

Host recognition and acceptance behaviour in *Cotesia sesamiae* and *C. flavipes* (Hymenoptera: Braconidae), parasitoids of gramineous stemborers in Africa

MESHACK OBONYO^{1,2}, FRITZ SCHULTHESS³, BRUNO LE RU², JOHNNIE VAN DEN BERG¹
and PAUL-ANDRÉ CALATAYUD^{2*}

¹School of Environmental Science and Development, North-West University, Potchefstroom, 2520, South Africa

²Institut de Recherche pour le Développement (IRD), UR 072, c/o International Centre of Insect Physiology and Ecology (ICIPE), Noctuid Stemborer Biodiversity (NSBB) Project, PO Box 30772-00100, Nairobi, Kenya and Université Paris-Sud 11, 91405 Orsay, France

³ICIPE, Stemborer Biocontrol Program, PO Box 30772-00100, Nairobi, Kenya

Key words. Hymenoptera, Braconidae, *Cotesia sesamiae*, *C. flavipes*, Lepidoptera, Pyralidae, *Eldana saccharina*, Noctuidae, *Busseola fusca*, *Chilo partellus*, parasitoids, host recognition, host acceptance, stemborers, Africa

Abstract. The host recognition and acceptance behaviour of two braconid larval parasitoids (*Cotesia sesamiae* and *C. flavipes*) were studied using natural stemborer hosts (i.e., the noctuid *Busseola fusca* for *C. sesamiae*, and the crambid *Chilo partellus* for *C. flavipes*) and a non-host (the pyralid *Eldana saccharina*). A single larva was introduced into an arena together with a female parasitoid and the behaviour of the wasp recorded until it either stung the larva or for a maximum of 5 min if it did not sting the larva. There was a clear hierarchy of behavioural steps, which was similar for both parasitoid species. In the presence of suitable host larvae, after a latency period of 16–17 s, the wasp walked rapidly drumming the surface with its antennae until it located the larva. After location and antennal examination of the host, which lasted 60–70 s and 30 s, respectively, the parasitoid inserted its ovipositor. Stinging that resulted in successful oviposition usually lasted 5–6 s. In the presence of non-host larvae, the latency period was between 25–70 s, and parasitoids spent significantly more time walking and antennal drumming on larvae without ovipositing. It is likely that these two parasitoid species use their antennae for host recognition, and both their antennae and tarsi for final acceptance of a host for oviposition. In both *C. sesamiae* and *C. flavipes* tactile and contact-chemoreception stimuli from the hosts seemed to play a major role in the decision to oviposit.

INTRODUCTION

In sub-Saharan Africa, crambid, pyralid and noctuid stemborers are the economically most important pests of maize and sorghum (Harris, 1990; Polaszek, 1998; Kfir et al., 2002). The most cited species are the crambid *Chilo partellus* (Swinhoe), the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson, and the pyralid *Eldana saccharina* (Walker) (Polaszek, 1998). With exception of *C. partellus*, which was accidentally introduced from Asia into Southern Africa sometime before the 1930s (Tams, 1932), these species are indigenous to the African continent.

In the early 90s, the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, increased its research into biological control. The most abundant larval parasitoid recovered from stemborers in East and Southern Africa is the gregarious braconid *Cotesia sesamiae* (Cameron). Parasitism is usually below 5%, though in some localities it can attain 75% (Kfir, 1995; Sallam et al., 1999; Jiang et al., 2006; Songa et al., 2007). Several strains of *C. sesamiae* from Kenya were introduced into west Africa, where *C. sesamiae* is very rare, to control *B. fusca* and *S. calamistis* (Schulthess et al., 1997; Ndemah et al., 2007). *Cotesia flavipes*

Cameron, a close relative of *C. sesamiae*, was introduced into Kenya from Asia in 1991, and released against *C. partellus* in the coastal area in 1993 (Overholt et al., 1994b), where it reduced *C. partellus* densities by over 50% (Zhou et al., 2001; Jiang et al., 2006).

In order to become established in a new habitat a parasitoid must first locate and be able to discriminate suitable from unsuitable hosts. Behavioural events leading to successful parasitism include host habitat location, host location, host acceptance and suitability (Vinson, 1976, 1985; Godfray, 1994). During location of hosts, the parasitoids typically exploit long and short range stimuli emanating from the host habitat (Vinson, 1975; Godfray, 1994), followed by stimuli directly associated with the host and its products (Vinson, 1985; Vet & Dicke 1992; Godfray, 1994). However, stimuli from the habitat do not convey sufficiently reliable information on the suitability of host species but are mere indicators of the presence of herbivores (Ngi-Song & Overholt, 1997). As a result, *C. sesamiae* and *C. flavipes* are often attracted to plants infested with unsuitable species of stemborer (Potting et al., 1993; Ngi-Song et al., 1996; Obonyo et al., 2008). Therefore, it is suggested that *C. sesamiae* and *C. flavipes*, though not capable of recognizing a host species

* Corresponding author; current address: IRD c/o CNRS, LEGS, 91198 Gif-sur-Yvette, France and Université Paris-Sud 11, 91405 Orsay, France; e-mail: paul-andre.calatayud@legs.cnrs-gif.fr

from a distance, are able to distinguish suitable from non-suitable hosts on contact. This is especially crucial for the establishment of the exotic *C. flavipes*, which encountered many new suitable and unsuitable hosts in its new environment in Africa (Le Ru et al., 2006).

Host recognition and acceptance behaviour has been studied in various parasitoid species (e.g., Vinson, 1985; Godfray, 1994; Vinson, 1998; Canale & Raspi, 2000). There exists some information on the host searching and selection behaviour of *C. sesamiae* and *C. flavipes* parasitizing larvae feeding inside plants (Potting et al., 1993; Gohole et al., 2005), but there are no direct observations on the step-by-step process leading to oviposition in a larval host. The present study tried to identify the behavioural steps leading to the identification, acceptance and successful parasitism of a host using host and non-host borer species. Through detailed direct observations, the role of receptors present on the antennae, tarsi and ovipositor, and the host cues involved in host discrimination and acceptance by the two parasitoids, were elucidated.

