repeated releases, the central concern is that genetic variation for attributes that affect success is either not collected or is lost during the importation and colonization process (Roush, 1990; Hopper et al., 1993). Among the criteria considered to be important in introduction programs are physiological compatibility (i.e. parasitoid virulence) and climatic and phenological (ecological) adaptability.

PHENOTYPIC PLASTICITY

The behavior of parasitoids is not fixed, but variable within and among individuals of a population. The proximate basis for this behavioural plasticity includes the physiological state and the informational state (i.e. learning processes) of the animal. Among the physiological factors influencing female parasitoid foraging decisions are: eggload (e.g. Rosenheim and Rosen, 1991), hunger status (e.g. Wäckers, 1994; Heimpel and Rosenheim, 1995) and disease (Hamm et al., 1988). The informational state (i.e. internal view of external environment) of a parasitoid is influenced by its foraging experiences. For instance, parasitoid behavior may change after an experience with parasitized hosts (e.g. Visser et al., 1992), the presence of conspecifics (Visser et al., 1990) or related species (Janssen et al., 1995) and after experiencing different day-lengths during its development (Roitberg et al., 1992). The

role of learning in parasitoid host foraging is well studied (recent reviews: Vet and Groenewold, 1990; Turlings et al., 1993; Vet et al., 1995) and considered as an important factor influencing parasitoid foraging behavior (Lewis et al., 1990; Vet et al., 1990). It has been demonstrated that parasitoid females can learn to associate odors and/or visual cues with successful host location (e.g. Arthur, 1966; Lewis and Tumlinson, 1988). Odor learning in host foraging is very common in parasitoids and is sometimes triggered by pre-adult learning, as a consequence of development in a particular host and its food, but more commonly as an adult by experience during foraging.

GENETIC DIVERSITY

Parasitoid populations utilize different resources over the species' entire geographical range and local populations may experience different selection pressures. Populations from different regions may thus each be adapted to utilize specific resources and a generalist species can actually consist of specialist local populations (Fox & Morrow, 1981; Futuyma & Peterson, 1985). The differentiation of populations within a species may result in populations that differ biologically from other conspecific populations and are referred to as a strain or race (Diehl & Bush, 1984). The identification of strains is based on biological parameters such as host and habitat specificity, diapause habit and climatic adaptability (Roush, 1990; Hopper et al., 1993). Well known and studied examples of biological differences in morphologically identical species or strains are *Aphytis* spp. (Rosen, 1994) and *Trichogramma* spp. (Pak, 1988; Pinto and Stouthamer, 1994). For instance, recognition of biological differences between morphologically identical strains of *Aphytis maculicornis* Masi was the key to successful biological control of olive scale, in California (DeBach et al., 1971).

An uncritical list of reports on intraspecific variation in parasitoid traits is given by Ruberson et al. (1989). Using iso-female lines, Prevost and Lewis (1990) made an attempt to reveal genetic variation for olfactory responses within a parasitoid population. Reviews of reports on between-population variation in parasitoid (life history) traits in relation to biological control are given by González et al. (1979) and Caltagirone (1985) and more detailed lists are given by Hopper et al. (1993) and Unruh and Messing (1993). Although often discussed as genetic differences, inter-strain variation is only weak evidence for genetic differences, as emphasized by Hopper et al. (1993). Populations often originate from different environments and/or are tested under different conditions, hence conditioning effects (i.e. early-adult learning) can mimic genetic differences. Furthermore, what was originally thought to be variation among strains has sometimes turned out to be variation among sibling species (e.g. Vet et al., 1984; Haardt and Höller, 1992).

What follows is a selective overview of the available evidence of the existence of parasitoid strains, with emphasis on the relation to biological control.

Micro-habitat-adapted strains

For phytophagous insects there is ample evidence for intraspecific variation in physiological adaptation to specific host plants and differential selection of host plants as oviposition site (Futuyma and Peterson, 1985; Jaenike, 1990; Jaenike and Holt, 1991; Via, 1990).

Natural enemies have evolved in a multitrophic system and the behavior and physiology can be influenced by the habitat (i.e. food plant) of their hosts. Thus, natural enemy populations may become locally adapted to a particular micro-habitat of their host. Populations may differ in their behavioral response to stimuli from the hosts's micro-habitat or they may differ in their physiological adaptation to a particular food of their host. There are only a few studies which have studied plant-adaptation in parasitoid populations.

One example of physiological adaptation of parasitoids to plants is the relation between *Cotesia congregata* (Say), its host *Manduca sexta* L. and the food plants tomato and tobacco. The survival of *C. congregata* is directly affected by host-ingested nicotine, a major alkaloid of tobacco (Barbosa et al., 1991). Kester and Barbosa (1991) showed that two populations of *C. congregata*, which differed in their present and historical exposure to tobacco, differed in their tolerance to dietary nicotine and willingness to search on tobacco plants (Kester and Barbosa, 1994), suggesting an adaptation to tobacco by the (Marlboro!) parasitoid population with the longest exposure to tobacco.

Differentiation in behavioral response to stimuli from the host's micro-habitat has been demonstrated for populations of the *Drosophila* parasitoid *Asobara tabida* (Mollema, 1988; Kraaijeveld et al., 1994) and the existence of plant-specific strains has been postulated for the stemborer parasitoid *Cotesia flavipes* (Mohyuddin et al., 1981).

Host-adapted strains

One of the key factors of a successful biological control program is the ability of the released parasitoid population to successfully parasitize the local host population. Parasitoid populations may be physiologically adapted to particular host species (or local populations of a particular host species). Simmonds (1963) gives several examples of host-adapted geographic strains of the stemborer parasitoids *Paratheresia claripalpis* Wulp, *Palpozenilla palpalis* (Ald.) and *Lixophaga diatraea*. To give a typical example, Mexican and Trinidad strains of *P. claripalpis* were both able to develop on *D. saccharalis* larvae, but only the Mexican strain was able to develop on its sympatric host *Zeodiatraea lineolata* (Simmonds, 1963). Other well studied examples

of a differential preference, acceptance and host suitability between parasitoid populations have been demonstrated for *Leptopilina boulaudi* (Bouletreau, 1986) and *Asobara tabida* (Kraaijeveld and van der Wel, 1995; Kraaijeveld et al., 1995).

Climate adapted / seasonal synchronized strains

In classical biological control, the released parasitoid population should be adapted to the local (new) climate and the biology of the parasitoid must be well synchronized with that of the host, so that it is in phase with the susceptible stages of the host. This synchronization must extend through quiescent stages of the host's annual cycle (as in diapause) or there must be alternative hosts that can carry the natural enemy through such phases. It has been demonstrated that parasitoid populations from different altitudes or regions may differ in their diapause behavior (e.g. Kenis, 1994; Kraaijeveld and van Alphen, 1995) or that specific diapause behavior may be obtained through selective breeding (e.g. Van Houten et al., 1995). There are several examples of the application of climate adapted parasitoid strains in biological control programs (e.g. Flint, 1980; Pak and Heiningen, 1985).

A multiple strain approach has been applied in a (classical) biological control project against the Argentine stem weevil *Listronotus bonariensis* (Coleoptera) in New Zealand. Here several strains from a range of ecoclimatic zones in South America of the parasitoid *Microctonus hyperdodae* (Braconidae) were released in equal numbers at different sites (Goldson et al., 1993). Using morphometric identification it was demonstrated that particular strains established in particular regions, reflecting differences in diapause ability (Phillips et al., 1994).

Insecticide resistant strains

Several authors have advocated selective breeding in the laboratory to obtain strains of a parasitoid adapted to given environmental conditions (e.g. Hoy, 1979). Especially in the field of insecticide resistance some progress has been made in obtaining insecticide resistant strains of predators (e.g. Hoy, 1985) and parasitoids (e.g. Spollen and Hoy, 1992).

OBJECTIVE OF RESEARCH

The aim of the research presented in this thesis was to provide insight in the foraging behavior of a parasitoid used in biological control of stemborers. *Cotesia flavipes*, a larval parasitoid used worldwide in biological control against tropical stemborers, has a wide host range in diverse habitats. The existence of different geographic, host and/or plant specific strains in *C. flavipes* has been postulated. This hypothesis is based on laboratory studies on plant preference behavior of different

strains (Mohyuddin et al., 1981; Shami & Mohyuddin, 1992), and on anecdotal notes of increased local (field) parasitism rates after release of particular parasitoid strains (Mohyuddin, 1990b). One of the initial objectives of the present research was to achieve insight into the factors causing the assumed differences between *C. flavipes* strains. Therefore we determined to what extent the reported plant and host specificity in *C. flavipes* has a genetic basis or is due to phenotypic plasticity through learning. We determined the behavioral plasticity (i.e. learning processes) and genetic diversity (between population variation) in the preference for and performance on particular plant-host-complexes. In addition to this, the behavioral ecology of this stemborer parasitoid was addressed from a more theoretical perspective. It was investigated how the life history of the parasitoid and its foraging environment may have shaped its foraging strategies, with respect to the role of learning, the pattern of clutch size allocation and the acceptance of low quality (i.e. already parasitized or unsuitable) hosts.

OUTLINE OF THESIS

The behavioral ecology of *C. flavipes* was investigated from an applied as well as a theoretical perspective. In the first part gaps in the knowledge on behavioral ecology of *C. flavipes* are studied which include the long- and short-range searching behavior, some aspects of the life history and host discrimination abilities. The second part focuses on the intraspecific variability in *C. flavipes* behavior and here we determine to what extent the reported plant and host specificity in *C. flavipes* has a genetic basis or is due to phenotypic plasticity through learning.

Elucidation of volatile stimuli involved in host location

Earlier reports on the foraging behavior of *C. flavipes* mainly focused on the short-range behavior, in particular the contact-response to larval frass (Kajita and Drake, 1969; Mohyuddin, 1971; Mohyuddin et al., 1981; Leerdam et al., 1985). The use of volatile infochemicals in the long-range host micro-habitat location is well documented for several parasitoid species (Vet and Dicke, 1992), but was never investigated for *C. flavipes*. Recent studies have demonstrated that several herbivore-infested plant species release volatiles attractive to insect predators and parasitoids. The release of these chemical signals (i.e. herbivore induced synomones) is not restricted to the infested plant parts, but occurs systemically throughout the plant (for review see Dicke, 1994). The experiments described in chapter 2 determined the plant and/or host origin of olfactory stimuli involved in location of stemborer infested plants. It was further investigated whether the endophytic feeding larvae in the stem part of the plant could induce the systemic release of herbivore induced

synomones by the undamaged leaves of the plant.

Short range host location and life history

It is generally accepted that *C. flavipes* females attack the stemborer larvae by ingressing the stemborer tunnel (Smith et al., 1993). However, the actual foraging behavior on an infested plant and inside a stemborer tunnel was never investigated. Chapter 3 focuses on some aspects of the life history, such as initial eggload, clutch size allocation and longevity, and describes the foraging behavior of female *C. flavipes* on stemborer infested maize plants. Furthermore, the behavior of the parasitoid and host inside a stemborer tunnel was investigated using artificial transparent tunnels. The experiments revealed that attacking a defending stemborer larva in the confined space of a tunnel is very risky for the parasitoid female. A considerable proportion of the parasitoids is killed by the defending host. The possible consequences of this risky foraging strategy for the evolution of life history traits and foraging decisions in *C. flavipes* are discussed.

Utilization of previous visited sites and hosts

Superparasitism is the deposition of eggs in a host that has already been parasitized by the female itself or by a conspecific. Many parasitoid species are able to detect the previous presence of conspecifics, a phenomenon referred to as host discrimination (van Lenteren, 1981; van Alphen and Visser, 1990). In chapter 4 the fitness consequences of superparasitism and the behavioral mechanism of host discrimination are described for *C. flavipes*. Considering the risky foraging strategy it is hypothesized that *C. flavipes* has evolved an innate ability to discriminate and that the time-saving and life-saving benefit of discrimination is greatest if a female can parasitized hosts by means of external cues, before contacting the offensive host.

Phenotypic variation: behavioral plasticity through learning

Theory on the adaptive value of learning in foraging suggests that an animal foraging in a predictable homogeneous environment and/or making only a few foraging decisions is not expected to use learning in foraging (Papaj and Lewis, 1993). For insect parasitoids, learning during foraging is well documented (Turlings et al., 1993; Vet et al., 1995). Host seeking females can learn olfactory and visual cues associated with their hosts or the hosts micro-habitat and experience can strongly affect preference for major foraging cues. In chapter 5 the role of odor learning in host foraging in *C. flavipes* is discussed, using experimental procedures similar to other parasitoid learning studies. The learning mechanisms priming (i.e. increase in response) and preference-induction in *C. flavipes* were determined at two stages in the life cycle of the parasitoid: upon emergence from the stemborer tunnel and at oviposition. It is hypothesized that early-adult learning could be the basis for the

plant-specific strains in *C. flavipes* that Mohyuddin et al. (1981) postulated. Taking the ecology of *C. flavipes* into account it is further hypothesized that adult learning does not play a significant role in host foraging in this parasitoid species.

Genetic variation: behavioral and physiological strain comparison

Local parasitoid populations may be specifically adapted to their sympatric major plant host complex. Local variation in the use of resources has important implications for the evolution of ecological specialization and is relevant to host race formation and speciation (Futuyma and Peterson, 1985; Bush, 1994). It also has important implications for biological control, because the selection of appropriate strains is a significant factor in successful biological control (Roush, 1990; Lewis et al., 1990).

The existence of plant and/or host specific strains in *C. flavipes* has been postulated by Mohyuddin et al. (1981). In chapter 6 experiments are presented that investigated the between-population variation in plant-host-complex preference and reproductive success in *C. flavipes*. The behavior and physiology (i.e. virulence) was compared of six different geographic strains of *C. flavipes* that differed in the plant-host-complex from which they were obtained. Two strains originated from *C. partellus* on maize and four strains from other stemborers species on sugarcane. The comparisons were made under controlled conditions in a comparative setup. First it was established if the strains differed in micro-habitat preference and in host species acceptance and subsequently if the strains differed in their reproductive success on different host species.

Genetic variation: molecular identification of strains and cryptic species

The differentiation of populations within species in biologically different populations are referred to as a strain or race (Diehl and Bush, 1984). Species can be seen as a common gene pool that is variable due to the different (environmental) local circumstances. Using modern molecular techniques the variability within a gene pool can be characterized (Avise, 1994). An important genetic marker used to separate species and strains is ribosomal DNA (rDNA) and in particular the (non coding) internal transcribed spacer (ITS). In comparison with coding regions, spacer regions evolve rapidly and can be used for comparison of closely related species or populations (e.g. Sappal et al., 1995; Van Kan et al., 1996). The objective of the study was to see whether we could differentiate the six geographic *C. flavipes* strains used in the present study with the use of molecular techniques, based on PCR followed by nucleotide sequencing of rDNA-ITS. Furthermore, it was tested whether the cryptic *Cotesia* spp. in the *Cotesia flavipes* complex (*C. flavipes*, *C. chilomis* and *C. sesamiae*) could be distinguished with this technique. If successful, the origin of a

particular field population may be elucidated and a fast identification technique for the cryptic *C. flavipes* complex species may be available. Unfortunately, the experiments are still in progress and due to time constraints the results are not presented in this thesis but will be published later.

PLANT HOST PARASITOID SYSTEMS

The research presented in this thesis deals with the braconid parasitoid *C. flavipes*, a natural enemy of gramineous stemborers. What follows is an overview of the biology of stemborers, their host plants and a review of the research done on *C. flavipes*.

The hosts

Stemborers are chiefly Lepidoptera belonging to the families Pyralidae (e.g. *Chilo* Zincken; *Diatraea* Guelding; *Ostrinia* Hübner) and Noctuidae (e.g. *Busseola* Thunberg; *Sesamia* Guenée) (reviews: Bleszynski, 1969; Harris, 1990). The life cycle of these stemborer species is very similar. Adult moths usually deposit their eggs in clusters on plant leaves and stems. Early-instar larvae feed cryptically in the leaf whorl before tunnelling into the stem. Older larvae feed in excavated tunnels inside the plant stem. The feeding tunnel is maintained relatively clean and stemborer larvae deposit their frass outside the entrance of the tunnel. Pupation normally occurs in the stem in a pupal chamber constructed near the tunnel entrance.

The plants

The host range of lepidopteran stemborers consists mainly of grasses (*Graminae*), sedges (*Cyperaceae*) and cat-tails (*Typhaceae*). Economically important plants attacked by stemborers include the staple food crops of maize (*Zea mays*), millet (*Pennisetum spp.*), sorghum (*Sorghum bicolor*) and sugarcane (*Saccharum officinarum*). These cultivated plants were each domesticated from their wild relatives between 7000-12000 years ago. Through domestication the small seeded perennial wild grasses were converted into large-grained annual plants that may have lost resistance/compensation mechanisms against stemborers. For instance, maize has lost its ability to tiller (i.e. formation of young shoots after damage) and stemborer survival is much higher on maize compared to selected wild grasses (Rosenthal and Welter, 1995), probably due to a lower silica content in maize compared to wild grasses (McNaughton et al., 1985; Setamou et al., 1993).

Stemborers are considered as one of the most economical important pests of maize, sorghum and sugarcane. Crop losses are attributed to the stem tunnelling habit of the later-instar larvae. Tunnelling in young gramineous plants usually destroys the

apical meristem and stops growth of the injured shoot, creating a 'deadheart' condition. Extensive larval tunnelling weakens the stem and provides sites for invasion by plant pathogens (Seshu Reddy and Walker, 1990). The concealed lifestyle of stemborer larvae inside stems has certainly enhanced their potential to be transported around the world on propagative plant material. There is an anecdotal example related to the work presented in this thesis. In 1639 the Dutch introduced several plant species of economic interest on the island of Mauritius, including sugarcane from Indonesia (Batavia). In 1850 the sugarcane stemborer *Chilo sacchariphagus* was reported for the first time on the island. In the same year a shipment from Java of about a million sugarcane cuttings of sugarcane was received in Mauritius and with it apparently arrived the sugarcane stemborer *C. sacchariphagus*, which is now the major pest species in sugarcane on Mauritius (Williams, 1983).

The parasitoids

Despite their hidden lifestyle stemborers do not escape parasitism. Parasitoids have evolved several amazing strategies to attack their concealed hosts inside the plant stem. Smith et al. (1993) divided the attack tactics of parasitoids of stemborer larvae in six categories. Parasitoids with the 'Probe and sting' tactic probe with their ovipositor into the leaf sheath to find early-instar larvae. Other species probe through the exit hole of the tunnel to find mature larva. A related tactic is the 'wait and sting' strategy, where the parasitoid inserts her long ovipositor through one of the tunnel holes and then waits till the host larva passes by and is close enough for oviposition. Parasitoids with the 'Drill and sting' strategy have long and strong ovipositors and drill through the plant stem with their ovipositor to parasitize the host. Several Tachinid parasites have the 'Planidial ingress' tactic, where the female larviposits a mobile maggot at the tunnel entrance that will actively search for the host larva. Other tachinid parasitoids with the 'Bait and wait' tactic lay their eggs at the tunnel entrance and require that the eggs are ingested by the host for parasitism to occur. Finally some small parasitoid species with the 'Ingress and sting' tactic are small enough to enter the tunnel and parasitize the host there.

Cotesia flavipes: Biology

The research described in this thesis deals with the behavioral ecology of *Cotesia* (= *Apanteles*) *flavipes*, a parasitoid with the 'ingress and sting' tactic. There are three morphologically similar species of *Cotesia* which attack tropical stem borers. *Cotesia chilonis* is native to Japan, *Cotesia flavipes* originates from the Indo-Australian region and *Cotesia sesamiae* is native to Africa. Polaszek and Walker (1991) grouped the three species as the '*Cotesia flavipes* complex' after the most well known species used

in biological control.

Basic studies on the behavioral and physiological aspects of parasitism by *C. flavipes* are reported by Moutia and Courtois (1952), Gifford and Mann (1967), Kajita and Drake (1969), Mohyuddin (1971), Wiedenmann et al. (1992) and Ngi Song et al. (1995a). These studies revealed that the gregarious endoparasitoid *C. flavipes* has a short lifespan of a few days and an initial eggload of around 150 eggs. A female *C. flavipes* allocates around 40 eggs in a host and the highest reproductive success is on the later larval instars (4-6th). The egg to adult development time is around 20 days and the sex ratio is usually female biased (60-70%). Arakaki and Ganaha (1986) studied the mating behavior of *C. flavipes* and found a high level of sib mating directly after emergence from the stemborer tunnel.

There are only a few reports on the host foraging behavior of *C. flavipes* and they mainly focus on the contact response to larval frass (Kajita and Drake, 1969; Mohyuddin et al., 1981; Inayatullah, 1983). The only detailed report on the foraging behavior of *C. flavipes* is the study by van Leerdam et al. (1985). They investigated the role of larval frass in the short range host location behavior in *C. flavipes*. The characteristic response of *C. flavipes* to larval frass consists of a decreased rate of locomotion coupled with increased searching on and around the frass, with the parasite exhibiting close antennal contact with the faecal substrate (van Leerdam et al., 1985). Fresh frass can elicit oviposition attempts by the parasite (Mohyuddin et al., 1981). Van Leerdam et al. (1985) found that the substance in frass that elicited a response was water soluble: aqueous frass extracts elicited positive responses in contact experiments with *C. flavipes*. They further found that oral secretions of *D. saccharalis* larvae elicited a response and suggested that mandibular gland stimulants could be involved in the attractiveness of the frass. Recently, Ngi-Song et al. (1995b) investigated the role of volatile chemicals in the host-micro-habitat location in *C. flavipes*.

Biological control with *C. flavipes*

Classical biological control involves the introduction and establishment of exotic natural enemies against introduced pest species. Sometimes, exotic parasitoids are introduced against endemic pests. The latter is referred to as the 'new association' approach (Hokkanen and Pimentel, 1989). *C. flavipes* has been used in new and old association approaches and has been introduced into more than 40 countries in the tropics for biological control of pyralid stemborers in the genera *Chilo* and *Diatraea* (Polaszek and Walker, 1991). The movement of *C. flavipes* material around the world is complex and often difficult to uncover. The major movements are presented in figure 1.1. The main basis of the worldwide introductions are field populations collected and redistributed by the IIBC (International Institute for Biological Control) station in Pakistan. The *C. flavipes* population in Pakistan

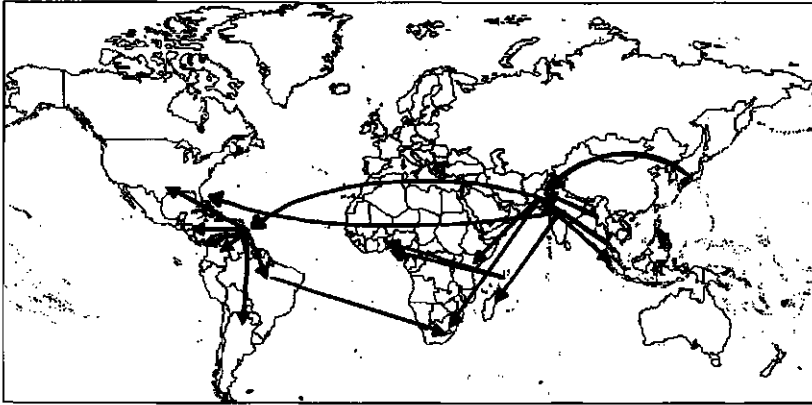


Fig 1.1. Overview of major movements of *Cotesia flavipes* material around the world.

probably originates from imported Japanese material (Alam et al., 1972). An important basis for the distribution of *C. flavipes* in the new world is the IIBC station in Trinidad, which used material from Pakistan to establish a colony of *C. flavipes* on the neotropical host *D. saccharalis*. This formed the basis for introductions in North, Central and South America against *Diatraea* spp. in sugarcane and maize.

At the moment *C. flavipes* is widespread in the Indo-Australian region and due to introductions against *Chilo partellus* (Alam et al. 1972; Overholt et al. 1994), *Diatraea saccharalis* (Gifford & Mann, 1967; Alam et al., 1971; Fuchs et al., 1979; Macedo et al., 1993) and *Chilo sacchariphagus* (Betbeder-Matibet & Malinge, 1968), *C. flavipes* now also occurs in the Caribbean, major parts of North and South America (Polaszek & Walker, 1991) and recently in East-Africa (Omwega et al., 1995). From field studies it is known to successfully parasitize more than 20 host species in more than 15 plant species, including economically important stemborers in maize, sorghum, sugarcane and rice (table 1.1).

The research project described in this thesis complemented a collaborative project of the Department of Entomology of the Wageningen Agricultural University and the International Centre for Insect Physiology and Ecology (ICIPE) on the biological control of stemborers in Africa (Overholt et al., 1994). One of the main stemborer species in Africa is *C. partellus* (Swinhoe). *C. partellus* is an Asian species that was first reported in Africa from Malawi around 1930, and which has now spread to most countries in eastern and southern Africa (Harris, 1990). In many of the areas it has invaded, *C. partellus* is considered to be the most economically important pest of maize and sorghum (Seshu Reddy and Walker, 1990). *C. flavipes* was imported into Kenya from Pakistan in 1991 to investigate its potential for

Table 1.1 Reported field populations of *C. flavipes*, where plant species and host species are mentioned.

Host species	Plant species	Country
PYRALIDAE		
<i>Chilo auricilius</i> Dudgeon	<i>Saccharum officinarum</i> <i>Sacciolepis interrupta</i>	India ¹ , Indonesia ² India ³
<i>Chilo batri</i> Fletcher	<i>Vetiveria zizamoides</i>	India ³
<i>Chilo ceylonicus</i> Hampson	<i>Vetiveria zizamoides</i>	India ³
<i>Chilo infuscatellus</i> Snellen	<i>Saccharum officinarum</i> <i>Vetiveria zizamoides</i>	Taiwan ⁴ , India ⁵ India ³
<i>Chilo orichociliellus</i>	<i>Zea mays</i> L.	Kenya ⁴⁴
<i>Chilo partellus</i> (Swinhoe)	<i>Zea mays</i> L.	Nepal ⁶ , India ^{7,8} , Kenya ⁹ Pakistan ¹⁰ , Comoro Islands ¹¹
	<i>Sorghum bicolor</i> L.	India ^{3,7}
	<i>Coix lachryma-jobi</i> L.	India ¹
	<i>Saccharum spontaneum</i>	India ³
<i>Chilo polychrysus</i> (Meyrick)	<i>Oryza sativa</i>	Malaysia ¹²
<i>Chilo sacchariphagus</i> (Bojer)	<i>Saccharum officinarum</i>	Indonesia ² , Madagascar ¹³ , Mauritius ¹⁴ , Taiwan ⁴
<i>Chilo suppressalis</i> (Walker)	<i>Oryza sativa</i> <i>Saccharum officinarum</i>	Japan ¹⁵ Taiwan ⁴
<i>Cnaphalocrocis medinalis</i> (Gue- nee)	<i>Oryza sativa</i>	Sri Lanka ¹⁶
<i>Diatraea centrella</i> (Moschl.)	<i>Saccharum officinarum</i>	Trinidad ¹⁷
<i>Diatraea flavipennella</i> (Box)	<i>Saccharum officinarum</i>	Brazil ¹⁸
<i>Diatraea impersonatella</i> (Walker)	<i>Saccharum officinarum</i>	Guadeloupe ³⁰ , Trinidad ^{17,20}
<i>Diatraea lineolata</i> (Walker)	<i>Zea mays</i>	Mexico ²¹ , USA ²²
<i>Diatraea saccharalis</i> (Fabricius)	<i>Saccharum officinarum</i>	Brazil ^{23,24} , USA ^{25,26} , Peru ^{27,28} , Caribbean ²⁹ , Guadeloupe ³⁰ , Tri- nidad ^{17,20} , Costa Rica ³¹ , Argen- tina ⁴³ , Barbados ³²

Host species	Plant species	Country
	<i>Sorghum vulgare</i>	USA ²⁵
	<i>Zea Mays</i>	USA ²² , Mexico ²¹
<i>Donacoscaptes steniellus</i> (Hampson) (= <i>Acigona</i> (= <i>Bissetia</i>) <i>steniellus</i>)	<i>Saccharum officinarum</i>	India ^{33,34,35}
<i>Elasmopalpus lignosella</i> (Zeller)	<i>Zea mays</i>	USA ²²
<i>Eoreuma loftini</i> Dyar	<i>Zea mays</i>	USA ²² , Mexico ²¹
<i>Scirpophaga incertulas</i> (Walker) (= <i>Tryporya incertulas</i>)	<i>Oryza sativa</i>	India ³⁶ , Hong Kong ³⁷
	<i>Saccharum officinarum</i>	Taiwan ⁴
NOCTUIDAE		
<i>Mythimna loreyi</i> Duponchel	<i>Saccharum officinarum</i>	Taiwan ⁴
<i>Sesamia calamistis</i> Hmps	<i>Zea mays</i>	Kenya ⁴⁴
<i>Sesamia inferens</i> (Wlk.)	<i>Saccharum officinarum</i>	Taiwan ⁴ , India ³⁸ , Japan ³⁹
	<i>Oryza sativa</i>	India ³ , Indonesia ⁴⁰
	<i>Erianthus arundinaceus</i>	India ³
	<i>Typha angustata</i>	Pakistan ⁴¹
<i>Sesamia grisescens</i> Walker	<i>Saccharum officinarum</i>	Papua New Guinea ⁴²
TORTRICIDAE		
<i>Tetramoera schistaceana</i> (Snellen)	<i>Saccharum officinarum</i>	Taiwan ⁴

References: 1= Nair 1988; 2= Sunaryo & Suryanto 1986; 3= Nagarkatti & Nair 1973; 4= Cheng et al. 1987; 5= Maninder & Varma 1982; 6= Neupane et al. 1985; 7= Subba Rao et al. 1969; 8= Singh et al. 1975; 9= Omwega et al. 1995; 10= Mohyuddin 1990a; 11= Breniere et al. 1985; 12= Ooi 1974; 13= Betbeder-Matibet & Malinge 1968; 14= Moutia & Courtois 1952; 15= Kajita & Drake 1969; 16= Rajapakse & Kulasekare 1982; 17= Vignes 1981; 18= Planalsucar 1979; 19= Overholt & Smith 1990; 20= Mahadeo 1985; 21= Rodriguez-del-Bosque et al. 1990; 22= Youm et al. 1990; 23= Macedo 1978; Macedo et al. 1984; 24= Macedo et al. 1993; 25= Fuchs et al. 1979; 26= Charpentier et al. 1971; 27= Ayquipa et al. 1979; 28= Cueva et al. 1981; 29= Cock 1985; 30= Delattre 1978; 31= Badilla et al. 1991; 32= Alam et al. 1971; 33= Mathur 1967; 34= Chaudhary & Chand 1973; 35= Varma et al. 1981; 36= Nath & Hikim 1978; 37= Thorton et al. 1975; 38= Kumar & Kalra 1965; 39= Arakaki & Ganaha 1986; 40= Rothschild 1970; 41= Carl 1962; 42= Kuniata & Sweet 1994; 43= Willink et al. 1983. 44= Overholt pers. comm.

suppressing *C. partellus* populations in East-Africa (Overholt et al., 1994). It was released in 1992 and is now considered to be established in Kenya and Tanzania (Omwega et al., 1995; Overholt pers. comm.).

C. flavipes strains

The existence of different geographic, host and/or plant specific strains in *C. flavipes* has been postulated. This hypothesis is based on laboratory studies on plant preference behavior of different strains (Mohyuddin et al., 1981; Shami & Mohyuddin, 1992), on anecdotal notes of increased local field parasitism rates after release of particular parasitoid strains (Mohyuddin, 1990b) and on variation in reproductive success on *D. saccharalis* among strains of *C. flavipes* (Wiedenmann and Smith, 1995).

The publication of Mohyuddin et al. (1981) formed the basis for the frequently cited existence of plant specific strains in *C. flavipes*. In a comparison of the USA-strain of *C. flavipes* (reared from *D. saccharalis* on sugarcane) and the Pakistan-strain (reared on *C. partellus* on maize), Mohyuddin et al. (1981) found that the USA-strain had a higher response upon contact to sugarcane frass and the Pakistan-strain a higher response to maize frass. A differential response to volatile stimuli emanating from frass was reported by Shami and Mohyuddin (1992). In olfactometer experiments Shami and Mohyuddin (1992) found that a maize strain was more attracted to maize frass and a sugarcane strain was more attracted to sugarcane frass. In selective breeding experiments Shami and Mohyuddin (1992) tried to reveal the genetic basis for this differential preference. They showed that the preference of the maize-strain for maize frass could be reversed in a preference for sugarcane frass when reared on *Chilo infuscatellus* fed on sugarcane for five successive generations. The reverse could also be shown: the preference of the sugarcane strain for sugarcane frass could be reversed to a preference for maize frass after a selective breeding for a preference for maize frass for five generations only. The results of these artificial selection experiments indicated that there could be a genetic basis for the host-plant preference. However, Shami and Mohyuddin (1992) found that reciprocal crosses between the two strains did not alter the preference in the predicted direction: the progeny of the crosses preferred the host-plant of their mother. This result indicated that there could be a learning-induced preference for the host or host-plant the wasps developed on and emerged from. The research presented in this thesis aimed to elucidate the origin of the postulated host-plant preferences.

REFERENCES

- Alam, M.M., Bennett, F.D. and Carl, K.P. 1971. Biological control of *Diatraea saccharalis* (F.) in Barbados by *Apanteles flavipes* Cam. and *Lixophaga diatraea* T.T. *Entomophaga* 16: 151-158.

- Alam, M.M., Beg, M.N. and Ghani, M.A., 1972. Introduction of *Apanteles* spp. against graminaceous borers into Pakistan. *Technical Bulletin, CIBC* 15: 1-10.
- Alphen, J.J.M. van and Vet, L.E.M. 1986. An evolutionary approach to host finding and selection. In: J.K. Waage & D.J. Greathead (eds.), *Insect parasitoids*, pp. 23-61. Acad. Press, London.
- Alphen, J.J.M. van and Visser, M.E. 1990. Superparasitism as an adaptive strategy for insect parasitoids. *Annu. Rev. Entomol.* 35: 59-79.
- Arakaki, N. and Ganaha, Y. 1986. Emergence pattern and mating behavior of *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae). *Appl. Ent. Zool.* 21: 382-388.
- Arthur, A.P. 1966. Associative learning in *Itopectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *Can. Entomol.* 98: 213-233.
- Avise, J.C. 1994. *Molecular markers, natural history and evolution*. Chapman & Hall, New York.
- Ayquipa, A.G., Cueva, C.M.A. and Sirlopu, R.J. 1979. Introduction of *Apanteles flavipes* Cameron (Hym.: Braconidae) for biological control of the sugarcane stemborer *Diatraea saccharalis* Fabr. (Lep.: Crambidae). *Entomology Newsletter (ISST)* 7: 8 (RAE 69: 679).
- Badilla, F., Solis, A.I. and Alfaro, D., 1991. Control biológico del taladrador de la cana de azúcar *Diatraea* spp. (Lepidoptera: Pyralidae) en Costa Rica. *Manejo Integrado de Plagas (Costa Rica)* No.20-21: 39-44 (RAE 81: 3991).
- Barbosa, P., Gross, P. and Kemper, J. 1991. Influence of plant allelochemicals on the tobacco hornworm and its parasitoid *Cotesia congregata*. *Ecology* 72: 1567-1575.
- Betbeder-Matibet, M. and Malinge, P. 1968. Un succès de la lutte biologique: contrôle de *Proceras sacchariphagus* Boj. «Borer ponctué» de la canne à sucre à Madagascar par un parasite introduit: *Apanteles flavipes* Cam. *Agron. Tropicale* 22: 1196-1220.
- Bigler, F. 1989. Quality assessment and control in entomophagous insects used for biological control. *J. Appl. Entomol.* 108: 390-400.
- Bleszynski, S. 1969. The taxonomy of the Crambine moth borers of sugarcane. In: Williams et al. (Eds.) *Pests of sugarcane*, pp. 11-41. Elsevier, Amsterdam.
- Bouletreau, M. 1986. Coevolution between parasitoids and hosts. In: *Insect parasitoids* (eds. J.K. Waage and D. Greathead), Academic Press, London, pp. 169-200.
- Breniere, J., Bordat, D., Vercambre, B., Hamza, H. and Renand, M. 1985. Les opérations de lutte biologique contre le foreur du maïs *Chilo partellus* (Swinhoe) (Lepidoptera), dans l'île de Ngazidja. *Agron. Tropicale* 40: 157-166.
- Bush, G.L. 1994. Sympatric speciation in animals: new wine in old bottles. *TREE* 9: 285-288.
- Caltagirone, L.E. 1985. Identifying and discriminating among biotypes of parasites and predators. In: M.A. Hoy & D.C. Herzog (eds.), *Biological control in agricultural IPM systems*, pp. 189-200. Acad. Press, New York.
- Carl, K.P. 1962. Gramineous moth borers in west pakistan. *Tech. Bull. CIBC* 2: 29-76 (RAE 51: p.277).
- Chambers, D.L. 1977. Quality control in mass rearing. *Annu. Rev. Entomol.* 22: 289-308.
- Charpentier, L.J., Gifford, J.R., Summers, T.E. and Jackson, R.D. 1971. Biological control of sugarcane insects in continental United States - a historical review. *Proc. 14th congress Int. Soc. Sugarcane Techn.* 4665-476.
- Chaudhary, J.P. and Chand, N. 1973. First record of *Ceraphron fijiensis* Ferriere (Ceraphronidae: Hymenoptera) - a hyperparasite of *Apanteles flavipes* Cameron (Braconidae: Hymenoptera) from India. *Indian J. Entomol.* 34: 179-180.
- Cheng, W.Y., Chang, C.H. and Wang, Z.T. 1987. Occurrence of *Cotesia flavipes* Cameron (Hym.: Braconidae) in autumn sugarcane fields. *Report of the Taiwan Sugar Research Institute* 117: 31-41 (RAE 77: 6530).
- Cock, M.J.W. 1985. A review of biological control of pests in the Commonwealth Caribbean and Bermuda up to 1982. *Techn. Comm. CIBC* 9: 1-218.
- Cueva, C.M., Ayquipa, A.G. and Mescua, B.V. 1981. [Studies on *Apanteles flavipes* (Cameron), introduced to control *Diatraea saccharalis* (F.) in Peru.] *Revista Peruana de Entomologia* 23: 73-76

- (RAE 70: 3891).
- De Bach, P., Rosen, D. and Kennet, C.E. 1971. Biological control of coccids by introduced natural enemies. In: *Biological Control* (Huffaker, C.B. Ed.), pp. 165-194. Plenum, New York.
- Delattre, P. 1978. Conditions d'établissement et de dispersion en Guadeloupe d'*Apanteles flavipes* (Hym.: Braconidae), parasite des Pyrales de la canne à sucre du genre *Diatraea* (Lep.: Pyralidae). *Entomophaga* 23: 43-50.
- Diehl, S.R. and Bush, G.L. 1984. An evolutionary and applied perspective of insect biotypes. *Annu. Rev. Entomol.* 29: 471-504.
- Flint, M.L. 1980. Climatic ecotypes in *Tryoxys complanatus*, a parasite of the spotted alfalfa aphid. *Environ. Entomol.* 9: 501-507.
- Fox, L.R. and Morrow, P.A. 1981. Specialization: species property or local phenomenon? *Science* 211: 887-893.
- Fuchs, T.W., Huffman, F.R. and Smith, J.W. 1979. Introduction and establishment of *Apanteles flavipes* [Hym.: Braconidae] on *Diatraea saccharalis* [Lep.: Pyralidae] in Texas. *Entomophaga* 24: 109-114.
- Futuyma, D.J. and Peterson, S.C. 1985. Genetic variation in the use of resources by insects. *Annu. Rev. Entomol.* 30: 217-238.
- Gifford, J.R. and Mann, G.A. 1967. Biology, rearing, and a trial release of *Apanteles flavipes* in the Florida Everglades to control the sugarcane borer. *J. Econ. Entomol.* 60: 44-47.
- Godfray, H.C.J. 1987. The evolution of clutch size in parasitic wasps. *Am. Nat.* 129: 221-233.
- Godfray, H.C.J. 1994. *Parasitoids, behavioral and evolutionary ecology*. Princeton University Press, New Jersey.
- Goldson, S.L., McNeil, M.R., Profitt, J.R., Barker, G.M., Addison, P.J., Barratt, B.I.P. and Ferguson, C.M. 1993. Systematic mass rearing and release of *Microctonus hyperodae* (Hym.: Braconidae, Euphorinae), a parasitoid of the Argentine stem weevil *Listronotus bonariensis* (Col.: Curculionidae) and records of its establishment in New Zealand. *Entomophaga*: 38: 527-536.
- Gonzalez, D.; Gordh, G.; Thompson, S.N. and Adler, J. 1979. Biotype discrimination and its importance to biological control. In: Hoy, M.A. & J.J. McKelvey Jr. (Eds.) *Genetics in relation to insect management*. Rockefeller foundation. pp. 129-136.
- Gross, H.R., Lewis, W.J., Jones, R.L. and Nordlund, D.A. 1975. Kairomones and their use for management of entomophagous insects: III. Stimulation of *Trichogramma achaeae*, *T. pretiosum* and *Microplitis croceipes* with host seeking stimuli at time of release to improve their efficiency. *J. Chem. Ecol.* 1: 431-438.
- Haardt, H. and Holler, C. 1992. Differences in life history traits between isofemale lines of the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). *Bull. Entomol. Res.* 82: 479-484.
- Hamm, J.J., Styler, E.L. and Lewis, W.J. 1988. A baculovirus pathogenic to the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). *J. Invertebr. Pathol.* 52: 189-191.
- Harris, K.M. 1990. Bioecology of *Chilo* species. *Insect Sci. Appl.* 11: 467-477.
- Heimpel, G.E. and Rosenheim, J.A. 1995. Dynamic host feeding by the parasitoid *Aphytis melinus*: the balance between current and future reproduction. *J. Anim. Ecol.* 64: 153-167.
- Hokkanen, H.M. and Pimentel, D. 1989. New associations in biological control: theory and practice. *Can. Entomol.* 121: 829-840.
- Hopper, K.R., Roush, R.T. and Powell, W. 1993. Management of genetics of biological control introductions. *Annu. Rev. Entomol.* 38: 27-51.
- Houten, Y. van, Stratum, P. van, Bruin, J. and Veerman, A. 1995. Selection for non-diapause in *Amblyseius cucumeris* and *Amblyseius barkeri* and exploration of the effectiveness of selected strains for thrips control. *Entomol. Exp. Appl.* 77: 289-295.
- Hoy, M.A. 1979. The potential for genetic improvement of predators for pest management programs. In: M.A. Hoy & J.J. McKelvey (eds.) *Genetics in relation to insect management*. Rockefeller Found. press, N.Y. pp. 106-115.

