University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Publications from USDA-ARS / UNL Faculty

U.S. Department of Agriculture: Agricultural
Research Service, Lincoln, Nebraska

1998

Gas Chromatography/Mass Spectrometry Analysis of the Cuticular Hydrocarbons from Parasitic Wasps of the Genus *Muscidifurax*

Ulrich R. Bernier

University of Florida, ubernier@gainesville.usda.ufl.edu

David A. Carlson

United States Department of Agriculture—Agricultural Research Service

Christopher J. Geden

United States Department of Agriculture—Agricultural Research Service, Chris.geden@ars.usda.gov

Follow this and additional works at: <https://digitalcommons.unl.edu/usdaarsfacpub>

Part of the [Agricultural Science Commons](#)

Bernier, Ulrich R.; Carlson, David A.; and Geden, Christopher J., "Gas Chromatography/Mass Spectrometry Analysis of the Cuticular Hydrocarbons from Parasitic Wasps of the Genus *Muscidifurax*" (1998).

Publications from USDA-ARS / UNL Faculty. 955.

<https://digitalcommons.unl.edu/usdaarsfacpub/955>

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Gas Chromatography/Mass Spectrometry Analysis of the Cuticular Hydrocarbons from Parasitic Wasps of the Genus *Muscidifurax*

Ulrich R. Bernier, David A. Carlson, and Christopher J. Geden

United States Department of Agriculture–Agricultural Research Service, Center for Medical, Agricultural,
and Veterinary Entomology, Gainesville, Florida, USA

Parasitic Hymenoptera can be difficult to identify by conventional taxonomic techniques. Examination of the cuticular hydrocarbons (CHCs) provides a basis for chemotaxonomic differentiation, which may lead to the discovery of pheromones, and can be a means of examining colonies for species cross-contamination. The parasitic wasps examined were *Muscidifurax raptor*, *M. zaraptor*, *M. uniraptor*, and the gregarious form of *M. raptorellus*. Species within the genus *Muscidifurax*, as well as the sex, can clearly be differentiated by examining the gas chromatograms of the CHCs. Identification of the alkanes by mass spectrometry shows uncommon dimethylalkanes and trimethylalkanes for members of the genus. The methyl branched cuticular hydrocarbons of these insects are rare compared to those found on insects reported in the literature, but are present in significant amounts on these insects. Additionally, sexual dimorphism is observed in long chain alkanes (C_{21} – C_{39}) present on male and female cuticular surfaces for these species. Females tend to have cuticular hydrocarbons with methyl branches located externally on the carbon backbone chain for dimethyl-, trimethyl-, and tetramethylalkanes, whereas males tend to have dimethyl- and trimethylalkanes located internally on the hydrocarbon backbone chains. Mass spectra of novel and rare methyl branched compounds identified on these parasitoids are presented. (J Am Soc Mass Spectrom 1998, 9, 320–332)

I ncreasing concern about the use of synthetic organic pesticides to control agricultural insect pests is prompting the development of integrated pest management strategies that emphasize biological control. A method of biological control for house flies, widely used on poultry and dairy farms, is the disbursement of pteromalid wasps [1, 2]. Wasps in this family (Pteromalidae) do not sting or bite humans or animals but are entirely host specific for flies, attacking the puparia [3]. Pteromalid species vary in effectiveness against flies and are influenced by environmental and climatic effects.

Most of the indigenous parasitoids can readily be identified by morphological characters [3]. There is a need, however, for clearer taxonomic characters of exotic species contemplated for release in the United States. The wasps studied in this work are *Muscidifurax* spp. that are difficult to identify by taxonomic techniques [4]. Several are proven or potential biological control agents for filth fly infestations in the United States. There are five species that comprise this genus. *M. raptor* and *M. zaraptor* are of North American origin and are morphologically and biologically similar. *M.*

uniraptor is a unisexual female species originally identified in Puerto Rico. *M. raptorellus* is native to South America and has both a gregarious and solitary form. However, a gregarious strain of *M. raptorellus* was discovered recently in Nebraska [2]. *M. rapteroides* is restricted to Central America; therefore, this species was not examined here.

Starch gel electrophoresis of allozymes has been examined as a means of differentiating species in this genus, but a satisfactory degree of resolution was not achieved [5, 6]. Techniques such as RAPD-PCR [7] and mitochondrial DNA [8] have been successfully used to differentiate these closely related species. However, differentiation of *M. uniraptor* and *M. raptorellus* was difficult using mitochondrial DNA. Single insects were differentiated by quantitation from gas chromatography (GC), and variants of species, such as gregarious versus solitary forms of *M. raptorellus*, were differentiated by careful selection of the GC peaks as markers in a companion study [9]. The focus of this work is the identification of hydrocarbon components present on males and females of this genus, specifically examining patterns in the compounds that demonstrate the sexual dimorphism.

Demonstration of sexual dimorphism in the cuticular hydrocarbons (CHCs) of female and male insects can provide clues for the determination of a sex pheromone

Address reprint requests to Dr. Ulrich R. Bernier, USDA-ARS-CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608. E-mail: ubernier@gainesville.usda.ufl.edu

[10–13]. Additionally, CHCs have been used to differentiate species of beetles [14], mosquitoes [15], cockroaches [16], grasshoppers [17, 18], termites [19, 20], bees [21, 22], fruit flies [23], tsetse flies [11–13, 24], and others. The identification of CHCs by gas chromatography/mass spectrometry (GC/MS) allows the differentiation of four species of *Muscidifurax* by examining not only the gas chromatograms but also the mass spectra which clearly contain different compounds that demonstrate the sexual dimorphism in this species.

