Journal of Chemical Ecology, Vol. 25, No. 12, 1999

IDENTIFICATION AND FIELD EVALUATION OF COMPONENTS OF FEMALE SEX PHEROMONE OF MILLET STEM BORER, *Coniesta ignefusalis*

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(Received March 26, 1998; accepted July 31, 1999)

Abstract—Five active compounds were detected during analyses of ovipositor washings and effluvia from virgin female *Coniesta ignefusalis* moths by gas chromatography (GC) linked to electroantennographic (EAG) recording from a male moth. These were identified as (*Z*)-7-dodecen-1-ol (*Z*7–12 : OH), (*Z*)-5-decen-1-ol (*Z*5–10 : OH), (*Z*)-7-dodecenal (*Z*7–12 : Ald), (*Z*)-7-dodecenyl acetate (*Z*7–12 : Ac), and (*Z*)-9-tetradecen-1-ol (*Z*9–14 : OH) by comparison of their GC retention times, mass spectra, and EAG activities with those of synthetic standards. Laboratory tests of dispensers for these compounds showed that release rates from polyethylene vials increased to relatively uniform values after three to four days, but release from septa was very rapid and nonuniform and decreased to low levels after two to three days. Trapping tests in Niger showed that the major component, *Z*7–12 : OH, and two of the minor components, *Z*5–10 : OH and *Z*7–12 : Ald, were essential for attraction of male *C. ignefusalis* moths. The most attractive blend contained these three components in a 100 : 5 : 3.3 ratio in a polyethylene vial, which emitted the components in similar proportions to those produced by the female *C. ignefusalis* moth. Water traps baited with this blend containing 1 mg of *Z*7–12 : OH caught more male *C. ignefusalis* moths than traps baited with newly emerged female moths. Addition of up to 10% of the corresponding *E* isomers of the pheromone components had no effect on catches, but addition of the other two minor components detected, *Z*7–12 : Ac and/or *Z*9–14 : OH, to the attractive blend at naturally occurring levels caused significant reductions in trap catch.

Key Words—*Coniesta ignefusalis*, *Acigona ignefusalis*, Lepidoptera, Pyra-

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0098-0331/99/1200-2643$16.00/0

lidae, sex pheromone, (Z)-7-dodecen-1-ol, (Z)-5-decen-1-ol, (Z)-7-dodecenal, (Z)-7-dodecenyl acetate, (Z)-9-tetradecen-1-ol.

INTRODUCTION

The millet stem borer, *Coniesta* (= *Acigona*) *ignefusalis* Hampson (Lepidoptera: Pyralidae), is an important pest of pearl millet, *Pennisetum glaucum* (L.) R. Br. throughout the West African Sahelian and Soudanian zones (Harris, 1962; N'doye et al., 1984; N'doye and Gahukar, 1987; Youm et al., 1996). During feeding and development, *C. ignefusalis* larvae cause different types of damage, depending upon plant age and the generation. First-generation larvae attack small plants causing "dead heart" and stand loss, whereas second and third generations cause lodging, disruption of the plant vascular system, and inhibition of grain formation due to tunneling (Harris, 1962). In the sub-Saharan African region where pearl millet is the major staple crop grown by subsistence farmers, yield losses due to attack by *C. ignefusalis* range from 15% to total crop failure (Harris, 1962; Ajayi, 1990). In Niger, over 90% of stem borer infestation and damage on millet is caused by *C. ignefusalis* (Youm and Gilstrap, 1993). During research into methods for control of *C. ignefusalis*, the presence of a female sex pheromone was demonstrated in millet fields in Niger (Bako, 1977; ICRISAT, 1989) and seen to be of potential use in an integrated pest management strategy.

This paper reports the results of studies leading to the identification of five compounds produced by virgin female *C. ignefusalis* moths that elicit an antennal response from *C. ignefusalis* male moths and field evaluation of the synthetic compounds as attractants for the male moths.

METHODS AND MATERIALS

Insect Material. Millet stems from the previous season which contained fourth- and fifth-instar diapausing larvae were soaked with water to break diapause and promote further development to pupae. These stems were dissected and pupae dispatched by air from Niger to England where they were sexed, transfered to Perspex containers, and maintained in an environmental cabinet at 32°C, 60% relative humidity during a reversed 12-hr light period and 24°C, 80% relative humidity during the 12-hr dark period.

Pheromone Collection. Ovipositor washings were prepared in hexane from virgin moths 0–2 days old, 7–10 hr into the scotophase, as described by Sower et al. (1973). Effluvia were collected on filters containing activated charcoal (5 mg) by passing charcoal-filtered air (2 liter/ml) over one to three female moths held

in silanized glass containers (12 cm × 4 cm diameter) at 3–12 hr into the scotophase, as previously described (Grob and Zurcher, 1976; Nesbitt et al., 1979; Tumlinson et al., 1982). Trapped volatiles were eluted with dichloromethane (2 × 10 μl).

Release rates of synthetic compounds from dispensers were measured similarly, placing the dispenser in a glass vessel (4 cm × 2 cm diameter) constructed from Quickfit adapters, maintained in a room held at constant temperature (27°C). Volatiles trapped on the charcoal filter were eluted with dichloromethane (3 × 10 μl), pentadecyl acetate (1 μg) added as internal standard, and components were assayed by gas chromatography (GC) as below. Both vials and septa were initially loaded with a mixture containing approximately 0.5 mg each of all five components. Release rates were corrected for the actual amounts of the components in the lures as determined by GC analysis in order to give release rates for all components corresponding to an initial loading of 0.5 mg.

