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IDENTIFICATION OF THE CUTICULAR HYDROCARBONS OF THE HORN FLY[1] AND ASSAYS FOR ATTRACTION[6]

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Abstract—Horn fly cuticular paraffin and monoolefin hydrocarbons were chemically identified and assayed for biological activity as attractants. The majority of the paraffins were odd-numbered, straight-chain molecules 21–29 carbons in length; much smaller amounts of even-numbered, straight-chain molecules 22–28 carbons in length and methyl-branched compounds were also present. At least 80% of the monoolefin consisted of straight-chain molecules 23, 25, and 27 carbons in length, two of which have been identified as sex pheromones in other muscoid species. The hydrocarbon profiles among sexes and strains (laboratory and wild) were very similar except for wild females, which showed quantitative differences from the other sources. However, only females showed significant (albeit low) responses to some test materials, both synthetic and natural, and activity appeared to be centered in the monoolefins.

Key Words—Horn fly, olefins, mating pheromone, paraffins, *Musca autumnalis*, sex attractant.

[1]Diptera: Muscidae.
[4]A portion of a dissertation by the first author to the Graduate School of the University of Florida in partial fulfillment of the requirements for the PhD degree.
[6]Mention of a proprietary or commercial product does not constitute an endorsement by either the University of Florida or the U.S. Department of Agriculture.

INTRODUCTION

The horn fly, *Haematobia irritans* (L.), is a serious pest of cattle in the U.S. and elsewhere. The blood-feeding habits of the adults cause irritation, worry, and blood loss in host animals, and this commonly results in reductions in weight gain and milk production (Bruce, 1942, 1964). In recent years, control of this pest has become increasingly difficult because of legal restrictions placed on insecticide use.

The utilization of sex attractants in insect control programs offers promise for reducing dependence on insecticides. To date, certain of the cuticular hydrocarbons from a number of related muscoid flies have been isolated and identified as sex attractants and/or mating stimulants (Carlson et al., 1971, 1978; Muhammed et al., 1975; Uebel et al., 1975a–c, 1978a,b). Furthermore, Mayer et al. (1972) found attraction of male house flies, Musca domestica L., in an olfactometer to horn fly cuticular hydrocarbons.

In view of these findings, we determined the chemical nature of horn fly cuticular hydrocarbons, and investigated their potential as horn fly attractants.

METHODS AND MATERIALS

Chemical Analyses. Crude lipid extracts were obtained separately from 3- to 4-day-old sexually mature, virgin, male and female flies from the colony at the University of Florida, hereafter called Florida Laboratory Strain (L), that were immobilized by freezing at −20° C and rinsed with *n*-hexane (Phillips, 20 flies/ml). The hexane washes were reduced to dryness on a rotary evaporator, weighed, and then reconstituted with *n*-hexane. The hydrocarbons were obtained by open-column liquid chromatography (LC) by eluting 1–2 g of extract from a 2 × 45-cm column of silica gel (60–200 mesh, J.T. Baker Chemical Co.) with 200 ml *n*-hexane. The hydrocarbons were then eluted on a 1.3 × 36-cm column of 20% $AgNO_3$-impregnated silica gel (60–200 mesh, Hi-Flosil-Ag, Applied Science Laboratories) with 60 ml *n*-hexane to obtain the paraffins, followed by 100 ml of 2% ether in hexane to obtain the olefins. Separation and purity of all eluted fractions were confirmed by thin-layer chromatography (TLC) on silica gel plates (250 μm Anasil, Analabs, or 150 μm Uniplates, Analtech) or $AgNO_3$-impregnated silica gel plates (250 μm AG Anasil, Analtech). No polyolefins were detected.

The hydrocarbons were analyzed by gas chromatography (GC) using a Varian model 2100 with a 1.8-m × 2-mm ID glass column containing 3 or 5% SE-30 on 120–140 mesh Gas Chrom Q, and flame ionization detection. The major *n*-paraffins and chain lengths of branched paraffins were determined by coinjection of known paraffin standards. Chain lengths of the major monoolefins were similarly determined and retention indices (KI) assigned

(Kovats, 1966). Quantitations were obtained by comparing peak height or peak areas with standards of known concentration; peak areas were measured on a Hewlett-Packard model 3380A integrator.

