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COMPONENTS OF THE SEX PHEROMONE OF THE FEMALE SPOTTED STALK BORER, *Chilo partellus* (Swinhoe) (LEPIDOPTERA: PYRALIDAE): IDENTIFICATION AND PRELIMINARY FIELD TRIALS

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Abstract—Female *Chilo partellus* (Swinhoe) abdominal tip extracts were examined by gas–liquid chromatography (GLC) combined with simultaneous electroantennographic (EAG) recording from the male moth. Two olfactory stimulants were detected and identified as (*Z*)-11-hexadecenal (I) and (*Z*)-11-hexadecen-1-ol (II) by their GLC behavior, microchemical reactions, and comparison with synthetic materials. Both compounds were detected in volatiles emitted by the "calling" female moth. Synthetic (*Z*)-9-tetradecenyl formate, a structural analog of aldehyde (I), also elicited a significant EAG response from the male moth. Field trials carried out in India using synthetic (I) and (II) as bait in water traps showed that compound (I) was highly attractive to male *C. partellus*; compound (II) was not attractive, and its addition to (I) significantly reduced trap catches.

Key Words—Sex pheromone, sex attractant, Lepidoptera, Pyralidae, *Chilo partellus*, spotted stalk borer, electroantennography, (*Z*)-11-hexadecenal, (*Z*)-11-hexadecen-1-ol, (*Z*)-9-tetradecenyl formate.

INTRODUCTION

The spotted stalk borer moth, *Chilo partellus* (Swinhoe), is a pest of major economic importance in India and East Africa, the larvae attacking maize, sorghum, sugar cane, and rice. An investigation of the female sex pheromone was undertaken to provide a synthetic attractant for population

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monitoring, and we report here the identification of the pheromone components and preliminary field testing of the synthetic compounds carried out in India. This is part of a continuing program of work on the sex pheromones of *Chilo* pest species which, it is hoped, will also provide information on the role of pheromones in premating reproductive isolation in this genus.

METHODS AND MATERIALS

Insect Material and Preparation of Extracts. A laboratory culture of *C. partellus* based on insect material of Indian origin was maintained at the Centre for Overseas Pest Research, London. Larvae were reared for the first 10 days on maize (approx. 6 weeks old) and from then on until pupation on an artificial wheat germ diet similar to that of Chatterjee et al. (1968). The culture was maintained on a 14-hr light:10-hr dark cycle with photophase temperatures of 26–28° C and scotophase temperatures of 20–22° C. Pupae for pheromone work were kept at 24–26° C under constant light, and adult moths were sexed on emergence. On the first day after emergence, females were placed in the dark for 2 hr. After freezing for 10 min at −20° C, the abdominal tips (terminal 3–4 segments) were clipped and extracted by soaking in purified dichloromethane for 15 min at room temperature. The extracts were filtered through glass wool and concentrated to approximately 1 tip equivalent per microliter by rotary evaporation without heating. Later work indicated that the yield of pheromone could be increased by placing the females in the dark for 5–6 hr before clipping.

Entrainment of Pheromone. The composition of the pheromone actually emitted by a single female moth was examined using charcoal air filters similar to those described by Grob and Zürcher (1976) to trap the airborne volatiles. The moth was contained in a glass chamber (12 × 4 cm) through which air was passed at 2 liters/min (equivalent to 0.02 m/sec), after purification by passage through active carbon and humidification by bubbling through distilled water. Moths were used within 24 hr of emergence, and placed for 1 hr in the entrainment apparatus under full light. The lights were then switched off and observations made with a small safe-light at hourly intervals. The air filter was changed every 2 hr, and absorbed materials were extracted with four 10-μl portions of carbon disulfide. Aliquots of this extract were examined by GLC and by GLC linked to EAG recording.

GLC and EAG Analyses. GLC instrumentation and conditions were similar to those described previously (Nesbitt et al., 1975b). In an attempt to prevent degradation of one of the components of the pheromone during analysis, a glass SCOT Carbowax 20M column (50 m × 0.5 mm ID) was used instead of the original stainless-steel column. The isomeric composition of synthetic compounds was determined by analysis on a packed OV 275

column (10 m × 2 mm ID glass column, packed with 20% OV 275 on 100–120 mesh Gas-Chrom RZ).

GLC analysis combined with simultaneous recording of male moth EAG responses to the column effluent was carried out as described by Moorhouse et al. (1969). The "puffing" method for measuring EAG responses to test compounds by blowing them directly over the insect's antenna was carried out as described previously (Nesbitt et al., 1977).