Gas Chromatography. Analyses of natural and synthetic compounds were conducted on a Carlo Erba Mega series 5300 instrument fitted with two Grob split/splitless injectors (200°C) and a flame ionization detector (FID, 240°C). Fused silica capillary columns (25 m × 0.32 mm ID; Chrompack, London, UK) were used, coated with either nonpolar CP Sil5CB (chemically bonded methylsilicone) or polar CP Wax 52CB (chemically bonded Carbowax 20 M equivalent). The carrier gas was helium and inlet pressures of 0.50 and 0.45 kg/cm^2, respectively, were used to maintain a gas velocity of 25 cm/sec in each column. All injections were made in the splitless mode with the split valve closed for 40 sec. Oven temperature for the nonpolar column was held at 60°C for two min, then programmed at 20°C/min to 90°C, then at 1°C/min to 124°C, and then at 4°C/min until the end of the analysis. Conditions for the polar column were the same except that the 1°C/min program was maintained until the end of the analysis.

Electroantennography (EAG). EAG preparations were set up as described in Cork et al. (1990) by using the whole insect and intact antennae and inserting glass microelectrodes filled with saline into the interstitial membranes between the annuli. Coupled GC-EAG analyses were carried out essentially as described by Cork et al. (1990) by splitting the analytical column effluent into two parts, in the present case by using a four-port, zero dead volume connector (Chrompack) instead of push-fit Y tubes. One outlet was attached to the FID and the other to a glass reservoir. Column effluent was pulsed from the reservoir with nitrogen (500 ml/min for 3 sec) at 17-sec intervals over the male *C. ignefusalis* EAG preparation for the duration of the GC analysis.

EAG response profiles from male *C. ignefusalis* to synthetic compounds were recorded essentially as described by Beevor et al. (1986). The test compound was deposited on the inner wall of a glass Pasteur pipet and delivered directly over the antenna in nitrogen (500 ml/min for 3 sec). The interval between successive stimuli was 2 min.

Mass Spectrometry (MS). GC-MS analyses were carried out in electron impact mode on a Finnigan-MAT ITD 700 Ion Trap Detector with open split interface (230°C) to a Carlo Erba Mega series 5300 GC fitted with a fused silica capillary column (25 m × 0.32 mm ID) coated with BP 20 (Carbowax 20 M equivalent; SGE). Carrier gas was helium (0.4 kg/cm^2), splitless injection (200°C), and the GC oven was held at 70°C for 2 min then programmed at 20°C to 120°C, then at 4°C to 230°C.

Synthetic Compounds. Monounsaturated compounds were prepared at NRI by standard acetylenic and Wittig coupling routes followed by argentation chromatography to give material of greater than 99.9% chemical and stereochemical purity.

Field Trials. Field trials of synthetic compounds were carried out in farmers' fields near Sadore, Niger. Initial trials showed water traps to be the most effective trap design for *C. ignefusalis* and were used throughout the studies described here. They consisted of an aluminum tray (28 cm diameter) containing water with a small amount of mineral oil to reduce surface tension, and a plastic lid supported 10 cm above the water surface (Youm et al., 1993). Traps were positioned 0.5 m above ground level (Youm and Beevor, 1995). Pheromone dispensers used in field trials were closed polyethylene vials (22 × 9 × 1.5 mm thick; Just Plastics, London, U.K.), and rubber septa (Aldrich, Gillingham, Dorset, UK; catalog No. Z10,072-2, white) were also evaluated as dispensers in the laboratory. Dispensers were loaded by adding the synthetic compounds and an equal amount by weight of 2,6-di-*tert*-butyl-4-methylphenol (BHT) as antioxidant dissolved in 0.1 ml of petroleum spirit (bp 40–60°C) and allowing the solvent to evaporate. The dispensers were prepared at least two days before use and were mounted in the trap immediately below the lid and above the water surface. In virgin female-baited traps, a single virgin female moth that had emerged in the laboratory from field collected pupae was housed in a metal mesh container (5 × 4 cm) in place of the pheromone dispenser. Female moths were used the first night after emergence and renewed each night.

Traps were positioned approximately 25 m apart in a circular array within a replicate. Moth catches were recorded each day when dispensers were moved clockwise one position within a replicate. Five to seven replicates separated by at least 100 m were carried out for each experiment, and the experiment was run until each treatment had occupied each position once (three to five nights). Mean catches per trap per night in each replicate (x) were transformed to $\log(x + 1)$ to normalize the variance and subjected to analysis of variance (ANOVA). Treatments with zero catches were omitted from the analyses since these have no variance and violate the assumptions of ANOVA. Differences between treatment means were tested for significance by Duncan's multiple-range test (DMRT). Actual mean catches and standard errors are shown in the tables.

RESULTS

Structural Determination. In analyses of ovipositor washings and entrained volatiles from virgin female *C. ignefusalis* moths by GC-EAG, up to five compounds producing EAG activity of double the baseline noise were detected. These were labeled I–V in decreasing order of abundance in entrained volatiles (Table 1), with up to 100 ng of compound I being obtained from a female moth per night by entrainment. GC retention data for the active compounds are given in Table 1 as equivalent chain lengths (ECLs) relative to the retention times of straight-chain acetates (Christie, 1988; Harris and Habgood, 1966). By comparison with ECLs of standard compounds, the differences in ECLs on the two phases (Table 1) suggested that III and V were monounsaturated acetates or aldehydes with 12 carbon atoms, III most probably being an acetate and V with the greater difference in ECLs being an aldehyde. The corresponding differences in ECLs for I, II, and IV were much greater, and these data were characteristic of straight chain, monounsaturated alcohols with 12, 10, and 14 carbon atoms, respectively.

The retention time of each EAG-active compound was compared with those of available isomers on both GC phases. Retention data for component I were consistent only with those for (*Z*)-7-dodecen-1-ol (*Z*7–12 : OH) (Figure 1), data for II were consistent only with those for (*Z*)-5-decen-1-ol (*Z*5–10 : OH) (Figure 2), data for III were consistent only with those for (*Z*)-7-dodecenal (*Z*7–12 : Ald) (Figure 3), data for IV were consistent only with those for (*Z*)-9-tetradecen-1-ol (*Z*9–14 : OH) (Figure 4) and data for V were consistent only with those for (*Z*)-7-dodecen-1-yl acetate (*Z*7–12 : Ac) (Figure 5) (Nesbitt et al., 1986).

