

Calling behaviour and male response towards sex pheromone of poplar moth *Condylorrhiza vestigialis* (Lepidoptera: Crambidae)

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Abstract The calling behaviour of virgin females *Condylorrhiza vestigialis* Guenée, 1854 (Lepidoptera: Crambidae) and the female pheromone gland extract activity were studied under laboratory conditions. Most of the females started calling from their first scotophase period after emergence. Maximum calling occurred between the seventh and tenth hours of the scotophase period. The length of the calling increased with age until the fourth scotophase, but the onset of calling time did not differ with age. The number of calling bouts increased significantly with age, but the mean duration of each calling bout (20.8 min) did not vary with age. Extracts of pheromone glands evaluated in a Y-tube olfactometer attracted significantly more males than control, 70 and 30%, respectively. Gas chromatographic–electroantennogram detection (GC–EAD) analysis of these extracts indicated the presence of a single EAD-active peak, the putative sex pheromone of the species. This pheromone compound may be suitable for monitoring populations densities of *C. vestigialis*, and for detection of the onset of the seasonal flight period. An efficient pheromone is of importance also with respect to current attempts to develop new control methods for this important pest of *Populus* spp. in Brazil.

Keywords GC–EAD · Gland extract · Olfactometer · Poplar moth · *Populus* spp.

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Introduction

Crops areas of *Populus* spp. (Salicaceae) are increasing in south of Brazil due to the extensive use of this species by the match, tooth picks, and boxes factories (Castro et al. 2004), but its production is affected by the presence of several insect species. The poplar moth, *Condylorrhiza vestigialis* Guenée, 1854 (Lepidoptera: Crambidae) is the most important defoliator of the *Populus* spp. crop in Brazil (Castro et al. 2004). Poplar moth control is done by the use of insecticides, however, due to the fact that *Populus* spp. is cultivated in wet lands, new monitoring and control methods have been studied to control this insect and decrease the impact on the environment (Trefflich and Sousa 2000).

Mating in moths generally depend on the expression of a series of behaviour patterns (Hou and Sheng 2000). In females, these behaviours include production of volatile sex pheromones, emission of sex pheromones via calling behaviour, which leads to attraction of potential mates, and the receptivity to males that attempt mating (Kingan et al. 1993). Among these, pheromone emission is a crucial stage that deserves further studies (Hou and Sheng 2000). Hence, this paper aims to describe the age-related calling behaviour of *C. vestigialis* females, and to evaluate the male activity towards the female sex pheromone gland extract.

Materials and methods

Insects

Pupae of *C. vestigialis* were obtained from a laboratory culture maintained at the Federal University of Parana, Brazil. Pupae were sexed and held separately in plastic recipients (7 × 8 cm), so that emerging females and males did not

have contact with each other. Pupae and adults were kept at $23 \pm 2^\circ\text{C}$, $75 \pm 5\%$ RH and 14L:10D photoperiod. Adults were fed with 10% sucrose solution on cotton wad. The age of the moths was designated as age 0 on the day of emergence and age 1, age 2, etc. on subsequent days.

Observation of calling behaviour

Previous observations showed that there was no calling posture during the entire photophase. Due to that, the experiments started during the first complete scotophase after emergence. Twenty females were observed every 10 min during all scotophase period of seven successive days to determine the pattern of calling behaviour. The observations were done during only 7 days because according to Corrêa (2006) the females have a life period of 9 days and lay most of their eggs up to the sixth day. Moths were considered calling if the ovipositor was extended. A calling bout was each event of exposition of the ovipositor. If a female called in only one observation, the calling bout was considered to have lasted 10 min. However, if a female was calling during two consecutive observations, it was considered as a calling bout of 20 min. In all experiments, observations were done using a flashlight covered with six layers of red cellophane.

Gland extracts

The terminal abdominal segments were gently pressed with pincers to extrude the pheromone gland. Then only the pheromone gland was excised with pincers and kept 20 min in hexane at room temperature. The extracts were transferred into glass tubes, concentrated to one insect equivalent (1 IE/ μl) under an argon stream and maintained in freezer (-20°C) to be used at the behavioural tests.

Gas chromatography–electroantennographic detection

Extracts of ten females glands at the dose of 1 IE/ μl were analyzed by coupled gas chromatographic–electroantennographic detector (GC–EAD) with a system based on that of Leal et al. (1992, 1994, 2003). A Varian 3800 gas chromatograph, equipped with a DB-5 column (30 m \times 0.25 mm \times 0.25 μm ; Agilent Technologies, Santa Clara, CA, USA) and a splitless injector, was used in these analyses. The oven was programmed from 70°C (1 min hold) to 250°C at $7^\circ\text{C}/\text{min}$ and kept at this temperature for 5 min. One arm of the split column led to a glass tube (diameter 8 mm), with a charcoal-filtered and humidified air stream (0.5 l/min). Male antennae of *C. vestigialis* were 0.5 cm from the end of this glass tube and 30 cm from the EAD-outlet of the GC. The antennae were mounted between two glass pipette electrodes containing Ringer

solution, one electrode was connected to the ground and the other to an amplifier (Syntech).