Experimental

The insects were collected from the following locations: *M. uniraptor* and *M. zaraptor* were taken from Nebraskan cattle feedlots and established as colonies in Nebraska in 1992, *M. raptor* was collected from a Florida dairy farm in 1994, and *M. raptorellus* (gregarious form) was obtained in 1995 from the USDA-ARS, Midwest Livestock Insects Laboratory, Lincoln, NE. Insects were dried and frozen prior to lipid extraction with hexane. Twenty insects of each sex and species (identified previously by morphological characters) were soaked in hexane for approximately 2 h. The hexane fraction was placed on a silica gel column; the first 3 mL was eluted off the column with additional hexane and collected [15]. Nonhydrocarbon lipids remained on the column and were discarded. The hydrocarbon-containing fraction was concentrated to dryness with a stream of nitrogen. Each sample was injected in duplicate. The first sample contained approximately 2–3 insect equivalents (an insect equivalent represents the amount of material collected from a hexane rinse of a single insect) in 1.0 μ L hexane, accomplished by reconstitution of the hydrocarbon residue with 10–15 μ L hexane. This provided a chromatogram without overloaded peaks, such that the Kovat's indices (KIs) values could accurately be determined. The second injection was performed after sample evaporation followed by reconstitution with 2.0–2.5 μ L hexane. A 1.0 μ L injection was made, equaling 8 to 10 insect equivalents. The second analysis gave quality mass spectra for trace level compounds although the column was overloaded for the larger peaks.

Mass spectra and chromatograms were obtained by a HP 5988A mass spectrometer interfaced to a HP 5890 series II GC (Hewlett-Packard, Atlanta, GA); the GC injection port was fitted with an OCI-3 cold on-column injector (SIS, Inc., Ringoes, NJ). The column was a 30 m $\times$ 0.32 mm i.d. DB-1 FSOT column (d_f = 0.25 μ m) (J&W Scientific, Folsom, CA) attached to a retention gap with a 5 m $\times$ 0.53 mm i.d. deactivated FSOT guard column (Restek, Bellefonte, PA). The column linear velocity was set to approximately 50 cm/s at 60 °C. The GC ramp consisted of a 2 min hold at 60 °C, a ramp at 10 °C/min to 220 °C, then 3 °C/min to 310 °C, and a hold at 310 °C to elute all components.

The ion source was operated in electron ionization (EI) mode with an electron energy of 70 eV and ion source temperature of 150 °C. The mass spectrometer

tune parameters were optimized with perfluorotributylamine (PFTBA) to enhance the m/z 200–500 region, containing the m/z 219 and m/z 502 ions, relative to the m/z 69 ion. The mass spectral scan range extended from m/z 50 to m/z 700 with a scan rate of 1.8 s per scan. Interpretation of the mass spectra followed previously established conventions [11–13, 25–27]. These references describe the interpretation process and location of methyl branched positions for natural hydrocarbons, synthesized de novo, for which there are no synthetic equivalents.

Full names of the compounds identified will not be used throughout this manuscript. Instead, a shorthand nomenclature will be used based on locations of methyl substitutions and the number of carbons in the backbone chain length, reported as (m_1 , m_2 , m_3 , m_4 , -Me $_y$ C $_x$) where m_1 – m_4 represent the locations of methyl branches on the carbon backbone chain (numbered by IUPAC convention), y represents the number of methyl branches, and x represents the number of carbons in the chain backbone. For example, 3,7,11,15-tetramethylpentatriacontane is written as 3,7,11,15-Me $_4$ C $_{35}$ with the understanding that there are a total of 39 carbons in this compound, 35 of which are in the backbone chain.

The use of retention indices or Kovat's indices provides a rapid means of identifying homologous series of branched methylalkanes. It should be noted that these four digit values are not true KIs because they were not obtained under isothermal GC conditions. Instead, they are calculated from interpolation between the peak retention times of a standard containing n -alkanes. The standard used for this work consisted of n -alkanes from C $_{12}$ –C $_{36}$, plus 15,19,23-Me $_3$ C $_{37}$ (KI 3770), at concentrations of 50–300 ng/ μ L each in hexane. The standard was analyzed prior to acquisition of data as a means of diagnosing instrument operation conditions as well as providing the template for calculating KI values. Homologous series of methylalkanes shift to lower KI values as the carbon backbone chain increases in length [28]. In this work, the reported values are adjusted slightly to maintain consistent values for each series of methyl substitutions. An example of the KI values used in this manuscript is the series of internally branched dimethylalkanes, such as the 13,17-dimethylalkanes. The earliest eluting 13,17-isomer occurs at KI 2955 (13,17-dimethylnonacosane). The convention used here represents similar 13,17-dimethylalkanes as KI XX55, where the first two digits change according to the carbon backbone chain length and the significance of the last two digits represents the methyl branch pattern. The series of internal dimethylalkanes continues at peaks labeled KI 3155, 3355, and 3555 having carbon backbone chain lengths of 31, 33, and 35 carbons, respectively.

Results and Discussion

The total ion chromatograms for the 4 female *Muscidifurax* spp. and 3 male spp. are presented in Figure 1a

and 1b, respectively. The KI values for the larger peaks are labeled on the chromatograms. The displayed chromatograms contain some overloading of major components and represent the analyses used to provide quality mass spectra of trace level hydrocarbons. Comparison of the sets of chromatograms reveals that each is distinct, and that the females contain more components than the males in each case.

Table 1 contains the listing of compounds identified in *Muscidifurax* species, from 29 to 39 carbon backbone chain lengths; however, there are minor components below C_{29} and above C_{39} . This table contains qualitative data only, i.e., the presence or absence of a compound. The approximate quantitative amounts and structures can be inferred from Figure 1 and Table 1, which show that the CHCs of females are similar and the CHCs of males resemble each other, but with some differences within the sexes.