Further evidence for the presence of *Z*7–12 : Ac in ovipositor washings from

TABLE 1. RELATIVE RETENTION TIMES (ECL) AND ABUNDANCE OF EAG-ACTIVE COMPOUNDS DETECTED IN *C. ignefusalis* OVIPOSITOR WASHINGS AND FEMALE ENTRAINMENT BY LINKED GC-EAG ANALYSIS ON POLAR (CP WAX 52CB) AND NONPOLAR (CP SIL 5CB) COLUMNS

		Relative abundance		Relative retention time (ECL)		
Component	Compound	Washings	Entrainment	CPWax52	CPSil5	Δ^a
I	*Z*7–12:OH	100	100	13.16	10.41	2.75
II	*Z*5–10:OH	2	10	11.16	8.35	2.81
III	*Z*7–12:Ald	2	6	10.54	9.66	0.88
IV	*Z*9–14:OH	3	1	15.08	12.67	2.41
V	*Z*7–12:Ac	1	1	12.31	11.78	0.53

[a](ECL on CPWax52) – (ECL on CPSil5).

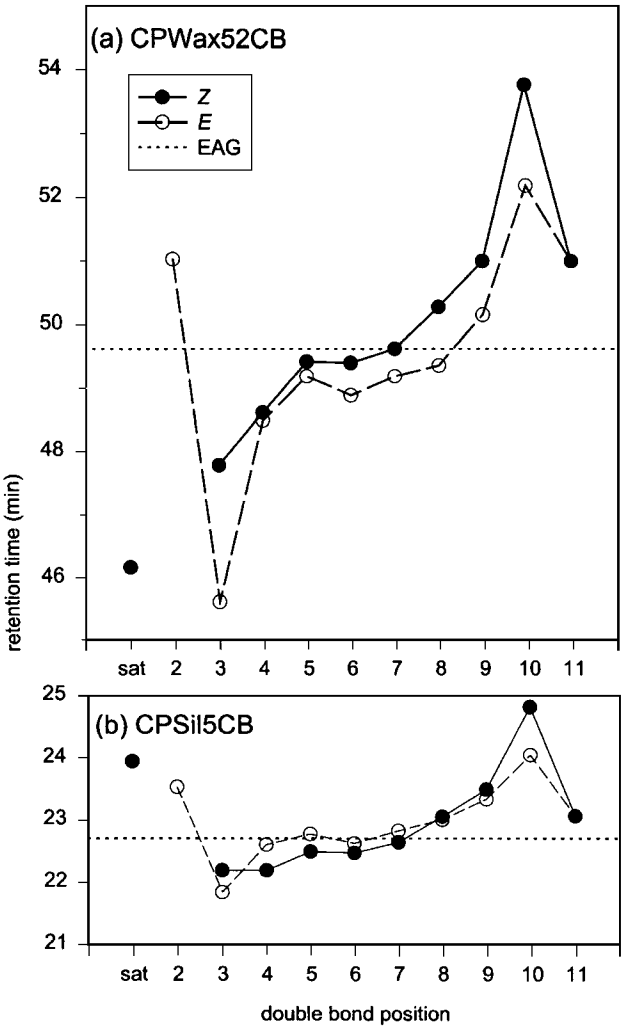

FIG. 1. GC retention times of dodecen-1-ol isomers and EAG-active component I on fused silica capillary columns coated with (a) CP Wax52CB and (b) CP Sil5CB.

female *C. ignefusalis* was obtained by analyzing the washings by GC-EAG using a male *Trichoplusia ni* (Lepidoptera: Noctuidae) moth for the EAG preparation. The main component of the pheromone of this species is Z7–12 : Ac (Berger, 1966; Bjostad et al., 1984), and a significant EAG response was obtained at the retention time corresponding to this compound.

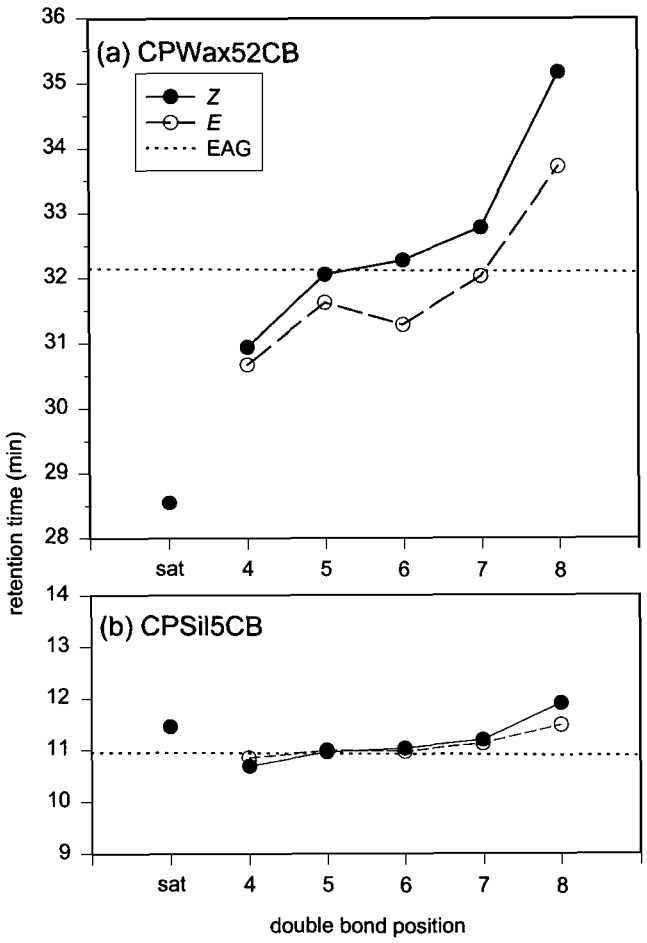

FIG. 2. GC retention times of decen-1-ol isomers and EAG-active component II on fused silica capillary columns coated with (a) CP Wax52CB and (b) CP Sil5CB.