MATERIAL AND METHODS

Insects

The adults of *C. sesamiae* and *C. flavipes* were obtained from laboratory-reared colonies established and maintained at ICIPE. The *C. sesamiae* colony was initiated with adults reared from *B. fusca* collected from maize fields at Kitale, western Kenya, in 2006, while *C. flavipes* was reared from *C. partellus* from coastal Kenya in 2005. Twice a year, field collected parasitoids were added to rejuvenate the colonies. *Cotesia sesamiae* and *C. flavipes* were reared on larvae of their hosts, *B. fusca* and *C. partellus*, respectively, according to the method described by Overholt et al. (1994a). Parasitoid cocoons were kept in Perspex cages (30 cm × 30 cm × 30 cm) until emergence. Adults were fed on a 20% honey-water solution in a cotton wool pad and kept under an artificial source of light for 24 h to mate. In all experiments, only 1-day-old, naïve, mated females were used. The experiments were carried out at 25 ± 2°C and 50–80% RH, and under a 12L : 12D photoperiod.

Of the three stemborer species used in this study, *B. fusca* and *C. partellus* are the hosts of *C. sesamiae* and *C. flavipes*, respectively, and *E. saccharina*, is not a host of either of the parasitoids.

Preliminary tests showed that neither parasitoid attacked *E. saccharina* (M. Obonyo, pers. observ.). *Eldana saccharina* and *B. fusca* were collected from maize fields in the Western Province, while *C. partellus* originated from maize grown in the coastal region. The larvae were reared on the artificial diets described by Ochieng et al. (1985) (for *C. partellus*) and Onyango & Ochieng-Odero (1994) (for *B. fusca* and *E. saccharina*). Thrice a year feral stemborer larvae from their respective locations were added to rejuvenate the colonies.

Experimental procedure

Third and fourth instar larvae were introduced into jars (10 cm × 20 cm) containing pieces of maize stem and left for 24 h to feed and produce frass. Then, a single larva was placed in an arena consisting of a glass Petri dish, 7 cm in diameter and 1 cm in height, together with a wasp. The behaviour of the female wasp was immediately recorded and continued until it either stung the larva or for a maximum of 5 min if the wasp did not sting the larva.

If a wasp was killed by a stemborer before it could sting a larva, both wasp and larva were excluded from the analyses.

After each replicate the arena was cleaned in distilled water and air-dried. Similarly, each wasp and larva was used only once. There were 50 replicates of each parasitoid/stemborer species combination. Larvae that were stung by parasitoids were reared on artificial diet and monitored daily until cocoon formation, pupation or death.

A digital video camera (Panasonic, Japan) with an optical zoom system (Computer TV zoom Lens, 8–80 mm) connected to a VHS video recorder (time lapse video cassette recorder, Panasonic, AG-6730) was used to record the parasitoids' behaviour.

In nature, adult *C. sesamiae* and *C. flavipes* are generally active throughout the day, especially around 1200 h, when light intensity and temperature are high (Mohyuddin, 1971). Thus, the experiments were conducted between 1000 and 1400 h, in arenas kept at 25 ± 2°C and illuminated from above by means of a white 60 W light bulb (Overholt, 1993; Smith et al., 1993).

Behaviour of female wasps

The behaviour of a female wasp was described in terms of her body posture and movement on encountering a larva. The behavioural steps are mutually exclusive events described as a combination of orientation and movement of different body parts, especially those bearing sensory structures (i.e., antennae, tarsi and ovipositor). These steps form part of a pattern, which is consistently and repeatedly observed. The six behavioural steps (Fig. 1) were as follows: (1) stationary with antennae upright and apart (ST); (2) grooming of the rear legs and/or antennae (G); (3) walking and antennal drumming in the arena (W); (4) walking on and drumming the body of a larva with its antennae (WB); (5) an attempt at stinging (SA), i.e., oviposition was disrupted before the ovipositor could be inserted; (6) oviposition (O), i.e., ovipositor successfully inserted, followed by the parasitoid leaving the host. The duration of each behavioural step was also recorded.

Data analysis

The analysis of the behaviour of female parasitoids on encountering stemborer larvae was done using Proc CATMOD in the SAS system (SAS Institute, 2003). The analysis was conducted on the total number of transitions between all possible pairs of behavioural steps and the data pooled for all females using transitional frequencies of all transitions between pairs of behavioural patterns. Transitional frequencies were used to form a contingency table ($p \times q \times r$), in which the first variable p represents the possible "preceding" behaviour and the second variable q the possible "following" behaviour, while r represents the host larva. "Logical zeroes" occurred in the table, where one behavioural pattern could not follow another, or between identical preceding and following behaviour.

Log-linear models were constructed based on specific combinations of interactions to provide expected values of transitional frequencies for the contingency table. The rationale and method of analysis is described by Parr et al. (1996) and Hora & Roessingh (1999). The goodness-of-fit of the log-linear model was assessed using a likelihood ratio statistic (G tests).

Where the expected values of a model were not significantly different from the observed, the assumptions of that model were accepted as necessary and sufficient to explain the observed data. In addition, an index was calculated to quantify the dissimilarity between the expected and observed values. The probability of each transition given the preceding behaviour was also calculated. To identify the transitions that were a significant part of a sequence of behavioural steps, standardized residuals of the observed transitions were calculated. When compared to a model that did not include the assumption of dependence of fol-

