Cuticular Hydrocarbons of Anastrepha obliqua (Diptera: Tephritidae) as Influenced by Extraction Method, Natal Host, and Age

Authors: Caravantes-Villatoro, Luis Alexis, Cruz-Esteban, Samuel, and Rojas, Julio C.

Source: Florida Entomologist, 104(4) : 289-296

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.104.0406

Cuticular hydrocarbons of *Anastrepha obliqua* (Diptera: Tephritidae) as influenced by extraction method, natal host, and age

Luis Alexis Caravantes-Villatoro[1], Samuel Cruz-Esteban[2,3], and Julio C. Rojas[1,]*

Abstract

The primary function of cuticular hydrocarbons is to keep insects from losing water. However, cuticular hydrocarbons also may mediate chemical communication in a number of species. In this study, we investigated the effect of the extraction method, natal host, and age (maturation) on the cuticular hydrocarbon profiles of the West Indian fruit fly, *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae). Cuticular hydrocarbons from female and male adults of different natal hosts (*Mangifera indica* L. or *Spondias mombin* L. [both Anacardiaceae]) and age were extracted by solvent extraction and direct contact solid-phase microextraction. Cuticular hydrocarbons were identified by gas chromatography coupled to mass spectrometry. In total, we recorded 12 compounds, but only 9 of them were identified. The identified cuticular hydrocarbons were linear alkanes (n-heneicosane, n-nonacosane, and n-hentriacontane), alkenes (n-heneicosene, n-tricosene, n-nonacosene, and n-hentriacontene), and branched alkanes (2-methyl-octacosane and 2-methyl-triacontane). There were no qualitative differences between sampling techniques. The solvent extraction method extracted more cuticular hydrocarbons from flies reared on mango compared to those extracted from flies reared on hog plum. In contrast, solid-phase microextraction extracted a higher concentration of cuticular hydrocarbons from flies reared on hog plum than to those extracted from flies reared on mango. Higher levels of 2-methyl-octacosane and 2-methyl-triacontane were detected with solvent extraction than with solid-phase microextraction; the opposite occurred with hentriacontane and unknown compound 2. The compounds n-heneicosene, n-heneicosane, and n-tricosene were present in mature males but not in mature females; n-nonacosene was found only in the mature flies of both sexes.

Key Words: West Indian fruit fly; *Spondias mombin*; *Mangifera indica*; solvent extraction; solid-phase microextraction; gas chromatography-mass spectrometry

Resumen

La función primaria de los hidrocarburos cuticulares es evitar que los insectos pierdan agua. Sin embargo, los hidrocarburos cuticulares también pueden mediar la comunicación química de diversas especies. En este estudio investigamos el efecto del método de extracción, fruto hospedero y la edad (madurez) sobre el perfil de los hidrocarburos cuticulares de la mosca de la fruta de las Indias Occidentales, *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae). Los hidrocarburos cuticulares de machos y hembras de diferente hospedero (*Manguifera indica* L. or *Spondias mombin* L. [ambos Anacardiaceae]) y edad fueron extraídos con disolvente y con microextracción en fase solida. Los hidrocarburos cuticulares fueron identificados por cromatografía de gases acoplada a espectrometría de masas. En total, 12 compuestos fueron encontrados, pero solo 9 fueron identificados. Los hidrocarburos cuticulares identificados fueron alcanos lineales (n-heneicosano, n-nonacosano, y n-hentriacontano), alquenos (n-heneicoseno, n-tricoseno, n-nonacoseno, y n-hentriaconteno) y alcanos ramificados (2-methyl-octacosano y 2-metil-triacontano). No se encontraron diferencias cualitativas entre las técnicas de muestreo. El método de extracción con solvente extrajo más hidrocarburos cuticulares de moscas criadas en mango en comparación con las extraídas de moscas criadas en jocote. Por el contrario, microextracción en fase solida extrajo una mayor concentración de hidrocarburos cuticulares de moscas criadas en jocote que de las extraídas de moscas criadas en mango. Los mayores niveles de 2-methyl-octacosano y 2-metil-triacontano fueron detectados con la extracción con disolvente que con microextracción en fase solida; lo opuesto ocurrió con el n-hentriaconteno y el compuesto 2 no identificado. Los compuestos n-heneicoseno, n-heneicosano, y n-tricoseno fueron detectados en machos maduros, pero no en hembras maduras; n-nonacoseno fue encontrado solamente en moscas maduras de ambos sexos.

Palabras Clave: mosca de la fruta de las Indias Occidentales; *Spondias mombin*; *Mangifera indica*; extracción con disolvente; microextracción en fase salida; cromatografía de gases-espectrometría de masas

Insect cuticular lipids consist of hydrocarbons, fatty acids, alcohols, esters, aldehydes, and ketones (Blomquist et al. 1987). Among cuticular lipids, hydrocarbons have received considerable attention because they are the most abundant compounds on the cuticular surface of insects, they are easy to extract, and there are standardized techniques to study them (Nation 2002). Cuticular hydrocarbons usually are found as complex mixtures of a saturated linear chain (n-alkanes), unsaturated (n-alkenes), and methyl branched (r-alkanes) components. In

[1]Departamento de Agricultura, Sociedad y Ambiente, Grupo de Ecología de Artrópodos y Manejo de Plagas, El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico; Email: luis.caravantes@estudianteposgrado.ecosur.ms (L. A. C. V.); jrojas@ecosur.mx (J. C. R.)
[2]Instituto de Ecología, A.C. Red de Diversidad Biológica del Occidente Mexicano, Avenida Lázaro Cárdenas 253, 61600 Pátzcuaro, Michoacán, Mexico; Email: samuel.cruz@inecol.mx (S. C. E.)
[3]CONACYT, Avenida Insurgentes Sur 1582, 03940, Ciudad de Mexico, Mexico
*Corresponding author; Email: jrojas@ecosur.mx

insects, a primary function of cuticular hydrocarbons is to reduce the transpiration of water through the cuticle (Gibbs 1998), but they also have toxic or inhibitory effects on entomopathogens (Crespo et al. 2000; Ortiz-Urquiza & Keyhani 2013). Additionally, in some species, cuticular hydrocarbons serve as recognition signals between individuals from the same species or different species, including mimicry, reproductive division of labor in social insects, and courtship (Howard 1993; Blomquist & Bagnères 2010; Chung & Carroll 2015). Cuticular hydrocarbons have been studied principally in insects from Diptera, Hymenoptera, and Coleoptera (Blomquist & Bagnères 2010). For instance, studies have identified the cuticular hydrocarbons of larvae and adults from tephritid fruit flies, including species of the genera *Ceratitis*, *Bactrocera*, and *Anastrepha* (Carlson & Yocom 1986; Sutton & Carlson 1993; Vaníčková et al. 2014, 2015, 2017; Bosa et al. 2018). The principal objective of these studies was to identify the cuticular hydrocarbons as a tool for chemotaxonomical discrimination against the cryptic species (Carlson & Yocom 1986; Vaníčková et al. 2014, 2015, 2017). In contrast, relatively few studies have investigated the dynamic of these compounds in relation to abiotic and biotic factors (Sutton & Carlson 1993; Vaníčková et al. 2012; Bosa et al. 2018).