Recordings were done with antennae of 3 days old virgin males ($n = 10$), between the seventh and tenth hours of scotophase. All antennae showed response towards the gland extracts.

Laboratory bioassay

The behavioural response of males ($n = 33$) was tested in a binary choice Y-tube glass olfactometer (4-cm diameter, 40-cm main tube, 20-cm each arm), operated at airflow of 4 l/min, previously humidified and filtered on active charcoal, at $23 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH. Each male was introduced into the olfactometer and its behaviour was observed for 5 min between the seventh and tenth hours of the scotophase. Observations were done under red light. It was recorded as no-choice if the male remained in the main tube and as choice if it had entered in one of the arms. Individuals that did not choose a particular arm were excluded from the statistical analysis. Each individual was tested only once. Pieces of filter paper (2 \times 2 cm) loaded with pheromone gland extract at the dose of 1 IE or with a hexane control were used as stimulus choices. The position of the odour sources was rotated for every four insects, to avoid positional bias (Ambrogi and Zarbin 2008; Zarbin et al. 2003, 2007a, b).

Statistical analysis

The data of length of calling, temporal pattern of calling, number of calling bouts, bout length, and onset of calling were analysed using ANOVA, followed by a Tukey test. Results of olfactometer bioassays were analysed using binomial test. Significance level was set at 0.05 in all tests. All analyses were performed using program BioEstat 3.0 (Ayres et al. 2003).

Results

During the calling behaviour, *C. vestigialis* females presented two calling positions. They either curved the abdomen downward to touch or press against the substrate or maintained the abdomen straight in a horizontal position. In both the calling positions, the ovipositor was extruded beyond the abdomen tip. Regardless the calling position, the females were moving their antennae up and down frequently and with occasional walking. It was observed that 65% of the 20 females already called on the first scotophase after emergence, but almost all females were calling between the third and seventh scotophase.

The length of calling was influenced by the female age ($F_{6,111} = 3.3$, $P = 0.005$) (Fig. 1A). There was an increase

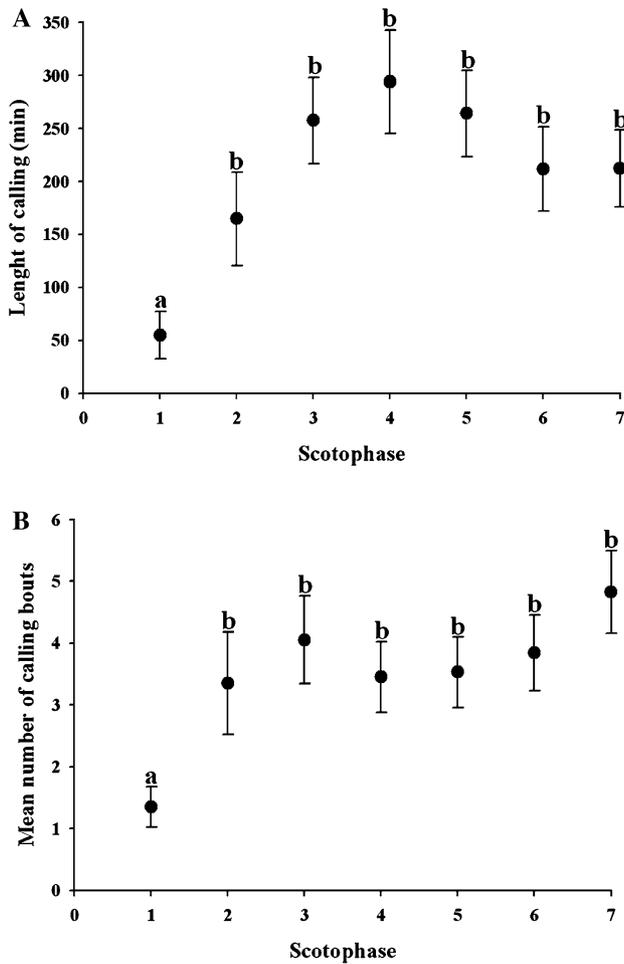


Fig. 1 **A** Mean duration (min) of calling by *C. vestigialis* females during seven scotophases ($n = 20$). **B** Mean number of calling bouts presented by *C. vestigialis* females during seven scotophases ($n = 20$). The same letters represent no significant difference ($P < 0.05$; Tukey's test)

in length through the fourth scotophase period and decreases thereafter. The number of calling bouts increased significantly with age ($F_{6,133} = 2.3, P = 0.03$), from 1.3 ± 0.3 (mean \pm SEM) bouts on scotophase 1 to 4.8 ± 0.7 bouts on scotophase 7 (Fig. 1B). However, the bout length did not differ with age.