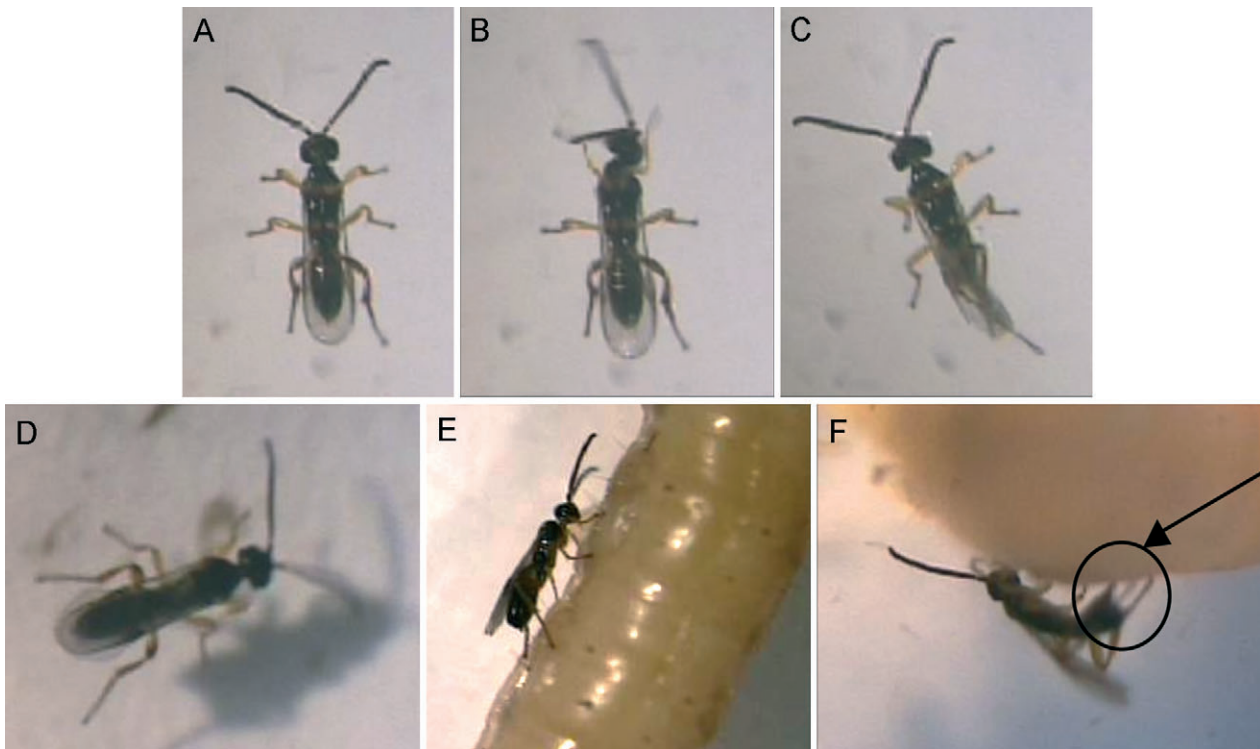


Fig. 1. Behavioural steps preceding oviposition displayed by female wasps (e.g., *Cotesia sesamiae* searching for *Busseola fusca*). A – stationary with antennae upright and apart (ST); B, C – grooming legs and/or the antennae (G); D – walking in an arena while drumming the surface with the tips of its antennae, which are curved downwards (W); E – walking on the body of larva while drumming its surface with its antennae (WB); F – oviposition (O), i.e., ovipositor insertion followed by egg-laying.

lowing and preceding behavioural steps, significant positive transitions – i.e., those for which positive standardized residuals were obtained, which were greater than the calculated threshold – were used to construct kinetograms of the host recognition and acceptance behaviour. χ^2 -tests were used to compare the percentage occurrence of behavioural steps with respect to stem-borer species. The procedure used to separate proportions in Table 1 is that described by Marascuilo (1966). For the data on the duration of each of the behavioural steps, Tukey's studentized range test was used to separate the means using the GLM procedure in the SAS system (SAS Institute, 2003).

RESULTS

When female *C. sesamiae* and *C. flavipes* encountered a larva, their behaviour depended on whether it was a host or not; the two parasitoids behaved similarly. When approaching a host larva, the wasp walked drumming the

surface with its antennae until it located the larva. It then jumped on the larva, briefly drummed the body of the larva with its antennae and then inserted its ovipositor. On a host larva, WB culminating in O was more frequent than on non-hosts (Figs 2 and 3). During walking and drumming and on the body of a larva (WB), the wasp directed its antennae forwards, with the apical portion curved down and backwards. Only the distal ends of the arena were observed to contact either the surface of the arena or that of a larva.

When *C. sesamiae* females were presented with *B. fusca*, they frequently displayed four pairs of behavioural transitions (probability of transitions > 0.01), i.e., (i) standing still (ST) – walking and antennal drumming in the arena (W), (ii) W – walking and antennal drumming on the body of a larva (WB), (iii) WB – successful inser-

TABLE 1. Fate of the host after an attempted stinging (SA) or successful ovipositor insertion (O) by *Cotesia sesamiae* and *Cotesia flavipes* (n = 50).

Parasitoid species	Host tested	% larvae stung	% hosts producing cocoons	% host larvae killed	% hosts pupating
<i>C. sesamiae</i>	<i>Busseola fusca</i>	82b	52b	16b	14b
	<i>Chilo partellus</i>	2a	0a	2a	0a
	<i>Eldana saccharina</i>	2a	0a	2a	0a
<i>C. flavipes</i>	<i>Chilo partellus</i>	62b	46b	6a	10a
	<i>Busseola fusca</i>	62b	0a	10a	52b
	<i>Eldana saccharina</i>	6a	0a	2a	4a

Percentages within a column followed by different letters are significantly different at $P \leq 0.05$ (Marascuilo procedure for multiple percentage comparisons).

