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RESEARCH ARTICLE

Doha Hesham El-Gashingy
El-Saeid Ahmed Naiem
Mervat Rafeik Abou Seada
Amal Ibrahim Seif

Age-related changes in the chemical composition of female pheromone glands of the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae)

ABSTRACT:

The Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) is a serious pest that attacks a wide range of crops in Egypt. To relate the age with the chemical composition of the pheromone glands of the female *S. littoralis,* the chemical components of the glands were analyzed using gas chromatography coupled to mass spectrometry (GC-MS). The pheromone glands of the females were excised at four different ages: newly emerged (D_0v), one-day-old (D_1v), two-day-old (D_2v), three-day-old (D_3v) and three-day-old mated females (D_3m). The pheromone glands contained 54 compounds in the hexane extract, (1 ketone, 1 acetate, 2 fatty acids, 3 esters, 6 aldehydes, 12 alcohols and 29 hydrocarbons). The presence and relative amounts of these compounds in the gland varied with age. Few compounds were observed in the glands of D_0v, increased in D_1v, D_2v, and D_3v, and remained almost constant in D_3m. Fifteen compounds were previously identified as sex pheromone components in other Lepidoptera; the major compounds were the two hydrocarbons, hentriacontane and heptacosane. Another fifteen components were previously identified from different insect orders other than Lepidoptera. The other twenty-four compounds were identified for the first time in insects. Determination and identification the sex pheromones of *S. littoralis* may present safe biologically active compounds that could be used in the management programs for this pest in Egypt.

KEY WORDS:

Spodoptera littoralis, Pheromone glands, Gas chromatography and Lepidoptera

CORRESPONDENCE:

Doha Hesham El-Gashingy

Entomology Section, Zoology Department, Faculty of Science, Tanta University, Egypt

E-mail: dohahesham44@yahoo.com

El-Saeid Ahmed Naiem

Mervat Rafeik Abou Seada

Amal Ibrahim Seif

Entomology Section, Zoology Department, Faculty of Science, Tanta University, Egypt

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INTRODUCTION:

In the last four decades, there have been extensive researches on insect semiochemicals “The Pherobase” (El-Sayed, 2014). They are chemical compounds implicated in the transfer of information from one individual to another to trigger a behavioral and physiological response in one or both insects (Dicke and Sabelis, 1988). The semiochemicals are subdivided into allelochemicals, involved in interspecific communications and pheromones, involved in intraspecific communication. Pheromones are chemicals or a multi-component blend of chemicals that mediate interactions of individuals of the same species. According to their behavioral function, pheromones can be classified according to their function into, territory marking, alarm, trail marking, aggregation, dispersal and sex pheromones (Nordlund, 1981). In most nocturnal Lepidoptera, several pheromone compounds are biosynthesized /stored in specialized bulbous eversible glands which are usually located near the ovipositor at the posterior tips of their abdominal segments

(Roelofs and Wolf, 1988; Ando *et al.*, 2004). In Lepidoptera these compounds "The Pherobase" (El-Sayed, 2014) play different roles.

Virgin Lepidoptera females emit sex pheromones which cause attraction of males to the location of the female and subsequently, mating takes place (Kingan *et al.*, 1993).

In moths pheromone communication signals, both quantitative and qualitative intraspecific differences have been found across geographic regions (Klun, 1975; Roelofs *et al.*, 1985). Such variation has generally been hypothesized to be due to selection and genetic control of these differences (Vogel *et al*., 2010). The composition of the female pheromone glands *S. littoralis* is highly dependent on the origin of the strain and its geographical distribution (Campion *et al*., 1980; Quero *et al*., 1996; Muñoz *et al*., 2008; Carot-Sans *et al*., 2015).

Indeed the sex pheromones have been determined for more than a thousand moth species aimed to identify optimal blends to attract males (Ando and Yamakawa, 2011; El-Sayed, 2014). However, there are surprisingly few attempts in the literature that covered the total chemical composition of the female pheromone glands. For instance, Altamar-Varón *et al.* (2016) identified and characterized the chemical components of the female sexual glands of *Copitarsia uncilata* (Lepidoptera: Noctuidae) at three different age stages in order to relate the effect of age on the gland extract composition. Rebouças *et al.* (1999) identified the chemical composition of the female pheromone gland of *Castnia licus* (Lepidoptera: Castniidae).

Variation in the total pheromone gland chemical composition of the Egyptian strain of *S. littoralis* is largely undocumented. Therefore, the present study analyzes and identifies the chemical components of the female pheromone gland at four different ages to assess their variation in amount and composition with age of adult *S. littoralis.* Data extracted herein is not only important for the basic understanding of the productive behavior of the cotton leafworm, but also may help us when developing control strategies that integrate sex pheromone to combat this pest.