The onset of calling did not differ with age. Calling behaviour, regardless of insect age, occurred from the first hour until the end of the scotophase, with highest calling occurring at the last hour of scotophase ($F_{9,50} = 12.1, P < 0.0001$) (Fig. 2).

Extracts of pheromone glands attracted significantly more *C. vestigialis* males than the control in the olfactometer ($X^2 = 4.36, df = 1, P = 0.036$). It was observed that 70% of the males were attracted to the filter paper containing pheromone gland extract, while only 30% of the males reached the control. When released in the olfactometer, the males remained quiet for some minutes, and then opened the hairbrushes and wing fanning while walking in

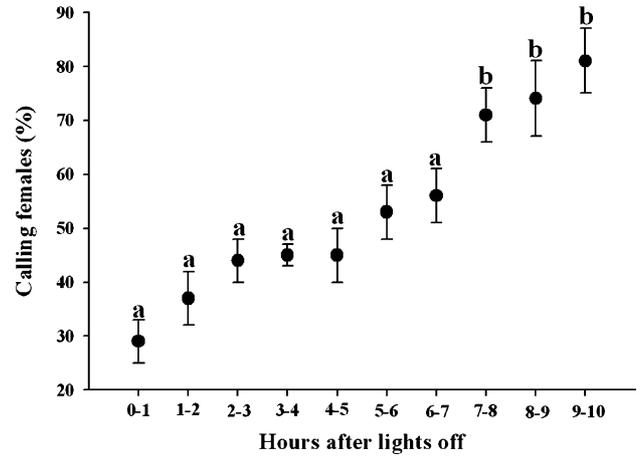


Fig. 2 Temporal pattern of calling behaviour exhibited by *C. vestigialis* females throughout the entire scotophase ($n = 20$). The same letters represent no significant difference ($P < 0.05$; Tukey's test)

response to the source. All the males tested displayed vigorous antennal movements during the tests.

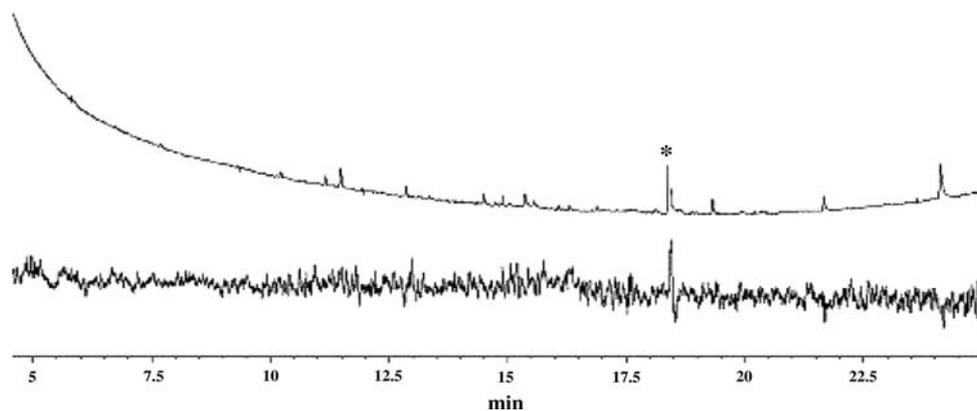
GC–EAD analysis of extract by using male antennae showed antennal response to one component present in the gland extract (Fig. 3), with a retention time of 23.3 min and Kovats index of 1856.

Discussion

The presence of different positions in *C. vestigialis* females during calling behaviour is typical for several species of moths such as *Thyrinteina arnobia* (Lepidoptera: Geometridae) (Batista-Pereira et al. 2004) and *Hydraecia micacea* (Lepidoptera: Noctuidae) (West et al. 1984). The extension of the ovipositor to expose the cuticle overlying the pheromone glands is the main characteristic of the calling posture, as in other lepidopterans (Turgeon and McNeil 1982; West et al. 1984; Howlader and Gerber 1986).