Purification of Tip Extracts. After initial GLC–EAG work had established that female tip extracts contained two olfactory stimulants for the male moth—an aldehyde (I) and an alcohol (II)—these were purified by a method involving conversion of (I) into its bisulfite adduct, prior to various microchemical reactions.

Solvent was removed from female tip extract by rotary evaporation at room temperature, and saturated aqueous sodium metabisulfite solution was added to the residue. After 30 min at room temperature, extraction of the reaction mixture with dichloromethane gave (II) contaminated with only traces of (I). Pure (II) was obtained by collection of the appropriate peaks from Carbowax 20M and Apiezon L GLC columns as described previously (Nesbitt et al., 1975a). Component (I) (95% pure by GLC analysis) was recovered by addition of sodium carbonate to the aqueous phase remaining after extraction of (II), followed by reextraction with dichloromethane.

Microozonolysis. Component (I) was ozonolyzed in carbon disulfide by a method based on that of Beroza and Bierl (1967). The ozonides were reductively cleaved with triphenylphosphine, and the reaction product was examined by GLC on the following packed columns: (A) 10% SP-216-PS on 100–120 mesh Supelcoport; (B) 9% Silar-5CP on 80–100 mesh Chromosorb W; (C) 2.5% Apiezon L on 80–100 mesh Chromosorb G. Aliphatic dialdehydes used as GLC reference compounds were prepared by reductive ozonolysis of the corresponding cycloalkenes or monounsaturated aldehydes.

Oxidation. The alcohol component (II) was oxidized by a method based on that of Corey and Suggs (1975). Following purification as described above, (II) was treated with a freshly prepared, saturated solution of pyridinium chlorochromate in dichloromethane. After 30 min at room temperature, the reaction mixture was examined by GLC–EAG on SCOT Carbowax 20M and Apiezon L columns.

Synthesis of the Pheromone Components and Related Compounds. The *Z* and *E* isomers of 11-hexadecen-1-ol and 11-hexadecenal were prepared from 1,10-decanediol and 1-hexyne as described previously (Nesbitt et al., 1975b), and (*Z*)-9-tetradecenyl formate was prepared by an analogous acetylenic route (Beevor et al., 1977). For all these compounds the *Z* isomers contained 1–2% of the corresponding *E* isomer, and the *E* isomers contained less than 1% of the *Z* isomer.

Field Attractancy Tests. The traps used for field tests were square galvanized metal pans (60 × 60 × 7.5 cm deep) containing water and a trace of

detergent, fitted with lids and metal legs 0.5 m high (Campion et al., 1974; Marks, 1976). Synthetic test compounds, combined with an equal weight of 2,6-di-*tert*-butyl-*p*-cresol (BHT) as antioxidant, were dispensed from sealed polyethylene vials (36 × 16 mm with 1.5-mm-thick walls) suspended from the underside of the trap lids about 5 cm above the surface of the water. At an early stage in the work it was observed that the aldehyde (I) and alcohol (II) reacted together even in dilute hexane solution at −20° C, and these two compounds were subsequently dispensed from separate vials. The corresponding acetal, a possible interaction product, could not be detected by thin-layer chromatographic comparison with an authentic sample.

Virgin female moths used in traps were aged 20 hr or less and were renewed nightly.

The traps were set out 30 m apart between the plant rows in a 6-hectare field of almost-mature sorghum. The experimental design for the first test described was a randomized complete block of three replicates run over 15 nights; replicates were separated by 100 m. The data obtained was analyzed using a $\log(x + 1)$ transformation.

RESULTS

When female abdominal tip extract was examined by GLC–EAG using packed columns of varying polarity, two compounds were detected which elicited strong responses from the male moth antennal preparation. The major component (I) had the lower retention temperature on all columns and was present in amounts of approx. 20 ng/tip equivalent; the amount of the minor component (II) was approx. 3 ng/tip equivalent. From previous experience, these compounds were assumed to be sex pheromone components, and their GLC retention temperatures on five packed columns are given in Table 1.

The GLC behavior of (I) was recognized as being that of a C_{16}, monounsaturated, straight-chain aldehyde by its similarity to that of the major component of the female sex pheromone of *Chilo suppressalis*, (*Z*)-11-hexadecenal (Nesbitt et al., 1975b). Since lepidopterous species of the same genus often have pheromone components in common, (*Z*)-11-hexadecenal was examined under the same conditions used for the *C. partellus* female tip extract. This compound was found to chromatograph in the same way as component (I) on all five columns (Table 1), and it elicited a comparable EAG response from the male moth (responses to 1 ng of material presented through the GLC–EAG link: component (I), 0.60 mV; (*Z*)-11-hexadecenal, 0.54 mV). As expected for an aldehyde, component (I) was almost completely removed from tip extracts by treatment with saturated aqueous sodium metabisulfite solution, and this was used to separate and purify the two pheromone components.

