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Host location behaviour of gravid females in the Mediterranean corn borer *Sesamia nonagrioides*: external morphology of antennae and ovipositor sensilla

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Abstract

The behaviour of gravid females of *Sesamia nonagrioides* (Lefebvre) during host plant location in a wind tunnel is described using an ethogram and compared with unmated females, males and gravid females deprived of antennae. The sequence of behaviours exhibited by the majority of the intact gravid females began with a resting period followed by the unfolding of the antennae towards the plant plume and wing buzzing. The females then started to walk while fanning their wings, displayed erratic flights striking the walls of the wind tunnel, and finally executed a zigzagging flight oriented towards the maize plant. The behavioural sequence was disrupted in the absence of both antennae. One third of the amputated females took flight; although the flights were not oriented, they attempted to fly towards the plant. The external morphology of the antennae's sensilla in females and males and of the ovipositor was studied using scanning electron microscopy and staining with silver nitrate to detect the presence of pores. Males and females showed sexual dimorphism in length, size, shape, and the presence and distribution of sensilla along the antennae, which may be related to differences in the odor sources that each sex seeks. The distribution of sensilla chaetica and sensilla trichoidea showed a type of compartmentalization with structures for the detection of volatiles concentrated on the middle flagellomeres and structures for mechano- and gustative-reception concentrated on the distal flagellomeres. The only argyrophilic sensilla on the ovipositor were type I, located primarily on the ventral surface of the ovipositor to detect chemical stimuli on the substrate.

Key words: host location behaviour, host acceptance, antennal morphology, ovipositor sensilla, scanning electron microscope, antennectomy.

Introduction

After mating, lepidopteran females must find an appropriate host plant for laying their eggs, and this plant may sometimes be located at some distance from the females. To find a suitable oviposition site, chemical, visual and mechanical cues are crucial. Gravid lepidopteran females follow a behavioural sequence that includes searching, orientation, encounter, landing, surface evaluation and acceptance of the host (Renwick and Chew, 1994). The pre-landing process includes searching, orientation and encounter with the host; visual and olfactory volatile chemical cues govern these three steps, whereas chemical contact cues and the physical characteristics of the plant are important during landing, evaluation and acceptance (Renwick and Chew, 1994; Hora and Roessingh, 1999; Finch and Collier, 2000; Calatayud *et al.*, 2008; Heinz, 2008). These processes are mediated within the insect's central nervous system through the integration of numerous sensory inputs including olfactory and gustatory semiochemical cues and physical information such as plant colour, shape and texture (Bruce *et al.*, 2005). The sensory receptors involved are present on the tarsi, antennae, proboscides and ovipositors of lepidopterans (Renwick and Chew, 1994; Hallberg and Hansson, 1999), and the different types of sensilla located on these organs play a key role in behaviour during adult life (Schneider, 1964; Hu *et al.*, 2009). The role of vision in insect interactions with host plants has received relatively little attention because it is assumed that chemical cues are the ultimate sensory determinants of host location (Reeves, 2011).

The Mediterranean corn borer, *Sesamia nonagrioides* (Lefebvre), is the most important borer pest of maize crops in northeast Spain. It is an oligophagous insect that feeds primarily on the Gramineae family, and maize is its major host plant in Mediterranean countries (Prota and Cavalloro, 1973; Konstantopoulou *et al.*, 2004; Margaritopoulos *et al.*, 2007). *S. nonagrioides* larvae overwinter in maize stubble, and in the spring, they pupate to produce the first flight of adults. Regardless of crop rotation, females usually spend the first nights in the emergence field until mating, ovipositing in the same field if young maize plants are present or flying to nearby crop fields to find a suitable host if they are not (López *et al.*, 1999). The eggs of *S. nonagrioides* are laid in small clusters under the sheath of lower leaves. Neonate larvae move immediately into the stem, where they complete their development endophytically, and after 1-2 months, depending on the weather, the emerging adults initiate a new cycle (Anglade, 1972). Damage is inflicted by the second or third larval generations occurring in the study region. Maize yield is affected by corn borer tunnelling, which interferes with movement of nutrients within the plant and increases the risk of stalk lodging. In addition, corn borer damage can affect plant health by vectoring *Fusarium moniliforme* and facilitating fungal infections (Sobek and Munkvold, 1999).

Although *S. nonagrioides* causes major damage to maize crops in several Mediterranean countries and its sex pheromone composition is well known (Sreng *et al.*, 1985; Mazomenos, 1989; Sans *et al.*, 1997; Krokos *et al.*, 2002), it is a species that has been relatively poorly studied, particularly with respect to its behaviour. Lopez *et al.* (2003) studied its mating behaviour, Solinas and Trona (2002) studied antennal sensilla using scanning electron microscopy and transmission electron microscopy, Robert and Frerot (1998) described certain aspects of the oviposition behaviour, and Konstantopoulou *et al.* (2002; 2004) studied the corn chemical cues that stimulate oviposition in gravid females. However, despite the above studies, the relation between behaviour, chemical cues, vision, and antennal and ovipositor sensilla morphology remains unclear.

In previous works, we demonstrated that gravid females detect and respond to their own pheromone and to the pheromone components of other sympatric species (Cruz and Eizaguirre, 2015a) and that gravid females without visual cues are able to discriminate between corn varieties by relying predominantly on olfactory cues in response to the plant volatile components (Cruz and Eizaguirre, 2015b).

To further our knowledge of the factors that allow host location and determine acceptance by gravid females, it would useful to determine the steps involved in host location behaviour and how females respond to different olfactory stimuli. A comparison between female and male behaviour and antenna morphology will help to discriminate the behavioural characteristics of the gravid female from the responses of unmated females or males and the sensilla involved in these responses.

The aim of this work was to identify the behavioural sequence performed by gravid females of *S. nonagrioides* during host plant location and acceptance, thus increasing our knowledge of the role of olfaction and mechanoreception in them. To this end, we describe the host location behaviour of intact gravid females, comparing their behaviour with unmated females and males. To assess the importance of the visual cues, we also compared the behaviour of intact gravid females with gravid females in which one or both antennae were amputated. Finally, we compared the types, density and distribution of sensilla on male antennae, female antennae and on the ovipositor. This study will further the understanding of the sensory systems involved in chemical communication and host location by *S. nonagrioides.*

Materials and methods

Plants

Maize (*Zea mays*) seeds of the cultivar Dracma® (Syngenta Seeds, Switzerland) were sown in regular potting soil in ceramic pots (diameter 8 cm, depth 9 cm). The plants were maintained in a greenhouse under a 16:8 h L:D photoperiod at 25 ± 2 °C and 70% RH. Between 15 and 20 days after planting, seedlings with three to five leaves were used for the experiments.