The West Indian fruit fly, *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae), is a Neotropical species distributed from Mexico to South America (Hernández-Ortiz 2007). This species is a generalist insect because it feeds on tropical fruits from different families, including species of economic importance such as mango (*Mangifera indica* L.), mombin (*Spondias* spp.) (both Anacardiaceae), carambola (*Averrhoa carambola* L.; Oxalidaceae), and occasionally guava (*Psidium guajava* L.; Myrtaceae) (Norrbom & Kim 1988; Toledo & Lara 1996; Birke et al. 2013). Under laboratory conditions, copulation occurs from 7 to 10 h when adults reach sexual maturity (7–8 d old). Females oviposit their eggs in the epidermis or mesocarp area of fruits. After hatching, larvae feed on the fruit pulp (Birke et al. 2013).

Here, we investigated whether cuticular hydrocarbon profiles are influenced by the extraction method, natal host, and age of *A. obliqua*. We sampled the cuticular hydrocarbons using solvent extraction and solid-phase microextraction; we identified cuticular hydrocarbons with gas chromatography coupled with mass spectrometry. A previous study has identified the cuticular hydrocarbons of *A. obliqua* larvae (Sutton & Carlson 1993).

Materials and Methods

BIOLOGICAL MATERIAL

Infested fruits of mango (*M. indica* cv. 'Coche') and hog plum (*Spondias mombin* L.) were collected in the municipality of Tapachula (14.8577778°N, 92.2827778°W), Chiapas, Mexico. Mangoes and hog plums were collected in May and Sep, respectively. Fruits were transported to the laboratory and maintained at 26 to 27 °C, 75 ± 5% RH, and a photoperiod regimen of 12:12 h (L:D). When larvae reached the third-instar, they were deposited in plastic containers (8 cm H × 11 cm diam) with humid vermiculite as a substrate for pupation. About 2 d before the adult emergence, the pupae were separated from the vermiculite with a sieve (mesh 18) and were placed inside plexiglass cages (30 × 30 × 30 cm). Upon adult emergence, flies were separated by sex and kept at 26 to 27 °C, 75 ± 5% RH, and a photoperiod regimen of 12:12 h (L:D). Flies were fed ad libitum with a mixture of enzymatic yeast hydrolysate (MP Biomedical, Irvine, California, USA), sucrose (1:3), and water provided in tubes covered with cotton wicks. Female and male adults were separated according to their natal host plant (mango or hog plum), and age (immature or mature) for the experiment. Immature flies were 5 d old, while mature females were 17 d old. About 10 flies from each treatment were placed in glass vials (8.5 cm H × 2 cm diam). Flies were killed by freezing and maintained at −20 °C until the extraction of their cuticular hydrocarbons.

CUTICULAR HYDROCARBON EXTRACTION

Cuticular hydrocarbons from the different treatments were extracted by 2 different sampling techniques. In the first technique, the cuticular hydrocarbons were sampled using solid-phase microextraction. A 100-µm polydimethylxylosane fiber (Supelco, Toluca, Mexico) was rubbed softly for 40 s on the principal parts of the fly body (head, thorax, wings, and abdomen). The fiber was first conditioned for 3 min at 250 °C in the injector of a gas chromatograph. The samples were desorbed for 1 min in the gas chromatograph injector for analysis. In total, 7 replicates were performed, with an individual considered as a replicate. In the second technique, cuticular hydrocarbons were sampled with solvent extraction. For each treatment, 3 flies were immersed in hexane for 5 min. Preliminary trials showed that using 1 or 2 flies was insufficient to extract the requisite amounts of cuticular hydrocarbons. The hexane extracts were concentrated at 100 µL under a gentle stream of nitrogen. One µL of these extracts were analyzed by gas chromatograph-mass spectrometer. The concentrated extracts were stored at −20 °C until analysis. In total, 6 replicates were performed for each treatment.

CHEMICAL ANALYSIS

Cuticular hydrocarbons were analyzed using a Varian CP-3800 gas chromatograph coupled with a Varian Saturn 2200 mass spectrometer fitted with a Varian VF-5MS (30 m L × 0.25 mm ID) fused silica capillary column (Varian, Palo Alto, California, USA). All samples were analyzed in the splitless mode with the injector port at 250 °C. Helium was used as a carrier gas at a constant flow of 1 mL per min. The oven temperature was programmed at 150 °C for 2 min, increased at 10 °C per min to 300 °C, and maintained at this temperature for 12 min. The samples were ionized by electronic impact at 70 eV. The detected compounds were identified based on diagnostic ions and retention indices. To obtain the retention indices, an aliquot (1 µL) of an alkane standard solution (C12–C26) and synthetic standards (C28, C30, C32) were injected on the gas chromatograph-mass spectrometer. The relative abundance of a particular compound was calculated as the proportion of its area to all gas chromatograph peak areas combined.

STATISTICAL ANALYSIS

The relative concentrations of the cuticular hydrocarbons were analyzed by a factorial analysis of variance (ANOVA) with extraction method, natal host, age (maturation), and compound as independent variables. Original data were transformed using Box-Cox transformation in order to ensure normality and homoscedasticity of the data. Data also were analyzed by a multivariate analysis (MANOVA) with a canonical analysis, which reduces the dimensionality of the data and, by means of a 2-dimensional graph, shows the grouping patterns of each of the treatments studied. All analyses were performed using the statistical software R version 3.6.2 (R Core Team 2019).

Results

In total, we found 12 compounds, but only 9 of them were identified. The identified compounds were linear alkanes (n-heneicosane, n-nonacosane, and n-hentriacontane), alkenes (n-heneicosene, n-tri-

cosene, n-nonacosene, and n-hentriacontene), and branched alkanes (2-methyl-octacosane and 2-methyl-triacontane) (Fig. 1). Our results showed that n-heneicosene, n-heneicosane, and n-tricosene were present only in mature males (sex-specific), while n-nonacosene was found only in mature flies of both sexes (age-specific).

