

# Effects of male age and mating status on response to the female sex pheromone of *Copitarsia decolora* (Lepidoptera: Noctuidae)

Humberto Reyes, René Arzuffi\* and Norma Robledo

---

## Abstract

The effects of male age and mating status on the behavioral response of *Copitarsia decolora* (Guenée) males to the glandular extract of the female sex pheromone were studied by conducting wind tunnel bioassays and electroantennography (EAG). To study the effect of age, 2-3, 4-5, 6-7, 8-9, 10-11 day old males were used to measure attraction in the wind tunnel. To study the effect of age on virgin male antenna depolarization, individual males 3-, 6- and 9-days old were used. The effect of male mating status was studied with 4-6 day old males and subsequent pheromone response was measured after a period of 24 and 48 h. Both male age and mating status significantly affected male response to the female sex pheromone. In the wind tunnel, male attraction to the extract reached its maximum in males 4-7 days old and then decreased as moth age increased. The glandular extract provoked a significantly greater depolarization of the antennae of 6 day old males than in 3 and 9 day old males. In the wind tunnel, virgin males were more attracted to female extracts than mated males. Significantly greater depolarization was observed in the antennae of virgin than mated males.

Key Words: *Copitarsia decolora*; sex pheromone; age; mating; wind tunnel; electroantennography

## Resumen

Se estudió el efecto de la edad del macho y el estado de apareamiento sobre la respuesta de comportamiento de machos de *Copitarsia decolora* (Guenée) al extracto glandular de la feromona sexual de la hembra, mediante bioensayos en túnel de viento y electroantenografía (EAG). Para medir la atracción en túnel de viento se utilizaron machos de 2-3, 4-5, 6-7, 8-9, 10-11 días de edad y para estudiar el efecto de la edad sobre la despolarización de sus antenas, se utilizaron individuos de 3, 6 y 9 días de edad. Para estudiar el efecto del apareamiento se utilizaron machos de 4-6 días de edad y su respuesta a la feromona se midió 24 y 48 horas después. Ambos, la edad del macho y el estado de apareamiento, afectan de manera significativa la respuesta de los machos a la feromona sexual de las hembras. En el túnel de viento, se observó que la atracción de los machos al extracto fue mayor a los 4-7 días y después disminuyó conforme incrementó la edad. El extracto glandular provocó una despolarización significativamente mayor en las antenas de los machos de 6 días de edad que en los machos de 3 y 9 días de edad. En túnel de viento los machos no apareados fueron más atraídos al extracto glandular de las hembras que los machos apareados. Se observó una despolarización significativamente mayor en las antenas de los machos no apareados que en los machos apareados.

Palabras Clave: *Copitarsia decolora*; feromona sexual; edad, apareamiento; túnel de viento; electroantenografía

---

Males moths use sex pheromones emitted by females to locate a mate. However, male response can be affected by numerous factors, including the physiological state of the insect (Anton et al. 2007; Evenden & Gries 2008) and experience (Anton et al. 2007). Pheromones are detected by olfactory receptor neurons (ORN) housed within cuticular sensilla mainly on the antennae (Keil 1999). The axons of ORNs project via the antennal nerve into the antennal lobe (AL). There they make synaptic contact with intrinsic AL neurons, the local interneurons, and with AL projection neurons, which transfer information to higher brain centers (de Belle & Kanzaki 1999). The AL consists of a species-specific number of glomeruli (Hansson & Anton 2000). Male moths have large numbers of olfactory receptor neurons tuned to single compounds of the sex pheromone produced by females, and large glomeruli, called

the macroglomerular complex, are entirely dedicated to the processing signals pertaining to these sex pheromones (Hansson & Anton 2000).

For some moth species such as *Plutella xylostella* (L.) (Zhang et al. 2009), *Agrotis ipsilon* (Hufnagel) (Anton & Gadenne 1999; Gadenne & Anton 2000; Gadenne et al. 2001; Barrozo et al. 2010a, 2010b, 2011), *Heliothis virescens* (F.) and *Heliothis subflexa* (Guenée) (Soques et al. 2010) it has been demonstrated that age and mating status can modify olfactory reception and sexual pheromone processing in males. In these species, the male response to the female sex pheromone is regulated by hemolymph titers of juvenile hormone (JH) (Gadenne et al. 1993; Dupontets et al. 1998; Anton & Gadenne 1999; Gadenne & Anton 2000), and by neuromodulators that act on ORNs and AL neurons (Anton et al. 2007).