Our results show that poplar moth females started to call from the first scotophase suggesting that females are reproductively mature on emergence, since previous studies demonstrated that there is a correlation between female calling and pheromone production in various lepidopteran species (Delisle and Royer 1994). Similar results were observed in calling behaviour studies done for *Zamagiria dixolophella* (Lepidoptera: Pyralidae) (Gómez and Rojas 2006), *Estigmene acrea* (Lepidoptera: Arctiidae) (Mazo-Cancino et al. 2004), *Cerconota anonella* (Lepidoptera: Oecophoridae) (Silva et al. 2006), *Sesamia nonagrioides* (Lepidoptera: Noctuidae) (Babilis and Mazomenos 1992) and *Helicoverpa assulta* (Lepidoptera: Noctuidae) (Kamimura and Tatsuki 1993). However, females of many moth species are not always reproductively mature or capable of calling immediately after emergence. For example,

Fig. 3 Coupled gas chromatograph–electroantennogram detection (GC–EAD) analysis of *C. vestigialis* pheromone gland extract; the flame ionisation detector response is above the EAD response ($n = 10$). Asterisk EAD-active peak



females of *Mamestra configurata* (Lepidoptera: Noctuidae) (Howlader and Gerber 1986) and *Copitarsia consueta* (Lepidoptera: Noctuidae) (Rojas and Cibrián-Tovar 1994) call for the first time during the second or third scotophase after emergence.

In our experiments, we found that age had significant influence on calling patterns with respect to calling length and number of bouts. Older females presented higher number of calling bouts and longer calling periods than younger females. Similar results were obtained for some lepidopteran species, such as *Agrotis ipsilon* (Lepidoptera: Noctuidae) (Swier et al. 1977), *H. micacea* (West et al. 1984), and *Chilo suppressalis* (Lepidoptera: Pyralidae) (Kanno 1979). This greater amount of calling with age can be correlated with reproductive maturity (Swier et al. 1977).

The onset of calling period of poplar moth was not affected by female age. Similar behaviour was recorded for potato stem borer, *H. micacea* (West et al. 1984). In contrast to our results, changes in the onset of calling behaviour with age have been recorded for other lepidopteran species (Turgeon and McNeil 1982; Batista-Pereira et al. 2004; Zarbin et al. 2007a, b). Swier et al. (1977) suggested that by calling earlier *A. ipsilon* females increased their probability of mating by being the first to attract the males. Regarding the *C. vestigialis* females, competition for males may be reflected in a different manner, perhaps by increasing the length of calling and the number of bouts.

As seen in Fig. 3, calling activity occurs more towards the end of the scotophase. This result suggests that the best time to extract poplar moth sex pheromone glands should be between the seventh and tenth hours of scotophase. Similar data have been presented for *C. consueta* (Rojas and Cibrián-Tovar 1994) and *Neuleucinodes elegantalis* (Lepidoptera: Crambidae) (Eiras 2000). The majority of Lepidoptera mate during the scotophase (Roelofs and Cardé 1987) and the fact that several species emit pheromone at specific time intervals has been regarded as an important mechanism to ensure reproductive isolation (Cardé and Roelofs 1973).

The observations in the olfactometer showed that the pheromone gland extracts attracted *C. vestigialis* males, and the gland extract also elicited an EAD response for male antennae. The male attractivity towards females pheromone gland extracts in Y olfactometer was obtained for the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) (Parra-Pedrazzoli et al. 2006), and for *Lonomia obliqua* (Lepidoptera: Saturniidae) (Zarbin et al. 2007a, b). Altogether, these data suggest the existence of a female-emitted sex pheromone and the single GC–EAD active component present in the gland extract is the putative sexual pheromone of the species. Single volatile compounds are rarely unique, only blends of volatiles encode specificity. This has been studied in detail with respect to lepidopteran sex pheromones. Species-specific communication within the phylogenetic groups with only a few, structurally similar, compounds is enabled by pheromone synergists and antagonists, which function to enhance and to inhibit, respectively, the behavioural response to the main pheromone component (Linn et al. 1986; Witzgall et al. 1996; Cardé and Minks 1997; Arn et al. 2000). This unique GC–EAD active peak detected in *C. vestigialis* gland extract is probably the main pheromone component, and further studies on structural identification will verify the existence of possible minor pheromone components.

This study shows that *C. vestigialis* calling behaviour occurs from the first scotophase, with a broad peak occurring between the seventh and tenth hours of the scotophase. The pheromone gland extracts were attractive to males, and the GC–EAD showed that there is, at least, one component in the extracts that promoted male antennal response. The identification of this molecule is now in progress in our laboratory. Reliable and species-specific trap lures are important tools for insect management. Populations of *C. vestigialis* are present in the areas of *Populus* spp., and calendar sprays can be avoided if outbreaks can be detected in time. Defoliators are more exposed to natural enemies than insects feeding inside the twigs or the steam. Long-term use of pheromone-blend methods and a concurrent reduction of insecticide sprays favour natural

enemies (Biddinger et al. 1994, Epstein et al. 2000) and thus may facilitate control of orchard leafrollers. Our results can contribute to the development of a pheromone to be used for monitoring and control of *C. vestigialis* in Brazil.

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