- Hoy, M.A. 1985. Recent advances in genetics and genetic improvement of the phytoseiidae. *Annu. Rev. Entomol.* 30: 345-370.
- Inayatullah, C. 1983. Host selection by *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae): influence of host and host plant. *J. Econ. Entomol.* 76: 1086-1087.
- Jaenike, J. and Holt, R.D. 1991. Genetic variation for habitat preference: evidence and explanations. *Am. Nat.* 137: S67-S90.
- Jaenike, J. 1990. Host specialization in phytophagous insects. *Annu. Rev. Ecol. Syst.* 21: 243-273.
- Janssen, A., van Alphen, J.J.M., Sabelis, M.W. and Bakker, K. 1995. Odour-mediated avoidance of competition in *Drosophila* parasitoids: the ghost of competition. *Oikos* 73: 356-366.
- Kajita, H. and Drake, E.F. 1969. Biology of *Apanteles chilonis* and *A. flavipes* (Hymenoptera: Braconidae), parasites of *Chilo suppressalis*. *Mushi* 42 : 163-179.
- Kenis, M. 1994. Variations in diapause among populations of *Eubazus semirugosus* (Nees) (Hym.: Braconidae), a parasitoid of *Pissodes* spp. (Col.: Curculionidae). *Norw. J. Agr. Sc. Suppl.* 16: 77-82.
- Kester, K.M. and Barbosa, R. 1991. Behavioral and ecological constraints imposed by plants on insect parasitoids: implications for biological control. *Biol. Control* 1: 94-106.
- Kester, K.M. and Barbosa, P. 1994. Behavioral responses to host foodplants of two populations of the insect parasitoid *Cotesia congregata* (Say). *Oecologia* 99: 151-157.
- Kraaijeveld, A.R., Voet, S. and Van Alphen, J.J.M. 1994. Geographical variation in habitat choice and host suitability in the parasitoid *Asobara rufescens*. *Entomol. Exp. Appl.* 72: 109-114.
- Kraaijeveld, A.R., Nowee, B., and Najem, R.W. 1995. Adaptive variation in host selection behaviour of *Asobara tabida*, a parasitoid of *Drosophila* larvae. *Funct. Ecol.* 9: 113-118.
- Kraaijeveld, A.R. and van Alphen, J.J.M. 1995. Variation in diapause and sex ratio in the parasitoid *Asobara tabida*. *Entomol. Exp. Appl.* 74: 259-265.
- Kraaijeveld, A.R. and van der Wel, N.N. 1995. Geographic variation in reproductive success of the parasitoid *Asobara tabida* in larvae of several *Drosophila* species. *Ecol. Entomol.* (in press).
- Krebs, J.R. and Davies, N.B. 1981. An introduction to behavioural ecology. Blackwell Scientific Oxford.
- Kumar, S. and Kalra, A.N. 1965. Attack of the pink borer, *Sesamia inferens* Wlk. as cane borer in Rajasthan. *Indian SugCane* 9: 154-155 (RAE 55: 474).
- Kuniata, L.S. and Sweet, C.P.M. 1994. Management of *Sesamia griseocens* Walker (Lep.: Noctuidae), a sugarcane borer in Papua New Guinea. *Crop Protec.* 13: 488-493.
- Leerdam, M.B. van ; Smith, J.W. Jr. and Fuchs, T.W. 1985. Frass-mediated, host finding behavior of *Cotesia flavipes*, a Braconid parasite of *Diatraea saccharalis* (Lepidoptera: Pyralidae). *Ann. Ent. Soc. Am.* 78: 647-650.
- Lenteren, J.C. van. 1980. Evaluation of control capabilities of natural enemies: does art have to become science. *Neth. J. Zool.* 30: 369-381.
- Lenteren, J.C. van. 1981. Host discrimination by parasitoids. In: Nordlund, D.A.; Jones, R.L. & Lewis, W.J. (eds.) *Semiochemicals, their role in pest control*. New York: Wiley. pp. 153-180.
- Lenteren, J.C. van. 1991. Quality control of natural enemies: hope or illusion ? In: Bigler, F. (Ed.) *Proc. 5th workshop IOBC global working group "quality control of mass reared arthropods"*, pp. 1-14.
- Lewis, W.J.; Vet, L.E.M.; Tumlinson, J.H.; Lenteren, J.C. van and Papaj, D.R. 1990. Variations in parasitoid foraging behavior: essential element of a sound biological control theory. *Environ. Entomol.* 19: 1183-1193.
- Lewis, W.J. and Martin, W.R. Jr. 1990. Semiochemicals for use with parasitoids: status and future. *J. Chem. Ecol.* 16: 3067-3089.
- Lewis, W.J. & Tumlinson, J.H. 1988. Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* 331: 257-259.
- Luck, R.F. 1990. Evaluation of natural enemies for biological control: a behavioral approach. *TREE* 5: 196-199.
- Macedo, N. 1978. New strain of *Cotesia flavipes* imported to increase its adaptive potential in the

- southern region of Brazil. *Entomology Newsletter (ISST)* 4: 11-12.
- Macedo, N., Mendoca Filho, A.F., Moreno, J.A. and Pinazza, A.H. 1984. Evaluation of the economic advantages of 10 years of biological control of *Diatraea* spp. through *Apanteles flavipes* Cameron, in the state of Alagoas (Brazil). *Entomology Newsletter (ISST)* 16: 9-10 (RAE 73: 3184).
- Macedo, N., Araujo, J.R. and Bothelo, P.S.M. 1993. Sixteen years of biological control of *Diatraea saccharalis* (Fabr.) (Lepidoptera: Pyralidae) by *Cotesia flavipes* (Cam.) (Hymenoptera: Braconidae), in the state of Sao Paulo, Brazil. *Ann. Soc. Entomol. Brasil* 22: 441-448.
- Mackauer, M., Ehler, L.E. and Roland, J. (eds.) 1990. *Critical issues in biological control*. Intercept, Andover, U.K.
- Mahadeo, C.R. 1985. Present status of indigenous and imported parasites for the control of the sugarcane stemborer complex (*Diatraea* spp.) in Trinidad. *Entomology Newsletter (ISST)* 17: 7-8 (RAE 75: 1095).
- Maninder and Varma, G.C. 1982. Record of a hyperparasitoid *Eupteromalus* sp. near *parnae* Gahan (Hymenoptera: Pteromalidae) from the Punjab, India. *Entomon.* 7: 335-336 (RAE 71: 5511).
- Mathur, K.C. 1967. Contribution to the knowledge of the natural enemies of *Bissetia steniellus*. *Entomophaga* 12: 443-448.
- McNaughton, S.J., Tarrant, J.T., McNaughton, M.M. and Davis, R.H. 1985. Silica as a defense against herbivory and a growth promotor in African grasses. *Ecology* 66: 528-535.
- Mohyuddin, A.I. 1971. Comparative biology and ecology of *Apanteles flavipes* (Cam.) and *A. sesamiae* Cam. as parasites of graminaceous borers. *Bull. Entomol. Res.* 61: 33-39.
- Mohyuddin A.I. 1990a. Biological control of *Chilo* spp. in maize, sorghum and millet. *Insect Sci. Applic.* 11: 721-732.
- Mohyuddin A.I. 1990b. Utilization of natural enemies for the control of insect pests of sugarcane. *Insect Sci. Applic.* 12: 19-26.
- Mohyuddin, A.I., Inayatullah, C. and King, E.G. 1981. Host selection and strain occurrence in *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) and its bearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* 71: 575-581.
- Mollegaard, C. 1988. *Genetical aspects of resistance in a host-parasitoid interaction*. Phd-thesis, University of Leiden.
- Moutia, L.A. and Courtois, C.M. 1952. Parasites of the moth-borers of sugarcane in Mauritius. *Bull. Entomol. Res.* 43: 325-363.
- Nagarkatti, S. and Nair, K.R. 1973. The influence of wild and cultivated Graminae and Cyperaceae on populations of sugarcane borers and their parasites in north India. *Entomophaga* 18: 419-430.
- Nair, K.R. 1988. Field parasitism by *Apanteles flavipes* Cam. (Hymenoptera: Braconidae) on *Chilo partellus* Swinh. in *Coix lachryma-jobi* L. and *Chilo auricilius* (Dudgn.) in sugarcane in India. *Entomon* 13: 283-287.
- Nath, D.K. and Hikim, I.S. 1978. Braconid parasites of rice yellow borer *Tryporyza incertulas* in West Bengal, India. *Int. Rice Res. Newsl.* 3: 21 (RAE 68: 3910).
- Neupane, F.P., Coppel, H.C. and Chapman, R.K. 1985. Bionomics of the maize borer *Chilo partellus* (Swinhoe) in Nepal. *Insect Sci. Appl.* 6: 547-553.
- Ngi-Song, A.J., Overholt, W.A. and Ayerty, J.N. 1995a. Suitability of African gramineous stemborers for development of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). *Environ. Entomol.* 24: 978-984.
- Ngi-Song, A.J., Overholt, W.A., Njagi, P.G.N., Dicke, M., Ayerty, J.N. and Lwande, W. 1995b. Volatile infochemicals in host and host habitat location by *Cotesia flavipes* and *C. sesamiae* (Cameron) (Hymenoptera: Braconidae), two larval parasitoids of gramineous stemborers. *J. Chem Ecol.* (in press).
- Omweiga, C.O., Kimani, S.W., Overholt, W.A. and Ogot, C.K.P.O. 1995. Evidence of the establishment of *Cotesia flavipes* (Hymenoptera: Braconidae) in continental Africa. *Bull. Entomol. Res.* (in press).

- Ooi, A.C.P. 1974. A padi stemborer survey in the Muda scheme, Kedah. *Malaysian Agricultural Journal* 49: 525-531 (RAE 64: 4849).
- Overholt, W.A. and Smith, J.W., Jr. 1990. Comparative evaluation of three exotic insect parasites (Hymenoptera: Braconidae) against the southwestern corn borer (Lepidoptera: Pyralidae) in corn. *Environ. Entomol.* 19: 1155-1162.
- Overholt, W.A., Ngi-Song, A.J., Kimani, S.K., Mbatila, J., Lammers, P. and Kioko, E. 1994. Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), in Africa. *Biocontrol news and Inf.* 15: 19-24.
- Pak, G.A. 1988. *Selection of Trichogramma for inundative biological control*. PhD thesis, Agricultural University Wageningen, The Netherlands.
- Pak, G.A. and Heiningen, T.G. 1985. Behavioral variations among strains of *Trichogramma* spp.: adaptability to field temperature conditions. *Entomol. Exp. Appl.* 38: 3-13.
- Papaj, D.R. and Lewis, A.C. 1993. *Insect learning. Ecological and evolutionary perspectives*. Chapman and Hall, New York.
- Phillips, C.B., Baird, D.B. and Goldson, S.L. 1994. The south american origins of New Zealand *Microtonus hyperodae* parasitoid as indicated by morphometric analysis. *Proc. 47th N.Z. Plant Protect. Conf.* 1994: 220-226.
- Pinto, J.H. and Stouthamer, R. 1994. Systematics of the Trichogrammatidae with emphasis on *Trichogramma*. In: Wajnberg, E. and Hassan, S.A. (eds.) *Biological control with egg parasitoids*, pp. 1-36. CAB International, Montpellier.
- Planalsucar. *Annual report 1979* (RAE 69: 5860).
- Polaszek, A. and Walker, A.K. 1991. The *Cotesia flavipes* species-complex: parasitoids of cereal stemborers in the tropics. *REDIA* 74: 335-341.
- Prévost, G. and Lewis, W.J. 1990. Genetic differences in the response of *Microplitis croceipes* to volatile semiochemicals. *J. Insect Behav.* 3: 277-287.
- Rajapakse, R.H.S. and Kulasekare, V.L. 1982. Larval parasites of rice leaffolder in southern Sri Lanka. *Int. Rice Res. Newsletter* 7: 11 (RAE 71: 1076).
- Rodriguez-del-Bosque, L.A., Browning, H.W. and J.W. Smith Jr. 1990. Seasonal parasitism of cornstalk borers (Lepidoptera: Pyralidae) by indigenous and introduced parasites in Northeastern Mexico. *Environ. Entomol.* 19: 393-402.
- Roitberg, B.D., Mangel, M., Lalonde, R.G., Roitberg, C.A., van Alphen, J.J.M. and Vet, L.E.M. 1992. Seasonal dynamic shifts in patch exploitation by parasitic wasps. *Behav. Ecol.* 3: 156-165.
- Rosen, D. 1994. *Advances in the study of Aphytis (Hymenoptera: Aphelinidae)*. Andover, Intercept.
- Rosenheim, J.A. and Rosen D. 1991. Foraging and oviposition decisions in the parasitoid *Aphys lingnanensis*: distinguishing the influences of egg load and experience. *J. Anim. Ecol.* 60: 873-893.
- Rosenthal, J.P. and Welter, S.C. 1995. Tolerance to herbivory by a stem-boring caterpillar in architecturally distinct maizes and wild relatives. *Oecologia* 102: 146-155.
- Rothschild, G.H.L. 1970. Parasites of rice stemborers in Sarawak (Malaysian Borneo). *Entomophaga* 15: 21-51.
- Roush, R.T. 1990. Genetic variation in natural enemies: critical issues for colonization in biological control. In: Mackauer, M., Ehler, L.E., Roland (eds.). *Critical issues in biological control*, pp. 563-588. Intercept, Andover, U.K.
- Ruberson, J.R., Tauber, M.J. and Tauber, C.A. 1989. Intraspecific variability in hymenopteran parasitoids: comparative studies of two biotypes of the egg parasitoid *Edovum putileri* (Hymenoptera: Eulophidae). *J. Kansas Ent. Soc.* 62: 189-202.
- Sappal, N.P., Jeng, R.S., Hubbes, M. and Liu, F. 1995. Restriction fragment length polymorphisms in polymerase chain reaction amplified ribosomal DNAs of three *Trichogramma* (Hymenoptera: Trichogrammatidae) species. *Genome* 38: 419-425.
- Seshu Reddy, K.V. and Walker, P.T. 1990. A review of the yield losses in graminaceous crops caused by *Chilo* spp. *Insect Sci. Applic.* 11: 563-569.

- Setamou, M. Schulthess, F., Bosque-Perez, N.A. and Odjo-Thomas, A. 1993. Effect of plant nitrogen and silica on the bionomics of *Sesamia calamistis* (Lepidoptera: Noctuidae). *Bull. Entomol. Res.* 83: 405-411.
- Shami, S. and Mohyuddin, A.I. 1992. Studies on host plant preference of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) an important parasitoid of graminaceous stalk borers. *Pakistan J. Zool.* 24: 313-316.
- Simmonds, F.J. 1963. Genetics and biological control. *Can. Entomol.* 95: 561-567.
- Singh, B., Dhaliwal, J.S., Battu, G.S. and Atwal, A.S. 1975. Population studies on the maize-borer *Chilo partellus* (Swinhoe) in the Punjab. III. Role of parasitization by *Apanteles flavipes* (Cameron) in the population build-up. *Indian J. Ecol.* 2: 115-124.
- Smith, J.W. Jr., Wiedemann, R.N. and Overholt, W.A. 1993. *Parasites of Lepidopteran stemborers of tropical gramineous plants*. ICIPE Science Press, Nairobi, pp.1-89.
- Spollen, K.M. and Hoy, M.A. 1992. Genetic improvement of an arthropod natural enemy: relative fitness of a carbaryl-resistant strain of the California red scale parasite *Aphytis melinus* DeBach. *Biol. Control* 2: 87-94.
- Subba Rao, B.R., Singh, R.N., Saxena, J.D. and Sharma, A.K. 1969. Bionomics of *Apanteles flavipes* (Cameron) a parasite of *Chilo zonellus* (Swinhoe) at Delhi with special reference to the mode of overwintering of the parasite. *Indian J. Entomol.* 31: 7-12.
- Sunaryo and Suryanto, S.J. 1986. Augmentation of local and introduction of Thailand strain of *Cotesia flavipes* (Cam.) in Gunung Madu Plantations, Lampung, Indonesia. *Proc. Pak. Sug. Techn.* 22nd Ann. Conv.: 153-159.
- Thorton, I.W.B., Marshal, A.T., Kwan, W.H., and Ma, Q. 1975. Studies on lepidopterous pests of rice crops in Hong Kong, with particular reference to the yellow stem-borer, *Tryporyza incertulas* (Wlk.). *PANS* 21: 239-252 (RAE 64: 2809).
- Turlings, T.C.J., Wäckers, F., Vet, L.E.M., Lewis, W.J. and Tumlinson, J.H. 1993. Learning of host-finding cues by hymenopterous parasitoids. In: Papaj, D.R. & Lewis, A.C. (eds.) *Insect learning: ecological and evolutionary perspectives*. pp. 51-78.
- Unruh, T.R. and Messing, R.H. 1993. Intraspecific biodiversity in Hymenoptera: implications for conservation and biological control. In: LaSalle, J. and Gauld, I.D. (eds.) *Hymenoptera and biodiversity*, pp. 27-52. CAB International, Wallingford.
- Varma, G.C., Mohanty, S.N. and Maninder 1981. Natural enemies of *Acigona steniella* (Hmps.) (Crambidae: Lepidoptera) in the Punjab. *Journal of Research, Punjab Agr. Univ.* 18: 163-169 (RAE 71:6908).
- Van Kan, F.J.P.M., Silva, I.M.M.S., Schilthuizen, M., Pinto, J.D. and Stouthamer, R. 1996. Use of DNA-based methods for the identification of minute wasps of the genus *Trichogramma*. *Proc. Exper. Appl. Entomol.* NEV 7: (in press).
- Vet, L.E.M., Janse, C., Achterberg, C. van and Alphen, J.J.M. van. 1984. Microhabitat location and niche segregation in two sibling species of Drosophilid parasitoids: *Asobara tabida* (Nees) and *A. rufescens* (Foerster) (Braconidae, Alysiniinae). *Oecologia* 61: 182-188.
- Vet, L.E.M., Lewis, W.J., Papaj, D.R., Lenteren, J.C. van. 1990. A variable-response model for parasitoid foraging behavior. *J. Insect Behav.* 3: 471-490.
- Vet, L.E.M. and Groenewold, A.W. 1990. Semiochemicals and learning in parasitoids. *J. Chem. Ecol.* 16: 3119-3135.
- Vet, L.E.M. and Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37: 141-172.
- Vet, L.E.M., Lewis, W.J. and Cardé, R.T. 1995. Parasitoid foraging and learning In: *Chemical ecology of insects* (eds. W.J. Bell and R.T. Cardé), Chapman Hall, New York, pp. 65-101.
- Via, S. 1990. Ecological genetics and host adaptation in herbivorous insects: The experimental study of evolution in natural and agricultural systems. *Annu. Rev. Entomol.* 35: 421-446.
- Vignes, W.G. des. 1981. Biological control of small moth borer (*Diatraea* species) on sugarcane in Trinidad. *Entomology Newsletter (ISST)* 10: 5-6 (RAE 70: 3254).

- Visser, M.E., Alphen, J.J.M. van and Nell, H. 1990. Adaptive superparasitism and patch time allocation in solitary parasitoids: the influence of the number of parasitoids depleting a patch. *Behaviour* 114: 21-36.
- Visser, M.E., Alphen, J.J.M. van and Nell, H. 1992. Adaptive superparasitism in solitary parasitoids: the influence of pre-patch experience. *Behav. Ecol. Sociobio.* 31: 163-171. 21-36.
- Waage, J.K. 1986. Family planning in insect parasitoids. In: *Insect parasitoids* (eds. J.K. Waage and D. Greathead), Academic Press, London, pp. 63-95.
- Waage, J.K. 1990. Ecological theory and the selection of biological control agents. In: Mackauer, M., Ehler, L.E., Roland (eds). *Critical issues in biological control*, pp. 135-157. Intercept, Andover, U.K.
- Wäckers, F.L. 1994. The effect of food deprivation on the innate visual and olfactory preferences in the parasitoid *Cotesia rubecula*. *J. Insect Physiol.* 40: 641-649.
- Wiedenmann, R.N.; Smith, J.W. Jr. and Darnell, P.O. 1992. Laboratory rearing and biology of the parasite *Cotesia flavipes* (Hymenoptera: Braconidae) using *Diatraea saccharalis* (Lepidoptera: Pyralidae) as a host. *Environ. Entomol.* 21: 1161-1167.
- Wiedenmann, R.N. and Smith, J.W. Jr. 1995. Parasitization of *Diatraea saccharalis* by *Cotesia chilonis* and *Cotesia flavipes*. *Environ Entomol.* 24: 950-961.
- Williams, J.R. 1983. The sugarcane stemborer (*Chilo sacchariphagus*) in Mauritius. *Revue Agricole et Sucri. de l'île Maurice* 62: 5-23.
- Willink, E., Costilla, M.A. and Osoreo, V.M. 1983. [Sugarcane. Towards the integrated control of the borer.] *Avance AgroIndustrial* 2: 9-13 (RAE 71:1003).
- Youm, O., Gilstrap, F.E. and Browning, H.W. 1990. Parasitism of stem borers (Lepidoptera: Pyralidae) associated with corn and sorghum in the Lower Rio Grande Valley of Texas. *J. Econ. Entomol.* 83: 84-88.

Host microhabitat location by the stemborer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systemically induced plant volatiles.

ABSTRACT The origin of olfactory stimuli involved in the host-microhabitat location in *Cotesia flavipes*, a parasitoid of stemborer larvae, was investigated in a Y-tube olfactometer. The response of female *C. flavipes* towards different components of the plant-host-complex, consisting of a maize plant infested with two or more larvae of the stemborer *Chilo partellus*, was tested in dual choice tests. The concealed lifestyle of the stemborer larvae did not limit the emission of volatiles attractive to a parasitoid. A major source of the attractive volatiles from the plant-host-complex was the stemborer-injured stem, including the frass produced by the feeding larvae. Moreover, the production of volatiles attractive to a parasitoid was not restricted to the infested stem-part but occurs systemically throughout the plant. The uninfested leaves of a stemborer-infested plant were found to emit volatiles that attract female *C. flavipes*. We further demonstrate that an exogenous elicitor of this systemic plant response is situated in the regurgitate of a stemborer larva. When a minor amount of regurgitate is inoculated into the stem of an uninfested plant, the leaves of the treated plant emit volatiles which attract female *C. flavipes*.

INTRODUCTION

In the evolutionary arms race between herbivores and their natural enemies, herbivores have evolved several traits to reduce their vulnerability to enemies. One of these traits can be a specific behavior that reduces the recognition and detection by natural enemies. It has been argued that the stem-boring habit of several herbivore species avoids proximity of feeding damage cues and reduces accessibility for natural enemies, which may have been one of the reasons for its evolution (Strong et al., 1984). However, an absolutely safety from natural enemies is virtually impossible. Although attack by enemies imposes selection on victims to reduce vulnerability, counter-selection works on enemies to improve their efficiency in finding hiding hosts (Jeffries & Lawton, 1984). Despite their hidden lifestyle, stemborer larvae have a broad range of natural enemies, which are able to locate and attack the larvae

which feed inside the plant tissue (Baker et al., 1949; Smith et al., 1993). The response of stemborer parasitoids to contact chemicals is well described (Leerdam et al., 1985), but there are only a few studies that focus on the volatile stimuli involved in the host searching by stemborer parasitoids (Roth et al., 1982; Ding et al., 1989a,b; Ma et al., 1992).

For other parasitoids the use of volatile infochemicals in their long-range search for suitable hosts is well documented (Vinson, 1976; Nordlund et al., 1988; Vet & Dicke, 1992). Many natural enemies are known to discriminate between volatile chemicals emitted by uninfested and herbivore-infested plants. Chemical stimuli emanating from the plant-host-complex can originate from the herbivore, the plant or from interactions between the herbivore and the plant. The response to volatile cues from the plant-host-complex is well described for parasitoids of leaf feeding caterpillars. For instance, the braconids *Cotesia marginiventris*, *Cotesia glomerata* and *Cotesia rubecula* are highly attracted by odors emanating from herbivore-damaged plants, whereas odors from larval frass or the larvae themselves are far less attractive to these parasitoids (Turlings et al., 1991a; Steinberg et al., 1992; Geervliet et al., 1994). Although odors from the host or host-products are very reliable indicators for host presence, their long range detectability is very low (Vet et al., 1991). Turlings et al. (1991b) demonstrated that larvae and frass only release volatiles in very low quantities, whereas plant volatiles are released in relatively large amounts. Plant odors play an important role in the foraging behavior of larval parasitoids, especially plant volatiles that are released by plants infested by herbivores. Damage by herbivores can increase the emission of plant volatiles enormously (Dicke et al., 1990a; Turlings et al., 1990) and these plant volatiles can be specific indicators of herbivore identity (Dicke et al., 1990a,b). If these chemicals attract natural enemies whose activities are favourable to the plant, they are called herbivore-induced synomones (Vet & Dicke, 1992). Recent studies have demonstrated that the plant has an active role in the production of such herbivore-induced synomones (for review see Dicke, 1994). The release of these chemical signals is not restricted to the infested plant parts, but occurs systemically throughout the plant (Dicke et al., 1990b; Takabayashi et al., 1991; Turlings & Tumlinson, 1992; Dicke & Dijkman, 1992).

In contrast to leaf-feeding herbivores, the larvae of stemborers have a concealed lifestyle and feed inside the plant. This present research deals with the foraging behavior of *Cotesia flavipes* (Hymenoptera: Braconidae), a gregarious larval endoparasitoid of tropical stemborers. *C. flavipes* attacks the last larval instars, by ingressing the stem through the entrance hole of the stemborer tunnel (Smith et al., 1993). *C. flavipes* is indigenous to South-East Asia where it attacks several stemborer

species in maize, sorghum, rice, sugarcane and various wild grasses. This parasitoid has been extensively used in classical biological control programmes against *Chilo partellus* (Alam et al., 1972; Overholt et al., 1994), *Diatraea saccharalis* (Gifford & Mann, 1967; Alam et al., 1971; Fuchs et al., 1979) and *Chilo sacchariphagus* (Betbeder-Matibet & Malinge, 1968). Earlier reports on the searching behavior of *C. flavipes* mainly focused on the short range searching behavior, in particular the contact-response to larval frass (Kajita & Drake, 1969; Mohyuddin 1971; Mohyuddin et al., 1981; Van Leerdam et al., 1985). Potting et al. (1993) and Ngi-Song et al. (1994) demonstrated that female *C. flavipes* are attracted to odors emanating from stemborer-infested maize plants.

The purpose of the present research was to elucidate the sources of the volatile stimuli responsible for this attraction. This was achieved by testing the attractiveness of different components of the plant-host-complex, which consisted of a maize plant with two or more feeding *C. partellus* larvae in the stem. We provide evidence that the production of volatiles attractive to a parasitoid is not restricted to the infested part of a plant but occurs systemically throughout the plant. We further demonstrate that an exogenous elicitor of this systemic plant response is present in the regurgitate of a stemborer larva.

MATERIALS AND METHODS

Insects

The *Chilo partellus* culture originated from a colony maintained by the International Centre for Insect Physiology and Ecology in Nairobi Kenya. The culture was maintained in temperature controlled rooms (25 °C) at 50-70% RH and a light/dark regime of 16L:8D. Adult moths were kept in an oviposition cage lined with waxed paper to provide suitable oviposition sites. Egg masses were cut from the paper daily and transferred to a closed petri dish containing moist cotton wool to maintain high humidity. Three to four egg masses (blackhead-stage) were transferred to one litera glass jars with 125 ml artificial diet prepared according to the procedure described by Seshu Reddy & Davies (1978) and Ochieng et al. (1985). The covers of the jars were provided with a hole and were lined with stainless steel screen to prevent escape of the larvae. As moths emerged they were transferred to an oviposition cage. The larvae used in the experiments and for the maintenance of the parasitoid culture (see below) were always removed from the artificial diet as fourth instar and allowed to feed on fresh maize stems for at least 24 h.

Cotesia flavipes was obtained from the International Institute for Biological Control, Pakistan, where it was collected from *C. partellus* in maize. In Wageningen, the

parasitoid culture was maintained on *C. partellus* at 25 °C, 70% RH and a 16-h photophase. Individual, mated, females (1 day old) were offered one host larva with some fresh larval frass to stimulate oviposition. Parasitized larvae were reared on cut maize stems kept in one litera jars.

Cocoons were collected from the stem pieces one to two days prior to emergence and transferred to a 250 ml bottle with agar medium lined with a polyester top with a drop of honey. One day after emergence the adult wasps were placed in an incubator at 15 °C. All experiments were conducted with naive (no oviposition experience), mated females that were two to four days old.

Plants

Maize plants (*Zea mays* cv. Anjo) were grown in a greenhouse at 20-25 °C, 50-80 %RH and a 16L:8D light regime. When the light intensity dropped below 500 lux in the photophase artificial light was used. Plants were watered daily and when necessary treated with AA Kasaerosol (Schering AAgrunol B.V.) against aphid infestation.

Olfactometer setup

Responses of *C. flavipes* females to volatile chemicals emitted by different odor sources were measured in a Y-tube olfactometer. The olfactometer consisted of a glass Y-shaped tube. The two arms of the Y-tube were connected to a flow meter and an odor source container. Odor sources were placed in containers, which consisted of 500 ml glass bottles. Air was blown through the odor source containers and introduced into the arms of the Y-tube. By balancing the input through the arms (4 l/min) and the output through the common tube (8 l/min) two laminary air flows were created. For more details of the olfactometer see Steinberg et al. (1992). The environmental conditions in the bioassay room were 25-27 °C, 40-70% RH.

Bioassay procedure

Individual female parasitoids were introduced into the base tube of the Y-tube olfactometer and their behavior was observed during a five min period. If the test female walked towards an odor source and crossed the 'choice line' (4 cm after division of the base tube) and stayed there for more than 15 s, it was recorded as a choice for the odor source in that arm. To circumvent any asymmetrical bias in the setup the odor sources were exchanged after having tested five parasitoids. For each combination 40-50 wasps were tested. The choice of the females was analyzed with a Chi-square test ($\alpha=0.05$).

Odor sources

Uninfested vs. infested maize plants.

Uninfested plants consisted of four to six weeks old plants (40-50 cm tall). An infested plant was obtained by introducing two fourth-instar *C. partellus* larvae into two holes bored in the stem of a plant at a distance of 10 cm from each other. The larvae were allowed to feed overnight. Prior to the bioassay, the test plant was cut into four parts: three pieces of stem and the remaining part with the leaves of the plant. Care was taken that the tunnel of the stemborer larvae never reached the part with the leaves. The four parts of each test plant were introduced into the odor source container. Three odor sources were tested: 1. clean air, which consisted of an empty odor source container (AIR); 2. the complete plant-host-complex (PHC) with two feeding larvae and 3. an uninfested plant (UNINFEST). The combinations tested were AIR versus UNINFEST and UNINFEST versus PHC. In an additional experiment it was tested whether an artificially damaged maize plant emitted attractive volatiles. In this test the plants were not cut into pieces. An artificially damaged plant (ART-DAMAGE) was obtained by puncturing 5 holes in the stem. To mimic feeding behavior of the stemborer larvae this was repeated every 15 minutes. The combination tested was ART-DAMAGE versus AIR. Forty wasps were tested in each choice situation.

Stem vs. leaves.

To investigate which part of the PHC emitted attractive volatiles the following combinations were tested. 1. Stem pieces of an uninfested plant (UNINF-STEM) versus stem pieces of an infested plant (PHC-STEM), 2. leaves of an uninfested plant (UNINF-LEAF) versus leaves of an infested plant (PHC-LEAF) and 3. leaves of an infested plant (PHC-LEAF) versus stem pieces of the same infested plant (PHC-STEM). All three combinations were tested on the same day and 47 wasps were tested in each choice situation.

Stem.

To determine the origin of attractive volatile chemicals, the different components of an infested stem were tested in dual choice tests. These were infested stem pieces with or without feeding larvae, larvae, larval frass, and artificially damaged (uninfested) stem-pieces. The stem of two months old maize plants (flowering stage) was cut in pieces of 12 cm. Five pieces of stem were used as odor source. A standard infested stem-piece was obtained by boring a hole on each side of the stem-piece and introducing one larva in each hole. The ten larvae were allowed to feed for 60 h.

The following odor sources were tested:

- INFESTED Five stemborer infested stem pieces, consisting of maize stems with two larvae in their stemborer tunnel filled with frass.
- HERB-DAM As INFESTED but larvae removed prior to experiment.
- DAM-STEM As HERB-DAM but all frass removed prior to experiment.
- LARVAE 10 water-washed larvae, removed from INFESTED.
- FRASS Frass removed from INFESTED.
- ART-DAM Artificially damaged stem pieces (10 holes bored in each stem piece, 12 hours prior to the start of the experiment).

The following choice tests were made:

1) INFESTED vs. ART-DAM; 2) HERB-DAM vs. ART-DAM; 3) INFESTED vs. HERB-DAM; 4) DAM-STEM vs. FRASS+LARVAE; 5) LARVAE vs. FRASS (in this test both odor sources placed directly in Y-tube). The choice tests were performed on separate days and 40 wasps were tested in each choice situation.

Leaves

In this series of experiments the odor sources consisted of the leaves of treated plants. All plants used consisted of singly potted maize plants, four to six weeks old. In the stem of each plant two holes were bored, 10 cm apart. The leaves were obtained by cutting the stem just below the whorl, in such a way that all the leaves were still attached to the stem part.

The treatments tested were: undamaged leaves which were removed from a host-infested plant, 24 h or 48 h after infestation (LARVAE-24h; LARVAE-48h) and undamaged leaves from a regurgitate-treated plant (REGURGITATE).

To obtain a regurgitate-treated plant, five μ l regurgitate from fourth instar *C. partellus* larvae was inoculated in each hole and the test plant was incubated for 24 h at 25 °C before removing the leaves. The regurgitate was collected following the method described by Mattiacci et al. (1994). A larva was held with a pair of soft forceps and teased at the mouthparts with a microcapillary tube. In this way five to ten μ l regurgitate could be obtained from one larva. In all tests the control (CONTROL) odor source consisted of the leaves of a standard treated plant (with two artificially bored holes), incubated as long as the treatment it was tested against. In the first series the choice tests CONTROL vs. LARVAE-24h and CONTROL vs. LARVAE-48h were tested on the same day (40 wasps tested in each combination). In a second series the choice tests CONTROL vs. REGURGITATE and CONTROL vs. LARVAE-24h were tested on the same day (50 wasps tested in each combination).

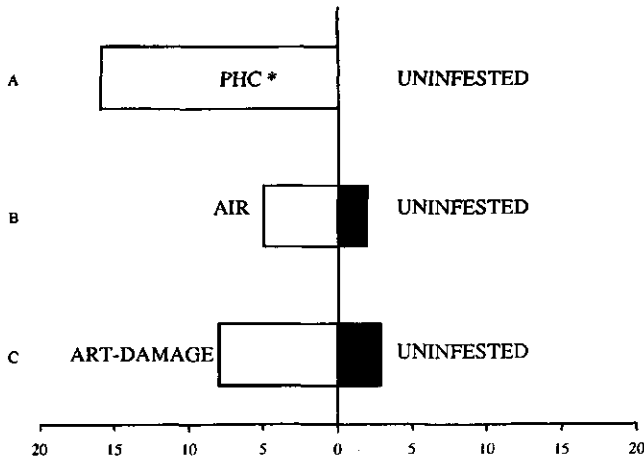


Figure 2.1 The response of *C. flavipes* to volatile chemicals emitted by *C. partellus*-infested and uninfested maize plants in a Y-tube olfactometer. In each dual choice test 40 females were tested. The bars indicate the number choosing for a particular odor source. Asterisks indicate statistically significant preferences within tests (chi-square, $P < 0.05$).

RESULTS

Uninfested vs. infested maize plants.

Volatiles emanating from a stemborer-infested maize plant attracted all responding parasitoids, when offered versus an uninfested plant (Fig 2.1a). Parasitoids were not attracted to odors emanating from uninfested maize plants or artificially damaged maize plants (Fig. 2.1b,c).

Stem vs. leaves.

Although the response level was low, the females that made a choice preferred the odors emanating from the undamaged leaves of a stemborer-infested plant, when offered together with undamaged leaves from an uninfested plant (Fig. 2.2a), hence the emission of attractive volatiles is not restricted to the site of damage, but occurs throughout the plant. Volatiles emanating from the infested stem part of the plant (with the feeding larvae) attracted the majority of the parasitoids, when tested against undamaged stems or the leaves of the very same infested plant (Fig. 2.2b,c).

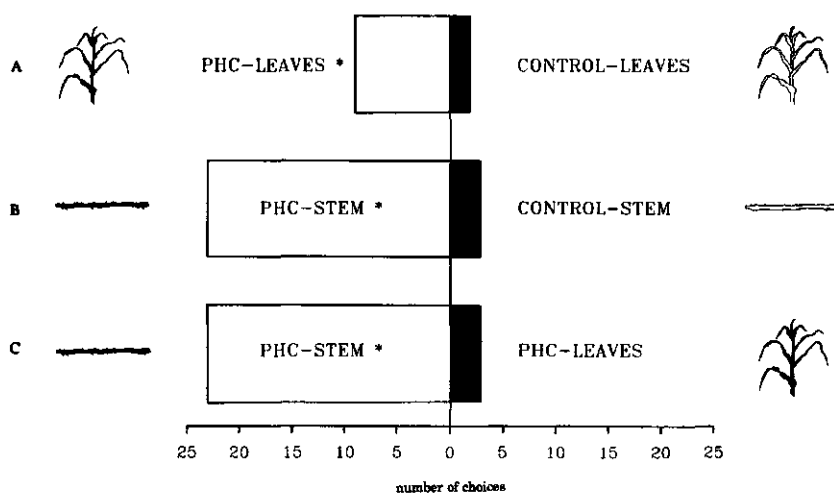


Figure 2.2 The response of *C. flavipes* to volatile chemicals emitted by different parts of the Plant-Host-Complex in a Y-tube olfactometer. In each dual choice test 47 females were tested. The bars indicate the number choosing for a particular odor source. Asterisks indicate statistically significant preferences within tests (chi-square, $P < 0.05$).

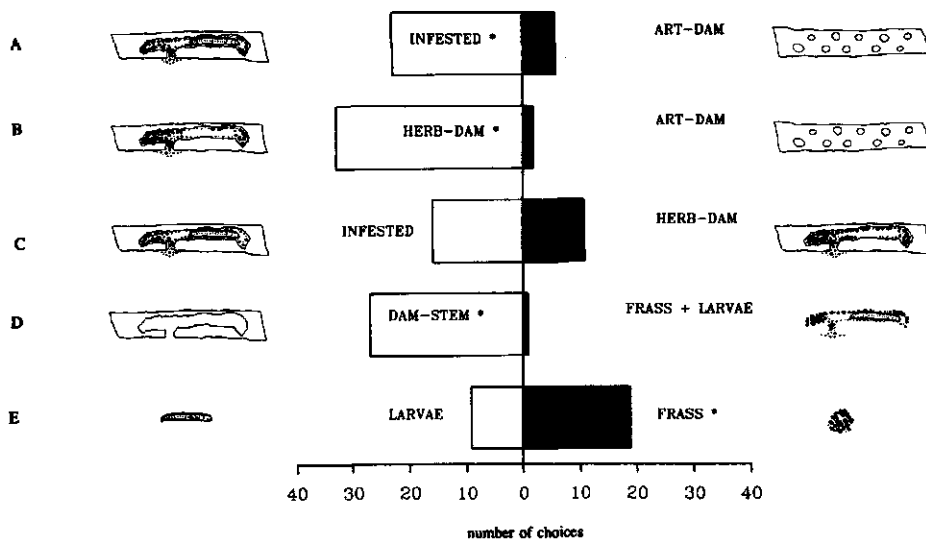


Figure 2.3 The response of *C. flavipes* to volatile chemicals emitted by different components of *C. partellus*-infested stem pieces. In each dual choice test 40 females were tested. The bars indicate the number choosing for a particular odor source. Asterisks indicate statistically significant preferences within tests (chi-square, $P < 0.05$). For more information on odor sources see Materials and Methods.

