Vol. 6(2003) 37 - 43

Periodicity in the Quantity and Ratio of Pheromone Components in Volatile Emissions from Virgin Females of the Spotted Stalk Borer Moth *Chilo partellus* (Swinhoe)

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Volatile sex pheromone was collected from the extruded pheromone gland of females of the spotted stalk borer moth *Chilo partellus* and trapped on glass wool. The pheromone was collected from females on the night of eclosion, 1st, 2nd, 3rd and 5th scotophases thereafter. The female sex pheromone components, (*Z*)-11-hexadecenal and (*Z*)-11-hexadecen-1-ol were identified by gas chromatography GC co-injection with synthetic authentic compounds and confirmed by GC-mass spectrometry. The quantity of the pheromone components was determined by comparison of GC peak areas with that of (*E*,*Z*)-3,13-octadecadienyl acetate as an internal standard. Periodicity in the pheromone emission was uni-modal with a peak about the 7-10 h into the scotophase. During the peak period, (Z) – 11 - hexadecenal was emitted at a rate of 43.1, 30.9, 21.5 and 16.5 ng/30 min on the day of eclosion, 1st, 3rd and 5th scotophases, respectively. A marked reduction in the release rate of the pheromone components was recorded with progressing age of females. This decrease was faster for (Z) – 11 – hexadecen – I - ol than for (Z) – 11 - hexadecenal which resulted in a spectacular shift in the ratio (Z) – 11 – hexadecen – I - ol ranging from about 1:1 at eclosion to 9:1, 22:1 and 32:1 in the 1st, 3rd and 5th scotophases, respectively. The age-dependent shift in both release rate and ratio of pheromone components corresponds to the change in attractiveness of females to mate-searching males. The periodicity in the quantity and blend ratios of *C. partellus* pheromone is discussed in light of development of a pheromone-based bait for the management of this pest.

Key words: Periodicity, Lepidoptera, Pyralidae, Chilo partellus, sex pheromone, pheromone emission.

INTRODUCTION

In Lepidoptera, behavioral and physiological processes that are involved in reproduction are usually co-ordinated, so that they occur synchronously under optimal conditions in a heterogeneous and variable environment. In many female Lepidoptera, occurrence and timing of sexual behavior such as calling and pheromone release is regulated by endogenous and exogenous factors [1]. The endogenous factors include mating status and stage of egg development while exogenous factors include photoperiod and temperature [1 – 7]. The effect of periodicity on behavioral processes such as female calling and pheromone release has been studied for a number of species [1]. Knowledge of the periodicity of these processes and how this essential co-ordination is achieved can be useful in the development and rational implementation of pheromone-based pest management techniques [1].

The spotted stalk borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) is an important pest of maize, sorghum, rice, sugarcane, pearl millet, wheat, foxtail, finger millet and various other grasses [8]. Previous work on the reproduction of *C. partellus* has included studies on various aspects of mating, longevity, fecundity, ovipositon and egg fertility [9 – 18]. The major components of the pheromone of *C. partellus* females were identified as (*Z*)-11-hexadecenal (*Z*11-16:ALD) and (*Z*)-11-hexadecen-1-ol (*Z*11-16:OH) [19] and the attractiveness of their synthetic blend was re-evaluated and confirmed in the laboratory and field conditions [17, 20]. Precise relationships between the periodicity of pheromone emission and female attractiveness to males, has however not been established for

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C. partellus. In this paper, we report on the periodicity in the quantity and blend ratios of the pheromone components in volatile emissions from sex pheromone glands of female *C. partellus.*

MATERIALS AND METHODS

Insects

Chilo partellus moths were drawn from cultures maintained in two locations in Kenya: Nairobi and at Mbita Point, a field station of the International Centre of Insect Physiology and Ecology (ICIPE), in western Kenya. The insects were reared under a natural light-dark 12:12 h photoperiod on a semi-artificial diet containing, among other ingredients, sorghum leaf powder [21]. For additional comparisons, insects were field-collected from maize in the coastal area of Kenya near Mombasa

Trapping of Pheromone Emissions

For collection of pheromone emissions, female pupae were placed in 20 x 15 x 15 cm cages and kept in the laboratory at 27±3 ^{o} C and 65±5 % relative humidity under a reversed light-dark 12:12 h photoperiod, with light starting at 18.00 h. The experiment was conducted in Nairobi using insects from a colony maintained in Nairobi. Emerged adult females were removed daily and transferred to separate cages where they were fed on a 5% sucrose-water solution and maintained under the same conditions.