Insects

Adults of *S. nonagrioides* were obtained from cultures maintained at the Entomology laboratory (Agrotecnio Center, University of Lleida, Spain) that originated from larvae collected on commercial maize fields in Lleida Province. They were maintained at a temperature of 20 °C with a 16:8 h L:D photoperiod. Individuals were separated as pupa and sexed based on external morphological differences. To obtain mated females, newly emerged males and females in a ratio of 2♂:1♀ were transferred to a plastic container (50 × 50 × 50 cm) containing a corn plant with 3-4 leaves. After 24 h, the moths were assumed to have mated and were used in the experiments. The females were later dissected to check for the presence of spermatophores in the bursa copulatrix, which indicates successful mating. Unmated 24-hour-old females were also tested.

Host location behaviour

The experiments on the host location behaviour of *S. nonagrioides* were conducted in a wind tunnel with laminar flow (length 151 cm, width 47 cm, height 44 cm) (Gemeno *et al.*, 2006) at 25 ± 2 °C, 50% RH and a wind velocity of 0.3 m/s. The adults were placed into the tunnel 10 minutes prior to the onset of scotophase, and they were observed from the onset until the third hour of scotophase. The timing (timetable and duration) of the experiments was determined in a preliminary experiment that entailed observing the behaviour of gravid females every fifteen minutes during the entire scotophase (results not shown). The tunnel was illuminated with a red light resulting in a lux intensity of 4 and 5 at the ceiling and floor of the tunnel, respectively.

Groups of 4 to 6 gravid females were placed on the floor, at the downwind end of the tunnel. The insects were located at a distance of 125 cm from a maize plant, which was located at the upwind end of the tunnel and served as the odour source. Observations were made on a total of 35 females. Moth behaviour was divided into two phases according to Thompson and Pellmyr (1991) and Ramaswamy (1988): pre-landing and landing. Pre-landing included the following behaviours: resting (Quiet), antennal movement (AM), wing buzzing (WB), walking and wing fanning (WWF), erratic flights and abdomen circles (AC), and upwind flying toward the plant (FLY). Landing included the following behaviours: leaf contact (LC); moving from the leaves to the stem and testing the maize plant with the antennae, tarsi and ovipositor (DC); moving around the stem and checking repeatedly with the ovipositor, antennae and tarsi until a suitable place to lay the eggs was found (SC); and oviposition (O). The response behaviours of 20 unmated females and males to plant odour was also tested. Calling and ovipositing females were removed from the wind tunnel. For the host location behaviour, the length of the pre-landing and landing periods and the percentage of gravid females that completed each behaviour in the sequence was recorded.

Roles of olfaction, vision, and mechanoreception

To compare the roles of olfaction, vision, and mechanoreception during the host location process, a wind tunnel experiment was designed. In this experiment, the response of intact gravid females to the maize plant was compared with the response of gravid females deprived of one or both antennae. Mated females were anesthetized with CO_2 24 hours before the onset of scotophase, and one or both antennae were amputated with fine forceps; control mated females with intact antennae were also anesthetized.

Wind tunnel conditions were similar to those in the host location experiment. A minimum of 35 intact females with one or both antennae amputated were used per treatment, and each insect was tested only once. For each mated female, the following behaviours were recorded: wing buzzing and fanning (WB), flying upwind (FLY), landing at the source (LS), and oviposition (O). Data were subjected to a χ^2 test using JMP Version 9 (SAS Institute Inc., Cary, NC). For all comparisons, the level of $P = 0.05$ was considered significant.

SEM

The ovipositors of females and the antennae of females and males were carefully excised from the base with fine forceps under a stereomicroscope. The number of flagellomeres, the length of each flagellomere and the total length of the antenna were determined for 10 individuals of each sex. The dissected antennae and ovipositors were dried at room temperature for three days. They were then mounted on stubs with conductive double-sided black adhesive tape, coated, using a sputter coated (Balzer SCD 050, Leica microsystems - Wetzlar, Germany, with gold and examined and photographed with a Zeiss DSM 940 - Oberkochen, Germany). For each sex, the average length and number of each type of sensillum in the scape-pedicel, and the first, intermediate, and distal flagellomeres was determined for 10 antennae from different individuals. All data obtained on the length and abundance of sensilla on the antennae and ovipositors were analyzed using a *t*-test; the level of $P = 0.05$ was considered significant.

Presence of pores: silver nitrate staining

The presence of pores on the antennae and ovipositor of 10 individuals of each sex was detected by staining with silver nitrate. The antennae of both sexes and the ovipositors of *S. nonagrioides* females were carefully excised with fine forceps, immersed for 48 h in 70% ethanol containing 1 M silver nitrate and then cleared overnight in xylene and mounted in DPX for light microscope observations following the methodology used by Maherand Thiery (2004).

Results

Host location behaviour

The host location process performed by gravid females of *S. nonagrioides* was divided in the two phases: pre-landing and landing (figure 1). Ten minutes before the onset of scotophase, gravid females were placed at the downwind end of the wind tunnel and remained resting (Quiet) for at least five minutes after the beginning of scotophase before showing any activity. The quiet period lasted 5-90 minutes depending on the female. The first sign of activity involved the unfolding of the antenna (separation) from the body towards the plant volatile plume (AM); during this step the females remained in the initial location for approximately 6 minutes. The majority of the females (57%) then began to buzz their wings (WB), keeping the ventral portion of the antennae directed towards the plant odour source for an average of 5 minutes and 50 seconds. After WB, 40% of the females started to walk while simultaneously fanning their wings (WWF); during this phase, the WB stopped but the AM continued, and the insects began to walk a few centimetres into the first third of the wind tunnel for an average of 13 seconds. The females then exhibited the next step in the behavioural sequence: for approximately eight minutes, 52.8% of females performed erratic flights, striking the walls of the wind tunnel, with some pauses during which they performed circular movements with the abdomen (AC) against the wind tunnel walls and drummed the substrate with their antennae. Finally, the females (52.8%) executed a zigzagging flight (FLY) oriented towards the maize plant, ending the pre-landing behaviour. Although 40% of the females showed this complete sequence, some individu-

