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Importance of contact chemical cues in host recognition and acceptance by the braconid larval endoparasitoids *Cotesia sesamiae* and *Cotesia flavipes*

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ABSTRACT

The ability of the congeneric braconid parasitoids *Cotesia sesamiae* (Cameron) and *Cotesia flavipes* Cameron to discriminate between stemborer larval cues upon contact was studied using their natural hosts, namely the noctuid *Busseola fusca* (Fuller) and the crambid *Chilo partellus* (Swinhoe), respectively, and the pyralid non-host *Eldana saccharina* (Walker). When the natural host larvae were washed in distilled water, parasitoid behavior was similar to that displayed when in contact with *E. saccharina*, characterized by the absence of ovipositor insertion. When washed host or non-host larvae were bathed with water extracts of their natural host, the parasitoids showed a significant increase in ovipositor insertions. However, the water extracts of host-larvae deposited on cotton wool balls did not induce ovipositor insertion in either *C. sesamiae* or *C. flavipes*. Nevertheless, the extracts enabled the parasitoids to discriminate between natural and non-hosts as indicated by the intensive antennating of the former. For both parasitoids, frass was found to be important in short-range host recognition as indicated by differences in the time spent on antennating between frass sources. In addition, the regurgitants of *B. fusca* and *C. partellus* induced ovipositor insertion in *C. flavipes* only. These results indicated that *C. sesamiae* and *C. flavipes* used different chemical cues for acceptation and oviposition in a stemborer larva, and that *B. fusca* and *C. partellus* shared the same chemical cues to induce oviposition in *C. flavipes*.

1. Introduction

In sub-Saharan Africa, lepidopteran stemborers of the Crambidae, Pyralidae and Noctuidae families are economically important pests of maize and sorghum (Harris, 1990; Polaszek, 1998; Kfir et al., 2002). Due to their widespread distribution and destructive nature, stemborers have been the subject of extensive research in Africa (Calatayud et al., 2006). 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 Africa before the 1930s (Kfir, 1992), they are indigenous to Africa. In East and Southern Africa (ESA), *B. fusca* and *C. partellus* are the most important pests of cereal crops (Seshu Reddy, 1983; Zhou et al., 2001a).

During the early 1990s, the International Centre of Insect Physiology and Ecology (ICIPE) renewed emphasis on biological control with the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) into Kenya from Asia. The parasitoid was released against *C. partellus* in the coastal area in 1993 (Overholt et al., 1994a), where it reduced *C. partellus* densities by over 50% (Zhou et al., 2001b; Jiang et al., 2006). This was to complement the action of the closely related *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), which is the most abundant indigenous larval parasitoid of lepidopteran stemborers in ESA. Parasitism by *C. sesamiae* 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).

The ability of parasitic wasps to successfully utilize cues arising from their habitat in location and discrimination between suitable and unsuitable hosts is vital in determining their efficiency (Vinson, 1985; Godfray, 1994). This involves host habitat and host location, host acceptance and suitability (Vinson, 1976, 1985; Godfray,

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1994). During location of hosts, they 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 and Dicke, 1992; Godfray, 1994).

However, stimuli from the habitat do not convey sufficiently reliable information on the suitability of a host species but are mere indicators of the presence of an herbivore (Vet, 1999). As a result, *C. sesamiae* and *C. flavipes* may often be attracted to plants harboring unsuitable stemborer species (Potting et al., 1993, 1995; Ngi-Song et al., 1996; Obonyo et al., 2008). Since the parasitoids are not able to recognize from a distance their suitable hosts, we hypothesize that they are able to do that at close contact.

In the laboratory, host acceptance and oviposition by the two congeneric wasps are induced by contact with host frass and other host products as well as chemicals emanating from the larval body (Ngi-Song and Overholt, 1997). Therefore, the purpose of this study was to elucidate the role of chemical cues produced by or emanating from larval body surface in the acceptance of hosts by searching female parasitoids.