In GC-MS analyses, components with mass spectra matching those of the above five synthetic compounds were recorded at the appropriate retention times, and the presence of dodecan-1-ol at 10–20% of the major component was also established. In particular, the four alcohols all showed a strong ion at m/z 31 (Attygalle et al., 1987).

EAG responses of male *C. ignefusalis* moths to synthetic dodecen-1-ol isomers were recorded by using 5 ng at source off glass. The response of Z7–12 : OH was significantly greater than that to the other isomers tested (Fig-

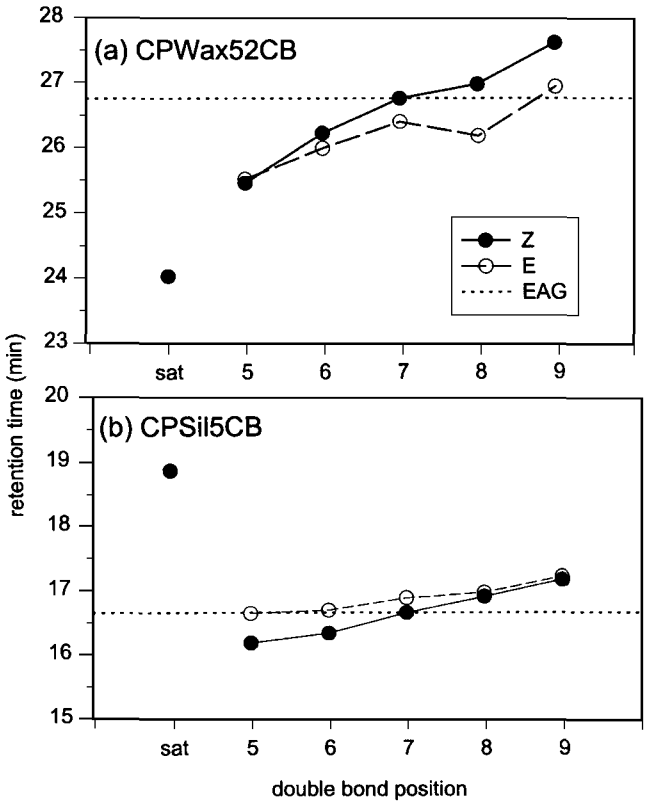

FIG. 3. GC retention times of dodecenal isomers and EAG-active component III on fused silica capillary columns coated with (a) CP Wax52CB and (b) CP Sil5CB.

ure 6). Similarly the EAG response to Z7–12 : Ac was higher than the responses elicited by other (Z)-dodecen-1-yl acetate isomers (Figure 7).

EAG responses of male *C. ignefusalis* to Z5–10 : OH and Z7–10 : OH measured by using linked GC-EAG were 2.61 mV and 0.31 mV above background, respectively, to 4 ng injected (2 ng at the antenna). Similarly, the EAG response to Z7–12 : Ald was 2.72 mV. EAG responses to (Z)-8- and (Z)-9-tetradecen-1-ol at the 5-ng level were similar at 0.42 ± 0.06 mV above blank, but the (Z)-7, (Z)-10, and all four corresponding *E* isomers were inactive at this level.

Pheromone Dispensers. Polyethylene vials and rubber septa were evaluated in the laboratory as dispensers for the five components identified. Results (Figure 8) showed that release was initially rapid from the septa but dropped almost to zero as the contents were exhaused within a few days. The relative ratios of

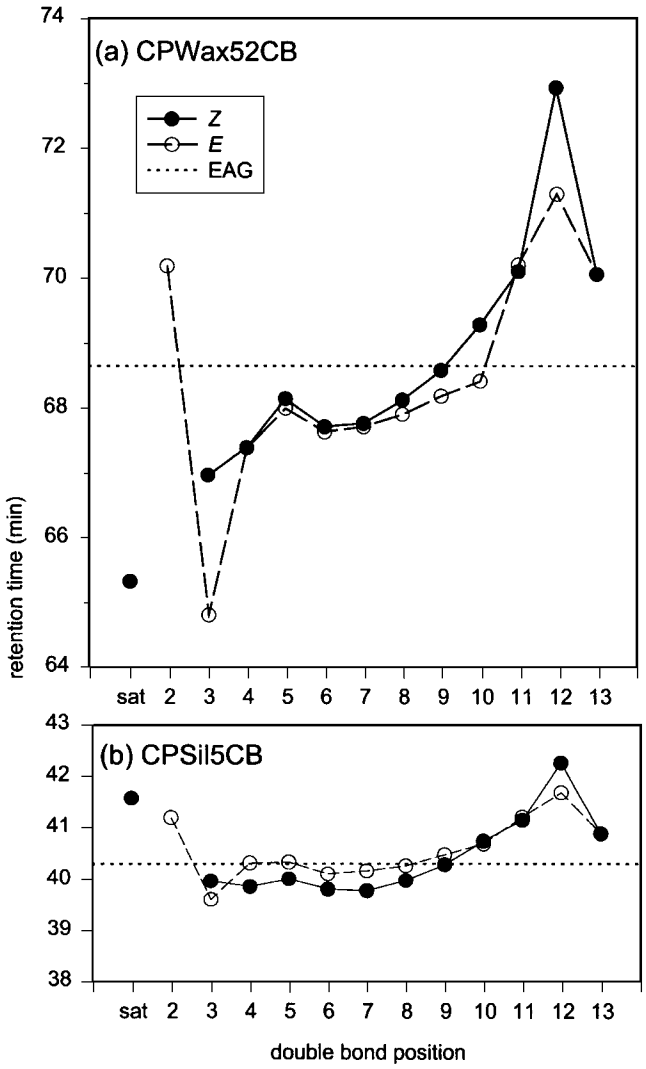

FIG. 4. GC retention times of tetradecen-1-ol isomers and EAG-active component IV on fused silica capillary columns coated with (a) CP Wax52CB and (b) CP Sil5CB.