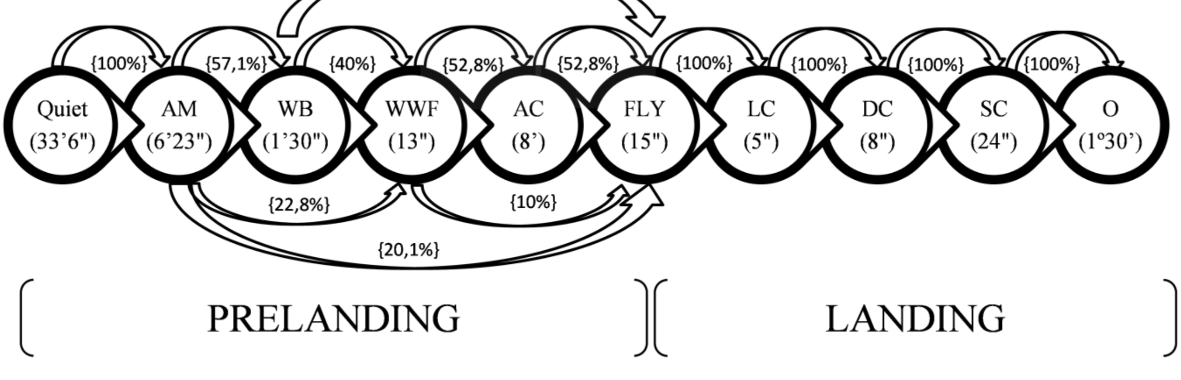

Figure 1. Ethogram of oviposition behaviour of *S. nonagrioides* in the wind tunnel. Behaviours described: Quiet, resting; AM, antennal movement; WB, wing buzzing; WWF, walking and wing fanning; AC, abdomen circles; FLY, upwind flying toward plant; LC, leaf contact; DC, contact of antennae, tarsi and abdomen with stem; SC, stem contact; O, oviposition. Numbers in parenthesis represent the average time in seconds spent in each behaviour. Numbers in brackets represent the percentage of females passing to the next behavioural step; n = 35 females.

als did not show all of the described steps: 20.1% of the females flew immediately after unfolding the antennae; 17.1% flew after buzzing their wings; 22.8% walked while fanning their wings immediately after unfolding the antennae and 10% flew after walking while fanning their wings.

The landing behaviour was more homogeneous than the pre-landing behaviour; during landing, all behaviours were performed by all females in a similar and consecutive manner. Following the oriented flight, all *S. nonagrioides* females landed on the upper leaves of the maize plant (LC), and then began to walk down, sweeping and drumming the plant with the antennae, tarsi and ovipositor (DC). When the females reached the lower part of the stem they circled the plant, touching it primarily with the ovipositors and antennae until they found an appropriate oviposition site (SC), inserted the ovipositor underneath the stem sheath and laid the eggs (O); this final step took approximately 90 minutes. Finally, the females flew to the wind tunnel walls and folded their antennae, remaining quiet during the rest of scotophase. Unmated females performed none of the behaviours described above, showing only a small erratic flight before initiating calling behaviour. Males also did not respond to the plant odour.

Roles of olfaction, vision, and mechanoreception

As in the host location experiment, the females with two intact antennae used their antennae in all of the behavioural steps; we wanted to determine whether gravid females were able to locate the maize plant and to lay eggs in the absence of one or both antennae using visual cues.

The unilateral antennectomy did not significantly affect the behavioural sequence in comparison with the females with two antennae (χ^2 test = 0.156, 0.62, 0.9 and 0.9 for WB, FLY, LS and O, respectively; df = 1, $P > 0.05$). In contrast, all behavioural steps were disrupted in the females without antennae in comparison with the intact females (χ^2 test = 19.6, 16.6, 24 and 28.5 for WB, FLY, LS, and O, respectively; df = 1, $P < 0.05$). A small proportion of females deprived of both antennae (22%) showed wing-fanning behaviour. They then began to walk rapidly in circles in the first third of the tunnel followed by a quick flight in a random manner (28%) along the entire tunnel, striking against the walls; sometimes they landed and remained on the walls and sometimes they recommenced the random flights. Occasionally, the amputated females flew close to the maize plant, striking the leaves without landing on them. In no instance did we observe the zigzagging flight typical of positive anemotaxis or the landing induced by plume-oriented flight. Of these females, 12% managed to land on the maize plant, attempting to test the plant and showing the (DC) behaviour described in the host location behaviour sequence. Only 3% of these females reached the stem and laid the eggs; the remaining 9% stayed on the upper leaves or recommenced the random flights (figure 2).

Antennal external morphology and presence of pores in sensilla

S. nonagrioides antennae showed sexual dimorphism in both size and shape. The total length of the antenna was greater in females than in males (7.52 mm and 6.62 mm, respectively), although females had fewer flagel-

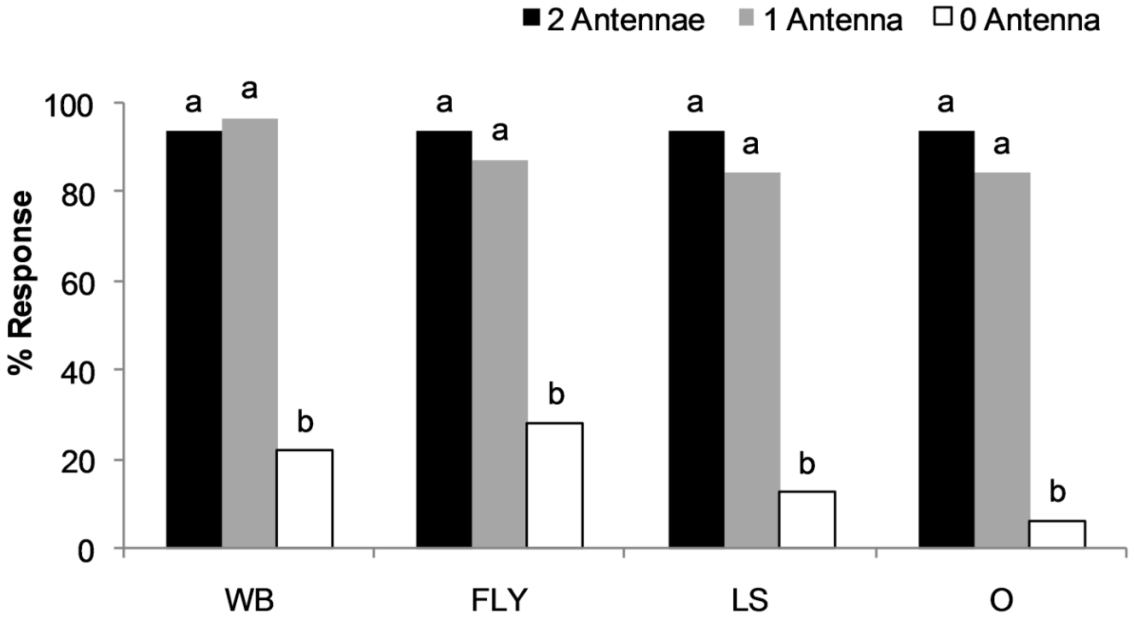

Figure 2. Host location behaviour of gravid females of *S. nonagrioides*, intact or deprived of one or two antenna, in the wind tunnel. Black bars indicate the percent response with both antennae, grey bars represent the percent response with unilateral antennectomy, and white bars indicate the percent response with total antennectomy; n = 35. Columns with different lowercase letters are significantly different for a specific behaviour; columns with different capital letters are significantly different between behaviours (χ^2 test, $P < 0.05$). Behavioural responses include wing buzzing (WB), flying (FLY), landing on the source (LS) and oviposition on the maize plant (O).