2. Materials and methods

2.1. Insects

Females of *C. sesamiae* and *C. flavipes* were obtained from laboratory-reared colonies established at ICIPE, Nairobi, Kenya. The *C. sesamiae* colony was obtained from *B. fusca* larvae collected from maize fields in Kitale, Western Kenya, in 2006, while *C. flavipes* was obtained from *C. partellus* larvae collected in coastal Kenya in 2005. Twice a year, field collected parasitoids were added to rejuvenate the colonies. *C. sesamiae* and *C. flavipes* were reared on larvae of their natural hosts, *B. fusca* and *C. partellus*, respectively, according to the method described by Overholt et al. (1994b). Parasitoid cocoons were kept in Perspex cages (30 × 30 × 30 cm) until emergence.

Adults were fed a 20% honey–water solution imbibed in a cotton wool pad and kept under artificial light for 24 h to mate. In all experiments, only 1-day-old, Naïve, putatively mated females were used. The experiments were carried out at 25 ± 2 °C, 50–80% RH, and a 12:12 h (L:D) photoperiod.

Three stemborer species were used in the study: *B. fusca* and *C. partellus* as natural hosts of *C. sesamiae* and *C. flavipes* respectively, and *E. saccharina* as a non-host to either parasitoid (Overholt et al., 1997). Preliminary tests showed that neither parasitoid attacked *E. saccharina* larvae (Obonyo M., personal observation). By contrast, over 50% of *C. flavipes* inserted the ovipositor into the non-host *B. fusca* (Obonyo, 2009).

E. saccharina and *B. fusca* were collected from maize fields in the Western Province, while *C. partellus* originated from maize grown in the coastal region of Kenya. The larvae were reared on artificial diet according to the methods described by Ochieng et al. (1985) and Onyango and Ochieng'-Odero (1994). Three times a year feral stemborer larvae from their respective locations were added to rejuvenate the colonies. Third and fourth larval instars were introduced into jars (10 × 20 cm) each containing pieces of maize stem and left for 48 h to feed and produce frass. Thereafter, the larvae and the frass were used in the experiments.

2.2. Experimental procedure

2.2.1. Influence of washing and bathing of larvae

In order to assess the influence of chemical cues on the larval cuticle, for each stemborer species a total of 200 larvae were washed together in 200 ml of distilled water. The 200 ml of extract was filtered (Whatman No. 1) to remove debris and used in the experiments "to bathe" individually the larvae previously washed. All "body extracts" were collected in a cold room maintained at 10 °C and kept frozen before being used in the assays.

Prior to bathing, third and fourth larval instars from maize stems were individually introduced into glass vials (7.5 × 2.5 cm) half filled with distilled water, gently swirled before pouring out the water. This procedure was repeated 5 times. Afterwards, the larvae were let to dry on a paper towel. The bathing of larvae was done as follows: an aliquot of the stock solution containing the "body extract" was used to bathe the previously washed larva. A single larva was dipped in the extracts five times for about two seconds. The larva was then placed on paper towel to drain-off excess water and transferred into an arena of 5.5 cm diameter × 1.5 cm height for use in the bioassays. The behavior of the wasp encountering the larva (washed or bathed) was monitored immediately after its release into the arena for a maximum of 120 s (in a pre-experiment, it was shown that the activity of the extracts rapidly diminished after 120 s). Unwashed host and non-host larvae were also tested as controls. Twenty replicates consisting of a single wasp and larva were used. Each wasp and larva was used only once. The percentage of wasps probing the larvae with their ovipositor was calculated.

2.2.2. Influence of larval "body extracts"

Twenty μl of "body extract" were deposited on a 3 mm cotton wool ball and presented to a single wasp in a glass Petri dish of 5.5 cm diameter and 1.5 cm height. The behavior of the wasp was then monitored for a maximum of 120 s. For each wasp, the duration of antennation, which indicates host recognition (Obonyo, 2009), was recorded. The percentage of stinging was calculated from 20 wasps tested per extract. The wasp, the cotton wool ball with extracts and the arena were replaced each time.

2.2.3. Influence of fresh frass

About 0.01 g of fresh frass collected directly from the aforementioned larvae fed on maize stems for 48 h was added to a glass Petri dish of 5.5 cm diameter × 1.5 cm height together with a female wasp. Each wasp was given a maximum of 120 s to respond to the frass. Only the time the wasp was in contact with the frass was considered in the analysis. For each wasp, the duration of antennation of the frass was recorded, after which the wasp, the frass and the arena were replaced. The mean duration of antennation and the percentage of stinging were calculated from 20 replicates each consisting of a wasp and frass.