Olefins from males and females consisted of four major peaks eluting at KI 2272 and KI 2292 (C_{23}), KI 2477 (C_{25}), and KI 2677 (C_{27}) and hereafter designated compounds I, II, III, and IV, respectively. Each was collected from samples of olefin from L females by preparative GC using a Varian model 90-P aerograph with a thermal conductivity detector and stainless-steel (SS) columns. The C_{23} compounds were collected from a 6-m × 4-mm ID column containing 5% SE-30 on 100–120 mesh Gas Chrom Q, and the C_{25} and C_{27} compounds collected from a 3-m × 2-mm ID column containing 3% SE-30. The individually trapped compounds were ozonized to determine sites of unsaturation and the resulting aldehyde fragments identified by GC after the method of Beroza and Bierl (1967). Aldehydes resulting from ozonolysis of compounds I and II were identified on three gas chromatographs (F and M model 810 with a 3-m × 2.2-mm ID SS column containing 5% Carbowax 20 M on 100–120 mesh Gas Chrom Q; Varian model 1200 with a 2-m × 2.2-mm ID SS column containing 5% Hi-EFF-1BP on 80–100 mesh Chromosorb W AW; and the previously described Varian model 2100 with the 5% SE-30 glass column). The short-chain aldehyde obtained from ozonolysis of compound II could not be detected on the above columns or on SS or glass columns of Porapak Q. The ozonolysis products of III and IV were determined with the 5% SE-30 column.

Electron impact mass spectra (EI-MS) of paraffins from L males and females and from wild females were obtained using a Varian MAT CH5 mass spectrometer interfaced via a membrane separator to a Varian 1400 GC equipped with 3.2-m × 2-mm ID glass columns of 3% OV-1 on 100–120 mesh Gas Chrom Q.

Chemical ionization mass spectra (CI-MS) of II and the ozonolysis products of the four olefins were obtained with a Finnigan 1015 S/L mass spectrometer, interfaced to a Systems Industries 150 data system. A Varian model 1400 GC, equipped with either a 1.8-m × 2-mm ID glass column containing 3% OV-1 on 100–120 mesh Gas Chrom Q or a 1.8-m × 2-mm ID SS column containing 5% SE-30 on Gas Chrom Q (100–120 mesh), was used as the inlet. Methane, used as the carrier and ionizing gas, was passed directly into the ion source where pressure was maintained at 1.0 torr, and the GC oven was temperature programed. The computer data system provided mass spectra, reconstructed gas chromatograms (RGC), and limited mass range searches (LMS), representing ions of specific masses plotted versus spectrum number.

Extracts from all sources were taken through the same scheme. These were (1) 3- to 4-day-old, virgin male or female flies reared from eggs obtained

from Florida wild flies (hereafter, W) and (2) hexane washes from cages heavily contaminated with fly feces.