Stem.

The parasitoids were clearly more attracted to odors emanating from stemborer-damaged stem pieces, when tested against artificially damaged stem pieces (Fig. 2.3a). A stemborer damaged stem consists of feeding larvae in their tunnel filled with larval frass. The infested stems remained attractive even when the feeding larvae were removed (Fig. 2.3b). The feeding activity of the larvae did not significantly enhance the attractiveness of the infested stems. The parasitoids did not make a distinction between infested stems with or without larvae (Fig. 2.3c). When the larvae and their frass were removed from the infested stems and tested against the remaining stem, the latter was the most attractive (Fig. 2.3d). Frass by itself is also an attractive component of a stemborer infested stem. The majority of the parasitoids was attracted to frass when tested against larvae (Fig. 2.3e).

Leaves

Leaves of a stemborer-damaged plant, which had not been in contact with the feeding larvae in the stem, emitted attractive volatiles (Fig. 2.4). Leaves from a plant, where two stemborer larvae were allowed to feed for 24 h or 48 h attracted the majority of the parasitoids when tested against leaves from a plant with two artificially bored holes. The induced effect was clear after 24 h of feeding and was not significantly enhanced by a longer feeding time (Fig. 2.4). An elicitor of the systemic production of the plant produced synomones seems to be present in the regurgitate of the feeding larva. If larval regurgitate was inoculated into the stem, the leaves of the treated plant started to emit attractive volatiles and a similar response was obtained as with the leaves from a plant with two feeding larvae inside the stem (Fig. 2.4).

DISCUSSION

Plants have evolved several characteristics to defend themselves against herbivore attack. These direct defense mechanisms include plant structures that prevent herbivore settlement, detrimental chemicals in the plant tissue or the systemic production of defensive chemicals (Juniper & Southwood, 1986; Tallamy & Raupp, 1991). An indirect defense mechanism of plants is to promote the effectiveness of natural enemies of the herbivore (Price et al. 1980; Dicke & Sabelis, 1988). This may be achieved by mediating an interaction between the herbivore and its natural enemies. Evidence is accumulating that plants are actively involved in the release of chemical signals which attract natural enemies of the herbivore (see Dicke 1994 for review). The emission of these volatiles by infested plants is not limited to the damaged sites but occurs throughout the plant, even in undamaged leaves (Dicke et

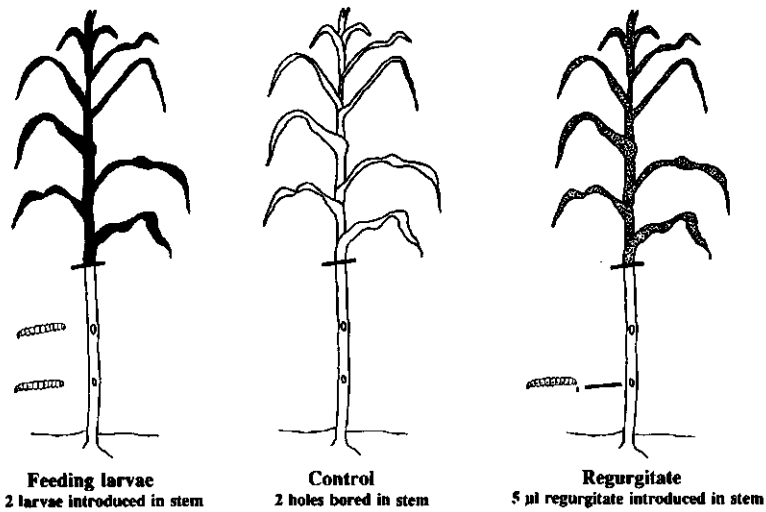


Figure 2.4 The response of *C. flavipes* to volatile chemicals emanating from the undamaged leaves of stemborer infested maize plants vs control leaves and to undamaged leaves from a regurgitate treated plant vs control leaves. For more information on odor sources see Materials and Methods. The bars indicate the number choosing for a particular odor source. Asterisks indicate statistically significant preferences within tests (chi-square, $P < 0.05$).

al. 1990b, 1993; Takabayashi et al. 1991; Turlings & Tumlinson, 1992). All this research involves externally feeding herbivores. The present study provides evidence that even when a plant is injured by a herbivore that feeds *inside* the stem, the whole plant emits chemical signals which attract a natural enemy of the herbivore. The concealed lifestyle of the stemborer larvae was found not to limit the emission of plant synomones. Female *C. flavipes* can discriminate between odors emitted by uninfested and stemborer-infested maize plants (Potting et al. 1993; Fig. 2.1). A major source of the attractive volatiles from the plant-host-complex is the stemborer-infested stem (Fig. 2.2). A stemborer-infested stem consists of a feeding larva inside a tunnel filled with larval frass. The larvae feeding inside the stem appear to trigger the emission of attractants by the plant, but do not emit attractants themselves. The frass produced by the feeding larvae was an attractive component, but odors emanating from a stemborer-injured stem are even more attractive (Fig 2.3). This parallels results from other studies in which herbivore-damaged plants or plant parts were more attractive to natural enemies than the herbivore or its products (Roth et al. 1982; Turlings et al. 1991a; Steinberg et al. 1993; McCall et al. 1993; Geervliet et al. 1994).

The release of the attractive volatiles is not restricted to the infested stem part but occurs systemically throughout the plant. Female *C. flavipes* are attracted to

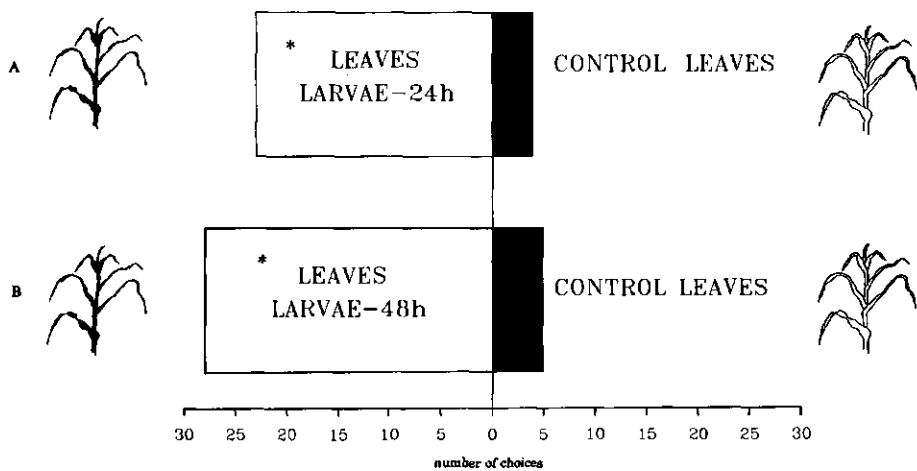
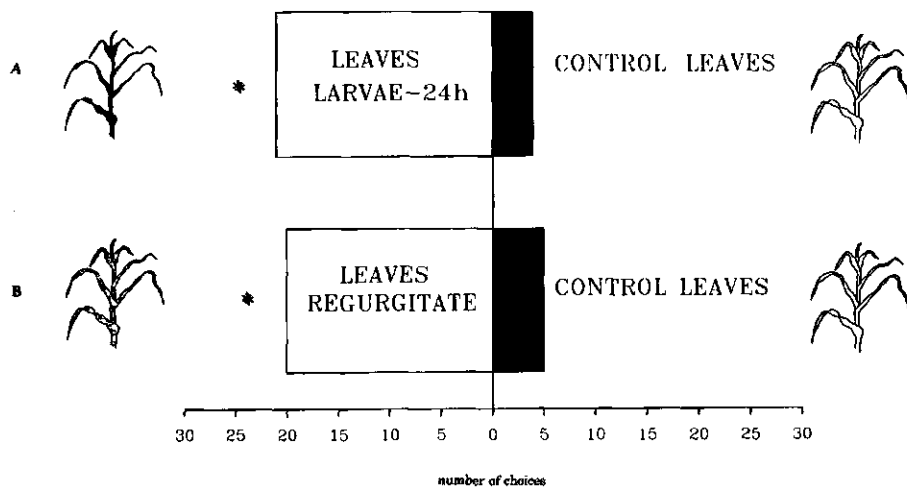


Fig 2.4 Continued

volatiles released by the undamaged leaves of a stemborer-infested plant (Fig. 2.4). The systemic release of these volatiles probably enhances the detectability of the infested plant for natural enemies. However, when given a choice between the odors emanating from the leaves and from the stem of an infested plant, the majority of the parasitoids was attracted to the infested stem. This may indicate that at close range the parasitoid is able to find the infested part of the plant. A similar result was reported by Dicke et al. (1993) who found that predatory mites preferred host-damaged leaves when offered versus undamaged leaves which emitted herbivore-induced synomones in response to an endogenous elicitor. A factor that induces the systemic response seems to be present in the regurgitate of the larva. When regurgitate was inoculated into the stem, the leaves of the treated plant emitted volatiles which attracted female *C. flavipes*. It remains to be investigated whether a regurgitate component itself or an endogenous elicitor induced by the regurgitate is transported through the stem to the leaves which start to produce attractive volatiles. Some recent studies have focused on the elicitor that triggers the systemic response of herbivore-infested plants. Dicke et al. (1993) extracted an elicitor that is transported from infested leaves to uninfested leaves. Turlings et al. (1993) could induce the production of plant volatiles by placing maize seedlings in diluted regurgitate from several species of caterpillars and a grasshopper. Recently Mattiacci et al. (1994, 1995) shed more light on the elucidation of the factor responsible for the induced plant response. They showed that an elicitor present in the gut regurgitant of *Pieris brassicae* caterpillars induced the release of synomones by mechanically damaged cabbage leaves (Mattiacci et al. 1994). They identified the enzyme β -glucosidase, a component of the regurgitant of *P. brassicae* caterpillars, as an elicitor of the synomone release by *P. brassicae* infested leaves (Mattiacci et al. 1995). Glucosidases seem to be common enzymes among herbivorous insects (Mattiacci et al. 1995) and it remains to be investigated if they are responsible for the induced plant responses in different plant-host-complexes, which could indicate that the plant response is a general response to attack by herbivorous insects.

A detailed analysis of the specific chemicals involved can establish whether there are qualitative and/or quantitative differences in the volatile blends emanating from the stem or the leaves of an infested plant. An identification of the specific chemicals can furthermore reveal whether the same terpenoids are involved as is shown for the maize - *Spodoptera exigua* - *C. marginiventris* system (Turlings et al. 1990; 1991b) and the maize - *Pseudaletia* - *Cotesia kariyai* system (Takabayashi et al. 1994).

In their search for the host habitat, parasitoids may exploit volatile cues from uninfested plants (Vinson, 1976; Nordlund et al. 1988; Vinson & Williams, 1991). For example, a response towards volatiles from uninfested maize plants was

demonstrated for *Macrocentrus grandii* (Ding et al. 1989a; Udayagiri & Jones, 1992) and *Eriborus terebans* (Ma et al. 1992), two larval parasitoids of *Ostrinia nubilalis*. In the present study female *C. flavipes* were not attracted to odors from uninfested or artificially damaged maize plants (Fig. 2.1). However, Ngi-Song et al. (1994) reported that *C. flavipes* was attracted to uninfested maize plants in a bioassay similar to the one used in our study. The discrepancy in these results could be due to different maize cultivars or to a different method of rearing the plants. In our study plants were grown in a greenhouse and treated regularly against aphids and other herbivores when necessary. Ngi-Song et al. (1994) used plants grown under field conditions and it is likely that they were infested with herbivores, which could have resulted in non-host damage and subsequent release of herbivore-induced synomones. Vinson & Williams (1991) reported a similar finding: the parasitoid *Campeletis sonorensis* was attracted to uninfested plants grown in a greenhouse, which were exposed to aphids and thrips, whereas it did not respond to uninfested plants grown in a herbivore free environmental chamber.

Through their concealed lifestyle stemborer larvae have created a partially enemy free space, especially for predators like birds and spiders. However, especially parasitoids have evolved the ability to successfully locate and attack the larvae inside the plant tissue (Smith et al. 1993). Stemborer larvae have to feed and defecate, and although they do this inside the plant tissue it does not prevent the release of attractive volatiles either through the frass they produce or through the volatiles which are produced systemically by the plant as a response to the feeding activity of the larvae. This study demonstrates that a stemborer parasitoid can exploit these plant-produced volatiles to locate the host-microhabitat.

ACKNOWLEDGMENTS

We thank Leo Koopman, Henk Snellen and André Gidding for insect rearing and Mariëlle Wintgens for the drawings. Bill Overholt, Joop van Lenteren, Jacqueline Geervliet, Letizia Mattiacci and Nicky Agelopoulos are acknowledged for valuable comments on a previous version of the manuscript. This work was supported by the Netherlands foundation for the Advancement of Tropical Research (WOTRO).

REFERENCES

- Alam, M.M., Bennett, F.D. and Carl, K.P. 1971. Biological control of *Diatraea saccharalis* (F.) in Barbados by *Apanteles flavipes* Cam. and *Lixophaga diatraeae* T.T. *Entomophaga* 16: 151-158.
- Alam, M.M., Beg, M.N., and Ghani, M.A. 1972. Introduction of *Apanteles* spp. against graminaceous borers into Pakistan. *Technical Bulletin CIBC* 15: 1-10.
- Baker, W.A., Bradley, W.G. and Clark, C.A. 1949. Biological control of the European corn borer in the United States. *USDA Tech. Bull.* 983: 1-185.
- Betbeder-Matibet, M. and Malinge, P. 1968. Un succès de la lutte biologique: contrôle de *Proceras*

- sacchariphagus* Boj. «Borer ponctué» de la canne à sucre à Madagascar par un parasite introduit: *Apanteles flavipes* Cam. *Agron. Tropicale* 22: 1196-1220.
- Dicke, M. 1994. Local and systemic production of volatile herbivore-induced terpenoids: their role in plant-carnivore mutualism. *J. Plant Physiol.* 143: 465-472.
- Dicke, M. and Sabelis, M.W. 1988. How plants obtain predatory mites as body guards. *Neth. J. Zool.* 38: 148-165.
- Dicke, M., Van Beek, T.A., Posthumus, M.A., Ben Dom, N., Van Bokhoven, H. and De Groot, A.E. 1990a. Isolation and identification of volatile kairomone that affects acarine predator-prey interactions. Involvement of host plant in its production. *J. Chem. Ecol.* 16: 381-396.
- Dicke, M., Sabelis, M.W., Takabayashi, J., Bruin, J. and Posthumus, M.A. 1990b. Plant strategies of manipulating predator-prey interactions through allelochemicals: prospects for application in pest control. *J. Chem. Ecol.* 16: 3091-3118.
- Dicke, M. and Dijkman, H. 1992. Induced defence in detached uninfested leaves: effects on behaviour of herbivores and their predators. *Oecologia* 91: 554-560.
- Dicke, M., Van Baarlen, P., Wessels, R. and Dijkman, H. 1993. Herbivory induces systemic production of plant volatiles that attract predators of the herbivore: extraction of endogenous elicitor. *J. Chem. Ecol.* 19: 581-599.
- Ding, D., Swedenborg, P.D. and Jones, R.L. 1989a. Chemical stimuli in host-seeking behavior of *Macrocentrus grandii* (Hymenoptera: Braconidae). *Ann. Ent. Soc. Am.* 82: 232-236.
- Ding, D., Swedenborg, P.D. and Jones R.L. 1989b. Plant odor preferences and learning in *Macrocentrus grandii* (Hymenoptera: Braconidae), a larval parasitoid of the european corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J. Kansas Ent. Soc.* 62: 164-176
- Fuchs, T.W., Huffman, F.R. and Smith, J.W. 1979. Introduction and establishment of *Apanteles flavipes* (Hym.: Braconidae) on *Diatraea saccharalis* (Lep.: Pyralidae) in Texas. *Entomophaga* 24: 109-114.
- Geervliet, J.B.F., Vet, L.E.M. and Dicke, M. 1994. Volatiles from damaged plants as major cues in long-range-host-searching by the specialist parasitoid *Cotesia rubecula*. *Entomol. Exp. Appl.* 73: 289-297.
- Gifford, J.R. and Mann, G.A. 1967. Biology, rearing, and a trial release of *Apanteles flavipes* in the Florida Everglades to control the sugarcane borer. *J. Econ. Entomol.* 60: 44-47.
- Jeffries, M.J. and Lawton, J.H. 1984. Enemy free space and the structure of ecological communities. *Biol. J. Linn. Soc.* 23: 269-286.
- Juniper, B.E. and Southwood, T.R.E. (eds.) 1986. Insects and the plant surface. Edward Arnold, London.
- Kajita, H. and Drake, E.F. 1969. Biology of *Apanteles chilonis* and *A. flavipes* (Hymenoptera: Braconidae), parasites of *Chilo suppressalis*. *Mushi* 42: 163-179.
- Leerdam, M.B. van, Smith J.W. Jr. and Fuchs, T.W. 1985. Frass-mediated host finding behavior of *Cotesia flavipes*, a Braconid parasite of *Diatraea saccharalis* (Lepidoptera: Pyralidae). *Ann. Ent. Soc. Am.* 78: 647-650.
- Ma, R.Z., Swedenborg, P.D. and Jones, R.L. 1992. Host-seeking behavior of *Eriborus terebrans* (Hymenoptera: Ichneumonidae) toward the european corn borer and the role of chemical stimuli. *Ann. Ent. Soc. Am.* 85: 72-79.
- Mattiacci, L., Dicke, M. and Posthumus, M.A. 1994. Induction of parasitoid attracting synomone in brussels sprouts plants by feeding of feeding *Pieris brassicae* larvae: role of mechanical damage and herbivore elicitor. *J. Chem. Ecol.* 20: 2229-2247.
- Mattiacci, L., Dicke, M. and Posthumus, M.A. 1995. β -glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proc. Natl. Acad. Sci. USA* 92: 2036-2040.
- McCall, P.J., Turlings, T.C.J., Lewis, W.J. and Tumlinson, J.H. 1993. The role of plant volatiles in host location by the specialist parasitoid *Microplitis croceipes* Cresson (Hymenoptera: Braconidae). *J. Insect Behav.* 6: 625-639.

- Mohyuddin, A.I. 1971. Comparative biology and ecology of *Apanteles flavipes* (Cam.) and *A. sesamiae* Cam. as parasites of graminaceous borers. *Bull. Entomol. Res.* 61: 33-39.
- Mohyuddin, A.I., Inayatullah, C. and King, E.G. 1981. Host selection and strain occurrence in *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) and its bearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* 71: 575-581.
- Ngi Song, A.J., Overholt, W.A., Lwande, W. and Njagi, P.G.N. 1994. Effect of plant produced and host related allelochemicals on the host selection behaviour of *Cotesia flavipes* and *Cotesia sesamiae*. *Insect Sci. Appl.* (in press).
- Nordlund, D.A.; Lewis, W.J. and Altieri, M.A. 1988. Influences of plant produced allelochemicals on the host and prey selection behavior of entomophagous insects, pp.65-90, in: P. Barbosa and D. Letourneau (eds.), *Novel Aspects of Insect-Plant Interactions*, Wiley and Sons, New York.
- Ochieng, R.S., Onyango, F.O. and Bungu, M.D.O. 1985. Improvement of techniques for mass-culture of *Chilo partellus* (Swinhoe). *Insect Sci. Appl.* 6: 425-428.
- Overholt, W.A., Ngi-Song, A.J., Kimani, S.K., Mbapila, J., Lammers, P. and Kioko, E. 1994. Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), in Africa. *Biocontrol News Inf.* 15: 19-24.
- Potting, R.P.J., Osa-Danso, F., Overholt, W.A. and Ngi-Song, A.J. 1993. Host selection in *Cotesia flavipes*, parasitoid of tropical stemborers. *Proc. Exper. Appl. Entomol. NEV* 4: 47-52.
- Price, P.W., Bouton, C.E., Gross, P., McPherson, B.A., Thompson, J.N. and Weis, A.E. 1980. Interactions among three trophic levels: influence of plant on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11: 41-65
- Roth, J.P., King, E.G. and Hensley, S.D. 1982. Plant, host and parasite interactions in the host selection sequence of the Tachinid *Lixophaga diatraea*. *Environ. Entomol.* 11: 273-277.
- Seshu Reddy, K.V. and Davies, J.C. 1978. A new medium for mass rearing of the sorghum stem borer *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) and its use in resistance screening. *Ind. J. Plant Protection* 6: 48-55.
- Smith, J.W., Jr., Wiedenmann, R.N. and Overholt, W.A. 1993. Parasites of lepidopteran stemborers of tropical graminaceous plants. ICIPE Sciences Press, Nairobi. 89 pp.
- Steinberg, S., Dicke, M., Vet, L.E.M. and Wainwright, R. 1992. Response of the braconid *Cotesia* (= *Apanteles*) *glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomol. exp. appl.* 63: 163-175.
- Steinberg, S., Dicke, M. and Vet, L.E.M. 1993. Relative importance of infochemicals from first and second trophic level in long-range host location by the larval parasitoid *Cotesia glomerata*. *J. Chem. Ecol.* 19: 47-59.
- Strong, D.R., Lawton, J.H., and Southwood, T.R.E. 1984. Insects on plants. Community patterns and mechanisms. Blackwell Scientific Publications, Oxford.
- Takabayashi, J., Dicke, M. and Posthumus, M.A. 1991. Induction of indirect defence against spider-mites in uninfested Lima bean leaves. *Phytochemistry* 30: 1459-1462.
- Takabayashi, J., Takahashi, S., Dicke, M. and Posthumus, M.A. 1994. Effect of time and developmental stage of the herbivore *Pseudaletia separata* in the production of herbivore-induced synomones by corn plants. *J. Chem. Ecol.* 21: 273-287.
- Tallamy, D.W. and Raupp, M.J. (eds.). 1991. Phytochemical induction by herbivores. John Wiley and Sons, New York.
- Turlings, T.C.J., Tumlinson, J.H. and Lewis, W.J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.
- Turlings, T.C.J., Tumlinson, J.H., Eller, F.J., Lewis, W.J. 1991a. Larval-damaged plants: source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the micro-habitat of its hosts. *Entomol. exp. appl.* 58: 75-82.
- Turlings, T.C.J., Tumlinson, J.H., Heath, R.R., Proveaus, A.T., Doolittle, R.E. 1991b. Isolation

- and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. *J. Chem. Ecol.* 17: 2235-2251.
- Turlings, T.C.J. and Tumlinson, J.H. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proc. Natl. Acad. Sci. USA* 89: 8399-8402.
- Turlings, T.C.J., McCall, P.J., Alborn, H.T. and Tumlinson, J.H. 1993. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *J. Chem. Ecol.* 19: 411-425.
- Udayagiri, S. and Jones, R.L. 1992. Role of plant odor in parasitism of european corn borer by braconid specialist parasitoid *Macrocentrus grandii* Goidanich: isolation and characterization of plant synomones eliciting parasitoid flight response. *J. Chem. Ecol.* 18: 1841-1855.
- Van Leerdam, M.B., Smith, J.W. and Fuchs, T.W. 1985. Frass-mediated, host finding behavior of *Cotesia flavipes*, a Braconid parasite of *Diatraea saccharalis* (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. Am.* 78: 647-650.
- Vet, L.E.M. and Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37: 141-172.
- Vet, L.E.M., Wäckers, F.L. and Dicke, M. 1991. How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. *Neth. J. Zool.* 41: 202-213.
- Vinson, S.B. 1976. Host selection by insect parasitoids. *Annu. Rev. Entomol.* 21: 109-134.
- Vinson, S.B. and Williams, H.J. 1991. Host selection behavior of *Campoletis sonorensis*: a model system. *Biol. control* 1: 107-117.

Foraging behavior and life history of the stemborer parasitoid *Cotesia flavipes*.

ABSTRACT The short range foraging behavior of female *C. flavipes* was studied on stemborer infested plants, in patches with host-related products and in artificial transparent tunnels. In addition, the longevity under specific conditions and the potential and realized fecundity of female *C. flavipes* was determined. Larval frass, caterpillar regurgitate and holes in the stem are used in host location by *C. flavipes*. No differences are found in the behavior of *C. flavipes* on maize plants infested with the suitable host, *Chilo partellus* (Lepidoptera: Pyralidae), or the unsuitable host, *Busseola fusca* (Lepidoptera: Noctuidae). Attacking a stemborer larva inside the stem is risky for the parasitoid. The mortality rate of the parasitoids inside the stem is high: 30-40% of the parasitoids are killed by the spitting and biting stemborer larva. *C. flavipes* is relatively short lived: without food the parasitoids die within two days, with food and under high humidity conditions they die within 5-6 days. *C. flavipes* is pro-ovigenic and has around 150 eggs available for oviposition. A relatively large proportion of the available egg load (20-25%) is allocated to each host, so female *C. flavipes* are egg depleted after parasitizing 5-6 hosts only.

INTRODUCTION

In their evolutionary game of hide and seek with natural enemies, some herbivores have adapted a concealed lifestyle, such as stemborers and root feeders. It has been hypothesized that an endophytic feeding style provides a physical refuge for certain herbivores. Feeding inside plant tissue makes it more difficult for enemies to locate and attack potential hosts (Askew and Shaw, 1986; Hawkins, 1994). However, being part of the evolutionary game, some parasitoids have developed behavioral and morphological characteristics allowing them to utilize hosts with these concealed lifestyles. Larval parasitoids of stemborers have evolved several fascinating strategies to attack their hosts inside the plant stem (Baker et al., 1949; Smith et al., 1993). For instance, some parasitoid species have long or strong ovipositors with which they drill through the plant tissue to attack their cryptic hosts. Some tachinid parasitoids larviposit a mobile maggot at the tunnel entrance that actively searches for the host. Other tachinid parasitoid species deposit their eggs in the tunnel, and these eggs only develop when they are ingested by the host (refs. in Bennett 1969). Finally, some

small parasitoid species ingress the stemborer tunnel and parasitize the host there. Here we study the parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae), which utilizes the last tactic. *C. flavipes* has been used worldwide for biological control of pyralid stemborers in maize, sorghum and sugarcane (Polaszek and Walker, 1991) and has recently been introduced in East Africa for the control of the introduced stemborer, *Chilo partellus* (Lepidoptera: Pyralidae), in maize and sorghum (Overholt et al., 1994a). Indigenous stemborers in maize and sorghum in East Africa include *Chilo orichalcociliellus* (Pyralidae), *Busseola fusca* and *Sesamia calamistis* (Noctuidae). Ngi-Song et al. (1995) demonstrated that these native stemborer species are suitable hosts for *C. flavipes* with the exception of *B. fusca*, in which the eggs of the parasitoid are encapsulated.

Although *C. flavipes* has been categorized as a parasitoid that enters the stemborer tunnel (Smith et al., 1993), the foraging behavior on infested plants has never been thoroughly studied. We investigated the foraging behavior of *C. flavipes* on maize plants infested by *C. partellus* or *B. fusca* and determined the mortality risks involved in attacking a stemborer larva inside the tunnel. In addition, the role of host related products in host location in *C. flavipes* was examined.

Kajita and Drake (1969) reported that the potential fecundity of *C. flavipes* was approximately 159 and that the mean clutch size was 36.5 eggs in *Chilo suppressalis* larvae. Theoretically, a female *C. flavipes* can thus be completely depleted of eggs after parasitizing four hosts. We investigated the progeny allocation among hosts of individual female parasitoids, and determined whether *C. flavipes* is depleted of eggs after parasitizing four hosts only. Furthermore, we measured the life history traits longevity and fecundity in *C. flavipes*. The possible consequences of the ingress tactic for the evolution of life history traits and foraging decisions in *C. flavipes* are discussed.

MATERIALS AND METHODS

Insects

A colony of *C. flavipes* originating from Pakistan was maintained on laboratory-reared fourth instar *C. partellus* larvae. Larvae of *C. partellus*, *C. orichalcociliellus*, *B. fusca* and *S. calamistis* were reared on artificial diets described by Ochieng et al. (1985) and Onyango and Ochieng-Odero (1994). For more details on the rearing procedures we refer to Ngi-Song et al. (1995). Larvae of *Spodoptera exempta* were reared according to the method described by Janssen (1994). For the experiments, 1-2 days old mated female *C. flavipes* were used, that were naive with respect to oviposition or contact with host related cues.

Experimental procedures

Adult Longevity

The longevity of mated females was measured at four different temperatures (22 °C, 25 °C, 28 °C and 31 °C), two humidities (35-40 % RH and 70-80% RH) and with or without food. Females were kept individually in glass vials (2.5 x 7 cm), with or without a drop of honey as food and with or without a layer of agar agar to maintain a high humidity. The lifespan of ten individuals was measured for each treatment combination. The light intensity in all incubators was 450 lux in the 12 h photophase. Mortality was recorded every two hours until all the insects had died. Treatment effects were tested using analysis of variance with temperature, humidity and food as main effects.

Fecundity

The potential fecundity of individual females was measured by dissecting one day old, mated females (n=35) and recording the number of ovarian eggs. The realized fecundity was determined by offering individual females two hosts (one in the morning and one in the afternoon) each day until they died. Fourth instar *C. partellus* larvae fed upon maize stems were used as hosts. Individual females (n=20) were kept in a glass vial at 25 °C, 70-80% RH and fed with a drop of honey. The experiment was terminated after five days, when all females had died. The parasitized larvae of each female were maintained individually on artificial diet and dissected 5-7 days after parasitization to assess the number of parasitoid progeny. The clutch size was assumed to be equal to the number of parasitoid larvae present 5-7 days after oviposition, as *C. flavipes* eggs or larvae are not encapsulated by *C. partellus* (Ngi-Song et al., 1995). Wasps that were killed by the host or escaped during the experiment were excluded from the analysis. The oviposition time on the first host was measured using a stopwatch. The oviposition time was defined as the duration of ovipositor insertion in the host.

Foraging behavior on plant

The foraging behavior of 20-23 *C. flavipes* females on uninfested maize plants and maize plants infested by *C. partellus* or *B. fusca* was observed in a temperature controlled room (23-26 °C, RH 65-75%). Uninfested maize plants (tassel stage, 70-110 cm) obtained from a farmer's field were used as a standard clean maize plant. Stemborer infested plants were obtained by introducing two larvae into holes bored horizontally in the stem, spaced 10 cm apart, with the first hole 5 cm from soil level. The larvae were introduced into the plant 18 h prior to the first experiment. During this time the larvae bored a small tunnel in the stem and most of them pushed some frass out of the artificially bored entrance hole.

A female wasp was released on the stem 25 cm above soil level, thus 10 cm above the top hole on infested plants. The behavior and the location of the parasitoid on the plant was observed continuously and recorded with a portable computer programmed as an eventrecorder. The following parameters were recorded: behavior: *Stand, Walk, Fly, Preen, Examine frass*; location: *Stem, Tunnel Entrance, Inside Tunnel, Whorl, Leaves*. The stem of the plant was divided into two parts. The lower part (20 cm above soil level) included the two holes and stemborer tunnels. The upper part included all parts of the plant higher than 20 cm above soil level. Experiments were terminated after 90 minutes or when the parasitoid left the plant by flying or walking away on the soil. After each replicate the plant was removed and replaced. If the parasitoid entered the stemborer tunnel, the larva in that hole was removed after the experiment, introduced into a vial containing artificial diet and dissected a few days later to determine if it was parasitized. For wasps that did not come out of the tunnel by the time the observation ended, the stem was opened to check if the wasp was dead or alive.

Contact stimuli involved in tunnel location

1. Response to holes in stem.

The behavior and residence time on untreated stems and stems with three artificially bored holes were recorded. The holes were bored 2.5 cm apart and the treatment zone was defined as the area 2.5 cm below the lowest hole and 2.5 cm above the highest hole. A female wasp (naive, 2 days old) was released at the centre of the treatment zone (10 cm) and the time spent in the treatment zone was measured. For the stems with holes the number of visits to the holes and the time spent inside the holes was recorded as well.

2. Response to host and non-host related products.

The response to frass and caterpillar regurgitate was tested in a petridish setup. Frass was collected from 4th-5th instar larvae of the stemborers *C. partellus*, *C. orichalcociliellus*, *B. fusca* and *S. calamistis* feeding on maize stems and from larvae of the leaf feeder *S. exempta* feeding on maize leaves. The regurgitate of larvae of *C. partellus* and *S. exempta* was collected by holding a larva with a soft forceps and teasing the mouthparts with a microcapillary pipet (Sigma, 5 μ l). The regurgitate (5 μ l) was applied to clean maize stem particles. The material to be tested (0.3 gr) was placed at the centre of a circular filterpaper (Whatman #3, 9 cm) that was placed in a glass Petri dish. The treatment zone was defined as the area 7 cm around the test-material, marked with a thin pencil line. The residence time of individual *C. flavipes* females inside the treatment zone was measured. In the first series the response to frass from different stemborer species (*C. partellus*, *C. orichalcociliellus*, *B. fusca* and *S. calamistis*) was compared. In the second series the response to regurgitate of

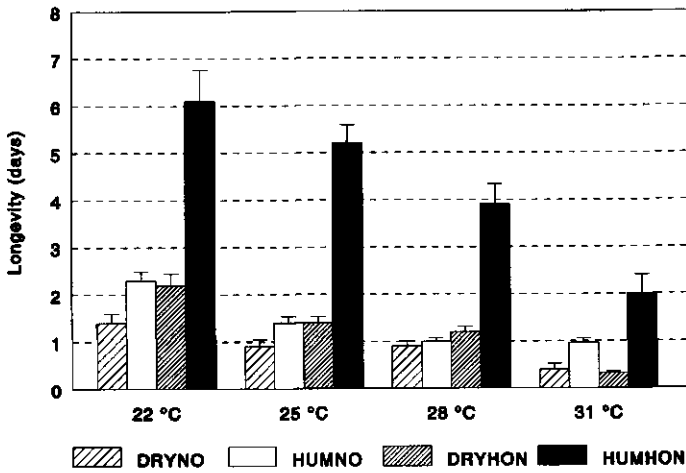


Figure 3.1 Average longevity (\pm SE, $n=10$) of *C. flavipes* females at four different temperatures and four different experimental conditions. DRYNO = Low humidity and no food; HUMNO = high humidity and no food; DRYHON = Low humidity with honey; HUMHON = high humidity with honey.

C. partellus was tested. *C. partellus* frass and clean maize stem particles served as controls. In the third series the response to frass and regurgitate of *S. exempta* was tested with *C. partellus* frass and clean maize stem particles as controls. The different test materials in the three series of assays were all tested on the same day.

Behavior inside tunnel

To record the behavior of the parasitoid inside the stemborer tunnel an artificial transparent tunnel with a feeding larva was created by using small glass tubes (6 x 0.5 cm). Glass tubes were filled with maize stem pieces and one fourth instar *C. partellus* larva was introduced into the tube. The tube was plugged with cotton wool at both ends and the larva was allowed to feed overnight and create a tunnel filled with larval frass. The behavior of individual female *C. flavipes* and the *C. partellus* larva inside the artificial tunnel was recorded.

RESULTS

Adult Longevity

There were significant effects of temperature ($F=62.8$, $DF=3$, $P<0.0001$), humidity ($F=262.8$, $DF=1$, $P<0.0001$) and food availability ($F=148.2$, $DF=1$, $P<0.0001$) on the longevity of *C. flavipes* females. Lower temperatures, a humid environment and the availability of honey as a food source increased the lifespan of *C. flavipes* females (fig 3.1). The average life span of fed females in a humid environment (70-

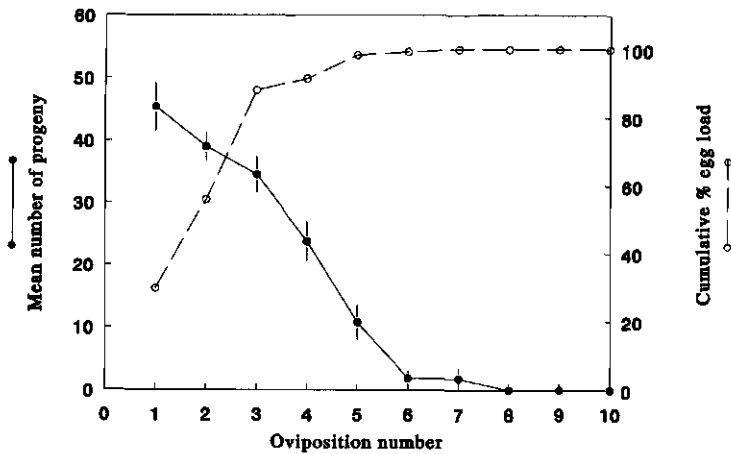


Figure 3.2 Clutch size allocation of *C. flavipes* in subsequently encountered hosts. Individual females were offered ten hosts in five days. Solid dots and line indicate the average number (\pm SE) of parasitoid larvae present 5-7 days after parasitization. Open dots and dashed line indicate cumulative percentage of individual realized fecundity. Numbers of females for oviposition number: 1,2,3 ($n=18$); 4,5,6 ($n=17$); 7 ($n=16$); 8 ($n=12$); 9 ($n=10$); 10 ($n=3$).

80% RH) at 22°C was 6.1 days, whereas fed parasitoids in a dry environment (35-40% RH) at 31°C lived an average of only 8 hours. There was a significant interaction between humidity and food ($F=65.2$, $DF=1$, $P<0.0001$). The availability of honey under humid conditions extended the lifespan of the parasitoids by a factor of 4 to 10, depending on temperature (fig 3.1).

Fecundity

C. flavipes is a pro-ovigenic species and has a fixed complement of eggs upon emergence. Dissection of 1 day old females revealed that the mean egg load was 144.2 ($SD=32.4$, $n=35$, range 84-212). For the first host the mean oviposition time was 7 s ($SD=2.9$, $n=17$, range 4-15) and the mean number of parasitoid progeny was 46.1 ($SD=4.0$, $n=17$, range 17-75). There was no significant correlation between oviposition time and clutch size ($r=0.43$, $n=17$, $P>0.05$). The realized fecundity, measured as the total number of progeny allocated to all attacked hosts, was 155.1 ($SD=39.9$, $n=18$, range 90-253). The clutch size allocated to hosts decreased with oviposition number (fig. 3.2). After the third host the females had allocated almost 80% of their initial egg load (fig. 3.2), which was estimated with their realized fecundity. Most females were depleted of eggs after they had parasitized four to five hosts. These females kept accepting hosts, with oviposition times upto 30-40 sec, but were unable to lay eggs.

Table 3.1 Behavior of *C. flavipes* females on uninfested maize plants and maize plants artificially infested with two fourth-instar larvae of *C. partellus* or *B. fusca*. Significant differences are indicated by different letters. Means (\pm SE) tested with Mann-Whitney U test, ratios with G-test ($P < 0.05$, Yates correction). ¹Excluding wasps that died inside stem. ²Of those wasps that located and entered tunnel.

Parameter	Treatment of maize plant		
	<i>Chilo partellus</i>	<i>Busseola fusca</i>	Uninfested
Mean residence time on plant (s) ¹	2517 a (\pm 559, n=19)	3513 a (\pm 691, n=13)	835 b (\pm 302, n=20)
Percentage visiting lower part of plant	61% a (n=23)	86% a (n=22)	25% b (n=20)
Percentage locating entrance tunnel	57% a (n=23)	77% a (n=22)	
Latency time location entrance tunnel (s)	418a (\pm 180, n=13)	239a (\pm 71, n=17)	
Mean time spent inside stem (s) ¹	2138a (\pm 656, n=9)	1670a (\pm 736, n=8)	
Percentage killed inside stem	31% a (n=13)	47% a (n=17)	
Parasitization perc. ²	23% a (n=13)	24% a (n=17)	

Foraging behavior on plants

The mean foraging time of *C. flavipes* on infested plants was significantly longer than on uninfested maize plants. There was no difference in the time spent on *C. partellus* or *B. fusca* infested maize plants (Table 3.1). After release on the stem the parasitoids immediately started walking, sometimes interrupted by periods of standing or preening. On infested plants a significantly higher fraction of the parasitoids visited the lower part of the stem compared to females on clean plants (Table 3.1). Most wasps found the spot of infestation within 5 min. The entrance hole of the tunnel was usually filled with frass pushed out of the tunnel by the feeding stemborer larva. After contacting the frass the female started antennating it and immediately tried to enter the tunnel by crawling through the frass. The time spent inside the tunnel varied considerably (3-5209 s). Sometimes the parasitoid re-entered the tunnel several times, probably when she was unable to reach the larva. The mortality rate of the parasitoids inside the tunnel was high: 31% of the wasps that entered a *C. partellus* tunnel and 47% of the wasps that entered a *B. fusca* tunnel died. The wasps that died were bitten or squeezed to death by the stemborer larva. Some wasps were found stuck to very wet frass inside the tunnel. When a female *C. flavipes* entered a tunnel, 23% of the *C. partellus* larvae and 24% of the *B. fusca* larvae were successfully parasitized. Only four of the seven parasitoids that successfully parasitized a larva survived the host attack. For these wasps the handling time and

their behavior after a successful attack could be calculated. The handling times, defined as the time spent inside the host tunnel, were 16 sec, 206 sec, 489 sec and 2765 sec. After exiting a tunnel in which larvae were successfully parasitized, the parasitoids remained 27 min, 40 min, 72 min and 88 minutes on the plant. Of the 45 wasps with which we initiated our experiments, only one found and entered the other tunnel on the same plant after a successful host attack.