---

CEPROBI, Instituto Politécnico Nacional, Km. 8.5 Carretera Yautepec-Jojutla Yautepec, Morelos C.P. 62731, México

\*Corresponding author; E-mail: rarzuffi@ipn.mx

*Copitarsia decolora* (Guenée) (Lepidoptera: Noctuidae) is an important pest of cruciferous crops in Mexico (Suarez-Vargas et al. 2006) and subject to quarantine in the United States (Venette & Gould 2006). Rojas et al. (2006) identified the sex pheromone of *C. decolora* and, although synthetic sex pheromones have been used as an alternative to synthetic insecticides (Rojas et al. 2006; Muñoz-Reyes et al. 2007; Barrientos-Hernández et al. 2011; Diaz-Gomez et al. 2012), the effect of environmental (Dumont & McNeil 1992) and physiological factors on male response to the sex pheromone have not been studied. However several factors, including of age and physiological status of males, should be evaluated before the pheromone compounds can be used effectively for *C. decolora* management. The efficiency of traps baited with pheromone could be affected importantly by these 2 factors.

This study examined the effect of age and male mating status on male attraction and antennal responses to sex pheromone extracts of *C. decolora* females. Understanding the effect of the factors on olfactory guided behavior of *C. decolora* males will contribute to improving current pheromone-based methods for the management of this pest species.

## Materials and Methods

### INSECTS

Insects used in the bioassays were obtained from a *C. decolora* colony at the Centro de Desarrollo de Productos Bióticos, National Polytechnic Institute (IPN) in Yauatepec, Morelos, Mexico. The insects were maintained at  $25 \pm 3$  °C,  $60 \pm 5\%$  RH and 12:12 h L:D, with the photophase and scotophase reversed with respect to the natural light cycle in order to allow pheromone extraction and bioassays during the day. Larvae were fed with a special artificial diet for lepidopterans (Cibrián-Tovar & Sugimoto 1992). Adults were fed with a 50% sucrose solution placed on cotton wool, which was replaced every 3rd day.

### PREPARATION OF SEX PHEROMONE EXTRACT

The gland that produces the sex pheromone, situated between the seventh and ninth abdominal segment (Rojas et al. 1995), was dissected from virgin 3-5 day old females that presented sexual calling behavior during the final third of the scotophase (Rojas et al. 2006). Sixty glands were placed in 2 mL glass vials containing 1 mL of dichloromethane as a solvent for 10 min. The supernatant was concentrated to 100  $\mu$ L under a nitrogen flow. Each extract had a concentration of 3 female equivalents (FE) for each 5  $\mu$ L. The gland extracts were stored at -4 °C until chemical analysis or use in the bioassays.

### ANALYSIS AND IDENTIFICATION OF VOLATILE COMPOUNDS

Two  $\mu$ L of extract were injected into a gas chromatograph (GC) (HP 6890) coupled with a mass spectrometer (MS) (HP 5972) (Agilent, USA). The samples were analysed using a non-polar HP 5MS column (30 m long, 250  $\mu$ m internal diameter and 0.25  $\mu$ m film thickness; Agilent Technologies, Santa Clara, California). The initial oven temperature was 60 °C, increasing 15 °C/min until it reached 280 °C. The carrier gas was helium at a constant flow of 1 mL/min. The injector temperature was 225 °C and the auxiliary was 280 °C; the injector functioned in splitless mode for 0.30 min. The MS functioned by electronic ionization (70 eV), in SCAN mode and at a mass interval of 35 to 550 AMU. Compound identification was carried out by considering retention time (RT) and by comparing mass spectra to spectral libraries (NIST/EPA/NIH 2002) and synthetic standards. The standards (Z)-9-tetradecenylacetate (Z9-14:Ac) and (Z)-9-tetradecenol (Z9-14:OH), with 98% purity were ob-

tained from Sigma Aldrich (Toluca, Mexico). In all of the extracts used in the bioassays, the presence of Z9-14:Ac and Z9-14:OH in similar proportions to those reported by Rojas et al. (2006) were verified.