The distinguishing characteristics for females are 4 sets of triple peaks at KI 3203/3210, 3237, 3260; 3403/3410, 3437, 3460; 3603, 3637, 3660; and 3803, 3837, 3860. Each set of peaks are homologous series of dimethylalkanes (XX03/XX10), trimethylalkanes (XX37), and tetramethylalkanes (XX60). A few of these are present in males, though at very low levels. *M. uniraptor* is a unisexual species, where all members are female. The female characteristics in this species are much weaker than the other females. Curiously, it contains characteristics of both sexes, although the characteristic male peaks are present at a weaker level. Similarity in characteristics is observed for females of *M. raptor*, *M. raptorellus*, and *M. uniraptor* below KI 3100 in the chromatograms, and between *M. raptorellus* males and *M. uniraptor* females above a retention time of KI 3600.

M. zaraptor females can readily be identified by the large KI 3203 and 3060 peaks found in the chromatograms. *M. raptor* have a large n - C_{31} alkane peak at 3100, a large 3-Me C_{31} at KI 3175, and large female characteristic peaks at 3437 and 3637. *M. raptorellus* females are characterized by a large KI 3637 trimethylalkane, followed by a KI 3437 peak. *M. uniraptor* contains a unique pattern compared to females, with KI 3100, 3175, and 3555–63 peaks as the most intense.

The males have up to 4 sets of triple peaks, some being homologous series, at KI 3135, 3175; 3335, 3355–63, 3375, 3380; 3535, 3555–63, 3580; and 3735, 3755–63; 3780, which tend to comprise internal mono- and dimethylalkane homologs, 3-methylalkanes, plus trimethylalkanes. These are less useful markers as females contain most of these, except for the XX80 trimethylalkanes. However, determining peak composition, especially for peaks in the KI XX55–63 range is difficult without GC/MS, or without long, narrow bore capillary columns. For example, the assignment of unseparated components from KI 3555 to 3563 is based solely on the results determined by EI GC/MS.

Males of *M. zaraptor* have internal monomethylalkanes at 3135 and 3-Me C_{31} at KI 3175 as the two most intense peaks. The largest peaks in *M. raptor* are KI 3100

and 3175, similar to *M. raptor* females, but devoid of the female characteristic peaks. *M. raptorellus* males are characterized by the KI 3563 dimethylalkane peak and 3580 internal 3/7-methylene interrupted trimethylalkane as the two largest peaks in the chromatogram.

Dimethylalkanes

Muscidifurax spp. females possess 11-methylene interrupted 3,15-dimethylalkanes (KI XX03) and structurally similar 3,X-isomers on odd carbon numbered backbone chains from C_{29} to C_{37} as well as less prevalent 4,16-dimethylalkanes (KI XX90) on even carbon backbone chains from C_{30} to C_{36} . Generally, insects produce hydrocarbons with odd methyl branch locations on odd carbon backbones (e.g., 3,15-Me $_2C_{33}$) and even branch locations on even backbones (e.g., 4,16-Me $_2C_{34}$). The occurrence of odd methyl branch locations on even backbones, although less common, still greatly outnumber the occasions where even branch locations are observed on an odd backbone chain. Exceptions do exist, such as 3,7-Me $_2C_{30}$ observed in *M. zaraptor* females at KI 3110.

Most methylene interruptions found in insect CHCs have three methylene units (e.g., 11,15- and 3,7-dimethylalkanes), and to a lesser extent, odd sequences greater than three units; however, a five methylene unit interruption is rare. Bridges with even numbers of methylene units, methyls on adjacent carbons in the backbone, or a dimethyl on a single carbon in the backbone are extremely rare and were not observed in these insects.

Reports in the literature of internally branched 11-methylene interrupted CHCs such as 11,23-Me $_2C_{35}$ are common [12, 13], but there are few reports of 3,15-dimethylalkanes, usually as a minor isomer within a peak containing other 3,X-isomers [14, 18, 29–32]. *Muscidifurax* spp. may be the first genus reported with a 3,15-isomer as a significant or sole 3,X-substituted dimethylalkane within the peak, rather than a predominant 3,7-dimethylalkane, as found typically at KI XX10 in other insects, such as *Blattella* cockroaches [16]. Additional 3,X-isomers with large methylene interruptions (greater than 3 methylene units) have been reported previously as 3,13- [14, 23, 30], 3,17- [14, 29], and 3,19- [33] dimethylalkanes. *Muscidifurax* spp. do contain these isomers, but at very low levels. Previously, isomers of 4,8-, 4,10-, 4,12-, and 4,14-dimethylalkanes have been reported [12, 26, 29, 34]. However, there is only one report of 4,16-dimethylalkanes, found in peaks containing 4,14- and 4,18-isomers [14]; it is not known whether the 4,16-isomer is the major component of this peak as is the case for *Muscidifurax*.

Figure 2 displays the EI mass spectra of 11-methylene interrupted 3,X- and 4,X-branched dimethylalkanes for backbone chain lengths from 33 to 35 carbons. In displaying these mass spectra, the mass-to-charge x axis is excluded below m/z 100. Generally, even/odd ion pairs at m/z 98/99, m/z 84/85, and sequentially lower

A

Figure 1. (Figure continued on next page)

B

Figure 1 (continued from previous page). Total ion chromatograms of pooled samples containing cuticular hydrocarbons extracted from 8 to 10 insects each of (a) female *Muscidifurax zaraptor*, *M. raptor*, *M. raptorellus*, and *M. uniraptor* and (b) males of *M. zaraptor*, *M. raptor*, and *M. raptorellus*. The largest peaks are labeled with retention indices. Homologous series are represented by KIs with the same last two digits.