Table 1. Length (mean ± SE) in mm of the pedicel, scape and selected antennal flagellomeres of *S. nonagrioides* (NP, no presence) (n = 10 antennae per sex). Means with different letters in the same column indicate significant differences between females and males (*t*-test, $P < 0.05$).

Sex	Scape	Pedicel	Flagellomere							
			10	20	30	40	50	51	59	Total
F	0.28 ± 0.01a	0.14 ± 0.01a	0.13 ± 0.01a	0.15 ± 0.01a	0.13 ± 0.01a	0.14 ± 0.01a	0.1 ± 0.0a	0.1 ± 0.0a	NP	7.5 ± 0.1a
M	0.25 ± 0.01b	0.15 ± 0.01a	0.11 ± 0.01b	0.13 ± 0.0b	0.13 ± 0.0a	0.11 ± 0.01a	0.09 ± 0.0a	0.1 ± 0.0a	0.12 ± 0.0	6.6 ± 0.1b

lomeres than males (54 and 59, respectively). The scape was longer in females than in males, but the pedicel was the same size in both sexes (table 1).

The females had filiform antennae (figure 3A, 3B), whereas the males had bipectinate antennae (figure 4A, 4B) with two lateral projections on all but the four distal flagellomeres. In both sexes, the dorsal surface of the antenna was covered with scales and the ventral surface was covered with different types of sensilla, which also exhibited sexual dimorphism in type, number and distribution. Table 2 shows the distribution, number and lengths of the different types of sensilla identified on the scape and pedicel and on the first, intermediate and distal flagellomeres of the antennae of males and females.

Böhm's bristles were present in both sexes only on the scape and the pedicel of the antenna (figure 3C) and were absent on the flagellomeres (table 2); the remaining sensilla identified were absent on the scape and the pedicel but present on the different flagellomeres of the antenna.

The sensilla chaetica (figure 3A, 4A), arising from a basal socket (figure 4C, 4E), were the largest and thickest of all the sensilla found (figure 3A). These sensilla were present on the antennae of both sexes. Females had five sensilla chaetica: two lateral, two on the mid-ventral surface, and one on the mid-dorsal surface of each flagellomere. There were eight additional sensilla chaetica on the distal flagellomere of the antenna (figure 3A). In contrast, in males four sensilla chaetica were located among the scales on each flagellomere: one on each lateral projection (figure 4B), one on the mid-ventral surface (figure 4C) and the fourth on the mid-

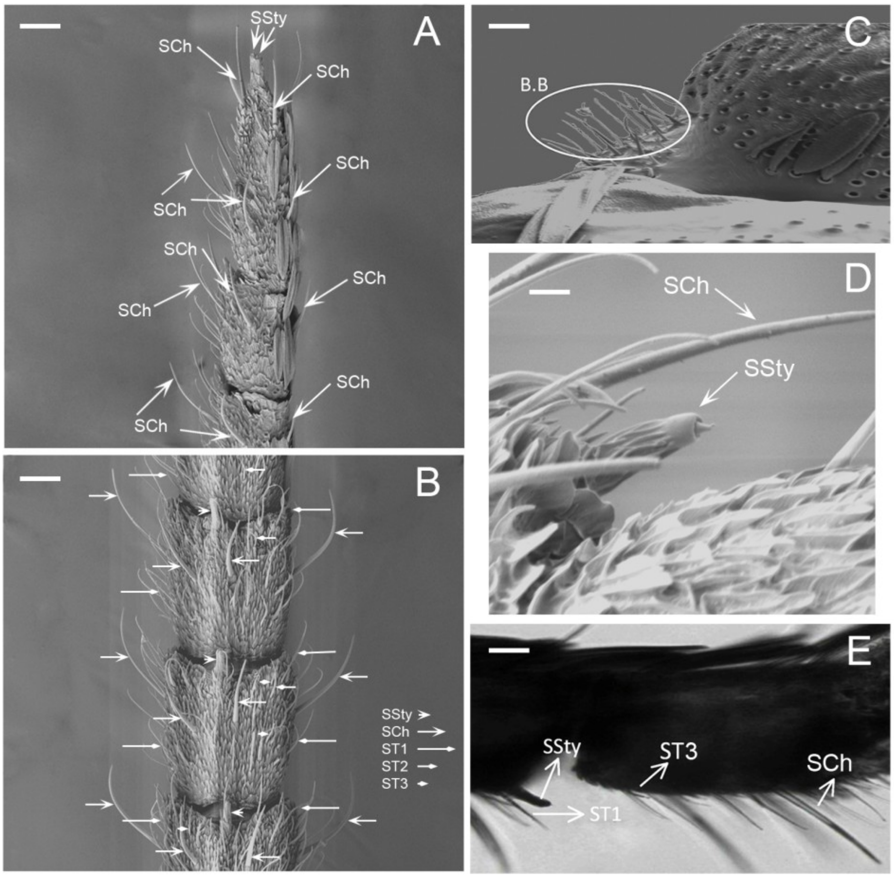

Figure 3. Scanning electron microscope images and light microscope image of silver nitrate staining of the filiforme antennae of female *S. nonagrioides*. A, distal flagellomeres; B, intermediate flagellomere; C, pedicel; D, sensilla styloconica in detail; E, silver staining of flagellomere; BB, Böhm´s bristles; SCh, sensilla chaetica; ST1, sensilla trichoidea type 1; ST2, sensilla trichoidea type 2; SCo, sensilla coeloconica; SSty, sensilla styloconica. Scale bars: A, B = 50 µm; C = 20 µm; D = 5 µm; E = 70 µm.