### WIND TUNNEL BIOASSAYS

In the wind tunnel bioassays, volatile chemicals from the glandular extract were carried to the insects by a current of air (0.4 m/s) produced by a fan (Siemens 1RA3055-4YK31, México). The experiments were conducted at  $25 \pm 3$  °C,  $60 \pm 5\%$  RH. In preliminary studies the following concentrations were tested: 3, 1.2, 0.12, 0.012 and 0.0012 FE. The 3FE concentration caused the greatest attraction response and landing, so this was used in both experiments (wind tunnel and EAG). 5  $\mu$ L (3 FE) of glandular extract was individually placed on filter paper (2.0  $\times$  2.0 cm, Whatman no. 1), then left for 20 s at the same height as the air flow entrance to allow the solvent to evaporate. A single male was released at this height but at the opposite end of the tunnel. Each moth was tested for response within 300 s and with one test per moth.

The following types of behavior were measured in the wind tunnel: no response, short flight (flight less than 100 cm), long flight (flight more than 100cm but less than 150 cm), and landing on the source. The number of males that displayed their claspers was also recorded. Furthermore, latency to activation (antenna and wing movement and displaying claspers) and latency to landing on the scent source were recorded.

### ELECTROANTENNOGRAPHY

The measurement and analysis of the EAG responses were conducted using Syntech EAG equipment (Kirchzarteng, Germany). Once the antenna of the insect was dissected, it was mounted between 2 silver (Ag) electrodes and conductor gel was added. The signal generated by the antenna was transmitted to an IDAC-2 amplifier and was observed on a monitor with software for EAG recording and analysis. Antenna stimulation continued for 1s with an air flow of 0.5 L/min transported to the antenna by a pump (stimulus controller SC-55) with a constant flow of humidified purified air (0.7 L/min). For stimulus application, 5  $\mu$ L (3 FE) of glandular extract of the sex pheromone were placed on filter paper (1  $\times$  0.5 cm, Whatman No. 1). In each experiment the solvent was allowed to evaporate for 20 s and the interval between each stimulus was 120 s. In these bioassays, the antenna polarization response to solvent was subtracted from response to glandular extract before analysis. Each antenna was used only once.

### TREATMENTS

To study the effect of age, the pupae were separated by sex. After emergence, adults were placed in plastic containers (20  $\times$  20  $\times$  20 cm) to obtain single sex groups of known age. In the wind tunnel bioassays, virgin males that were 2-3, 4-5, 6-7, 8-9 and 10-11 days old were used, with 10 moths tested per group. For EAG response, virgin males that were 3, 6 and 9 days old were tested, with 6 moths tested for each age. Selection of the age groups for EAG tests was based on results of wind tunnel tests.

For tests of the effect of mating, virgin males and females, 4-5 days after emergence, were individually paired and held in plastic containers (200 mL). The pairs were observed for mating and time when mating ended was recorded. Male responses in wind tunnel bioassays and EAG to the extracts were measured 24 and 48 h after mating. All of the evaluated mated males in both wind tunnel ( $n = 10$ ) and EAG ( $n = 6$ ) were 5-7 days old. The depolarization values obtained from the groups of mated males were compared against a group of virgin 4-6 day old males. The females from each mated pair were dissected and

the presence of the spermatophore used to confirm that matings were successful.

**STATISTICAL ANALYSIS**

The behaviors of the males in the wind tunnel were analysed by the Chi-square test with Yates correction. Regarding the latency of the responses observed in the wind tunnel, the median and interquartile range (Q1 to Q3) were determined for each group. The interquartile range is little affected by extreme scores, so it is a good measure of spread for skewed distributions. The latency data were analysed by a Kruskal-Wallis test and Tukey’s test ( $P = 0.05$ ) was used for comparison of means.

The data obtained from the depolarization in the male antennae were analysed by ANOVA. Comparisons of means were carried out using a Tukey’s test. Sigma Plot 11 (Systat Software Inc., Chicago, Illinois) was used for all statistical analyses.

**Results**

**EFFECT OF AGE**

Male attraction to the extract was greatest for 4-5 and 6-7 day old males and then decreased with age in wind tunnel tests (Table 1). These groups were the most responsive, although there was no difference between these 2 groups. There were also significant differences between 4-5, 6-7 and 2-3 (Chi square = 52.769;  $df = 1$ ;  $P < 0.001$ ), 8-9 (Chi square = 52.769;  $df = 1$ ;  $P < 0.001$ ) and 10-11 day old males (Chi square = 72.521;  $df = 1$ ;  $P < 0.001$ ) regarding flight behavior patterns when landing on the scent source. In addition, there were significant differences between 4-7 and 2-3 day old (Chi square = 20.056;  $df = 1$ ;  $P < 0.001$ ), 8-9 and 10-11 day old males (Chi square = 8.526;  $df = 1$ ;  $P = 0.004$ ) for displaying claspers (Table 1).