Contact stimuli involved in tunnel location

Larval frass offered in the Petri dish setup was intensively antennated and searched as previously described by Mohyuddin et al. (1981) and van Leerdam et al. (1985). Sometimes the parasitoid tried to find a way through the frass, a behavior similar to that observed on infested plants when the tunnel entrance was blocked with frass. *C. flavipes* females responded to the frass from the four stemborer species, and the only significant difference was the higher residence time on *C. partellus* frass compared to that on *B. fusca* frass (fig 3.2a). Regurgitate of *C. partellus* applied to maize particles also elicited a response in *C. flavipes* females. The parasitoids stayed significantly longer in a patch containing regurgitate than in a patch containing clean maize, and the response towards regurgitate was as high as the response to larval frass (fig 3.3b). Even frass and regurgitate from the leaf feeder *S. exempta* elicited a response in *C. flavipes*. The response to regurgitate of *S. exempta* was as strong as the response to *C. partellus* frass (fig 3.3c).

To investigate whether the presence of larval frass stimulated the parasitoid to enter a tunnel, the response of *C. flavipes* to an artificially bored hole in a clean maize stem was examined. The majority of wasps (80%) released on a stem with holes entered and examined the holes. The mean time spent examining artificial holes was 130 sec. Parasitoids stayed significantly longer (Mann-Whitney U test, $P < 0.05$) on the stem with three artificially bored holes (423 ± 73 sec, $n=30$) than on a clean maize stem (43 ± 8 sec, $n=30$).

Behavior inside tunnel

Because the behavior of the wasps inside a stemborer tunnel could not be observed, the behavior of wasps in an artificial transparent tunnel was investigated. All females immediately tried to enter the tunnel. The female wasp needed only very small openings in the larval frass filled tunnel to continue her search. Once the female contacted the host, she immediately inserted her ovipositor into the host and remained motionless for a few seconds, usually with raised wings. After oviposition, the female immediately turned around and walked out of the tunnel, and typically started preening once outside the tunnel. When contacted by the parasitoid, most larvae exhibited a defensive behavior by moving vigorously, spitting and attempting to bite the parasitoid. If the parasitoid attacked the head region of the host, it was

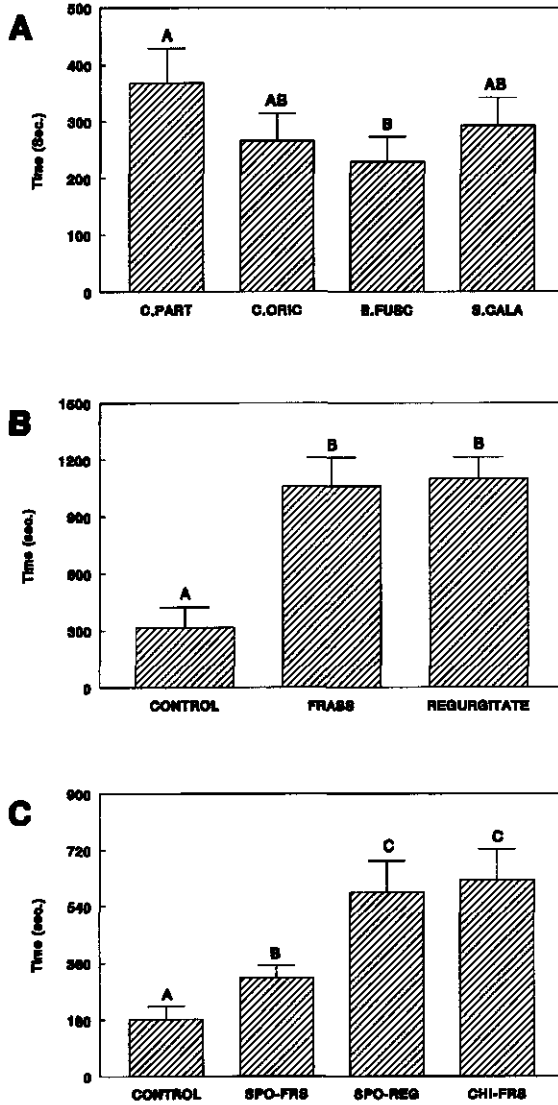


Figure 3.3 Response of *C. flavipes* females to larval frass and regurgitate. Indicated are average searching time (\pm SE) in treated patch. (A) Response to 0.3 g frass from four different stemborer species: C.PART = *C. partellus*, C.ORIC = *C. orichalcociliellus*, B.FUSC = *B. fusca* and S.CALA = *S. calamistis* (n=38). (B) Response to regurgitate of *C. partellus*. CONTROL = clean maize stem particles (n=23); FRASS = larval frass from *C. partellus* on maize (n=17); REGURGITATE = clean maize particles treated with *C. partellus* regurgitate (n=21). (C) Response to larval frass and regurgitate of *S. exempta* larvae. CONTROL = clean maize stem particles (n=21); SPO-FRS = larval frass from *S. Exempta* larvae on maize leaves (n=20); SPO-REG = clean maize treated with *S. exempta* regurgitate (n=20). CHI-FRS = larval frass from *C. partellus* on maize (n=16). Different letters above bars indicate significant different means (paired comparisons, Mann-Whitney U test, $\alpha=0.05$).

almost always bitten or squeezed to death by the agitated larva. Very often the wasps could not reach the host because the tunnel was blocked with larval frass. These wasps tried for 5-15 min to find an opening in the frass before leaving the tunnel.

DISCUSSION

C. flavipes has a relatively short lifespan compared to other stemborer parasitoids (Smith et al., 1993). Without food all parasitoids died within 48 hours, as was also reported by Wiedenmann et al. (1992). However, there was a synergistic effect of high humidity and honey: under these conditions *C. flavipes* females lived 4-10 times longer. Feeding increases longevity in numerous parasitoid species (Jervis and Kidd, 1993). It is unknown what food sources *C. flavipes* utilizes in nature. It may feed from extrafloral nectaries as a food source, or it may feed from wound fluids or larval frass on infested plants. In our observations we noticed that some wasps seemed to feed on plant juices and fresh larval frass.

The realized fecundity, measured as the total number of progeny allocated to the hosts, resembled the potential fecundity, measured as the egg load in one day old females that had never oviposited. If we take the realized fecundity as the indicator of the total eggload, *C. flavipes* has around 150 eggs available for oviposition. In each of the first three encountered hosts 35-45 eggs per host are laid. After oviposition in the third host, a female has allocated almost 80% of her available eggs, and approximately 40 eggs remain available for oviposition. If we assume that a clutch size of 40 eggs or more is the optimal clutch size in fitness terms, a female parasitoid can allocate all the remaining eggs to the fourth host, or decrease the clutch size and spread the progeny among several hosts. Under laboratory conditions *C. flavipes* females seem to do the latter and lay smaller clutches when they become egg limited. This is in agreement with clutch size models that predict small clutches when there is a risk that the parasitoid will run out of eggs (Parker and Courtney, 1984). However, under natural field conditions it may be very rare for a female to parasitize more than four hosts. After all, the probability that the female does not survive a successful parasitization is quite high. Around 40% of the wasps are killed inside the stem. Furthermore, the parasitoid may be time constrained to find another accessible host.

For a pro-ovigenic parasitoid, the number of expected host encounters in the lifetime can be estimated by dividing the potential fecundity (egg load upon emergence) by the optimal clutch size. An approximation of the optimal clutch size could be the mean clutch size found in the first encountered hosts. Reasoning this way, we expect a *C. flavipes* female to find 3-4 hosts only in her lifetime, if we

assume that the optimal clutch size is 40 and the potential fecundity is 150 eggs.

The probability of host encounter is influenced by factors such as the host range of the parasitoid, the availability of the susceptible host stage and the mortality rate at oviposition and between host encounters. One of the main factors responsible for the low number of expected host encounters in the lifetime of *C. flavipes* may be the high mortality rate at oviposition. Models for optimal progeny allocation predict that when host encounter rates are low, the optimal clutch size increases to a maximum value, which is the clutch size that maximizes fitness per host attacked (Parker and Courtney, 1984; Iwasa et al., 1984; Waage and Godfray, 1985). It remains to be investigated if this is true for *C. flavipes*. However, it is evident that *C. flavipes* allocates a relatively large proportion (20-25%) of the available eggload (155) to the first encountered host. As a consequence of the high mortality risk at oviposition the investment per host is high, as future opportunities to find other hosts may be low.

The role of the probability of host encounter in the evolution of progeny allocation is particularly expressed in parasitoid species that share the same foraging niche, such as parasitoids of larval stemborers. *Stenobracon deesae* is a parasitoid that also attacks the last larval stages of *C. partellus* larvae that feed inside the stem. However, this parasitoid has a different attack strategy, it stays outside and parasitizes the larva by ingressing her long ovipositor through the entrance of the stemborer tunnel. The mortality rate due to host defense is thus negligible. *S. deesae* allocates one egg per host and the reported mean realized fecundity is 23 (Narayanan and Chaudhuri, 1954). Reasoning the same way as above, the expected number of host encounters in the lifetime of *S. Deesae* is around 20 and the investment per host is low (4%) compared to *C. flavipes*.

To locate the host-microhabitat, female *C. flavipes* use olfactory stimuli that are emitted by the infested stem, but also by the undamaged leaves of infested plants (Potting et al., 1995). To locate the larva or pupa inside the tunnel, stemborer parasitoids use stimuli from frass (Roth et al., 1978; Leerdam et al., 1985), vibrations from the host (Hailemichael et al., 1994) or are stimulated by holes in the plant stem (Pfannenstiel et al., 1992). A host seeking response in *C. flavipes* was elicited by larval frass and holes in the stem. Holes in an uninfested stem elicited an entrance response in *C. flavipes*, so frass seems not to be essential for this. By examining holes without frass, the parasitoid may find other ways to enter to the stemborer tunnel if the main exit hole is blocked with frass. Van Leerdam et al. (1985) found that *C. flavipes* did not respond to dry frass and suggested that fresh frass could indicate to the parasitoid that it has found a tunnel with a feeding larva. A tunnel with dry frass could indicate that a tunnel was found where the larva has left (Berger, 1992) or has pupated. An active component in the frass may be situated in the gut regurgitate of

caterpillars. The regurgitate from its aboriginal host, *C. partellus*, as well as regurgitate of the leaf-feeder *S. exempta*, elicited a response in *C. flavipes* females. This similar response may have been induced by common gustatory enzyme(s) present in caterpillar regurgitate, such as glucosidases (Mattiacci et al., 1995). There are several reports that mandibular and gland secretions of lepidopterous hosts are an important source of short range attractants and arrestants for parasitoids (Vinson and Lewis, 1965; Corbet, 1971). Factors present in the regurgitate of larvae not only elicit a response upon contact, but also induces the release of volatiles by the leaves of an infested plant that attract female *C. flavipes* (Potting et al., 1995).

Once the parasitoid has found an entry to the stemborer tunnel it will try to reach the larva by crawling through the frass. The time spent inside the tunnel is probably dependent on the location of the larva and the amount of frass in the tunnel. The low parasitization rate (23-24%) of the larvae in tunnels that were visited by parasitoids was probably caused by larval frass blocking the tunnel and the larva being out of reach for the parasitoid.

It is a risky undertaking for *C. flavipes* to attack a stemborer larva inside the stem. Stemborer larvae do not passively undergo the parasitoid attack but defend themselves vigorously as was observed in the artificial glass tunnel. *C. flavipes* does not paralyze the larva before oviposition as some other ingressing stemborer parasitoids, such as *Microbracon* (= *Habrobracon*) *brevicornis* (Braconidae) (Genieys, 1925) and *Goniozus* spp. (Bethylidae) (Smith et al., 1993). Although oviposition only lasts for a few seconds in *C. flavipes*, the female can not always avoid being killed by the spitting and biting larva. Takasu and Overholt (unpublished data) studied the defense behavior of *C. partellus* in more detail, using the same artificial glass tunnel. They found that parasitoids approaching the larva towards the head had a high probability of being killed. However, the majority of the parasitoids that were killed were able to parasitize their host successfully.

When a parasitoid has a high mortality risk at each oviposition, life history theory predicts a high selectivity to avoid waste of progeny (Iwasa et al., 1984; Ward, 1992). The parasitoid should not risk her life for low quality hosts. In this study we found that *C. flavipes* did attack the unsuitable host *B. fusca* under semi-natural conditions. To risk your life and waste eggs in an unsuitable host does not seem to be an adaptive strategy. However, there is no co-evolutionary history between *C. flavipes* and *B. fusca*: *C. flavipes* originates from the Indo-australian region and *B. fusca* from the African continent. *C. flavipes* has not been under any selection pressure to avoid *B. fusca* or to circumvent its host defense mechanism.

As was discussed earlier, the number of expected host encounters is very low for *C. flavipes*. So, another possibility is that *C. flavipes* is so time constrained that she

will accept any host she finds in her foraging niche. The opportunity to attack another host may be limited. Furthermore, the probability of making a mistake by attacking an unsuitable host could be rare in the field. We would expect *C. flavipes* to have the ability to circumvent the host defense system of the prevailing stemborer species where it has evolved. Thus, *C. flavipes* may be a very opportunistic forager and accept any host it encounters inside the plant stem. However, it remains to be investigated whether *C. flavipes* avoids sympatric unsuitable hosts or already parasitized hosts. The response to host-related products by *C. flavipes* seems not be host-species specific. Although there was a difference in the response to frass from *C. partellus* and *B. fusca* in the petri dish setup (fig. 3.3), there were no significant differences in the behavior of *C. flavipes* on plants infested with *C. partellus* or *B. fusca* (table 3.1). Ngi-Song et al. (1995) found that *C. flavipes* accepted *C. partellus*, *C. orichalcociliellus*, *B. fusca* and *S. calamistis* at an equal rate for oviposition.

When an opportunistic forager is introduced into a foreign region, new parasitoid-host associations can develop when related host species occur in the same niche as the aboriginal host species (Hokkanen and Pimentel, 1989). An example of this is the successful biological control of the neotropical pyralid, *Diatraea saccharalis*, with *C. flavipes* in the Caribbean (Alam et al., 1971), North America (Fuchs et al., 1979) and Brazil (Macedo et al., 1993). However, this opportunistic foraging strategy could also pose problems in biological control programmes in areas where an unsuitable host occurs, such as *B. fusca* in Africa (Overholt et al., 1994a). In this study we have shown that *C. flavipes* will spend time and progeny and risk her life attacking this unsuitable host. *C. flavipes* is the most successful parasitoid that has been used against tropical gramineous stemborers (Overholt, 1994). At face value, *C. flavipes* has a remarkable life history for a successful biological control agent. It has a short lifespan and at best can parasitize only a few hosts. However, *C. flavipes* attacks the last larval stages of the host that are concealed in the stem and have a high survival rate (Overholt et al., 1994b). Furthermore, *C. flavipes* is gregarious, has a female biased sex ratio (Kajita and Drake, 1969) and sib mating seems to be common (Arakaki and Ganaha, 1986), so any problems in mate finding seem to be circumvented.

To conclude, the attack of a defending larva inside the stem is not without risk for a female *C. flavipes*. Due to the relatively high mortality rate inside the stem, the expected number of host encounters in the lifetime of *C. flavipes* is low. As a consequence, *C. flavipes* has an opportunistic foraging strategy and invests a high proportion of the available eggload in each encountered host.

ACKNOWLEDGMENTS

We thank John Onditi, Joseph Owino, Henk Snellen, Leo Koopman, Andre Gidding for the supply of stemborer larvae and parasitoids and Rieta Gols for the supply of *Spodoptera* larvae. Adele Ngi-Song is thanked for assistance in dissecting the parasitoids and Bart Knols for statistical advice. Marcel Dicke, Joop van Lenteren, Richard Stouthamer and Louise Vet are acknowledged for valuable comments on a previous version of the manuscript. This work was supported by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO).

REFERENCES

- Alam, M.M., Bennett, F.D. and Carl, K.P. 1971. Biological control of *Diatraea saccharalis* (F.) in Barbados by *Apanteles flavipes* Cam. and *Lixophaga diatraeae* T.T. *Entomophaga* 16: 151-158.
- Arakaki, N. and Ganaha, Y. 1986. Emergence pattern and mating behavior of *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae). *Appl. Entomol. Zool.* 21: 382-388.
- Askew, R.R. and Shaw, M.R. 1986. Parasitoid communities: Their size, structure and development. In: Waage, J.K. and Greathead D.J. (eds.), *Insect parasitoids*, pp. 225-264. Academic Press, London.
- Baker, W.A., Bradley, W.G., and Clark, C.A. 1949. Biological control of the European Corn Borer in the United States. *USDA Tech. Bull.* 983: 1-185.
- Bennett, F.D. 1969. Tachinid flies as biological control agents for sugarcane moth borers. In: Williams, J.R., Metcalfe, J.R., Mungomery, R.W. and Mathes, R. (eds.) *Pests of sugarcane*, pp. 116-148. Elsevier, Amsterdam.
- Berger, A. 1992. Larval movements of *Chilo partellus* (Lepidoptera: Pyralidae) within and between plants: timing, density responses and survival. *Bull. Entomol. Res.* 82: 441-448.
- Corbet, S.A. 1971. Mandibular gland secretion of larvae of the flour moth *Anagasta kuehniella*, contains an epideictic pheromone and elicits oviposition movement by a hymenopteran parasite. *Nature* 232: 481-484.
- Fuchs, T.W., Huffman, F.R. and Smith, J.W. 1979. Introduction and establishment of *Apanteles flavipes* [Hym.: Braconidae] on *Diatraea saccharalis* [Lep.: Pyralidae] in Texas. *Entomophaga* 24: 109-114.
- Genieys, P. 1925. *Habrobracon brevicornis* Wesm. *Ann. Entomol. Soc. Am.* 18: 143-202.
- Halelmech, Y., Smith, J.W. Jr., Wiedenmann, R.N. 1994. Host-finding behavior, host acceptance and host suitability of the parasite *Xanthopimpla stemmator*. *Entomol. Exp. Appl.* 71: 155-166.
- Hawkins, B.A. 1994. Pattern and process in host-parasitoid interactions, Cambridge University Press.
- Hokkanen, H.M. and Pimentel, D. 1989. New associations in biological control: theory and practice. *Can. Entomol.* 121: 829-840.
- Iwasa, Y., Suzuki, Y. and Matsuda, H. 1984. Theory of oviposition strategy of parasitoids. I. Effect of mortality and limited egg number. *Theor. Pop. Biol.* 26: 205-227.
- Janssen, J.A.M. 1994. Impact of the mineral composition and water content of excised maize leaf sections on fitness of the African armyworm, *Spodoptera exempta* (Lepidoptera: Noctuidae). *Bull. Entomol. Res.* 84: 233-245.
- Jervis, M.A. and Kidd, N.A.C. 1993. Flower visiting by hymenopteran parasitoids. *J. Nat. Hist.* 27: 67-105.
- Kajita, H. and Drake, E.F. 1969. Biology of *Apanteles chilonis* and *A. flavipes* (Hymenoptera: Braconidae), parasites of *Chilo suppressalis*. *Mushi* 42: 163-179.
- Leerdam, M.B. van ; Smith, J.W. Jr. and Fuchs, T.W. 1985. Frass-mediated, host finding behavior of *Cotesia flavipes*, a Braconid parasite of *Diatraea saccharalis* (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. Am.* 78: 647-650.

- Macedo, N., Araújo, J.R. de, Bothelo, P.S.M. 1993. Sixteen years of biological control of *Diatraea saccharalis* (Fabr.) (Lepidoptera: Pyralidae) by *Cotesia flavipes* (Hymenoptera: Braconidae), in the state of Sao Paulo, Brazil. *Anais Soc. Entomol. Brasil* 22: 441-448.
- Mattiacci, L., Dicke, M., Posthumus, M.A. 1995. β -glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proc. Natl. Acad. Sci. U.S.A.* 92: 2036-2040.
- Mohyuddin, A.I., Inayatullah, C. and King, E.G. 1981. Host selection and strain occurrence in *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) and its bearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* 71: 575-581.
- Narayanan, E.S. and Chaudhuri, R.P. 1954. Studies on *Stenobracon deesae* (Cam.), a parasite of certain lepidopterous borers of graminaceous crops in India. *Bull. Entomol. Res.* 45: 647-659.
- Ngi-Song, A.J., Overholt, W.A., Ayertey, J.N., 1995. Host suitability of African graminaceous stemborers for the development of *Cotesia flavipes* and *Cotesia sesamiae* (Hymenoptera: Braconidae). *Environ. Entomol.* 24: 978-984.
- Ochieng, R.S., Onyango, F.O. and Bungu, M.D.O. 1985. Improvement of techniques for mass-culture of *Chilo partellus* (Swinhoe). *Insect Sci. Appl.* 6: 425-428
- Onyango, F.O. and Ochieng-Odero, J.P.R. 1994. Continuous rearing of the maize stemborer *Busseola fusca* on an artificial diet. *Entomol. Exp. Appl.* 73: 139-144.
- Overholt, W.A. 1994. A review of classical biological control of graminaceous stemborers in Africa. In: Polaszek, A. (Ed.) *Cereal stemborers in Africa: taxonomy, natural enemies and control*, (in press).
- Overholt, W.A., Ngi-Song, A.J., Kimani, S.K., Mwapila, J., Lammers, P. and Kioko, E. 1994a. Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), in Africa. *Biocontrol news and Inform.* 15: 19-24.
- Overholt, W.A., Ogedah, K. and Lammers, P.M. 1994b. Distribution and sampling of *Chilo partellus* (Lepidoptera: Pyralidae) in maize and sorghum on the Kenya coast. *Bull. Entomol. Res.* 84: 367-378.
- Parker, G.A. and Courtney, S.P. 1984. Models of clutch size in insect oviposition. *Theor. Pop. Biol.* 26: 27-48.
- Pfannenstiel, R.S., Browning, H.W. and Smith, J.W. Jr. 1992. Searching behavior of *Pediobius furvus* (Hymenoptera: Eulophidae) for *Eoreuma loftini* (Lepidoptera: Pyralidae) in sugarcane. *J. Econ. Entomol.* 85: 384-388.
- Polaszek, A. and Walker, A.K. 1991. The *Cotesia flavipes* species-complex: parasitoids of cereal stemborers in the tropics. *REDIA* 74: 335-341.
- Potting, R.P.J., Vet, L.E.M. and Dicke, M. 1995. Host microhabitat location by the stemborer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systemically induced plant volatiles. *J. Chem. Ecol.* 21: 525-539.
- Roth, J.P., King, E.G. and Thompson, A.C. 1978. Host location behavior by the Tachinid *Lixophaga diatraea*. *Environ. Entomol.* 7: 794-798.
- Smith, J.W. Jr., Wiedenmann, R.N. and Overholt, W.A. 1993. Parasites of Lepidopteran stemborers of tropical graminaceous plants. ICIPE Science Press, Nairobi.
- Vinson, S.B. and Lewis, W.J. 1965. A method of host selection in *Cardiochiles nigriceps*. *J. Econ. Entomol.* 58: 869-871
- Waage, J.K. and Godfray, H.C.J. 1985. Reproductive strategies and population ecology of insect parasitoids. In: Sibly, R.M. and Smith, R.H. (eds.) *Behavioural ecology*, pp. 449-470. Blackwell Scientific, Oxford.
- Ward, S.A. 1992. Environmental uncertainty and polyphagy in herbivorous insects. *Oikos* 63: 506-512.
- Wiedenmann, R.N.; Smith, J.W. Jr. and Darnell, P.O. 1992. Laboratory rearing and biology of the

parasite *Cotesia flavipes* (Hymenoptera: Braconidae) using *Diatraea saccharalis* (Lepidoptera: Pyralidae) as a host. *Environ. Entomol.* 21: 1161-1167

4

Fitness consequences of superparasitism and mechanism of host discrimination in the stemborer parasitoid *Cotesia flavipes*.

ABSTRACT The fitness consequences of superparasitism and the mechanism of host discrimination in *Cotesia flavipes*, a larval parasitoid of concealed stemborer larvae was investigated. Naive females readily superparasitized and treated the already parasitized host as an unparasitized host by allocating the same amount of eggs as in an unparasitized host. However, there was no significant increase in the number of emerging parasitoids from superparasitized hosts due to substantial mortality of parasitoid offspring in superparasitized hosts. Furthermore, the developmental time of the parasitoids in a superparasitized host was significantly longer than in a singly parasitized host and the emerging progeny were significantly smaller (body length and head width). Naive females entered a tunnel in which the host was parasitized 4 hours previously and accepted it for oviposition. Experienced females (oviposition experience in unparasitized host) refused to enter a tunnel with a host parasitized by herself or by another female. In experiments where the tunnel and/or host was manipulated it was demonstrated that the female leaves a mark in the tunnel when she parasitizes a host. The role of patch marking in *C. flavipes* is discussed in relation to the ecology and life history of the parasitoid.

INTRODUCTION

In parasitoids lifetime reproductive success is tightly linked to the number and quality of hosts that are chosen to parasitize, since these hosts provide the resources for the parasitoid's offspring. Because hosts represent a limited discrete resource the fitness per offspring generally declines with increasing clutch size. Superparasitism, the deposition of eggs in or on a host that has already been parasitized by a conspecific, generally results in reduction in survivorship of larvae and/or size, longevity and fecundity of ensuing adults. In this respect parasitized hosts are often referred to as low quality hosts and foraging theory predicts that the optimal response to encountering an already parasitized host is often rejection of that host (Iwasa et al., 1984; Parker and Courtney, 1984). Such responses require that the parasite has the ability to distinguish parasitized from unparasitized hosts, a

Submitted as: Potting, R.P.J.; Snellen, H.M. and Vet, L.E.M. (1996). Fitness consequences of superparasitism and mechanism of host discrimination in the stemborer parasitoid *Cotesia flavipes*. *Behavioral Ecology and Sociobiology*.

phenomenon often referred to as host discrimination (van Lenteren, 1981). Mechanisms to avoid superparasitism are avoidance of previously searched areas and the rejection of already parasitized hosts.

Host discrimination is frequently mediated through employment of patch or host markers, chemical substances deposited by egg-laying females. Intraspecific communication with chemical markers left behind by ovipositing females is well described in insect parasitoids (van Lenteren, 1981; Van Alphen and Visser, 1990) and herbivorous insects (Roitberg and Prokopy, 1987). The avoidance of superparasitism is adaptive when sufficient unparasitized hosts can be found. However, under certain conditions superparasitism is an adaptive strategy, for example when hosts are scarce and individual parasitoids have to compete with others for hosts (e.g. Visser et al., 1990). Under these circumstances it may be beneficial for a female to distinguish between hosts attacked by itself or hosts attacked by others to avoid competition among its own progeny. The ability to distinguish between a host parasitized by itself or a conspecific, through the use of individualized chemical markers, has been demonstrated for several parasitoid species (refs. in van Dijken et al., 1992).

The ability to discriminate was examined in *Cotesia flavipes* (Hymenoptera: Braconidae), a gregarious endoparasitoid of stem-boring moth larvae in graminaceous plants. To attack the concealed host, *C. flavipes* enters the stem-borer tunnel. Handling a host inside a tunnel may be time consuming as the parasitoid has to enter the tunnel, crawl through the frass to reach the host, oviposit and then crawl back to exit the tunnel. The handling time, defined as the time spent inside the tunnel, is dependent on the accessibility of the larva and can be considerable (range 16-2765 sec ($n=17$), Potting et al. 1995a). The time taken to oviposit only lasts for 4-6 seconds, a fraction of the total handling time. Handling a host inside a tunnel is not only time consuming, but also risky. Attacking a defending larva in the confined space of a tunnel involves a substantial mortality risk to the female parasitoid. Potting et al. (1995a) showed that 30-40% of the parasitoids do not survive the attack, because they are killed by the spitting and biting stem-borer larva. However, the majority of the killed females have successfully parasitized their offensive host (Takasu and Overholt, 1995). Potting et al. (1995a) argue that, as a consequence of this high mortality risk at each host encounter of this parasitoid species, *C. flavipes* is expected to encounter only a few hosts in its lifetime.

Discrimination may confer an advantage on *C. flavipes* females by reducing the time spent in handling low quality hosts and by avoiding a superfluous mortality risk. Parasitoids can detect parasitized hosts or previously visited sites by detecting external cues on the host or substrate (e.g. Greany and Oatman, 1972; Wäckers et

al., 1995) or by detecting internal cues after oviposition (e.g. Vet et al., 1984). Internal stimuli are reliable indicators of previous parasitism but involve direct handling of the host before they are detected. External cues at the feeding site of the host can be quickly detected and are reliable indicators if host movement is restricted, as in feeding stemborer larvae. We hypothesize that the time-saving and life-saving benefit of discrimination would be greatest if *C. flavipes* could detect parasitized hosts by means of external cues, before contacting the offensive host.

Many parasitoid species avoid superparasitism after they have had an oviposition experience. Despite the vast amount of reports on host discrimination abilities of experienced individuals (van Lenteren, 1981; van Alphen and Visser, 1990), there is a scarcity on data whether truly naive wasps have an innate ability to discriminate (Henneman et al., 1995). It can be hypothesized that *C. flavipes* may have evolved an innate ability to discriminate, as there are considerable fitness penalties in attacking a previously parasitized host. Each host attack involves a mortality risk and furthermore the pro-ovigenic *C. flavipes* is egg-limited as it has only 150 eggs available for oviposition, with a normal clutch size of around 40 (Potting et al., 1995a).

In this study we examine the effect of superparasitism and mechanisms of host discrimination in *C. flavipes*. The host used in the experiments was one of its natural hosts, the stemborer *Chilo partellus* (Lepidoptera: Pyralidae). In the first experiment we determined the fitness consequences of superparasitism in *C. flavipes* by comparing the survival probability of progeny and size of ensuing adults in singly and superparasitized hosts. The second experimental series tested the ability of naive female *C. flavipes* to discriminate and tested whether experienced females discriminated between hosts parasitized by themselves and those parasitized by conspecifics. In the third experiment we investigated whether internal or external cues are used in host discrimination in *C. flavipes*.

MATERIALS AND METHODS

Insects

A colony of *C. flavipes* that originated from Pakistan was maintained on laboratory-reared fourth instar *C. partellus* larvae. For more details on the rearing procedures of parasitoid and host we refer to Potting et al. (1995b). For the experiments, 1 day old mated female *C. flavipes* were used that were naive with respect to oviposition or contact with host related cues.

Experiment 1: Reproductive success

To determine if there are any fitness consequences involved in superparasitism we determined the parasitoid's reproductive success when ovipositing in single parasitized and unparasitized hosts. Unparasitized fourth instar *C. partellus* larvae were individually exposed to individual naive female *C. flavipes*. These single parasitized larvae were divided in two groups. One group was used to estimate the parasitoid's clutch size. Clutch size was estimated by dissecting parasitized larvae and determining the number of progeny present 5 days after oviposition. We assumed that mortality during the egg stage was insignificant. The second group was used to determine the reproductive success in single parasitized hosts. The reproductive parameters determined were: development time, defined as period from oviposition until adult emergence from cocoon; number of produced cocoons and number, sex and size of emerged adults. Size of parasitoids was determined under a stereomicroscope, measuring body length from head to tail. To obtain superparasitized hosts once-parasitized hosts were exposed to a second attack by naive female *C. flavipes*. As above, one part of the superparasitized larvae was used to estimate the total number of parasitoid's eggs and the other part was used to determine the reproductive success. Parasitized larvae were reared on maize stems at 25 ± 1 °C, $70 \pm 5\%$ RH and 16L8D photoregime.

Experiment 2: Host discrimination

In this experiment we examined the ability of female *C. flavipes* to discriminate between parasitized and unparasitized larvae. We compared the response of naive (no oviposition experience) and experienced (one oviposition 2-4 h prior to bioassay) females to an unparasitized or a parasitized host. To determine if female *C. flavipes* avoid self-superparasitism the offered parasitized host consisted of a larva parasitized by herself or a larva parasitized by a conspecific.

C. flavipes attacks the concealed larvae ingressing the stemborer tunnel. To record the behaviour of the parasitoid inside the tunnel an artificial transparent tunnel with a feeding larva was created by using small glass tubes (fig 4.1). Glass tubes (6 x 0.5 cm) were filled with maize stem pieces and one fourth instar *C. partellus* larva was introduced into the tube. The tube was plugged with cotton wool at both ends and the larva was allowed to feed overnight to create a tunnel filled with larval frass. The behavior of individual female *C. flavipes* was observed for 5 minutes after they were placed near the opening of the glass tube. It was recorded whether the wasp contacted the larva and whether this resulted in oviposition. To obtain experienced females and parasitized hosts, individual one-day old wasps were allowed to parasitize a fresh host in a glass tube, 2-4 h prior to the bioassay. If the wasp was killed by the host larva, the glass tube and host were discarded. To allow for the possible role of chemical markers left on the substrate by parasitizing females,

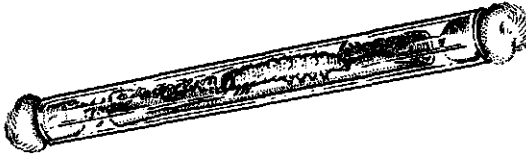


Figure 4.1 Schematic representation of artificial glass tunnel setup used in experiments.

parasitized hosts were offered in their original glass tube. In the bioassay the female was introduced from the side of the tunnel where the previous parasitizing female left the tunnel. The discrimination ability of naive females was tested by offering naive females an unparasitized host or a parasitized host (in a visited tunnel). Experienced females were distributed in three groups that were offered either an unparasitized host (to check their motivation to oviposit), a self-parasitized host, or a conspecific-parasitized host. Forty wasps were tested for each treatment. All treatment groups were always tested on the same experimental days.

Experiment 3: Marking of host or substrate

In this experiment we examined whether female *C. flavipes* leave a chemical mark in the tunnel and/or on the host after a successful parasitization. The same procedure and bioassay was used as in the previous experiment. In this experiment, experienced females (one oviposition) were offered four different tunnels. The tunnel was either not visited before by a conspecific female (FRESH) or previously visited by a female that parasitized a host there (VISIT). The host inside the tunnel was either unparasitized (UNPA) or parasitized (PARA). The following combinations were tested: FRESH/UNPA, FRESH/PARA, VISIT/PARA, VISIT/UNPA. Thirty five, one day old females were tested for each combination.

RESULTS

Reproductive success

Figure 4.2 shows the estimated number of eggs present in single and superparasitized hosts and the number of produced cocoons and surviving adults from single and superparasitized hosts. The mean number of eggs (estimated by the number of parasitoid progeny present five days after parasitization) in a superparasitized host was - almost exactly - doubled compared to single parasitized hosts. This indicates that naive females allocate the same amount of eggs in both host types. In spite of the higher initial number of parasitoid progeny in superparasitized hosts, this did

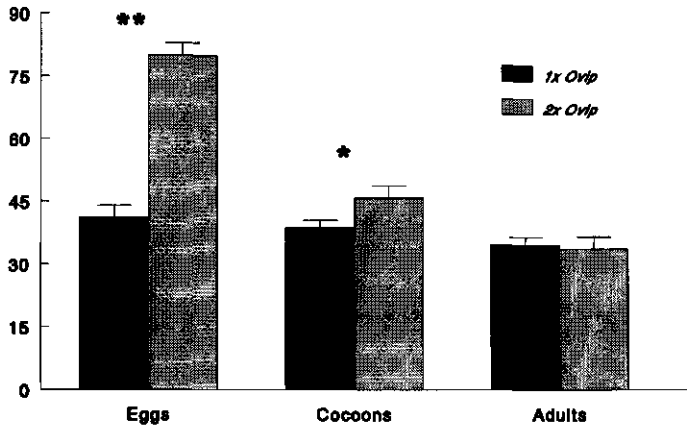


Figure 4.2 Mean numbers (\pm s.e.) of *C. flavipes* estimated eggs, produced cocoons and ensuing adults ($n=30$) from single (black bar) and superparasitized (shaded bar) fourth instar larvae of *C. partellus*. Asterisks indicate significant differences (t-test, $P < 0.05$).

not result in an increase in the number of produced parasitoids per host. Offspring egg to adult mortality was significantly higher in superparasitized hosts (58%) compared to single parasitized hosts (16%). Due to this substantial mortality of parasitoid offspring in superparasitized hosts, there was only a small significant increase in the number of produced cocoons and no significant increase in the number of emerging parasitoids (fig 4.2). In table 4.1 the other reproductive parameters that were measured are presented. The parasitoid development time from egg stage to emerging adult was significantly prolonged for one day in superparasitized hosts. Furthermore, the size of male and female parasitoids emerging from superparasitized hosts was significantly smaller. There was no significant difference in the sex ratio of surviving adults from single or superparasitized hosts.

Host discrimination

The previous experiment revealed that naive *C. flavipes* readily accept a parasitized host. However, in this experiment hosts were offered unconcealed in a petri dish, away from their feeding site, which could have excluded the role of possible external cues at the feeding site of the host. Therefore, in the following experiment the host acceptance behavior of naive and experienced females was observed in a semi-natural setup of transparent glass tunnels. In this bioassay the percentage of females making contact with the host is an indicator of the willingness of the female to enter and traverse the tunnel. Naive females readily accepted a previously parasitized host

Table 4.1 Mean egg-adult development time (days), sex ratio (% females), body length males and body length females ($\times 0.1\text{mm}$) of *C. flavipes* from single and superparasitized fourth instar larvae of *Chilo partellus*. $P < 0.05$ indicates significant differences (t-test).

	One oviposition		Two ovipositions		P (t-test)
	mean	(\pm SE, n)	mean	(\pm SE, n)	
Development time (egg-adult)	19.2	(0.8, 20)	20.1	(0.9, 25)	0.001
Sex ratio (% female)	52.9	(24.0, 31)	47.6	(22.7, 33)	n.s.
Body length female	18.0	(1.9, 28)	16.5	(1.6, 31)	0.003
Body length male	18.0	(0.7, 31)	16.5	(1.7, 32)	0.0009

inside its original tunnel. There was no significant difference in the percentage of naive females that made contact with or oviposited in an unparasitized host or parasitized host (fig 4.3: NAIVE UNPA and NAIVE CONS).

In contrast to naive females, experienced females were more reluctant to enter a previously visited tunnel with a parasitized host. This was evidenced by the significantly lower percentage of experienced wasps making contact with and ovipositing in a previously parasitized host in a previously visited tunnel (fig 4.3: EXPE-CONS and EXPE-SELF), compared to the percentage of experienced females contacting and accepting an unparasitized host in a fresh tunnel (fig 4.3: EXPE-UNPA). Experienced females were not only more reluctant to crawl through a previously visited tunnel but also the percentage of hosts accepted for oviposition after contact was significantly lower. The percentage of contacts resulting in oviposition was 47% for hosts parasitized by conspecifics and 50% for self-parasitized hosts, whereas the acceptance of an unparasitized hosts by experienced females was 90%. There was no significant difference in the behavior of experienced females in tunnels visited by herself or by a conspecific (paired G-tests EXPE-CONS vs EXPE-SELF for contact and ovipos., $P > 0.05$), indicating an inability of the wasp to recognize a tunnel or host which it has just visited as distinct from a tunnel or host visited previously by another female.