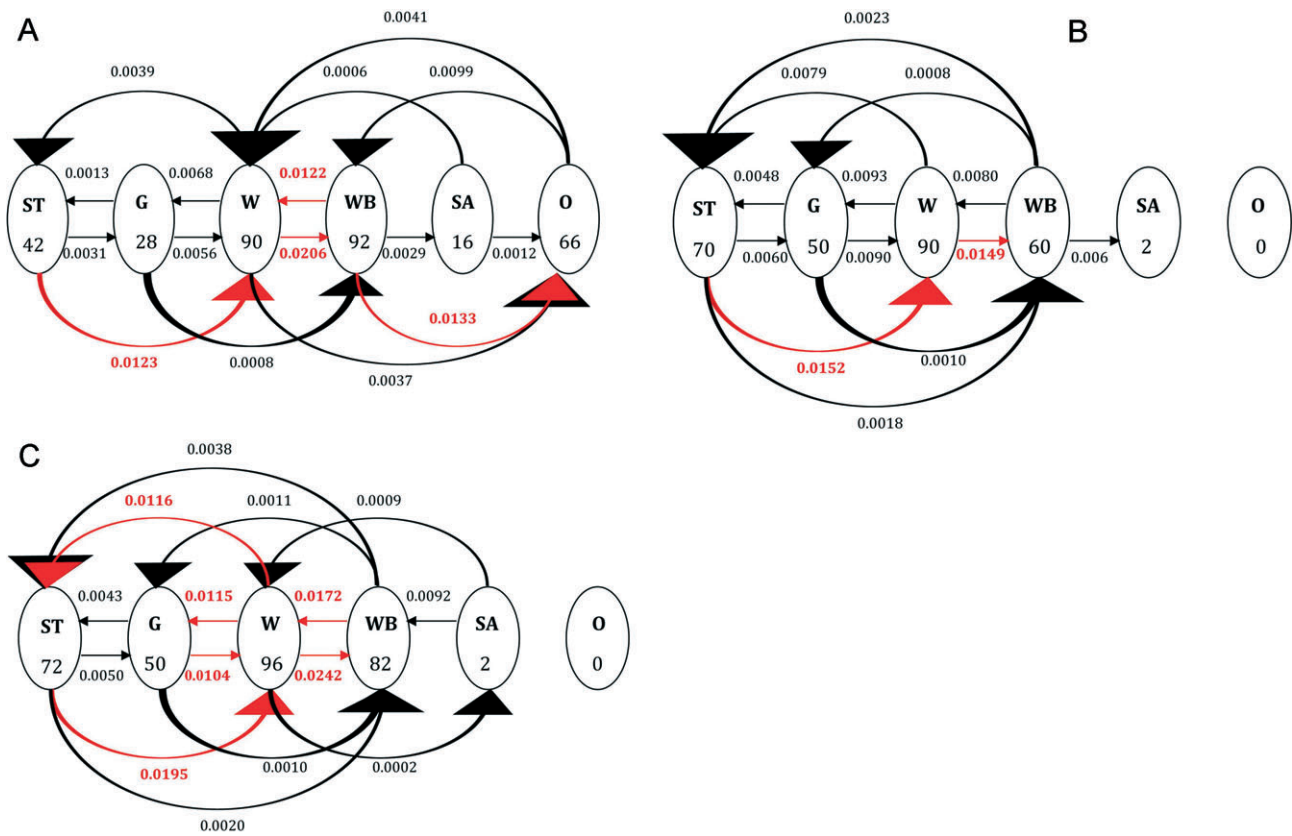


Fig. 2. Behavioural steps exhibited by a naive gravid female *Cotesia sesamiae* on encountering either *Busseola fusca* (A), *Chilo partellus* (B) or *Eldana saccharina* larvae (C). The values with arrows represent the probability of each significant transition given the preceding behaviour. For each experiment, fifty female wasps were observed. The probabilities of transition > 0.0100 are in red. The percentage of occurrence of each behavioural step is given below the category definition.

tion of ovipositor (O) and, (iv) WB – W (Fig. 2a). Among the females tested, the occurrence of O was 66% with most of the stung larvae producing cocoons (Table 1). In contrast, when they encountered *C. partellus* and *E. saccharina* they did not insert their ovipositors (O) (2×3 contingency table $\chi^2 = 84.6$, d.f. = 2, $P < 0.0001$) and consequently there was no transition from walking and drumming of the larval body with the antennae (WB) to successful ovipositor insertion (O) (Figs 2b and 2c). Moreover, the occurrence of a stinging attempt (SA) was significantly higher when *C. sesamiae* encountered *B. fusca* than either *C. partellus* or *E. saccharina* ($\chi^2 = 10.5$, d.f. = 2, $P = 0.005$). In addition, none of the 2% of the *C. partellus* or *E. saccharina* larvae that were stung produced cocoons (Table 1). For *C. partellus*, high probabilities of transitions ($P > 0.01$) were recorded only between two pairs of behavioural transitions, i.e., (i) standing still (ST) – walking and antennal drumming in the arena (W) and, (ii) W – walking and antennal drumming on the body of a larva (WB) (Fig. 2b). For *E. saccharina*, the kinetogram is more complex as the high probabilities of transition ($P > 0.01$) are not linked with oviposition behaviour. These were recorded between six pairs of behavioural transitions, i.e., (i) standing still (ST) – walking and antennal drumming in the arena (W), (ii) grooming of the rear legs and/or antennae (G) – W, (iii) W – walking and antennal drumming on the body of a

larva (WB), (iv) WB – W, (v) W – grooming of the rear legs and/or antennae (G), and, (vi) W – ST (Fig. 2c). With *B. fusca* as the host, 42% of the wasps stood still versus > 70% when *C. partellus* or *E. saccharina* larvae were offered ($\chi^2 = 11.0$, d.f. = 2, $P = 0.003$). Similarly, G was less frequent when *C. sesamiae* encountered *B. fusca* than either *C. partellus* or *E. saccharina* ($\chi^2 = 6.595$, d.f. = 2, $P = 0.0370$). Consequently, *C. sesamiae* females spent significantly less time stationary (ST) or in G when they encountered *B. fusca* than either *C. partellus* or *E. saccharina* (Table 2). They spent significantly more time in O when they encountered *B. fusca* than either *C. partellus* or *E. saccharina* (Table 2).

In the encounters between female *C. flavipes* and *C. partellus*, three pairs of behavioural transitions occurred with high probabilities ($P > 0.01$): (i) stationary (ST) – walking and antennal drumming in the arena (W), (ii) W – walking and antennal drumming on the body of the larva (WB) and, (iii) WB and successful oviposition (O). O was achieved by 58% of the females tested (Fig. 3a) and most of the *C. partellus* larvae parasitized produced cocoons (Table 1). For *B. fusca*, high probabilities of transition ($P > 0.01$) were recorded only for two transitions: (i) walking and antennal drumming in the arena (W) – walking and antennal drumming on the body of a larva (WB) and, (ii) WB – successful oviposition (O), with 52% of the wasps tested achieving O (Fig. 3b).