the different components also changed markedly during this time. In contrast, release rates for all the components from vials increased to reach a fairly constant level after three to four days, and these were maintained for at least the next six days. The release rate of Z7–12 : OH for an initial loading of 0.5 mg was 0.72

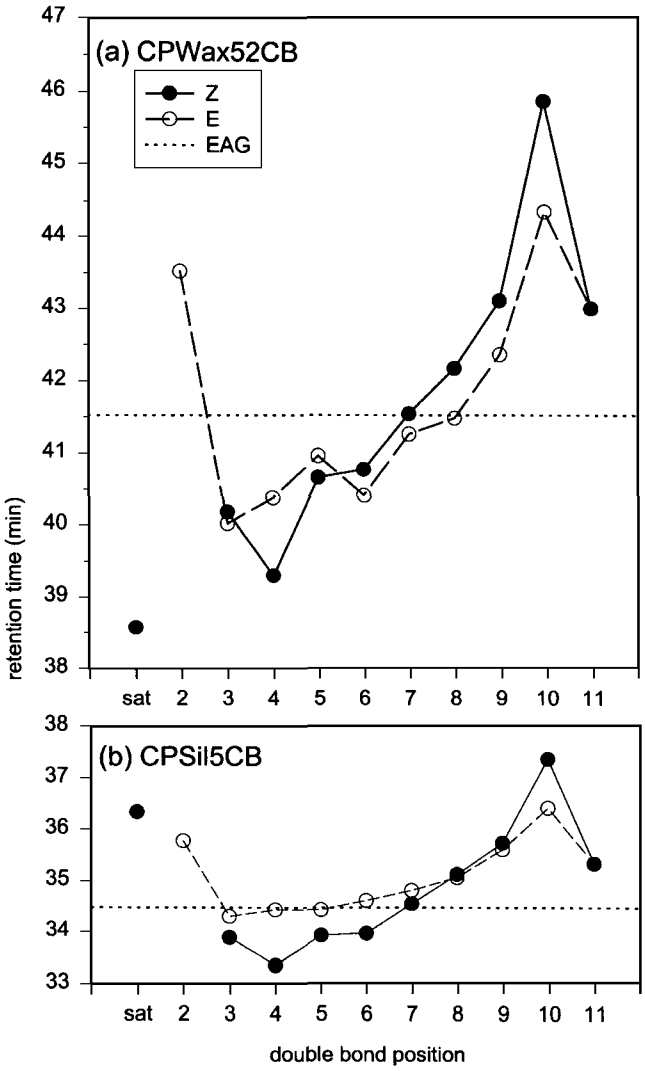

FIG. 5. GC retention times of dodecen-1-yl acetate isomers and EAG-active component V on fused silica capillary columns coated with (a) CP Wax52CB and (b) CP Sil5CB.

μg/hr at 27°C, and the release rates of Z7–12 : Ald, Z5–10 : OH, Z7–12 : Ac, and Z9–14 : OH relative to that of Z7–12 : OH (= 1.00) were 1.83, 1.79, 1.33, and 0.60, respectively.

Field Trials. Preliminary field trials had indicated that the major compo-

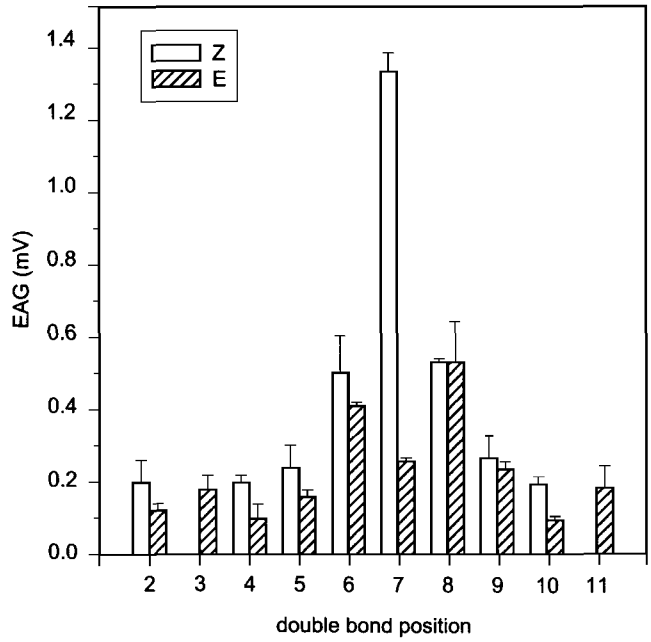

FIG. 6. EAG responses of male *C. ignefusalis* moth to dodecen-1-ol isomers (5 ng off glass; mean of two replicates on each of two insects ±SE).

nent, Z7–12 : OH, was unattractive alone to male *C. ignefusalis* moths but that addition of the minor components Z5–10 : OH and Z7–12 : Ald gave attractive blends. Both minor components were essential for attraction, when using relative amounts of the three components indicated in early analyses of ovipositor washings. (Table 2, experiment 1).

Increasing the amount of Z7–12 : Ald from 5% to 40% with respect to the major component, Z7–12 : OH, showed an optimum level of 5–10% with 20% and 40% significantly reducing catches (Table 2, experiment 2).

Similarly, increasing the amount of Z5–10 : OH from 7.5% to 15% with respect to the major component, Z7–12 : OH, had no effect on catches, but 30% and greater amounts significantly reduced catches (Table 2, experiment 3).