Marking of host or substrate

The avoidance behavior of experienced parasitoids in the previous experiment indicated that the wasp perceived a repellent mark (external cue) in a previously visited tunnel. To determine the source of the external cue, we manipulated the contents of the experimental tunnels and observed the acceptance behavior of experienced females. The results are summarized in figure 4.4. As control we first tested the willingness of experienced females to accept a fresh unparasitized host in a fresh unvisited tunnel (FRESH UNPA) and a parasitized host in its original tunnel

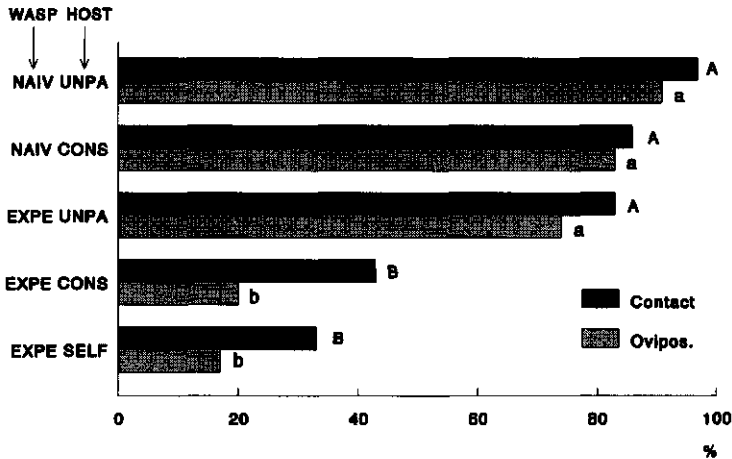


Figure 4.3 Discrimination by naive (NAIV) and oviposition experienced (EXPE) females of *C. flavipes* between unparasitized (UNPA), conspecific (CONS) and self parasitized (SELF) fourth instar larvae of *C. partellus* in artificial glass tunnels. Black bars indicate percentage of females ($n=35$) making contact with larva inside tunnel. Shaded bars indicate percentage ($n=35$) ovipositing in larva. Bars with different letters (capitals for contact; lower case for oviposition) are significantly different (paired G-tests, $P < 0.05$).

(VISIT PARA). As was found in the previous experiment, experienced females were reluctant to enter and traverse a tunnel previously visited by a parasitizing female. However in this experiment there was no significant reduction in the acceptance of a host after contact (83% for FRESH UNPA and 71% for VISIT PARA, G-test, $P > 0.5$). Replacing the parasitized host by a fresh unparasitized host (VISIT UNPA) does not significantly increase the willingness of experienced females to enter and traverse a previously visited tunnel, indicating that the female perceives a chemical mark on the substrate in the tunnel (left behind by the previous female). This was also evidenced by the fact that when a parasitized host was transferred to a fresh unvisited tunnel significantly more parasitoids traversed the tunnel and contacted the host (FRESH UNPA in fig 4.4), compared to the treatments with a previously visited tunnel (VISIT PARA and VISIT UNPA).

DISCUSSION

Fitness consequences of superparasitism

In general there are fitness penalties for a superparasitizing female parasitoid, due to increased competition for limited resources among progeny inside the host (van Lenteren, 1981; Waage, 1986). Clutch size theory predicts that gregarious parasitoids are (in general) expected to lay a clutch which maximizes (or nearly maximizes) the

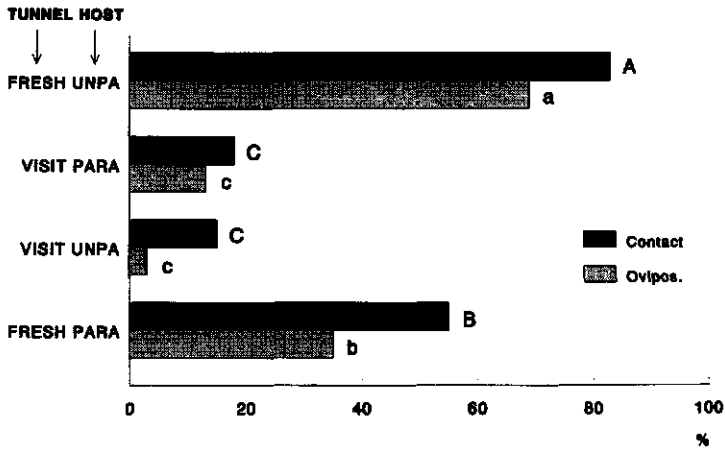


Figure 4.4 Behavior of oviposition experienced females of *C. flavipes* in manipulated artificial glass tunnels. FRESH = unvisited fresh tunnel; VISIT = tunnel visited previously by conspecific female parasitizing host; UNPA = unparasitized fourth instar *C. partellus* larva; PARA = parasitized fourth instar *C. partellus* larva. Black bars indicate percentage of females making contact with larva inside tunnel. Shaded bars indicate percentage ovipositing in larva. Bars with different letters (capitals for contact; lower case for oviposition) are significantly different (paired G-tests, $P < 0.05$).

number of surviving offspring per parasitized host (Godfray, 1987; 1994). In gregarious parasitoids superparasitism thus generally results in increased competition among progeny for resources. Our results indicate that the fitness consequences of superparasitism in *C. flavipes* are a prolonged development time, a decreased survival probability of the progeny and smaller size of the ensuing adults (table 4.1, fig 4.2). Our results support data from other studies, suggesting that superparasitism delays the development of the progeny (e.g. Wylie, 1983; Eller et al., 1990; Harvey et al., 1993), increases larval mortality (e.g. Vinson and Sroka, 1978; Vet et al., 1994) and results in smaller offspring (e.g. Harvey et al., 1993; Vet et al., 1994; but see for exception Bai and Mackauer, 1992).

For several parasitoid species it has been demonstrated that there is an inverse relationship between the number of progeny per host and their size (e.g. Hardy et al., 1992; Vet et al., 1994). There are several fitness penalties for being small. Smaller females may have a shorter lifespan and a smaller initial eggload (Waage, 1986; Godfray, 1994) and recently Visser (1994) showed that also searching ability of females can be related to their size.

Besides fitness consequences for the female's progeny, there are also direct fitness consequences for the superparasitizing female. Handling a host inside the stemborer tunnel is time consuming for the short lived *C. flavipes* female (2-4 days, Potting et al., 1995a), but also risky as 30-40% of the females is killed by the defending host

(Potting et al., 1995a). Thus, a female may waste searching time and risk her life in handling a previously parasitized host inside a tunnel. Handling a parasitized host may take even longer and be more risky than a unparasitized host. Parasitized *C. partellus* larvae have the tendency to block the tunnel with larval frass, complicating its accessibility to the parasitoid (Potting et al., 1995a). Takasu and Overholt (1995) showed that parasitized larvae are more aggressive than unparasitized larvae, resulting in a higher probability of parasitoid mortality.

To conclude, there are substantial fitness penalties for a female *C. flavipes* superparasitizing a host. Besides a lower fitness of offspring from superparasitized hosts the female wastes time in attacking a (low quality) host inside the tunnel, but more important, she exposes herself to a (superfluous) substantial mortality risk.

Optimal clutch size

Especially for animals whose lifetime reproductive success is limited by opportunities to reproduce, clutch size theory predicts a maximization of the fitness gain per clutch (Godfray, 1987). This may be true for *C. flavipes*, which has a short lifespan and a high mortality risk at each host encounter, resulting in a low number of expected lifetime host encounters (Potting et al., 1995a). It is clear that doubling the clutch size overshoots/exceeds the optimal clutch size in fourth instar *C. partellus* larvae. Due to mortality of supernumeraries in superparasitized *C. partellus* larvae the number of produced adults was equal to that of single parasitized hosts (fig 4.2). Although it needs more rigorous testing, our results indicate that female *C. flavipes* lay an optimal (or near optimal) clutch size in fourth instar *C. partellus* larvae. Wiedenmann and Smith (1995), using *Diatraea saccharalis* as a host, found that the number of produced adults from superparasitized hosts was twice the number produced from singly parasitized hosts. Superparasitism in this host species may thus be more profitable than in *C. partellus*. An explanation could be that the neotropical *D. saccharalis* is a relatively new host for the old world parasitoid *C. flavipes*. Therefore *C. flavipes* may not yet have adapted to lay an optimal clutch size in this new host species, but still shows the sub-optimal strategy to maximize progeny production on its ancestral (original) host species.

Experience induced discrimination

In spite of the substantial fitness penalties for superparasitizing a host naive *C. flavipes* readily superparasitize. In our experiments naive females readily accepted a parasitized host, either unconcealed or within an artificial tunnel. However, females with an oviposition experience were reluctant to enter a previously utilized stemborer tunnel and superparasitize. The finding that previous experience decreases the willingness to superparasitize seems to be common in insect parasitoids (Klomp et al., 1980). Whether a female has to learn to distinguish between a used or unused

host or whether the female is adopting a conditional strategy based on the perceived density of unparasitized hosts remains a matter of debate (for recent discussion see: Henneman et al., 1995). Recently Henneman et al. (1995) explored whether a differential egg load may be another factor responsible for the difference in behavior between naive and oviposition experienced females. As emphasized by Rosenheim (1993) it is difficult to distinguish learning from effects of changes in eggload on behavior. Especially for gregarious parasitoids the decrease in egg load with each oviposition can be considerable. A naive female *C. flavipes* allocates 30% of her total eggload to the first encountered host (Potting et al., 1995a). An egg-limited parasitoid may discount superparasitizing against the chance of finding sufficient unparasitized hosts in which to lay her few remaining eggs (Speirs et al., 1991). The increased choosiness after an oviposition experience may thus certainly be induced by the reduction in eggload and warrants more investigation.

Naive *C. flavipes* readily superparasitize, despite the fitness penalties involved in superparasitizing a host. Our initial hypothesis that *C. flavipes* may have evolved an innate ability and 'willingness' to avoid previously visited tunnels with parasitized hosts does not hold. We did not find any evidence of discrimination in naive *C. flavipes* females. There was no difference in acceptance behavior of parasitized or unparasitized hosts (fig 4.3) and there was no significant difference in the clutch size in both host types (fig 4.2). For several parasitoid species it has been demonstrated that naive females (with no previous oviposition experience) have the ability to discriminate between parasitized and unparasitized hosts. Naive females may reject parasitized hosts (Bai and Mackauer, 1990; Völkl and Mackauer, 1990) or may lay significantly smaller clutches in parasitized hosts compared to unparasitized hosts. The latter has been demonstrated for *Trichogramma evanescens* (Van Dijken and Waage, 1987), *Cotesia glomerata* (Ikawa and Suzuki, 1982) and *Pediobius foveolatus* (Hooker and Barrows, 1992).

The lifetime expectancy of host encounter rate is very low in *C. flavipes*. The pro-ovigenic *C. flavipes* is equipped with an eggload sufficient to parasitize 3-4 hosts only (Potting et al., 1995a). The lack of an innate ability or willingness to discriminate in *C. flavipes* may be due to these constrained opportunities to find and parasitize hosts. The best strategy for a (just emerged) naive female *C. flavipes* is to accept the first encountered host, irrespective its quality. This opportunistic behavior may change after a successful attack in an unparasitized host. The increased choosiness after an oviposition experience may be due to the fact that the fitness penalties of superparasitism may outweigh the risk of not finding another unparasitized host. The possible future (additional) fitness payoff is high when the female is able to find and successfully attack an additional unparasitized host.

self-parasitism vs conspecific superparasitism

For a superparasitizing female it can be adaptive to distinguish self-parasitized hosts from conspecific parasitized hosts. The experiments reported here suggest that experienced *C. flavipes* does not recognize the site and host which it has just utilized as distinct from the site and host utilized previously by another female. The ability to recognize individual-specific marks has been demonstrated in five parasitoid species (van Dijken et al., 1992; Hubbard et al., 1987; Visser, 1993; Völkl and Mackauer, 1990; Wäckers and Lewis 1995), while it was not found in four other species (Van Alphen and Nell, 1982; Bai and Mackauer 1990; Van Dijken and Waage, 1987; Gates, 1993). As argued previously, the lifetime host encounter rate is very low in *C. flavipes*. For parasitoids that encounter parasitized hosts only rarely, the selection pressure for identification of self-parasitized hosts will be small (Visser et al., 1992).

Marking of visited tunnel

The function of site and host discrimination in *C. flavipes* is clear: a discriminating female saves searching time, avoids the wastage of eggs and avoids a direct mortality risk. The mechanism of discrimination is the recognition of an external cue on the tunnel substrate. Our initial hypothesis that *C. flavipes* utilizes external cues on the substrate before contacting the defending (aggressive) host seems to hold. Experienced female *C. flavipes* are reluctant to enter and traverse a tunnel with a parasitized larva (fig 4.3,4.4) suggesting the parasitizing female marks the substrate. The exact source of the substance eliciting the avoidance behavior can not be elucidated conclusively from our experiments. We can not exclude the possibility that the mark is applied on the host and that through the movement of the host the tunnel is contaminated with the mark. However, (although we did not test this in detail) we observed several times that females were also reluctant to enter and traverse a tunnel where the host had not moved significantly after being attacked by the previous parasitoid, indicating active substrate marking. We can not exclude the existence and use of internal cues. However, we expect that internal marks are not used as these involve direct host handling, which imposes a direct mortality risk to the parasitoid.

Although the reports are scanty, host or substrate marking seems to be quite common in hymenopterous parasitoids (Godfray, 1994). Recently, the Dufour's gland has been established as the source of both internal and external chemical markers in the Ichneumonid *Venturia canescens* (Harrison et al., 1985; Hubbard et al., 1987). There are only a few reports of the discriminatory ability of stemborer parasitoids. The tachinid *Lixophaga diatreae*, an ectoparasitoid of stemborer larvae, larviposits a mobile maggot at the tunnel entrance that actively searches for the host inside the tunnel. Roth et al. (1982) reported that female *L. diatreae* readily

larviposited at previously larviposition sites, but that females started to avoid sites where a larva was deposited 24 hours previously. This could indicate that *L. diatræa* can or starts to discriminate a utilized site after the parasitoid grub has successfully located and attached to the host in the tunnel. The mechanisms of host discrimination in this interesting system warrants more investigation.

It is generally hypothesized that marking evolved primarily as a means for individuals to avoid superparasitizing hosts they themselves previously parasitized, and that secondary benefits accrue when other individuals also avoid such marked hosts (Roitberg and Prokopy, 1987). Our study indicates that a utilized stemborer tunnel is recognized at least 4 hours after previous parasitization. It remains to be investigated how long this mark lasts, before it evaporates or wears off.

ACKNOWLEDGMENTS

We thank Leo Koopman, Andre Giddink and Joseph Owino for the supply of stemborer larvae and parasitoids. Bill Overholt of the International Centre for Insect Physiology and Ecology, Nairobi, Kenya is kindly thanked for providing working space for part of the experiments. Marcel Visser and Joop van Lenteren are acknowledged for valuable comments on a previous version of the manuscript. This work was supported by the Netherlands foundation for the Advancement of Tropical Research (W84-325).

REFERENCES

- Bai, B. and Mackauer, M. 1990. Host discrimination by the aphid parasitoid *Aphelinus asychis* (Hymenoptera: Aphelinidae): when superparasitism is not adaptive. *Can. Entomol.* 122: 363-372.
- Eller, F.J., Tumlinson, J.H. and Lewis, W.J. 1990. Intraspecific competition in *Microplitis croceipes* (Hymenoptera: Braconidae), a parasitoid of *Heliothis* species (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 83: 504-508.
- Gates, S. 1993. Self and conspecific superparasitism by the solitary parasitoid *Antrocephalus pandens*. *Ecol. Entomol.* 18: 303-309.
- Godfray, H.C.J. 1987. The evolution of clutch size in parasitic wasps. *Am. Nat.* 129: 221-233.
- Godfray, H.C.J. 1994. *Parasitoids, behavioral and evolutionary ecology*. Princeton University Press, New Jersey.
- Greany, P.D. and Oatman, E.R. 1972. Analysis of host discrimination in the parasite *Orgilus lepidus* (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.* 65: 377-383.
- Hardy, I.C.W., Griffiths, N.T. and Godfray, H.C.J. 1992. Clutch size in parasitoid wasp: a manipulation experiment. *J. Anim. Ecol.* 61: 121-129.
- Harrison, E., Fisher, R.C. and Ross, K.M. 1985. The temporal effects of dufour's gland secretion in host discrimination by *Nemeritis canescens*. *Entomol. Exp. Appl.* 38: 215-220.
- Harvey, J.A., Harvey, I.F. and Thompson, D.J. 1993. The effect of superparasitism on development of the solitary wasp parasitoid wasp *Venturia canescens* (Hymenoptera: Ichneumonidae). *Ecol. Entomol.* 18: 203-208.
- Henneman, M.L., Papaj, D.R., Figueredo, A.J. and Vet, L.E.M. 1995. Egg-laying experience and acceptance of parasitized and unparasitized hosts by the parasitoid, *Leptopilina heterotoma* (Hymenoptera: Eucoilidae). *J. Insect Behav.* 8: 331-342.
- Hooker, M.E. and Barrows, E.M. 1992. Clutch size reduction and host discrimination in the

- superparasitizing gregarious endoparasitic wasp *Pediobius foveolatus* (Hymenoptera: Eulophidae). *Ann. Entomol. Soc. Am.* 85: 207-213.
- Hubbard, S.F., Marris, G., Reynolds, A. and Rowe, G.W. 1987. Adaptive patterns in the avoidance of superparasitism by solitary parasitic wasps. *J. Anim. Ecol.* 56: 387-401.
- Ikawa, T. and Suzuki, Y. 1982. Ovipositional experience of the gregarious parasitoid *Apanteles glomeratus* (Hymenoptera: Braconidae), influencing her discrimination of the host larvae, *Pieris rapae crucivora*. *Appl. Entomol. Zool.* 17: 119-126.
- Iwasa, Y., Suzuki, Y. and Matsuda, H. 1984. Theory of oviposition strategy of parasitoids. I. Effect of mortality and limited egg number. *Theor. Pop. Biol.* 26: 205-227
- Klomp, H. Teerink, B.J. and Ma, W.C. 1980. Discrimination between parasitized and unparasitized hosts in the egg parasite *Trichogramma embryophagum* (Hym.: Trichogrammatidae): a matter of learning and forgetting. *Neth. J. Zool.* 30: 254-277.
- Parker, G.A. and Courtney, S.P. 1984. Models of clutch size in insect oviposition. *Theor. Pop. Biol.* 26: 27-48.
- Potting, R.P.J., Overholt, W.A., Danso, F.O. and Takasu, K., 1995a. Foraging behavior and life history of the stemborer parasitoid *Cotesia flavipes*. *J. Insect Behav.* (submitted)
- Potting, R.P.J., Vet, L.E.M. and Dicke, M. 1995b. Host microhabitat location by stemborer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systemically induced plant volatiles. *J. Chem. Ecol.* 21: 525-539
- Roitberg, B.D. and Prokopy, R.J. 1987. Insects that mark host plants. An ecological, evolutionary perspective on host-marking chemicals. *BioScience* 37: 400-406.
- Rosenheim, J.A. 1992. Comparative and experimental approaches to understanding insect learning In: Papaj, D.R. & Lewis, A.C. (eds.) *Insect learning: ecological and evolutionary perspectives*. Chapman and Hall, New York, pp. 273-307.
- Roth, J.P., King, E.G. and Hensley, S.D, 1982. Plant, host and parasite interactions in the host selection sequence of the Tachinid *Lixophaga diatraea*. *Environ. Entomol.* 11: 273-277
- Speirs, D.C., Sherratt, T.N. and Hubbard, S.F. 1991. Parasitoid diets: does superparasitism pay ? *TREE* 6: 22-25.
- Takasu, K. and Overholt, W.A. 1995. Defense behavior of *Chilo partellus* against parasitization by *Cotesia flavipes*. *Jpn. J. Entomol. Zool.* (submitted).
- Van Alphen, J.J.M. and Nell, H. 1982. Superparasitism and host discrimination by *Asobara tabida* Nees (Braconidae: Alysiinae), a larval parasitoid of Drosophilidae. *Neth. J. Zool.* 32: 232-260.
- Van Alphen, J.J.M. and Visser, M.E. 1990. Superparasitism as an adaptive strategy for insect parasitoids. *Annu. Rev. Entomol.* 35: 59-79.
- Van Dijken, M.J. and Waage, J.K. 1987. Self and conspecific superparasitism by the egg parasitoid *Trichogramma evanescens*. *Entomol. Exp. Appl.* 43: 183-192.
- Van Dijken, M.J., van Stratum, P. and van Alphen, J.J.M. 1992. Recognition of individual-specific marked parasitized hosts by the solitary parasitoid *Epidinocarsis lopezi*. *Behav. Ecol. Sociobio.* 30: 77-82.
- Van Lenteren, J.C. 1976. The development of host discrimination and the prevention of superparasitism in the parasite *Pseudocoila bochei* (Hym.: Cynipidae). *Neth. J. Zool.* 26: 1-83.
- Van Lenteren, J.C. 1981. Host discrimination by parasitoids. In: Nordlund, D.A.; Jones, R.L. & Lewis, W.J. (eds.) *Semiochemicals, their role in pest control*. New York: Wiley. pp. 153-180.
- Vet, L.E.M., Datema, A., Janssen, A. and Snellen, H. 1994. Clutch size in a larval-pupal endoparasitoid: consequences for fitness. *J. Anim. Ecol.* 63: 807-815.
- Vinson, S.B. and Sroka, P. 1978. Effects of superparasitism by a solitary endoparasitoid on the host, parasitoid and field samplings. *Southwestern Entomol.* 3: 299-301.
- Visser, M.E. 1993. Adaptive self- and conspecific superparasitism in the solitary parasitoid *Leptopilina heterotoma*. *Behav. Ecol.* 4: 22-28.
- Visser, M.E. 1994. The importance of being large: the relationship between size and fitness in

- females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *J. Anim. Ecol.* 63: 963-978.
- Visser, M.E., Alphen, J.J.M. van and Nell, H. 1990. Adaptive superparasitism and patch time allocation in solitary parasitoids: the influence of the number of parasitoids depleting a patch. *Behaviour* 114: 21-36.
- Visser, M.E., Luyckx, B., Nell, H. and Boskamp, G.J.F. 1992. Adaptive superparasitism in solitary parasitoids: marking of parasitized hosts in relation to the pay-off from superparasitism. *Ecol. Entomol.* 17: 76-82.
- Völkl, W. and Mackauer, M. 1990. Age specific pattern of host discrimination by the aphid parasitoid *Ephedrus californicus* Baker (Hymenoptera, Aphidiidae). *Can. Entomol.* 122: 349-361.
- Waage, J.K. 1986. Family planning in insect parasitoids. In: *Insect parasitoids* (eds. J.K. Waage and D. Greathead), Academic Press, London, pp. 63-95.
- Wäckers, F.L. and Lewis, W.J. 1995. Host discrimination in flight and following alightment by the parasitoid *Microplitis croceipes*: a study of sensory mechanisms. (submitted).
- Wiedenmann, R.N. and Smith, J.W. Jr. 1995. Parasitization of *Diatraea saccharalis* by *Cotesia chilonis* and *Cotesia flavipes*. *Ann. Entomol. Soc. Am.* 24: 950-961.
- Wylie, H.G. 1983. Delayed development of *Microctonus vittatae* (Hymenoptera: Braconidae) in superparasitized adults of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Can. Entomol.* 115: 441-442.

The relation between parasitoid ecology and learning: the absence of learning in the stemborer parasitoid *Cotesia flavipes*.

ABSTRACT Theory on the adaptive value of learning in foraging suggests that an animal foraging in a predictable homogeneous environment and/or making only a few foraging decisions is not expected to use learning in foraging. For insect parasitoids, learning during foraging is well documented. Host seeking females can learn olfactory and visual cues associated with their hosts or the hosts micro-habitat and experience can strongly affect preference for major foraging cues. We studied the role of learning in host foraging in *Cotesia flavipes* (Hymenoptera: Braconidae), a parasitoid of stemborer larvae with an ecology where learning is expected to be of low adaptive value. Using experimental procedures similar to other parasitoid learning studies, we determined the role of the learning mechanisms priming and preference-induction in the foraging of *C. flavipes*. We did not find any evidence that *C. flavipes* uses odour learning in host-microhabitat location. There was no significant effect of the development and emergence environment on the response level or preference towards infested plant odours. Neither did we find evidence that experience with a particular plant-host-complex during foraging influences subsequent foraging decisions in *C. flavipes* females. The absence of learning in *C. flavipes*, which seems an exception among the parasitoids studied, is discussed in relation to its ecology.

INTRODUCTION

In the past decade, awareness has grown of the importance of learning in the foraging behaviour of insects (Papaj & Lewis, 1993). Apart from a theoretical perspective, where the main research interest is focused on the mechanisms and functions of learning (Gould and Marler, 1984; Real, 1994), there are also several practical considerations that have raised the interest in insect learning (Papaj & Prokopy, 1989; Prokopy & Lewis, 1993). The role of learning in the foraging behaviour of parasitoid wasps is well studied (Turlings et al., 1993), not only because they are used in biological control (Lewis et al., 1990), but also because they are ideal laboratory animals to test theoretical questions in behavioural ecology (Vet et al., 1995).

In parasitoids the lifetime reproductive success is tightly linked to the number and

quality of parasitized hosts, since these hosts provide the resources for the parasitoid's offspring. As a consequence there is a tight linkage between successful host location and parasitoid fitness, and we expect that parasitoids have evolved the ability to use or learn to use those stimuli that enhance the probability of finding suitable hosts. Insect parasitoids use olfactory and visual cues in their search for hosts. Experience with a host or host-related products can have various effects on parasitoid behaviour (Turlings et al., 1993; Vet et al., 1995). Experience can make the wasps more responsive to certain odours (*priming*) or the wasps can learn to respond to the specific odours that they encounter during the experience (*preference induction*). Both learning mechanisms can be induced by experiences at various stages of the life cycle of the parasitoid. Parasitoids develop in and emerge from a host that is feeding in a particular micro-habitat. They can thus use information acquired immediately upon emergence, which can originate either from their cocoon (e.g. Hérard et al., 1988) and/or from the host-microhabitat from which they emerge (e.g. Vet, 1983; Kester & Barbosa, 1991). Many studies have shown that parasitoids can learn visual or olfactory stimuli associated with successful host location and use these odours in subsequent foraging decisions (reviewed by Turlings et al., 1993; Vet et al., 1995). In these olfactometer and windtunnel studies the response towards and preference for a particular resource is tested for individuals with different rearing histories or different pre-bioassay experiences. To give a typical example, *Cotesia marginiventris* is a generalist parasitoid that attacks the larvae of many Lepidoptera. Turlings et al. (1989, 1990) demonstrated that an experience with host-infested leaves dramatically increased the subsequent response by *C. marginiventris* females to host related cues in olfactometric bioassays. Furthermore, Turlings et al., (1990, 1993) demonstrated preference shifts in favor of the experienced plant-host combination. The ability to learn has now been demonstrated in more than 20 different parasitoid species and learning in parasitoids seems to be the rule rather than the exception (Turlings et al., 1993).

Several authors have emphasized the importance of considering an animals ecology when studying and interpreting its learning abilities (Johnston, 1982; Bolles & Beecher, 1988; Kamil, 1994). If learning is of adaptive significance in foraging we may expect correlations between ecology and learning mechanisms. It has been hypothesized that the adaptive value of learning in foraging is dependent on the predictability of the environment (Stephens, 1993) and the number of lifetime foraging decisions (Roitberg et al., 1993). Stephens (1993) argues that the value of learning about a resource depends upon its variability or patchiness in space and time. If the resource is constant, then a fixed or innate response is favoured, while intermediate variability favors assessment of learning. Roitberg et al. (1993) proposed

that the frequency of decisions an animal makes while foraging for a particular resource may also be a factor to be considered in the evolution of learning. They argue that the value of learning in animal decision making is dependent upon the frequency of (reproductive) decisions. Animals making only a few decisions are not expected to learn. Both hypotheses are not mutually exclusive, for instance a polyphagous parasitoid foraging in a heterogeneous variable environment is expected to be faced with more foraging decisions than a parasitoid foraging in a homogeneous habitat. Two conditions where learning may not be found are thus a predictable homogeneous foraging environment and a low number of lifetime foraging decisions. We studied the role of learning in the foraging behaviour of a parasitoid with an ecology which seems to meet both conditions for the absence of learning.

Cotesia flavipes (Hymenoptera: Braconidae) is a gregarious endoparasitoid of lepidopterous stemborer larvae in gramineous crops as maize, sugarcane and sorghum and several wild perennial grasses (Nagarkatti & Nair, 1973). It can be envisaged that *C. flavipes* has evolved its foraging strategy in a homogeneous environment, consisting of stemborer larvae in huge fields of perennial grasses. Besides foraging in a homogeneous environment, *C. flavipes* is expected to encounter only a few hosts in its lifetime. *C. flavipes* ingresses the stemborer tunnel and parasitizes the larva inside the stem. This ingress tactic is not without risk for the parasitoid, as 30-40% of the parasitoids do not survive the attack, because they are killed by the defending stemborer larva (Potting et al., 1996). However, the majority of females that are killed have successfully parasitized their host (Potting et al., 1996). As a consequence of the high mortality rate at each oviposition, the number of reproductive decisions is very low in *C. flavipes*.

To locate the host-microhabitat, *C. flavipes* females use olfactory stimuli that are released by infested plants (Potting et al., 1995). We studied the learning mechanisms priming and preference-induction in *C. flavipes*, at two stages in the life cycle of the parasitoid: upon emergence from the stemborer tunnel and during adult life at oviposition. Taking the ecology of *C. flavipes* into account we hypothesize that adult learning does not play a significant role in host foraging in this parasitoid species.

MATERIALS AND METHODS

Insects

Female *C. flavipes*, aged 2-4 days, were obtained from a laboratory culture established from wasps collected in Pakistan from *Chilo partellus* (Lepidoptera: Pyralidae). Parasitoids were reared on fourth instar *C. partellus* feeding on maize stems or artificial diet. Unless stated otherwise 1-2 days after cocoon formation the

cocoon masses were transferred to a bottle topped with a polyester stop. Parasitoids were fed honey and kept at 15 °C. The hosts *C. partellus* and *Sesamia calamistis* (Lepidoptera: Noctuidae) were reared on an artificial diet according to the method described by Ochieng et al. (1985). For all experiments fourth instar host larvae were used that were allowed to feed for at least 48 hr on fresh plant stems.

Plants

Maize (cv Anjo) and sugarcane (cv POJ 8043) were grown under greenhouse conditions in Wageningen, the Netherlands. For the experiments with wild sorghum and host species selection experiments (see below) wild sorghum and maize plants were obtained from the field in Nairobi, Kenya. The diameter of stems used was 1.5 cm for maize and wild sorghum and 3 cm for sugarcane.

Bioassay

The preference of *C. flavipes* females for particular odour sources was examined in dual choice tests in a Y-tube olfactometer. For details of the Y-tube olfactometer we refer to Steinberg et al. (1992) and Potting et al. (1995). Parasitoid females were individually introduced in the base tube of the olfactometer and given a maximum of 5 min to make a choice for one of the arms of the olfactometer. The observation was counted as a choice when the wasp passed the finish line in one of the arms for a period of 15 seconds. As odour sources we used stemborer infested maize plants or stem pieces. Infested maize plants were obtained by boring two holes in the stem and introducing two fourth instar larvae. The larvae were allowed to feed overnight. Infested stem pieces were obtained by introducing 2 larvae into holes bored in a stem piece of 10 cm. Five stem pieces were used as one odour source and the ten larvae were allowed to feed for 60h prior to the experiment. Parasitoids with different experience histories were tested alternately on the same day. To test for experience effects the G-test with Yates correction ($\alpha=0.05$) was used (Sokal and Rolf, 1981).

Treatments / experiments

1. INCREASE IN RESPONSIVENESS (PRIMING)

Early adult experience upon emergence

To determine whether experience with a stemborer infested stem upon emergence influences the responsiveness of the parasitoids we compared the response of *C. flavipes* females that experienced different emergence environments. Three different

cocoon formation and emergence environments were tested. 1 CONTROL: parasitoid cocoon formation in and emergence from stemborer tunnel in maize stem. 2 GLASS: Emergence on glass from cocoon that was formed from parasitized larva that was transferred from maize stem to glass bottle 1-2 days prior to parasitoid cocoon formation. 3 ISOLATED: same treatment as GLASS but parasitoids were dissected out of and isolated from their cocoon 1-2 days before emergence. The response to an infested maize plant was tested, with an uninfested maize plant as control. The treatment (experience) groups were tested in two blocks: CONTROL vs GLASS (n=56) and CONTROL vs ISOLATED (n=103).

Adult experience

To determine whether an experience with a host or host product increased the responsiveness of female *C. flavipes* towards infested plant odours three different experience groups were compared. 1 NAIVE: parasitoids that had not contacted frass or a host. 2 FRASS: 15-30 minutes prior to the test parasitoids were allowed to contact fresh larval frass (from *C. partellus* fed upon maize) for 30 sec. 3 FRASS+HOST: parasitoids were allowed to oviposit in a *C. partellus* larva fed upon maize 15-30 min prior to the bioassay. The response to infested maize stem pieces was tested, with uninfested maize stem pieces as control (n=40).

2. PREFERENCE INDUCTION

Early adult experience upon emergence

To determine whether the development and emergence environment could induce a preference for the experienced plant-host-complex we conducted choice experiments with different plant species and host species. Treatment groups consisted of parasitoids that had developed on and emerged from *C. partellus* (Cp) feeding inside maize, sugarcane or wild sorghum stems and parasitoids that had developed on and emerged from *S. calamistis* (Sc) feeding on maize. Treatment groups were compared in three blocks: 1 Cp-maize vs Cp-sugarcane (n=62), 2 Cp-maize vs Cp-wild sorghum (n=40) and 3 Cp-maize vs Sc-maize (n=40). Due to logistic reasons we used parasitoids reared from larvae feeding on artificial diet for the Cp-Maize group in the second block (Cp-maize vs Cp-wild sorghum).

In the first two blocks we first tested the response potential to odours from infested stems of the two plant-host-complexes under consideration (uninfested vs infested). The same infested stem pieces were then offered simultaneously to test for preference for a plant-host-complex.

Adult experience at oviposition

This experiment tested whether an oviposition experience with a particular plant-host-complex induced a preference for this plant-host-complex. Parasitoids used for this experiment had developed on and emerged from *C. partellus* (Cp) feeding on maize, except for block 2 (see below), where larvae had fed on artificial diet. One hour prior to being tested a female was allowed to oviposit in a *C. partellus* larva that had been feeding on maize, sugarcane or wild sorghum or a *S. calamistis* (Sc) larva that had fed upon maize. Larvae were offered together with some fresh frass produced on the plant they were fed upon. Wasps with the following experiences were compared in three blocks (n=40): 1 Cp-maize vs Cp-sugarcane, 2 Cp-maize vs Cp-wild sorghum and 3 Cp-maize vs Sc-maize. Preference for a plant-host-complex was tested for both treatment groups in a choice situation. As control, females with no oviposition experience were used (naive).

RESULTS

Effect of experience on responsiveness

We tested the response level, defined as the percentage of the tested females that walked upwind and made a choice for one of the odour fields, for females that had a different emergence environment or adult experience. The results are summarized in figure 5.1. In all the tests the females had a significant preference for the odour field with the infested plant odours (chi-square, $P < 0.05$). Parasitoids that had formed their cocoon outside a stemborer tunnel on glass and emerged in this environment had the same response level as the control group that had formed their cocoon in and emerged from a stemborer tunnel in maize (fig 5.1a). There was no significant difference in responsiveness (chi-square, $P = 0.92$, $DF = 1$) and choice distribution for infested or uninfested plant odours (chi-square, $P = 0.70$, $DF = 1$) between parasitoids from different development and emergence environments.

Even parasitoids that were dissected out of and isolated from their cocoon as a pupa and emerged in a glass environment had a similar response as the control group (fig 5.1b). There was no significant difference in responsiveness (chi-square, $P = 0.67$, $DF = 1$) and choice distribution (chi-square, $P = 0.87$, $DF = 1$).

Giving the parasitoids a contact experience with larval frass or an oviposition experience prior to the bioassay did not increase the responsiveness towards infested plant odours (fig 5.1c). There was no significant difference in the response level (chi-square, $P = 0.89$, $DF = 2$) and choice distribution (chi-square, $P = 0.77$, $DF = 2$) between the different experience groups.

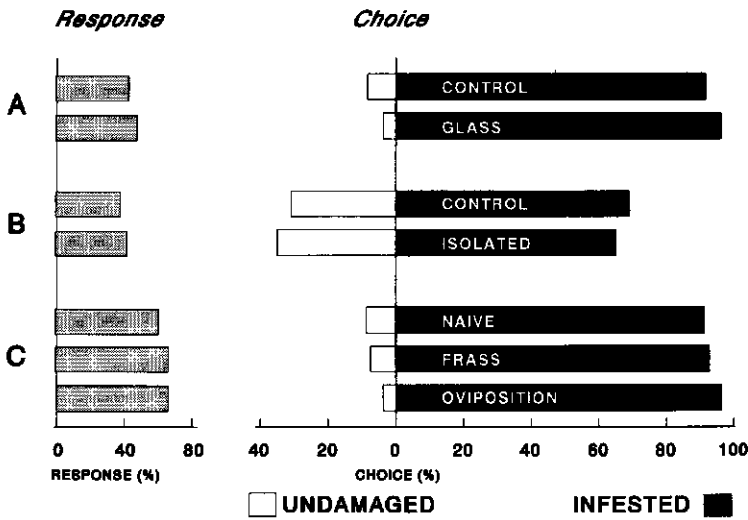


Figure 5.1 The role of priming in the response to odours in *C. flavipes*. Grey bars indicate overall response level (percentage making choice for one of odour fields) and black and white bars indicate choice (percentage of choice for particular odour field) of *C. flavipes* females with different emergence environments (A & B) or different adult experiences (C) to odours from a uninfested (UNDAMAGED) or *C. partellus* infested maize plant (INFESTED) in Y-tube olfactometer. Emergence environments: CONTROL = cocoon formation in and emergence from maize stem, GLASS = cocoon formation on and emergence from glass, ISOLATED = cocoon formation on glass, emergence dissected out of and isolated from cocoon on glass. Adult experience: NAIVE = no contact with frass or host; FRASS = experience with *C. partellus* frass; OVIPOSITION = as FRASS and with oviposition in *C. partellus* larva. There were no significant differences in response levels and choice distributions in experiment A ($n = 56$), B ($n = 103$) and C ($n = 40$).

Preference induction for experienced plant-host-complex

In the preference induction experiments we tested if there was a preference shift for the experienced plant-host-complex in two phases of the parasitoid life: upon emergence and during adult life at an oviposition. The response potentials to the odour sources used in the plant preference experiments are summarized in figure 5.2. Parasitoids responded to odours from stemborer infested stems of maize, sugarcane and wild sorghum, irrespective of their previous development and emergence environment. There were no significant differences in responsiveness (paired G-tests, $\alpha = 0.05$) and choice distributions (paired G-tests, $\alpha = 0.05$) between the treatment groups.

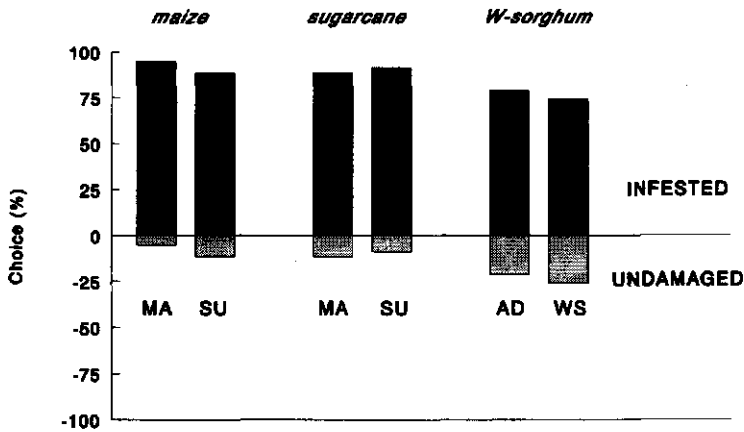


Figure 5.2 Response to odours from *C. partellus*-infested maize, sugarcane and wild sorghum stems by *C. flavipes* females with different development and emergence environments. Black bars indicate choice percentage for odours from *C. partellus* INFESTED stem pieces. Grey bars indicate choice for uninfested (UNDAMAGED) stem pieces. Treatment groups developed on and emerged from larva feeding inside stems of maize (MA), sugarcane (SU), wild sorghum (WS) or a vial containing artificial diet (AD). There were no significant differences in choice distributions in response to infested maize ($n = 53$), sugarcane ($n = 63$) and wild sorghum ($n = 40$).

maize vs sugarcane

In the preference test with infested maize and infested sugarcane we found no significant preference for the plant-host-complex the parasitoids had developed on and emerged from (fig 5.3a) (chi-square, $P = 0.26$, $DF = 1$). An oviposition experience with either a host in maize or sugarcane did not induce a significant preference either (fig 5.3b) (chi-square, $P = 0.60$, $DF = 2$).

maize vs wild sorghum

In the preference test with infested maize and infested wild sorghum both groups were attracted more to infested maize than to infested wild sorghum (fig 5.4a). There was no significant preference induction for the environment the parasitoids had developed in and emerged from (chi-square, $P = 1$, $DF = 1$). There was also no preference induction found after an oviposition experience with a larva in maize or wild sorghum (fig 5.4b). No significant difference was found in the choice distributions between the experience groups (chi-square, $P = 0.21$, $DF = 2$).