Compound I had been synthesized previously (Carlson et al., 1971), while III and IV were obtained from other sources, for GC and TLC analysis, ozonolysis, and bioassay. Compound II [(*Z*)-5-tricosene] was prepared by the Wittig reaction as follows. 1-Bromooctadecane (Aldrich, 52 g, 0.156 mol) and 52 g (0.2 mol) of triphenylphosphine (Aldrich) were dissolved in 200 ml of acetonitrile and refluxed overnight in N_2 atmosphere. The acetonitrile was removed on a rotary evaporator, and the residue was poured into anhydrous ether. The white solid that precipitated upon stirring was collected and dried thoroughly under vacuum to give octadecyltriphenylphosphonium bromide. Octadecyltriphenylphosphonium bromide (34 g, 0.057 mol) was dissolved in 100 ml of anhydrous tetrahydrofuran (THF) in a dry flask under N_2. The solution was stirred, cooled in an ice bath, and held between 10 and 20° C, while 30 ml of butyllithium (15.16% solution in hexane, Foote Mineral Co.) was added slowly. The dark red solution was held at 15–20° C for 1 hr and then cooled to 10° C. Freshly distilled pentanal [5.6 g, 0.065 mol, bp 102–103° C (760 mm)] was added dropwise with stirring; the solution was allowed to warm to room temperature overnight. Two days later, the reaction mixture was shaken with water and hexane and separated. The hexane layer was washed with brine and dried. Rotary evaporation gave 25 g of crude oil and a few crystals of triphenylphosphine oxide. The crude oil was diluted with hexane and portions were passed 3 times through 2 × 50-cm columns of silica gel (60–200 mesh, Baker) with 200 ml hexane each time. Removal of solvents gave 13.5 g (54%) of olefin. The *cis* and *trans* isomers from a 2-g sample of the olefin were separated by $AgNO_3$-LC; the *trans* isomer was eluted with 120 ml of hexane and the *cis* isomer with 150 ml of 1% ether in hexane. Separation and purity of the eluents were confirmed by $AgNO_3$-TLC. The olefin as synthesized was estimated to contain 85% *cis* and 15% *trans* isomer of 5-tricosene by weighing the $AgNO_3$-LC eluents after removal of solvent.

Bioassays. The movement and choice response of flies over a distance of ca. 50 cm to test materials was assayed in an olfactometer. The olfactometer was a modification of the vertical types used to assay stable fly, *Stomoxys calcitrans* (L.) (Muhammed et al., 1975) and screwworm fly, *Cochliomyia hominivorax* (Coquerel) (Adams et al., 1979) attractants. It consisted of four independent units, each comprised of an insect-holding chamber (30 cm long, 7 cm ID) and two choice chambers (each 30 cm long, 3.5 cm ID) (Figure 1). Insects placed in the holding chambers moved vertically against the air flow, through the choice ports, and into treatment or control choice chambers. Partially conditioned air was directed through an activated charcoal filter to the small mixing chamber (21 × 21 × 9.5 cm); situated here was a heating element connected with a proportional temperature controller (RFL model

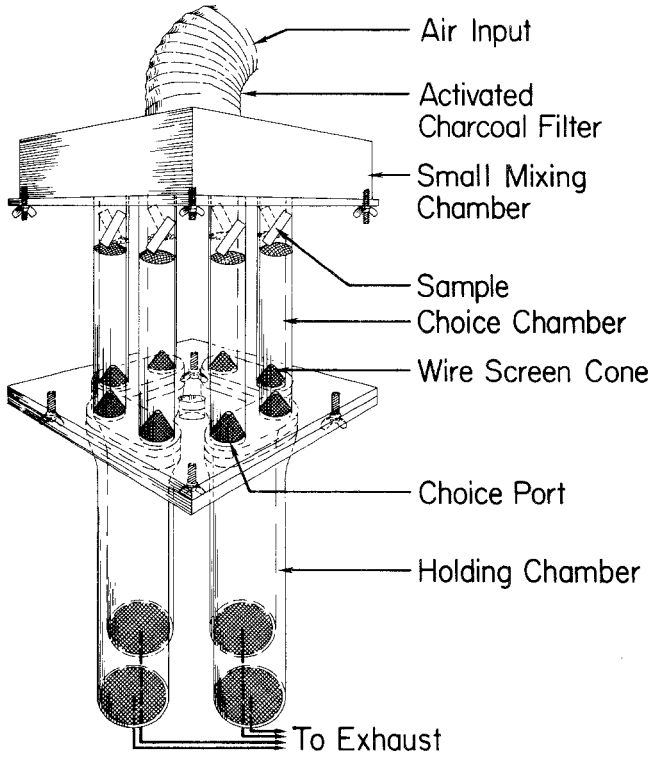

FIG. 1. The horn fly olfactometer.