The factorial analysis showed that the relative concentrations of cuticular hydrocarbons of *A. obliqua* were affected by the extraction method, natal host, age, and compound (Table 1). Moreover, most of the first, second, and order interactions were significant, except method × host, method × age, and method × host × age (Table 1). The solvent extraction method extracted more cuticular hydrocarbons from flies reared on mango compared to those extracted from flies reared on hog plum. In contrast, solid-phase microextraction extracted a higher concentration of cuticular hydrocarbons from flies reared on hog plum than those extracted from flies reared on mango (Fig. 2A). The differences between the 2 extraction techniques with respect to the age of the flies were not apparent. However, immature flies had higher levels of cuticular hydrocarbons than mature flies; mature females had higher concentrations of cuticular hydrocarbons than mature males (Fig. 2B). The solvent extraction method performed better for most of the compounds, except with unknown 1, hentriacontane, and unknown 2, which were extracted more easily

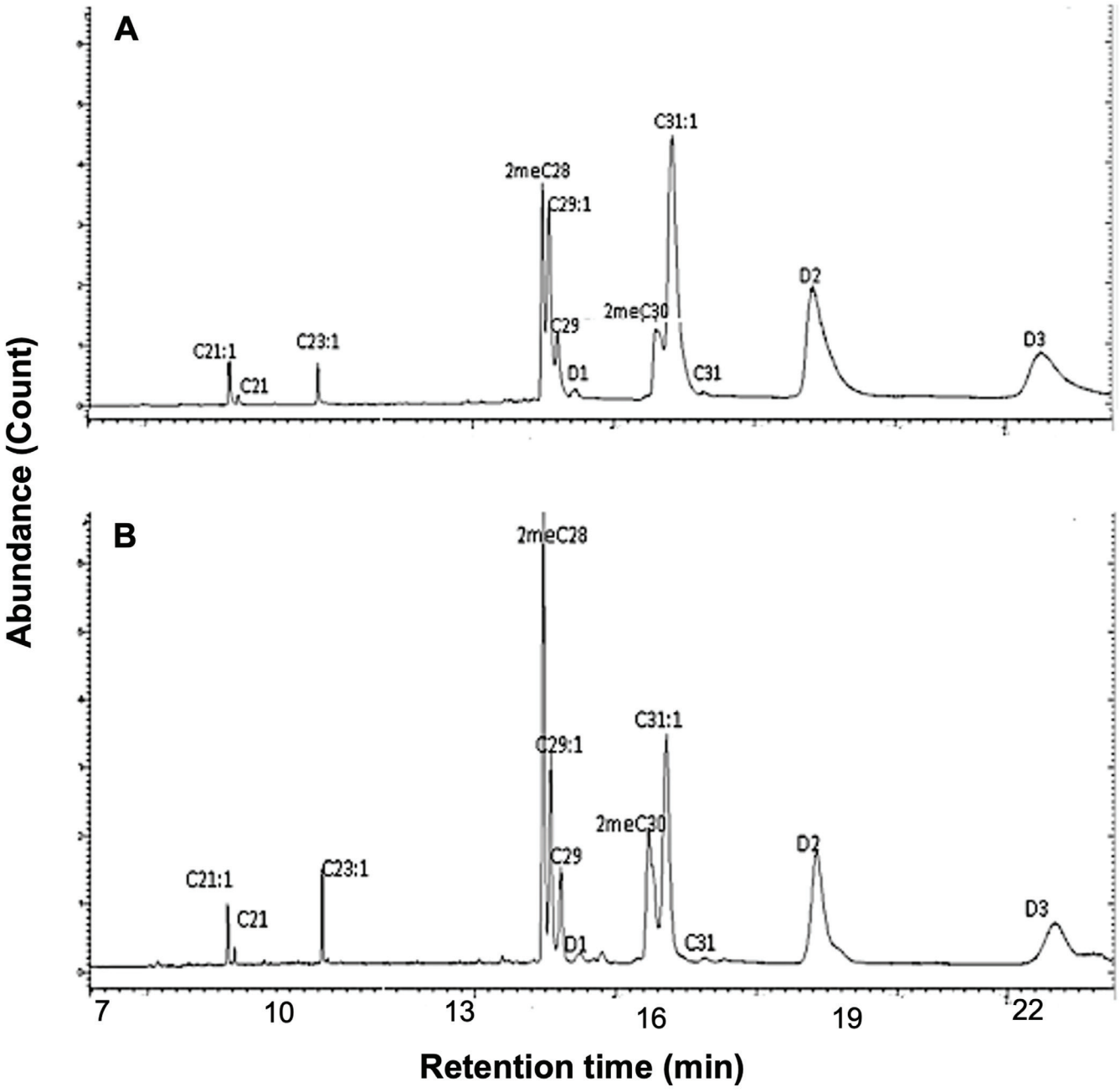

Fig. 1. Representative gas chromatograms of hexane extracts (A) and solid-phase microextraction (B) of *Anastrepha obliqua* males from mango and *Spondias mombin*, respectively. 1: n-heneicosene; 2: n-heneicosane; 3: n-tricosene; 4: 2-methyl-octacosane; 5: n-nonacosene; 6: n-nonacosane; 7: unknown compound 1; 8: 2-methyl-triacontane; 9: n-hentriacontene; 10: n-hentriacontane; 11: unknown compound 2; 12: unknown compound 3.

Table 1. Factorial analysis of the effect of the extraction method, natal host, age (maturation), and identified compounds on the cuticular hydrocarbons of *Anastrepha obliqua* adults.

	Sum sq	Df	F value	Pr (> F)	
Method	0.135	1	19.144	1.40E–05	***
Host	0.111	1	15.8156	7.71E–05	***
Age	0.213	3	10.0642	1.69E–06	***
Compound	140.593	7	2,852.7871	< 2.20E–16	***
Method*Host	0.001	1	0.1312	7.17E–01	
Method*Age	0.054	3	2.5616	0.0538647	
Method*Compound	3.000	7	60.8785	< 2.20E–16	***
Host*Age	0.084	3	3.9573	0.0081713	**
Host*Compound	0.821	7	16.6671	< 2.20E–16	***
Compound*Age	8.032	21	54.3271	< 2.20E–16	***
Method*Host*Age	0.034	3	1.5943	0.1894330	
Method*Host*Compound	0.128	7	2.6055	0.0116589	*
Method*Compound*Age	0.365	21	2.4670	0.0002952	***
Host*Compound*Age	0.674	21	4.5556	1.13E–10	***
Method*Host*Compound*Age	0.299	21	2.0227	0.0044622	**
Residuals	4.956	704			

with the solid-phase microextraction technique (Fig. 2C). The natal host did not strongly influence the relative amount of cuticular hydrocarbons on the flies of different ages. However, immature flies, males, and females presented a higher relative amount of cuticular hydrocarbons compared to both sexes of mature flies (Fig. 2D). Unknown 3, unknown 2, hentriacontane, and 2-methyl-octacosane were the most abundant compounds, while n-hentriacontane, unknown 1, n-nonacosene, and 2-methyl-triacontene were the less abundant compounds, independent of the fly origin (Fig. 2E). The relative amounts of unknown 1, n-hentriacontane, and unknown 2 were similar in mature and immature flies. However, the relative amount of 2-methyl-triacontane and hentriacontane was higher in mature flies than in immature flies (Fig. 2F). The relative amounts of 2-methyl-octacosane, n-nonacosane, and unknown 3 were higher in immature flies than in mature flies. The relative amounts of unknown compound 3 was higher markedly in immature females, as opposed to its low relative amount in mature males.