Volatile emissions were collected from forcibly extruded pheromone glands (located at the abdominal tip) of *C. partellus*, following the method by Du *et al*. [22]. Every hour, a female moth was inserted abdomen first into a female-holder made from a micropipette tip. The female was made to extrude its pheromone gland by applying pressure to its abdomen using cotton wool. The holder was then mounted into a glass collector. Charcoal-filtered nitrogen was allowed to flow at 50 ml/min over the extruded gland and through a plug of activated glass wool (15 mg) placed at the distal end of the collector. After 30 min of collection, 100ng of an internal standard [(*E,Z*)-3, 13-octadecadienyl acetate] was introduced into the glass wool, which was then rinsed with four aliquots of 50 µl of purified n-hexane into a micro sample tube. The resulting solution was then condensed to 1-2 µl using a gentle stream of pure nitrogen and then injected into a gas chromatograph for analysis. Otherwise, the sample was stored in a freezer at –20 ^{0}C.

During the night of eclosion (emergence) under normal photoperiod, pupae were observed under a red light for emergence of adults at every hour up to the 12th h into scotophase. Moths that emerged up to the 7th h into the scotophase were kept in one group while those that emerged thereafter were isolated on an hourly basis. For each group, determination of pheromone emission was conducted starting one hour after emergence on an hourly basis until daybreak.

On the first scotophase (1 day after emergence), the volatile pheromone was trapped from females at one-hour intervals, starting from the 1st h to the 12th h into scotophase. During the 2nd, 3rd and 5th scotophases, the volatile pheromone was trapped from the 7th to the 10th hour into scotophase, which from the results of the 1st scotophase, was found to be the peak period of pheromone release. For comparisons, the pheromone was also trapped from the field-collected insects from 8th to 9th hours of their 1st and 3rd scotophases.

Quantification of Volatile Pheromone by Gas Chromatography

The quantity of the pheromone components of female *C. partellus*, Z11-16:ALD and Z11-16:OH, was determined by gas chromatography (GC). The pheromone components were identified through GC co-injection of authentic synthetic compounds (purchased from Aldrich Chemicals, UK) with volatile trappings from pheromone glands of the moths. The compounds were then confirmed by analyzing the pheromone extract using a gas chromatograph coupled to a mass spectrometer (GC-MS). GC analyses were conducted on a Hewlett Packard model 5890 GC (USA) equipped with a splitless capillary injector system, a flame ionization detector and a Hewlett Packard 3393A integrator. Nitrogen was used as the carrier gas at a flow rate of 0.6 ml/min. A Hewlett Packard fused silica capillary column; 50 m x 0.32 mm i.d. coated with Carbowax 20 M (0.3 µm film thickness) was used. The column temperature was maintained at 60 ^{o} C for 4 min following injection and then programmed to a gradient at 10 ^{o} C/min to 220 ^{o} C where it was held for 10

minutes. All injections were made in the splitless mode with a 45 seconds delay before injection purging. Injector and detector temperatures were held at 220 ^{o}C. Quantification of the pheromone components was done by comparison of the peak areas with that of the internal standard, (*E, Z*)-3,13-octadecadienyl acetate.

GC-MS analysis was carried out on a VG Masslab 12-250 mass spectrometer (EI, 70 eV) coupled to an HP 5790 Series A gas chromatograph. The GC column and temperature programming were the same as those described for GC analysis.

RESULTS

Periodicity in sex pheromone emission from the gland surface of virgin females was examined during the night of eclosion and one, two, three and five scotophases thereafter. On the night of eclosion the pheromone components, the aldehyde, Z11-16:ALD and the alcohol, Z11-16:OH, were emitted at high, almost constant and equal rate throughout the night, irrespective of the time into the scotophase when the moths emerged (Table 1). The average release rates of Z11-16:ALD (ALD) and Z11-16:OH (OH) for the entire night was about 43 ng/ 30 min and 34 ng/30 min per female respectively, representing the ratio ALD:OH of about 1.2 : 1 (Table 2). Remarkably, both the release pattern and the ratio of the main pheromone components changed substantially the following night. The release rate was no longer constant and, while the quantity of the aldehyde remained similar (reduced by about 25 %) to that of the night of eclosion, the release rate for the alcohol dropped over 10-fold. On the 1st scotophase, a build up of pheromone emission occurred from the fifth hour and increased to a maximum in the 8th and 9th hours into the scotophase (Table 2). During the peak period (8 - 9 th h), Z11-16:ALD was emitted at a rate of about 35 ng/30 min and Z11-16:OH at a rate of about 4 ng/30min per female, representing a ratio ALD:OH of about 9:1. On the 2nd scotophase, the release rate of the aldehyde dropped further to about 20 ng/30 min per female and did not change significantly up to the 5th scotophase. However, a significant reduction in the quantity of emitted alcohol continued over the same period (Table 2). Generally, moths showed a reduction in the pheromone emission from the day of eclosion to the 5th scotophase with the alcohol getting depleted faster. In some cases the alcohol was completely undetected by GC. The level of emitted aldehyde remained relatively high throughout the lifetime of moths. This resulted in a progressing shift in the ALD:OH ratio of about 1:1, 9:1, 21:1, 32:1 for the night of eclosion, 1st, 3rd and 5th scotophase, respectively. The quantity of emitted pheromone from the field-collected moths, trapped during the peak hours (8th and 9th h), was similar to that of laboratory-reared insects (Table 3).