879, RFL Industries), and wet and dry bulb thermistor probes to monitor temperature and humidity. Temperature and humidity were maintained at 32.5 ± 1.5° C and 60 ± 10% relative humidity, respectively. The fully conditioned air then passed into the eight choice chambers, over glass microscope slides treated with given test materials (Figure 1, Sample), through the choice ports and holding chambers, and finally was exhausted to the outside. Air velocity at the choice ports was maintained at 15 m/min. A fluorescent lamp, either a General Electric Plant Light or a Sears Cool White, was ensheathed in red plastic and illuminated the olfactometer from its central position along the axis of the choice and holding chambers.

For a given bioassay, test materials in 6- to 50-μl solutions of hexane were applied by syringe to one side of glass microscope slides, which were then placed in the treatment choice chambers. Slides treated with 25 μl hexane only were placed in control choice chambers. Lots of 20–40 virgin, 3- to 4-day-old male or female test insects, which had been offered a blood meal up until 1 hr before, were anesthetized briefly with N_2 and introduced to each of the

holding chambers. Flies entering treatment and control choice chambers were counted at the end of 30 min. Flies for a given test were used only once and then discarded. Between each test the holding and choice chambers were thoroughly washed with hot water and detergent. The materials tested included male and female crude lipids; mixed-sex hydrocarbons, nonhydrocarbons, olefins, and paraffins; and synthesized olefins. Since the hydrocarbon profiles of males and females as identified were very similar, the large quantities of extracted natural materials which were needed were recovered from mixed-sex rather than virgin flies.

Tests run concurrently in the independent units of the olfactometer were considered to be replicates. The assay of a given test material was replicated 8–43 times. Data transformations were performed ($\sqrt{X} \pm 0.5$) owing to large variation in test insect response, and the transformed values were analyzed with the paired t test.

RESULTS AND DISCUSSION

Chemical Analyses. The bulk of the paraffins from L flies were odd-numbered, straight-chain molecules 21–29 carbons in length; much smaller amounts of even-numbered, straight-chain molecules 22–28 carbons in length and methyl-branched compounds were present. Major branched isomers are listed first, and others in descending order, as estimated from heights of fragments in the EI-MS data. The lack of parent ions does not affect identification for these materials, as the KI data and characteristic mass spectra are unequivocal (Tables 1 and 2). The methods of Nelson et al. (1972) and Pomonis et al. (1978) were followed to determine the lengths of paraffins and the number of methyl branches by GC-MS. Identification was possible for all major and most branched components. The identified branched paraffins were either mono- or dimethyl, with the most common sites of methyl substitution being the 9, 11, 13, and 15 positions, with three methylene units present between branches in dimethyl components. A unique dimethyl-branched paraffin was found at KI 2750 only in the mass spectra of W females, and it eluted with the last traces of 5-methylheptacosane. The fragments at 168, 169, 196, 197, 239, and 393 (M-15) are consistent with 11,15-dimethyl-heptacosane. GC analyses showed that the latter was the most prevalent branched paraffin in W females, and other samples had less of it. Otherwise, male and female paraffins from L and W flies were qualitatively similar, since they eluted together by GC, but quantitative differences were seen between the strains and the sexes (Table 3). Paraffins in Table 3 were present at 2.8, 5.8, 3.9, and 5.4 μg/fly for L males, L females, W males, and W females, respectively, and branched paraffins comprised only 5.8, 9.6, 9.3, and 10.0% of these totals, respectively. GC profiles of paraffins recovered from cage washes (feces) were very similar to those of L flies.