MATERIAL AND METHODS:

Spodoptera littoralis was obtained from a stock culture continuously reared in the laboratory at the Ministry of Agriculture, Giza, Egypt. Larvae were reared in cadges 30 x 30 x 30 cm under laboratory conditions at 25 ± 2ºC, 70% relative humidity and 16L-8D photoperiod regime and were fed daily on fresh castor oil leaves, *Ricinus communis.* Healthy and uniform size pupae were collected from the stock colony, sexed, held separately and noticed until adult emergence. Adults were fed on 10% aqueous solution of honey using a piece of cotton. After mating, adult females were let to lay eggs to keep the life cycle run. Only the female moths were chosen to investigate the chemical composition of the pheromone gland and the age-related changes. The female moths were divided into five groups: 4 virgins and one mated; each cohort consists of 20 females.

The virgin groups were newly emerged females about 4-5 hours (D_0v), one-day-old virgin females (D_1v), two-day-old virgin females (D_2v) and three-day-old virgin females (D_3v). The fifth group was three-day-old mated females (D_3m). Mating occurred mainly 0 - 3 hrs into scotophase (Dunkelblum *et al.*, 1987). Thirty virgin females were coupled with fifty virgin males in a mating box directly before the onset of scotophase. Observations were made using a flashlight covered by several pieces of red sheets. Moths were observed every 30 min of scotophase upon which all mated pairs were picked and put in another mating box until the end of the mating process.

The pheromone glands extraction:

The extraction of the pheromone glands had been done at the 4th hr after onset of the scotophase period. The females were captured and chilled. Gentle presser with pincers was performed between the 8th and 9th lateral-posterior abdominal segments of the female until protrusion of pheromone glands (Fig. 1 A & B). The glands were excised from the abdomen with pincers. The adjacent tissues and the residual of the alimentary canal and the reproductive system were removed using micro scissors and fine forceps. Usually, for chemical analysis, ten females were used (El-Sayed *et al.*, 2005). Twenty glands were collected into a glass vial containing 2 ml hexane and kept at 4˚C overnight, filtered and stored at -20˚C until analysis.

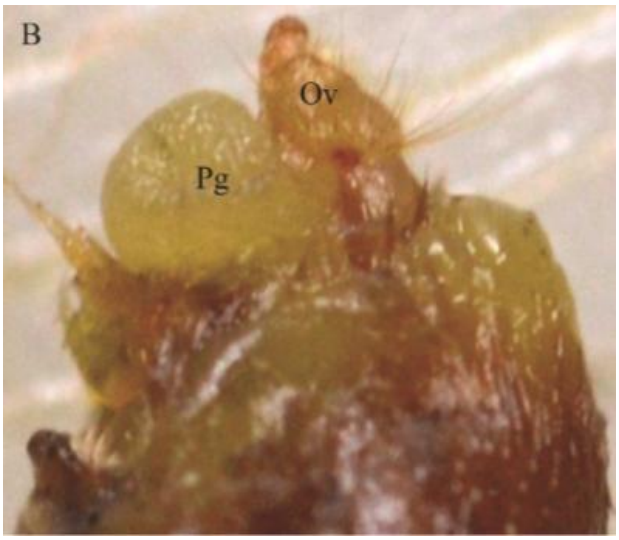

Fig. 1. The pheromone gland of one-day old virgin female *Spodoptera littoralis* forced out by gentle presser with forceps. A. Ventral view. B. Lateral view. Pg: Pheromone gland; Ov: sclerotized ovipositor valve. Photograph shot was taken by Nikon Digital Camera with 8 x magnification and Mikro Nikon 62 mm lens.

Gas chromatography coupled to mass spectrometry (GC-MS) analyses of extracts:

Conventional GC/MS analyses were carried out using a Thermo Scientific TRACE 1310 gas chromatography combined with ISQ LT single quadrupole mass spectrometer as described in (Dunkelblum *et al.*, 1982). The gas chromatography was equipped with fused silica capillary column DB1(15 m × 0.25 mm). The helium flow was set at 1.5 ml He/min. One microliter of the extract was injected using a splitless injector (split-less time: 0.80 min). The temperature of the injector was fixed at 200˚C and the detector at 300˚C. The column temperature program began at 115˚C for 1 min, and increased to 280˚C at a rate of 7.5˚C min. The ionization was EI mode and the voltage was 70 ev. The identification of the compounds was performed by comparing the obtained mass spectra fragmentation patterns with those in the WILEY and NIST mass spectral database library and by comparing their retention indices to "The Pherobase" (El-Sayed, 2014).