The MANOVA analysis explained 83.6% of the total variability of the data, with 6 possible groups (Pillai: 3.55; $F = 704$; $P < 0.001$). The separations were influenced more by the extraction method and age, independent of sex (Fig. 3). The largest group was found in the 2 left quadrants. This group was influenced highly by 2-methyl-octacosane, n-nonacosane, n-hentriacontane, and unknown 3, which were extracted at a high level in immature flies. Under that same group (lower left quadrant of Fig. 3), there was a group formed by treatments of immature flies, whose cuticular hydrocarbons were extracted with solid-phase microextraction. This subgroup was influenced highly by unknown 1 (Fig. 3). In the upper right quadrant

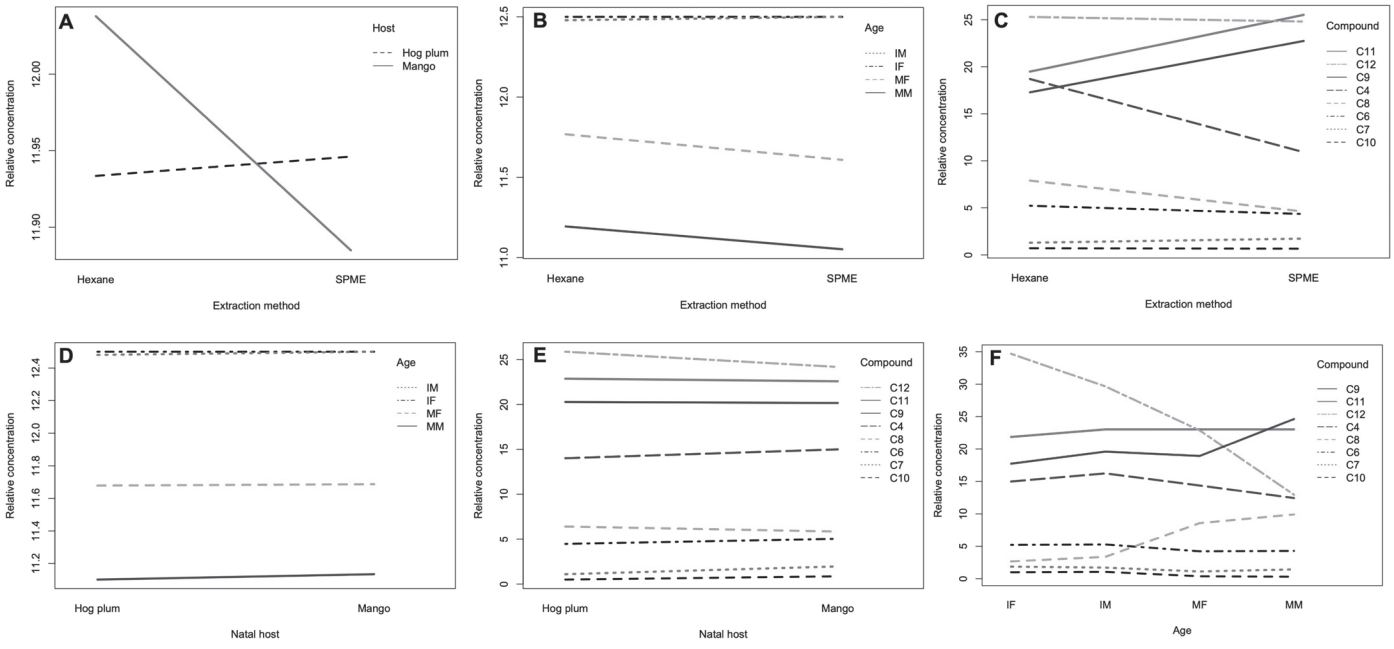

Fig. 2. Interaction graph of the analysis of the cuticular compounds of *Anastrepha obliqua* with different extraction methods, natal host, and age. IM = immature male, IF = immature female, MM = mature male, MF = mature female. C4: 2-methyl-octacosane; C6: n-nonacosane; C7: unknown compound 1; C8: 2-methyl-triacontane; C9: n-hentriacontene; C10: n-hentriacontane; C11: unknown compound 2; C12: unknown compound 3.