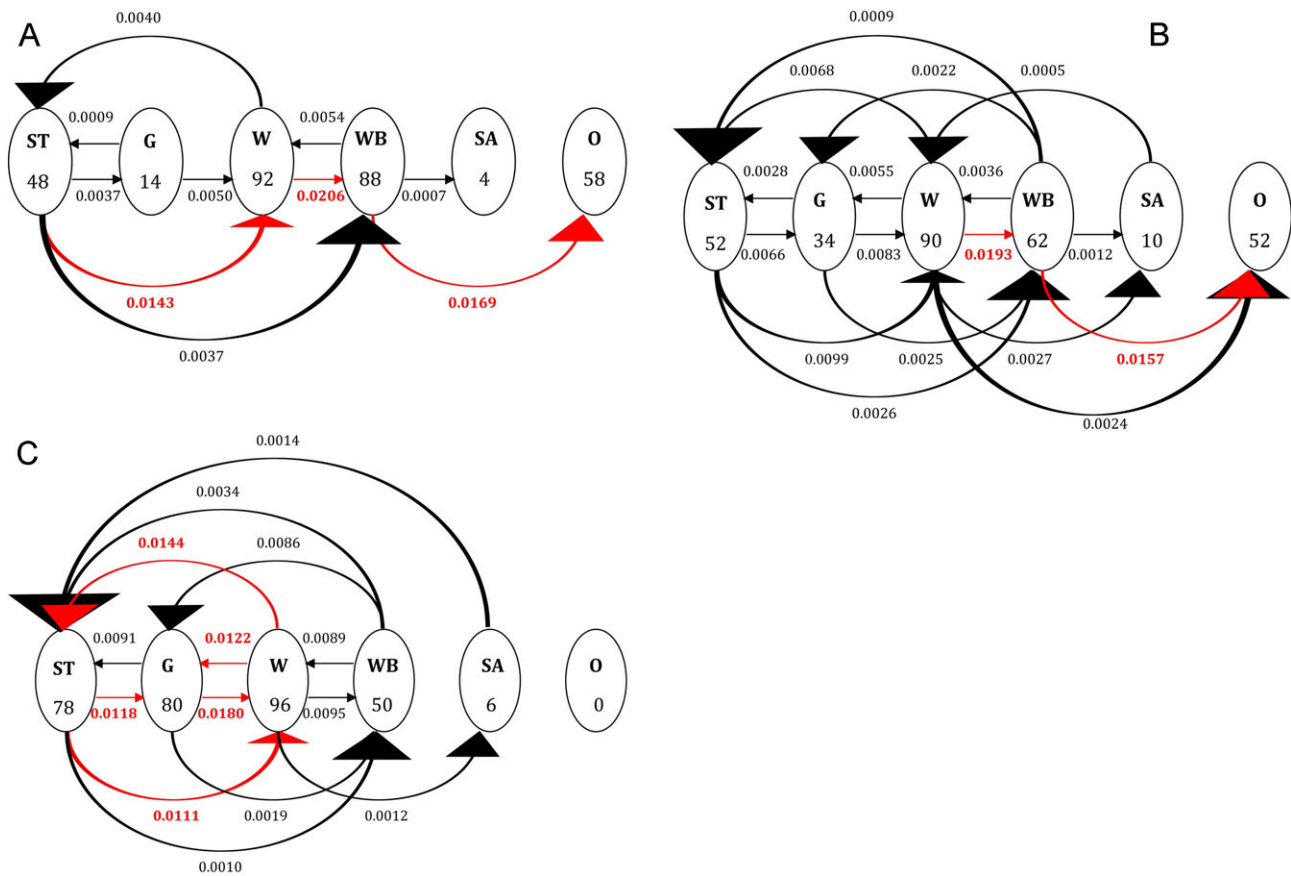


Fig. 3. Behavioural steps exhibited by a naive gravid female *Cotesia flavipes* on encountering either *Chilo partellus* (A), *Busseola fusca* (B) or *Eldana saccharina* larvae (C). The values with arrows represent the probability of each significant transition given the preceding behaviour. For each experiment, fifty female wasps were observed. The probabilities of transition > 0.0100 are in red. The percentage of occurrence of each behavioural step is given below the category definition.

However, the kinetogram when *B. fusca* is the host is more complex and has a higher number of transitions than when *C. partellus* is the host. Most of the parasitized larvae of *B. fusca* pupated without producing cocoons (Table 1). When a female *C. flavipes* encountered the non-host *E. saccharina*, the wasps were rarely able to insert their ovipositor (O), and the few larvae that were stung either pupated or died (Table 1). High transition probabilities ($P > 0.01$) were recorded for: (i) stationary (ST) – grooming of the rear legs and/or antennae (G), (ii) ST – walking and antennal drumming in the arena (W),

(iii) grooming of the rear legs and/or antennae (G) – W, (iv) W – G and (v) W – ST (Fig. 3c). In the presence of *E. saccharina* larvae, the percentage of females showing WB behaviour decreased and of those that groomed their rear legs and/or antennae (G) and remained stationary (ST) increased (for walking and antennal drumming on the body of a larva (WB): $\chi^2 = 16.9$, d.f. = 2, $P < 0.0001$; for G: $\chi^2 = 46.8$, d.f. = 2, $P < 0.0001$; for ST: $\chi^2 = 11.0$, d.f. = 2, $P = 0.0041$). When *C. partellus* was the host, female *C. flavipes* spent less time ovipositing (O) than stationary (ST) and grooming their rear legs and/or

TABLE 2. Total duration (seconds, mean \pm SE, n = 50) of each behavioural steps exhibited by the female wasps of *Cotesia sesamiae* and *Cotesia flavipes* on host or non-host larvae.