TABLE 1. FEMALE L HORN FLY PARAFFINS

Kovats' index	Structure	Observed fragments		
		m/e	M-15	M
2330	9-$C_{23}H_{47}(CH_3)$	140, 141	323	none
		224, 225		
	11-$C_{23}H_{47}(CH_3)$	168, 169		
		196, 197		
2530	11-$C_{25}H_{51}(CH_3)$	168, 169	351	none
		224, 225		
	13-$C_{25}H_{51}(CH_3)$	196, 197		
2550	11,15-$C_{25}H_{50}(CH_3)_2$	168, 169	none	none
		239		
2730	11-$C_{27}H_{55}(CH_3)$	168, 169	379	none
		252, 253		
	13-$C_{27}H_{55}(CH_3)$	196, 197		
		224, 225		
	5-$C_{27}H_{55}(CH_3)$	337	379	none
	7-$C_{27}H_{55}(CH_3)$	309		
2930	11-$C_{29}H_{59}(CH_3)$	168, 169	407	422
		281, 282		
	13-$C_{33}H_{67}(CH_3)$	196, 197		
		252, 253		
	15-$C_{29}H_{59}(CH_3)$	224, 225		
2950	11,15-$C_{29}H_{58}(CH_3)_2$	168, 169	421	none
		224, 225		
		238, 239		
		295		
	13,17-$C_{29}H_{58}(CH_3)_2$	196, 197		
		266, 267		
3130	11-$C_{31}H_{63}(CH_3)$	168, 169	435	450
		308, 309		
	13-$C_{31}H_{63}(CH_3)$	196, 197		
		280, 281		
	15-$C_{31}H_{63}(CH_3)$	224, 225		
		252, 253		
3150	9,13-$C_{31}H_{62}(CH_3)_2$	140, 141	449	464
		210, 211		
		280, 281		
		352, 353		
	11,15-$C_{31}H_{62}(CH_3)_2$	238, 239		
		252, 253		
		322, 323		
	13,17-$C_{31}H_{62}(CH_3)_2$	196, 197		
		266, 267		
		294, 295		
3330	11-$C_{33}H_{67}(CH_3)$	168, 169	463	478
		336, 337		

TABLE 1. (*cont.*)

Kovats' index	Structure	Observed fragments		
		m/e	M-15	M
	13-$C_{33}H_{67}(CH_3)$	196, 197		
		308, 309		
	15-$C_{33}H_{67}(CH_3)$	224, 225		
		280, 281		
3350	11,15-$C_{33}H_{66}(CH_3)_2$	169, 169	none	none
		238, 239		
		280, 281		
		350, 351		
	13,17-$C_{33}H_{66}(CH_3)_2$	196, 197		
		252, 253		
		266, 267		
		322, 323		
3530	11-$C_{35}H_{71}(CH_3)$	168, 169	491	none

TABLE 2. MALE L HORN FLY PARAFFINS

Kovats' index	Structure	Observed fragments		
		m/e	M-15	M
2130	9-$C_{21}H_{43}(CH_3)$	140, 196		
	11-$C_{21}H_{43}(CH_3)$	169		
2200	$C_{22}H_{46}$		none	310
2300	$C_{23}H_{48}$		none	324
2330	9-$C_{23}H_{47}(CH_3)$	140, 224	none	none
	11-$C_{23}H_{47}(CH_3)$	168, 196		
2400	$C_{24}H_{50}$		none	338
2500	$C_{25}H_{52}$		none	none
2530	7-$C_{25}H_{51}(CH_3)$	113, 281	none	none
	9-$C_{25}H_{51}(CH_3)$	252, 253		
		141, 142		
	11-$C_{25}H_{51}(CH_3)$	168, 169		
		224, 225		
	13-$C_{25}H_{51}(CH_3)$	196, 197		
2550	9,13-$C_{25}H_{50}(CH_3)_2$	141, 197	none	none
		211, 267		
	11,15-$C_{25}H_{50}(CH_3)_2$	169, 239		
2600	$C_{26}H_{54}$		none	none
2730	7-$C_{27}H_{55}(CH_3)$	112, 308	379	none
	11-$C_{27}H_{55}(CH_3)$	169, 253		
	13-$C_{27}H_{55}(CH_3)$	196, 197		
		225		

TABLE 2. (*cont.*)