2.2.4. Influence of frass extract

For each stemborer species, about 1.0 g of fresh frass was suspended in 10 ml distilled water, vortexed and then filtered (Whatman No. 1). The extracts were collected in a cold room (around 10 °C) and freeze dried. The freeze-drying process does not alter the activity of the extracts (Obonyo, 2009). Thereafter each extract was resuspended in 1 ml of distilled water to form a stock solution. The stock solution was divided into portions of working solution (100 μl) in 0.2 ml tubes, while the rest was kept frozen. During the experiments, 20 μl of the frass extract of a particular stemborer species was deposited on a 3 mm ball of cotton wool and introduced in a glass Petri dish of 5.5 cm diameter × 1.5 cm height together with a wasp and covered. Observations were carried out for 120 s, after which the wasp, the extract source and the arena were replaced. For each wasp, the duration of antennation on the cotton wool ball was recorded. The mean duration of antennation and the percent of cotton balls stung were calculated from 20 replicates each consisting of a wasp and a frass extract.

2.2.5. Influence of regurgitant

For each stemborer species, third and fourth instar larvae were recovered from maize stems after 48 h of feeding. A single larva held by a soft forceps was gently squeezed behind the head and a capillary tube was used to collect oral extracts. The process was repeated for several larvae and the total mass of the extract recorded each time. The volume of regurgitant was estimated by weighing.

The collection of regurgitant was done in a cold room at 10 °C. The extract was then diluted into 200 µl distilled water in a 1 ml tube, filtered (Whatman No. 1) and freeze dried. The freeze-drying process does not alter the activity of the extracts (Obonyo, 2009). It was later resuspended in distilled water to obtain a solution of about 20 µl of regurgitant per 100 µl for each stemborer species. The 20 µl aliquot was deposited on a 3 mm ball of cotton wool, which was exposed to the parasitoid. Observations were done for a maximum of 120 s, after which the wasp, the extract source and the arena were replaced. For each wasp, the duration of antennation on the ball of cotton wool was recorded. The mean duration of antennation and the percentage of stinging were calculated from 20 replicates each consisting of a wasp and a regurgitant.

2.3. Statistical analysis

All means were separated by Student–Newman–Keuls test following one-way analysis of variance (ANOVA). Percentages of stinging and durations were arcsin and log transformed, respectively, then subjected to ANOVA (SAS Institute Inc., 2003). Untransformed results are presented in the tables.

3. Results

3.1. Washed and bathed larvae

As controls, 0% and 85% of the larvae were stung by *C. sesamiae* when using unwashed non-host (i.e. *E. saccharina*) and host (i.e. *B. fusca*) larvae, respectively (Table 1). When the host larvae were washed no stinging or ovipositor insertion was observed. The stinging activity was recovered when they were bathed with *B. fusca* extracts. There was a significant reduction in the incidence of stinging when *B. fusca* was bathed with the "body extracts" of the non-host *E. saccharina* compared to washed *B. fusca* larvae bathed with the "body extracts" of *B. fusca*. In addition, the number of stung *E. saccharina* bathed with *B. fusca* extracts was similar to the number of stung washed *B. fusca* bathed with *B. fusca* extracts.

Similarly, as controls, 0% and 85% of the larvae were stung by *C. flavipes* when using unwashed non-host (i.e. *E. saccharina*) and host (i.e. *C. partellus*) larvae, respectively (Table 1). When the host larvae were washed no stinging or ovipositor insertion was observed. The stinging activity was recovered when they were bathed with *C. partellus* extracts. The percentage of stinging decreased significantly when washed *C. partellus* larvae were bathed with *E. saccharina* extracts compared to washed *C. partellus* larvae bathed with the "body extracts" of *C. partellus*. There was no significant difference between *E. saccharina* larvae bathed with *C. partellus* extracts and washed *C. partellus* larvae bathed with *C. partellus* extracts.

In this experiment, each time the wasp was stinging the larva, antennation on the larval body was not clearly seen or too fast to estimate correctly its duration. Moreover, it was not occurring on the larval body in the absence of stinging behavior by the parasitic wasp.

3.2. Influence of larval "body extracts"

When exposed to cotton wool imbibed in larval "body extracts", no stinging activity was recorded among the wasps regardless of the host species tested. Only antennation was observed and thus considered.