DISCUSSION

Periodicity in quantity of pheromone emitted by female of *C. partellus* was uni–modal with a distinct peak around the 7-10 h into the scotophase. In many moths the pheromone release rate and the ratio of its components have been shown to vary with female age [23 - 26]. Based on pheromone gland extracts, the quantity of pheromone from *Holomelina lamae* females increased from day 1 and peaked on day 4 after emergence and then decreased thereafter [27]. Similarly, in virgin females of *Heliothis virescens,* the highest quantity of pheromone was recorded on the 3rd day and then decreased drastically by the 5th day after eclosion [28]. However, similar to our results, the quantity of pheromone decreased with age in females of the potato tuberworm moth *Phthorimaea operculella* [26]. Maximal titers of pheromone from *P. operculella* occurred at the onset of scotophase and then declined to a minimum at the beginning of photophase. Similar to our results, the pheromone production in virgin females of *H. virescens* peaked on the 4th - 7th h into scotophase in the 2nd to 5th days following eclosion [28]. Although the ratios of pheromone components emitted by individual female carnation tortrix *Cacoecimorpha pronubana* were constant, significant interindividual variations was observed [29]. However, in the cabbage looper moth *Trichoplusiani,* the ratios of pheromone components in individual moths were found to change during the photoperiod [30]. In the case of *C. partellus*, the quantity of Z11-16:OH varied significantly among moths, such that it was undetected by GC in some older females.

Table 1: Rate of pheromone emission (ng/30min ± s.e.)* by virgin females of *Chilo. partellus* on the night of eclosion under normal photoperiod

Time of emergence (hours into scotophase)	Volatile trapping time (hours into scotophase)														
	7			8			9			10			11		
	ALD	OH	ALD/ OH	ALD	OH	ALD/ OH	ALD	OH	ALD/ OH	ALD	OH	ALD/ OH	ALD	OH	ALD/ OH
6	48.75 ±5.56	37.09 ±3.99	1.3	45.31 ±3.73	27.62 ±2.70	1.6	29.60 ±3.26	34.28 ±4.26	0.9	44.37 ±4.14	32.01 ±5.18	1.4	44.39 ±1.73	26.67 ±3.99	1.7
7				37.16 ±8.36	36.91 ±6.08	1.0	45.00 ±8.23	34.54 ±4.58	1.3	35.16 ±4.64	25.91 ±4.41	1.4	36.50 ±4.83	29.61 ±3.15	1.2
8							55.43 ±8.55	52.08 ±16.41	1.1	37.70 ±5.95	27.20 ±2.16	1.4	38.62 ±5.52	35.58 ±4.76	1.1
9										53.42 ±7.63	53.16 ±14.18	1.0	44.02 ±11.28	29.55 ±4.20	1.5
10													38.10 ±7.01	43.43 ±5.76	0.9
Mean rate ± s.e.	48.75 ±5.56	37.09 ±3.99	1.3	41.24 ±4.54	32.27 ±3.47	1.3	43.34 ±4.64	40.30 ±5.86	1.1	41.91 ±3.12	34.57 ±4.30	1.2	40.45 ±2.99	32.78 ±2.19	1.2

*** Number of replicates is 6, ALD: (Z)-11-hexadecenal, OH: (Z)-11-hexadecen-1-ol**

Table 2: Pheromone emission (ng/30min ± s.e.)* by virgin females of *Chilo partellus*.