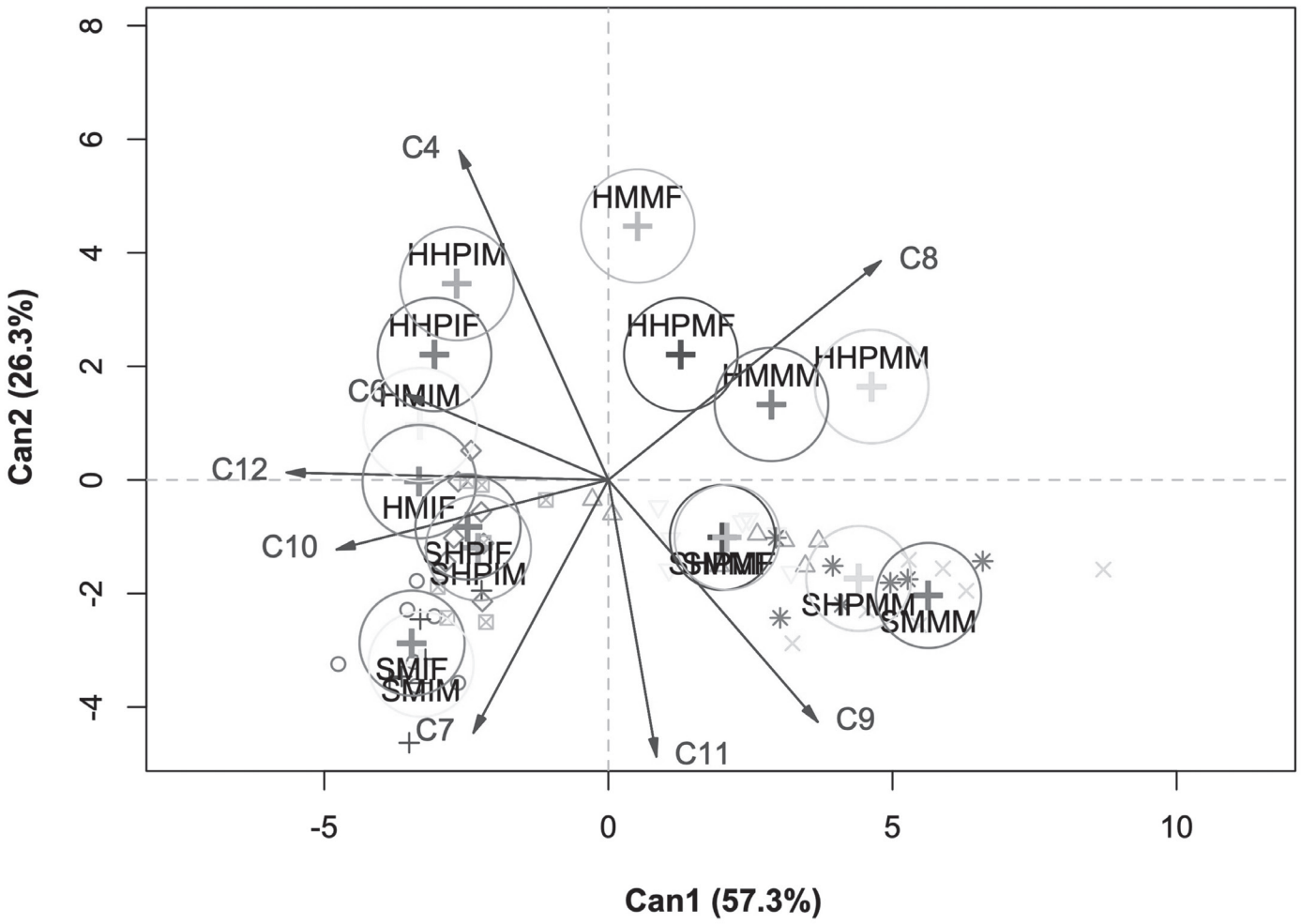

Fig. 3. Canonical discriminant analysis plot of the association between the cuticular compounds of *Anastrepha obliqua* with different extraction methods, natal host, and age. C4: 2-methyl-octacosane; C6: n-nonacosane; C7: unkown compound 1; C8: 2-methyl-triacontane; C9: n-hentriacontene; C10: 10, n-hentriacontane; C11: unknown compound 2; C12: unknown compound 3. HMIF = hexane, mango, immature female; HMIM = hexane, mango, immature male; HMMF = hexane, mango mature female; HMMM = hexane, mango mature male; SMIF = solid-phase microextraction, mango, immature female; SMIM = solid-phase microextraction, mango, immature male; SMMF = solid-phase microextraction, mango, mature female; SMMM = solid-phase microextraction, mango, mature male; HHPIF = hexane, hog plum, immature female; HHPIM = hexane, hog plum, immature male; HHPMM = hexane, hog plum, mature female; HHPMM = hexane, hog plum, mature male; SHPIF = solid-phase microextraction, hog plum, immature female; SHPIM = solid-phase microextraction, hog plum, immature male; SHPMF = solid-phase microextraction, hog plum, mature female; SHPMM = solid-phase, hog plum, mature male.

of Figure 3, there is a group composed of the hexane, mango mature female treatment. This group was influenced by 2-methyl-octacosane and 2-methyl-triacontene; both compounds were extracted mostly with hexane. The other group, in the same quadrant of Figure 3, was influenced highly by 2-methyl-triacontene. This group was made up of cuticular hydrocarbons of mature flies extracted with hexane. The last group was influenced by unknown 2 and n-hentriacontene, and it was composed of treatments of mature flies. These compounds mostly were extracted by solid-phase microextraction (Fig. 3).

The correlation matrix showed a positive relationship between 2-methyl-octacosane and n-nonacosane, n-nonacosene, and n-hentriacontene, and unknown 1 and n-hentriacontane. In Figure 3, the vectors of these compounds are close to each other. A negative correlation also was found between 2-methyl-octacosane and n-hentriacontene, 2-methyl-octacosane and unknown 2, and 2-methyl-triacontane and unknown 2. In Figure 3, the vectors of these compounds are in opposite directions.

Discussion

In this study, using 2 extraction techniques, we identified the cuticular hydrocarbons of female and male *A. obliqua*. Traditionally, insect cuticular hydrocarbons have been extracted by rinsing whole specimens in pentane, hexane, or heptane (Carlson & Service 1979; Haverty et al. 1996). One of the issues with solvent extraction is that non-cuticular hydrocarbons can be extracted either from the cuticle or from the internal organs. The extraction of non-cuticular hydrocarbons may be minimized by reducing the extraction time (Haverty et al. 1996), although this may affect the amount of cuticular hydrocarbons extracted (Drijfhout et al. 2009). The sole extraction of cuticular hydrocarbons is crucial when the study aims to determine what compounds are involved in insect communication. The solid-phase microextraction technique also has been used to extract cuticular hydrocarbons from insects (Moneti et al. 1997). It has been suggested that hydrocarbon profiles obtained from solid-phase microextraction samples are more characteristic of the insect's cuticular compounds compared with sol-

vent extraction (Ginzel et al. 2003; Kather & Martin 2012). One of the advantages of the solid-phase microextraction is that model insects are not killed and can be used for further assays (Monnin et al. 1998; Everaerts et al. 2010; Ginzel 2010); however, solid-phase microextraction has some disadvantages, including high cost and fragility of fibers (Turillazzi et al. 1998). Some authors suggest corroborating the hydrocarbon profiles with solvent extractions because solid-phase microextraction is a non-exhaustive extraction technique (De Pasquele et al. 2005, 2007).

In our study, solvent extraction and solid-phase microextraction extracted the same compounds, although there were quantitative differences in the relative amounts of the extracted cuticular hydrocarbons. In this manner, the solvent extraction technique extracted a higher amount of 2-methyl-octacosane and 2-methyl-triacontane than when solid-phase microextraction was used. The opposite was found with n-hentriacontene and unknown compound 2. The quantitative differences observed between the techniques might be explained by several reasons. For instance, the solid-phase microextraction fiber contacted only the thorax, abdomen, and wings of flies, whereas the entire fly was submerged in hexane. Also, the number of individuals and the sampling time were different for each technique. Thus, the solid-phase microextraction technique extracted only the superficial cuticular hydrocarbons from specific parts of the body, whereas the solvent extraction technique can extract cuticular hydrocarbons from external and internal parts of the whole body. It is possible that cuticular hydrocarbons were extracted in greater amounts by the solid-phase microextraction technique because it is more abundant in the body surface. In general, our results agree with other published studies that have evaluated the performance of both techniques in the extraction of cuticular hydrocarbons in a number of insect orders (Moneti et al. 1997; Roux et al. 2002; Bland et al. 2003; Geiselhardt et al. 2009; Everaerts et al. 2010). For example, Moneti et al. (1997) found that cuticular hydrocarbon profiles of the social wasps *Vespa crabro* L., *Vespa orientalis* F., and *Polistes dominulus* (Christ) (all Hymenoptera: Vespidae) were qualitatively similar when extracted with hexane and solid-phase microextraction; however, they reported that greater amounts of high-molecular-weight hydrocarbons of *V. crabro* were extracted with solid-phase microextraction compared to the solvent extraction technique. The cuticular hydrocarbon profiles of *Nauphoeta cinerea* (Olivier) (Blattodea: Blaberidae) dominant and subordinate males did not differ qualitatively using solid-phase microextraction and pentane extraction (Roux et al. 2002). The cuticular hydrocarbon profiles of cockroach males were dissimilar between solid-phase microextraction and solvent extraction regarding the ratio of some compounds (Roux et al. 2002). In contrast, Ferreira-Caliman et al. (2012) found qualitative and quantitative differences in the cuticular hydrocarbon profiles of *Melipona marginata* Lepeletier and *Apis mellifera* L. (both Hymenoptea: Apidae) workers when sampled with hexane and solid-phase microextraction.