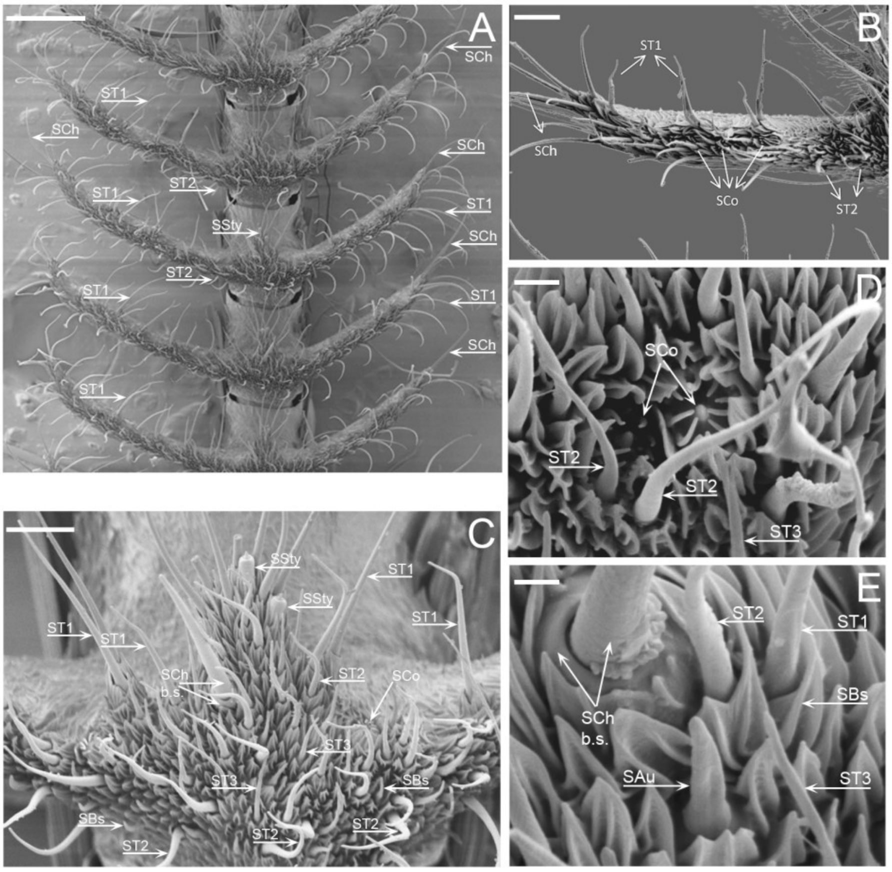

Figure 4. Scanning electron microscope image showing the bipectinate male antenna of *S. nonagrioides*. A, intermediate portion of the antenna; B, lateral projection; C, middle portion of the flagellomere; D, sensilla coeloconica in detail; E, sensilla auricillica in detail; SCh, sensilla chaetica; b.s., basal socket; ST1, sensilla trichoidea type 1; ST2, sensilla trichoidea type 2; ST3 sensilla trichoidea type 3; SCo, sensilla coeloconica; SSty, sensilla styloconica; SBs, sensilla basiconica; SAu, sensilla auricillica. Scale bars: A = 50 µm; B, C = 20 µm; D, E = 5 µm.

Table 2. Number (mean ± SE) and length in µm (mean ± SE) of the different types of sensilla on the pedicel and the first, intermediate and distal antennal flagellomeres (n = 10 antennae per sex). BB, Böhm's bristles; SCh, sensilla chaetica; ST1, sensilla trichoidea type 1; ST2, sensilla trichoidea type 2; ST3, sensilla trichoidea type 3; SCo, sensilla coeloconica; SSty, sensilla styloconica; SBs, sensilla basiconica; SAu, sensilla auricillica; NP, no presence. Means with different letters in the same column indicate significant differences between females and males for this type of sensilla (*t*-test, $P < 0.05$).

Type	Sex	Scape-Pedicel		First Flagellomere		Intermediate Flagellomere		Distal Flagellomere	
		Number	Length	Number	Length	Number	Length	Number	Length
BB	M	35.0 ± 1.7 a	23.2 ± 1.4 a	NP	NP	NP	NP	NP	NP
	F	37.0 ± 1.4 a	25.7 ± 1.2 a	NP	NP	NP	NP	NP	NP
SCh	M	NP	NP	4.0 ± 0.0b	71.6 ± 6.4a	4.0 ± 0.0b	107.1 ± 8.7a	6.0 ± 0.0b	92.6 ± 3.0a
	F	NP	NP	5.0 ± 0.0a	60.0 ± 3.5b	5.0 ± 0.0a	73.2 ± 4.2b	8.0 ± 0.0a	69.5 ± 3.3b
ST1	M	NP	NP	56.3 ± 2.0a	67.2 ± 1.7a	63.0 ± 1.6a	67.5 ± 1.2a	8.0 ± 1.1	61 ± 1.8a
	F	NP	NP	40.1 ± 2.0b	42.3 ± 1.8b	51.8 ± 1.5b	41.6 ± 1.7b	9.1 ± 0.8	50 ± 2.0b
ST2	M	NP	NP	12.0 ± 0.3a	25.5 ± 0.2	50.0 ± 0.4a	28.0 ± 0.5a	3.4 ± 0.1	27.7 ± 0.4
	F	NP	NP	10.0 ± 0.3b	24.7 ± 0.3	15.0 ± 0.5b	25.3 ± 0.3b	NP	NP
ST3	M	NP	NP	5.3 ± 0.3	17.5 ± 0.3	8.9 ± 0.4a	13.1 ± 0.6	NP	NP
	F	NP	NP	NP	NP	4.6 ± 0.5b	5.4 ± 0.8	NP	NP
SCo	M	NP	NP	3.5 ± 0.2b	8.2 ± 0.4	13.0 ± 0.2a	7.3 ± 0.5	4.0 ± 0.2	7.6 ± 0.5
	F	NP	NP	5.0 ± 0.2a	8.3 ± 0.4	5.0 ± 0.2b	8.4 ± 0.4	4.0 ± 0.2	7.6 ± 0.4
SSty	M	NP	NP	2.0 ± 0.0	13.8 ± 0.2	2.0 ± 0.0	4.7 ± 0.5	2.0 ± 0.0	17.9 ±0.4
	F	NP	NP	2.0 ± 0.0	14.0 ± 0.2	2.0 ± 0.0	4.5 ± 0.5	2.0 ± 0.0	18.5 ± 0.4
SBs	M	NP	NP	NP	NP	3.5 ± 0.1	7.3 ± 0.1	NP	NP
	F	NP	NP	NP	NP	NP	NP	NP	NP
SAu	M	NP	NP	NP	NP	1	8.9 ± 0.1	NP	NP
	F	NP	NP	NP	NP	NP	NP	NP	NP

dorsal surface. There were two additional sensilla chaetica on the mid-ventral surface of the distal portion of the flagellomere. These sensilla were longer in the males than in the females on the first, intermediate and distal flagellomeres ($P < 0.001$, *t*-test). With regard to function, the presence of silver nitrate staining inside (figure 3E) and within the basal socket suggested a role in chemo- and mechanoreception.