Parasitoid species	Host tested	Behavioural steps					
		ST	G	W	WB	SA	O
<i>C. sesamiae</i>	<i>Busseola fusca</i>	16.5 \pm 6.5a	10.7 \pm 3.6a	73.0 \pm 8.6a	32.1 \pm 6.3b	0.5 \pm 0.2	6.5 \pm 0.9b
	<i>Chilo partellus</i>	64.5 \pm 10.3b	38.4 \pm 8.4b	95.5 \pm 11.3a	17.8 \pm 4.1a	0.2 \pm 0.2	0a
	<i>Eldana saccharina</i>	24.4 \pm 4.0b	14.6 \pm 3.0ab	156.3 \pm 11.0b	71.7 \pm 8.5c	2.4 \pm 2.4	0a
<i>C. flavipes</i>	<i>Chilo partellus</i>	17.2 \pm 4.5a	2.6 \pm 1.4a	63.6 \pm 10.0a	30.0 \pm 7.6b	0.1 \pm 0.1	4.9 \pm 0.7b
	<i>Busseola fusca</i>	31.8 \pm 6.9a	12.4 \pm 3.4a	54.9 \pm 8.4a	11.8 \pm 2.4a	1.5 \pm 0.7	5.6 \pm 0.8b
	<i>Eldana saccharina</i>	69.7 \pm 8.2b	60.6 \pm 8.4b	90.0 \pm 8.4b	37.0 \pm 6.7b	3.5 \pm 2.0	0a

Means within a column and parasitoid species followed by different letter are significantly different at $P \leq 0.05$ (Tukey's studentized range test). ST – standing still; G – grooming the legs and/or the antennae; W – walking in the arena; WB – walking on the larval body; SA – stinging attempt; O – oviposition.

antennae (G) than when the host was *B. fusca*. The wasp also spent significantly more time ($P < 0.05$) in ST, G and walking and antennal drumming in the arena (W) in the presence of *E. saccharina* than in the presence of *C. partellus* (Table 2).

Generally, for both parasitoid species, there was no relationship between the time they spent walking and antennal drumming on the body of a larva (WB) and successful oviposition (O); more time was spent in WB with non-host larvae but this was not followed by O (Table 2).

DISCUSSION

The stationary behaviour displayed by *C. sesamiae* and *C. flavipes* females appears to be linked to the detection of the presence of larvae in the arena. During this stationary phase, the wasps appear to sense the odour emanating from the larvae or residues from feeding activities (e.g., frass). Although females of *C. sesamiae* and *C. flavipes* can respond to the smell of larval frass they are unable to discriminate between the smell of frass of host and non-host species (Ngi-Song & Overholt, 1997). In the current study, female wasps remained stationary for a shorter time when in the presence of larvae, in which they subsequently oviposited, suggesting that they were able to identify a host at this stage in the host handling process.

Antennal drumming played an essential role in the location, recognition and acceptance of hosts. More time was generally spent in antennal grooming when in the presence of a larva they did not subsequently oviposit in. Grooming in insects, other than cleaning the outer body surface or organs, may indicate displacement activity for host location and recognition (Spruijt et al., 1992).

The use of antennae by female parasitoids in host examination prior to ovipositor insertion is well documented in the literature (Vinson, 1985, 1998; Godfray, 1994; Canale & Raspi, 2000). In both *Cotesia* species, the distal antennomeres appeared to be intimately involved in host location and recognition since they made direct contact with the body surface of the larvae. This observation corroborates findings that the distal antennomeres of braconid parasitoids possess gustatory sensillae that detect chemical stimuli associated with the host (Navasero & Elzen, 1991; Barbarossa-Tomassini et al., 1998; Canale & Raspi, 2000; Ochieng et al., 2000).

Compared to other braconids, oviposition in *C. sesamiae* and *C. flavipes* occurred very rapidly (5–6 s) and soon after antennal examination of the larval cuticle. In other braconids, such as *Opius concolor* Szepilgeti, oviposition lasted 30–40 s (Canale & Raspi, 2000) and in *Cotesia glomeratus* (Linnaeus) 16–20 s (Tagawa et al., 1987). The behaviour of attacked larvae may influence the behaviour of a parasitoid during parasitisation. The time spent penetrating the cuticle usually varies greatly among species; it may be long, when a sessile host is attacked but often very short, when mobile or aggressive hosts are attacked (Vinson, 1985). Lepidopteran larvae that feed on plants, whether externally or internally, defend themselves against parasitoids either indirectly by hiding during times when vulnerable to attack (Gross,

1993) or directly by biting, spitting or flicking the parasitoid off (Potting et al., 1993; Takasu & Overholt, 1997). Thus, due to this aggressive behaviour of their hosts, *C. sesamiae* and *C. flavipes* females are under pressure to oviposit quickly once they contact a larva.

Both *C. sesamiae* and *C. flavipes* appeared to accept a host after examining it externally with their antennae. In contrast, *O. concolor* stings a larva before accepting or rejecting it for oviposition indicating that the ovipositor plays an important role in host discrimination (Canale & Raspi, 2000). Parasitoids are thought to use their ovipositors to determine the internal suitability of a host (Godfray, 1994). Thus, host acceptance or rejection appears to be guided by internal cues perceived by the ovipositor of the female wasp (Godfray, 1994; Le Ralec et al., 1996; Van Lenteren et al., 2007). Such processing may also occur in *C. sesamiae* and *C. flavipes* and may explain why some females rejected the non-host species *E. saccharina* after inserting their ovipositor for a short period. Only 2% and 6% of the larvae that *C. sesamiae* and *C. flavipes*, respectively, inserted their ovipositors into did not produce parasitoid cocoons. However, in cases where no parasitoids developed it was not established whether the wasp deposited any eggs.

In contrast to the results obtained by Ngi-Song et al. (1995) and Mochiah et al. (2001), *C. sesamiae* did not successfully develop in *C. partellus*. This is probably due to the population origin of *C. sesamiae* used. In Kenya, there are two populations of this endoparasitoid, which differ in their ability to develop in *B. fusca*. Unlike the coastal population, the inland population is able to complete its development in this host, and both can develop in *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), the main host of *C. sesamiae* in coastal Kenya (Ngi-Song et al., 1998). Ngi-Song et al. (1995) used the coastal population of *C. sesamiae* while Mochiah et al. (2001) used the inland population from Kitale. However, the Kitale strain of *C. sesamiae* used in our study did not develop in *C. partellus* indicating that it was different from that used by Mochiah et al. (2001).

The behaviour of *C. flavipes* varied significantly when presented with the two non-hosts, *B. fusca* and *E. saccharina*. In contrast to *B. fusca* very few larvae of *E. saccharina* were stung. Since both stemborers have recently come into contact with *C. flavipes* as compared to *C. sesamiae*, it is suggested that unlike *E. saccharina*, *B. fusca* and *C. partellus* share the same stimuli for host acceptance by *C. flavipes*.