Statistical analysis:

The relative content of the chemical components of the pheromone glands at different ages were checked for normal distribution using Anderson-Darling test. In case of non-normally distributed, data were SQRT transformed. The relative content of each component was analyzed using the linear regression test to find the trend with increasing time (ages).

RESULTS:

Analysis of the pheromone glands of *S. littoralis* females, revealed that the total number of components identified at ages D_0v, D_1v, D_2v, D_3v and D_3m were 35, 46, 33, 29, and 29 compounds, respectively. The compounds included were classified according to their chemical nature and formula as the number of carbon atoms and the functional group to 1 ketone, 1 acetate, 2 fatty acids, 3 esters, 6 aldehydes, 12 alcohols and 29 hydrocarbons. The components detected varied according to the female age categories.

Ketone, acetate and fatty acids:

Both the friedelin ketone and epilupeol acetate only appeared in D_1v (Table 1). The fatty acids octadecanoic acid and (Z)-9-octadecenoic acid (Table 1) were identified in the glands of all age categories. The highest percentages of them were in the glands of D_3v and D_3m females, and the lowest in D_1v.

Table 1. Relative contents of ketone, acetate and fatty acids identified in the pheromone glands extraction of 20 *Spodoptera littoralis* females

Constituents Identification	Formula	Relative content %				
		D_0v	D_1v	D_2v	D_3v	D_3m
Friedelin ketone	$C_{30}H_{50}O$	-	5.58	-	-	-
Epilupeol acetate	$C_{32}H_{52}O_2$	-	11.75	-	-	-
Octadecanoic acid	$C_{18}H_{36}O_2$	1.28	0.10	1.95	3.61	3.67
(Z)-9-Octadecenoic acid	$C_{18}H_{34}O_2$	0.26	0.12	0.36	0.70	0.71

D_0v: Newly emerged females, D_1v: one-day-old virgin females, D_2v: two-day-old virgin females, D_3v: three-day-old virgin females and D_3m: three-day-old mated females.

Esters:

The three esters dibutyl phthalate, decylpentyl phthalate and diisooctyl phthalate were detected in all age categories (Table 2). Decylpentyl phthalate and diisooctyl phthalate showed the highest percentages in the glands of D_3v and D_3m females, and the lowest in D_1v. However, the highest percentage of dibutyl phthalate ester was in D_2v.

Table 2. Relative contents of esters identified in the pheromone glands extraction of 20 *Spodoptera littoralis* females

Constituents Identification	Formula	Relative content %				
		D_0v	D_1v	D_2v	D_3v	D_3m
Dibutyl phthalate	$C_{16}H_{22}O_4$	2.41	0.23	23.13	17.52	16.68
Decylpentyl phthalate	$C_{23}H_{36}O_4$	0.06	0.014	1.11	3.88	3.95
Diisooctyl phthalate	$C_{24}H_{38}O_4$	2.30	0.01	2.34	5.83	5.93

D_0v: Newly emerged females, D_1v: one-day-old virgin females, D_2v two-day-old virgin females, D_3v: three-day-old virgin females and D_3m: three-day-old mated females.

Aldehydes:

(E,E)-2,4-Heptadienal, nonanal and 7-hexadecenal were detected in the glands of all age categories (Table 3). Nonanal showed an upward trend with age (P = 0.037). The 2-decenal was absent in D_0v. Both (E, E)-2,4-decadienal and (E)-2-undecenal were only detected in D_2v glands.

Table 3. Relative contents of aldehydes identified in the pheromone glands extraction of 20 *Spodoptera littoralis* females

Constituents Identification	Formula	Relative content %				
		D_0v	D_1v	D_2v	D_3v	D_3m
(E,E)-2,4-Heptadienal	$C_7H_{10}O$	0.07	0.23	1.82	0.20	0.21
Nonanal	$C_9H_{18}O$	0.05	0.18	1.43	2.26	2.30
2-Decenal	$C_{10}H_{18}O$	-	0.014	1.08	0.29	0.30
(E,E)- 2,4-Decadienal	$C_{10}H_{16}O$	-	-	0.76	-	-
(E) -2-Undecenal	$C_{11}H_{20}O$	-	-	0.64	-	-
7-Hexadecenal	$C_{16}H_{30}O$	0.08	0.03	0.73	1.77	1.57

D_0v: Newly emerged females, D_1v: one-day-old virgin females, D_2v: two-day-old virgin females, D_3v: three-day-old virgin females and D_3m: three-day-old mated females.