The cuticular hydrocarbons found in *A. obliqua* have been reported in a number of insect species (Lockey 1976; Page et al. 1997; Ferreira-Caliman et al. 2012; Curtis et al. 2013). For instance, n-heneicosane, n-tricosene, n-nonacosene, n-nonacosane, n-hentriacontene, and n-hentriacontane have been found in *A. mellifera* workers (Ferreira-Caliman et al. 2012). Compounds 2-methyl-octacosane and 2-methyl-tricontane have been reported to be present in the epicuticle of both sexes of *Drosophila subquinaria* Spencer and *Drosophila recens* Wheeler (both Diptera: Drosophilidae) (Curtis et al. 2013). Interestingly, we found a low number of cuticular hydrocarbons in *A. obliqua* compared to those reported for other tephritid fruit flies (Vaníčková et al. 2012, 2014, 2015). For instance, almost 60 cuticular hydrocarbons were reported on the body surfaces of 4 species of *Ceratitis* (Diptera: Tephritidae), consisting of straight-chained and methyl-branched alkanes, alkenes, alkadienes, and alkatrienes (Vaníčková et al. 2014). A total of 69 cuticular hydrocarbons were identified in the epicuticle of *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) species complex, including 15 n-alkanes (C11–C29), 28 monomethyl alkanes, 17 alkenes, 7 alkadienes, and 1 alkatriene (Vaníčková et al. 2015). In contrast, a recent study showed that cuticular hydrocarbon profiles from wild and mass-reared *Anastrepha ludens* Loew (Diptera: Tephritidae) males contained 2 n-alkanes, 5 monomethyl alkanes, and 2 alkanes (Bosa et al. 2018). The cuticular hydrocarbon profile of *A. obliqua* more closely resembles that of *A. ludens* than *A. fraterculus*. *Anastrepha ludens*, *A. obliqua*, and *A. fraterculus* belong to the *A. fraterculus* species group; therefore, the difference in the number of cuticular hydrocarbons among these 3 species is noteworthy. However, interspecies relationships among the *A. fraterculus* group are not yet resolved; this group is composed of 8 taxonomically recognized morphotypes (Hernández-Ortiz et al. 2012, 2015).

Intrinsic and extrinsic factors can affect the cuticular hydrocarbon profiles of insects. In our case, we found that the natal host, age, and sex qualitatively and quantitatively affected the cuticular hydrocarbon profiles of wild *A. obliqua* adults. Previously, studies have shown that diet significantly influences the cuticular hydrocarbon profiles in insects (Francis et al. 1989; Stennett & Etges 1997; Liang & Silverman 2000). In the only study carried out so far that investigated the effect of the natal host on the cuticular hydrocarbon profiles with a species of Tephritidae, Carlson and Yocom (1986) reported a quantitative variation in the profile of *A. ludens* adults reared from loquat, guava, cattley guava, and laboratory diet. Stennett and Etges (1997) found that the cuticular hydrocarbons of *Drosophila mojavensis* Patterson and *Drosophila arizonae* Ruiz and Wasserman (both Diptera: Drosophilidae) varied qualitatively and quantitatively, depending on whether they were reared on fermenting agria or organ pipe cactus tissues and laboratory food. Furthermore, Stennett and Etges (1997) reported that the abundance of C29-branched alkanes was greater in flies reared on pipe cactus than those reared on agria or laboratory food. A recent study with *A. obliqua* has shown that the natal host can affect the qualitative and quantitative profile of volatile compounds emitted by calling males (Aluja et al. 2020). For instance, males reared on *P. guajava*, an occasional host of *A. obliqua*, released 8 compounds, while males reared on *S. mombin*, the ancestral host of *A. obliqua*, emitted only 4 compounds.

Besides the quantitative differences observed in the cuticular hydrocarbon profiles of *A. obliqua*, we found that n-nonacosene is a compound that appears only in older flies of both sexes. Vaníčková et al. (2012) reported quantitative age-dependent dynamics in the cuticular hydrocarbon profiles of *A. fraterculus* adults. Young females and males have a similar cuticular composition. However, the cuticular hydrocarbon profiles of both sexes began to deviate from d 7 after eclosion; the profiles were different between sexes in older flies (20–30 d after eclosion). In *A. ludens*, 2-methyl-hexacosane, n-nonacosene, and 2-methyl-triacontane varied significantly among wild males of different ages (Bosa et al. 2018). Total hydrocarbon abundance of *D. mojavensis* changed from eclosion to 14 to 18 d, past the age at sexual maturity, and then decreased in older flies (Etges & de Oliveira 2014). The abundance of most saturated hydrocarbons and methyl-branched hydrocarbons decreased in 4 to 6 d old *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) males; in females, most short-chain hydrocarbons decreased but (*Z*)-5-pentacosene and 2-methyl-octacosane increased with age (Everaerts et al. 2010).

We noted also that n-heneicosene, n-heneicosane, and n-tricosene were present only in older *A. obliqua* males. A similar observation was observed in *A. fraterculus* (Vaníčková et al. 2012), where it was reported that the cuticle of mature males had a series of 7-monoenes,

including 7-henicosene, 7-docosene, 7-tricosene, 7-pentacosene, and 7-tritriacontene, which were absent in mature females. Our results and those of Vaníčková et al. (2012) suggest that these cuticular hydrocarbons may have a sexual function, possibly playing a role as a contact sex pheromone. However, to our knowledge, it is unknown whether contact chemical signals play a role in the courtship and mating in a tephritid fruit fly species. Cuticular hydrocarbons can play a significant role in the courtship and mating of Diptera species (Ferveur & Cobb 2010). For instance, chemical and behavioral analyses showed that a fraction of 4 monoenes mediated the courtship behavior of *Drosophila virilis* Sturtevant (Diptera: Drosophilidae); however, only (*Z*)-11-pentacosene evoked all courtship acts of this species (Oguma et al. 1992). The male copulatory response of *Glossina austeni* (Newstead) (Diptera: Glossinidae) is evoked by a blend of 5 alkenes present on the cuticle of conspecific females (Carlson et al. 2005). In contrast, 9-tricosene, 7-tricosene, 5-tricosene, and tricosane adversely regulated courtship and mating of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), although all of these hydrocarbons were present in sexually mature flies (Snellings et al. 2018).