TABLE 1. GLC DATA FOR *Chilo partellus* FEMALE TIP EXTRACT AND SYNTHETIC STANDARDS ON PACKED COLUMNS

	Retention temperatures (°C)				
	C[a]	D	E	F	G
Female tip extract					
Component (I)	208.9	181.9	180.6	147.0	162.5
Component (II)	218.9	205.0	189.5	165.6	165.4
Synthetic standards					
Tetradecyl acetate	208.8	175.1	182.0	140.6	159.2
Hexadecyl acetate	232.5	194.7	202.8	156.2	174.9
(*Z*)-11-Hexadecenal	208.8	182.3	180.7	146.9	162.4
(*Z*)-11-Hexadecen-1-ol	218.8	204.8	189.5	166.2	164.8

[a]Stationary phases: (C) Apiezon L; (D) Carbowax 20M; (E) Silicone gum SE 30; (F) EGSS-X; (G) Fluorosilicone oil QF1.

The GLC behavior of component (II) relative to that of hexadecyl acetate, particularly its high retention temperature on Carbowax 20M, suggested it was a C_{16}, monounsaturated, straight-chain alcohol. The compounds making up multicomponent pheromone systems have generally been found to possess related chemical structures. Hence (*Z*)-11-hexadecen-1-ol, which has the same ω distance and double-bond configuration as the major component (I), was examined by GLC–EAG under the conditions used for the tip extract. As shown in Table 1, its chromatographic behavior was very similar to that of component (II), and the synthetic compound also elicited an EAG response from male *C. partellus* at the nanogram level (responses to 1 ng of material: component (II), 0.40 mV; (*Z*)-11-hexadecen-1-ol, 0.41 mV).

The 11- position of the double bond in component (I) was confirmed by reductive ozonolysis. This gave a product which cochromatographed with synthetic 1,11-undecanedial on the three columns used [retention temperatures: column (A), 163.9°C; (B), 184.8°C; (C), 155.7°C). The configuration of the double bond was determined by comparison of the retention data for (I) on SCOT columns with that for synthetic (*Z*)- and (*E*)-11-hexadecenal (Table 2). In addition, component (I) was shown to cochromatograph with the *Z* isomer on the three stationary phases used. No GLC peaks and no EAG responses were observed at the retention temperatures for the *E* isomer during linked GLC–EAG analyses of female tip extract.

Insufficient amounts of component (II) were available for microozonolysis to confirm the double bond position, and chromatography on both metal and glass SCOT columns resulted in loss of this component (standard unsaturated alcohols were also totally destroyed when chromatographed on

TABLE 2. GLC DATA FOR *Chilo partellus* FEMALE TIP EXTRACT AND SYNTHETIC STANDARDS ON SCOT COLUMNS

	Retention temperatures (°C)		
	H[a]	J	K
Female tip extract			
Component (I)	152.8	188.1	175.4
Synthetic standards			
Dodecyl acetate	143.2	176.9	164.4
Tetradecyl acetate	150.7	184.7	174.8
(*Z*)-11-Hexadecenal	152.8	188.1	175.3
(*E*)-11-Hexadecenal	152.7	187.8	175.9

[a]Stationary phases: (H) DEGS; (J) Carbowax 20M; (K) Apiezon L.

these columns at low loadings). However, it was possible to oxidize purified (II) with pyridinium chlorochromate and analyze the product by GLC–EAG. A compound was obtained which cochromatographed with (*Z*)-11-hexadecenal on the three SCOT columns and had similar EAG activity.

Two compounds with the same GLC behavior and EAG activity as components (I) and (II) were obtained by collection of airborne volatiles from a virgin female moth. They were collected in amounts corresponding to 5–40 ng/hr of (I) and less than 1–3 ng/hr of (II) during the period 5–9 hr after lights-off. Yields were highest on those occasions when the female moth was seen to adopt a classical "calling" position.

EAG responses to (*Z*)-9-tetradecenyl formate, a compound in which the α-methylene group of (*Z*)-11-hexadecenal is replaced by oxygen, were lower than those elicited by this aldehyde, but comparable in magnitude to responses to (*Z*)-11-hexadecen-1-ol. The average responses to 2 ng of test compound in six replicate "puff" tests were: (*Z*)-11-hexadecenal, 1.29 mV; (*Z*)-11-hexadecen-1-ol, 0.82 mV; (*Z*)-9-tetradecenyl formate, 0.88 mV.