Alcohols:

Only butylated hydroxytoluene showed a linear increase with age (P = 0.049), however, it was absent in D_0v (Table 4). The two alcohols, docosan-1-ol and 6,10,13-trimethyltetradecan-1-ol were recorded in all virgin and mated female glands. The relative amounts of 6,10,13-trimethyltetradecan-1-ol were higher in D_3v and D_3m. The alcohols (Z)-3-decen-1-ol, lupeol, 1-heptatriacontanol, lanosterol and 24-methylene-24,25- dihydrolanosterol were only identified in D_1v. Hexadecan-1-ol was detected in both D_3v and D_3m glands. Eiocosan-1-ol was absent in D_1v females. Cholest-5-en-3beta-ol (Cholesterol) was identified only in both D_1v and D_2v females. Geranylgeraniol was present in D_0v and D_1v glands.

Table 4. Relative amounts of alcohols identified in the pheromone glands of 20 *Spodoptera littoralis* females

Constituents Identification	Formula	Relative content %				
		D_0v	D_1v	D_2v	D_3v	D_3m
(Z)-3-Decen-1-ol	$C_{10}H_{20}O$	-	0.15	-	-	-
Hexadecan-1-ol	$C_{16}H_{34}O$	-	-	-	0.50	0.37
Docosan-1-ol	$C_{22}H_{46}O$	0.02	0.014	1.58	0.57	0.58
6,10,13-Trimethyl tetradecan-1-ol	$C_{17}H_{36}O$	1.21	0.01	0.90	3.1	3.14
Eiocosan-1-ol	$C_{20}H_{42}O$	0.09	-	0.61	0.50	0.50
Cholest-5-en-3beta -ol(Cholesterol)	$C_{27}H_{46}O$	-	1.9	2.40	-	-
Geranylgeraniol	$C_{20}H_{34}O$	0.16	0.07	-	-	-
Lupeol	$C_{30}H_{50}O$	-	5.79	-	-	-
1-Heptatriacontanol	$C_{37}H_{76}O$	-	1.82	-	-	-
Butylated hydroxy -toluene	$C_{15}H_{24}O$	-	0.08	0.97	5.83	5.92
Lanosterol	$C_{30}H_{50}O$	-	5.14	-	-	-
24-Methylene-24,25-dihydrolanosterol	$C_{31}H_{52}O$	-	10.62	-	-	-

D_0v: Newly emerged females, D_1v: one-day-old virgin females, D_2v: two-day-old virgin females, D_3v: three-day-old virgin females and D_3m: three-day-old mated females.

Hydrocarbons:

The chemical analysis of *S. littoralis* glands showed the presence of 29 hydrocarbons (Table 5). They were the most abundant chemical category found in pheromone glands. The 1,3-dimethyl cyclopentane, methylcyclohexane and ethylcyclopentane were only found in D_0v females.

Table 5. Relative amounts of hydrocarbons identified in the pheromone glands extraction of 20 *Spodoptera littoralis* females

Constituents Identification	Formula	Relative content %				
		D_0v	D_1v	D_2v	D_3v	D_3m
1,3–Dimethylcyclopentane	C_7H_{14}	31.61	-	-	-	-
Methylcyclohexane	C_7H_{14}	9.26	-	-	-	-
Ethylcyclopentane	C_7H_{14}	11.33	-	-	-	-
Trans -1,4-dimethylcyclohexane	C_8H_{16}	6.02	0.16	6.54	-	-
Octane	C_8H_{18}	14.00	3.06	4.21	2.26	2.30
2-Methyloctane	C_9H_{20}	0.24	9.17	6.93	0.20	0.21
1,4-Dimethylbenzene (*p*-Xylene)	C_8H_{10}	2.25	11.11	0.52	-	-
Nonane	C_9H_{20}	0.37	7.29	-	-	-
2,3-Dimethylheptane	C_9H_{20}	0.023	3.26	-	-	-
1,2,4-Trimethylcyclohexane	C_9H_{18}	0.046	3.25	-	-	-
1-Ethyl, 3-methylbenzene	C_9H_{12}	-	1.07	-	-	-
Propylcyclohexane	C_9H_{18}	-	1.37	-	-	-
Decane	$C_{10}H_{22}$	-	2.68	-	-	-
Nonadecane	$C_{19}H_{40}$	-	0.14	3.31	3.00	3.05
Heptacosane	$C_{27}H_{56}$	0.09	0.24	6.00	7.03	7.14
2-Methyloctadecane	$C_{19}H_{40}$	0.01	0.05	3.42	5.12	5.20
(E,E,E,E)-6,8-Diethyl-4-methyl-3,5,7,9-dodecatetraene	$C_{17}H_{28}$	-	0.15	-	-	-
Farnesane	$C_{15}H_{32}$	-	0.11	0.61	0.24	0.24
1-Chlorooctadecane	$C_{18}H_{37}Cl$	0.04	0.03	1.06	1.56	1.38
Pentatriacontane	$C_{35}H_{72}$	3.89	0.10	2.05	1.29	1.31
Heneicosane	$C_{21}H_{44}$	0.7	-	3.62	7.06	7.07
1-Docosene	$C_{22}H_{44}$	0.11	3.93	4.33	1.27	1.29
2-Methyldocosane	$C_{23}H_{48}$	0.01	0.01	0.79	0.35	0.54
Hentriacontane	$C_{31}H_{64}$	7.55	0.01	5.96	11.98	12.17
2,6,10 - Trimethyldodecane	$C_{15}H_{32}$	0.03	0.08	1.79	1.40	1.42
Hexacosane	$C_{26}H_{54}$	0.22	0.014	1.93	0.96	0.97
Triethylbenzene	$C_{12}H_{18}$	0.85	4.57	-	-	-
Ethylcyclohexane	C_8H_{16}	2.58	1.43	-	-	-
Dotriacontane	$C_{32}H_{66}$	0.15	0.054	4.5	5.39	5.47