are difficult to observe due to the increased low mass fragments characteristically produced from hydrocarbons. Detecting a m/z 56/57 ion pair, theoretically indicative of a 3,X-terminal fragment from the end with the 3-methyl branch, is virtually impossible to interpret and assign with certainty. Instead, the interpretation relies heavily on the remaining three observable secondary ion fragments. The expected fragments are listed on the structural representation included at the right side of each mass spectrum in the figure. All cleavages occur next to the tertiary carbon; the bars above and below the structure indicate which bond is

cleaved and the expected fragment ion mass produced from the cleavage. In addition to these expected secondary fragmentation ions, there are additional minor primary fragment ions observed [27]. The ion pairs at m/z 252/253 in Figure 2a of 3,15-Me₂C₃₃ and in Figure 2b of 4,16-Me₂C₃₄, as well as the m/z 280/281 pair in Figure 2c of 3,15-Me₂C₃₅ are primary ions produced from α -cleavage at the methyl branch adjacent to the terminal end that contains the larger chain.

Anomalous ions are sometimes generated by the fragmentation of large methylene interrupted hydrocarbons [12, 17, 35, 36]. In particular, an artifact is pro-

Table 1. Cuticular hydrocarbons identified in *Muscidifurax uniraptor*, *M. raptor*, *M. zaraptor*, and *M. raptorellus*^a

KI	Hydrocarbons		<i>M.</i> <i>uniraptor</i>	<i>M.</i> <i>raptor</i>	<i>M.</i> <i>raptor</i>	<i>M.</i> <i>zaraptor</i>	<i>M.</i> <i>zaraptor</i>	<i>M.</i> <i>raptorellus</i>	<i>M.</i> <i>raptorellus</i>
			♀	♀	♂	♀	♂	♀	♂
2900		<i>n</i> -C ₂₉	+	+	+	+	+	+	+
2935	(S)15-, 13-, 11-, 9-	MeC ₂₉	++++	---+	++++	---+	++++	t+++	++++
2940	7-	MeC ₂₉	+	+	+	+	+	+	+
2950	5-	MeC ₂₉	t	+	+		+		+
2955	(S)13,17-	Me ₂ C ₂₉							t
2975	3-	MeC ₂₉	+	+	+	+	+	+	+
3000		<i>n</i> -C ₃₀	+	+	+		t	+	+
3003	3,15-	Me ₂ C ₂₉				+			
3010	3,9-, 3,7-	Me ₂ C ₂₉	t+	t+	t+	t+	t+	—+	
3035	14-, 12-, 10-	MeC ₃₀	++t		+++		—t		—+—
3037	3,7,15-, 3,7,11-	Me ₃ C ₂₉		—t		+t	tt	+—	
3040	8-	MeC ₃₀	t		t		t		+
3045	6-	MeC ₃₀	+		t				
3060	3,7,11,15-	Me ₄ C ₂₉				+	+		
3065	4-	MeC ₃₀	+	+	t?	+	+	+	+
3070	6,10-	Me ₂ C ₃₀	+						
3075	3-	MeC ₃₀	+	+	+	+	+	+	+
3090	4,16-, 4,10-, 4,8-	Me ₃ C ₃₀	—t+	—+—		+—+	—+—	—t+	
3100		<i>n</i> -C ₃₁	+	+	+	+	+	+	+
3110	3,7-	Me ₂ C ₃₀				+			
3135	15-, 13-, 11-, 9-	MeC ₃₁	++++	++++	++++	++++	++++	++++	++++
3140	7-	MeC ₃₁	+	+	+	+	+	+	+
3150	5-	MeC ₃₁	+	+	+	+	+	+	+
3155	13,17-, 11,15-, 9,13-	Me ₂ C ₃₁	++—		—t+	—+—	tt+	+t—	+++
3163	7,11-	Me ₂ C ₃₁	+		+	+	+	+	+
3163	(S)11,21-, 9,19-	Me ₂ C ₃₁	++						
3175	3-	MeC ₃₁	+	+	+	+	+	+	+
3183	5,9-	Me ₂ C ₃₁	+	+		+	+		
3200		<i>n</i> -C ₃₂	+	+	+		+		+
3203	3,19-, 3,17-, 3,15-	Me ₂ C ₃₁		—++	+++	—+—	—+—		
3210	3,11-, 3,9-, 3,7-	Me ₂ C ₃₁	—t+	—t+	—t+	tt+	t—+	+++	—t+
3235	16-, 14-, 12-, 10-	MeC ₃₂	++++		—+—		—t—		—+++
3237	3,7,15-, 3,7,11-	Me ₃ C ₃₁	—+	—+	—+	++	++	++	
3240	8-	MeC ₃₂	t						
3245	6-	MeC ₃₂	+	+					
3260	8,12-	Me ₂ C ₃₂	+						
3260	3,7,11,15-	Me ₄ C ₃₁				+	t		
3265	10,22-, 9,21-, 8,20-	Me ₂ C ₃₂							+++
3265	4-	MeC ₃₂	+	+	t			t	
3270	6,10-	Me ₂ C ₃₂	+						
3275	3-	MeC ₃₂		t	+				
3290	4,16-, 4,8-	Me ₂ C ₃₂	—+	++		++	t+	—+	
3300		<i>n</i> -C ₃₃	+	+	+	+		+	
3320	4,8,16-, 4,8,12-	Me ₃ C ₃₂	—+	++		—+		++	
3335	(S)17-, 15-, 13-, 11-, 9-	MeC ₃₃	+++++	+++++	+++++	+++++	+++++	++++—	++++—
3340	7-	MeC ₃₃	+	+	+	+			
3350	5-	MeC ₃₃	+	+	+				
3355	(S)15,19-, 13,17-, 11,15-, 9,13-	Me ₂ C ₃₃	++++	++++	++++	++++	++++	++++	—+—+
3363	7,11-	Me ₂ C ₃₃	+		+		+		
3363	11,21-, (S)11,23-, 9,21-	Me ₂ C ₃₃	+++		+++	+—	+—	+—	++t
3375	3-	MeC ₃₃	+	+	+	+	+	+	+
3380	11,15,23-, 9,13,21-, 9,13,23-	Me ₃ C ₃₃	—+t		—t—	tt—	t+—	t—	++—
3383	5,9-	Me ₂ C ₃₃		+		+			
3385	7,11,15-	Me ₃ C ₃₃	t?					t?	
3400		<i>n</i> -C ₃₄					t		
3403	3,15-, 3,13-	Me ₂ C ₃₃	++	+t	+—	+t	+—	+—	
3410	3,9-, 3,7-	Me ₂ C ₃₃	t+	++	t+	t+		t+	
3435	17-, 16-, 14-, 12-	MeC ₃₄	+++—		—+—				—+—+
3437	3,7,15-, 3,7,13-, 3,7,11-	Me ₃ C ₃₃	tt+	+t—	+—	+—	t—	+—	+—t
3440	8-	MeC ₃₄	+						
3455	15,19-, 14,18-, 13,17-, 12,16-	Me ₂ C ₃₄	t+—	—+—	—t—			—tt	