Field testing of (*Z*)-11-hexadecenal and (*Z*)-11-hexadecen-1-ol was carried out at ICRISAT, Hyderabad, India, and two experiments conducted in 1977 are described here. Table 3 gives the results of an experiment comparing catches of male *C. partellus* moths in water traps baited with the aldehyde alone, the alcohol alone, the aldehyde plus the alcohol (in the same trap but in different vials) and a virgin female moth, and catches in control, unbaited water traps. The aldehyde and alcohol were tested together in the ratio found in tip extracts, i.e., 7:1, but the aldehyde alone caught significantly more moths than any other treatment ($P < 0.001$). The catches in traps baited with (*Z*)-11-hexadecen-1-ol were not significantly different from those in the control traps, and addition of this compound to the aldehyde caused a signifi-

cant reduction in catches. Very few female moths were caught in any of the traps.

The coefficient of variance in this experiment, using transformed data, was 32%. Further analysis of the data showed that there were significant differences in catches over nights, and these were not due to aging of the attractant sources since fluctuations were also observed in the catches in virgin female and control traps. Peak catches were associated with rain, drizzle, or irrigation of the fields.

A longer experiment, in which the three synthetic pheromone treatments—aldehyde alone, alcohol alone, and aldehyde plus alcohol—were tested at five sites, gave similar results, shown in Figure 1. Traps baited with the aldehyde alone caught more than four times as many moths as those baited with the aldehyde and alcohol in a 7:1 ratio.

These observations and further field experiments will be reported in detail elsewhere.

DISCUSSION

We believe that the GLC, EAG, and chemical data taken in conjunction with the preliminary field results establish that (*Z*)-11-hexadecenal and (*Z*)-11-hexadecen-1-ol constitute the female sex pheromone of *C. partellus*. The major aldehydic component alone, when dispensed from polyethylene vials, is comparable in attractiveness with the female moth and is currently being used in traps to monitor populations of *C. partellus* at ICRISAT.

TABLE 3. CATCHES OF *Chilo partellus* MOTHS IN TRAPS BAITED WITH (*Z*)-11-HEXADECENAL (Z11-16:CHO) AND (*Z*)-11-HEXADECEN-1-OL (Z11-16:OH) COMPARED WITH THOSE IN VIRGIN FEMALE AND UNBAITED TRAPS: 3 TRAPS PER TREATMENT RUN OVER 15 NIGHTS, MARCH-APRIL 1977

Treatment	Total catch		Male moth catch per trap night	
	Males	Females	Mean	Transformed mean[a]
437.5 μg Z11-16:CHO	2194	14	48.8	3.68 a
437.5 μg Z11-16:CHO + 62.5 μg Z11-16:OH	848	9	18.8	2.55 b
62.5 μg Z11-16:OH	74	9	1.6	0.76 c
Virgin female moth	1381	13	30.7	2.64 b
Unbaited trap	102	8	2.3	0.85 c

[a]Means followed by the same letter are not significantly different at the 5% level.