In addition to chemical stimuli, various other physical characteristics of the larvae, such as size, shape, texture or movement can be involved in host recognition (Vinson, 1991; Godfray, 1994). During host encounter, female parasitoids often jumped on a larva and walked all over it and probably reacted to cues emanating from the larva. This suggests reception of vibrations via contact mechanoreceptors on the tarsi of the parasitoid are used in host recognition (Godfray, 1994). In addition, when the larvae remained motionless during examination, the wasp took longer before ovipositing than when the larvae were

moving or reacted to being contacted by the wasp. Similarly, for *O. concolor*, when in the presence of immobilised host larvae, the females took longer to locate a host and frequently failed to do so (Canale & Raspi, 2000).

During external examination, the labial and maxillary palpi of *C. sesamiae* and *C. flavipes* were held perpendicular to the body surface of a larva and they did not appear to contact the larva during the examination process. In contrast to *O. concolor*, which uses its palps in host searching (Canale & Raspi, 2000), *C. sesamiae* and *C. flavipes* used their palps for feeding only and they do not appear to have a role in host recognition.

In conclusion, *C. sesamiae* and *C. flavipes* females are able to detect the presence of a larva close by, most probably by the odour emanating from the frass produced by the larva. This detection allows the female wasp to locate a potential host when it is close by. Thereafter, antennal examination plays a crucial role in host recognition and acceptance. The tarsi are probably important for final host acceptance before stinging, very likely through the reception of vibrations. The contact cues associated with stemborer larvae (both physical and chemical) very likely play a role in host acceptance for oviposition by female wasps. The identification of these cues will facilitate the isolation of kairomone(s) involved in host recognition and acceptance by *C. sesamiae* and *C. flavipes*.

ACKNOWLEDGEMENTS. The authors are grateful to A. Wanjoya and J. Voise for their statistical help and L. Kaiser-Arnauld for critical revision of earlier versions of this manuscript. This work was funded by IRD, France and the German academic exchange DAAD for which the authors are very grateful.

REFERENCES

- BARBAROSSA-TOMASSINI I., MURONI P., DARDANI M., CASULA P. & ANGIOY A.M. 1998: New insight into the antennal chemosensory function of *Opius concolor* (Hymenoptera: Braconidae). *Ital. J. Zool.* **65**: 367–370.
- CANALE A. & RASPI A. 2000: Host location and oviposition behaviour in *Opius concolor* (Hymenoptera: Braconidae). *Entomol. Probl.* **31**: 25–32.
- GODFRAY H.C.J. 1994: *Parasitoids: Behavioural and Evolutionary Ecology*. Princeton University Press, Princeton, 473 pp.
- GOHOLE L.S., OVERHOLT W.A., KHAN Z.R. & VET L.E.M. 2005: Close-range host searching behavior of the stemborer parasitoids *Cotesia sesamiae* and *Denticasmius busseolae*: influence of a non-host plant *Melinis minutiflora*. *J. Insect Behav.* **18**: 149–169.
- GROSS P. 1993: Insect behavioural and morphological defences against parasitoids. *Annu. Rev. Entomol.* **38**: 251–273.
- HARRIS K.M. 1990: Bioecology of sorghum stemborers. *Insect Sci. Appl.* **11**: 467–477.
- HORA K.H. & ROESSINGH P. 1999: Oviposition in *Yponomeuta cagnagellus*: the importance of contact cues for host plant acceptance. *Physiol. Entomol.* **24**: 109–120.
- JIANG N., ZHOU G., OVERHOLT W.A. & SCHULTHESS F. 2006: The synchrony of the stemborer and parasitoid populations of coastal Kenya. *Ann. Soc. Entomol. Fr.* **42**: 381–388.
- KFIR R. 1995: Parasitoids of the African stemborer *Busseola fusca* (Lepidoptera: Noctuidae) in South Africa. *Bull. Entomol. Res.* **85**: 369–377.
- KFIR R., OVERHOLT W.A., KHAN Z.R. & POLASZEK A. 2002: Biology and management of economically important lepidopteran cereal stemborers in Africa. *Annu. Rev. Entomol.* **47**: 701–731.
- LE RALEC A., RABASE J.M. & WAINBERG E. 1996: Comparative morphology of the ovipositor of some parasitic Hymenoptera in relation to characteristics of their hosts. *Can. Entomol.* **128**: 413–433.
- LE RÛ B.P., ONG'AMO G.O., MOYAL P., NGALA L., MUSYOKA B., ABDULLAH Z., CUGALA D., DEFABACHEW B., HAILE T.A., MATAMA-KAUMA T., LADA V.Y., NEGASSI B., PALLANGYO B., RAVOLONANDRIANINA J., SIDUMO A., OMWEGA C.O., SCHULTHESS F., CALATAYUD P.A. & SILVAIN J.F. 2006: Major ecological characteristics of East African noctuid stemborers. *Bull. Entomol. Res.* **96**: 555–563.
- MARASCUULO L. 1966: Large-sample multiple comparisons. *Psychol. Bull.* **65**: 289–299.
- MOCHIAH M.B., NGI-SONG A.J., OVERHOLT W.A. & BOTCHEY M. 2001: Host suitability of four cereal stem borers (Lepidoptera: Crambidae, Noctuidae) for different geographic populations of *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) in Kenya. *Biol. Control* **21**: 285–292.
- MOHYUDDIN A.I. 1971: Comparative biology and ecology of *Apanteles flavipes* and *A. sesamiae* Cameron as parasites of gramineous borers. *Bull. Entomol. Res.* **61**: 33–39.
- NAVASERO R.C. & ELZEN G.W. 1991: Sensilla on the antennae, foretarsi and palpi of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). *Proc. Entomol. Soc. Wash.* **93**: 737–747.
- NDEMAH R., SCHULTHESS F., LE RÛ B. & BAME I. 2007: Lepidopteran cereal stemborers and associated natural enemies on maize and wild grass hosts in Cameroon. *J. Appl. Entomol.* **131**: 658–668.
- NGI-SONG A.J. & OVERHOLT W.A. 1997: Host location and acceptance of *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), parasitoids of African stemborers: Role of frass and other cues. *Biol. Control* **9**: 136–142.
- NGI-SONG A.J., OVERHOLT W.A. & AYERTEY J.N. 1995: Suitability of African gramineous stemborers for development of *Cotesia flavipes* and *C. sesamiae*. *Environ. Entomol.* **24**: 978–984.
- NGI-SONG A.J., OVERHOLT W.A., NJAGI P.G.N., DICKE M., AYERTEY J.N. & LWANDE W. 1996: Volatile infochemicals used in host habitat location by *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), larval parasitoids of stemborers on graminiae. *J. Chem. Ecol.* **22**: 307–323.
- NGI-SONG A.J., OVERHOLT W.A. & STOUTHAMER R. 1998: Suitability of *Busseola fusca* and *Sesamia calamistis* (Lepidoptera: Noctuidae) for the development of two populations of *Cotesia sesamiae* (Hymenoptera: Braconidae) in Kenya. *Biol. Control* **12**: 208–214.
- OBONYO M., SCHULTHESS F., GERALD J., WANYAMA O., LE RÛ B. & CALATAYUD P.-A. 2008: Location, acceptance and suitability of lepidopteran stemborers feeding on a cultivated and wild host-plant to the endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). *Biol. Control* **45**: 36–47.
- 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.
- OCHIENG S.A., PARK K.C., ZHU J.W. & BAKER T.C. 2000: Functional morphology of antennal chemoreceptors of the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). *Arthr. Struct. Dev.* **29**: 231–240.