D_0v: Newly emerged females, D_1v: one-day-old virgin females, D_2v: two-day-old virgin females, D_3v: three-day-old virgin females and D_3m: three-day-old mated females.

Octane, 2-methyloctane, heptacosane, 2-methyloctadecane, 1-chloro-octadecane, pentatriacontane, 1-docosene, 2-methyldocosane, hentriacontane, 2,6,10-trimethyldodecane, hexacosane, and

dotriacontane were consistently found in all females. 2-Methyloctadecane and 1-chloro-octadecane ($P > 0.05$), did not show any trend with ages. Octane and pentatriacontane were found in the highest relative amounts in D_0v females. On the other hand, 2-methyloctane its higher relative amounts were in both D_1v and D_2v females (9.17 and 6.93%, respectively).

The highest relative amounts of heptacosane, 2-methyloctadecane and hentriacontane were found in D_3v and D_3m females, while 1-chloro-octadecane relative abundance was almost similar in the pheromone glands of D_2v, D_3v and D_3m (1.06, 1.56, and 1.38%, respectively). The highest relative amount of 1-docosene was detected in glands of D_2v (4.33%) followed by D_1v (3.93%). Similar relative amounts of 2,6,10 trimethyldodecane were recorded in the glands of D_2v, D_3v and D_3m females (1.79, 1.40, and 1.42%, respectively). Hexacosane highest relative amount (1.93%) was detected in D_2v females.

Both D_3v and D_3m glands contained similar relative amounts of dotriacontane (5.39 and 5.47%, respectively) followed by D_2v (4.5%). Trans-1,4- dimethylcyclohexane and 1,4-dimethylbenzene (*p*-xylene) were detected only in D_0v, D_1v and D_2v females.

Nonane, 2,3-dimethylheptane, 1,2,4-trimethylcyclohexane, triethylbenzene and ethylcyclohexanewere only detected in glands of D_0v and D_1v females. 1-Ethyl,3-methylbenzen, propylcyclohexane, decane and (E, E, E, E)-6,8-diethyl-4-methyl-3,5,7,9-dodecatetraene were only detected in D_1v females. Both nonadecane and farnesane were detected in all female categories except those of D_0v females. Heneicosane was present in the glands of all females except those of D_1v females.

DISCUSSION:

The total number of chemical compounds identified in the pheromone glands of D_0v, D_1v, D_2v, D_3v virgin and D_3m mated females were 35, 46, 33, 29, and 29 compounds respectively. These variations might provide information that may indicate when and why males choose a female "The Pherobase" (El-Sayed, 2014). The components identified in *S. littoralis* female glands may include non-pheromone components. Some components could be only the products of the pheromone biosynthesis pathway (Jurenka, 2004) or tissue (Tumlinson *et al.*, 1986).

GC-MS analysis revealed the presence of 54 compounds in *S. littoralis* female glands. 15 were previously identified as sex pheromones in Lepidoptera, another 15 were detected as semiochemicals from different insect orders and 24 were recorded for the first time from insects according to "The Pherobase" (El-Sayed, 2014). The fifteen compounds identified from Lepidoptera were the fatty acids octadecanoic acid and (Z)-9-octadecenoic acid, the aldehydes nonanal and 7-hexadecenal, the alcohols hexadecan-1-ol, eiocosan-1-ol and cholest-5-en-3beta-ol (cholesterol), and the hydrocarbons nonadecane, heptacosane, 2-methyloctadecane, heneicosane,1-docosene, hentriacontane, hexacosane, and dotriacontane.