Sensilla without a flexible socket were classified according to their size as sensilla trichoidea types 1, 2, and 3. All flagellomeres of both sexes were ventrally covered with different types of these sensilla (figure 3B, 4C). All of them were argyrophilic, indicating the presence of pores and suggesting a role in chemoreception (figure 3E). Sensilla trichoidea types 1 and 2 were present on the antennae of both sexes (table 2). Type 1 sensilla trichoidea were the longest (41.6 to 67.5 µm) and were more abundant in males than in females on the first and the intermediate flagellomeres. On the distal flagellomere, they were longer in males, but the number was similar in both sexes ($P < 0.001$, *t*-test). Sensilla trichoidea type 2 occurred along the entire flagellum; their morphology and distribution were similar to the type 1 but they were smaller in size (24.7 to 28.0 µm). In males, they could be distinguished from the other sensilla trichoidea by a characteristic curved tip (figure 4C). These sensilla had a rounded tip and a non-flexible socket. Sensilla trichoidea type 3 were the shortest (13.12 to 17.5 µm) of the three sensilla trichoidea; they were only observed on the intermediate flagellomeres of both sexes (figure 3B, 4C) and on the first flagellomere of the males, being absent from the distal flagellomeres of both sexes (table 2).

Sensilla coeloconica consisted of a short, conical peg (7.34 to 8.36 µm) surrounded by 10 to 12 cuticular fingers; they were observed on each flagellomere of both sexes (figure 4B, 4C, 4D). On the ventral surface of the female antennae, four to six of these sensilla were observed on each flagellomere. In males, the number of sensilla coeloconica depended on the flagellomere: 3 to 4 were observed on the first, 12 to 14 on the intermediate flagellomeres (5 to 6 on each lateral projection and 2 on the mid-ventral surface), and 4 to 6 were observed on the distal flagellomere (figure 4B). The number of these sensilla on the first and intermediate flagellomeres was significantly different ($P < 0.001$, *t*-test) between males and females but was similar for males and females on the distal flagellomere ($P > 0.05$, *t*-test) (table 2). Staining was not detected; there were no significant differences between males and females in the length of these sensilla.

Both sexes presented two sensilla styloconica, consisting of a solid cylindrical base with a peg (and a conical structure on the tip), located in the centre of the distal edge on the ventral surface of each flagellomere (figure 3A, 3B, 3D, 4A, 4C). There were no differences in the lengths of these sensilla between the sexes ($P > 0.05$, *t*-test). These sensilla were well-stained with silver, indicating the presence of pores.

The sensilla basiconica were shorter than sensilla trichoidea (7.3 ± 1.4 µm, mean ± SD) (table2) and lacked the flexible socket at the base. They were found only in males (figure 4C, 4E); 3 to 4 were observed in the centre of the intermediate flagellomeres, but they were not found on the basal or distal flagellomeres of the males or on any of the flagellomeres of the females. Staining of these sensilla was not detected.

Finally, the sensilla auricillica, with a typical rabbit-ear shape (figure 4E) and a length of 8.9 ± 0.3 µm (table 2), were observed between the cuticular sculptures only in the intermediate flagellomeres of the males. Staining of these sensilla was not detected.

Ovipositor morphology and presence of pores in sensilla

The ovipositor consisted of two sclerotized valves with a conical shape. Their length was 1.45 ± 0.03 mm (mean ± SD), and their diameter was 0.86 ± 0.01 mm and 0.11 ± 0.01 mm at the base and tip, respectively. On the paired valves of the ovipositor, four types of sensilla and four claws (table 3) were observed on each valve at the tip of the ovipositor (figure 5A).

Type I sensilla chaetica were located on the ventral surface of the ovipositor at high density. Each sensillum arose from an elastic membrane and had a length of 78 ± 23 µm (mean ± SD), a grooved texture and a rounded tip (figure 5B). These sensilla were the only argyrophilic sensilla on the ovipositor (figure 5C).

Type II sensilla chaetica were observed covering the lateral surfaces of the ovipositor. They had a length of 12.5 ± 1.9 µm (mean ± SD). Each sensillum was inserted into a socket at the base (figure 5C). They had a sharp tip and a grooved texture and were not argyrophilic (figure 5E).

The type III sensilla chaetica were the longest sensilla found on the ovipositor. Eight to ten of these sensilla were located on the lateral surfaces of the distal third of the ovipositor. They had a smooth surface, straight hair with a sharp tip (figure 5A, 5D) and were not argyrophilic (figure 5E).

Table 3. Description of the ovipositor sensilla of *S. nonagrioides* (n = 5). Values are mean (± SE) length in µm of ovipositor sensilla.

Sensilla Type	Localization	Extremity	Length	Texture	Apical Pore	Function
Chaetica I	Ventral side	Rounded	78 ± 23	Grooved	Present	Chemo and mechanoreception
Chaetica II	Lateral sides	Sharp	12.5 ± 1.9	Grooved	Absent	Mechanoreception
Chaetica III	2/3 distal portion	Sharp	295 ± 52	Smooth	Absent	Mechanoreception
Microtrichia	Between valves	Sharp	6.2 ± 2,4	Smooth	Absent	Mechanoreception
Claws	Distal portion	Rounded	143 ± 37	Smooth	Absent	Abrasive