The 4-5 and 6-7 day old males presented activation and landing latencies significantly less than those displayed by 2-3, 8-9 and 10-11 day old males ( $H = 28.557$ ,  $df = 4$ ,  $P < 0.001$  and  $H = 24.167$ ,  $df = 4$ ,  $P < 0.001$ ; respectively) (Fig. 1).

The EAG responses by age group are shown in Fig. 2. The extract elicited a significantly greater depolarization in antennae of 6 day old males than in 3 and 9 day old males ( $F = 9.965$ ;  $df = 2,17$ ;  $P = 0.002$ ).

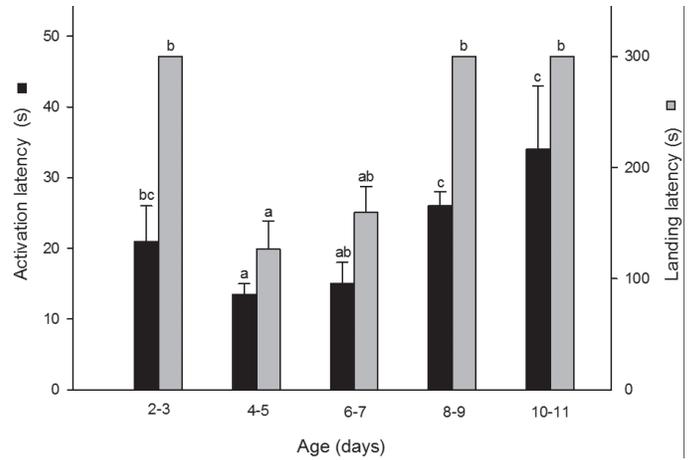
**EFFECT OF MATING**

In the wind tunnel bioassays, the virgin males were more attracted (both long flight and landing) than males evaluated 24 and 48 h after mating (AM). Differences were found in source landing behavior for 24 AM males (Chi square =18.484;  $df = 1$ ;  $P < 0.001$ ) and 48 AM males (Chi square = 48.505;  $df = 1$ ;  $P < 0.001$ ); as well as in long flight behavior for

**Table 1.** Response (percentage) by age group of virgin *Copitarsia decolora* males to female sex pheromone extract (3fe) in wind tunnel bioassays.

Behavior	Age (days)				
	2-3	4-5	6-7	8-9	10-11
No response	20 b	0 a	0 a	10 b	50 b
Short flight	30 b	0 a	10 a	30 b	10 a
Long flight	10 a	10 a	10 a	20 a	10 a
Landed	40 a	90 b	80 b	40 a	30 a
Claspers displayed	80 a	100 b	100 b	90 a	90 a

Percentages followed by the same letter within a behavioral category are not significantly different (Chi-square,  $n = 10$ ,  $P < 0.05$ ).



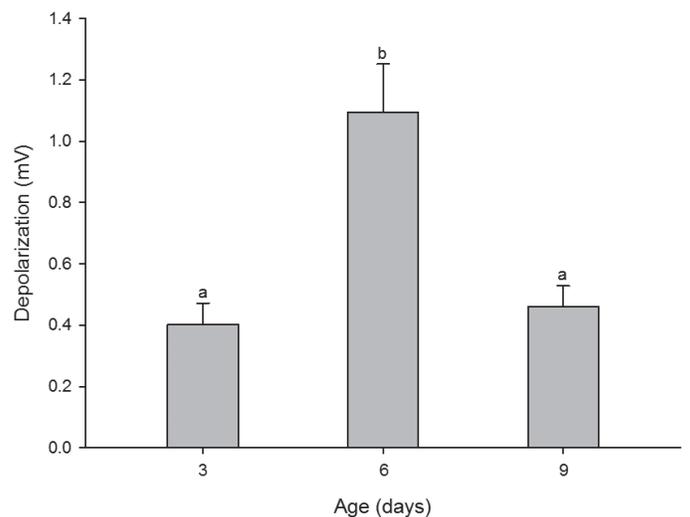
**Fig. 1.** Activation latencies (black bars) and landing latencies (gray bars) ( $Q_1 < Median < Q_3$ ) by age group of virgin males in response to a glandular extract of female sex pheromone (3FE) in wind tunnel bioassays. Bars within a behavior headed by the same letter are not significantly different (Tukey’s mean separation test,  $n = 10$ ,  $P < 0.05$ ).