For *C. sesamiae*, there was no significant difference in duration of antennation with either *C. partellus* or *E. saccharina* as the host, while significantly more time was spent antennating the "body extracts" of their natural host *B. fusca* (Table 2). *C. flavipes* females spent more time antennating "body extracts" of *B. fusca* and *C. partellus* than those of *E.* saccharina; it spent significantly more time antennating *C. partellus* extracts than *C. sesamiae*.

3.3. Influence of fresh frass, frass extracts and regurgitant

C. sesamiae females spent significantly more time antennating the fresh frass of *B. fusca* than that of *C. partellus* and *E. saccharina*, while *C. flavipes* females spent more time on fresh frass of *C. partellus* and *B. fusca* than that of *E. saccharina* (Table 3). No stinging activity was recorded on fresh frass regardless of host and parasitoid species tested.

For *C. sesamiae* females, the longest antennation time was spent on frass extracts of *B. fusca* and the shortest on that of *C. partellus*, while for *C. flavipes*, there was no significant difference among the frass extracts (Table 3). Overall, the duration of antennation on frass extract was significantly higher for *C. flavipes* than *C. sesamiae*

Table 1
Percentage stinging (%, mean ± SE, $n = 20$) by female wasps in response to the larval treatment.

Parasitoid species	Larval treatment	% Stinging
Cotesia sesamiae	Unwashed *B. fusca*	85 ± 10d
	Washed *B. fusca*	0a
	Unwashed *E. saccharina*	0a
	Washed *B. fusca* painted with *B. fusca* extract	65 ± 11c
	Washed *B. fusca* painted with *E. saccharina* extract	25 ± 10ab
	Unwashed *E. saccharina* painted with *B. fusca* extract	50 ± 11bc
		($F = 16.3$, $df = 5$, $P < 0.0001$)
Cotesia flavipes	Unwashed *C. partellus*	85 ± 6c
	Washed *C. partellus*	0a
	Unwashed *E. saccharina*	0a
	Washed *C. partellus* painted with *C. partellus* extract	75 ± 10c
	Washed *C. partellus* painted with *E. saccharina* extract	30 ± 10b
	Unwashed *E. saccharina* painted with *C. partellus* extract	65 ± 11c
		($F = 13.6$, $df = 5$, $P < 0.0001$)

For each parasitoid species, means within a column followed by the same letter(s) are not different at $P \leqslant 0.05$ (Student–Newman–Keuls test following ANOVA).

Table 2
Duration of antennation (in seconds, mean ± SE, $n = 20$) by female wasps on cotton wool treated with "body extracts" from stemborer larvae.

Extract tested	Parasitoid species		(F, df, P)[a]
	Cotesia sesamiae	*Cotesia flavipes*	
H_2O	0a	0a	(0, 1, 0)
B. fusca body	31.6 ± 6.3bA	20.9 ± 5.3cA	(1.7, 1, 0.2030)
C. partellus body	0.2 ± 0.1aA	39.5 ± 9.7cB	(16.5, 1, 0.0002)
E. saccharina body	1.0 ± 0.6aA	2.8 ± 1.0bA	(2.5, 1, 0.1195)
(F, df, P)[a]	(24.1, 3, 0.0001)	(10.9, 3, 0.0001)	

[a] Means within a column followed by the same lower case letter(s) and means within row followed by the same capital letter(s) are not different at $P \leqslant 0.05$ (Student–Newman–Keuls test following ANOVA).

Table 3
Duration of antennation (in seconds, mean ± SE, $n = 20$) by female parasitoids on fresh frass, frass extracts and regurgitants.