(Continued)

Table 1. (continued from previous page) Cuticular hydrocarbons identified in *Muscidifurax uniraptor*, *M. raptor*, *M. zaraptor*, and *M. raptorellus*

KI	Hydrocarbons		<i>M.</i> <i>uniraptor</i>	<i>M.</i> <i>raptor</i>	<i>M.</i> <i>raptor</i>	<i>M.</i> <i>zaraptor</i>	<i>M.</i> <i>zaraptor</i>	<i>M.</i> <i>raptorellus</i>	<i>M.</i> <i>raptorellus</i>
			♀	♀	♂	♀	♂	♀	♂
3460	8,12-	Me ₂ C ₃₄	t						
3460	3,7,11,15-	Me ₄ C ₃₃	+	+		+		+	
3465	12,22-, 11,21-	Me ₂ C ₃₄							++
3465	4-	MeC ₃₄		+					
3470	6,16-, 6,14-, 6,10-	Me ₃ C ₃₄	-t+	+++					
3490	4,16-, 4,14-, 4,8-	Me ₃ C ₃₄	+++	+++		t--		t-+	
3495	10,14,22-, 6,10,14-	Me ₃ C ₃₄	-+						+-
3520	4,8,16-, 4,8,14-, 4,8,12-	Me ₃ C ₃₄	-t+	+-				+-+	
3535	17-, 15-, 13-, 11-, 9-	MeC ₃₅	+++++	+++++	+++++	+++++	+++++	+++++	+++++
3540	7-	MeC ₃₅		+					
3555	15,19-, 13,17-, 11,15-	Me ₂ C ₃₅	++t	++t	+++	+++	+++	+++	
3563	(S)13,23-, 11,21-, 9,19-	Me ₂ C ₃₅		+++	++-			++-	++-
3563	11,23-, 9,21-	Me ₂ C ₃₅	++						+-
3570	7,21-, 7,19-, 7,15-	Me ₂ C ₃₅	-+-		+-	--t			
3575	3-	MeC ₃₅		+	+				
3580	11,15,25-, 11,15,23-, 11,19,23-, 11,15,19-	Me ₃ C ₃₅	-+--	-+--	-+--	-+--	-+--	t?++t	-+--
3583	5,21-, 5,19-, 5,17-	Me ₂ C ₃₅		ttt					
3603	3,15-	Me ₂ C ₃₅	+	+	+	+	+	+	
3610	3,9-, 3,7-	Me ₂ C ₃₅		++				-+	
3635	16-	MeC ₃₆	+						
3637	3,7,15-, 3,7,13-	Me ₃ C ₃₅	+t	+-	+-	+-	+-	+-	+-
3655	16,20-, 15,19-, 14,18-	Me ₂ C ₃₆		t++				-++	
3660	3,7,11,15-	Me ₄ C ₃₅	+	+		+			
3665	13,23-, 12,22-	Me ₂ C ₃₆							++
3690	4,16-	Me ₂ C ₃₆		+					
3720	4,8,16-	Me ₃ C ₃₆		+				+	
3735	(S)19-, 17-, 15-, 13-, 11-, 9-	MeC ₃₇	+++++	+++++	+++++	+++++	+++++	+++++	+++++
3755	(S)17,21-, 15,19-, 13,17-, 11,15-	Me ₂ C ₃₇	++++	++++	++t-	-tt-	++++	++t-	++++
3763	(S)13,25-, 11,23-	Me ₂ C ₃₇	++					++	++
3780	(S)15,19,23-, 13,17,21-, 11,15,19-	Me ₃ C ₃₇		+t+				+-+	
3780	13,17,25-, 11,15,23-, 11,19,23-	Me ₃ C ₃₇	+++						+++
3803	3,19-, 3,17-, 3,15-	Me ₂ C ₃₇		+t+		--+		--t	
3837	3,7,15-	Me ₃ C ₃₇		+		+		+	
3855	15,19-	Me ₂ C ₃₈						+	
3860	3,7,11,15-	Me ₄ C ₃₇				t			
3955	17,21-, 15,19-, 13,17-, 11,15-	Me ₂ C ₃₉	+-	+++				+++	+++
3963	13,25-, 15,25-, 13,23-	Me ₂ C ₃₉	+-						-tt
3980	13,17,25-, 13,21,25-, 15,19,23-, 13,17,21-	Me ₃ C ₃₉	-+--					+++t	