These data suggested that the minor components Z5–10 : OH and Z7–12 : Ald might be optimally combined in the ratio 3 : 2. Results from laboratory determination of release rates from the vials (above) also became available at this time. Together with the amounts of the minor components found in volatiles collected from the female *C. ignefusalis* moth (Table 1), these indicated that Z7–12 : OH, Z5–10 : OH, and Z7–12 : Ald should be present initially in the

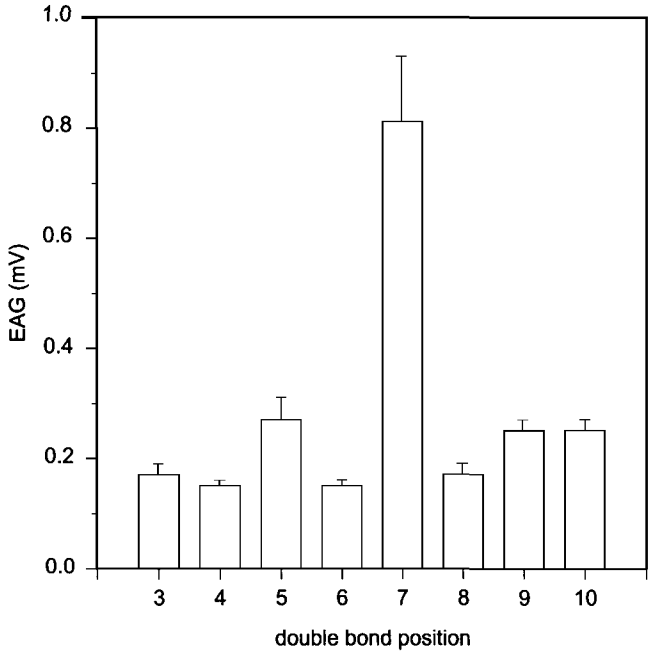

FIG. 7. EAG responses of male *C. ignefusalis* moth to (Z)-dodecen-1-yl acetate isomers (5 ng off glass; mean of two replicates on each of two insects ±SE).

vial at relative amounts of 1000 : 50 : 33, respectively. Further field trials with blends based on these results confirmed that the predicted blend was as attractive as any others tested (Table 3). Increasing or decreasing the relative amounts of the two minor components decreased catches (Table 3).

Addition of either Z7–12 : Ac or Z9–14 : OH to the optimum attractive blend at levels similar to those found in the natural extract and entrained material resulted in significant decreases in catch even at 0.5% and 1.5%, respectively, with respect to the major component (Table 4, experiments 1 and 2). These inhibitory effects seemed to be additive, such that a significant reduction in catches of male moths by the optimum blend was caused by addition of both Z7–12 : Ac and Z9–14 : OH at 0.25% and 0.75%, respectively, with respect to Z7–12 : OH (Table 4, Experiment 3).

Increasing loadings of the optimum pheromone blend in the lures from 0.01 mg to 1.0 mg increased catches of male *C. ignefusalis*, although the difference in catches with 0.1 mg and 1.0 mg was not statistically significant at the 5% level (Table 5, experiment 1).

In a comparison of the attractiveness of the optimum synthetic pheromone

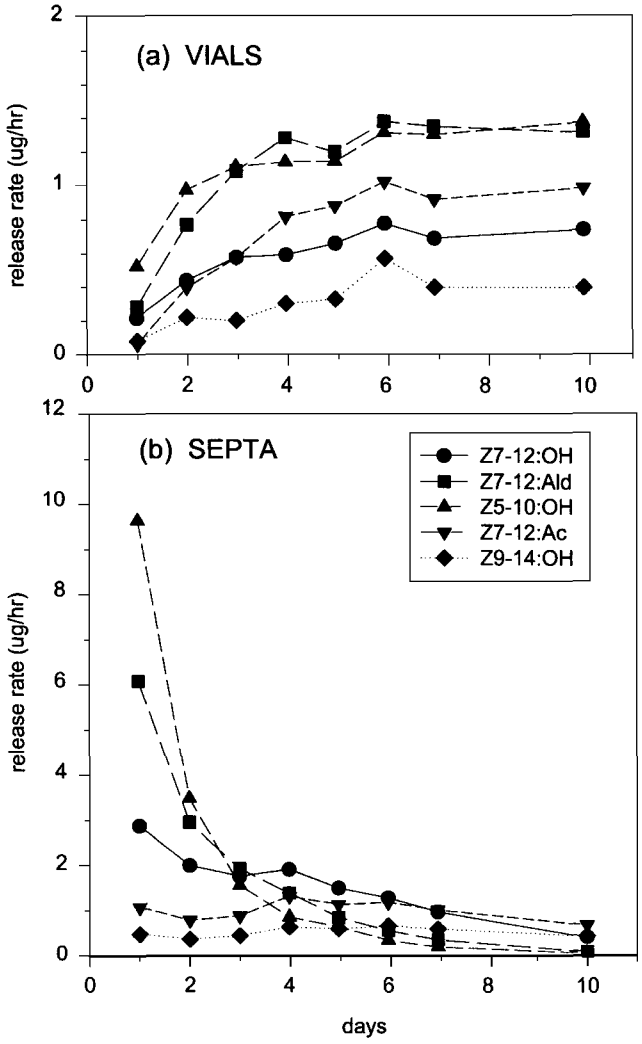

FIG. 8. Release rates of synthetic pheromone components from (a) polyethylene vials and (b) rubber septa at 27°C.

blend and virgin female *C. ignefusalis* moths, water traps baited with the synthetic blend dispensed from a polyethylene vial at a loading of 1 mg *Z*7–12 : OH caught more than twice as many male *C. ignefusalis* moths as traps baited with a single virgin female moth (Table 5, experiment 2).

The effects of the presence of the corresponding *E* isomers on the attrac-

TABLE 2. CATCHES OF MALE *C. ignefusalis* IN TRAPS BAITED WITH *Z*7–12:OH AND VARIOUS COMBINATIONS OF *Z*5–10:OH AND *Z*7–12:ALD.