- ONYANGO F.O. & 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. 1993: Laboratory rearing procedure for *Cotesia flavipes*. In Overholt W.A. (ed.): *Proceedings, Group Training Course on Identification of Cotesia spp. Stemborer Parasitoids*. ICIPE Science Press, Nairobi, pp. 29–36.
- OVERHOLT W.A., OCHIENG J.O., LAMMERS P.M. & OGEDAH K. 1994a: Rearing and field release methods for *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a parasitoid of tropical gramineous stemborers. *Insect Sci. Appl.* **15**: 253–259.
- OVERHOLT W.A., OGEDAH K. & LAMMERS P.M. 1994b: Distribution and sampling of *Chilo partellus* (Swinhoe) on the Kenya coast. *Bull. Entomol. Res.* **84**: 367–378.
- PARR M.J., TRAN B.M.D., SIMMONDS M.S.J. & CREDLAND P.F. 1996: Oviposition behavior of the cowpea seed beetle, *Callosobruchus maculatus*. *Physiol. Entomol.* **21**: 107–117.
- POLASZEK A. 1998: *African Cereal Stemborers: Economic Importance, Taxonomy, Natural Enemies and Control*. CABI, Wallingford, 530 pp.
- POTTING R.P.J., OSANE-DANSO F., OVERHOLT W.A. & NGI-SONG A.J. 1993: Host selection in *Cotesia flavipes* parasitoid of tropical stemborers. *Proc. Exp. Appl. Entomol.* **4**: 47–62.
- SALLAM N.M., OVERHOLT W.A. & KAIRU E. 1999: Comparative evaluation of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae) for the management of *Chilo partellus* (Lepidoptera: Pyralidae) in Kenya. *Bull. Entomol. Res.* **89**: 185–191.
- SAS INSTITUTE INC. 2003: *SAS OnlineDoc*[®], version 9.1. SAS Institute Inc., Cary, NC.
- SCHULTHESS F., BOSQUE-PÉREZ N.A., CHABI-OLAYE A., GOUNOU S., NDEMAH R. & GOERGEN G. 1997: Exchanging natural enemies species of lepidopterous cereal stemborers between African regions. *Insect Sci. Appl.* **17**: 97–108.
- SMITH J.W. JR., WEIDENMAN R.N. & OVERHOLT W.A. 1993: *Parasites of Lepidopteran Stemborers of Tropical Gramineous Plants*. ICIPE Science Press, Nairobi, 89 pp.
- SONGA J.M., JIANG N., SCHULTHESS F. & OMWEGA C. 2007: The role of intercropping different cereal species in controlling lepidopteran stemborers on maize in Kenya. *J. Appl. Entomol.* **131**: 40–49.
- SPRUIJT B.M., VAN HOOFF J.A. & GISPEN W.H. 1992: Ethology and neurobiology of grooming behavior. *Physiol. Rev.* **72**: 825–852.
- TAGAWA J., YOSHIDA C., HASHIMOTO T. & SUDARE A. 1987: Effects of mating on the oviposition behaviour of the parasitic wasp, *Apanteles glomeratus* L. (Hymenoptera: Braconidae). *J. Ethol.* **5**: 37–41.
- TAKASU K. & OVERHOLT W.A. 1997: Aggressive behaviour of *Chilo partellus* (Swinhoe) larvae against the parasitoid *Cotesia flavipes* Cameron. *Insect Sci. Appl.* **17**: 131–136.
- TAMS W.H.T. 1932: New species of African Heterocera. *Entomologist* **65**: 1241–1249.
- VAN LENTEREN J.C., RUSCHIONI S., ROMANI R., VAN LOON J.J.A., QIU Y.T., SMID H.M., ISIDORO N. & BIN F. 2007: Structure and electrophysiological responses of gustatory organs on the ovipositor of the parasitoid *Leptopilina heterotoma*. *Arthr. Struct. Dev.* **36**: 271–276.
- VET L.E.M. & DICKE M. 1992: Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* **37**: 141–172.
- VINSON S.B. 1975: Biochemical convolution between parasitoids and their hosts. In Price P.W. (ed.): *Evolutionary Strategies of Parasitic Insects and Mites*. Plenum Press, New York, pp. 14–48.
- VINSON S.B. 1976: Host selection by insect parasitoids. *Annu. Rev. Entomol.* **21**: 109–134.
- VINSON S.B. 1985: The behaviour of parasitoids. In Kerkut G.A. & Gilbert L.I. (eds): *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Pergamon Press, New York, 417–469.
- VINSON S.B. 1991: Chemical signals used by insect parasitoids. *Redia* **74**: 15–42.
- VINSON S.B. 1998: The general host selection behaviour of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biol. Control* **11**: 79–96.
- ZHOU G., BAUMGARTNER J. & OVERHOLT W.A. 2001: Impact of an exotic parasitoid on stemborer (Lepidoptera) population dynamics in Kenya. *Ecol. Entomol.* **11**: 1554–1561.

Received December 1, 2009; revised and accepted January 21, 2010