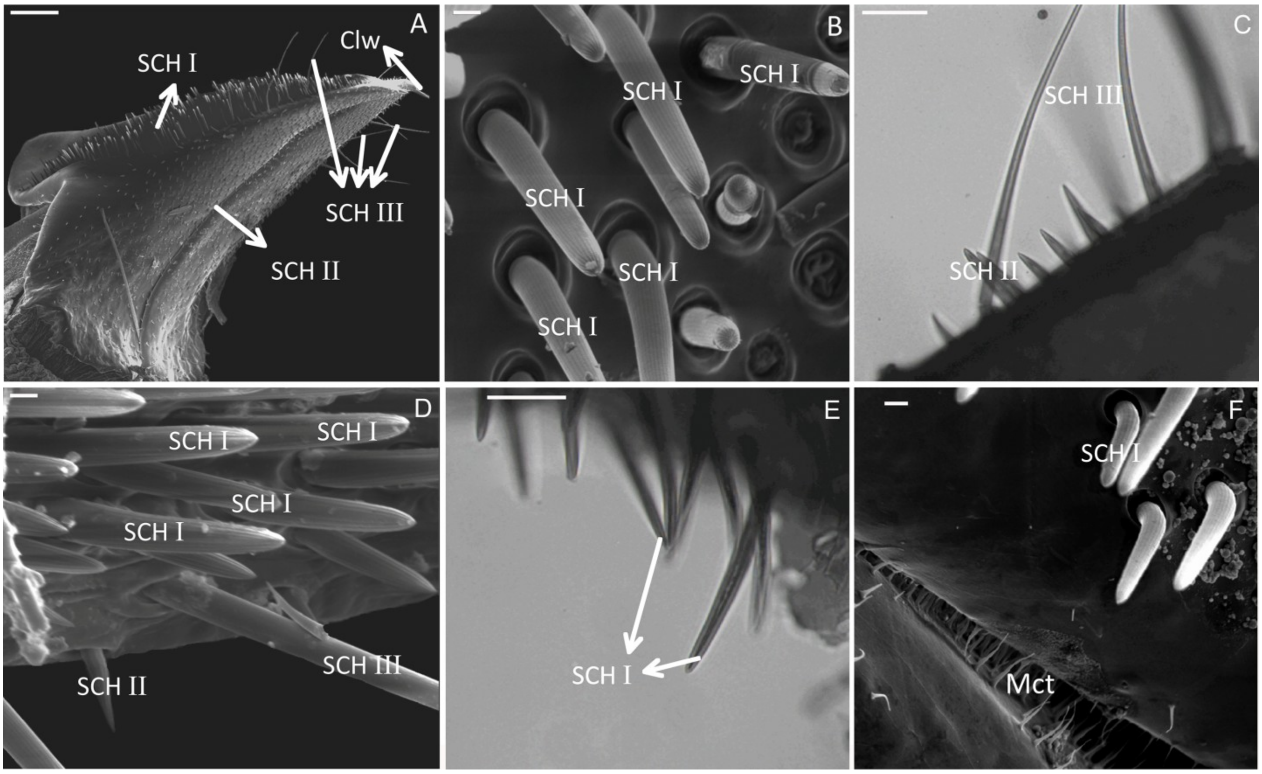

Figure 5. Scanning electron microscope images and light microscope image of silver nitrate staining of the ovipositor sensilla of *S. nonagrioides*. Silver-stained sensilla are indicated by an arrow. SCH I, sensilla chaetica type I; SCH II, sensilla chaetica type II; SCH III, sensilla chaetica type III; Mct, microtrichia; Clw, claws. Scale bars, A = 200 µm; B = 50 µm; C, E =70 µm; D =5 µm.

The microtrichia were 6.2 ± 2.4 µm in length. This type of sensilla had a smooth surface and a sharp tip, was located in the center of the ovipositor valves (figure 5F) and was not argyrophilic.

Discussion and conclusions

The behavioural sequences leading to oviposition in the Lepidoptera were divided by Renwick and Chew (1994) into three main steps: searching, orientation and encounter. The short-range host localization described in this paper corresponds to the orientation and encounter steps. The presence of a maize plant triggered behavioural steps in mated females of *S. nonagrioides* similar to those observed by Ramaswamy (1988), Hora and Roessing (1999), Rojas and Waytt (1999), and Calatayud *et al*. (2008). Furthermore, the first steps of the host location behaviour (pre-landing) of the gravid females were quite similar to the mating behaviour of *S. nonagrioides* males as described by Lopez *et al*. (2003). In both sexes, 30 minutes after scotophase, individuals unfolded their antennae and performed walks and erratic flights while buzzing their wings, as if they were testing and evaluating their surroundings. They then detected the odor plume, pheromones in the case of males (Lopez *et al*., 2003) and plant odours in the case of gravid females, and displayed oriented flight toward the odour source. Males and unmated females did not respond to the host plant odour. Some authors (Saver *et al*., 2012) have suggested that the stimulus to which some females of lepidopterans respond changes after mating; whereas unmated females respond to a feeding stimulus, i.e., floral scents, mated females respond to host volatiles. The reason why unmated females and males do not respond to any plant volatile may be because *S. nonagrioides* adults have atrophied mouth parts and they do not feed (Lopez *et al*., 2003). The wing buzzing of the males has been related to vibrational and acoustic communication during courtship (Ramaswamy, 1988; Mazzoni *et al*., 2011; Cocroft, 2011; Benelli *et al*., 2012), but the behavioural significance of wing buzzing by gravid females of *S. nonagrioides* during host location remains unclear.

Gravid females of *S. nonagrioides* deprived of one antenna were capable of completing the host location and oviposition behaviour in the same manner as the intact females. Vickers and Baker (1991) achieved similar results for the oriented flight of *Heliothis virescens* F. males. The absence of both antennae disrupted all of the behavioural steps of the females. Almost one third of the amputated females took flight, but the flights were not oriented, and in all cases the females collided with the wind tunnel walls, ceiling and floor. Sane *et al*. (2007) observed a similar disrupted flight when removing the antennal flagellum in *Manduca sexta* L. and suggested that the effect could also be due to the reduction of mechanical inputs. However, the majority of the amputated *S. nonagrioides* females that took flight attempted to fly towards the plant, implying that although the olfactory cues predominated when the gravid females looked for a suitable host on which to lay eggs (Cruz and Eizaguirre, 2015b), vision may play a role in the flight towards the plant. A synergism between visual and olfactory cues was found in *M. sexta*, when Raguso and Willis (2002) compared the responses to real flowers and dummy flowers with and without fragrance.

Half of the females with amputated antennae that landed on the plant managed to lay eggs, showing the importance of the chemo- and mechanoreceptor sensilla located on the ovipositor. The absence of antennae eliminated the antennal drumming but allowed ovipositor sweeping and tarsal tasting (Chadha and Roome, 1980; Marion-Poll *et al*., 1992; Maher and Thiery, 2004; Calatayud *et al*., 2006), explaining why some of these landed females were able to lay eggs.