^a Key: ((S)) symmetrical, (+) indicates that this isomer was observed, (-) indicates that this isomer was not observed (t) trace amount of component, (?) some uncertainty about the identity, KI adjusted to produce uniform indices for C₃₅ and above, (♀) denotes female and (♂) denotes male.

duced at m/z 209 in 11-methylene interrupted hydrocarbons. This ion has been observed previously in published mass spectra [35, 36]. Normally, an ion pair would be expected at m/z 210/211 as the primary ion, having weaker intensity than the corresponding secondary ion at m/z 239; however, the observed ion is 1–2 Da lower than expected. It is thought to be produced from α -cleavage exterior to the methyl branches on each end of the 11-methylene sequence rather than being produced from the m/z 211 primary ion. This reasoning is based on the observation of the mass-to-charge values for anomalous ions produced from other large methylene interrupted methylalkanes [12, 17]. The resulting

fragment from this process may be a distonic radical cation, providing a favorable pathway for H₂ elimination to produce an alkene ion, observed at m/z 209. It is not known whether the charge is retained on the end where the shorter or longer chain was cleaved.

The males of this genus, as well as *M. uniraptor* females, also have dimethylalkanes with 11-methylene unit interruptions; however, these branches are more symmetrical (internal). Figure 3 displays two such isomers, eluting at the same KI, found in *M. uniraptor*. The major component is the 11,23-dimethylheptatriacontane (11,23-Me₂C₃₇) isomer. The minor component of this peak is the symmetrical 13,25-Me₂C₃₇.

Figure 2. Mass spectra of external dimethylalkanes with 11-methylene interrupted sequences between methyl branches. These compounds are characteristic dimethylalkanes from the cuticle of *Muscidifurax* females. This mass spectra shown are: (a) 3,15-Me₂C₃₃ from the KI 3403 peak of *M. raptor*, (b) 4,16-Me₂C₃₄ from the KI 3490 peak and of *M. raptor*, and (c) 3,15-Me₂C₃₅ from the KI 3603 peak of *M. raptor*.

Trimethylalkanes

Females of *Muscidifurax*, with the exception of *M. uniraptor*, have large 3/7-methylene interrupted 3,7,15-trimethylalkanes. The pattern demonstrates retention of

methyl branches at carbons 3 and 15 from the dimethylalkane, with the addition of a methyl branch 3-methylene units inside the external 3-position to produce the trimethylalkane. If this were a random process, the presence of 7/3-methylene sequenced 3,11,15-trimeth-

Figure 3. Mass spectrum of internal dimethylalkanes with 11-methylene interrupted sequences between methyl branches. These compounds are characteristic dimethylalkanes from the cuticle of *Muscidifurax* males and *M. uniraptor* females. The mass spectrum shown is from the KI 3763 peak of *M. uniraptor* containing a mixture of 13,25-Me₂C₃₇ and 11,23-Me₂C₃₇.

ylalkanes would be expected; however, isomers with this branching pattern were not observed in these insects.

The 3/7-methylene interrupted examples presented here are 4,8,16-Me₃C₃₄ (Figure 4a), 3,7,15-Me₃C₃₅ (Figure 4b), and 4,8,16-Me₃C₃₆ (Figure 4c). These examples are drawn from *M. raptor* and *M. raptorellus* females. Even-backboned analogs are generally less prevalent than the odd-backboned species, but usually follow a similar methylene interruption pattern. Because of the lower abundance of even-backboned species typically observed in insect CHCs, mass spectra of these even analogs are reported less often. Insects of the genus *Muscidifurax* are one of the two genera reported to-date which have a clearly definable 3,7,15-trimethylalkane series as well as corresponding 4,8,16-trimethylalkanes on even carbon backbone chains. The only other insects reported to produce a 3,7,15-trimethylalkane are *lps* pine engraver beetles [14]. Although not presented in these figures, 3,7,11- and 4,8,12-trimethylalkanes were observed in *Muscidifurax*; however, these are present at low levels and have been found in several other insects [12, 26, 29, 37].

The males also contain 3/7-methylene interrupted trimethylalkanes, characteristic of this genus, but there are two important factors that differentiate them from the females. Males possess internally branched trimethylalkanes rather than an external 3,7,15-isomer as found in the females. For example, chromatograms of male *M. raptorellus* have a peak at KI 3380 containing two isomers: 11,15,23-Me₃C₃₃ and 9,13,21-Me₃C₃₃ (Figure 5a).

Secondly, multiple 3/7-methylene interrupted isomers of comparable abundance may be found under one peak. There are some exceptions where males contain only one isomer, such as for the KI 3580 peak in *M. zaraptor* males (Figure 5b), just as there are cases where females may contain trace amounts of other

3/X-methylene interrupted isomers in addition to the 3,7,15-trimethylalkane. The KI 3780 peak in *M. raptorellus* males consists of three isomers (Figure 5c). In this case, a third isomer of weaker intensity has a 7/3-methylene interruption pattern. This pattern was not observed in female trimethylalkanes, but has been observed in the CHCs and cuticular alcohols of other insects as a 5,13,17-methyl branched isomer [26, 36]. Males do not have even-backbone analogs of these 3/7-methylene interrupted internal methyl-branched alkanes except for 10,14,22-Me₃C₃₄ found at KI 3495 in *M. raptorellus*. Trimethylalkanes with 3/3-methylene interruptions, such as 13,17,21- and 11,15,19-isomers in *Muscidifurax*, are very common in insects.