24 AM males (Chi square = 11.281;  $df = 1$ ;  $P < 0.001$ ) and 48 AM males (Chi square =36.214;  $df = 1$ ;  $P < 0.001$ ) (Table 2).

Mating had an effect on both activation and landing latencies (activation latency  $H = 17.867$ ,  $df = 2$ ,  $P < 0.001$ ; landing latency  $H = 14.885$ ,  $df = 2$ ,  $P < 0.001$ ). The males from the 24 AM and 48 AM groups spent more time in activation and landing on the scent source than the virgin males group (Fig. 3). Similarly, a significantly greater depolarization was observed EAG responses of virgin males than mated males (24 AM and 48 AM groups) ( $F = 9.814$ ;  $df = 2, 17$ ;  $P = 0.002$ ) (Fig. 4).

**Discussion**

In this study, we found that antennal sensitivity to the sex pheromone by male *C. decolora* moths varies in relation to age, reaching a maximum at 6 days. Similar changes in antennal sensitivity due to age have been



**Fig. 2.** Depolarization (mean  $\pm$  SEM) of virgin male antennae in response to a glandular extract of female sex pheromone (3FE) by male age. Bars headed by the same letter are not significantly different (Tukey’s mean separation test,  $n = 6$ ,  $P < 0.05$ ).

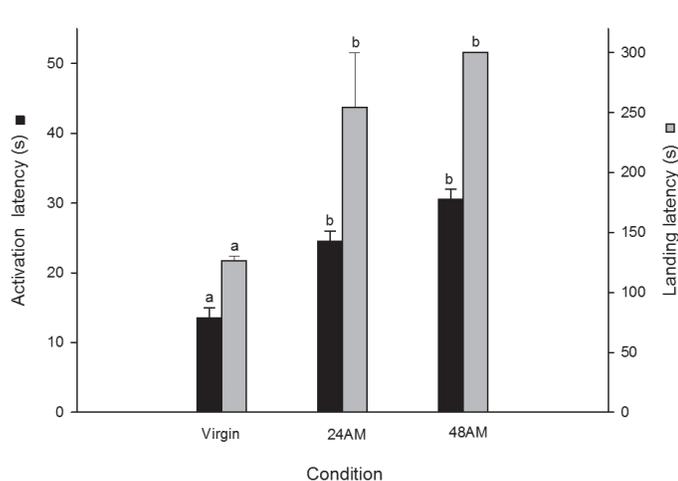
**Table 2.** Response (percentage) of virgin and mated *Copitarsia decolora* males to female sex pheromone extract (3FE) in wind tunnel bioassays. Mated males were tested 24 h and 48 h after mating (AM).

Behavior	Condition		
	Virgin	Mated, 24 h AM	Mated, 48 h AM
Short flight	10 a	20 a	20 a
Long flight	10 a	30 b	50 c
Landing	80 c	50 b	30 a
Claspers displayed	100 a	100 a	100 a

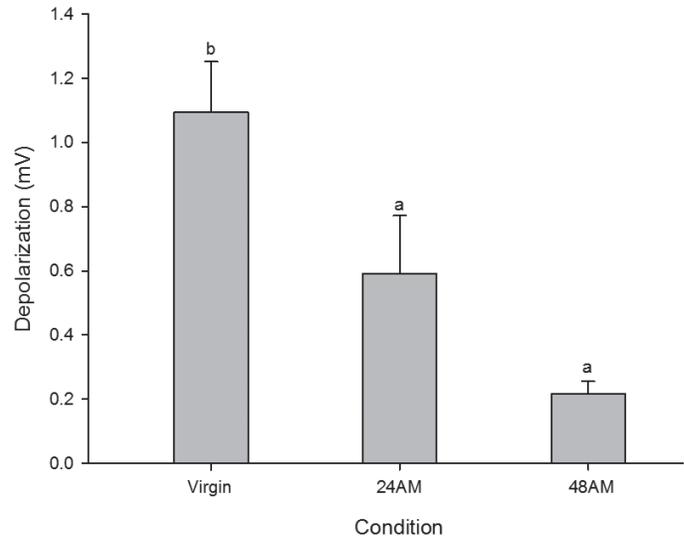
Percentages in each row followed by the same letter within a behavior are not significantly different (Chi-square,  $n = 10$ ,  $P < 0.05$ ).