Meanwhile, the other fifteen components identified as semiochemicals from different insect orders except Lepidoptera were ; aldehydes (E)- 2-decenal , (E,E)-2-4-decadienal, (E)-2-undecenal, alcohols (Z)-3-decen-1-ol, 6-10-13-trimethyltetradecanol,docosan-1-ol, geranylgeraniol and hydrocarbons methylcyclohexane, octane, 1,4-dimethylbenzene (*p*-xylene), nonane, decane, (E,E,E,E)-6-8-diethyl-4-methyl-3-5-7-9-dodecatetraene, pentatriacontane and 2-methyldocosane "The Pherobase" (El-Sayed, 2014).

The other twenty-four compounds identified in the pheromone gland of *S. littoralis* have not been reported in insects; the friedelin ketone, epilupeol acetate, esters dibutyl phthalate, decylpentyl phthalate and diisooctyl phthalate, aldehyde (E,E)-2,4-heptadienal, alcohols lupeol, 1-heptatriacontanol, butylated hydroxytoluene, lanosterol and 24-methlene-24,25-dihydrolanosterol, hydrocarbons 1,3-dimethylcyclopentane, ethylcyclopentane, trans-1,4- dimethylcyclohexane, 2-methyloctane , 2,3-dimethylheptane, 1,2,4-trimethylcyclohexane, 1-ethyl, 3-methylbenzene, propylcyclohexane, farnesane, 1-chloro-octadecane, 2,6,10-trimethyldodecane, triethylbenzene and ethylcyclohexane. To our knowledge, this is the first time that they have been recorded in the pheromone gland of *S. littoralis*.

Chemicals previously identified as sex pheromones in Lepidoptera:

The present study revealed that both hentriacontane and heptacosane were major compounds. The most abundant compounds were dotriacontane, 2-methyloctadecane, octadecanoic acid, nonanal, 7-hexadecenal, 1-docosene and a number of compounds presented in minor relative amounts, such as hexacosane and (Z)-9-octadecenoic acid. Hexadecan-1-ol was identified only from the pheromone glands of D_3v and D_3m females. Both eiocosan-1-ol and heneicosane were detected in virgin and mated females except D_1v females. Cholest-5-en-3beta-ol was identified only in both D_1v and D_2v females. Nonadecane was detected in all virgin and mated females except D_0v females.

S. littoralis in this study showed slight changes in the relative abundance of chemical compounds of pheromone glands. Pheromone relative abundance was very low in the pheromone glands on the first night after eclosion (D_0v and D_1v), but increased in

D_2v and D_3v and remain constant in D_3m. It appeared that D_0v adult females were reproductively immature within 1 day after eclosion and were ready to mate after the second and the third day after eclosion, therefore this coincides with the calling behavior that was maximal on the 2^{nd} –3^{rd} day old females (Dunkelblum *et al.*, 1987). Moreover, females are polyandrous (multiple maters) and ready to mate at any time throughout her life (Kehat and Gordon, 1975).

For a particular species, the blend components of sex pheromone usually belong to only one of two general structural classes (Type I and Type II). However, recent studies have revealed that some species use a combination of Type I and Type II pheromone compounds for mate attraction (Ando and Yamakawa, 2011). In the present study, only two components (the aldehyde 7-hexadecenal and the alcohol hexadecan-1-ol) have the typical structure of the type I pheromones, which comprise 75% of the known pheromones and are usually present in females of the Noctuoidea super family (Ando and Yamakawa, 2011). Straight-chain alkanes nonadecane, heptacosane, 2-methyloctadecane, heneicosane, 1-docosene, hentriacontane, hexacosane, and dotriacontane were also identified as pheromone gland components of *S. littoralis.* Straight- and branched-chain alkanes have been shown to function as lepidopteran pheromones (Tillman *et al.*, 1999). Altamar-Varón *et al.* (2016) demonstrated straight alkanes in the eversible pheromone gland of *Copitarsia uncilata* (Noctuidae).

Hexadecan-1-ol was identified as sex pheromone of *Helicoverpa assulta* (Noctuidae) (Cork *et al.*, 1992) and *Heliothis subflexa* (Teal *et al.*, 1981). Moreover, the aldehyde 7-hexadecenal was identified as one of the minor pheromone components of the 3 to 4 days old virgin females *Helicoverpa armigera* (Zhang *et al.*, 2012). Nonanal has been identified in the sex pheromone glands of several other species from different Lepidoptera families including 3- 4 days old virgin females of *H. armigera* (Zhang *et al.*, 2012). Eiocosan-1-ol was detected in the sex pheromone glands of 2-3 old virgin females from an Italian strain of the grapevine moth *Lobesia botrana* (Witzgall *et al.*, 2005). Ômura *et al.* (2012) revealed the presence of cholest-5-en-3beta-ol among 17 major components of cuticular lipids in the extraction of the whole individual of both male and female *Papilio protenor* butterflies.