			Scotophase after emergence									
			Eclosion		1		2		3		5	
			rate	ALD/OH	Rate	ALD/OH	rate	ALD/OH	rate	ALD/OH	rate	ALD/OH
Volatile trapping time (hours into scotophase)	**1**	ALD			6.20 ±1.55	9.4						
		OH			0.66 ±0.10							
	2	ALD			6.25 ±0.96	7.1						
		OH			0.88 ±0.12							
	3	ALD			4.78 ±0.57	3.7						
		OH			1.28 ±0.28							
	4	ALD			10.83 ±2.45	5.8						
		OH			1.88 ±0.25							
	5	ALD			18.10 ±1.42	7.8						
		OH			2.31 ±0.26							
	6	ALD			22.28 ±2.46	9.3						
		OH			2.39 ±0.62							
	7	ALD	48.75 ±5.56		28.58 ±2.69	10.8	13.72 ±4.11		23.28 ±4.51		16.00 ±3.77	
		OH	37.09 ±3.99	1.3	2.64 ±0.58		0.49 ±0.08	28.0	1.10 ±0.27	21.2	0.53 ±0.17	30.2
	8	ALD	41.24 ±4.54		35.58 ±2.88	9.7	26.42 ±5.95		18.68 ±4.57		14.89 ±2.66	
		OH	32.27 ±3.47	1.3	3.66 ±0.69		0.89 ±0.27	29.7	0.54 ±0.31	34.6	0.52 ±0.18	28.6
	9	ALD	43.34 ±4.64		34.32 ±3.21	8.4	18.21 ±4.99		25.00 ±7.96		14.75 ±4.20	
		OH	40.30 ±5.86	1.1	4.10 ±1.04		0.51 ±0.22	35.7	0.98 ±0.34	25.5	0.39 ±0.15	37.8
	10	ALD	41.91 ±3.12		25.11 ±1.70	8.4	24.09 ±3.89		19.00 ±3.88		20.38 ±5.25	
		OH	34.57 ±4.30	1.2	2.98 ±0.62		0.86 ±0.35	28.0	1.33 ±0.34	14.3	0.60 ±0.23	34.0
	11	ALD	40.45 ±2.99		18.50 ±2.57	9.0						
		OH	32.78 ±2.19	1.2	2.05 ±0.36							
	12	ALD			14.18 ±2.60	14.2						
		OH			1.00 ±0.28							
Mean rate		ALD	43.14	1.2	30.90**	9.2	20.61	29.9	21.49	21.7	16.51	32.4
		OH	35.40		3.35**		0.69		0.99		0.51	

*** Number of replicates ranges from 6 to 13, **: Mean is calculated from 7 - 10 h into scotophase, ALD: (Z)-11-hexadecenal, OH: (Z)-11-hexadecen-1-ol.**

Table 3: Pheromone emission (ng/30min ±s.e.)* by virgin females of *C. partellus* from field collected pupae.

		Scotophase after emergence			
		1		3	
		rate	ALD/ OH	rate	ALD/ OH
8 & 9 Hour into scotophase	ALD	37.17 ± 3.70	11.8	31.13 ± 3.20	16.4
	OH	3.14 ± 0.32		1.90 ± 0.16	

***: Number of replicates is 8, ALD: (Z)-11-hexadecenal, OH: (Z)-11-hexadecen-1-ol**.

Our results revealed distinct age-dependent patterns in the rate of pheromone release and ratio of its components. In general, with the progressing age of females, a marked reduction in the release rate of the pheromone was noticed, especially during the first three days after eclosion. The decrease was much faster in the case of the alcohol than in the case of the aldehyde, which resulted in a spectacular shift in the ratio of the pheromone components. Potentially, such an age-dependent shift in the ratio of pheromone components may serve as a powerful indicator enabling males to assess the age of calling females from a distance. Age-related modulation in the pheromone release is correlated to differential attractiveness of females to males. Such age-dependent shifts in attractiveness of females have been reported for many moth species. In *Diatrea grandiosella*, the attractiveness of females was high on the night of emergence, but dropped sharply after the fourth day [31]. Banerjee [32] reported that *Crubus trisectus* and *Crubus teterrellus* females were most attractive during the first 2-3 days of their life and their attractiveness decreased with age. A very similar trend was recorded for the sugar cane borer, *Diatraea saccharalis* [33].

For *C. partellus*, young females (at eclosion and 1 day old) are more attractive than older ones to the mate-searching males. Mating in *C. partellus* peaked on the 7^{th} – 9^{th} h into scotophase on the night of eclosion and on the 1^{st} scotophase [12], but declined markedly during successive nights [11]. The synchrony of pheromone titer and mating in *C. partellus* indicates that reproduction in this moth can be successfully disrupted using pheromone-baited traps. However, the pheromone bait to be developed for such a program should sufficiently resemble newly emerged and 1-day-old females in its composition and release rate. This means that the ratio of Z11-16:ALD to Z11-16:OH should remain within the range 1:1 - 1:10. The combination of a limited life span of moths and the decrease in female attractiveness with age through diminishing pheromone emission is favorable for sex pheromone based traps.

ACKNOWLEDGEMENT

This study was supported by grants from Swedish Agency for Research Co-operation with Developing Countries (SAREC). We thank Peter Njoroge for the supply of *C. partellus* pupae and Slawomir lux for comments on this paper.

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