observed in other species of moths such as *A. ipsilon* (Gadenne et al. 1993), *Spodoptera littoralis* (Boisduval) (Martel et al. 2009) and *Mamestra brassica* (L.) (Pophof 2000). In these species, this has been attributed to the maturation of the ORNs (Martel et al. 2009), changes in hemolymph titers of the juvenile hormone (Gadenne et al. 1993; Gadenne & Anton 2000) and neuromodulation of biogenic amines that act on ORNs sensitivity (Pophof 2000; Grosmaître et al. 2001). These 3 mechanisms could explain the results observed in *C. decolora* as well; however studies are needed on these variables for this species.

In *C. decolora*, the observed changes in antennal sensitivity to the sex pheromone correspond to observed behavioral changes. During the initial days after emergence, male attraction response is low and it then begins to increase with age until reaching its maximum level at days 4-5 and 6-7, subsequently decreasing with age. In some species of moths, such as *A. ipsilon*, male response to the sex pheromone starts to increase in 3 day old individuals (Gadenne et al. 1993). In other moths such as *Spodoptera frugiperda* (Smith), the optimal age is 2 days (Rogers & Marti 1994), while for *Spodoptera exigua* (Hübner) it is reached at 1-2 days (Rogers & Marti 1996). It seems that young *C. decolora* males (2-3 days old) do not have sufficient sexual maturity to respond to the sex pheromone, and this also seems to be the case regarding recently emerged *A. ipsilon* males (Gadenne et al. 1993; Duportets et al. 1998). Across species, the olfactory response increases until the males reach sexual maturity, and then decreases (Evenden & Gries 2008).



**Fig. 3.** Activation latencies (black bars) and landing latencies (gray bars) ( $Q_1 < \text{Median} < Q_3$ ) of virgin and mated *Copitarsia decolora* males to female sex pheromone extract (3FE) in wind tunnel bioassays. Mated males were tested 24 h and 48 h after mating (24 AM and 48 AM, respectively). Bars within a behavior headed by the same letter are not significantly different (Tukey's mean separation test,  $n = 10$ ,  $P < 0.05$ ).



**Fig. 4.** Depolarization (mean  $\pm$  SEM) of antennae in response to a glandular extract of female sex pheromone (3FE) of virgin and mated males. Mated males were tested 24 h and 48 h after mating (24 AM and 48 AM, respectively). Bars headed by the same letter are not significantly different (Tukey's mean separation test,  $n = 6$ ,  $P < 0.05$ ).

Mating in *C. decolora* males was associated with a decrease in behavioral responses, similar to those observed in *A. ipsilon* mated males (Gadenne et al. 2001). In *A. ipsilon*, mating triggers the release of neuromodulators that have the ability to modulate antennal lobe neurons in response to female sex pheromones and affect the development of the sex accessory glands, but antennal sensitivity is not affected (Duportets et al. 1998; Gadenne et al. 2001). In contrast, in *C. decolora* the antennal sensitivity is affected by mating. The decrease in pheromone response could be related to the fact that *C. decolora* males do not mate more than once during the night (Rojas & Cibrián-Tovar 1994) and can mate a total of 2 to 3 times during their lifetime (Castrejón-Gómez et al. 2000). This inhibition in the antennal and behavioral responses, combined with the effect of age, could represent a mechanism for temporally separated matings.

*Copitarsia decolora* males possibly need to recover from increased energy expenditure during mating and store sperm again (Gillott 2003). During this period of inhibition, the males can focus on searching for food to re-establish reserves in their accessory glands to allow sperm production and therefore mate again, as observed in *A. ipsilon* (Barrozo et al. 2011).

The attraction response of male *C. decolora* to pheromone not only depends on the chemical composition (type of compounds and their relative proportions) (Rojas et al. 2006), but is also influenced by insect age and mating status, which affect the sensory input or processing that generates the necessary locomotion pattern to land on the stimulation source (Daly et al. 2007; Anton et al. 2007; Lemmen & Evenden 2009). In *C. decolora* males, neural plasticity induced by the physiological state allows males to modulate their response to the sex pheromone, which must be considered before that synthetic pheromone is used to manage this insect.