Kovats' index	Structure	Observed fragments		
		m/e	M-15	M
2900	$C_{29}H_{60}$		none	none
2930	7-$C_{29}H_{59}(CH_3)$	112, 337	none	none
	9-$C_{29}H_{59}(CH_3)$	141, 309		
	11-$C_{29}H_{59}(CH_3)$	168, 281		
	13-$C_{29}H_{59}(CH_3)$	196, 252		
	15-$C_{29}H_{59}(CH_3)$	225		
3130	11-$C_{31}H_{63}(CH_3)$	169, 309	435	450
	13-$C_{31}H_{63}(CH_3)$	196, 281		
	15-$C_{31}H_{63}(CH_3)$	225, 253		

TABLE 3. QUANTITIES[a] OF PARAFFINS RECOVERED FROM L AND W HORN FLIES

Kovats' index[b]	L male	L female	W male	W female
2100	19.7	10.5	7.9	1.0
2130	0.2	0	1.9	0.4
2200	1.1	0.9	2.1	0.8
2300	36.3	27.5	11.6	3.1
2330	0.6	0.4	0.4	0.1
2350	0.1	0.1	0.1	0.4
2400	0.6	0.7	1.5	1.7
2500	15.7	18.9	20.0	22.8
2550	0.3	0.2	0.4	0.4
2600	0.6	0.8	2.2	2.9
2700	13.7	22.4	25.6	42.9
2730	0.5	0.7	2.0	2.5
2750	0.2	1.7	3.6	5.3
2800	0.2	0.5	1.7	2.6
2900	4.8	6.4	3.6	5.6
2930	2.0	3.0	0.9	0.9
3100	0.5			
3130	1.9	3.5		

[a]Percent of total.

[b]Unbranched (normal) paraffins have KI values of 2100, 2200, 2300, etc., by definition; KI values of 30 (eg, 2130) are internally monomethyl branched; KI values of 50 (eg, 2150) are dimethyl branched.

All olefins were of the *cis* configuration. The major longer aldehydes resulting from ozonolysis of compounds I, II, III, and IV coeluted on GC with C_{14}, C_{18}, C_{16}, and C_{18} aldehyde standards, respectively. The shorter aldehydes from ozonized I, III, and IV coeluted with the C_9 aldehyde nonanal. Pentanal from ozonized II was not detected. CI-MS confirmed the identities of the detected aldehyde fragments (Table 4). All the major aldehydes had fragments M + 1, M − 1, M + 1–18; for some aldehydes, M + 29 and M + 41 fragment ions (addition of $C_2H_5^+$ and $C_3H_5^+$, respectively) were observed as noted. The aldehyde fragments account for the following structures: (*Z*)-9-tricosene (I), (*Z*)-5-tricosene (II), (*Z*)-9-pentacosene (III), and (*Z*)-9-heptacosene (IV). Compounds I–IV coeluted by GC with their respective synthetic counterparts as did the ozonolysis products of I and II with the ozonolysis products of their respective synthetic counterparts.

Olefins were present at 5.13, 7.17, 2.12, and 2.34 μg/fly for L males, L females, W males, and W females, respectively. The four major compounds comprised 95% of the total olefins in L flies and W males, and 80% of the total in W females. Table 5 summarizes the quantities of these compounds in each group of flies. Among L flies and W males, I was the major component (38–83% of the total); however, in W females it was only 2% of the total. In W females III and IV were 22 and 55% of the total, respectively, while two other unidentified compounds of higher molecular weight made up most of the remainder. Natural materials were not collected by preparative GC for bioassay because the response to natural olefin was low. Monoolefins extracted from fly feces showed GC profiles quite similar to those of L flies.

Bioassays. Males showed a barely significant, positive response to synthesized II but did not respond to the crude lipids, the natural olefin, or other materials containing this compound (Table 6). Female response was more consistent and significant, as positive responses to the female crude lipid, hydrocarbons, olefins, and compound I were elicited. Neither sex responded to other lipids, but no obvious repellency was observed. The lack of stronger responses to the individual olefins suggests that combinations of the compounds were necessary, that synthesized compounds were impure or too pure for the assay, or that essential compounds were missing.