Octadecanoic acid was identified as one of the major constituents of the cuticular lipids from *Papilio protenor* (Ômura *et al.*, 2012) and from the hair pencil extracts of male African milkweed butterflies (Schulz *et al.,* 1993). (Z)-9-Octadecenoic acid was determined as one of the major components of the pheromone glands of virgin females *Castnia licus* (Drury) (Castniidae) (Rebouças *et al*., 1999).

Nonadecane has an important role as sex pheromone and allomone in several species. This component has been isolated from the sex pheromone glands of *Spodoptera exigua* (Mujiono *et al.*, 2015). Heptacosane has been recorded from the faint scent of both male and female adults extracts of swallowtail *Papilio polytes* (Papilionidae) (Ômura and Honda, 2005), *Papilio protenor* (Ômura *et al.*, 2012) and from the female gland of *Ostrinia nubilalis* (Kalinova *et al.*, 1994).

The hydrocarbons 2-methyloctadecane, 1-docosene, hexacosane and hentriacontane were identified in the glands of all virgin and mated females, while heneicosane was reported in all female classes except D_1v females. 2-Mehyloctadecane was reported as one of sex pheromones extracted of *Holomelina lamae* (Schal *et al.*, 1987). Heneicosane, 1-docosene, and hexacosane were identified from the hairpencil extracts of various males African milkweed butterfly species (Schulz *et al.*, 1993). Hentriacontane and dotricontane both appeared in the extraction of adult *Papilio protenor* butterflies cuticular lipids (Ômura *et al.*, 2012).

Chemicals identified in different insect orders except Lepidoptera:

The aldehyde (E)-2-decenal was identified as adefensive compound of the staphylinid Omaliinae and Proteininae (Dettner and Reissenweber, 1991), meanwhile nonane, decane and octane were detected from the carabid beetle *Galerita lecontei* abdominal defensive glands (Rossini *et al.*, 1997). Bartelt *et al.* (1993) found that males of the coleopteran *Carpophilus antiquus* emit E-E-E-E-6-8-diethyl-4-methyl-3-5-7-9-dodecatetraene as aggregation pheromone, (Z)-3-decen-1-ol was recognized from male long-horned cerambycid beetle *Rosalia funebris* (Ray *et al.*, 2009) and 6-10-13-trimethyl-tetradecan-1-ol was confirmed from the stink bug, *Stiretrus anchorago* (Kochansky *et al.*, 1989) as well.

Methylcyclohexane and nonane were identified as volatile semiochemicals used in host location by the coffee berry borer *Hypothenemus hampei* (Mendesil *et al.*, 2009). Geranylgeraniol was investigated as an active sex pheromone compound from cephalic extracts of eusocial bumblebee *Bombus terrestris* (Apidae) (Krieger *et al.*, 2006).

Pentatriacontane was reported as one of n-alkanes from the cuticular hydrocarbons of the coleopteran *Lepidochora discoidalis* (Lockey, 1985). The fatty alcohol docosan-1-ol was found from hexane extracts of virgin males and females triatomine *Triatoma infestans* epicuticular lipids (Cocchiararo-Bastias *et al.*, 2011). Moreover, the mono methylalkane 2-methyl- docosane was

detected as one of the cuticular lipids of different species of termites (Haverty *et al.*, 2000) and from tarsal secretions of the beetles *Phaedon cochleariae and Nicrophoru snepalensis*as (Geiselhardt *et al.*, 2011).

1,4-Dimethylbenzene (*p*-xylene) was identified from the analysis of the female pecan weevil *Curculio caryae* essential oil (Mody *et al.*, 1973). The aldehyde (E)-2-undecenal was detected among other compounds at the relatively low range in either whole-body and/or metathoracic scent gland extracts of females insidious flower bug *Orius insidiosus* (Say) (Aldrich *et al.*, 2007). Moreover (E,E)-2,4-decadienal was reported from the metathoracic glands (MTGs) of both males and females of the predatory stink bug *Eocanthecona furcellata* (Ho *et al.*, 2003).

Chemicals not reported in insects:

Twenty four compounds were identified in the pheromone glands of *S. littoralis* that have not been reported in insects. As previous Schulz *et al.* (2004) found 6 compounds in male hairpencel components of 54 species in genera of Ithomiinae butterflies that have not been reported before from insects. In insects semiochmicals are either synthesized *de novo* or derived from dietary precursors utilized directly or altered minimally by insect enzymatic systems (Tillman *et al.*, 1999). Several reports indicated that host precursors are converted to pheromone component through a simple chemical transformation. Alkaloids (Bell *et al.*, 1984; Bell and Meinwald, 1986; Schulz *et al.*, 1993; Eisner and Meinwald, 2003), fatty acids (Charlton and Roelofs, 1991) and plant isoprenoids (Hendry *et al.*, 1980) can serve as insect pheromone precursors.