## Acknowledgments

R. Arzuffi and N. Robledo are COFAA and EDI, IPN fellows. H. Reyes would like to thank CONACYT and IPN for a scholarship. We are also very grateful to SIP, IPN (Projects 20121192 and 20130883).

## References Cited

- Anton S, Gadenne C. 1999. Effect of juvenile hormone on the central nervous processing of sex pheromone in an insect. *Proceedings of the National Academy of Science, USA* 96: 5764-5767.
- Anton S, Dufour MC, Gadenne C. 2007. Plasticity of olfactory guide behaviour and its neurobiological basis: lessons from moths and locusts. *Entomologia Experimentalis et Applicata* 123:1-11.
- Barrientos-Hernández I, Hernández-Ramírez G, Cibrián-Tovar J. 2011. Tasa de liberación de la feromona sexual de *Copitarsia decolora* (Lepidoptera: Noctuidae) y capturas de machos con trampas en brócoli. *Entomotropica* 26: 47-58.
- Barrozo RB, Jarriault D, Simeone X, Gaertner C, Gadenne C, Anton S. 2010a. Mating-induced transient inhibition of responses to sex pheromone in a male moth is not mediated by octopamine or serotonin. *Journal of Experimental Biology* 213: 1100-1106.
- Barrozo RB, Gadenne C, Anton S. 2010b. Switching attraction to inhibition: Mating-induced reversed role of sex pheromone in an insect. *Journal of Experimental Biology* 213: 2933-2939.
- Barrozo RB, Jarriault D, Deisig N, Gemeno C, Monsempe C, Lucas P, Gadenne C, Anton S. 2011. Mating-induced differential coding of plant odour and sex pheromone in a male moth. *European Journal of Neuroscience* 33: 1841-1850.
- Castrejón-Gómez VR, Cibrián-Tovar J, Romero-Napoles J, Camino-Lavin M. 2000. Mating frequency in wild females of *Copitarsia consueta* (Lepidoptera: Noctuidae). *Florida Entomologist* 83: 374-376.
- Cibrián-Tovar J, Sugimoto A. 1992. Elaboración de una dieta artificial para la cría de *Copitarsia consueta* Walker (Lepidoptera:Noctuidae), pp. 416 *In* Martínez CG, Lastras AR, Valle JM., Medellín PM., Hernández JG., Loza RJM., García RL [eds.], *Memorias del XXVII Congreso Nacional de Entomología. Sociedad Mexicana de Entomología. México.*
- Daly KC, Carrell LA, Mwilaria E. 2007. Detection versus perception: physiological and behavioral analysis of olfactory sensitivity in the moth (*Manduca sexta*). *Behavioral Neuroscience* 121: 794-807.
- De Belle JS, Kanzaki R. 1999. Protocerebral olfactory processing. pp. 243–282 *In* Hansson BS [ed.], *Insect Olfaction*. Springer, Berlin, Germany.
- Diaz-Gomez O, Malo EA, Patiño-Arellano SA, Rojas JC. 2012. Pheromone trap for monitoring *Copitarsia decolora* (Lepidoptera:Noctuidae) activity in cruciferous crops in Mexico. *Florida Entomologist* 95: 602-609.
- Dumont S, McNeil JN. 1992. Responsiveness of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) males, maintained as adults under different temperature and photoperiodic conditions, to female sex pheromone. *Journal of Chemical Ecology* 18: 1797-1807.
- Duportets L, Dufour MC, Couillaud F, Gadenne C. 1998. Biosynthetic activity of corpora allata growth of sex accessory glands and mating in the male moth *Agrotis ipsilon*. *Journal of Experimental Biology* 201: 2425-2432.
- Evenden ML, Gries R. 2008. Plasticity of male response to sex pheromone depends on physiological state in a long-lived moth. *Animal Behavior* 75: 663-672.
- Gadenne C, Anton S. 2000. Central processing of sex pheromone stimuli is differentially regulated by juvenile hormone in a male moth. *Journal of Insect Physiology* 46: 1195-1206.
- Gadenne C, Renou M, Sreng L. 1993. Hormonal control of pheromone responsiveness in the male black cutworm *Agrotis ipsilon*. *Experientia* 49: 721-724.