(*Z*)-9-Tricosene has been previously identified as a house fly attractant pheromone (Carlson et al., 1971), and (*Z*)-9-pentacosene has been identified as the copulatory sex pheromone of the little house fly, *Fannia canicularis* (L.) (Uebel et al., 1975a). In olfactometer tests performed by Mayer et al. (1972), compound I was apparently responsible for the attraction of male house flies to horn fly hydrocarbons. Because of the success of these approaches, we felt justified in assaying synthetic olefins. We did not test olefins from horn flies other than compounds I–IV because it has been our experience that no highly potent attractants in flies have been found that work at submicrogram

TABLE 4. CI-MS OF MONOOLEFIN OZONIDES FROM FEMALE L HORN FLIES

Kovats' index	Compound	Aldehydes	Observed fragments						
			M-1-18	M + 1-18	M-1	M	M + 1	M + 29	M + 41
2272	(Z)-9-Tricosene (I)	C_9[a]	123	125	141		143[b]	171	183
		C_{14}[a]	193	195	211	212	213	241	
2292	(Z)-5-Tricosene (II)	C_{16}	221		239		241[b]	269	281
		C_{17}	235		253		255	283	
		C_{18}[a]	249		267		269	297	
2477	(Z)-9-Pentacosene (III)	C_9[a]	123	125	141		143[b]	171	183
		C_{13}			197		199[b]	227	
		C_{14}			211		213[b]		
		C_{16}[a]	221	223	239	240	241[b]	269	281
		C_{18}	249		267		269[b]	297	
2677	(Z)-9-Heptacosene (IV)	C_8		111[b]			129	157	169
		C_9[a]	123	125	141		143	171	183
		C_{15}		209	225	226		255	
		C_{16}	221	223	239		241[b]	269	281
		C_{17}	235	237	253	254	255	283	295
		C_{18}[a]	249	251	267	268	269	297	309
		C_{20}	277		295	296	297	325	

[a]Major products.
[b]Base peak.

TABLE 5. QUANTITIES OF 4 MAJOR MONOOLEFINS RECOVERED FROM L AND W HORN FLIES

Kovats' index	Compound[a]	Component/fly (μg)			
		L male	L female	W male	W female
2272	Z-9-Tricosene	1.20	0.64	0.18	0.01
2292	Z-5-Tricosene	0.76	1.04	0.35	0.02
2477	Z-9-Pentacosene	0.34	0.54	0.42	0.37
2677	Z-9-Heptacosene	0.12	0.34	0.37	0.90

[a]Compounds identified via CI-MS from L females only.

quantities. No *trans* isomers were found in flies, nor were any *trans* synthetics tested, but *trans* isomers do not appear to play an inhibitory role in response of other muscids studied (Carlson et al., 1974).

At this point, the nature of attraction of horn flies to these hydrocarbons is not clear, especially as no striking sexual dimorphism was observed in the quantity or identity of cuticular components of L flies (Tables 1, 2, 3, and 5).