Tetramethylalkanes

The final pair of mass spectra (Figure 6) clearly show the presence of tetramethylalkanes in *Muscidifurax* females. The 3,7,11,15-Me₄C₃₃ of *M. raptor* (KI 3460) (Figure 6a) and 3,7,11,15-Me₄C₃₃ of *M. zaraptor* (KI 3660) (Figure 6b) are presented as excellent examples of the unmistakable mass spectra obtained. Observations of tetramethylalkanes in insects are rare, and when present, these hydrocarbons are typically of low abundance. Female *M. zaraptor* contain the largest amounts of tetramethylalkanes, starting with a large peak at KI 3060 and ending at KI 3860. These represent tetramethylalkanes on carbon backbone chains of C₂₉ to C₃₇. Only two other insects have been reported to contain 3,7,11,15-tetramethylalkanes; these are the tsetse fly *Glossina brevipalpis* [13] and the pine engraver beetle *lps paraconfusus* [14].

An artifact is observed in the mass spectra displayed in Figure 6b. Usually, the even mass of an even/odd pair of fragments is indicative of a terminal hydrocarbon fragment. The *m/z* 266/267 ion pair results from α -cleavage external to the methyl branch at carbon 15 in

Figure 4. Mass spectra of external trimethylalkanes with 3/7-methylene interrupted sequences between methyl branches. These compounds are characteristic trimethylalkanes from the cuticle of *Muscidifurax* females. The mass spectra shown are: (a) 4,8,16-Me₃C₃₄ from the KI 3520 peak of *M. raptor*, (b) 3,7,15-Me₃C₃₅ from the KI 3637 peak of *M. raptor*, and (c) 4,8,16-Me₃C₃₆ from the KI 3720 peak of *M. raptorellus*.

3,7,11,15-Me₄C₃₅ and therefore, is expected to produce a m/z 267 ion of greater intensity than m/z 266. This artifact is also observed in the mass spectrum of the *lps* pine engraver beetle for a homologous tetramethylalkane [14]. Therefore, this anomaly is most likely consistent in the mass spectrum of this compound and is not produced by the mass spectrometer or conditions of the analysis. Tetramethylalkanes were not detected in the males.

Conclusions

Both sexes of *Muscidifurax* contain prominent CHCs with rare methylene interrupted patterns. Sexual dimorphism is clearly seen by GC/MS. Female CHCs (except for those of *M. uniraptor*) are predominated by external 11-methylene interrupted 3,15-dimethylalkanes and 3/7-methylene interrupted 3,7,15-trimethylalkanes, culminating in 3/3/3-methylene interrupted

Figure 5. Mass spectra of internal trimethylalkanes with 3/7-methylene interrupted sequences between methyl branches. These compounds are characteristic trimethylalkanes from the cuticle of *Muscidifurax* males. The mass spectra shown are: (a) a mixture of 11,15,23-Me₃C₃₃ and 9,13,21-Me₃C₃₃ from the KI 3380 peak of *M. raptorellus*, (b) 11,15,23-Me₃C₃₅ from the KI 3580 peak of *M. zaraptor*, and (c) a mixture of 13,17,25-Me₃C₃₇, 11,15,23-Me₃C₃₇, and a rare 7/3-methylene sequenced 11,19,23-Me₃C₃₇ from the KI 3780 peak of *M. raptorellus*.

3,7,11,15-tetramethylalkanes, all with odd carbon chain backbones. Some of the even backbone analogs of these can be found in the females, such as 4,16-dimethylalkanes and 4,8,16-trimethylalkanes; however, evidence was not found for an even backbone tetramethylalkane. Male CHCs have internal 11-methylene interrupted 11,23-dimethylalkanes, and 3/7-methylene

interrupted 11,15,23-trimethylalkanes, but no tetramethylalkanes are observed in the males.

M. uniraptor is the most difficult member of the genus to identify morphologically and is thought to have originated when populations of a related species, perhaps *M. raptorellus*, acquired a *Wohlbachia* bacterial symbiont that produces the unisexual trait [38, 39].

Figure 6. Mass spectra of external tetramethylalkanes observed in females of *Muscidifurax*. The mass spectra shown are: (a) 3,7,11,15-Me₄C₃₃ from the KI 3460 peak of *M. raptor* and (b) 3,7,11,15-Me₄C₃₅ from the KI 3660 peak of *M. zaraptor*.

Some characters or components link *M. uniraptor* to the females, whereas other characters link this species to males, in particular *M. raptorellus* males. For example, the composition of internal trimethylalkanes are identical at KI 3780 in *M. uniraptor* females and *M. raptorellus* males. It is possible that, given the absence of males in this species, selection has been relaxed for the presence of the full compliment of hydrocarbon components involved in mate recognition. If this is the case, *M. uniraptor* females may be in transition back towards the simpler CHC pattern found in males of the other species.

The mass spectral data from females indicate that the biochemical processes leading to production of CHCs are most similar in *M. raptor* and *M. zaraptor*, while *M. raptorellus* appear more distantly related; *M. uniraptor* are least similar but appear to be related more to *M. raptorellus* than *M. raptor* or *M. zaraptor*. This tends to agree with the results of mitochondrial DNA [8]. Neither the diet of parasitoids nor seasonal effects are expected to significantly alter the observed CHCs. It is believed that the production of CHCs is genetically based. Using characteristics and quantitation from GC for speciation of single females as well as examining patterns from various collection locations is addressed

more closely with the analysis of single *Muscidifurax* by Geden et al. [9]. Care should be taken, however, in basing phylogenetic classification solely on compounds that are observed.

This work may ultimately prove most useful in the examination of hydrocarbons from larvae, where an assessment of colony species contamination can be made prior to eclosion of adult wasps. Chemotaxonomic characters derived solely from GC peak areas can also be used to produce a key to examine parasitic wasps for accidental mixing of species among colonies [9].