- Gadenne C, Dufour MC, Anton S. 2001. Transient post-mating inhibition of behavioural and central nervous responses to sex pheromone in an insect. *Proceedings of the Royal Society of London B* 268: 1631-1635.
- Gillott C. 2003. Male accessory gland secretions: Modulators of female reproductive physiology and behavior. *Annual Review of Entomology* 48: 163-184.
- Grosmaître X, Marion-Poll F, Renou M. 2001. Biogenic amines modulate olfactory receptor neurons firing activity in *Mamestra brassicae*. *Chemical Senses* 26: 653-661.
- Hansson BS, Anton S. 2000. Function and morphology of the antennal lobe: New developments. *Annual Review of Entomology* 45: 203–231.
- Keil TA. 1999. Morphology and development of the peripheral olfactory organs. pp. 5-48 *In* Hansson BS [ed.], *Insect Olfaction*. Springer, Berlin, Germany.
- Lemmen J, Evenden M. 2009. Peripheral and behavioral plasticity of pheromone response and its hormonal control in a long-lived moth. *Journal of Experimental Biology* 212: 2000-2006.
- Martel V, Anderson P, Hansson BS, Schlyter F. 2009. Peripheral modulation of olfaction by physiological state in the Egyptian leafworm *Spodoptera littoralis* (Lepidoptera:Noctuidae). *Journal of Insect Physiology* 55: 793-797.
- Muñiz-Reyes E, Cibrián-Tovar J, Rojas-Leon J, Díaz-Gómez O, Valdés-Carrasco J, Bautista-Martínez N. 2007. Capturas de *Copitarsia decolora* (Lepidoptera: Noctuidae) en trampas cebadas con diferentes proporciones de feromona sexual. *Agrociencia* 41: 575-581.
- NIST/EPA/NIH. 2002. Mass spectral library. Mass Spectral Library with Search Program (Data Version: NIST05, Software Version 2.0), USA.
- Pophof B. 2000. Octopamine modulates the sensitivity of silk moth pheromone receptor neurons. *Journal of Comparative Physiology A* 186: 307-313.
- Rogers CE, Marti OG. Jr. 1994. Reproductive potential of once-mated moths of fall armyworm (Lepidoptera: Noctuidae). *Florida Entomologist* 77: 402-410.
- Rogers CE, Marti OG. Jr. 1996. Beet armyworm (Lepidoptera: Noctuidae): Effects of age at first mating on reproductive potential. *Florida Entomologist* 79: 343-352.
- Rojas JC, Cibrián-Tovar J. 1994. Reproductive behavior of *Copitarsia consueta* (Lepidoptera: Noctuidae): mating frequency, effect of age on mating, and influence of delayed mating on fecundity and egg fertility. *Pan-Pacific Entomologist* 70: 276-282.
- Rojas JC, Valdez-Carrasco J, Cibrián-Tovar J. 1995. Morphological evidence for possible sites of production of the female sex pheromone of *Copitarsia consueta* (Lepidoptera: Noctuidae). *Florida Entomologist* 78: 360-363.
- Rojas JC, Cruz-Lopez C, Malo EA, Díaz-Gómez O, Calyecac G, Cibrián-Tovar J. 2006. Identification of the sex pheromone of *Copitarsia decolora*. *Journal of Economic Entomology* 99: 797-802.
- Soques S, Vazquez GM, Grozinger CM, Gould F. 2010. Age and mating status do not affect transcript levels of odorant receptor genes in male antennae of *Heliothis virescens* and *Heliothis subflexa*. *Journal of Chemical Ecology* 36: 1226-1233.
- Suarez-Vargas AD, Bautista-Martinez N, Valdez-Carrasco J, Angulo-Ormeño A, Alatorre-Rosas R, Vera-Graziano J, Equihua-Martinez A, Manuel-Pinto V. 2006. Fluctuación poblacional de *Copitarsia decolora* (Gueéne) y su asociación con crucíferas comerciales. *Agrociencia* 40: 501-509.
- Venette CR, Gould JR. 2006. A pest risk assessment for *Copitarsia* spp.: Insects associated with importation of commodities into the United States. *Euphytica* 148: 165-183.
- Zhang ZC, Wang MQ, Zhang G. 2009. Molecular cloning and expression of pheromone-binding protein1 from the diamondback moth, *Plutella xylostella*. *Entomologia Experimentalis et Applicata* 133: 136-145.