TABLE 6. AVERAGE RESPONSE OF VIRGIN, 3- TO 4-DAY-OLD HORN FLIES TO TEST MATERIALS AND CHECKS IN THE OLFACTOMETER

Test material	Quantity	Sex tested	*N*	T[a]	C[b]	*t* statistic[c]
Natural products						
♂ crude lipid	20 FE	♀	16	7.9 ± 4.5	7.4 ± 5.1	0.32
		♂	16	9.3 ± 4.4	5.5 ± 4.4	1.80
♀ crude lipid	20 FE	♀	16	6.3 ± 4.5	5.6 ± 3.6	0.23
		♂	16	9.0 ± 4.7	4.8 ± 2.9	2.58**
Hydrocarbons	100 μg	♀	8	12.4 ± 5.1	8.9 ± 4.6	1.12
		♂	10	6.7 ± 3.8	2.6 ± 2.6	3.08**
Nonhydrocarbons	20 FE	♀	16	8.8 ± 4.6	7.5 ± 4.0	0.46
		♂	16	6.5 ± 4.7	6.8 ± 3.7	0.40
Paraffins	1 mg	♀	15	3.3 ± 2.1	4.0 ± 2.5	0.89
		♂	15	4.8 ± 6.7	5.6 ± 4.0	0.60
Olefins	1 mg	♀	43	5.7 ± 5.2	4.5 ± 4.9	1.44
		♂	35	7.8 ± 4.7	4.6 ± 3.0	4.77***
Synthetics						
I	50 μg	♀	18	6.4 ± 4.1	4.0 ± 3.2	2.89**
	1 mg		32	5.6 ± 4.4	8.2 ± 5.4	1.96
		♂	13	9.3 ± 4.6	7.0 ± 3.1	1.40
II	1 mg	♀	40	8.2 ± 4.5	5.8 ± 3.8	2.02*
		♀	27	6.1 ± 4.9	6.3 ± 4.6	0.25

TABLE 6. (*cont.*)

Test material	Quantity	Sex tested	*N*	T[a]	C[b]	*t* statistic[c]
Synthetics (*cont.*)						
III	1 mg	♂	16	7.3 ± 3.5	8.0 ± 4.2	0.31
		♀	16	7.9 ± 4.5	5.6 ± 3.7	1.28
IV	1 mg	♂	16	7.9 ± 4.2	9.1 ± 4.4	0.53
		♀	16	6.8 ± 5.2	8.5 ± 5.2	0.61
I:II (60:40)[d]	1 mg	♂	16	9.2 ± 4.1	7.8 ± 3.9	0.79
		♀	16	7.5 ± 3.9	7.8 ± 4.1	0.14
I:II (40:60)[e]	1 mg	♂	16	9.1 ± 3.8	7.7 ± 3.3	0.85
		♀	16	8.9 ± 3.8	8.3 ± 4.2	0.36

[a]Test materials as 6-50 μl solutions in hexane on glass microscope slides, means ($\bar{x}$) followed by standard deviation (±SD).
[b]25 μl hexane only on glass microscope slides as control, $\bar{x}$ ± SD.
[c]*, **, and *** paired *t* values at the 0.05, 0.025, and 0.001 levels of confidence, respectively.
[d]Reflects the ratio of these two compounds in virgin, mature lab-reared male horn flies.
[e]Reflects the ratio of these two compounds in virgin, mature lab-reared female horn flies.

However, Bolton et al. (1980) have shown in horn fly mating stimulant tests that males respond to female cuticular hydrocarbons but not to male cuticular hydrocarbons and crude lipids. Also, activity was shown using olefin blends which did not necessarily include I. Differences in mode of action (mating stimulation of males vs. attraction of females) and minor differences between male and female hydrocarbons may account for the different conclusions drawn by us and Bolton et al. (1980) concerning biological activity. In both efforts, branched paraffins were a minor complement to the total of compounds found, and never comprised more than 10% of the paraffin fractions. Thus, it is difficult to believe that they could contribute much to attractant activity, although a role in sex stimulant activity is possible as in the house fly where synergism of olefins by branched paraffins was observed by Uebel et al. (1976). While our L females had relatively large amounts of the more volatile olefins I and II, in which activity could reasonably be expected, W females had very little of them, but mostly III and IV, for which no activity was seen. Lower paraffins were also lacking in W females, suggesting that an essentially laboratory phenomenon was tested and both data from L and W flies must be considered. Perhaps, as some researchers have concluded about the tsetse fly, *Glossina* spp. (Bursell, 1961; Dean et al., 1969), the host animal serves not only as a food source but as an aggregation site for the sexes, thus eliminating the need for a sex attractant which operates over distance. However, the observation of similar hydrocarbons in horn fly feces suggest that the deposition and build-up of these

materials on a host's body surface is quite possible, which may affect flies coming near and/or alighting on the surface.

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