Acknowledgments

The authors thank M. Y. Hosack for sample preparation, J. J. Petersen for supplying *M. raptorellus*, and S. Penn for supplying *M. zaraptor*. The authors also thank C. C. Grimm, M. M. Booth, and J. A. Hogsette for critically reviewing this manuscript.

References

- Geden, C. J.; Steinkraus, D. C.; Miller, R. W.; Rutz, D. A. *Environ. Entomol.* **1992**, 21, 1419–1426.
- Petersen, J. J.; Cawthra, J. K. *Biol. Control* **1995**, 5, 279–84.

3. Rueda, L. M.; Axtell, R. C. N. C. *Agric. Res. Serv. Tech. Bull.* **1985**, 278, 1–88.
4. Kogan, M.; Legner, E. F. *Can. Entomol.* **1970**, 102, 1268–90.
5. Kawooya, J. K. Ph.D. Dissertation, University of Illinois at Champaign-Urbana, 1983.
6. Propp, G. D. *Misc. Publ. Entomol. Soc. Am.* **1986**, 61, 164–174.
7. Antolin, M. F.; Guertin, D. S.; Petersen, J. J. *Biol. Control* **1996**, 6, 76–82.
8. Taylor, D. B.; Peterson, R. D.; Szalanski, A. L.; Petersen, J. J. *Ann. Entomol. Soc. Am.* **1997**, 90, 814–824.
9. Geden, C. J.; Bernier, U. R.; Carlson, D. A.; Sutton, B. D. *Biol. Control*, in press.
10. Carlson, D. A.; Mayer, M. S.; Silhacek, D. L.; James, J. D.; Beroza, M.; Bierl, B. A. *Science* **1971**, 174, 76–78.
11. Carlson, D. A.; Nelson, D. R.; Langley, P. A.; Coates, T. W.; Davis, T. L.; Leegwater-van der Linden, M. J. *Chem. Ecol.* **1984**, 10, 429–450.
12. Nelson, D. R.; Carlson, D. A. *Insect Biochem.* **1986**, 16, 403–416.
13. Nelson, D. R.; Carlson, D. A.; Fatland, C. L. *J. Chem. Ecol.* **1988**, 14, 963–987.
14. Page, M.; Nelson, L. J.; Blomquist, G. J.; Seybold, S. J. *J. Chem. Ecol.* **1997**, 23, 1053–99.
15. Carlson, D. A.; Service, M. W. *Science* **1980**, 207, 1089–1091.
16. Brenner, R. J.; Carlson, D. A.; Roth, L. M.; Patterson, R. S. *Invert. Taxon.* **1993**, 7, 1205–1219.
17. Nelson, D. R.; Nunn, N. J.; Jackson, L. L. *Insect Biochem.* **1984**, 6, 677–683.
18. Sutton, B. D.; Carlson, D. A.; Lockwood, J. A.; Nunamaker, R. A. *J. Orthop. Res.* **1996**, 5, 1–12.
19. Takahashi, S.; Grassa, A. J. *Chem. Ecol.* **1985**, 21, 1837–45.
20. Haverty, M. I.; Collins, M. C.; Nelson, L. J.; Thorne, B. L. *J. Chem. Ecol.* **1997**, 23, 927–965.
21. Lavine, B.; Carlson, D. A. *Anal. Chem.* **1987**, 59, 469A–470A.
22. Lavine, B.; Carlson, D. A.; Henry, D.; Jurs, P. C. *J. Chemometrics* **1988**, 2, 29–37.
23. Sutton, B. D.; Carlson, D. A. *Arch. Insect Biochem Physiol.* **1993**, 23, 53–65.
24. Carlson, D. A.; Milstrey, S. K.; Narang, S. K. *Bull. Entomol. Soc.* **1993**, 83, 507–515.
25. Nelson, D. R.; Sukkestad, D. R. *Biochemistry* **1970**, 9, 4601–4611.
26. Nelson, D. R.; Fatland, C. L.; Howard, R. W.; McDaniel, C. A.; Blomquist, G. J. *Insect Biochem.* **1980**, 10, 409–418.
27. Nelson, D. R.; Sukkestad, D. R.; Zaylskie, R. G. *J. Lipid Res.* **1972**, 13, 413–421.
28. Kissin, Y. V.; Feulmer, G. P. *J. Chromatogr. Sci.* **1986**, 24, 53–59.
29. Lockey, K. H. *Insect Biochem.* **1984**, 14, 645–656.
30. Lockey, K. H. *Insect Biochem.* **1984**, 14, 65–75.
31. Lockey, K. H. *Comp. Biochem. Physiol.* **1985**, 80B, 633–640.
32. Yoder, J. A.; Blomquist, G. J.; Denlinger, D. L. *Arch. Insect Biochem. Physiol.* **1995**, 28, 377–385.
33. Kruger, E. L.; Pappas, C. D.; Howard, R. W. *J. Med. Entomol.* **1991**, 28, 859–864.
34. Nelson, D. R.; Dillwith, J. W.; Blomquist, G. J. *Insect Biochem.* **1981**, 11, 187–197.
35. Nelson, D. R.; Fatland, C. L. *Insect Biochem. Mol. Biol.* **1992**, 22, 111–123.
36. Nelson, D. R.; de Renobales, M.; Dwyer, L. A.; Zamboni, A. C.; Fatland, C. L.; Blomquist, G. J. *Insect Biochem.* **1989**, 19, 197–208.
37. Martin, M. M.; MacConnell, J. G. *Tetrahedron* **1970**, 26, 307–319.
38. Stouhammer, R. S.; Breeuwer, J. A. J.; Luck, R. F.; Werren, J. H. *Nature* **1993**, 361, 66–68.
39. Stouhammer, R. S.; Luko, S.; Mak, F. *Norwegian J. Agric. Sci.* **1994**, 16, 117–122.