The host plant used in this study to rear *S. littoralis* larvae was *R. communis.*, the glands of all virgin and mated *S. littoralis* comprised several compounds such as hexacosane, hentriacontane, heptacosane, dotriacontane, pentatriacontane and octadecanoic fatty acid that were also present in *R. communis* (de Araújo Silva *et al.*, 2017). Moreover, the glands contained friedelin, lupeol, lanosterol, cholesterol and farnesane; all known as plant triterpens (Seigler, 1998; Arimura *et al.*, 2005; Silva *et al.*, 2011; de Araújo Silva *et al.*, 2017) and dibutyl phthalate ester (Li *et al.*, 2013).

The findings of this investigation appeared to be close to the chemical composition previously found in the pheromone glands of the mideteranean *S. littoralis* that fed on similar host plant. Surprisingly, the relative content of major components of the pheromone glands appeared in this analysis did not show significant changes with increasing time (age). However, two components nonanal and butylated hydrohytoluene have upward trend with age. From the previous literature, nonanal was identified as a novel compound in the sex pheromone blend of female *Helicoverpa armigera.* Moreover, it had strong electrophysiological responses in male antennae (Zhang *et al.*, 2012). This aldehyde also was recorded from male *Galleria mellonella* , pheromone glands (Romel *et al.*, 1992) and is known to be an attractant for females (Payne and Finn, 1977). Butylated hydroxytoluene has been recorded for the first time in insects and its function is not known yet.

The possible roles of the components identified in the extracts of the pheromone glands of the Egyptian strain of *S. littorals* female are required to be elucidated in further studies of the pheromone communication system. The results may contribute to the development of sex pheromone compounds to be used for management and control of this important pest in Egypt.

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التغيرات المرتبطة بالعمر فى التركيب الكيميائي لغدة الفرمون في أنثي دودة ورق القطن المصرية، سبودوبترا ليتوراليس (ليبيدوبترا: نوكتويدى)

ضحي هشام الجاشنجي، السعيد أحمد نعيم، ميرفت رفيق أبوسعدة، آمال إبراهيم سيف

شعبة علم الحشرات، قسم علم الحيوان، كلية العلوم، جامعة طنطا، مصر

تعتبر دودة ورق القطن المصرية، سبودوبترا ليتوراليس (ليبيدوبترا: نوكتويدى) آفة خطيرة فهي تهاجم المحاصيل علي نطاق واسع فى مصر. هدفت هذه الدراسة إلى ربط العمر بالتركيب الكيميائي لغدة الفرمون لأنثي سبودوبترا ليتوراليس. تم تحليل المركبات الكيميائية للغدة باستخدام كروماتوغرافيا الغاز إلى جانب مطياف الكتلة (GC-MS) . تم اختيار غدد الفرمون للإناث على أربع أعمار مختلفة: التي ظهرت حديثا (D_0v) ، عمر يوم واحد (D_1v)، عمر يومان (D_2v)، عمر ثلاثة أيام (D_3v)، عمر ثلاثة أيام متزوجة (D_3m). ولقد أظهرت النتائج احتواء الغدد علي 54 مركب في مستخرج الهكسان. هذه المركبات صنفت كالتالى: 1 كيتون، 1 خلات، 2 أحماض دهنية، 3 إسترات، 6 ألدهيدات، 12 كحولات و 29 هيدروكربونات . إن وجود هذه المركبات وتغيرها النسبي في الغدة يختلف بالعمر. فقد ظهر عدد قليل من المركبات في غدة (D_0v) ثم زاد العدد في (D_1v)، (D_2v)،(D_3v) و ظل ثابتا في (D_3m). 15 مركب تم تعريفهم سابقا كفرمونات جنسية من رتبة Lepidoptera ؛ وكانت أعلى نسب لاثنان من الهيدروكربونات hentriacontane و heptacosane . 15 مركب آخر تم تعريفه سابقا من رتب أخرى خلاف رتبة Lepidoptera. أما عن 24 مركب الباقين تم تعريفهم لأول مرة في الحشرات. لذلك فإن تحديد وتعريف الفرمونات الجنسية من سبودوبترا ليتوراليس من الممكن أن يوفر مركبات آمنة للمكافحة البيولوجية لهذه الآفة في مصر.