Sample tested	Host species	*C. sesamiae*	*C. flavipes*	(*F*, *df*, *P*)[a]
Fresh frass	*B. fusca*	117.9 ± 19.0bA	112.6 ± 15.7bA	0.05, 1, 0.8324
	C. partellus	21.7 ± 6.4aA	142.9 ± 25.6bB	21.1, 1, 0.0001
	E. saccharina	18.0 ± 5.6aA	61.3 ± 17.8aB	5.4, 1, 0.0260
	(*F*, *df*, *P*)[a]	6.75, 2, 0.0023	4.26, 2, 0.0188	
Frass extracts	*B. fusca*	2.7 ± 0.6bA	26.1 ± 11.6aB	6.4, 1, 0.0160
	C. partellus	1.1 ± 0.3aA	21.7 ± 5.9aB	34.5, 1, 0.0001
	E. saccharina	1.7 ± 0.4abA	10.1 ± 3.6aB	8.7, 1, 0.0054
	(*F*, *df*, *P*)[a]	3.54, 2, 0.0354	1.12, 2, 0.3330	
Regurgitants	H_2O	0a	0a	
	B. fusca	1.4 ± 0.2bA	16.7 ± 5.3bB	8.3, 1, 0.0066
	C. partellus	1.4 ± 0.6bA	25.3 ± 7.1bB	11.2, 1, 0.0019
	E. saccharina	0a	0a	
	(*F*, *df*, *P*)[a]	8.0, 3, 0.0001	6.8, 3, 0.0004	

[a] For each sample tested, means within a column followed by the same lower case letter(s) and means within row followed by the same capital letter(s) are not different at $P \leqslant 0.05$ (Student–Newman–Keuls test following ANOVA).

regardless of the host species tested. In addition, no stinging activity was recorded on the cotton wool ball imbibed in the frass extract regardless of the host and parasitoid species tested.

The regurgitant of *E. saccharina* elicited less response by either parasitoid than that of *B. fusca* and *C. partellus* with no significant difference between the latter two (Table 3). The regurgitant of *C. partellus* and *B. fusca* elicited a longer duration of antennation in *C. flavipes* than *C. sesamiae*. There was no difference in the stinging behavior of *C. sesamiae* among host species offered. Forty percent of the *C. flavipes* females attempted to sting the balls of cotton wool containing the regurgitant of *C. partellus* or *B. fusca*, whereas no stinging was observed with the regurgitant of *E. saccharina*.

4. Discussion

The present findings show that the chemical cues present on the stemborer larval body are involved in host recognition and acceptance by *C. sesamiae* and *C. flavipes*. The wasps recognized the "body extracts" of their natural hosts, which elicited ovipositor probing. Both parasitoids also inserted their ovipositors in the non-host *E. saccharina* when it was bathed with extracts of their natural hosts, whereas there was a marked reduction in ovipositor probing on their natural hosts when they had been bathed with *E. saccharina* extracts. In addition, as also reported by Ngi-Song and Overholt (1997), the wasp did not recognize or sting its natural host when it has been washed.

The wasps did not probe the body extracts of their natural hosts on cotton wool balls. However, the same extracts used on mobile larvae induced oviposition. Thus, the cotton wool probably lacked certain tactile stimuli required for final acceptance and oviposition.

Olfactometric studies by Ngi-Song and Overholt (1997) and Obonyo et al. (2008) showed that at short to medium distance the two parasitoids did not discriminate between host plants infested by suitable and unsuitable stemborer species. For *C. sesamiae*, the present findings indicated that at close contact the parasitoid was able to distinguish between host species as antennation time was longer on cotton wool imbibed with body extracts and on frass from the natural host when compared to that of the non-host. However, *C. flavipes* was not able to distinguish between body extracts or frass of the suitable *C. partellus* and the unsuitable *B. fusca*, though it avoided the non-host *E. saccharina*. This indicates that *C. partellus* and *B. fusca* share the same contact chemical stimuli from the body for host acceptance by *C. flavipes*. In contrast to *C. sesamiae,* which coevolved with *B. fusca* (the host, from which it was recovered), the results obtained on *C. flavipes* cannot be explained by looking at the evolutionary history of the parasitoids: the exotic wasp *C. flavipes* coevolving with *C. partellus* (originated from the same geographic area [Asia] and from which it was recovered). The ability to discriminate between hosts is crucial for the establishment of the exotic *C. flavipes*, which encounters many new suitable and unsuitable hosts in its new environment in Africa (Le Rü et al., 2006a,b). According to Takasu and Overholt (1997) up to 50% of the foraging wasps are killed in stem tunnels due to the aggressive behavior of the host. The life history theory predicts that when a parasitoid has a high mortality risk at each oviposition, there would be a high selectivity to avoid waste of progeny (Ward, 1992). Thus, the wasps come under selection pressure to recognize their hosts with minimal risk of injury, i.e. before they enter the feeding tunnel of the host larvae. In fact, during stem tunneling, the frass is usually pushed outside. Thus, the parasitoid has to be able to recognize the cues of a suitable host feeding inside the stem before entering the tunnel, which reduces its risk of being killed by an unsuitable host. Such risk can be limited for *C. sesamiae* but not for *C. flavipes* not able to distinguish frass of *C. partellus* to those of *B. fusca*. In this case, the unsuitable borer species would form a reproductive sink to *C. flavipes* thereby negatively affect the efficiency of the parasitoid used as biological control agent. This may explain the regional variability of parasitism rates of *C. flavipes* (Jiang et al., 2006) as the speed of establishment would depend on the stemborer species composition in a given area. For example, in the mid-altitude of Kenya, where both *B. fusca* and *C. partellus* occur, parasitism by *C. flavipes* are typically below 10% (Songa et al., 2007) compared to 20–30% at the coast where the prevalent borer species are all suitable to the parasitoid (Jiang et al., 2006). On the other hand, the low ability to discriminate between hosts may also explain why *C. flavipes* was such a successful new association biological control agent (Hokkanen and Pimentel, 1984) in the Americas (Weidenmann and Smith, 1997).