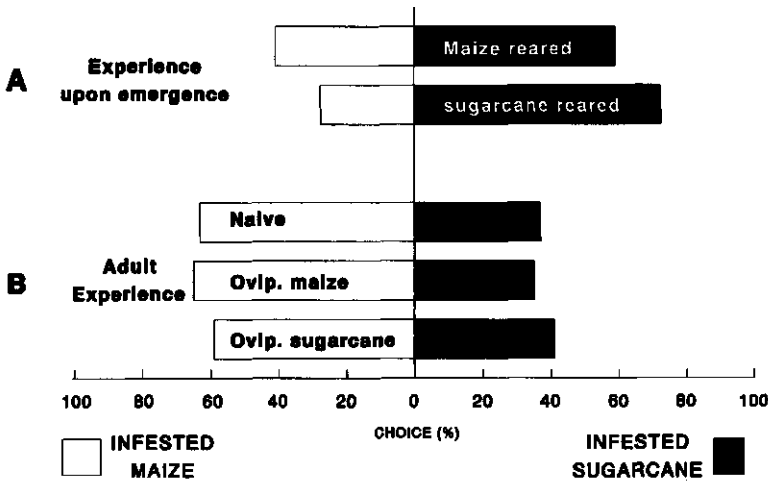


Figure 5.3 The role of development and emergence environment in preference induction in *C. flavipes*. Choice between *C. partellus*-infested maize vs *C. partellus*-infested sugarcane. (A) Early adult experience: development on and emergence from larva feeding inside sugarcane stem or maize stem. (B) Adult experience: no oviposition experience (NAIVE) or oviposition experience with larva feeding on maize (ovip. maize) or sugarcane (ovip. sugarcane). No significant preference induction for experienced plant-host-complex in experiment A (n=62) and B (n=40).

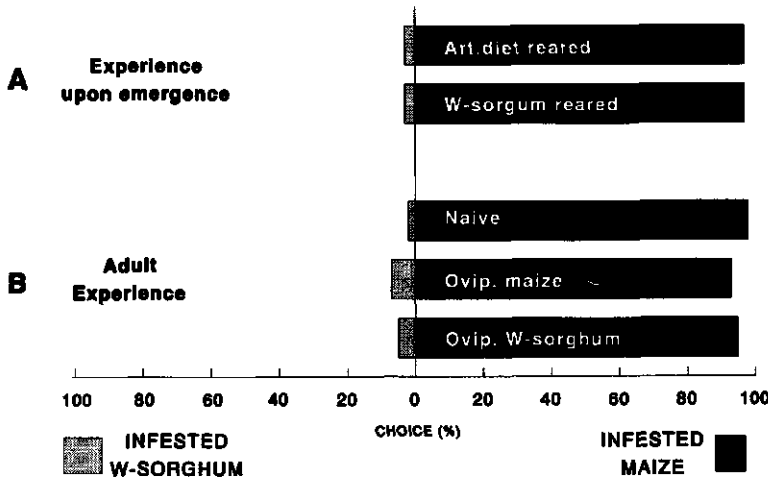


Figure 5.4 The role of development and emergence environment in preference induction in *C. flavipes*. Choice between *C. partellus*-infested maize vs *C. partellus*-infested wild sorghum. (A) Early adult experience: development on and emergence from larva feeding on wild sorghum (W-sorghum reared) or artificial diet (Art. diet reared). (B) Adult experience: no oviposition experience (NAIVE) or oviposition experience with larva feeding on maize (ovip. maize) or wild sorghum (ovip. W-sorghum). No significant preference induction for experienced plant-host-complex in experiment A (n=40) and B (n=40).

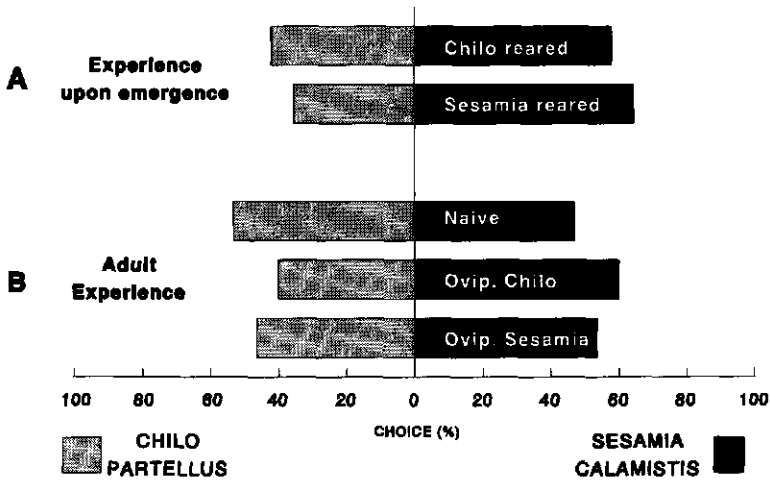


Figure 5.5 The role of development and emergence environment in preference induction in *C. flavipes*. Choice between *C. partellus*-infested maize vs *S. calamistis*-infested maize. (A) Early adult experience: development on and emergence from *Chilo partellus* (Chilo reared) or *Sesamia calamistis* (Sesamia reared) larva feeding on maize. (B) Adult experience: no oviposition experience (NAIVE) or oviposition experience with *C. partellus* larva (Ovip. Chilo) or *S. calamistis* larva (ovip. Sesamia). No significant preference induction for experienced plant-host-complex in experiment A ($n=40$) and B ($n=40$).

C. partellus vs *S. calamistis*

The results of the experiments that tested for a host species preference induction are summarized in figure 5.5. There was no significant preference induction for the host species the parasitoids had developed in and emerged from (fig 5.5a; chi-square, $P=0.77$, $DF=1$). There was also no preference induction for the host species experienced at oviposition (fig 5.5b). No significant difference was found in the choice distributions between the experience groups (chi-square, $P=0.56$, $DF=2$).

DISCUSSION

For an animal to optimize its foraging it is necessary to estimate the encounter rate distribution of the resources in its foraging environment. This estimation can be done by cognitive processes such as learning or through evolved "rules of thumb" in evolutionary time (Real, 1991; Stephens, 1993). Learning allows an individual to adapt its foraging effort to the most profitable resource sites. Also for an insect parasitoid, in search for hosts to lay its eggs, learning can be an adaptive mechanism to increase the encounter rate with suitable hosts (e.g. Papaj & Vet,

1990). A parasitoid may build up its information on the environment in which it is foraging by experience upon emergence and by learning stimuli that are associated with successful host location.

Early adult learning

If there is a correspondence between the foraging environment of the mother and her daughters, information obtained at the development and emergence site may be of adaptive value for emerging wasps. For several parasitoid species it has been demonstrated that cues encountered during or directly after emergence from their cocoon can be important in priming (e.g. Hérard et al., 1988) and in preference induction (e.g. Kester & Barbosa, 1991). Caubet & Jaisson (1991) and Cortesero & Monge (1994) demonstrated for two parasitoids of concealed hosts a learning sensitive phase prior to emergence from the host's feeding site. Potentially, a *C. flavipes* female that emerges inside the stemborer tunnel can acquire information from the host larva, its own cocoon or the larval frass encountered while exiting the tunnel. We did not find any evidence for early-adult learning in *C. flavipes*. Wasps that had emerged from a cocoon, that was formed outside the stemborer tunnel, responded equally well as wasps that emerged from a stemborer-tunnel in a plant stem (fig 5.1a). The response level was also not decreased when wasps were isolated from their cocoon (fig 5.1b). Furthermore, we did not find a preference induction for the plant species or host species from which *C. flavipes* emerged (fig 5.3a, 5.4a, 5.5a). Mohyuddin et al. (1981) postulated the existence of plant specific strains in *C. flavipes*. They reported that *C. flavipes* strains from sugarcane or maize had a preference for the plant and host species from which they originated. Our results indicate that the reported existence of plant-specific strains in *C. flavipes* (Mohyuddin et al., 1981) is thus not induced by early adult conditioning, but may originate by genetically induced preferences.

Adult learning

Numerous studies with insect parasitoids have shown that adult learning (i.e. oviposition experience) can strongly modify the response to host-related cues (Turlings et al., 1993; Vet et al., 1995). One of the effects of adult learning is an increase in responsiveness to odour cues, a much appreciated and described effect by students of parasitoid foraging behaviour. Furthermore, parasitoids can learn to associate odours with successful host location (e.g. Lewis & Tumlinson, 1988; Vet & Groenewold, 1990). In the present study we did not find any evidence that adult learning can influence behavioural decisions in *C. flavipes*. Naive females had a high innate response towards infested plant odours. Contact with larval frass or an oviposition experience prior to the bioassay did not increase the response level

further (fig 5.1c). Neither could we demonstrate a preference induction. *C. flavipes* females did not prefer the plant species or host species, with which they had an oviposition experience (fig 5.3b, 5.4b, 5.5b).

We can conclude that *C. flavipes* (apparently) does not use odour learning in host-microhabitat location. Learning in host foraging seems to be widespread in parasitoids and has been demonstrated in egg, larval and pupal parasitoids from several different families such as Eucolidae, Braconidae, Ichneumonidae, Pteromalidae and Trichogrammatidae. Restricting ourselves to larval parasitoids of the family Braconidae to which *C. flavipes* belongs, preference odour learning has been demonstrated in: *Asobara rufescens*, *Asobara tabida* (Vet & Opzeeland, 1984), *Bracon Mellitor* (Vinson et al., 1977), *Cotesia congregata* (Kester & Barbosa, 1991), *Cotesia glomerata* (Geervliet et al., 1994), *Cotesia marginiventris* (Turlings et al., 1989, 1990), *Diaeretiella rapae* (Sheehan & Shelton, 1989), *Macrocentrus grandii* (Ding et al., 1989; Udayagiri & Jones 1993), *Microplitis croceipes* (Lewis & Tumlinson, 1988; Eller et al., 1992) and *Opius dissitus* (Petitt et al., 1992).

There are many pitfalls in comparing learning abilities of different species (Rosenheim, 1993; Kamil, 1994). The failure of a species to perform well in a particular test does not necessarily mean the species lacks the ability for which it is supposedly tested. As argued by Kamil (1994) it is theoretically impossible to prove that there is no set of circumstances in which an animal can learn a particular task. One could argue that we tested only a limited range of plant and host species or that *C. flavipes* might be able to learn other stimuli such as visual cues as has been demonstrated for other parasitoid species (Arthur, 1966; Wardle & Borden, 1989; Wäckers & Lewis, 1994). However, taking the risk that we discard learning while it may be expressed under a different set of circumstances, we are confronted with an interesting phenomenon that contrasts to the many occasions where learning is so easily demonstrated in other parasitoid species.

As predicted by the hypotheses stated earlier, two factors may be responsible for the unimportance of learning in foraging in *C. flavipes*: a predictable foraging environment and the restricted number of lifetime foraging decisions. It can be hypothesized that *C. flavipes* has evolved its foraging strategy in a homogeneous predictable environment that consists of large fields of perennial grasses with one or two prevailing stemborer species. The experienced world is thus highly predictable and the wasp does not need to acquire information from its environment to determine future encounter rates. Under these conditions we expect fixed responses that are not influenced by experience (Stephens, 1993). The second factor that may be responsible for the absence of learning may be the low frequency of host

encounter in the lifetime of the parasitoid. Potting et al. (1996) demonstrated that a female *C. flavipes* is expected to attack only 2-4 hosts in its lifetime.

To study the adaptive significance of learning in foraging one can compare the learning abilities of populations of the same species that are confronted with different foraging environments (micro-evolutionary approach) or compare the learning abilities of different species (macro-evolutionary approach). Only a few studies have used the micro-evolutionary approach to study the adaptiveness of learning. For example, Papaj (1986) studied the local adaptation for learning in a monophagous and a biphagous population of the butterfly *Battus philenor*. He found that the two populations of *B. philenor* were equally capable to learn visual cues (leaf shape) of a particular host species. The macro-evolutionary approach, where the learning abilities of different species are compared, has been used, for instance to compare the learning abilities of specialist and generalist parasitoids (Vet et al., 1995; Poolman Simons et al., 1992; Geervliet et al., 1993). A species comparative approach can also be used for parasitoids attacking stemborer larvae. For three parasitoid species, that all attack the stem-boring larva of *Ostrinia nubilalis* (Lepidoptera: Pyralidae), the role of learning in the foraging behaviour has been studied. Ma et al. (1992) found that the ichneumonid *Eriborus terebrans* (Gravenhorst) was more responsive to odours after an oviposition experience. A preference induction for the experienced micro-habitat was demonstrated for the braconid *Macrocentrus grandii* (Goidanich) (Ding et al., 1989; Udayagiri and Jones 1993) and the ichneumonid *Exeristis roborator* (F.). Learning was well studied for *E. roborator* and it has been demonstrated that it can learn the colour (Wardle, 1990), form upon contact (Wardle and Borden, 1990) and odour (Wardle and Borden, 1989) associated with a host-microhabitat.

Although these stemborer parasitoids attack their hosts in the same foraging niche as *C. flavipes*, they have a different life history and attack strategy, which influences the two factors that we hypothesize to be important for learning. Firstly, *E. terebrans*, *E. roborator* and *M. grandii* are all 2-3 times bigger and are relatively long lived compared to *C. flavipes* (few weeks vs several days). All three species allocate a single egg to each encountered host, whereas *C. flavipes* is gregarious. A rough estimate of the expected host encounter rate is the maximum number of hosts that an individual female can parasitize under laboratory conditions. *C. flavipes* is already egg depleted after parasitizing 5-6 hosts only (Potting et al., 1996), whereas the reported mean realized fecundity is 400 for *E. roborator* (Baker, 1949) and 200 for *M. grandii* (Parker, 1931). Furthermore, *C. flavipes* attacks the concealed larvae by ingressing the stemborer tunnel, exposing itself to a considerable mortality risk, whereas the other species reach the larva with their long ovipositor by drilling

through the plant tissue or probing through the exit hole of the tunnel. The longer lifespan and negligible mortality rate at oviposition enhances the host encounter rate compared to the small, short lived and ingressing *C. flavipes*. The frequency of host encounter may thus be the decisive factor in the evolution or retention of learning abilities in these stemborer parasitoids.

Secondly, concerning the environmental variability hypothesis it is likely that, although these four stemborer parasitoids all attack larvae in perennial grasses, the actual experienced environmental variability in these learning species is much higher than for *C. flavipes*. For instance, *E. roborator* is a polyphagous parasitoid attacking concealed hosts in a variety of micro-habitats such as maize (e.g. *Ostrinia nubilalis*), pine (i.e. *Rhyacionia buoliana*), cotton (e.g. *Pectinophora gossypiella*) and apple (e.g. *Cydia pomonella*). Due to its longer lifespan (and better flight capabilities) the area covered in the lifetime of *E. roborator* may thus be much larger and more diverse than that of *C. flavipes*. The area covered in the lifetime of a female *C. flavipes* (e.g. a field of sugarcane or maize) could be a patch in the foraging environment of *E. roborator*. The environmental variability a female *E. roborator* encounters in its lifetime is thus much higher than for *C. flavipes* that forages in a relatively homogeneous environment.

We can only speculate whether *C. flavipes* has never evolved the ability to use learning in host foraging, has lost the ability to learn or does not express the learned information. Papaj (1993b) hypothesizes that learning as an optimization mechanism may be an ancestral trait and may have persisted in environments where we do not expect it. Using simulation models Papaj showed that we may expect evolution towards congenital responses and/or evolution towards faster and faster learning for animals foraging in a predictable environments (Papaj 1993a) or for animals with a limited number of foraging experiences (Papaj 1994). Our results indicate that *C. flavipes* does not use learning (anymore) and fully relies on its congenital responses. Animals are not expected to express learning in situations in which the information needed to guide behaviour is completely predictable. There could be fitness penalties when learning is used in a homogeneous predictable environment. Learning can be less efficient and certain than reliable innate information: it takes time to learn and mistakes can be made by irrelevant correlations. Animals that do not learn have a fixed (innate) expectation of the available resources in their world and they may have evolved rules of thumb for host foraging decisions. One rule of thumb in *C. flavipes* could be: do not be choosy and respond to odours from an infested plant irrespective of the plant or host species. It remains to be investigated which common odour(s) are used by *C. flavipes* as host seeking cues.

ACKNOWLEDGMENTS

We thank Henk Snellen, Leo Koopman, Andre Giddink and Joseph Owino for the supply of stemborer larvae and parasitoids. Dr. W.A. Overholt of the International Centre of Insect Ecology and Physiology, Nairobi, Kenya kindly provided working space for part of the experiments. Dan Papaj, Felix Wäckers, Yvonne Drost, Jacqueline Geervliet and Joop van Lenteren are acknowledged for valuable comments on a previous version of the manuscript. This work was supported by the Netherlands foundation for the Advancement of Tropical Research (W84-325).

REFERENCES

- Arthur, A.P. 1966. Associative learning in *Itopectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *Can. Entomol.* 98: 213-233.
- Baker, W.A., Bradley, W.G. & Clark, C.A. 1949. Biological control of the European corn borer in the United States. *USDA Tech. Bull.* 983: 1-185.
- Bolles, R.C. & Beecher, M.S. 1988. Evolution and learning. Hillsdale: Lawrence Erlbaum Associates.
- Caubet, Y. & Jaisson, P. 1991. A post-eclosion early learning involved in host recognition by *Dinarmus basalis rondani* (Hymenoptera: Pteromalidae). *Anim. Behav.* 42: 977-980.
- Cortesero, A.M. & Monge, J.P. 1994. Influence of pre-emergence experience on response to host and host plant odours in the larval parasitoid *Eupelmus vullei*. *Entomol. Exp. Appl.* 72: 281-288.
- Ding, D., Swedenborg, P.D. & Jones R.L. 1989. Plant odor preferences and learning in *Macrocentrus grandii* (Hymenoptera: Braconidae), a larval parasitoid of the european corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J. Kansas Ent. Soc.* 62: 164-176.
- Eller, F.J., Tumlinson, J.H. & Lewis, W.J. 1992. Effect of host diet and preflight experience on the flight responses of *Microplitis croceipes* (Cresson). *Physiol. Entomol.* 17: 235-240.
- Geervliet, J.B.F., Aken, R. van, Savelkoul, C., Smitte, S.M., Brodeur, J., Vet, L.E.M. & Dicke, M. 1993. Comparative approach to infochemical use by parasitoids for the case of *Cotesia glomerata* and *C. rubecula*. *Proc. Exper. & Appl. Entomol.* 4: 33-38.
- Geervliet, J.B.F., Ariens, S.J.A., Vet, L.E.M. & Dicke, M. 1994. Learned odour preferences in the parasitoid *Cotesia glomerata*. *Norw. J. Agr. Sc. Suppl.* 16: 261-267.
- Gould, J.L. & Marler, P. 1984. Ethology and the natural history of learning. In: *The biology of learning* (Ed. by P. Marler & H.S. Terrace), pp. 47-77. Berlin: Springer Verlag.
- Hérard, F., Keller, M.A., Lewis, W.J. & Tumlinson, J.H. 1988. Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. *J. Chem. Ecol.* 14: 1597-1606.
- Johnston, T.D. 1982. Selective costs and benefits in the evolution of learning. *Adv. St. Behav.* 12: 65-106.
- Kamil, A.C. 1994. A synthetic approach to the study of animal intelligence. In: *Behavioural mechanisms in evolutionary ecology* (Ed. by L.A. Real), pp. 11-45. Chicago: University of Chicago Press.
- Kester, K.M. & Barbosa, P. 1991. Postemergence learning in the insect parasitoid *Cotesia congregata* (Say) (Hymenoptera: Braconidae). *J. Insect Behav.* 4: 727-742.
- Lewis, W.J. & Tumlinson, J.H. 1988. Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* 331: 257-259.
- Lewis, W.J., Vet, L.E.M., Tumlinson, J.H., van Lenteren, J.C. & Papaj, D.R. 1990. Variations in parasitoid foraging behavior: essential element of a sound biological control theory. *Environ. Entomol.* 19: 1183-1193.
- Ma, R.Z., Swedenborg, P.D. & Jones, R.L. 1992. Host-seeking behavior of *Eriborus terebrans*

- (Hymenoptera: Ichneumonidae) toward the European corn borer and the role of chemical stimuli. *Ann. Ent. Soc. Am.* 85: 72-79.
- Mohyuddin, A.I., Inayatullah, C. & King, E.G. 1981. Host selection and strain occurrence in *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) and its bearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* 71: 575-581.
- Nagarkatti, S. & Nair, K.R. 1973. The influence of wild and cultivated Graminae and Cyperaceae on populations of sugarcane borers and their parasites in North India. *Entomophaga* 18: 419-430.
- Ochieng, R.S., Onyango, F.O. & Bungu, M.D.O. 1985. Improvement of techniques for mass-culture of *Chilo partellus* (Swinhoe). *Insect Sci. Appl.* 6: 425-428.
- Papaj, D.R. 1986. Interpopulation differences in host preference and the evolution of learning in the butterfly *Battus philenor*. *Evolution* 40: 518-530.
- Papaj, D.R. 1993a. Automatic behavior and the evolution of instinct: lessons from learning in parasitoids. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis), pp. 243-272. New York: Chapman and Hall.
- Papaj, D.R. 1993b. Afterword: learning, adaptation, and the lessons of O. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis), pp. 374-386. New York: Chapman and Hall.
- Papaj, D.R. 1994. Optimizing learning and its effect on evolutionary change in behavior. In: *Behavioral mechanisms in evolutionary ecology* (Ed. by L.A. Real), pp. 133-153. Chicago: University of Chicago Press.
- Papaj, D.R. & Lewis, A.C. 1993. Insect learning. Ecological and evolutionary perspectives. New York: Chapman and Hall.
- Papaj, D.R. & Prokopy, R.J. 1989. Ecological and evolutionary aspects of learning in phytophagous insects. *Annu. Rev. Entomol.* 34: 315-350.
- Papaj, D.R. & Vet, L.E.M. 1990. Odor learning and foraging success in the parasitoid *Leptopilina heterotoma*. *J. Chem. Ecol.* 16: 3137-3150.
- Parker, H.L. 1931. *Macrocentrus gifuensis* Ashmead, a polyembryonic braconid parasite of the European Corn Borer. *USDA Tech. Bull.* 230: 1-62.
- Pettitt, F.L., Turlings, T.C.J. & Wolf, S.P. 1992. Adult experience modifies attraction of the leafminer parasitoid *Opisus dissitus* (Hymenoptera: Braconidae) to volatile semiochemicals. *J. Insect Behav.* 5: 623-634
- Poolman Simons, M.T.T., Suverkropp, B.P., Vet, L.E.M. & de Moed, G. 1992. Comparisons of learning in related generalist and specialist eucoilid parasitoids. *Entomol. exp. appl.* 64: 117-124.
- Potting, R.P.J., Vet, L.E.M. & Dicke, M. 1995. Host microhabitat location by stemborer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systemically induced plant volatiles. *J. Chem. Ecol.* 21: 525-539.
- Potting, R.P.J., Overholt, W.A., Danso, F.O. & Takasu, K., 1996. Foraging behavior and life history of the stemborer parasitoid *Cotesia flavipes*. *J. Insect Behav.* (submitted).
- Prokopy, R.J. & Lewis, W.J., 1993. Application of learning to pest management. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis), pp. 308-342. New York: Chapman and Hall.
- Real, L.A. 1991. Animal choice behaviour and the evolution of cognitive architecture. *Science* 253: 980-986.
- Real, L.A. 1994. Behavioural mechanisms in evolutionary ecology. Chicago: University of Chicago Press.
- Roitberg, B.D., Reid, M.L. & Li, C. 1993. Choosing hosts and mates: the value of learning. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis), pp. 174-194. New York: Chapman and Hall.
- Rosenheim, J.A. 1993. Comparative and experimental approaches to understanding insect learning. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis),

- pp. 273-307. New York: Chapman and Hall.
- Sheehan, W. & Shelton, A.M. 1989. The role of experience in plant foraging by the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *J. Insect Behav.* 2: 743-759.
- Sokal, R.R. & Rolf, F.J., 1981. *Biometry*, 2nd edn. San Francisco: Freeman.
- Steinberg, S., Dicke, M., Vet, L.E.M. & Wainwright, R. 1992. Response of the braconid *Cotesia (=Apanteles) glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomol. exp. appl.* 63: 163-175.
- Stephens, D.W. 1993. Learning and behavioral ecology: incomplete information and environmental predictability. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis), pp. 195-218. New York: Chapman and Hall.
- Turlings, T.C.J., Tumlinson, J.H., Lewis, W.J. & Vet, L.E.M. 1989. Beneficial arthropod behavior mediated by airborne semiochemicals. VIII. Learning of host-related odours induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *J. Insect Behav.* 2: 217-225.
- Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.
- Turlings, T.C.J., Wäckers, F., Vet, L.E.M., Lewis, W.J. & Tumlinson, J.H. 1993. Learning of host-finding cues by hymenopterous parasitoids. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis), pp. 51-78. New York: Chapman and Hall.
- Udayagiri, S. & Jones, R.L. 1993. Variation in response of the specialist parasitoid *Macrocentrus grandii* Goidanich to food plants of its European corn borer host. *Entomol. exp. appl.* 69: 183-193.
- Vet, L.E.M. 1983. Host-habitat location through olfactory cues by *Leptopilina clavipes* (Hartig) (Hym: Eucolidae), a parasitoid of fungivorous *Drosophila*: the influence of conditioning. *Neth. J. Zool.* 33: 225-248.
- Vet, L.E.M. & Opzeeland, K. 1984. The influence of conditioning on olfactory microhabitat and host location in *Asobara tabida* (Nees) and *A. rufescens* (Foerster) (Braconidae, Alysiinae), larval parasitoids of *Drosophila*. *Oecologia* 63: 171-177.
- Vet, L.E.M. & Groenewold, A.W. 1990. Semiochemicals and learning in parasitoids. *J. Chem. Ecol.* 16: 3119-3135.
- Vet, L.E.M., Lewis, W.J. & Cardé, R.T. 1995. Parasitoid foraging and learning. In: *Chemical ecology of insects* (Ed. by W.J. Bell and R.T. Cardé), pp. 65-101. New York: Chapman Hall.
- Vinson, S.B., Barfield, C.S. & Henson, R.D. 1977. Oviposition behaviour of *Bracon mellitor*, a parasitoid of the boll weevil (*Anthonomus grandis*). II. Associative learning. *Phys. Entomol.* 2: 157-164.
- Wäckers, F.L. & Lewis, W.J. 1994. Olfactory and visual learning and their combined influence on host site location by *Microplitis croceipes*. *Biol. Control* 4: 105-112.
- Wardle, A.R. 1990. Learning of host microhabitat colour by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *Anim. Behav.* 39: 914-923.
- Wardle, A.R. & Borden, J.H. 1989. Learning of an olfactory stimulus associated with a host microhabitat by *Exeristes roborator*. *Entomol. exp. appl.* 52: 271-279.
- Wardle, A.R. & Borden, J.H. 1990. Learning of host microhabitat form by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *J. Insect. Behav.* 3: 251-263.

Geographic variation in host selection behavior and reproductive success in the stemborer parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae).

ABSTRACT Local parasitoid populations may be adapted to their sympatric major plant host complex. Parasitoid strains may thus differ in their propensity to search for a particular host or micro-habitat or they may differ in their physiological compatibility with particular host species. *Cotesia flavipes*, a larval parasitoid used worldwide in biological control against tropical stemborers, has a wide host range in diverse habitats. The existence of plant and/or host specific strains in *C. flavipes* has been postulated. To provide insight in the existence of strains in *C. flavipes* we compared the host selection behavior and physiological compatibility with different stemborers of six different geographic strains of *C. flavipes* that differed in the plant-host-complex they were obtained from. The results of the host selection experiments indicate that there is no interspecific variation in host selection behavior among *C. flavipes* strains. However, our comparative experiments show variation in reproductive success among strains. The most significant result was that the strain with the longest period of co-existence with the new host *D. saccharalis*, had the highest reproductive success on this host species. We argue that the earlier reported existence of *C. flavipes* strains is not based on a differential host selection behavior, but on differences in physiological compatibility between local parasitoid and host population.

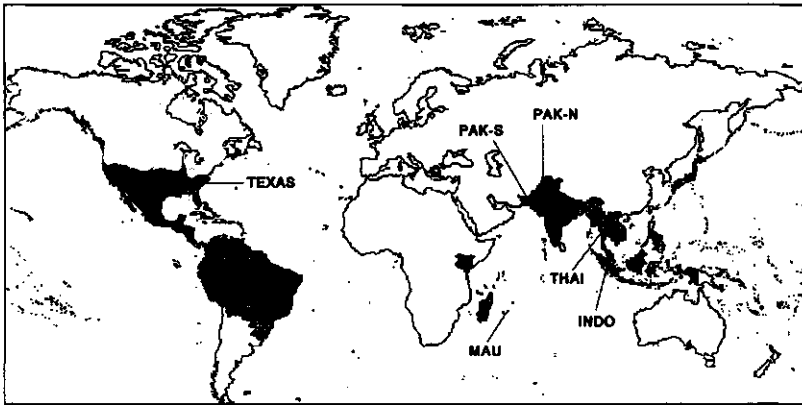
INTRODUCTION

Many insects utilize diverse resources over the species' entire geographical range and local populations may experience different selection pressures. Populations from distinct regions may be adapted to utilize specific resources. A putatively generalist species can actually consist of specialist local populations (Fox & Morrow, 1981; Futuyma & Peterson, 1985). The differentiation of populations within species may result in populations that differ biologically from other conspecific populations and are referred to as a strain or race (Diehl and Bush, 1984; Kim and McPheron, 1993). Intraspecific variability in hymenopteran parasitoids is well known and has been reported for ecological, behavioral and physiological traits, such as climatic adaptability, diapause, host selection and virulence (reviewed by: Hopper et al.,

Submitted as: Potting, R.P.J., Vet, L.E.M. and Overholt, W.A. (1996). Geographic variation in host selection behavior and reproductive success in the stemborer parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae). *Bulletin of Entomological Research*.

1993; Unruh and Messing, 1993). Local variation in the use of resources has important implications for the evolution of ecological specialization and is relevant to host race formation and parasitoid speciation (Diehl and Bush, 1984; Bush, 1994). Plant and/or host specific strains may result from the combination of selection pressures on physiological (i.e. virulence) and behavioral traits (host selection). For instance, strain specific variation in host selection and virulence has been reported for the hymenopterous parasitoids *Cotesia congregata* (Kester and Barbosa, 1991, 1994) and *Asobara tabida* (Kraaijeveld and van der Wel, 1995; Kraaijeveld et al., 1995). Over time resource specific strains may evolve towards distinct species as suggested by Vet and Janse (1984) for *A. tabida* and *A. rufescens*. Local variation in the use of resources also has important implications for biological control, because the selection of appropriate strains is a significant factor in successful biological control (Roush, 1990; Lewis et al., 1990). For example, it is important to choose a strain that ensures seasonal synchronization with its host (e.g. Kenis, 1994) as well as a strain that has the ability (potential) to search for and utilize the target host species in its micro-habitat (Lewis et al., 1990).

We studied the variation in host selection and virulence among strains of a parasitoid used worldwide in biological control against stemborers. Following Diehl and Bush (1984) we define a strain here as 'the cultured offspring of a sample taken from a field population at a certain time and locality'. The braconid *Cotesia flavipes* Cameron is a gregarious endoparasitoid of lepidopterous stemborer larvae that is indigenous and widespread in the Indo-Australian region (fig 6.1). Due to introductions against *Chilo partellus* (Alam et al., 1972; Overholt et al., 1994), *Diatraea saccharalis* (Gifford & Mann, 1967; Alam et al., 1971; Fuchs et al., 1979; Macedo et al., 1993) and *Chilo sacchariphagus* (Betbeder-Matibet & Malinge, 1968) *C. flavipes* now also occurs in the Caribbean, major parts of North and South America (Polaszek & Walker, 1991), several Indian Ocean islands and recently in East-Africa (Omwege et al., 1995). Across its geographic range *C. flavipes* attacks noctuid and pyralid stem boring larvae. From field studies it is known to successfully parasitize more than 20 host species in more than 15 plant species, including economically important stemborers in maize, sorghum, sugarcane and rice (Potting and Polaszek, unpubl.). The existence of different geographic, host and/or plant specific strains in *C. flavipes* has been postulated. This hypothesis is based on laboratory studies on plant preference behavior of different strains (Mohyuddin et al., 1981; Shami & Mohyuddin, 1992), on anecdotal notes of increased local (field) parasitism rates after release of particular parasitoid strains (Mohyuddin, 1990) and on variation in reproductive success on *D. saccharalis* among strains of *C. flavipes* (Wiedenmann and Smith, 1995).



Name	Initial year and site of collection	Original host species / plant species	Laboratory host / diet
INDO	1994, Sumatra, Indonesia	<i>C. sacchariphagus</i> / sugarcane	-
MAU	1994, Mauritius	<i>C. sacchariphagus</i> / sugarcane	-
PAK-N	1991, Rawalpindi, North Pakistan	<i>C. partellus</i> / maize	<i>C. partellus</i> / Maize
PAK-S	1993, South Pakistan	<i>C. partellus</i> / maize	<i>C. partellus</i> / AD1
TEXAS	1993, Rio-Grande Valley, Texas, USA	<i>D. saccharalis</i> / sugarcane	<i>D. saccharalis</i> / AD2
THAI	1990, Thailand	<i>C. sacchariphagus</i> / <i>C. infuscatellus</i> / sugarcane	<i>D. saccharalis</i> / AD2

Figure 6.1 Origins and original host species and plant species of six *Cotesia flavipes* strains used in present study. AD1 = sorghum based artificial diet (Ochieng et al., 1985); AD2 = sugarcane based artificial diet (Martinez et al., 1988). World map shows approximate geographic range of *C. flavipes* (shaded areas) and site of initial collection.

In the present study we compared the behavior and virulence of six geographic strains of *C. flavipes* that differed in the plant-host-complex from which they were obtained. Two strains originated from *C. partellus* on maize and four strains from other stemborer species on sugarcane. In this paper we first report experiments to establish if strains differ in microhabitat preference and in host species acceptance, and subsequently if the strains differ in their reproductive success on different host species. To locate the host-microhabitat, *C. flavipes* females use olfactory stimuli that are released by stemborer-infested plants (Potting et al., 1995b; Ngi-Song et al., 1995b). One of the sources of attractive volatiles from the plant-host-complex is the frass produced by the feeding larvae. To determine variation in plant-host-complex preference among strains we tested the preference for a particular frass source in an olfactometer setup. Furthermore, we examined host acceptance and subsequent reproductive success of different host species by the different strains. To determine the reproductive success of the different strains on different host species, we

measured the development time, number and sex of progeny and, as a fitness measure, the weight of the emerging wasps. In our comparative experiments, a maize strain was tested against one or two sugarcane strains on the same experimental days with the same alternative odor sources or hosts, to rule out any day effect. The alternative odor sources or hosts consisted of the original plant and host species of the strains under consideration.

If strains are locally adapted to the prevailing plant-host-complex (PHC), we expect that the strains may exhibit a preference for the PHC from which they originated and a higher acceptance ratio for the aboriginal host species. Furthermore, we expect the highest physiological compatibility (i.e. reproductive success) with the strain's original host species.

MATERIALS AND METHODS

Parasitoids

Six geographic *C. flavipes* strains were used for the experiments. The origin and plant-host-complex from which they were collected are given in figure 6.1. The wasps of the Mauritius and Indonesia strain were collected in the field and not maintained in the laboratory. The other strains had been reared in the laboratory for several years on *C. partellus* (Pakistan-South) or *D. saccharalis* (Texas and Thailand) feeding on artificial diet. For rearing methods of these strains we refer to Ngi-Song et al. (1995a) and Wiedenmann et al. (1992). The Pakistan-North strain was maintained on *C. partellus* feeding on maize stems as described by Potting et al. (1995b). Prior to emergence parasitoid cocoon masses were transferred to 150 ml. bottles with an agar medium to maintain a high humidity. Adult parasitoids were fed with undiluted honey and stored at 15 ± 0.5 °C until used for the experiments. For the experiments, 1-3 days old females were used, which were naive with respect to frass and host contact.

Hosts

The pyralids *Chilo partellus*, *Chilo auricilius* and *Diatraea saccharalis* larvae were reared on artificial diets (Ochieng et al., 1985; Martinez et al., 1988). *Chilo sacchariphagus* larvae were field collected from sugarcane plantations in Mauritius. For the experiments, mature stemborer larvae (approx. 4th instar) were used that had fed for at least 48 h on maize (*C. partellus*) or sugarcane (*C. auricilius*, *C. sacchariphagus*, *D. saccharalis*).

Plants

Maize (*Zea mays*, cv Anjo) and sugarcane (*Saccharum officinarum*, cv POJ2878) were grown in a greenhouse. Stem pieces (15 cm) from maize in the flowering stage and

mature sugarcane plants (\varnothing 4-5 cm) were used in the experiments.

Plant-host-complex preference

The preference for odors from a particular plant-host-complex was tested in a Y-tube olfactometer (Steinberg et al., 1992). Odor sources were placed directly in the two arms of the Y-tube, behind the double screen mesh. Odor sources consisted of 2 g frass, produced by stemborer larvae feeding on maize stems or sugarcane stems. In one experiment (with Indonesia strain) the odor sources consisted of an uninfested maize plant and *C. partellus*-infested maize plant, prepared and tested as described by Potting et al. (1995b). Individual female parasitoids were introduced into the central olfactometer tube. Walking upwind the parasitoid could choose one of the olfactometer arms. The observation was counted as a choice when the female passed the finish line in one of the arms for a period of 15 seconds. Individuals that had not made a choice within 5 minutes were counted as 'no choice'. For more details on the test procedure, refer to Potting et al. (1995b). To rule out any day effect, the response of two or three strains were always tested on the same test days, where the maize strain from Pakistan-North served as a control. Prior to the dual choice tests, where maize frass was tested against sugarcane frass, the response potential to a particular frass source was measured, by testing the frass source against clean air. To determine whether the quantity or quality of volatiles from frass induced a preference for a particular frass source, a small dose-response experiment was carried out. In this test wasps of the PAK-N strain were offered a choice between different quantities (1 g vs 8 g) of different frass sources (*C. partellus*/maize vs *C. auricilius*/sugarcane). Prior to these choice tests the attractiveness of the lowest quantity (1 g) was tested with clean air as control.

Host acceptance

Individual female *C. flavipes* were introduced in a glass Petri dish (9 cm) containing a stemborer larva with 2-3 g fresh frass produced by the same stemborer species. The behavior of the female was observed for 5 min and the time taken to accept the larva as a host was recorded, as well as the oviposition time. For each test a new host and fresh frass was used. To rule out any day effect the acceptance ratio (i.e. percentage of hosts accepted for oviposition) of two strains for two host species were always tested on the same test days.

Reproductive success

The reproductive success of the *C. flavipes* strains was tested in two separate blocks. In the first series the reproductive success/parameters of PAK-N and INDO were determined with *C. partellus* on maize and *C. auricilius* on sugarcane as hosts species. In the second series the reproductive success of the strains from PAK-N, PAK-S,

TEXAS and THAI was determined with *C. partellus* on maize and *D. saccharalis* on sugarcane as hosts.

The reproductive parameters measured were: clutch size, number of produced cocoons and the number and sex ratio of emerged adults from parasitized larvae. Larvae were parasitized by individual females and reared on maize stem pieces (*C. partellus*) or sugarcane stem pieces (*C. auricilius*, *C. sacchariphagus*, *D. saccharalis*) at 25 ± 1 °C, $70 \pm 5\%$ RH and photoperiod regime of 16L:8D. Due to unexpected mortality of parasitized hosts as a result of a disease, no reliable data were obtained on the number of parasitized hosts producing parasitoid offspring. The reproductive success of the tested strains on the tested host species was therefore analyzed for hosts that produced at least more than one parasitoid. Clutch size was estimated by dissecting a part of the parasitized larvae 5-7 days after oviposition and counting the number of parasitoid larvae. The developmental time was measured as the period from oviposition until parasitoid adult emergence from their cocoon. In one comparison (PAK-N and INDO), the emerged parasitoids from each parasitized host were dried for two days at 65 °C and subsequently weighed on a micro-balance. The individual female dry weight was estimated from the total dry weight of all emerged parasitoids from one host. Because individual parasitoid weighings revealed that males were significantly (1.08x) heavier than females (t-test, $T = -2.03$, $P < 0.05$, $DF = 150$), individual female dry weight was corrected for sex ratio.