In summary, our results showed that the extraction technique, natal host, age, and sex affected the cuticular hydrocarbons of *A. obliqua*. Remarkably, we found that n-nonacosene is an age-specific compound because it appears only in mature females and males. Our results also showed that n-heneicosene, n-heneicosane, and n-tricosene are present in mature males but not in mature females. Further behavioral assays are needed to confirm any possible involvement of these compounds in the sexual behavior of *A. obliqua*.

Acknowledgments

We thank Jorge Toledo and Azucena Oropeza for providing biological material used in the cuticular extractions, and Leopoldo Cruz-López and Antonio Santiesteban for their valuable assistance in identifying the compounds.

References Cited

Aluja M, Cabagne G, Altúzar-Molina A, Pascacio-Villafán C, Enciso E, Guillén L. 2020. Host plant and antibiotic effects on scent bouquet composition of *Anastrepha ludens* and *Anastrepha obliqua* calling males, two polyphagous tephritid pests. Insects 11: 309. doi: 10.3390/insects11050309

Birke A, Guillén L, Midgarden D, Aluja M. 2013. Fruit flies, *Anastrepha ludens* (Loew), *A. obliqua* (Macquart) and *A. grandis* (Macquart) (Diptera: Tephritidae): three pestiferous tropical fruit flies that could potentially expand their range to temperate areas, pp. 192–213 *In* Peña JE [ed.], Potential Invasive Pests. CAB International, Wallingford, United Kingdom.

Bland JM, Osbrink WL, Cornelius ML, Lax AR, Craig V. 2003. Detection of termite cuticular hydrocarbons by solid-phase microextraction (SPME). Sociobiology 41: 91–104.

Blomquist GJ, Bagnères AG. 2010. Insect Hydrocarbons. Biology, Biochemistry and Chemical Ecology. Cambridge University Press, Cambridge, United Kingdom.

Blomquist GJ, Nelson D, De Renobales M. 1987. Chemistry, biochemistry and physiology of insect cuticular lipids. Archives of Insect Biochemistry and Physiology 6: 227–265.

Bosa CF, Cruz-López L, Guillén-Navarro K, Zepeda-Cisneros CS, Liedo P. 2018. Variation in the cuticular hydrocarbons of the Mexican fruit fly *Anastrepha ludens* males between strains and age classes. Archives of Insect Biochemistry and Physiology 99: e21513. doi: 10.1002/arch.21513

Carlson DA, Service MW. 1979. Differentiation between species of the *Anopheles gambiae* Giles complex (Diptera: Culicidae) by analysis of cuticular hydrocarbons. Annals of Tropical Medicine and Parasitology 73: 589–592.

Carlson DA, Yocom SR. 1986. Cuticular hydrocarbons from six species of tephritid fruit flies. Archives of Insect Biochemistry and Physiology 3: 397–412.

Carlson DA, Mramba F, Sutton BD, Bernier UR, Geden CJ. 2005. Sex pheromone of the tsetse species, *Glossina austeni*: isolation and identification of natural hydrocarbons, and bioassay of synthesized compounds. Medical and Veterinary Entomology 19: 470–479.

Chung H, Carroll SB. 2015. Wax, sex and the origin of species: dual roles of insect cuticular hydrocarbons in adaptation and mating. BioEssays 37: 822–830.

Crespo R, Juárez MP, Cafferata LF. 2000. Biochemical interaction between entomopathogenous fungi and their insect-host-like hydrocarbons. Mycology 92: 528–536.

Curtis S, Sztepanacz JL, White BE, Dyer KA, Rundle HD, Mayer P. 2013. Epicuticular compounds of *Drosophila subquinaria* and *D. recens*: identification, quantification, and their role in female mate choice. Journal of Chemical Ecology 39: 579–590.

De Pasquale C, Jones A, Charlton A, Alonzo G. 2005. Use of SPME extraction to determine organophosphorus pesticides adsorption phenomena in water and soil matrices. International Journal of Environmental Analytical Chemistry 85: 1101–1115.

De Pasquale C, Guarino S, Peri E, Alonzo G, Colazza S. 2007. Investigation of cuticular hydrocarbons from *Bagrada hilaris* genders by SPME/GC-MS. Analytical Bioanalytical Chemistry 389: 1259–1265.

Drijfhout F, Martin S, Kather R. 2009. The role of cuticular hydrocarbons in insects, pp. 91–114 *In* Zang W, Liu H [eds.], Behavioral and Chemical Ecology. Nova Science Publishers, Hauppauge, New York, USA.

Etges WJ, de Oliveira CC. 2014. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. X. Age-specific dynamics of adult epicuticular hydrocarbon expression in response to different host plants. Ecology and Evolution 4: 2033–2045.

Everaerts C, Farine JP, Cobb M, Ferveur JF. 2010. *Drosophila* cuticular hydrocarbons revisited: mating status alters cuticular profiles. PloS One 5: e9607. doi: 10.1371/journal.pone.0009607

Francis BR, Blanton WE, Littlefield JL, Nunamaker RA. 1989. Hydrocarbons of the cuticle and hemolymph of the adult honey bee (Hymenoptera: Apidae). Annals of Entomological Society of America 82: 486–494.

Ferreira-Caliman MJ, Turatti ICC, Lopes NP, Zucchi R, Nascimento FS. 2012. Analysis of insect cuticular compounds by non-lethal solid phase micro extraction with styrene-divinylbenzene copolymers. Journal Chemical of Ecology 38: 418–426.

Ferveur JF, Cobb M. 2010. Behavioral and evolutionary roles of cuticular hydrocarbons in Diptera, pp. 325–343 *In* Blomquist GJ, Bagnères AG [eds.], Insect Hydrocarbons. Biology, Biochemistry and Chemical Ecology. Cambridge University Press, Cambridge, United Kingdom.

Geiselhardt S, Otte T, Hilker M. 2009. The role of cuticular hydrocarbons in male mating behavior of the mustard leaf beetle, *Phaedon cochleariae* (F.). Journal of Chemical Ecology 35: 1162–1171.

Gibbs AG. 1998. Water-proofing properties of cuticular lipids. American Zoologist 38: 471–482.