Van Leerdam et al. (1985) reported that host searching in *C. flavipes* is mediated by water-soluble chemicals present in the frass, which, when extracted, elicited a characteristic host searching response. Similarly in the present study, *C. sesamiae* and *C. flavipes* antennated the ball of cotton wool containing frass extracts of their natural and suitable hosts. However, the shorter antennation activity on frass extracts for either parasitoid with their respective suitable hosts as compared to fresh frass might be the results of the low activity of the water extract due to insolubility in water of some components of frass such as fatty acids and lipids (Kuwahara et al., 1983; Takabayashi and Takahashi, 1989; Horikoshi et al., 1997; Roux et al., 2007).

Spiting among lepidopteran stemborer larvae is often a defense mechanism in response to attacks by parasitoids (Takasu and Overholt, 1997). However, host regurgitants can also be an important source of short-range attractants and arrestants for parasitoids (Cobert, 1971). These regurgitants are produced at the final stage of host encounter during oviposition attempts. Regurgitants of *B. fusca* and *C. partellus* but not that of *E. saccharina* induced stinging activity in *C. flavipes*. Thus, the parasitoid was able to distinguish between regurgitants of host species, thus the regurgitants appeared to be more or less host specific. This corroborates results by Potting et al. (1997) who showed that extracts from the mandibular glands of *C. partellus* elicited behavioral response among *C. flavipes* females. In contrast to *C. flavipes*, *C. sesamiae* did not sting the balls of cotton wool imbibed by the regurgitant of its natural host *B. fusca*. Thus, the compounds eliciting stinging behavior in *C. sesamiae* appeared not to be present. Previous reports have indicated that both plant juice and host regurgitant enhance host-searching behavior of *C. glomerata* (L.) (Hymenoptera: Braconidae) through production of infochemicals (Sato, 1979; Horikoshi et al., 1997). It is therefore probable that

for *C. sesamiae* plant juice associated with regurgitant should elicit such stinging behavior but not regurgitant alone. Moreover, since the activity of the regurgitants rapidly diminished after 120 s (Meshack Obonyo, Pers. Observ.) this can be the result of the presence of enzymes or thermo-labile compounds in the regurgitants, denaturing easily from ambient temperature, which are eliciting behavioral response among *C. flavipes* females. Enzymes in the regurgitant such as ß-glucosidases have been revealed in *Pieris brassicae* (L.) (Lepidoptera: Pieridae) larvae, and are causing a release of volatiles in Brassicacea plants that attract the parasitoids (Mattiacci et al., 1995). In our case, the stimuli eliciting antennation and stinging behavior of *C. flavipes* are probably not coming from volatiles but most probably from non-volatiles compounds, such as enzymes or thermo-labile compounds.

It can be concluded that the congeneric parasitoids *C. sesamiae* and *C. flavipes* primarily rely on chemical cues that are closely linked to the prospective host species in discriminating between natural host and non-host larvae. Furthermore, the importance of these chemicals appears to be directly related to the proximity of the parasitoid to the host larvae and to products of larval feeding activity. *C. sesamiae* and *C. flavipes* probably use different chemical cues for host recognition and acceptance. Thus, niche segregation for these two parasitoid species would have a chemical basis.

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