RESULTS

Plant-host-complex preference

In the control tests, where the initial response potential for a particular frass source was tested (frass vs air), all strains responded to the offered frass sources (fig 6.2). There was no significant difference in the percentage of females that responded to frass produced by *C. sacchariphagus* feeding on sugarcane or *C. partellus* feeding on maize for wasps of the strains from Pakistan (G-test, $P > 0.5$) Mauritius (G-test, $P > 0.1$) and Indonesia (G-test, $P > 0.1$) (joined response bars in fig 6.2). Wasps of the PAK-N strain responded to sugarcane frass produced by *D. saccharalis* and the TEXAS and THAI strain both responded to maize frass produced by *C. partellus* (fig 6.2). The INDO strain was attracted to odors from a *C. partellus* infested maize plant (fig 6.2). In the dual choice tests females were exposed to odors from maize frass (from *C. partellus*) or sugarcane frass (from either *C. auricilius*, *C. sacchariphagus*, or *D. saccharalis*). The results, as summarized in figure 6.3, show that there were no differences in preferences for maize vs sugarcane frass for the strains tested. With the PAK-N strain as control, no significant shifts in the choice

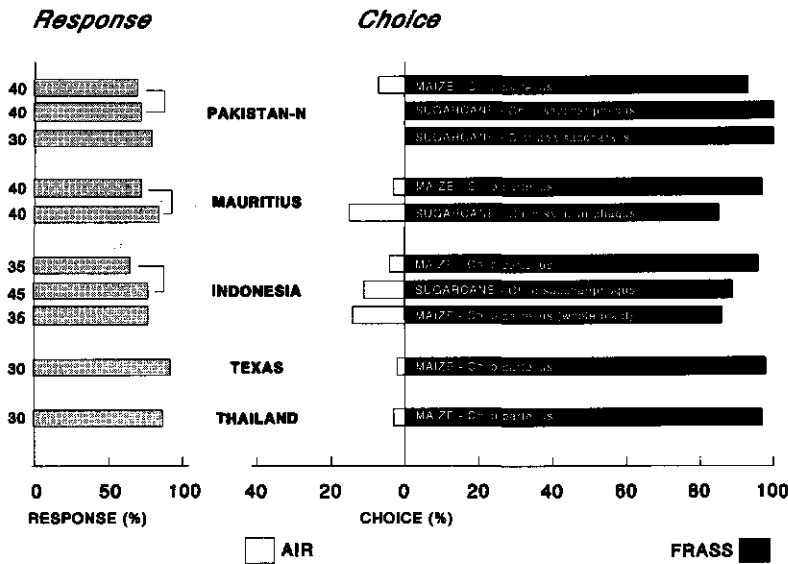


Figure 6.2 The percentage of *C. flavipes* females from different strains responding to odors from sugarcane or maize frass from different stemborer species in a Y-tube olfactometer. Bars indicate response level (grey) and percentage choosing for air (white) or frass (black). Connected bars indicate blocks tested on same experimental days. Numbers indicate number of females tested.

distribution were found for the strains: PAK-S ($G=0.08$, $df=1$, $P>0.5$), MAU ($G=0.77$, $df=1$, $P>0.5$) and INDO ($G=0.70$, $df=1$, $P>0.5$) for the choice between maize frass (*C. partellus*) and sugarcane frass (*C. sacchariphagus*); TEXAS and THAI ($G=2.06$, $df=2$, $P>0.1$) for the choice between maize frass (*C. partellus*) and sugarcane frass (*D. saccharalis*) and INDO ($G=0.08$, $df=1$, $P>0.5$) for the choice between maize frass (*C. partellus*) and sugarcane frass (*C. auricilius*).

The choice for maize or sugarcane frass seems to be dependent on the quantity rather than on the quality of the released odors. The choice for maize or sugarcane frass by the PAK-N strain could be manipulated by increasing the amount of frass (fig 6.4). Although 1 g of maize or sugarcane frass could attract wasps when tested against air (fig 6.4), the wasps significantly preferred ($G=10.3$, $df=1$, $P<0.01$) the arm in the olfactometer with 8 g maize or sugarcane frass in the dual choice tests (fig 6.4).

Host acceptance

The results of the host acceptance experiments are summarized in figure 6.5, where the bars indicate the time until host acceptance (acceptance speed) and the percentages within the bars the percentage of hosts accepted for oviposition (acceptance ratio). In the first series females from the PAK-N and MAU strain

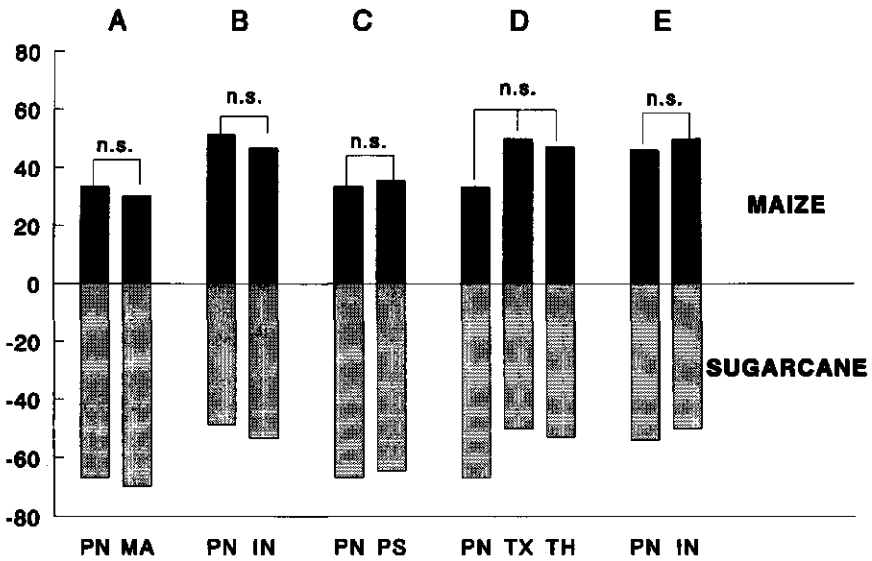


Figure 6.3 Choice of *C. flavipes* strains for odors from sugarcane or maize frass from different stemborer species in Y-tube olfactometer. Bars indicate percentage choosing for particular odor source. Strains are indicated by: PN: Pakistan North, MA: Mauritius, IN: Indonesia, PS: Pakistan South, TX: Texas, TH: Thailand. Maize stemborer in all tests *C. partellus*; sugarcane borer species used in test A, B, C: *C. sacchariphagus*, D: *D. saccharalis* and E: *C. auricilius*. Connected bars indicate blocks tested on same experimental days, significant preference differences within blocks were tested with G-test for goodness of fit ($P < 0.05$). Numbers of females for: A ($n=56$); B ($n=46$); C ($n=45$); D,E ($n=40$).

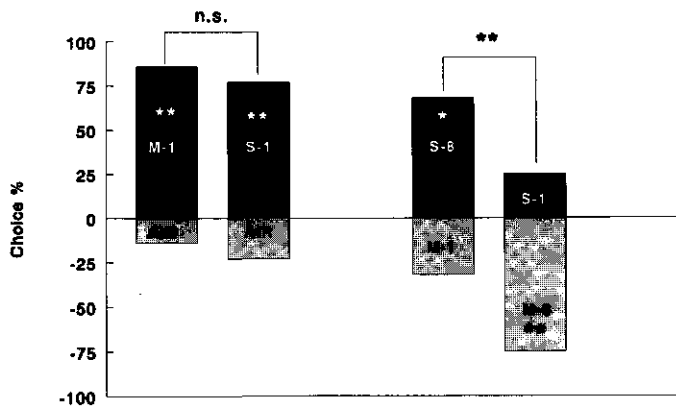


Figure 6.4 Response of *C. flavipes* to different quantities of frass in olfactometer. Odor sources consisted of AIR: clean air; M-1, M-8: 1 g or 8 g frass from *C. partellus* feeding on maize; S-1, S-8: 1 g or 8 g frass from *C. auricilius* feeding on sugarcane. Differences in choice distribution were tested with G-test for goodness of fit ($P < 0.05$) for connected bars. Numbers of females for: AIR vs 1 g frass ($n=50$); 1 g frass vs 8 g frass ($n=55$).

accepted *C. partellus* and *C. sacchariphagus* in an equal rate for oviposition (within strain pairwise G-tests, $P > 0.01$). For the PAK-N and MAU strain, there was no significant difference in the time until acceptance for the two host species (fig 6.5a). However, MAU females that originated directly from the field, accepted their hosts significantly slower than females from the laboratory strain from PAK-N (host species pooled, Mann Whitney U-test, $P < 0.0001$). A similar pattern was found in the second series where the acceptance speed and ratio for *C. partellus* on maize and *C. auricilius* on sugarcane were determined for the INDO and PAK-N strains (fig 6.5b). There were no significant differences in the acceptance speed (Mann Whitney U-tests, $P > 0.05$) and acceptance ratios (G-tests, $P > 0.5$) for the two host species within strains. However, INDO females accepted their hosts significantly slower (host species pooled, Mann Whitney U-test, $P < 0.01$) and at a significantly lower rate (host species pooled, G-test, $P < 0.0001$) compared to the PAK-N females (fig 6.5b).

In the third series, the acceptance speed and ratio for *C. partellus* on maize and *D. saccharalis* on sugarcane were determined for the TEXAS, THAI and PAK-N strain (fig 6.5c). There was no difference in acceptance ratio, because the three strains accepted all offered hosts. However, THAI (Mann Whitney U-test, $P < 0.0001$) and TEXAS (Mann Whitney U-test, $P < 0.01$) accepted *C. partellus* significantly slower than *D. saccharalis*. There was no significant difference in the acceptance speed of the two host species in the PAK-N strain (fig 6.5c).

Reproductive success

Chilo partellus vs *Chilo auricilius*

In the first series the reproductive success of the PAK-N and INDO strain was determined with *C. partellus* on maize and *C. auricilius* on sugarcane as host species. The results are summarized in table 6.1 and fig 6.6. There was no significant difference in the number of progeny allocated by the two strains in the two host species (Two way ANOVA: STRAIN ($F=0.64$, $P > 0.4$), HOST ($F=1.0$, $P > 0.3$) and STRAIN*HOST ($F=3.5$, $P > 0.05$). However, the survival rate (mean number emerged / mean clutch size) was higher for the PAK-N strain (fig 6.6). The PAK-N strain produced significantly more offspring on *C. partellus* than the INDO strain (table 6.1). Furthermore, the PAK-N strain developed significantly faster on both host species than the INDO strain (table 6.1). No significant differences were found in the sex ratio of the emerged progeny of the two strains on the two host species. However, ANOVA indicates there was a significant strain*host interaction. The PAK strain produced the lowest ratio of males on *C. partellus*, whereas the INDO strain produced the lowest ratio of males on *C. auricilius* (table 6.1). There was no

Host acceptance

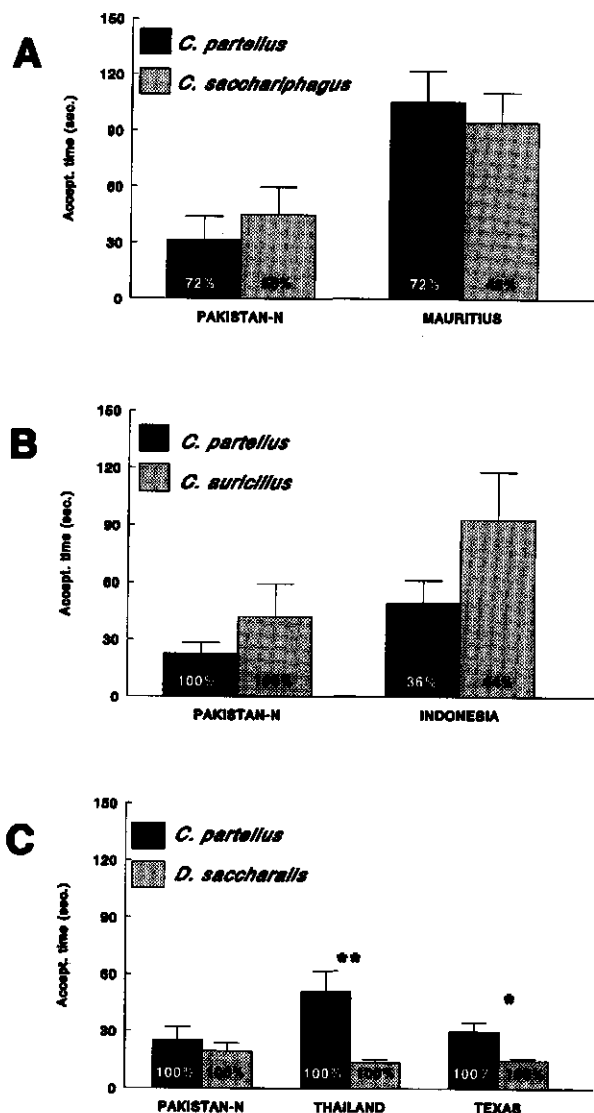


Figure 6.5 Host acceptance of different stemborer species by *C. flavipes* strains. (A) Acceptance of *C. partellus* and *C. sacchariphagus* by *C. flavipes* strains of Pakistan and Mauritius (n=25). (B) Acceptance of *C. partellus* and *C. auricilius* by *C. flavipes* strains of Pakistan and Indonesia (n=25). (C) Acceptance of *C. partellus* and *D. saccharalis* by *C. flavipes* strains of Pakistan, Thailand and Texas (n=20). Bars indicate time (mean (sec) \pm SE) till acceptance of host, percentage within bars indicate percentage of accepted hosts. Asterisks indicate significant differences in acceptance time (t-test, $P < 0.05$).

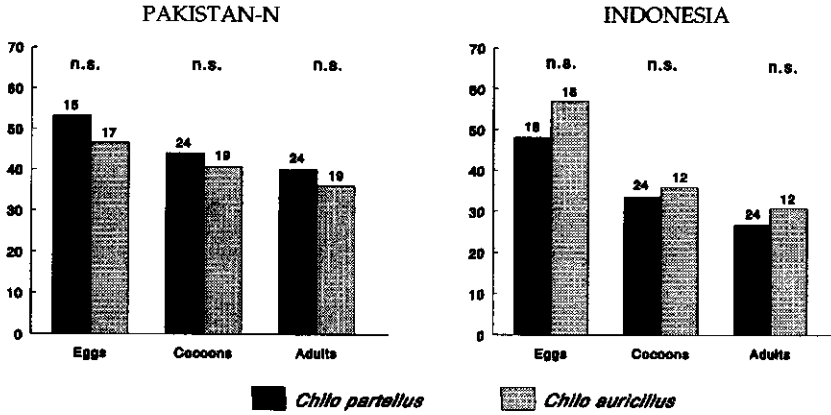


Figure 6.6 Estimated clutch size, number of produced cocoons and number of emerging adults of *C. flavipes* strains from Indonesia and Pakistan on *C. partellus* on maize (black bars) and *C. auricilius* on sugarcane (shaded bars). Differences between strains were tested with t-test for unequal variances ($P < 0.05$). Numbers above bars indicate sample size.

significant difference in the individual dry weight of parasitoids emerging from *C. partellus* or *C. auricilius* (table 6.1).

Chilo partellus vs *Diatraea saccharalis*

In this series the reproductive success of the strains from PAK-N, PAK-S, TEXAS and THAI was determined with *C. partellus* on maize and *D. saccharalis* on sugarcane as hosts. The results are summarized in fig 6.7 for TEXAS and PAK-N and table 6.2 for PAK-N, PAK-S, TEXAS and THAI. There was no significant difference in the clutch size (estimated by number of progeny present after five days) allocated to *C. partellus* or *D. saccharalis* larvae by *C. flavipes* females from TEXAS and PAK-N (fig 6.7). However, significant differences in the survival rate of the parasitoid progeny were found. Both TEXAS and PAK-N had a significantly higher survival rate in *C. partellus* compared to *D. saccharalis* (fig 6.7). All the strains tested in this series produced significantly more offspring on *C. partellus* than on *D. saccharalis* (table 6.2). There was no significant difference in the number of offspring produced among the strains on *C. partellus* ($F=1.85$, $P > 0.1$, $DF=3$), but a significant difference was found in the number of offspring produced among the strains on *D. saccharalis* ($F=9.14$, $P < 0.0001$, $DF=3$). The strain from TEXAS produced significantly more offspring on *D. saccharalis* than *C. flavipes* from PAK-N, PAK-S and THAI (Tukey multiple range test, $P < 0.05$). No significant difference was found in the egg to adult development time on the different host species (table 6.2) and between the strains (One way ANOVA, *C. partellus*: $F=1.21$, $P > 0.3$,

Table 6.1 Development time (days), number of progeny produced, sex ratio (% males) and individual female dry weight (mg) for *C. flavipes* strains from Indonesia and Pakistan on *C. partellus* on maize and *C. auricilius* on sugarcane. Two way analysis of variance results are indicated ($P < 0.05$). Means followed by different letters are significantly different (Tukey multiple comparison test, $P < 0.05$).

	Development time			Number of progeny produced			Sex ratio			Individual female		
	Egg-Adult (days)			per host			(% males)			dry weight (mg)		
	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
PAKISTAN-N												
<i>Chilo partellus</i> (maize)	16.5a	0.7	24	40.0a	14.8	24	27.4a	27.9	24	0.10a	0.04	23
<i>Chilo auricilius</i> (sugarcane)	16.8a	0.8	19	36.0ab	15.1	19	39.6a	33.1	19	0.10a	0.04	18
INDONESIA												
<i>Chilo partellus</i> (maize)	18.0b	0.9	24	26.7b	13.7	24	49.7a	41.1	24	0.09a	0.04	24
<i>Chilo auricilius</i> (sugarcane)	17.9b	0.5	12	30.8ab	14.5	12	30.3a	28.1	12	0.09a	0.03	12
Two way ANOVA, main effects:												
	F	P	DF	F	P	DF	F	P	DF	F	P	DF
STRAIN	69.0	0.0001	1	9.7	0.003	1	0.48	n.s.	1	1.58	n.s.	1
HOST	0.8	n.s.	1	0	n.s.	1	0.11	n.s.	1	0.08	n.s.	1
STRAIN*HOST	1.4	n.s.	1	1.9	n.s.	1	4.9	0.03	1	0.90	n.s.	1

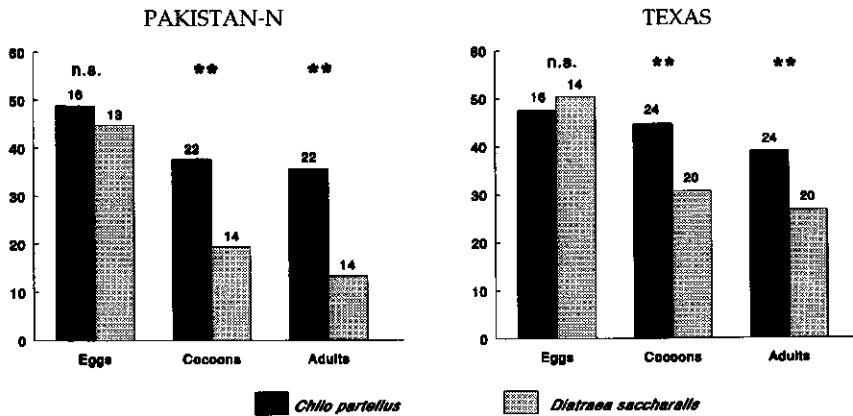


Figure 6.7 Estimated clutch size, number of produced cocoons and number of emerging adults of *C. flavipes* strains from Texas and Pakistan on *C. partellus* on maize and *D. saccharalis* on sugarcane. Differences between strains were tested with t-test for unequal variances ($P < 0.05$). Numbers above bars indicate sample size.

DF=3; *D. saccharalis*: $F=1.54$, $P > 0.2$, DF=3). There was no significant difference in sex ratio of emerged *C. flavipes* from the different host species within a strain (table 6.2) and between the strains (One way ANOVA, *C. partellus*: $F=1.37$, $P > 0.2$, DF=3; *D. saccharalis*: $F=1.93$, $P > 0.1$, DF=3).

DISCUSSION

Biological control programmes in which an exotic natural enemy is introduced into a new environment offer an opportunity to test ecological and evolutionary hypotheses (Greathead, 1986). Introduction of a parasitoid population can induce artificial allopatric speciation, by forcing the parasitoid to locally adapt to its new host or host-micro-habitat. This scenario could induce the formation of adapted parasitoid races and over time even distinct species. In biological control the performance of a released parasitoid population is dependent on its behaviour and physiology. The parasitoids not only must be able to locate the potential hosts successfully, but also must be able to develop in these hosts. The failure or success of a parasitoid introduction can thus be dependent on the behaviour of the parasitoids (i.e. host selection) and/or on the physiological compatibility between the introduced parasitoid strain and the local host population. In this study we compared the host selection behavior and physiological compatibility with different

Table 6.2 Development time (days), number of progeny produced, sex ratio (% males) for *C. flavipes* strains from Pakistan, Texas and Thailand on *C. partellus* on maize and *D. saccharalis* on sugarcane. Results of one way ANOVA are indicated ($P < 0.05$). Differences between means within a strain are indicated by different letters (t-Test for unequal variances, $P < 0.05$).

STRAIN / host species	n	Development time		Egg- Progeny produced		Sex ratio	
		Adult (days)	SD	per host	SD	(% males)	SD
PAKISTAN (NORTH)							
<i>Chilo partellus</i>	22	18.1a	0.5	35.6a	9.0	40.6a	34.7
<i>Diatraea saccharalis</i>	14	18.8a	1.8	14.3b	6.2	56.2a	42.8
TEXAS							
<i>Chilo partellus</i>	24	18.5a	1.1	39.0a	12.2	48.8a	39.5
<i>Diatraea saccharalis</i>	20	18.3a	0.7	26.7b	8.5	33.3a	29.2
THAILAND							
<i>Chilo partellus</i>	15	18.4a	0.8	31.1a	13.4	64.8a	37.6
<i>Diatraea saccharalis</i>	10	19.5a	2.2	18.4b	7.0	58.8a	29.7
PAKISTAN (SOUTH)							
<i>Chilo partellus</i>	12	18.5a	0.8	38.8a	7.4	45.6a	30.7
<i>Diatraea saccharalis</i>	7	18.4a	1.6	14.9b	7.8	44.2a	25.0
One way ANOVA for strain	DF	F	P	F	P	F	P
<i>Chilo partellus</i>	3	1.21	n.s	1.85	n.s.	1.37	n.s.
<i>Diatraea saccharalis</i>	3	1.54	n.s	9.14	0.0001	1.93	n.s.

stem-borers of six different geographic strains of *C. flavipes* that differed in the plant-host-complex they were obtained from.

Host selection

The existence of plant specific strains in *C. flavipes* has been postulated by previous researchers and is based on results of differential responses to various frass sources in contact experiments (Mohyuddin et al., 1981; Inayatullah, 1983) and in olfactometer experiments (Shami & Mohyuddin, 1992). Among-population variation in host selection may result from genetic differences between populations or from phenotypically plastic responses due to different development and emergence environments (i.e. early adult learning). Potting et al. (1995c) demonstrated that early adult learning does not play a significant role in plant and host species selection in *C. flavipes*, indicating that the proposed strain existence may be based on genetic differences.

However, we did not find any evidence for plant/host specific strains in our host selection and acceptance experiments. The populations that originated from *C. partellus* on maize (PAK-N and PAK-S) responded to all offered sugarcane frass sources in the olfactometer and (PAK-N) accepted the sugarcane borers (*C. auricilius*,

C. sacchariphagus, *Diatraea saccharalis*) as a host. The MAU, INDO, TEXAS and THAI populations, all originating from sugarcane stemborers, responded to maize frass and all accepted the maize stemborer *C. partellus*. In the dual choice tests in the olfactometer, where strains were tested at the same time in a sound comparative manner, no significant differences between the strains were found for the preference for maize or sugarcane frass (fig 6.3). The preference for maize or sugarcane (in our experimental setup) seems to be based on quantitative differences rather than qualitative differences between odors emanating from frass, as evidenced by the dose-response experiment (fig 6.4).

There was no significant difference in the acceptance ratio of the original host and the alternative host by the tested strains (fig 6.5). The only difference found was that TEXAS and THAI took a longer time before accepting *C. partellus* compared to their original host *D. saccharalis*, but there was no significant difference in the acceptance ratio. This low specificity in response towards odors from different frass sources and low specificity in host species acceptance was not due to loss of specificity of laboratory cultures (PAK-N, TEXAS, THAI) as the two tested field strains (INDO and MAU) also had a low specificity. The only significant difference found was that the two field strains were more reluctant to accept the hosts under the artificial laboratory conditions. Both strains took a longer time before they accepted the unconcealed host and in case of the INDO strain also at a significantly lower acceptance rate. It seems quite common that initiating a parasitoid culture with material from the field is difficult in the first generations (i.e. host acceptance) and alleviates after several generations, probably due to an (artificial) selection process for acceptance behavior in the laboratory.

To conclude, our results indicate that there is no strain specific variation in host selection behavior in *C. flavipes*. This contradicts the results of Mohyuddin et al. (1981) and Shami and Mohyuddin (1992). Reasons for this discrepancy may be due to differences in biological material, experimental setup and procedures. For instance, we used as sugarcane stemborer species *C. sacchariphagus*, *D. saccharalis* and *C. auricilius*, whereas Mohyuddin et al. (1981) used *Acigona steniellus* and Shami and Mohyuddin (1992) used *Chilo infuscatellus*. A second factor may be due to procedural differences. A differential preference among strains can only be demonstrated by testing the strains under similar conditions at the same time. For instance, our small dose-response experiment demonstrated that the preference for frass from maize or sugarcane source could be controlled by manipulating the quantity of frass (fig 6.4). In our experiments a maize strain was always tested at the same time against one or more sugarcane strains with the same odor sources to rule out any day or condition effects. It is not clear whether this was done by Mohyuddin et al. (1981) and Shami and Mohyuddin (1992).

Potting et al. (1995a) argue that, as a consequence of the high mortality rate at

each host attack inside the stem, *C. flavipes* is expected to encounter only a few hosts in its lifetime. They further argue that due to this low host encounter rate *C. flavipes* has a low specificity within its narrow foraging niche consisting of stem-boring larvae in gramineous plants. Besides the present study, this low specificity has been demonstrated several times in *C. flavipes*. For instance, Gifford and Man (1967) reported acceptance of seven unsuitable host species. The acceptance of a host and subsequent encapsulation of parasitoid progeny has been reported for the economically important stem-borers *Ostrinia nubilalis* (Chiang and Palmer, 1978), *Eldana saccharina* (Conlong unpubl.) and *Busseola fusca* (Ngi-Song et al., 1995a). When a parasitoid with a low specificity is introduced into a foreign region, new parasitoid-host associations can develop when related host species occur in the same niche as the aboriginal host species (Hokkanen & Pimentel, 1989). An example of this is the successful biological control of *Diatraea* spp. with *C. flavipes* in the new world (Alam et al., 1971; Fuchs et al., 1979).

Reproductive success

Our comparative experiments show variation in reproductive success among strains. For instance, the PAK-N strain developed faster and had a higher survival rate in both host species compared to INDO strain (table 6.1). This could indicate that the laboratory PAK-N strain is better adapted to the laboratory conditions than the INDO field strain. The only significant difference found in the comparison of the reproductive success of the INDO and PAK-N strains on *C. partellus* and *C. auricilius* was the lower percentage of males produced on the host species from which they originated (table 6.1). Whether this difference in sex ratio is based on a sex linked differential mortality needs further study. The differences in reproductive success among strains were more pronounced in the comparison of reproductive success on *C. partellus* and *D. saccharalis* (table 6.2, fig 6.7). In new host-parasitoid relations, selection will favor physiological adaptation of the parasitoid to its new host. The relative suitability of a certain host is likely to depend on the co-evolutionary history of the parasitoid with that particular host. For instance, the association between the old world parasitoid *C. flavipes* and the new world host *D. saccharalis* is novel. As expected, all strains had a lower survival on *D. saccharalis* compared to *C. partellus*, their ancestral host (table 6.2). Interestingly, the TEXAS strain had the highest survival rate on *D. saccharalis* compared to the other tested strains. The strain from Texas has the longest co-evolutionary history with the new host *D. saccharalis*, since it was introduced and established in 1977 in Texas, USA on *D. saccharalis* in sugarcane (Fuchs et al., 1979). Wiedenmann and Smith (1995) also found that the TEXAS strain had a higher reproductive success on *D. saccharalis* compared to the THAI strain.

Our results corroborate anecdotal reports of variation in virulence of *C. flavipes*

strains. Mohyuddin (1990) reported that in Pakistan *C. flavipes* established on *C. partellus* in maize, but did not successfully parasitize *C. infuscatellus* and *Acigona steniellus* in sugarcane. After release of sugarcane strains of *C. flavipes* from Indonesia, Thailand and Barbados, *C. flavipes* established successfully on *C. infuscatellus* and *A. steniellus*. However, it was not investigated whether this was due to a difference in virulence and/or a differential preference. Mohyuddin (1990) further reported that a local population of *C. flavipes* in Indonesia was found to parasitize *Chilo sacchariphagus* successfully, but was encapsulated by another sympatric host *Chilo auricilius*. After introduction of a *C. flavipes* sugarcane strain from Thailand, the field parasitization rate of *C. auricilius* increased from 0-15%. On Mauritius, *C. flavipes* is unable to develop on the sympatric *S. calamistis* (Rajabalee and Govendasamy, 1988), whereas a strain from Pakistan successfully attacks *S. calamistis* in Kenya (Ngi-Song et al., 1995a). Variation in physiological compatibility is also reported in a close relative of *C. flavipes*, the African stemborer parasitoid *Cotesia sesamiae* (Polaszek and Walker, 1992). Within its geographic range *C. sesamiae* encounters different host species complexes. For instance, in East and Southern Africa the stemborer *Busseola fusca* occurs primarily in areas 800 m above sea level. A population of *C. sesamiae* from the coastal area of Kenya is unable to develop in *B. fusca*, because their eggs are encapsulated (Ngi-song et al., 1995a), whereas *C. sesamiae* populations from higher altitudes in Kenya successfully attack the sympatric host *B. fusca* (Ngi-Song and Overholt, unpubl. data).

In comparing the virulence of parasitoid strains, it has to be stressed that a difference in physiological compatibility between strains may reflect a difference in virulence of the (tested) parasitoid populations, but may also reflect a difference in the ability of the host population in encapsulating the parasitoids progeny. In a detailed study of the relationship between the parasitoid *Asobara tabida* and its drosophilid hosts, Kraaijeveld and coworkers not only showed in controlled laboratory experiments that there was variation in virulence between parasitoid populations (Kraaijeveld and van der Wel, 1995), but that there was also variation in encapsulation ability between host populations (Kraaijeveld and van Alphen, 1995).

Although biological control programs offer an opportunity to evaluate the environmental impact on populations, few direct comparisons have been made of behavior and physiology of parasitoids before and after introduction on different host species. Le Masurier and Waage (1993) found that a recently introduced population of *Cotesia glomerata* in the USA had a higher attack rate on *Pieris rapae*, a host generally avoided in its aboriginal home Europe. It would be interesting to test under controlled conditions, whether the American *C. glomerata* population is also physiologically better adapted to *P. rapae* than its European congener, which

performs relatively poor on this host species (Geervliet and Brodeur 1992).

To conclude, our results indicate that there is no significant variation in host selection behavior among strains in *C. flavipes*, but we did find significant variation in virulence among strains. The reported anecdotal cases of increased parasitization rates of sugarcane stemborers after introduction of sugarcane strains (Mohyuddin, 1990) may thus not be due to a differential preference behavior, but to an increased physiological compatibility between the introduced parasitoid strain and the local host population.

ACKNOWLEDGMENTS

We would like to thank the following persons for their kind supply of *C. flavipes* strains and stemborer larvae: Dr. A. Rajabalee of the Mauritius Sugar Industry Research Institute, Mauritius; Dr. J.W. Smith Jr. and P. Darnell of the Department of entomology, Texas A&M University, USA; Dr. Widyatmoko of the Gunung Manung Plantations, Sumatra, Indonesia. A. Polaszek and J.K. Waage suggested names and sites for collection of strains. S. Kimani and A. Polaszek confirmed the identifications of *C. flavipes* strains. We thank Leo Koopman, Andre Giddink for maintaining insect cultures and Henk Snellen for assistance in the experimental work. Menno Schilthuisen, Antoon Loomans and Joop van Lenteren are acknowledged for valuable comments on a previous version of the manuscript. This work was supported by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO W84-325).

REFERENCES

- Alam, M.M., Bennett, F.D. and Carl, K.P. 1971. Biological control of *Diatraea saccharalis* (F.) in Barbados by *Apanteles flavipes* Cam. and *Lixophaga diatraeae* T.T. *Entomophaga* 16: 151-158.
- Alam, M.M., Beg, M.N., and Ghani, M.A. 1972. Introduction of *Apanteles* spp. against graminaceous borers into Pakistan. *Technical Bulletin CIBC* 15: 1-10.
- Betbeder-Matibet, M. and Malinge, P. 1968. Un succès de la lutte biologique: contrôle de *Proceras sacchariphagus* Boj. «Borer ponctué» de la canne à sucre à Madagascar par un parasite introduit: *Apanteles flavipes* Cam. *Agron. Tropicale* 22: 1196-1220.
- Bush, G.L. 1994. Sympatric speciation in animals: new wine in old bottles. *TREE* 9: 285-288.
- Chiang, H.C. and Palmer, D.F. 1978. Attempts to enrich the parasite fauna of the european corn borer in Minnesota. *J. Minnesota Academy of Science* 44: 15-17.
- Diehl, S.R. and Bush, G.L. 1984. An evolutionary and applied perspective of insect biotypes. *Annu. Rev. Entomol.* 29: 471-504.
- Fox, L.R. and Morrow, P.A. 1981. Specialization: species property or local phenomenon ? *Science* 211: 887-893.
- Fuchs, T.W., Huffman, F.R. and Smith, J.W. 1979. Introduction and establishment of *Apanteles flavipes* [Hym.: Braconidae] on *Diatraea saccharalis* [Lep.: Pyralidae] in Texas. *Entomophaga* 24: 109-114.
- Futuyma, D.J. and Peterson, S.C. 1985. Genetic variation in the use of resources by insects. *Annu. Rev. Entomol.* 30: 217-238.
- Geervliet, J.B.F. and Brodeur, J. 1992. Host species affecting the performance of larval parasitoids *Cotesia glomerata* and *Cotesia rubecula* (Hymenoptera: Braconidae). I. Comparative suitability of three Pieris species (Lepidoptera: Pieridae). *Med. Fac. Landbouw Univ. Gent* 57: 547-550.
- Gifford, J.R. and Mann, G.A. 1967. Biology, rearing, and a trial release of *Apanteles flavipes* in the

- Florida Everglades to control the sugarcane borer. *J. Econ. Entomol.* 60: 44-47.
- Greathead, D.J. 1986. Parasitoids in classical biological control. In: J.K. Waage and D.J. Greathead (eds) *Insect Parasitoids*, Academic Press, London, pp. 290-318.
- Hokkanen, H.M. and Pimentel, D. 1989. New associations in biological control: theory and practice. *Can. Entomol.* 121: 829-840.
- Hopper, K.R., Roush, R.T. and Powell, W. 1993. Management of genetics of biological control introductions. *Annu. Rev. Entomol.* 38: 27-51.
- Inayatullah, C. 1983. Host selection by *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). *J. Econ. Entomol.* 76: 1086-1087.
- Kenis, K. 1994. Variations in diapause among populations of *Eubazus semirugosus* (nass) (Hym.: Braconidae), a parasitoid of *Pissodes* spp. (Col.: Curculionidae). *Norw. J. Agr. Sci.* 16: 77-82.
- Kester, K.M. and Barbosa, P. 1991. Behavioral and ecological constraints imposed by plants on insect parasitoids: implications for biological control. *Biol. Control* 1: 94-106.
- Kester, K.M. and Barbosa, P. 1994. Behavioral responses to host foodplants of two populations of the insect parasitoid *Cotesia congregata* (Say). *Oecologia* 99: 151-157.
- Kim, K.C. and McPheron, B.A. 1993. Biology of variation: epilogue. In: *Evolution of insect pests, patterns of variation*. (Kim, K.C. & McPheron, B.A. Eds.). John Wiley, New York, pp. 453-468.
- Kraaijeveld, A.R. and van Alphen, J.A.M. 1995. Geographical variation in encapsulation ability of *Drosophila melanogaster* larvae and evidence for parasitoid specific components. *Evol. Ecol.* (in press).
- Kraaijeveld, A.R. and van der Wel, N.N. 1995. Geographic variation in reproductive success of the parasitoid *Asobara tabida* in larvae of several *Drosophila* species. *Ecol. Entomol.* (in press).
- Kraaijeveld, A.R., Nowee, B. and Najem, R.W., 1995. Adaptive variation in host selection behaviour of *Asobara tabida*, a parasitoid of *Drosophila* larvae. *Funct. Ecol.* 9: 113-118.
- Lewis, W.J., Vet, L.E.M., Tumlinson, J.H., Lenteren, J.C. van and Papaj, D.R. 1990. Variations in parasitoid foraging behavior: essential element of a sound biological control theory. *Environ. Entomol.* 19: 1183-1193.
- Macedo, N., Araújo, J.R. de, Bothelo, P.S.M. 1993. Sixteen years of biological control of *Diatraea saccharalis* (Fabr.) (Lepidoptera: Pyralidae) by *Cotesia flavipes* (Hymenoptera: Braconidae), in the state of Sao Paulo, Brazil. *Anais Soc. Entomol. Brasil* 22: 441-448.
- Martinez, A.J., Bard, J. and Holler, T. 1988. Mass rearing sugarcane borer and mexican rice borer for production of parasites *Allorhogas pyralophagus* and *Rhaconotus roslinensis*. *USDA-APHIS* 83-1.
- Masurier, A.D. le, Waage, J.K., 1993. A comparison of attack rates in a native and an introduced population of the parasitoid *Cotesia glomerata*. *Biocontrol Sc. Technol.* 3: 467-474.
- Mohyuddin, A.I. 1990. Utilization of natural enemies for the control of insect pests of sugarcane. *Insect Sci. Applic.* 12: 19-26.
- Mohyuddin, A.I., Inayatullah, C. and King, E.G. 1981. Host selection and strain occurrence in *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) and its bearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* 71: 575-581
- Ngi-Song, A.J., Overholt, W.A. and Ayerty, J.N. 1995a. Suitability of African graminaceous stemborers for development of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). *Environ. Entomol.* 24: 978-984.
- Ngi-Song, A.J., Overholt, W.A., Njagi, P.G.N., Dicke, M., Ayerty, J.N. and Lwande, W. 1995b. Volatile infochemicals in host and host habitat location by *Cotesia flavipes* and *C. sesamiae* (Cameron) (Hymenoptera: Braconidae), two larval parasitoids of graminaceous stemborers. *J. Chem Ecol.* (in press).
- Ochieng, R.S., Onyango, F.O. & Bungu, M.D.O., 1985. Improvement of techniques for mass-culture of *Chilo partellus* (Swinhoe). *Insect Sci. Appl.* 6: 425-428.
- Omwega, C.O., Kimani, S.W., Overholt, W.A. and Ogot, C.K.P.O. 1995. Evidence of the

- establishment of *Cotesia flavipes* (Hymenoptera: Braconidae) in continental Africa. *Bull. Entomol. Res.* (in press).
- Overholt, W.A., Ngi-Song, A.J., Kimani, S.K., Mbapila, J., Lammers, P. and Kioko, E. 1994. Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), in Africa. *Biocontrol News Inf.* 15: 19-24.
- Polaszek, A and Walker, A.K. 1991. The *Cotesia flavipes* species complex: parasitoids of cereal stemborers in the tropics. *REDIA* 74: 335-341.
- Potting, R.P.J., Overholt, W.A., Danso, F.O. and Takasu, K., 1995a. Foraging behavior and life history of the stemborer parasitoid *Cotesia flavipes*. *J. Insect Behav.* (submitted).
- Potting, R.P.J., Vet, L.E.M., Dicke, M., 1995b. Host microhabitat location by stemborer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systemically induced plant volatiles. *J. Chem. Ecol.* 21: 525-539.
- Potting, R.P.J., Vet, L.E.M., Otten, H. 1995c. The relation between parasitoid ecology and learning: absence of learning in the stemborer parasitoid *Cotesia flavipes*. *Anim. Behav.* (submitted).
- Rajabalee, M.A. and Govendasamy, M. 1988. Host specificity and efficacy of *Apanteles flavipes* (Cam.) and *A. sesamiae* (Cam.) (Hymenoptera: Braconidae) parasites of sugarcane moth borers in Mauritius. *Revue Agricole et Sucriere* 67: 78-80.
- Roush, R.T. 1990. Genetic variation in natural enemies: critical issues for colonization in biological control. In: M. Mackauer, L.E. Ehler and J. Roland (eds), *Critical issues in biological control*, pp. 263-288, Intercept, Andover.
- Shami, S. and Mohyuddin, A.I. 1992. Studies on host plant preference of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) an important parasitoid of graminaceous stalk borers. *Pakistan J. Zool.* 24: 313-316.
- Steinberg, S., Dicke, M., Vet, L.E.M. and Wanningen, R. 1992. Response of the braconid *Cotesia* (= *Apanteles*) *glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomol. exp. appl.* 63: 163-175.
- Unruh, T.R. and Messing, R.H. 1993. Intraspecific biodiversity in Hymenoptera: implications for conservation and biological control. In: J. LaSalle and I.D. Gauld (eds) *Hymenoptera and biodiversity*, CAB International, Wallingford, pp. 27-52.
- Vet, L.E.M. and Janse, C.J. 1984. Fitness of two sibling species of *Asobara* (Braconidae: Alysiinae), larval parasitoids of Drosophilidae in different microhabitats. *Ecol. Entomol.* 9: 345-354.
- Wiedenmann, R.N. and Smith, J.W. Jr. 1995. Parasitization of *Diatraea saccharalis* by *Cotesia chilonis* and *Cotesia flavipes*. *Environ. Entomol.* 24: 950-961.
- Wiedenmann, R.N.; Smith, J.W. Jr. & P. O. Darnell, 1992. Laboratory rearing and biology of the parasite *Cotesia flavipes* (Hymenoptera: Braconidae) using *Diatraea saccharalis* (Lepidoptera: Pyralidae) as a host. *Environ. Entomol.* 21: 1161-1167.