Pheromone component (μg)			Mean catch/ trap/night[a]
*Z*7–12:OH	*Z*5–10:OH	*Z*7–12:Ald	
Experiment 1 (6 replicates, 5 nights)			
500	75	50	41.2 ± 12.8 a
500	75	—	3.3 ± 0.9 b
500	—	50	0.3 ± 0.2 c
500	—	—	0.5 ± 0.1 c
—	—	—	0.0 c
Experiment 2 (5 replicates, 5 nights)			
500	75	0	1.4 ± 0.8 c
500	75	25	15.1 ± 4.4 a
500	75	50	12.8 ± 3.4 ab
500	75	100	10.1 ± 3.3 b
500	75	200	1.7 ± 0.5 c
Experiment 3 (6 replicates, 5 nights)			
500	0	50	0.3 ± 0.1 c
500	37.5	50	17.1 ± 6.1 a
500	75	50	17.8 ± 5.1 a
500	150	50	4.9 ± 1.7 b
500	300	50	3.8 ± 1.6 b

[a]Actual mean catches ±SE; for ANOVA data transformed to $\log(x + 1)$, means followed by the same letter in each experiment are not significantly different at the 5% confidence level by DMRT.

tiveness of the optimum synthetic pheromone blend were investigated. Addition of up to 10% of the *E* isomers of any of the three pheromone components either singly or together had no consistently significant effects on catches (Table 6). There was even an indication that addition of 2.5% of the *E* isomers increased catches slightly relative to those with the blend of isomerically pure components.

DISCUSSION

This study has shown the presence of five compounds produced and emitted by virgin female *C. ignefusalis*, that elicit an antennal response in male *C. ignefusalis*. These were identified as *Z*7–12 : OH, *Z*5–10 : OH *Z*7–12 : Ald, *Z*7–12 : Ac, and *Z*9–14 : OH, and field studies demonstrated that *Z*7–12 : OH, *Z*5–10 : OH, and *Z*7–12 : Ald are essential for attraction of male moths. Polyethylene vials impregnated with a blend of these three components in 100 : 5 : 3.3 ratio emit the components at similar relative rates to those measured from a virgin female moth, and this blend was found to be optimum for attraction of male

TABLE 3. CATCHES OF MALE *C. ignefusalis* IN TRAPS BAITED WITH *Z*7–12:OH AND VARIOUS AMOUNTS OF *Z*5–10:OH + *Z*7–12:ALD IN 3 : 2 RATIO (6 REPLICATES OVER 5 NIGHTS PER EXPERIMENT)

Pheromone component (μg)			Mean catch/ trap/night[a]
*Z*7–12:OH	*Z*5–10:OH	*Z*7–12:Ald	
Experiment 1			
500	0	0	0.8 ± 0.1 b
500	6.25	4.16	22.7 ± 5.1 a
500	12.5	8.33	26.1 ± 2.8 a
500	25	16.67	27.2 ± 6.9 a
—	—	—	0.0 c
Experiment 2			
500	25	16.7	27.9 ± 5.4 a
500	50	33.3	19.1 ± 4.3 ab
500	75	50	12.4 ± 3.0 b
500	100	66.7	10.8 ± 1.8 b
500	200	133.3	3.9 ± 1.0 c

[a]Actual mean catches ± SE; for ANOVA data transformed to $\log(x + 1)$, means followed by the same letter in each experiment are not significantly different at the 5% confidence level by DMRT.

moths and at least as attractive as a virgin female moth as measured by catches in baited water traps.

Release rates of the pheromone components from the polyethylene vial dispensers were found to increase over the first three days to reasonably steady levels for at least the next seven days at 27°C. Relative release rates for the two minor components are similar and faster than for the major component, *Z*7–12 : OH, and hence these components will be depleted more quickly and the relative amounts emitted will decrease over time. However, this will not lead to immediate reductions in catch, and the vials should be effective for at least two weeks in the field. In contrast, rubber septa were shown not to be good dispensers for these relatively volatile pheromone components. As found by Butler and McDonough (1981) and McDonough and Butler (1983), release rates were high and significantly different for the different components such that most of the pheromone was emitted during the first two to three days, and the blend released changed markedly in composition during this period.

Addition of the *E* isomers of the pheromone components to the synthetic blend at up to 10% of the *Z* isomer did not affect catches, so it is not necessary to use materials specially purified to high stereoisomeric purity in lures for *C. ignefusalis*. However, addition of the other minor components shown to be produced by the female moth, *Z*7–12 : Ac and *Z*9–14 : OH, markedly reduced catches by

TABLE 4. CATCHES OF MALE *C. ignefusalis* IN TRAPS BAITED WITH ATTRACTIVE BLEND (500 μg Z7–12:OH + 25 μg Z5–10:OH + 16.7 μg Z7–12:ALD) + Z7–12:AC AND/OR Z9–14:OH (6 REPLICATES OVER 5 NIGHTS PER EXPERIMENT)

Blend	Pheromone component (μg)		Mean catch/ trap/night[a]
	Z7–12:Ac	Z9–14:OH	
Experiment 1			
542	—	—	8.6 ± 1.3 a
542	1.25	—	7.3 ± 1.3 ab
542	2.5	—	4.1 ± 0.8 b
542	5.0	—	3.8 ± 0.8 b
—	—	—	0.0 ± 0.0 c
Experiment 2			
542	—	—	12.9 ± 2.6 a
542	—	3.75	7.5 ± 1.1 ab
542	—	7.5	6.2 ± 1.2 bc
542	—	15.0	4.5 ± 0.3 c
—	—	—	0.1 ± 0.1 d
Experiment 3			
542	—	—	11.1 ± 2.1 a
542	1.25	3.75	5.5 ± 1.6 b
542	2.5	7.5	3.4 ± 1.5 b
542	5.0	15.0	0.7 ± 0.2 b
—	—	—	0.0 c

[a]Actual mean catches ±SE; for ANOVA data transformed to $\log(x + 1)$, means followed by the same letter in each experiment are not significantly different at the 5% confidence level by DMRT.

the three-component attractive blend. When both were present at levels thought to be similar to those produced by the female moth, catches were significantly reduced. Z7–12 : Ac and Z9–14 : OH are potential by-products of the biosynthetic pathways to the components of the attractive blend in the female *C. ignefusalis* moth (e.g., Bjostad et al., 1984) and may act on the same antennal receptors in the male. However, it is not known whether these components have any real behavioral significance within the species and, as they are produced at such low levels, it seems unlikely that they are involved in hindering cross-attraction of other species to help ensure species specificity.