Ginzel MD. 2010. Hydrocarbons as contact pheromones of longhorned beetles (Coleoptera: Cerambycidae), pp. 375–389 *In* Blomquist GJ, Bagnères AG [eds.], Insect Hydrocarbons. Biology, Biochemistry and Chemical Ecology. Cambridge University Press, Cambridge, United Kingdom.

Ginzel MD, Blomquist GJ, Millar JG, Hanks LM. 2003. Role of contact pheromones in mate recognition in *Xylotrechus colonus*. Journal of Chemical Ecology 29: 533–545.

Haverty MI, Grace JK, Nelson LJ, Yamamoto RT. 1996. Intercaste, intercolony, and temporal variation in cuticular hydrocarbons of *Copotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Journal of Chemical Ecology 22: 1813–1834.

Hernández-Ortiz V. 2007. Diversidad y biogeografía del género *Anastrepha* en México, pp. 53–76 *In* Hernández-Ortiz V [ed.], Moscas de la Fruta en Latinoamérica (Diptera: Tephritidae): Diversidad, Biología y Manejo. S y G Editores, Distrito Federal, Mexico.

Hernández-Ortiz V, Bartolucci AF, Morales-Valles P, Frías D, Selivon D. 2012. Cryptic species of the *Anastrepha fraterculus* complex (Diptera: Tephritidae): a multivariate approach for the recognition of South American morphotypes. Annals of the Entomological Society of America 105: 305–318.

Hernández-Ortiz V, Canal NA, Trigero Salas JO, Ruíz-Hurtado FM, Duzul-Cauich D. 2015. Taxonomy and phenotypic relationships of the *Anastrepha fraterculus* complex in the Mesoamerican and Pacific Neotropical dominions (Diptera, Tephritidae). ZooKeys 540: 95–124.

Howard R. 1993. Cuticular hydrocarbons and chemical communication, pp. 179–226 *In* Stanley-Samuelson D, Nelson D [eds.], Insect Lipids: Chemistry, Biochemistry and Biology. University of Nebraska Press, Lincoln, Nebraska, USA.

Kather R, Martin SJ. 2012. Cuticular hydrocarbon profiles as a taxonomic tool: advantages, limitations and technical aspects. Physiological Entomology 37: 25–32.

Liang D, Silverman J. 2000. "You are what you eat": diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. Naturwissenschaften 87: 412–416.

Lockey KH. 1976. Cuticular hydrocarbons of *Locusta*, *Schistocerca*, and *Periplaneta*, and their role in waterproofing. Insect Biochemistry 6: 457–472.

Nation J. 2002. Insect Physiology and Biochemistry. CRC Press, Boca Raton, Florida, USA.

Moneti G, Dani F, Pieraccini G. 1997. Solid-phase microextraction of insect epicuticular hydrocarbons for gas chromatographic/mass spectrometric analysis. Rapid Communications in Mass Spectrometry 11: 857–862.

Monnin T, Malosse C, Peeters C. 1998. Solid-phase microextraction and cuticular hydrocarbon differences related to reproductive activity in queenless ant *Dinoponera quadriceps*. Journal of Chemical Ecology 24: 473–490.

Norrbom AL, Kim KC. 1988. A list of the reported host plants of the species of *Anastrepha* (Diptera: Tephritidae). USDA-APHIS-PPQ Publication #81-52. US Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Washington, DC, USA.

Oguma Y, Nemoto T, Kuwahara Y. 1992. (*Z*)-11-Pentacosene is the major sex pheromone component in *Drosophila virilis* (Diptera). Chemoecology 3: 60–64.

Ortiz-Urquiza A, Keyhani NO. 2013. Action on the surface: entomopathogenic fungi versus the insect cuticle. Insects 4: 357–374.

Page M, Nelson LJ, Blomquist GJ, Seybold SJ. 1997. Cuticular hydrocarbons as chemotaxonomic characters of pine engraver beetles (*Ips* spp.) in the *grandicollis* subgeneric group. Journal of Chemical Ecology 23: 1053–1099.

R Core Team. 2019. R*:* a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-proje ct.org

Roux E, Sreng L, Provost E, Roux M, Clement JL. 2002. Cuticular hydrocarbon profiles of dominant versus subordinate male *Nauphoeta cinerea* cockroaches. Journal of Chemical Ecology 28: 1221–1235.

Toledo J, Lara JR. 1996. Comparison in the biology of *Anastrepha obliqua* reared in mango and in mombin, pp. 359–362 *In* McPherson BA, Steck GJ [eds], Fruit Fly Pests: A World Assessment of their Biology and Management. St. Lucie Press, Sand Key, Florida, USA.

Snellings Y, Herrera B, Wildemann B, Beelen M, Zwarts L, Wenseelers T, Callaerts P. 2018. The role of cuticular hydrocarbons in mate recognition in *Drosophila suzukii*. Scientific Reports 8: 4996. doi: 10.1038/s41598-018_23189-6

Stennett MD, Etges WJ. 1997. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. III. Epicuticular hydrocarbon variation is determined by use of different host plants in *Drosophila mojavensis* and *Drosophila arizonae*. Journal of Chemical Ecology 23: 2803–2824.

Sutton BD, Carlson DA. 1993. Interspecific variation in tephritid fruit fly larvae surface hydrocarbons. Archives of Insect Biochemistry and Physiology 23: 53–65.

Turillazzi S, Sledge MF, Moneti G. 1998. Use of a simple method for sampling cuticular hydrocarbons from live social wasps. Ethology, Ecology and Evolution 10: 293–297.

Vaníčková L, Svatoš J, Kroiss J, Kaltenpoth M, Do Nascimiento RR, Hoskovec M, Břízová R, Kalinová B. 2012. Cuticular hydrocarbons of the South American fruit fly *Anastrepha fraterculus*: variability with sex and age. Journal of Chemical Ecology 38: 1133–1142.

Vaníčková L, Virgilio M, Tomčala A, Břízová R, Ekezi S, Hoskovec M, Kalinová B, Do Nascimiento RR, De Meyer M. 2014. Resolution of three cryptic agricultural pests (*Ceratitis fasciventris*, *C. anonae*, *C. rosa*, Diptera: Tephritidae) using cuticular hydrocarbon profiling. Bulletin of Entomological Research 104: 631–638.

Vaníčková L, Břízová R, Mendonça AL, Pompeiano A, Do Nascimiento RR. 2015. Intraspecific variation of cuticular hydrocarbon profiles in the *Anastrepha fraterculus* (Diptera: Tephritidae) species complex. Journal of Applied Entomology 139: 679–689.

Vaníčková L, Nagy R, Pompeiano A, Kalinová B. 2017. Epicuticular chemistry reinforces the new taxonomic classification of the *Bactrocera dorsalis* species complex (Diptera: Tephritidae, Dacinae). PLoS One 12: e0184102. doi: 10.1371/journal.pone.0184102