Summarizing Discussion

Classical biological control involves the introduction of an exotic natural enemy to control an introduced pest species. In 1991 the department of Entomology of the Wageningen Agricultural University started a collaborative project with the International Centre for Insect Physiology and Ecology (ICIPE) in Nairobi Kenya, on the biological control of stemborers in Africa. The gregarious endoparasitoid *Cotesia flavipes* (Hymenoptera: Braconidae) was chosen as the natural enemy to be introduced against the accidentally introduced pest *Chilo partellus* (Lepidoptera: Pyralidae) in East-Africa (Overholt et al., 1994; Omwega et al., 1996). The research described in this thesis was related to this project and addressed several aspects of the behavioral ecology of this parasitoid. In the first part, gaps in the knowledge on behavioral ecology of *C. flavipes* are studied which include the long- and short-range searching behavior, some aspects of the life history and host discrimination abilities. The second part focuses on the intraspecific variability in *C. flavipes* behavior and here we determine to what extent the reported plant and host specificity in *C. flavipes* has a genetic basis or is due to phenotypic plasticity through learning.

Micro-habitat and host location in Cotesia flavipes

The first part addresses the origin of the olfactory stimuli involved in host-microhabitat location (chapter 2) and the contact stimuli involved in tunnel and host location on a stemborer infested plant (chapter 3). In chapter 2 it is demonstrated that a major source of the attractive volatiles from the plant-host-complex is the stemborer-injured stem, including the frass produced by the feeding larvae. Moreover, the production of volatiles attractive to a parasitoid is not restricted to the infested stem-part but occurs systemically throughout the plant. The uninfested leaves of a stemborer-infested plant emit volatiles that attract female *C. flavipes*. An exogenous elicitor of this systemic plant response is situated in the regurgitate of a stemborer larva. When a minor amount of regurgitate is inoculated into the stem of an uninfested plant, the leaves of the treated plant emit volatiles which attract female *C. flavipes*. Evidence is accumulating that plants are actively involved in attracting natural enemies (Dicke, 1994). However, whether plants have specifically evolved the ability to release volatiles that attract natural

enemies of the herbivore that is attacking them remains a matter of debate (Bruin et al. 1995).

Foraging behavior on stemborer infested plant

Once a female *C. flavipes* has located a stemborer infested plant, it has to locate the concealed host inside the plant stem. In chapter 3 the behavior of female *C. flavipes* on stemborer infested plants was investigated. It is demonstrated that larval frass, caterpillar regurgitate and holes in the stem are used in host location by *C. flavipes*. After locating the exit hole of the stemborer tunnel, where larval frass has accumulated, the parasitoid female tries to enter the stemborer tunnel. This can take a long time because the tunnel is often blocked by larval frass and the female sometimes has to squeeze through small holes. The response to host products by *C. flavipes* seems not to be host species specific. Female *C. flavipes* respond to frass from four different stemborer species and one leaf feeder. No differences are found in the behavior of *C. flavipes* on maize plants infested with the suitable host, *Chilo partellus* (Lepidoptera: Pyralidae), or the unsuitable host, *Busseola fusca* (Lepidoptera: Noctuidae). Attacking a concealed stemborer larvae in the confined space of stemborer tunnel is not only time consuming but also risky. It is demonstrated that 30-40% of the parasitoids is killed by the spitting and biting stemborer larva. Takasu and Overholt (1996) showed that a female parasitoid has a high probability (0.9) to be bitten to death when it approaches the host towards the head. However, the majority of the females were first able to successfully parasitize its offensive host before being killed. A female *C. flavipes* needs only a few seconds to inject around 45 eggs into its host. The high probability of mortality at each host encounter results in a very low expectation of the number of lifetime host encounters. The possible consequences of this low lifetime host encounter rate for the evolution of life history- and foraging strategies formed the basis of a large part of this thesis.

Life history of Cotesia flavipes

C. flavipes is relatively short lived: without food the parasitoids die within two days, with food and under high humidity conditions they die within 5-6 days. In chapter 3 the fecundity and clutch size allocation of *C. flavipes* was investigated. It is demonstrated that *C. flavipes* is pro-ovigenic and has around 150 eggs available for oviposition. In the first encountered hosts 35-45 eggs per host are laid. Thus, a relatively large proportion of the available egg load (20-25%) is allocated to each host, and a female *C. flavipes* is equipped with an eggload to parasitize 3-4 hosts only. Especially for animals whose lifetime reproductive success is limited by opportunities to reproduce, clutch size theory predicts a maximization of the fitness gain per clutch (Godfray, 1987). This may be true for

C. flavipes, which has a short lifespan and a high mortality risk at each host encounter, resulting in a low number of expected lifetime host encounters (Chapter 3). In chapter 4 it is demonstrated that the number of produced adults from superparasitized hosts is equal to that of singly parasitized hosts. This indicates that female *C. flavipes* indeed lay an optimal clutch size in fourth instar *C. partellus* larvae.

Host- and host-site discrimination

The fitness consequences of superparasitism and the mechanism of host discrimination in *Cotesia flavipes* are described in chapter 4. Naïve females readily superparasitized and treated the already parasitized host as an unparasitized host by allocating the same amount of eggs as in an unparasitized host. However, there was no significant increase in the number of emerging parasitoids from superparasitized hosts due to substantial mortality of parasitoid offspring in superparasitized hosts. Furthermore, the developmental time of the parasitoids in a superparasitized host was significantly longer than in a singly parasitized host and the emerging progeny were significantly smaller (body length and head width). Naïve females entered a tunnel in which the host was parasitized 4 hours previously and accepted it for oviposition. Experienced females (oviposition experience in unparasitized host) refused to enter a tunnel with a host parasitized by herself or by another female. In experiments where the tunnel and/or host was manipulated it was demonstrated that the female leaves a mark in the tunnel when she has parasitized a host. The function of the avoidance of superparasitism in *C. flavipes* is clear: a discriminating female saves searching time, avoids the wastage of eggs and avoids a direct mortality risk. The mechanism of host discrimination is the recognition of a chemical mark on the tunnel substrate.

The role of learning in host foraging

Many studies have shown that parasitoids can learn visual or olfactory stimuli associated with successful host location and use these odours in subsequent foraging decisions (reviewed by Turlings et al., 1993; Vet et al., 1995). The ability to learn has now been demonstrated in more than 20 different parasitoid species and learning in parasitoids seems to be the rule rather than the exception (Turlings et al., 1993). In chapter 5 the role of learning in host foraging in *C. flavipes* was investigated. Using experimental procedures similar to other parasitoid learning studies, the role of the learning mechanisms priming (i.e. increase in response) and preference-induction in the foraging of *C. flavipes* was determined. No evidence was found that *C. flavipes* uses odour learning in host-microhabitat location. There was no significant effect of the development and emergence environment on the response level or preference towards infested

plant odours. Neither was any evidence found that experience with a particular plant-host-complex during foraging influences subsequent foraging decisions in *C. flavipes* females.

Recent discussions of animal learning emphasize the importance of considering an animals ecology when studying and interpreting its learning abilities. Recently, it has been hypothesized that the adaptive value of learning in foraging is dependent on the predictability of the environment (Stephens, 1993) and the number of lifetime foraging decisions (Roitberg et al., 1993). Learning is not expected when the foraging environment is highly predictable (i.e. the resource is constant) and when animals make only a few decisions while foraging. Taking the ecology of *C. flavipes* into account it is hypothesized that two factors may be responsible for the lack of learning in foraging in *C. flavipes*: a predictable foraging environment and the restricted number of lifetime foraging decisions.

EVOLUTION OF LIFE HISTORY AND FORAGING STRATEGIES

Evolutionary ecological theory concentrates on the interpretation of form and function of individuals as adaptations to their environment. Theories of life history evolution predict what sorts of life history should evolve in specified ecological circumstances (e.g. Stearns, 1992; Roff, 1992) and optimal foraging theory addresses the problem of choice among resources or habitats (e.g. Krebs and Davies, 1981; Stephens and Krebs, 1986). It is tempting to relate the ecology of *C. flavipes* with its life history characteristics and its foraging tactics. The stemborer parasitoid *C. flavipes* has a peculiar ecology. It not only forages for hosts in a relatively homogeneous and predictable habitat, but it also has a risky attack tactic resulting in a low number of expected lifetime host encounters.

The small *C. flavipes* attacks stemborer larvae by entering the stemborer tunnel (chapter 3). To reach the host, the parasitoid female has to squeeze through small holes in the tunnel which is filled with larval frass. It has been suggested that the dorso-ventral body shape, which is typical of the *Cotesia* species belonging to the *Cotesia flavipes* complex is an adaptation to this ingress behavior (Kimani, pers. comm.). Attacking a host in the confined space of a stemborer tunnel is not without risk for the female parasitoid. At each host attack the female has a considerable risk to be killed by its aggressive host (chapter 3). The short oviposition time (around 40 eggs in 3-4 seconds) may be an adaptation to this mortality risk. The majority of the females that are killed have already successfully parasitized their host.

The relatively high mortality risk at each host encounter in combination with the short lifespan results in a very low number of expected lifetime host encounters. This is reflected in the eggload of a female at emergence, which is just

enough to parasitize 3-4 hosts (chapter 3). When the probability of surviving to find another host is small, optimal progeny allocation models predicts an optimal 'Lack' clutch size, where fitness is maximized per host (Waage & Godfray, 1985; Godfray 1987). Although it was not tested in depth the results of the superparasitism experiments (chapter 4) indicated that *C. flavipes* lays an optimal clutch size.

The foraging environment of a female *C. flavipes* can be envisaged as a homogeneous and stable habitat, consisting of a field of perennial grasses with a few prevalent stemborer species. In chapter 5 it is hypothesized that this predictable foraging environment together with the low number of expected lifetime host encounters plays a part in the absence of (odor) learning in *C. flavipes* host foraging. In chapter 4 it is demonstrated that female *C. flavipes* leave an external mark on the tunnel substrate after parasitization. It is generally hypothesized that marking evolved as a means for individuals to avoid superparasitizing hosts they themselves previously parasitized (Roitberg and Prokopy, 1987). A female *C. flavipes* saves time and avoids a superfluous mortality risk by avoiding utilized host tunnels.

When a parasitoid has a high mortality risk at each oviposition, life history theory predicts a high selectivity to avoid waste of progeny (Iwasa et al., 1984; Ward, 1992). The parasitoid should not risk her life for low quality hosts, such as unsuitable hosts or already parasitized hosts. However, naive female *C. flavipes* (no oviposition experience) seem to have a very opportunistic host selection behavior. In chapter 3 it is demonstrated that *C. flavipes* did attack the (new) unsuitable host *B. fusca* and in chapter 5 it is found that naive females did utilize a previously parasitized host. The lack of an innate ability or willingness to avoid low quality hosts in *C. flavipes* may be due to the constrained opportunities to find and parasitize hosts. Each animal faces a evolutionary trade off between reproducing now or in the future, whereby survival chances play a determining role. The best strategy for a recently emerged naive female *C. flavipes* is to accept the first encountered host, irrespective its quality. Superparasitism pays when future expectancy of host encounter rate is very low. The lower fitness increment of superparasitism (in comparison with single parasitism) will always outweigh the fitness penalty of not finding any unparasitized host.

In chapter 5 it is demonstrated, however, that females with oviposition experience *do* avoid previously utilized stemborer tunnels. The increased choosiness after an oviposition experience in an unparasitized host may be due to the fact that, the parasitoids assessment of host availability has changed. When there is a high chance of finding unparasitized hosts it does pay to reject. Furthermore, in contrast to naive females, oviposition experienced females that superparasitize run the risk to encounter a host they themselves previously parasitized (Van Alphen

and Visser, 1990). A safe strategy of females that have already parasitized one or more hosts may be to avoid any already parasitized host to avoid competition among her own progeny.

VARIATION IN PARASITOID BEHAVIOR AND BIOLOGICAL CONTROL

In biological control the performance of a released parasitoid population in the field is dependent on the ability of individual females to locate hosts. The behaviour of parasitoids is not fixed and predictable, but most of the times highly variable. This variation in behaviour can be an obstacle in the effective use of natural enemies in biological control, so it is necessary to understand the sources of variation in behavior (Vet et al., 1990; Lewis et al., 1990). In this way we can predict the general behavior of the natural enemy population in the field better and we may even be able to manipulate it.

Behavioral variation may exist because individuals differ genetically in propensity to find or accept different hosts. Secondly, individuals may differ because they have experienced different environments (i.e. learning).

Local variation in parasitoid behavior and physiology

The existence of plant specific strains in *C. flavipes* has been postulated by Mohyuddin and coworkers (e.g. Mohyuddin et al., 1981; Mohyuddin, 1990). However, the genetic and/or phenotypic basis for this reported specificity has never been addressed thoroughly. For instance, early adult conditioning can mimic genetic differences between parasitoid strains. However, in chapter 5 we demonstrated that there is no early adult conditioning for the development and emergence environment in *C. flavipes*, indicating innate (genetic) differences among strains. Therefore, the between population variation in behavior and physiology of *C. flavipes* populations was investigated in more detail in chapter 6. The host selection behavior and physiological compatibility with different stemborers (i.e. parasitoid virulence) was compared for six different geographic strains of *C. flavipes* that differed in the plant-host-complex they were obtained from. The results of these host selection experiments indicated that there is no interspecific variation in host selection behavior among *C. flavipes* strains, which contradicts the finding of the Mohyuddin research group. However, the comparative experiments did show variation in reproductive success among strains. The most significant result was that the strain with the longest co-existence time with the new host *D. saccharalis*, had the highest reproductive success on this host species. It is argued that the earlier reported existence of *C. flavipes* strains is not based on a differential host selection behavior, but on differences in physiological compatibility between local parasitoid and host population. *C. flavipes* has been

used on a worldwide scale in biological control against stemborers with varying degree of success (Polaszek and Walker, 1991). The failure of biological control with *C. flavipes* may be due to the introduction of an inappropriate strain. The present study has demonstrated that there is no differential plant preference among strains, but that there are differences in parasitoid virulence among strains. For a reliable biological control with *C. flavipes* it is thus important to select a strain that is physiologically adapted to the target host population.

REFERENCES

- Bruin, J., Sabelis, M.W. and Dicke, M. 1995. Do plants tap SOS signals from their infested neighbours? *TREE* 10: 167-170.
- Dicke, M. 1994. Local and systemic production of volatile herbivore-induced terpenoids: their role in plant-carnivore mutualism. *J. Plant Physiol.* 143: 465-472.
- Godfray, H.C.J. 1987. The evolution of clutch size in parasitic wasps. *Am. Nat.* 129: 221-233.
- Gould, S.J. and Lewontin, R.C. 1979. The spandrels of San Marcos and the Panglossian paradigm: a critique of the adaptationist programme. *Proc. R. Soc. Lond. B.* 205: 581-598.
- Iwasa, Y., Suzuki, Y. and Matsuda, H. 1984. Theory of oviposition strategy of parasitoids. I. Effect of mortality and limited egg number. *Theor. Pop. Biol.* 26: 205-227.
- Krebs, J.R. and Davies, N.B. 1981. *An introduction to behavioural ecology*. Blackwell Scientific Oxford.
- Lewis, W.J., Vet, L.E.M., Tumlinson, J.H., van Lenteren, J.C. & Papaj, D.R. 1990. Variations in parasitoid foraging behavior: essential element of a sound biological control theory. *Environ. Entomol.* 19: 1183-1193.
- Mohyuddin A.I. 1990. Utilization of natural enemies for the control of insect pests of sugarcane. *Insect Sci. Applic.* 12: 19-26.
- Mohyuddin, A.I., Inayatullah, C. and King, E.G. 1981. Host selection and strain occurrence in *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) and its bearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* 71: 575-581.
- Omwega, C.O., Kimani, S.W., Overholt, W.A. and Ogol, C.K.P.O. 1996. Evidence of the establishment of *Cotesia flavipes* (Hymenoptera: Braconidae) in continental Africa. *Bull. Entomol. Res.* (in press).
- Overholt, W.A., Ngi-Song, A.J., Kimani, S.K., Mbapila, J., Lammers, P. and Kioko, E. 1994. Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), in Africa. *Biocontrol news and Inf.* 15: 19-24.
- Roff, D.A. 1992. *The evolution of life histories; theories and analysis*. Chapman and Hall, London.
- Roitberg, B.D. and Prokopy, R.J. 1987. Insects that mark host plants. An ecological, evolutionary perspective on host-marking chemicals. *BioScience* 37: 400-406.
- Roitberg, B.D., Reid, M.L. & Li, C. 1993. Choosing hosts and mates: the value of learning. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis), pp. 174-194. New York: Chapman and Hall.

- Stearns, S.C. 1992. *The evolution of life histories*. Oxford University Press.
- Stephens, D.W. 1993. Learning and behavioral ecology: incomplete information and environmental predictability. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis), pp. 195-218. New York: Chapman and Hall.
- Stephens, D.W. and Krebs, J.R. 1986. *Foraging theory*. Princetown University Press, Princeton.
- Takasu, K. and Overholt, W.A. 1995. Defense behavior of *Chilo partellus* against parasitization by *Cotesia flavipes*. (in prep.).
- Turlings, T.C.J., Wäckers, F., Vet, L.E.M., Lewis, W.J. & Tumlinson, J.H. 1993. Learning of host-finding cues by hymenopterous parasitoids. In: *Insect learning: ecological and evolutionary perspectives* (Ed. by D.R. Papaj & A.C. Lewis), pp. 51-78. New York: Chapman and Hall.
- Van Alphen, J.A. and Visser, M.E. 1990. Superparasitism as an adaptive strategy for insect parasitoids. *Annu. Rev. Entomol.* 35: 59-79.
- Vet, L.E.M., Lewis, W.J., Papaj, D.R., Lenteren, J.C. van. 1990. A variable-response model for parasitoid foraging behavior. *J. Insect Behav.* 3: 471-490.
- Vet, L.E.M., Lewis, W.J. and Carde, R.T. 1995. Parasitoid foraging and learning. In: *Chemical ecology of insects* (eds W.J. Bell and R.T. Carde), Chapman Hall, New York, pp. 65-101.
- Waage, J.K. and Godfray, H.C.J. 1985. Reproductive strategies and population ecology of insect parasitoids. In: R.M. Sibly and R.H. Smith (eds.) *Behavioural ecology*, pp. 449-470. Blackwell Scientific, Oxford.
- Ward, S.A. 1992. Environmental uncertainty and polyphagy in herbivorous insects *Oikos* 63: 506-512.
- Wardle, A.R. 1990. Learning of host microhabitat colour by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *Anim. Behav.* 39: 914-923.
- Wardle, A.R. and Borden, J.H. 1989. Learning of an olfactory stimulus associated with a host microhabitat by *Exeristes roborator*. *Entomol. exp. appl.* 52: 271-279.
- Wardle, A.R. and Borden, J.H. 1990. Learning of host microhabitat form by *Exeristes roborator* (F.) (Hymenoptera: Ichneumonidae). *J. Insect Behav.* 3: 251-263.

Samenvatting

Het foerageergedrag van parasitaire wespen is een interessant onderwerp voor gedragsoecologisch onderzoek. Ten eerste vanuit een evolutionair oecologisch gezichtspunt. Bij sluipwespen bestaat er een directe correlatie tussen foerageren en reproducieren. Het reproductieve succes van een sluipwesp is afhankelijk van het aantal en de kwaliteit van de gastheren, die ze in haar leven kan vinden en parasiteren. De natuurlijke selectiedruk op een zo efficiënt mogelijk foerageerproces is dus groot. Daardoor zijn sluipwespen dankbare onderzoeksobjecten om foerageertheorieën te testen, die onderzoeken of individuen zich als optimale besluitvormers gedragen bij de beslissingen die ze nemen tijdens het zoeken naar geschikte prooien of voedselbronnen.

Een tweede aspect, dat het onderzoek naar het foerageergedrag van sluipwespen aantrekkelijk maakt is dat parasitaire wespen steeds vaker ingezet worden bij de biologische bestrijding van insektenplagen. Om een betrouwbare en voorspelbare biologische bestrijding met sluipwespen te waarborgen is het van belang om een goed inzicht te verkrijgen in het foerageergedrag van de ingezette natuurlijke vijanden, bijvoorbeeld in de factoren die een rol spelen bij de voorkeur die een sluipwesp kan hebben voor een bepaalde gastheer op een bepaald gewas (plant-gastheer-complex).

Het onderzoek gepresenteerd in dit proefschrift richtte zich op de gedragsoecologie van *Cotesia flavipes* (Hymenoptera: Braconidae), een sluipwesp die wereldwijd meerdere malen is ingezet bij de biologische bestrijding van stengelboorders. Stengelboorders zijn rupsen (Lepidoptera: Pyralidae, Noctuidae), die zich in de door henzelf gemaakte tunnels voeden van het stengelweefsel van de plant. Door hun verborgen levenswijze, zijn stengelboorders moeilijk met conventionele (chemische) methodes te bestrijden en zijn ze beruchte plaaginsekten in bijvoorbeeld suikerriet (bijv. *Diatraea saccharalis*), maïs (bijv. *Ostrinia nubilalis*) en rijst (bijv. *Chilo suppressalis*). Een van de economisch gezien belangrijkste stengelboorders op het Afrikaanse continent is de maïs-stengelboorder *Chilo partellus*. In de zestiger jaren is *C. partellus* vermoedelijk vanuit zuidoost Azië per ongeluk geïntroduceerd op het Afrikaanse continent en breidt zich nog continu uit, waarschijnlijk door het ontbreken van een aangepaste natuurlijke vijand gemeenschap. In samenwerking met de vakgroep Entomologie van de Landbouw Universiteit Wageningen onderzoekt het International Centre For Insect Physiology and

Ecology (ICIPE) in Kenya, sinds 1992 de mogelijkheid om *C. partellus* biologisch te bestrijden met parasitaire wespen uit het oorsprongsgebied van *C. partellus*. Als hoofdkandidaat voor een introductie in Kenya werd *C. flavipes* gekozen, die al in andere delen van de wereld met succes is geïntroduceerd tegen verschillende soorten stengelboorders in suikerriet en mais. Het hier gepresenteerde onderzoek was gerelateerd aan bovenstaand project en richtte zich op enkele evolutionaire en toegepaste aspecten van de gedragsoecologie van *C. flavipes*.

In het eerste deel van dit proefschrift wordt de lokalisatie van stengelboorder aangetaste planten en het verdere foerageerproces op deze planten behandeld. Het tweede deel van het proefschrift richt zich op de in de literatuur gerapporteerde (intraspecifieke) variabiliteit van plant- en gastheerkeuze van *C. flavipes*. Hier wordt nagegaan in hoeverre deze variabiliteit fenotypisch bepaald is door leerprocessen. Daarnaast wordt door middel van een vergelijking van verschillende *C. flavipes* stammen bepaald in hoeverre deze variabiliteit een genetische basis heeft.

Lokalisatie van geïnfecteerde planten en gastheren

Met behulp van een Y-buis geurmeter wordt in **hoofdstuk 2** aangetoond dat *C. flavipes* de aanwezigheid van een gastheer in een maïsplant kan waarnemen door middel van vluchtige stoffen, die vrijgegeven worden door een aangetaste plant. Ondanks haar verborgen levenswijze kan de stengelboorderlarve niet verhinderen dat de plant vluchtige stoffen afgeeft die natuurlijke vijanden van de stengelboorder aantrekt. Deze voor *C. flavipes* attractieve stoffen worden niet alleen vrijgegeven op de plaats van aantasting (de stengel met daarin de stengelboorderlarven), maar door de hele plant heen. Ook de onbeschadigde bladeren van dezelfde plant geven attractieve stoffen af. Een stof die deze systemische plantrespons oproept bevindt zich in het speeksel van de stengelboorderlarve. Door het toedienen van enkele druppeltjes rupsenspuug in de stengel van een onaangetaste maïsplant kon dezelfde systemische reactie van de plant geïnduceerd worden en werden de bladeren van de behandelde plant attractief voor *C. flavipes*.

Het gedrag van *C. flavipes* op stengelboorder-geïnfecteerde planten wordt onderzocht in **hoofdstuk 3**. Na landing op een geïnfecteerde plant vindt de vrouwelijke sluipwesp de ingang van de stengelboorder tunnel vrij snel, waarschijnlijk door stimuli van de geaccumuleerde uitwerpselen bij de ingang/uitgang van de tunnel. Vervolgens kruipt ze naar binnen op zoek naar de stengelboorderlarve. Het gedrag in de tunnel werd geobserveerd met behulp van kunstmatige transparante tunnels. Zodra de wesp contact maakt met de larve steekt ze haar legboor in de larve en parasiteert die binnen enkele

seconden. De larve zelf reageert zeer agressief en begint onmiddellijk heftig te bewegen, te spugen en te bijten. Dit defensieve gedrag van de veel grotere gastheer maakt het parasiteren tot een risicovolle onderneming voor de sluipwesp: 30-40% van de sluipwespen wordt dood gebeten of dood gedrukt tegen de tunnelwand. Echter, het merendeel van de gedode sluipwespen is toch nog in staat geweest om de larve te parasiteren. Vervolg onderzoek door Takasu en Overholt heeft aangetoond dat 90% van de gedode wespen haar gastheer succesvol heeft geparasiteerd.

Voortplantingsstrategie Cotesia flavipes

In hoofdstuk 3 werd de fecunditeit en de verdeling van de legselgrootte onderzocht. Door middel van het uitprepareren van de ovaria van pas uitgekomen wespen en door het bepalen van de legselgrootte in opeenvolgende gastheren kon vastgesteld worden dat *C. flavipes* pro-ovigeen is. Dat wil zeggen, dat de eivoorraad van de sluipwesp bij uitkomst van de cocon compleet is (150 eieren) en er geen eieren tijdens het verdere leven afgerijpt worden. De legselgrootte per gastheer is gemiddeld 45 eieren. Na 5-6 parasiteringen heeft de sluipwesp geen eieren meer beschikbaar voor ovipositie. In hoofdstuk 3 wordt bediscussieerd dat de lage levensverwachting van het aantal te parasiteren gastheren als gevolg van de hoge mortaliteitskans per parasitering weerspiegeld wordt in een relatieve kleine eivoorraad en een hoge investering per geparasiteerde gastheer.

Herkenning van eerder bezochte locaties

In het voorgaande hoofdstuk werd aangetoond dat het parasiteren van een agressieve gastheer in een tunnel een risicovolle onderneming is. Men verwacht, dat de sluipwesp niet haar leven riskeert voor gastheren, die een lage reproductieve waarde hebben, zoals gastheren die al eerder geparasiteerd zijn. De risicospreiding zal het grootste zijn indien de wesp al geparasiteerde gastheren kan herkennen voordat ze contact maakt met de defensieve gastheer. In hoofdstuk 4 wordt de fitness consequentie van superparasitisme onderzocht en het mechanisme dat gebruikt wordt, om eerder geparasiteerde gastheren en eerder bezochte tunnels te herkennen. Naïeve vrouwtjes accepteerden een al eerder geparasiteerde gastheer en verdubbelden het aantal eieren in de al geparasiteerde gastheer. Dit resulteerde echter niet in een verhoging van het aantal uitgekomen wespen per gastheer, door de hoge mortaliteit van juvenielen in de gastheer. Bovendien was de ontwikkelingsduur significant langer en waren de uitgekomen wespen significant kleiner in dubbel geparasiteerde gastheren. In tegenstelling tot naïeve vrouwtjes, mijden vrouwtjes met een eileg-ervaring een tunnel die al eerder bezocht is door

henzelf of een ander vrouwtje. Het kon worden aangetoond dat sluipwespen dit onderscheid kunnen maken, door herkenning van een door de voorgangster achtergelaten chemische markering op het tunnelsubstraat.

De rol van leerprocessen in het zoekgedrag

Het is algemeen bekend dat sluipwespen beïnvloed worden door ervaringen die ze opdoen bij het kruipen uit hun cocon en vooral bij het vinden van een gastheer. Na het succesvol parasiteren van een gastheer hebben individuen vaak een verhoogde motivatie om op gastheer-gerelateerde stimuli te reageren, een verschijnsel dat sensitisatie genoemd wordt. Daarnaast kunnen sluipwespen vorm, kleur en vooral geur leren associëren met het vinden van een bepaalde gastheer of plantensoort, resulterend in een preferentie voor de ervaren stimulus. Vooral olfactorisch leren is goed onderzocht en beschreven voor meer dan 20 soorten sluipwespen.

In hoofdstuk 5 werd onderzocht in hoeverre leerprocessen een rol spelen bij het zoekgedrag van *C. flavipes*. De rol van sensitisatie en preferentieinductie werd onderzocht in een Y-buis opstelling, waarin twee verschillende geurvelden werden aangeboden aan individuen met een verschillende voorervaring. De test groepen verschilden in de gastheersoort waarin zij waren opgegroeid en/of de voedselplant waarop hun gastheer zich had gevoed (vroeg-adulte ervaring) of hadden een verschillende eileg-ervaring met een bepaald plant-gastheer-complex (adulte ervaring). Er werd geen enkele aanwijzing gevonden, dat leerprocessen een rol spelen bij het gastheerzoekgedrag van *C. flavipes*. Er was geen significant effect van de ontwikkelings- en uitkomst omgeving op het respons niveau (percentage dat door de geur wordt aangetrokken) en op de eventuele voorkeur voor een bepaald plant-gastheer-complex. Ook werd er geen sensitisatie of preferentie-inductie gevonden na een eileg-ervaring met een bepaald plant-gastheer-complex. Dit is een van de eerste studies waarbij het leren van geuren bij een hymenoptere parasitoid niet kon worden aangetoond, ondanks dat het grondig is onderzocht. In hoofdstuk 5 wordt beargumenteerd dat de adaptieve waarde van leerprocessen in het foerageergedrag van *C. flavipes* klein is. Een van de redenen is dat de levensverwachting van het aantal gastheerontmoetingen door de hoge mortaliteitskansen bij elke parasitering klein is. Hierdoor is de adaptieve waarde van de informatie, die de sluipwesp bij elke parasitering eventueel kan onthouden en gebruiken bij verdere foerageerbeslissingen (=leren) gering.

Lokale variatie in gedrag en fysiologie van populaties

Insektenpopulaties uit verschillende regionen of van verschillende habitats kunnen specifiek aangepast zijn aan hun ecologische omgeving. Indien er

duidelijke morfologische of biologische verschillen bestaan tussen populaties, spreekt men van biotypes of stammen. In de literatuur is verschillende malen het bestaan van plant-aangepaste stammen van *C. flavipes* geopperd. In hoofdstuk 6 is dit nader onderzocht, door het gedrag en de fysiologie van zes geografische populaties van *C. flavipes* te vergelijken. De zes stammen, uit Pakistan, Mauritius, Indonesië en de Verenigde Staten verschilden in de plantensoort (suikerriet of maïs) en/of de gastheersoort (*Chilo partellus*, *Chilo sacchariphagus* of *Diatraea saccharalis*) waarvan ze verzameld waren. In olfactometer experimenten werd onderzocht of een stam een voorkeur heeft voor de plantensoort van origine (suikerriet of maïs). Verder werd onderzocht of de stammen verschilden in de acceptatie van verschillende gastheersoorten en werd het reproductieve succes (aantal geproduceerde nakomelingen) op verschillende gastheersoorten vergeleken. Er werd geen verschil gevonden in de plant/gastheer preferenties tussen de onderzochte *C. flavipes* stammen in de olfactometer experimenten en de gastheer-acceptatie experimenten. Er werden echter wel verschillen gevonden in het reproductieve succes van de verschillende stammen op de verschillende gastheren. De populatie uit de Verenigde Staten, die de langste coëxistentie met een nieuwe gastheersoort (*D. saccharalis*) heeft gehad, had het hoogste reproductieve succes op deze gastheer. In de discussie van hoofdstuk 6 wordt beargumenteerd, dat het bestaan van plant-specifieke *C. flavipes* stammen niet berust op een gedifferentieerde plant- en/of gastheerselectie, maar eerder berust op een verschil in fysiologische aanpassing aan bepaalde gastheer soorten. Voor een betrouwbare biologische bestrijding van stengelboorders met *C. flavipes* is het dus van belang, om een populatie te selecteren, die goed fysiologisch is aangepast aan de lokale gastheersoort.

Afterword

The research described in this thesis was carried out between 1991 and 1995 at the Department of Entomology of the Wageningen Agricultural University (WAU), the Netherlands and at the International Centre for Insect Ecology and Physiology (ICIPE) in Kenya. At both places I worked with great pleasure and here I want to express my sincere thanks to everybody involved in this project and name a few specifically.

I am grateful to Louise Vet for initiating and supervising the project. Thank you for your trust in me and giving me ample space to develop my own ideas and to conduct the research in an independent way. I benefited a lot from your expertise in behavioral ecology and this is certainly reflected in the theoretical flavour of some chapters in this thesis. I would also like to thank Joop van Lenteren for being my 'promotor' and his useful comments to all the manuscripts. As a mediator for the projects in Kenya and Wageningen I thank Arnold van Huis for his interest and advice throughout the project.

In Wageningen the research depended on the assistance of numerous people. For rearing the plants I thank the Unifarm team from the 'Binnenhaven' greenhouse for the supply of maize and Casper Pillen and Mr. Stoker of the Department of Tropical Plant Breeding for the supply of sorghum and sugarcane. Rearing tropical stemborers in the Dutch Lowlands was often a nightmare and I thank Leo Koopman and Andre Gidding for all their efforts to keep the stemborer culture going. Henk Snellen was always there to help me to rear the parasitoids and to tackle the small practical problems inside and outside the 'Binnenhaven'. I thank all the PhD students, colleagues and friends for their pleasant company inside and outside 'Loburg'. For their administrative help I thank Ans Klunder, Ineke Buunk and Truus de Vries. Piet Kostense and Mariëlle Wintgens prepared several beautiful illustrations.

Not all the experimental work ended up in this thesis. Nevertheless, for their help in the molecular identification of the *C. flavipes* strains I would like to thank Richard Stouthamer, Menno Schilthuizen and in particular Frenk van Kan, who did put a lot of time and energy in the PCR analyses and who introduced me in a very pleasant way to the world of molecular techniques. I thank Marcel Dicke and Maarten Posthumus for their involvement in the chemical identification of the headspace volatiles of maize plants.

I enjoyed it very much to guide several Msc students that were involved in this project. The work of Felix Osaë Danso and Hedi Otten are included in this thesis and the experimental work of Menno van der Straten and Nicolene Vermeulen contributed significantly to the development of my ideas. I owe a

special thanks to Felix Osaë Danso for his friendship and the unforgettable evenings with lots of Pilsner baridi and nyama choma at Milimani Road, Nairobi.

A lot of the ideas expressed in this thesis were borne during my visits to Kenya. I would like to express my sincere thanks to Bill Overholt, program leader of the ICIPE/WAU project on biological control of stemborers, who gave me the opportunity to do part of the research in his inspiring laboratory at ICIPE. I thank you for your interest and support throughout the program and your valuable comments on the manuscripts. Without your logistic support I would not have been able to finish the experimental work in Wageningen. At the end of my research period I completely relied on your bi-weekly life line of *C. partellus* material. Among the inspiring and very friendly crowd at ICIPE were Adele Ngi-Song, Susan Kimani, Charles Omwega, Jacob Mbapila, Bill Budenburg and Keiji Takasu. I thank Joseph Owino, John Odhiambo and John Onditi for their assistance in the experiments and Bernadette Opiyo for her administrative help. I could escape many 'matatu' flights thanks to enjoyable daily rides with Baldwin Torto, Charles Omwega and Keiji Takasu.

The experiments of chapter 6 were entirely done with insect material flown in on a regular basis by DHL courier from around the world. For their kind regular supply and excellent shipping and handling of the insect material I thank Afzale Rajabalee of the Mauritius Sugarcane Research Institute (MSRI), Koko Widyatmoko of the Gunung Madu Plantations, Indonesia and Jim Smith of the Biocontrol Laboratory at Texas A&M University.

I was very lucky that I had the opportunity to experience stemborers and their natural enemies in the field and not only in the lab. For sharing their expertise and their kind hospitality I thank in Kenya Paul Lammers; in South Africa Des Conlong and Nicolene Vermeulen of the Experiment Station of the Sugarcane Association and Rami Kfir of the Plant Protection Institute and on Mauritius Afzale Rajabalee of the MSRI.

Tenslotte wil ik mijn ouders bedanken die me de gelegenheid gaven om bioloog te worden en die dit alles mogelijk gemaakt hebben en Mariëlle, voor al je geduld, hoop en liefde in de soms bange OIO dagen.

Roel

Leverkusen, Mei 1996

List of publications

- Balen, J.H. van & Potting, R.P.J. (1990). Comparative reproductive biology of four Blue Tit populations in the Netherlands. In: *Population biology of passerine birds: an integrated approach* (Eds. J. Blondel et al.), pp. 19-38. NATO ASI series Gvol 24. Springer, Berlin.
- Noldus, L.P.J.J. & Potting, R.P.J. (1990). Calling behaviour of *Mamestra brassicae*: effect of age and photoperiod. *Entomol. exp. appl.* 56: 23-30.
- Noldus, L.P.J.J., Potting, R.P.J. & Barendregt, H.E. (1991). Moth sex pheromone adsorption to leaf surface: bridge in time for chemical spies. *Phys. Entomol.* 16: 329-344
- Potting, R.P.J., Osae-Danso, F., Overholt, W.A. & Ngi-Song, A.J. (1993). Host selection in *Cotesia flavipes*, parasitoid of tropical stemborers. *Proc. Exper. & Appl. Entomol., N.E.V., Amsterdam* 4: 47-52.
- Potting, R.P.J., Vet, L.E.M. & Dicke, M. (1995). Host microhabitat location by the stemborer parasitoid *Cotesia flavipes*: the role of locally and systemically induced plant volatiles. *J. Chem. Ecol.* 21: 525-539.
- Potting, R.P.J., Overholt, W.A., Osae-Danso, F.O. & Takasu, K. (1995). Foraging behavior and life history of the stemborer parasitoid *Cotesia flavipes*. *J. Insect Behavior* (submitted).
- Potting, R.P.J., Otten, H. & Vet, L.E.M. (1995). The relation between parasitoid ecology and learning: the absence of learning in the stemborer parasitoid *Cotesia flavipes*. *Anim. Behav.* (submitted).
- Potting, R.P.J., Snellen, H.M. & Vet, L.E.M. (1995). Fitness consequences of superparasitism and mechanism of host discrimination in the stemborer parasitoid *Cotesia flavipes*. *Behav. Ecol. Sociobio.* (submitted).
- Potting, R.P.J., Vet, L.E.M. & Overholt, W.A. (1996) Geographic variation in host selection behaviour and reproductive success in the stemborer parasitoid *Cotesia flavipes*. *Bull. Entomol. Res.* (submitted).

Roland Peter Joseph Potting was born on April 2nd 1964 in Meerssen. He graduated from Sint Maartens College in 1982. In the same year he began his studies of biology at the Wageningen Agricultural University. He did his practical training period on tsetse ecology at the International Centre for Insect Physiology and Ecology in Kenya. During his graduate studies he was involved in research projects on bird ecology at the Netherlands Institute for Ecological Research and on semiochemical mediated behavior at the Department of Entomology at the Wageningen Agricultural University. He obtained his Msc degree in October 1988.

From 1989 to 1990 he worked as a Research Entomologist on parasitoid quality control at the Federal Research Institute for Crop Protection, Reckenholz, Switzerland, under the supervision of Dr. F. Bigler. From 1990 to 1991 he worked in Wageningen as a Software Development Engineer at Noldus Information Technology, a company specialized in recording and analyzing animal behavior.

The research described in this doctoral thesis was carried out during the years 1991 to 1995 at the Department of Entomology, Wageningen Agricultural University. Here he conducted research on the behavioral ecology of a stemborer parasitoid, under supervision of Prof. Dr. J.C. van Lenteren and Dr. L.E.M. Vet. Part of the work was carried out with Dr. W.A. Overholt at the International Centre for Insect Physiology and Ecology in Kenya.

Since March 1996 he is employed at the Central Research Division (section Behavior Modifying Chemicals) of Bayer AG in Germany.