The antennal morphology and the types of sensilla found in this study are quite similar to those described previously by Solinas and Trona (2002) and to those described by Calatayud *et al*., (2006) for an African corn borer, *Busseola fusca* (Fuller). However, we have found some differences from both of these studies. The antennae of male and female *S. nonagrioides* show sexual dimorphism in shape, being filiform in females and bipectinate in males, but in our population, in contrast to the results of Solinas and Trona (2002), the female's antennae had fewer flagellomeres but were longer than the male's antenna. Neither Solinas and Trona (2002) nor Calatayud *et al*. (2006) mentioned the Böhm's bristles, the mechanoreceptor organs present on the scape-pedicel that mediates antennal position during flight (Krishnan *et al*., 2012). Some of the sensilla identified in this study and the presence of pores in these sensilla correspond to the sensilla and their roles identified by Solinas and Trona (2002) based on transmission electron microscopy of the male's antennae. Thus, the sensilla chaeticae, more abundant in females than in males and more abundant on the distal flagellomere than on the other antennal flagellomeres, have a basal socket and are argyrophilic, which indicates a bimodal chemo(gustative)-mechanoreception role as Solinas and Trona (2002) determined for *S. nonagrioides*, as Calatayud *et al*. (2006) determined for *B. fusca* and other authors have identified for different Lepidoptera species (Frank *et al*., 2010; Diongue *et al*., 2013). The greater abundance of these sensilla on the distal flagellomere indicates that they play a role in evaluating the substrate and detecting plant surface chemicals during the host recognition and oviposition processes. Sensilla trichoidea have an olfactory function in the Lepidoptera (Halberg and Hansson, 1999), being involved in sex pheromone detection and orientation in males and in host volatile detection and orientation in females. However, *S. nonagrioides* females can also detect the pheromone released by females of their own species (Cruz and Eizaguirre, 2015a) as has been suggested for other species (Steinbrecht *et al*., 1995; Schneider *et al*., 1998; Judd *et al*., 2005; Stephens *et al*., 2008; Yang *et al*., 2009), whereas males respond only to pheromone compounds. This suggests that in the females of several species, the sensilla trichoidea can detect plant volatiles and/or pheromone components. All antennal flagellomeres of both sexes were ventrally covered with sensilla trichoidea types 1, 2, and 3, and all of them were argyrophilic, confirming the role in olfaction suggested by Solinas and Trona (2002). Unlike the sensilla chaetica, the sensilla trichoidea on *S. nonagrioides* antennae were more abundant on the intermediate flagellomeres than on the distal flagellomeres, demonstrating a type of compartmentalization in the antennae sensilla with structures for the detection of volatiles concentrated on the intermediate portion and the structures for mechano- and chemo-reception concentrated on the distal portion of the antennae. These hypotheses need to be confirmed through electrophysiological experiments that would also determine whether one type of sensilla responds to plant volatiles or to pheromone compounds or to both.

The sensilla coeloconica occurred on each flagellomere of the antenna in both sexes, but males had a significantly greater number than females, particularly on the lateral projections of the intermediate flagellomeres. These sensilla have been considered to be thermo- and hygroreceptive (Altner *et al*., 1981; Hansson *et al*., 1996; Ruchty *et al*., 2009) and detectors of host volatiles (Pophof, 1997; Hallberg and Hansson, 1999). We were unable to detect pores with silver nitrate staining owing to the distribution and short length of these sensilla, but Solinas and Trona (2002) found radial pores, suggesting an olfactory function. Two sensilla styloconica were located in the centre of the distal edge on the ventral surface of each flagellomere in both sexes, and showed a distribution and morphology similar to other noctuid species (Calatayud *et al*., 2006; Shun *et al*., 2010; Binyameen *et al*., 2012; Diongue *et al*., 2013). Although Solinas and Trona (2006) described these sensilla as poreless in *S. nonagrioides*, in our study, these sensilla were argyrophilic indicating a chemo-sensory function [as Calatayud *et al*. (2006) suggested for *B. fusca*] that needs to be confirmed. The sensilla basiconica were found in the centre of the intermediate flagellomeres only in males, suggesting their involvement in the perception of sexual pheromone by the males. Although we were unable to detect any staining; several studies have demonstrated that these sensilla have numerous pores and thin walls, indicating an olfactory function (Solinas and Trona, 2002; Koh *et al*., 1995; Anderson *et al*., 2000). Finally, sensilla auricillica were detected between the cuticular sculptures only in the intermediate flagellomeres of males, but Solinas and Trona (2002) also observed these sensilla in the lateral projections of male antennae. They have been considered olfactory receptors of plant odours (Anderson *et al*., 2000; Solinas and Trona, 2002; Frank *et al*., 2010), although we were unable to observe silver nitrate staining. The cuticular sculptures between the sensilla appear to have a protective function and also work to trap and concentrate the odour molecules (Wall, 1978).

Upon landing, the physical and chemicals cues perceived by the sensilla chaetica via contact through the antennae, ovipositor and tarsi provide the gravid females with sufficient information regarding whether the host plant is suitable for laying eggs. The type I sensilla chaeticae located primarily on the ventral surface of the ovipositor were the only argyrophilic sensilla present, as has been reported for other Lepidoptera (Chadha and Roome, 1980; Marion-Poll *et al*., 1992; Maher and Thiery, 2004; Calatayud *et al*., 2006). The sweeping behaviour during the final steps of the host location and oviposition behaviours allow these sensilla to detect chemical stimuli on the substrate and to give information to the brain about the suitability of the oviposition site. Non-argyrophilic sensilla chaetica types II and III are probably mechanoreceptors responsible for detecting pressure differences between the leaf sheet and the plant stem necessary for *S. nonagrioides* females to lay eggs. Although Renwick and Chew (1994) argued that plant surface and texture (e.g., pubescence) are important for moths evaluating oviposition sites, Konstantopoulou *et al*. (2002; 2004) found that stem texture was not important because an artificial substrate impregnated with leaf extracts stimulated *S. nonagrioides* oviposition. The microtrichia found between the ovipositor valves were not argyrophilic and probably also function as mechanoreceptors. Finally, Calatayud *et al.* (2006) observed lesions on the maize stem produced by the claws of *B. fusca* during oviposition and argued that these lesions liberate internal plant compounds that can be detected by chemoreceptors on the same ovipositor to elicit host recognition and acceptance, thus facilitating the choice of suitable oviposition sites. This situation could also apply to the claws of the ovipositor of *S. nonagrioides* females. Gravid females of *S. nonagrioides* need less than a minute to accept the maize plant as a suitable plant for ovipositing. The higher number of sensilla chaetica found at the antenna tip, along with the argyrophilic sensilla of the ovipositor, reveals a bimodal chemo- and mechanosensory function and their role in host acceptance.

Further studies are needed to clarify the role of visual cues in plant localization, and single-sensillum recordings are needed to determine the response of each type of sensilla trichoidea to the olfactory signals perceived by males and females.

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