Z7–12 : OH is produced by the female moths in *Autographa gamma* (Dunkelblum et al., 1983), *Eucosma womonana* (Underhill et al., 1987), and *Graphania insignis* (Frérot et al., 1993) and is an essential minor component of the attractive pheromone blends for these species. This compound has also been found in *Actebia fennica* (Struble et al., 1989) *Autographa nigrisigna* (Sugie et al., 1991), *Plusia chalcites* (Dunkelblum et al., 1987), *Cornutiplusia circumflexa*

TABLE 5. CATCHES OF MALE *C. ignefusalis* IN TRAPS BAITED WITH ATTRACTIVE BLEND AT VARIOUS LOADINGS AND IN COMPARISON WITH VIRGIN FEMALE MOTH

Pheromone component (μg)			Mean catch/ trap/night[a]
*Z*7–12:OH	Z5–10:OH	*Z*7–12:Ald	
Experiment 1 (6 replicates, 4 nights)			
1000	50	30	41.4 ± 3.8 a
100	5	3	33.4 ± 1.9 a
10	0.5	0.3	14.0 ± 1.8 b
—	—	—	0.0 c
Experiment 2 (7 replicates, 3 nights)			
1000	50	30	57.5 ± 5.6 a
Virgin female moth			23.8 ± 2.8 b
—	—	—	0.2 ± 0.1 c

[a]Actual mean catches ± SE; for ANOVA data transformed to log(x + 1), means followed by the same letter in each experiment are not significantly different at the 5% confidence level by DMRT.

(Mazor et al., 1991), and *Trichoplusia ni* (Bjostad et al., 1984), although it does not form part of their attractive pheromone blends and in some species actually reduces attraction. Although Z7–12 : OH is produced by female *Agrotis segetum* moths (Löfstedt et al., 1982, 1985, 1986), only Toth and Szõcs (1991) reported it to be attractive to the male moths at high doses in Bulgaria. *Z*7–12 : OH has also been reported as a component of attractive blends for 30 other species of Lepidoptera, although it has not been shown to be produced naturally in these cases (Arn et al., 1998). To the best of our knowledge, *C. ignefusalis* is the first species recorded in which *Z*7–12 : OH is produced by the female moth as the major component of the pheromone blend.

Similarly, the minor components Z5–10 : OH and *Z*7–12 : Ald have been reported previously, but only in *C. ignefusalis* have they been shown to be both produced by the female moths and essential for attraction of the male moths. Thus Z5–10 : OH was identified in the female pheromone gland extracts from several strains of *A. segetum* (Löfstedt et al., 1985, 1986) but was not necessary for attraction of male moths in the field, while field screening showed Z5–10 : OH either alone or in combination with Z5–10 : Ac to be attractive to 16 *Coleophora* species (Priesner et al., 1982; Priesner, 1987; Priesner and Zhang, 1991) and to *Batrachedra pinicolella* (Priesner, 1989). *Z*7–12 : Ald has previously only been reported as present in *Actebia fennica* (Struble et al., 1989), although it was not necessary for attraction of males in the field, and random field testing has shown blends containing this compound to be attractive to nine other species of Lepidoptera (Arn et al., 1998).

*Z*7–12 : Ac and *Z*9–14 : OH have been reported to be produced by many

TABLE 6. CATCHES OF MALE *C. ignefusalis* IN TRAPS BAITED WITH SYNTHETIC PHEROMONE BLEND (500 μg *Z*7–12:OH + 25 μg Z5–10:OH + 16.7 μg *Z*7–12:ALD) CONTAINING DIFFERENT AMOUNTS OF CORRESPONDING *E* ISOMERS, (6 REPLICATES OVER 5 NIGHTS PER EXPERIMENT)

Additional pheromone component (μg)			Mean catch/ trap/night[a]
*E*7–12:OH	*E*5–10:OH	*E*7–12:Ald	
Experiment 1			
—	—	—	13.5 ± 2.2 b
12.5	—	—	19.3 ± 3.5 a
25	—	—	19.4 ± 3.9 a
50	—	—	17.2 ± 2.4 ab
Unbaited			0.1 ± 0.1 c
Experiment 2			
—	—	—	16.3 ± 1.7 ab
—	0.625	—	20.4 ± 4.3 a
—	1.25	—	17.4 ± 2.5 ab
—	2.5	—	16.8 ± 3.1 b
Unbaited			0.1 ± 0.04 c
Experiment 3			
—	—	—	16.3 ± 3.5 b
—	—	0.42	20.8 ± 3.2 a
—	—	0.84	17.1 ± 3.4 ab
—	—	1.67	21.1 ± 3.7 a
Unbaited			0.1 ± 0.04 c
Experiment 4			
—	—	—	17.4 ± 4.9 b
12.5	0.625	0.42	19.9 ± 3.5 a
25	1.25	0.84	16.2 ± 2.1 ab
50	2.5	1.67	17.1 ± 2.6 ab
Unbaited			0.0 c

[a]Actual mean catches ± SE; for ANOVA data transformed to log(x + 1), means followed by the same letter in each experiment are not significantly different at the 5% confidence level by DMRT.

species of Lepidoptera, often being an essential component of an attractive pheromone blend (Arn et al., 1998).

Work is in progress to develop pheromone traps for monitoring *C. ignefusalis* in West Africa (Youm et al., 1993; 1997; Youm and Beevor, 1995).

Acknowledgments—The authors are grateful to Rose Robinson and Dudley Farman at NRI for painstaking purification of compounds used in this work. The work was funded jointly by ICRISAT and the Natural Resources Research Department of the UK Department for International Development (DFID) (Project R5281) for the benefit of developing countries, although the views expressed are not necessarily those of DFID. The manuscript was approved as Journal Article JA2100 by ICRISAT.

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