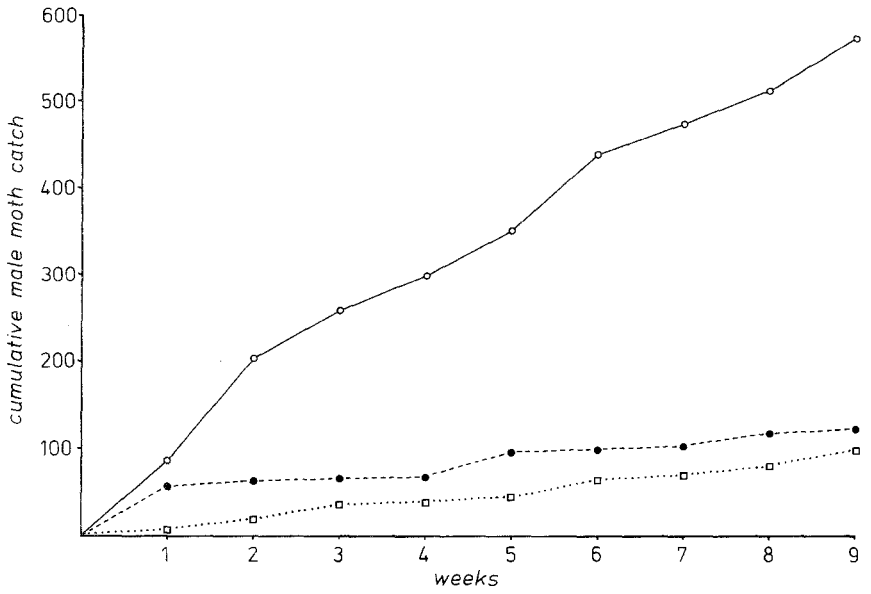

FIG. 1. Cumulative catches of male *Chilo partellus* moths in traps baited with 437.5 μg (*Z*)-11-hexadecenal (○——○), 437.5 μg (*Z*)-11-hexadecenal + 62.5 μg (*Z*)-11-hexadecen-1-ol (●------●), and 62.5 μg (*Z*)-11-hexadecen-1-ol (□·····□) over 9 weeks (January 20 to March 16, 1977); 5 sites; 1 set of 3 treatments per site; vials renewed weekly.

However, the exact function of the alcohol is uncertain. During the initial field trials reported here, addition of (*Z*)-11-hexadecen-1-ol to (*Z*)-11-hexadecenal in the 1:7 ratio found in tip extracts reduced trap catches to the level of the unbaited traps. The two compounds were dispensed separately from adjacent polyethylene vials, and our release rate data for moth pheromones with different functional groups would suggest that the alcohol-to-aldehyde ratio actually emitted by the combined source should also have been approximately 1:7 (Campion et al., 1978). This ratio was chosen for initial trials rather than the lower alcohol-to-aldehyde ratio found in entrained volatiles, because tests with nanogram quantities of synthetic (*Z*)-11-hexadecen-1-ol and (*Z*)-11-hexadecenal have shown that the percentage recovery of the alcohol is much lower than that of the aldehyde in our entrainment system. However, further field trials are in progress to compare the attractiveness of a range of alcohol/aldehyde ratios.

The detection of the alcohol in airborne volatiles from a "calling" female *C. partellus* moth indicates that the alcohol is actually emitted by the moth, and would suggest that it has a definite role in premating behavior, despite the observed effect on trap catches. The situation with *C. partellus* thus seems

to be different from that reported for the tortricid moth, *Choristoneura fumiferana*. In the latter, the main attractant component of the female sex pheromone was identified as (*E*)-11-tetradecenal (Weatherston et al., 1971) and the attractiveness of the synthetic aldehyde to male moths was shown to be reduced by addition of (*E*)-11-tetradecen-1-ol (Sanders et al., 1972). Both the aldehyde and alcohol were found in tip extracts, but only the aldehyde was detected in washings from jars that had held female moths (Weatherston and Maclean, 1974) and in volatiles entrained from female moths and trapped on Porapak Q (Weatherston et al., 1975). Weatherston and Maclean (1974) concluded that the alcohol was merely a biosynthetic precursor to the aldehyde. More recently, reexamination of the pheromone obtained by rinsing out female moth containers showed it to be a mixture of the *E* and *Z* isomers of 11-tetradecenal in a 96:4 ratio, and addition of a small percentage of the *Z* isomer to pure synthetic (*E*)-11-tetradecenal was found to be necessary to optimize attraction (Sanders and Weatherston, 1976). No comment was made on the isomeric composition of the 11-tetradecen-1-ol in tip extracts.

(*Z*)-11-Hexadecenal has also been identified as the major component of the female sex pheromone of *Chilo suppressalis*, where its attractiveness to male moths is synergized by a second component, the homologous aldehyde (*Z*)-13-octadecenal (Nesbitt et al., 1975b; Ohta et al., 1976; Beevor et al., 1977). In both *C. partellus* and *C. suppressalis*, we have found (*Z*)-9-tetradecenyl formate, a compound structurally related to (*Z*)-11-hexadecenal, to be a potent olfactory stimulant for the male moth (Nesbitt et al., 1975b; cf. also Nesbitt et al., 1977), and with *C. suppressalis* this formate has been shown to disrupt communication between male and female moths in the field (Beevor et al., 1977).

(*Z*)-11-Hexadecenal has also been reported as a female sex pheromone component in the noctuid species *Heliothis virescens*, *H. zea* (Roelofs et al., 1974; Tumlinson et al., 1975), and *H. armigera* (Piccardi et al., 1977; Nesbitt et al., unpublished), and in the plutellid *Plutella xylostella* (Tamaki et al., 1977; Chow et al., 1977). (*Z*)-11-Hexadecen-1-ol has been found as a pheromone component in the clover cutworm, *Scotogramma trifolii* (Underhill et al., 1976).

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